

41.



**Industria y Comercio**  
**SUPERINTENDENCIA**

**SOLICITUD**  
**PATENTE DE INVENCION**

21. EXPEDIENTE N°

**05-39485**

54. TÍTULO "PRODUCTO ALIMENTICIO A BASE DE HARINA QUE COMPRENDE ALFA-  
AMILASA TERMOESTABLE"

51. CLASIFICACIÓN INTERNACIONAL A21D 8/04

71. SOLICITANTE NESTEC S.A.

DOMICILIO VEVEY SUIZA

74. REPRESENTANTE LEGAL ALICIA LLOREDA RICAURTE  
T.P. 53.215

22. BOGOTÁ, D.C. 28-11-1985

2020 99

## PETITORIO



SUPERINDUSTRIA Y COMERCIO

Radicación: 05039485 00000000

Folios: 118

Fecha (MDD): 2005-04-25 16:00:21

Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTA

Dependencia: 0020 DIVISION DE NUEVAS CREACIONES

FORMULARIO UNICO DE SOLICITUD DE PATENTE  
PCT  
ENTRADA EN FASE NACIONAL

①

## SOLICITUD DE:

☒ Patente de Invención☐ Patente de Modelo de Utilidad☐ Capítulo I.☒ Capítulo II.

②

SOLICITANTE  
(71)

Nombre: NESTEC S.A.

Dirección: Avenue Nestlé 55, CH-1800  
Vevey, Suiza.

Nacionalidad o Domicilio: Vevey, Suiza.

Lugar de Constitución: Suiza.

Teléfono: 3264270 Fax: 6069701

E-mail: jllico@lloredacamacho.com

## IDENTIFICACION

C.C. ☐NIT ☐C.E. ☐Otro ☐

Cual \_\_\_\_\_

Número \_\_\_\_\_

③

REPRESENTANTE  
O APODERADO

Nombre: ALICIA LLOREDA RICAURTE

Dirección: Calle 72 No. 5-83 Piso 5º,  
Bogotá, Colombia

Teléfono: 3264270 Fax: 6069701

E-mail: jllico@lloredacamacho.com

C.C. ☒NIT ☐C.E. ☐Otro ☐

Cual \_\_\_\_\_

Número 39.690.713 TP 53.215

④

INVENTOR(ES)  
(72)

Nombre: NICOLAS, Pierre y HANSEN, Carl, Erik.

Dirección: 18, Route du Tirage, CH-1806 Saint-Legier y Ch. du Pré-d'Yverdon  
5E, CH-1066 Epalinges.

Nacionalidad o Domicilio: Suiza.

⑤

## PCT (86)

Solicitud Internacional No. PCT/EP2003/012053Fecha: 30/10/03Publicación Internacional No. WO 2004/039162 A1Fecha: 13/05/04

⑥

Título (54) "PRODUCTO ALIMENTICIO A BASE DE HARINA QUE COMPRENDE ALFA-AMILASA  
TERMOESTABLE"

⑦

Clasificación Internacional (51) A21D 8/04

⑧

## Prioridad

SI ☒ NO ☐

(33) País de Origen

(32) Fecha

(31) Número de Solicitud

EP

30/10/02

02024497.6

⑨

Comprobante de Pago No. 05 - 27.804Fecha: 15/04/05

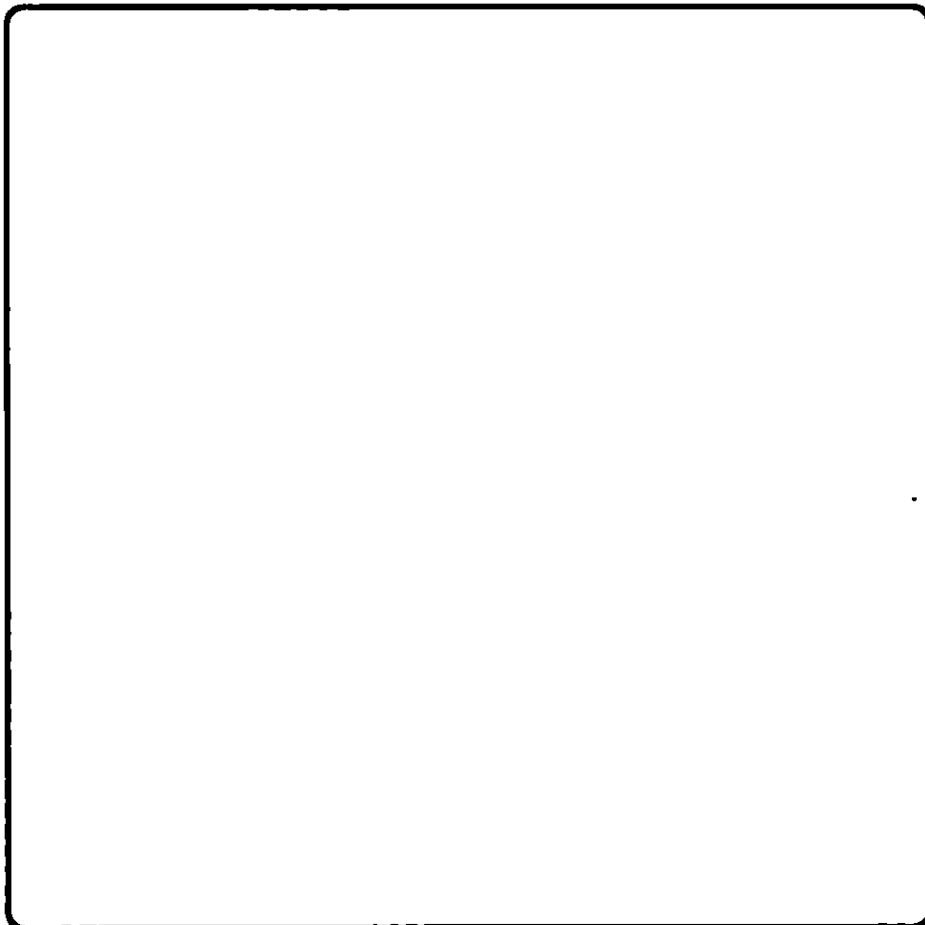
10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☒ Documento que acredite la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Documento de Representación Legal.
- ☒ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☐ Copia de la solicitud Internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud Internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☒ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ Arte Final 12X12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☒ Otros: Reporte de Búsqueda Internacional con su traducción, anexos del reporte de búsqueda internacional, capítulo reivindicatorio originalmente presentado en Fase Internacional con su traducción y anexo explicativo de modificaciones a las reivindicaciones.

11

FIGURA CARACTERISTICA:



12

Solicito la concesión de la patente

NOMBRE: ALICIA ILLOREDA RICAURTE

FIRMA:

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

SUPERINDUSTRIA Y COMERCIO  
Radicacion : 05039405 00000000 Folios: 118  
Fecha (AND): 2005-04-26 16:00:21  
Tramite : 011 PCT-PATENT 379 FASE NDCI 411 PRESENTA  
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 05 - 27,604  
FECHA : ABRIL 15 DE 2005

##### CONSIGNACION #####

| DEPOSITANTE        | TIPO PAGO    | BANCO         | CUENTA      | No. PAGO | FECHA PGO  | Vr. PAGO     |
|--------------------|--------------|---------------|-------------|----------|------------|--------------|
| LLOREDA Y CIA S. A | CONSIGNACION | BANCO POPULAR | 050-00110-6 | 29721127 | 15/04/2005 | 9,692,000.00 |

##### C O N C E P T O #####

| CANT. | RENTISTICO              | CONCEPTO                            | TOTAL CONCEPTO |
|-------|-------------------------|-------------------------------------|----------------|
| 1     | 50005-01-01 SOLICITUDES | 1 TRAMITES DE SOL. DE PATENTE DE IN | 443,000.00     |
|       |                         | TOTAL :                             | \$ 443,000.00  |

SON: CUATROCIENTOS CUARENTA Y TRES MIL PESOS

RESPONSABLE : \_\_\_\_\_

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. \_\_\_\_\_

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**ANEXO**

El documento que acredita la calidad de REPRESENTANTES LEGALES en Bogotá de los doctores **ALICIA LLOREDA y/o GUSTAVO TAMAYO y/ IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ** para TODOS LOS ASUNTOS RELACIONADOS CON LA PROPIEDAD INDUSTRIAL de la sociedad peticionaria, que faculta a cada uno de dichos abogados para actuar individualmente, se está presentando con esta solicitud. En consecuencia, ruego a usted reconocer la personería de cada uno de dichos Representantes Legales para actuar dentro del presente expediente, a fin de que cualquiera de ellos pueda intervenir en cualquier tiempo respecto de la solicitud en él contenida.



ALICIA LLOREDA RICAURTE

T.P. No. 53.215

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4.

TRADUCCIÓN OFICIAL No.283/04 DE UN DOCUMENTO ESCRITO EN FRANCÉS, EL CUAL PARA SU IDENTIFICACIÓN SE SELLA CON EL SELLO DEL TRADUCTOR. ESTA ES UNA TRADUCCIÓN OFICIAL EFECTUADA EL DIA 01 DE SEPTIEMBRE DE 2004 POR JEAN-JACQUES TURPIN, C.E.: 193849 DE BOGOTÁ, TRADUCTOR E INTÉRPRETE OFICIAL SEGÚN RESOLUCIÓN No. 2680/91 DEL 19 DE DICIEMBRE DE 1991, EXPEDIDA POR EL MINISTERIO DE JUSTICIA DE COLOMBIA.

Autenticaciones que se hallan a continuación de un documento de poder conferido en inglés y español por NESTEC S.A., una sociedad organizada y existe de conformidad con las leyes de Suiza, domiciliada en Avenue Nestlé 55, CH-1800 Vevey, Suiza, y otorgado a los Doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARÍA y/o ENRIQUE ÁLVAREZ, todos domiciliados en Bogotá, Colombia, Calle 72 No. 5 – 83 (Piso 6°).

Dado el 5 de agosto de 2004. Firma ilegible de la Señora Paula Nelson, Vice-Presidente de Nestec S.A.

#### CERTIFICADO NOTARIAL (SOCIEDAD)

Legalización por parte del suscrito Notario de la firma de la Señora Paula NELSON quien desempeña el cargo de Vice-Presidente de la compañía NESTEC S.A., una sociedad legalmente constituida y existente de acuerdo con las leyes de Suiza, domiciliada en Vevey (Suiza). Además, dicho Notario certifica que el signatario, según las leyes de Suiza, tiene facultad para otorgar el presente documento. Los hechos anteriores le constan por haber examinado los documentos respectivos el 24 de marzo de 1997, y de todo ello da fe.

Firma de Jean-Daniel RUMPF.

Sello: Jean-Daniel RUMPF – Notario. Estampilla de 4 Frs.

Traducción Oficial No.  
JEAN - JACQUES H. TURPIN  
Traductor e Intérprete Oficial  
FRANCÉS - ESPAÑOL  
Res. Ministerio No. 2680/91

283/0.

5

Legalización No. 7.135. El Notario Jean-Daniel RUMPF, en Vevey, Distrito de Vevey, Cantón de Vaud, Suiza, certifica la firma puesta en la página anterior por la Señora Paula Nelson, Directora adjunta de Nestec S.A., en Vevey, la cual compromete en forma válida con su firma individual.

Dado en Vevey, el once de agosto de dos mil cuatro. Firma ilegible del Notario.

Sello: Jean-Daniel RUMPF – Notario. Estampilla de 4 Frs.

#### APOSTILLA

(Convención de La Haya del 5 de octubre de 1961)

1. - País: Suiza

El presente documento público

2. - ha sido firmado por el Señor Jean-Daniel RUMPF

3. - obrando en calidad de Notario en Vevey

4. - con sello de Jean-Daniel RUMPF, Notario

#### CERTIFICADO

5. - En Lausana

6.- El 12 de agosto de 2004

7. - Por la Cancillería de Estado del Cantón de Vaud

8. - Bajo el número 6705

9. - Sello: Cancillería del Consejo de Estado – Libertad y Patria  
de 25 Fr.

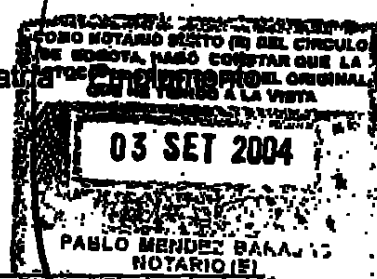
10. - Por el Canciller de Estado, firma de P. Equey.

Es traducción fiel y completa de la cual se deja copia en los archivos de esta oficina para confrontación y a la cual me remito.

Traducción oficial No. 283/04.

Traductor: Jean-Jacques Turpin.

Bogotá, D.C., 1 de septiembre de 2004.



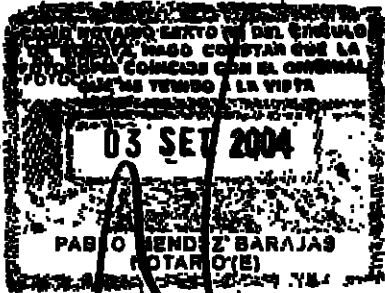
Traducción Oficial No. 283/04  
JEAN - JACQUES H. TURPIN  
Traductor e Interpreté Oficial  
FRANCOIS - ESPANOL  
Ministerio No. 2480/91

MINISTERIO DE RELACIONES  
EXTERIORES

*Fernando Tamayo*

661229

CUYA FIRMA APARECE EN EL  
DOCUMENTO DESEMPEÑA  
LAS FUNCIONES INDICADAS



NO SE ASUME  
RESPONSABILIDAD DEL TEXTO

2004 SEP -2 A 11: 09

*WLS*  
JEFE DE LEGALIZACIONES  
SANTAFE DE BOGOTA D.C.

Los abajo firmados, NESTEC S.A., una sociedad organizada y existente de conformidad con las leyes de Suiza

con domicilio en Avenue Nestlé 55, CH-1800  
Vevey, Suiza

nombramos por el presente a ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, domiciliados en Bogotá, Colombia, con oficinas en dicha ciudad, Calle 72 No. 5-83, piso 6o., nuestros representantes legales y verdaderos en Bogotá, con facultades para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales, con el fin de dar cumplimiento al parágrafo 1o. del artículo 543 del decreto 410 de 1971.

Los doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, o los apoderados que cualquiera de ellos designe, quedan especialmente facultados para representarnos ante las autoridades colombianas, administrativas, judiciales o de policía, en todos los trámites relativos a la propiedad Industrial, a cuyo efecto les facultamos para dar ante dichas autoridades todos los pasos necesarios al objeto indicado; elevar solicitudes, declaraciones, reclamos, recursos y presentar desistimientos; formular descripciones, enmiendas, limitar el alcance de las solicitudes, presentar oposiciones y apelaciones; instaurar cancelaciones, renunciar total o parcialmente a los registros, abonar todos los impuestos y cuotas y efectuar todo otro pago determinado por la ley; recibir todos los documentos y valores, dando el descargo respectivo; llenar cualesquiera otros requisitos y tomar, en fin, todas las medidas que creyeran conducentes al resguardo de nuestros intereses; y en caso de producirse oposición, pasando los antecedentes a los tribunales, quedan facultados para tomar intervención como demandantes o demandados ante los jueces que sean competentes, pudiendo transar, someter a árbitros, desistirse, percibir y apelar, con todas las demás facultades que resulten necesarias, y por el presente declaramos desde ahora válido y bueno cuanto dichos representantes y/o los apoderados que cualquiera de ellos nombre hicieren en nuestro beneficio.

Asimismo, facultamos a los doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, para solicitar en la Oficina de Propiedad Industrial la protocolización de este documento y de cualquier poder que cualquiera de ellos otorgue a nombre nuestro.

Dado y firmado

The undersigned, NESTEC S.A., a corporation organized and existing under the laws of Switzerland.

domiciled at Avenue Nestlé 55, CH-1800  
Vevey, Switzerland

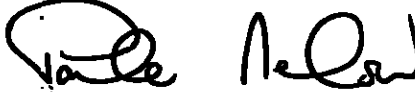
hereby appoint, ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ, of Bogotá, Colombia, with offices in said town, Calle 72 No. 5-83, 6th Floor, our true and legal representatives in Bogotá, with power to receive notifications and to appoint judicial or extrajudicial agents, so as to comply with paragraph 1o. of article 543 of decree 410 of 1971.

Doctors ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ, or the agents by any one of them appointed, are especially empowered to represent us before the administrative, judicial or police authorities of Colombia, in all proceedings pertaining to Industrial property, for which purpose we authorize them to take before the said authorities all the necessary steps for the said end; to file petitions, declarations, claims, recourses and withdrawals, to draw up specifications; to file amendments; to limit the scope of applications, file oppositions; file cancellations total or partially waive registrations, to pay all installments and effect all other payments prescribed by law; to receive proper receipts, to comply with all other requirements and, in short, to take all those measures which they may deem fit for the protection of our interests; and in case of opposition, where the proceedings be passed over to the courts, we authorize said agents to act on our behalf, as complainants as well as defendants, before the competent judges, with full powers to make arrangements, to submit to arbitrators, to desist, to collect, to appeal and, in all cases, to exercise all powers as may be required and as far as they are from now on as valid and good, that said representatives and/or the agents by any one of them appointed may do for us and in our name.

We also authorize doctors ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ, to apply for protocolization of this document and of any power of attorney by any one of them conferred in our name, before the Office of Industrial Property.

Given and signed this 5th August 2004

Paula NELSON - Vice President



NOTARY SHOULD EXECUTE OTHER SIDE

CERTIFICADO NOTARIAL (SOCIEDAD)

Sulza  
Vevey

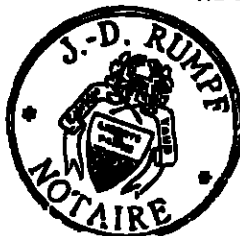
{ss

Hoy 11 de Agosto de 2004, compareció  
PAULA NELSON, a quien conozco y  
de quien me consta que firmó el anterior documento,  
y quien debidamente juramentado declaró y dijo que  
él es Vicepresidente

de NESTEC S.A., la  
compañía nombrada en el documento que antecede,  
una sociedad debidamente organizada y existente  
conforme a las leyes de Sulza, domiciliada en la  
ciudad de Vevey, Sulza legalmente constituida  
para un período de duración que aún no ha  
terminado, que ejerza su objeto social; conforme a  
las leyes de Sulza, que el declarante está autorizado  
por dicha Compañía para conferir este poder, que el  
sello estampado por él al pie de tal poder, en nombre  
y en representación de la expresada Compañía, es el  
sello oficial de ella; y que el objeto para el cual se  
confiere este Instrumento está comprendido dentro  
del de la sociedad.

El suscrito Notario ha visto los Estatutos de  
Incorporación firmados en Vevey, Sulza, con fecha  
24 Marzo, 1997, y certifica que de  
conformidad con la legislación de dicho País, la  
anterior declaración juramentada es prueba  
suficiente de que los hechos sobre que ella versa son  
clertos o verdaderos, de lo cual da fe.

Notary Public (Notario Público).



NOTARIAL CERTIFICATE (CORPORATION)

Switzerland  
Vevey

{ss

On this 11th day of August, 2004,  
personally came

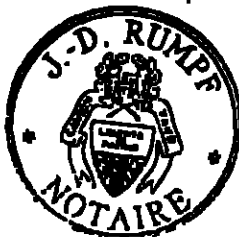
Paula NELSON  
to me known, and known to me to be the person who  
signed the foregoing instrument, who being by me  
duly sworn did depose and say that he/she is the

Vice President of NESTEC S.A., the  
company named within, a Corporation organized and  
existing under the laws of the Switzerland, domiciled  
at the City of Vevey, Switzerland legally constituted  
for a period of tenure not yet expired, which exercises  
its corporate purposes according to the laws of  
Switzerland, that the deponent is authorized by said  
Company to confer this power of attorney; that the  
seal affixed hereunto by the deponent in the name  
and on behalf of said Corporation is the corporate  
seal thereof; and that the purpose for which this  
Instrument is granted is included within the corporate  
purposes of the Company.

The undersigned Notary Public has seen the articles  
of Incorporation of said Corporation executed at  
Vevey, Switzerland, under date of March 24 1997  
and hereby certifies that under the laws of said  
County the foregoing acknowledgment is sufficient  
proof that the facts therein contained are true, which  
he attests.

Légalisation No 7'135.-

Le notaire JEAN-DANIEL RUMPF, à Vevey, District de Vevey, Canton de  
Vaud, Suisse, certifie authentique la signature apposée d'autre  
part par M<sup>me</sup> Paula NELSON, directrice-adjointe de la société  
NESTEC S.A., à Vevey, qu'elle engage valablement par sa signature  
individuelle. -----  
VEVEY, LE ONZE AOÛT DEUX MILLE QUATRE. -----



J.D. Rumpf



APOSTILLE  
(Convention de La Haye du 5 octobre 1961)

1. Pays: SUISSE

Le présent acte public

2. a été signé par Jean-Daniel Rumpf

3. agissant en qualité de notaire à Vevey

4. est revêtu du sceau/timbre de J.-D. Rumpf notaire



Attesté

5. à Lausanne

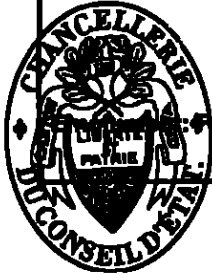
6. le 12 août 2004

7. par la Chancellerie d'Etat du Canton de Vaud

8. sous No 6705

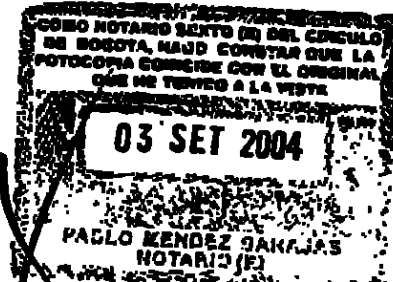
9. Sceau/timbre:

10. Signature  
pr le Chancelier d'Etat:



Fr. 25.-

*P. Equey*  
P. Equey



Retiro folios 8 y 9 Eduardo Luján

8

**TRATADO DE COOPERACION DE PATENTES**  
**PCT**  
**REPORTE DE BUSQUEDA INTERNACIONAL**

(PCT Artículo 18 y Reglas 43 y 44)

|                                                                          |                                                                                                                                                                          |                                                                 |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Registro de referencia del solicitante o agente<br><b>NO 7267/WO/PCT</b> | <b>PARA FUTURAS ACCIONES</b> ver Notificación o Transmisión del Reporte de Búsqueda Internacional (Forma PCT/ISA/220) así como, donde aplique, el ítem 5 a continuación. |                                                                 |
| Solicitud internacional No.<br><b>PCT/EP 03/12053</b>                    | Fecha de registro internacional (día/mes/año)<br><b>30/10/2003</b>                                                                                                       | Fecha de Prioridad (Primera) (día/mes/año)<br><b>30/10/2002</b> |
| Solicitante<br><b>NESTEC S.A.</b>                                        |                                                                                                                                                                          |                                                                 |

Este Reporte de Búsqueda Internacional ha sido preparado por esta Autoridad de Búsqueda Internacional y es transmitido al solicitante Según el Artículo 18. Una copia será transmitida a la Oficina Internacional.

Este Reporte de Búsqueda Internacional consta de un total de 5 hojas.

☒ También está acompañado de una copia de cada documento técnico anterior citado en este reporte.

**1. Bases del reporte**

a. Respecto al lenguaje, la búsqueda internacional se llevó a cabo según la solicitud internacional en el lenguaje en el cual se presentó, a menos que se indique de otro modo en este ítem.

☐ la búsqueda internacional se llevó a cabo según la traducción de la solicitud internacional proporcionada a esta Autoridad (Regla 23.1(b)).

b. Respecto a una secuencia de ácido nucleótico y/o amino revelado en la solicitud internacional, la búsqueda internacional se llevó a cabo según la lista de la secuencia:

☐ contenido en la solicitud internacional en forma escrita.

☐ presentada junto con la solicitud internacional en forma legible por computador.

☐ proporcionada subsiguientemente a esta Autoridad en forma escrita.

☐ proporcionada subsiguientemente a esta Autoridad en forma legible por computador.

☐ se ha proporcionado la afirmación de que el listado de secuencias escrito proporcionado subsiguientemente no va más allá de la revelación en la solicitud internacional según se presentó.

☐ se ha proporcionado la afirmación de que la información registrada en forma legible por computador es idéntica al listado de la secuencia escrita.

2. ☐ Ciertas reivindicaciones no pudieron localizarse (Ver Recuadro I).

3. ☐ Falta unidad de invención (ver Recuadro II).

**4. Respecto al título,**

☒ El texto es aprobado según lo presenta el solicitante.

☐ Esta Autoridad ha establecido que el texto se lee así:

**5. Respecto al resumen,**

☒ El texto es aprobado según lo presenta el solicitante.

☐ Esta Autoridad ha establecido el texto de acuerdo con la Norma 38.2(b), según aparece en el Recuadro III. El solicitante puede, un mes después de la fecha de haber enviado por correo este reporte de búsqueda internacional, presentar comentarios a esta Autoridad.

**6. La figura de los dibujos que se publicará con el resumen es la Figura No.**

☐ como lo sugirió el solicitante.

☐ porque el solicitante no sugirió una figura.

☐ porque esta figura caracteriza mejor a la invención-

☐ Ninguna de las figuras.

# REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.  
PCT/EP 03/12053

## A. CLASIFICACION DE MATERIA

IPC 7 A21D8/04 A21D13/08

Según la Clasificación de Patentes Internacional (CPI) o según la clasificación nacional y la CPI

## B. CAMPOS BUSCADOS

Documentación mínima buscada (sistema de clasificación seguido de símbolos de clasificación)

IPC 7 A21D A23L

Documentación buscada fuera de documentación mínima hasta donde dichos documentos se incluyan en los campos buscados

Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, donde sea práctico, buscar términos usados)

Dato WPI, EPO-Interno, PAJ, FSTA

## C. DOCUMENTOS CONSIDERADOS RELEVANTES

| Categoría * | Citación de documento, con indicación, donde sea apropiado, de los textos relevantes                                                                                                                                  | Relevante a la reivindicación No. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| X           | US 3 026 205 A (STONE IRWIN M)<br>20 de Marzo de 1962 (1962-03-20)<br><br>columna 2, línea 17-37<br>ejemplo VIII<br>reivindicaciones 1,4,7,8                                                                          | 1,2,4<br>6-10,<br>13-15           |
| X           | US 2 615 810 A (IRWIN STONE)<br>28 de Octubre de 1952 (1952-10-28)<br>citado en la solicitud<br>columna 1, línea 28-31, 39-50<br>columna 3, línea 9-13<br>ejemplo I, II<br>columna 4, línea 52-56<br>reivindicación 1 | 1,2,<br>6-10,<br>13-15            |

☒ En el recuadro C se enumeran más documentos.

☒ En el anexo se enumeran miembros de la familia de patentes.

\* Categorías especiales de documentos citados:

'A' documento que define el estado general de la técnica el cual no se considera de relevancia particular

'E' documento anterior pero publicado en o después de la fecha de presentación internacional

'L' documento que puede arrojar dudas sobre reivindicaciones de prioridad o que se cita para establecer la fecha de publicación de otra citación u otra razón especial (según se especifica)

'O' documento que se refiere a una revelación oral, uso, exhibición u otros medios

'P' documento publicado antes de la fecha de presentación internacional pero después de la fecha de prioridad reivindicada

'T' documento posterior publicado después de la fecha de presentación internacional o fecha de prioridad y que no está en conflicto con la solicitud pero citado para entender el principio o teoría que sustenta la invención

'X' documento de relevancia particular; la invención reivindicada no puede considerarse nueva o no puede considerarse que comprende un paso inventivo cuando el documento se toma solo

'Y' documento de relevancia particular; la invención reivindicada no puede considerarse que comprende un paso inventivo cuando el documento se combina con uno o más documentos, siendo dicha combinación obvia para una persona experta en la técnica

'Z' documento miembro de la misma familia de patentes

Fecha de la terminación real de la búsqueda internacional

1 de Marzo de 2004

Fecha de envío por correo del reporte de búsqueda internacional

11/03/2004

Nombre y dirección de correo de ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk

Tel. No. (+31-70) 340-2040, Tx. 31 651 epo nl,

Fax No. (+31-70) 340-3016

Funcionario autorizado

Piret-Viprey, E

# REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.  
PCT/EP 03/12053

## C. (Continuación) DOCUMENTOS CONSIDERADOS RELEVANTES

| Categoría * | Citación de documento, con indicación, donde sea apropiado, de los textos relevantes                                                                                                                                                                                                                                                                                                                                                  | Relevante a la reivindicación No. |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| X           | WO 99 53769 A (NOVONORDISK AS)<br>28 de Octubre de 1999 (1999-10-28)<br><br>página 2, línea 5-28<br>página 4, línea 28-32<br>página 5, línea 4-10, 18, 19, 34<br>ejemplo 1<br>reivindicaciones 1-4, 11, 16<br><br>---                                                                                                                                                                                                                 | 1,2,4<br>6-10,<br>12-15           |
| X           | DD 128 201 A (DRECHSEL WOLFGANG;<br>SCHLOBACH CARL RUDOLF; RUTTLOFF HEINZ;<br>TAEUFEL ALFRE)<br>2 de Noviembre de 1977 (1977-11-02)<br>ejemplo 2<br>reivindicación 4<br><br>---                                                                                                                                                                                                                                                       | 1-3, 5-7,<br>9-18                 |
| X           | TAUFEL, RUTTLOFF.EMMER: "Enzymeinsatz zur<br>Rationalisierung der Wfeelproduktion I. Zum Einsatz von<br>Proteasen und Amylasen"<br>BÄCKER UND KONDITOR,<br>Vol. 32, No. 1, 1984, páginas 22-24,<br>XP002237629<br>página 22, columna mano izquierda, línea 5-8<br>página 22, columna mano derecha, paragrafo 3<br>tabla 2<br>página 23, columna mano derecha, paragrafo 2<br><br>---                                                  | 1-3, 5-7,<br>9-18                 |
| X           | EP 0 445 585 A (LOTTE CO LTD)<br>11 de Septiembre de 1991 (1991-09-11)<br><br>página 3, línea 2-7<br>reivindicaciones 1,2,5<br><br>-----                                                                                                                                                                                                                                                                                              | 1,2,4<br>7-10, 12<br>14           |
| X           | US 2002/004084 A1 (ENGELS, HASEBORG,<br>HOOGLAND) 10 Enero 2002 (2002-01-10)<br><br>página 3, columna mano izquierda, paragrafo 2, 4<br>ejemplo 3<br>reivindicaciones 1, 3, 4, 6, 8, 14<br><br>---                                                                                                                                                                                                                                    | 1,2,6,7,<br>9,10,<br>12-15        |
| X           | US 3 919 434 A (TSEN CHO C ET AL)<br>11 de Noviembre de 1975 (1975-11-11)<br><br>ejemplo II<br><br>---                                                                                                                                                                                                                                                                                                                                | 1,2,6,7<br>9-10,<br>12-15         |
| X           | MANOHAR, RAO: "Efecto de la alfa-amilasa y proteasa<br>sobre las características de la pasta y la calidad de galletas"<br>ADVANCES IN FOOD SCIENCE,<br>vol. 19, No. 1/2, 1997, páginas 15-21,<br>XP008015977<br>página 16, columna mano izquierda, paragrafo 2<br>página 16, columna mano derecha, último paragrafo<br>página 20, columna mano izquierda, último paragrafo<br>- columna mano derecha, paragrafo 1<br><br>-----<br>-/- | 1,2,4,<br>6-10,<br>12-15          |

# REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.  
PCT/EP 03/12053

## C. (Continuación ) DOCUMENTOS CONSIDERADOS RELEVANTES

| Categoría * | Citación de documento, con indicación, donde sea apropiado, de los textos relevantes                                                                                                                             | Relevante a la reivindicación No. |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| X           | US 5 176 927 A (HAARASILTA SAMPSA ET AL)<br>5 de Enero de 1993 (1993-01-05)<br><br>ejemplo 5<br>columna 12, línea 49-53<br>reivindicaciones 1,7<br><br>---                                                       | 1,2,<br>6-10,12,<br>13            |
| X           | EP 0 487 122 A (UNILEVER PLC; UNILEVER NV (NL))<br>27 de Mayo de 1972 (1992-05-27)<br>ejemplo II, III<br>reivindicaciones 1, 2, 4, 6, 10, 13<br><br>---                                                          | 1,6-10,<br>12,13                  |
| X           | US 2002/142069 A1 (ELMUSA)<br>3 de Octubre de 2002 (2002-10-03)<br>página 2, columna mano izquierda, paragrafo 3,4<br>página 2, tabla 1<br>página 3, tabla 2<br>reivindicaciones 1, 9, 15, 17, 25, 26<br><br>--- | 1,6-10,<br>12,13                  |

**REPORTE DE BUSQUEDA INTERNACIONAL**  
Informe sobre miembros de familia de patentes

Solicitud Internacional No.  
PCT/EP 03/12053

| Documento de patente citado en el reporte de búsqueda |    | Fecha de publicación | Miembro(s) de la familia de patentes |               | Fecha de publicación |
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| US 3026205                                            | A  | 20-03-1962           | NINGUNO                              |               |                      |
| US 2615810                                            | A  | 28-10-1952           | NINGUNO                              |               |                      |
| WO 9953769                                            | A  | 28-10-1999           | AT                                   | 228307 T      | 15-12-2002           |
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|                                                       |    |                      | AU                                   | 3025399 A     | 08-11-1999           |
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| DD 128201                                             | A  | 02-11-1977           | DD                                   | 128201 A1     | 02-11-1977           |
| EP 0445585                                            | A  | 11-09-1991           | JP                                   | 1821569 C     | 10-02-1994           |
|                                                       |    |                      | JP                                   | 3244340 A     | 31-10-1991           |
|                                                       |    |                      | JP                                   | 5032008 B     | 14-05-1993           |
|                                                       |    |                      | EP                                   | 0445585 A1    | 11-09-1991           |
| US 2002004084                                         | A1 | 10-01-2002           | AU                                   | 696897 B2     | 24-09-1998           |
|                                                       |    |                      | AU                                   | 3981395 A     | 31-05-1996           |
|                                                       |    |                      | CA                                   | 2204634 A1    | 17-05-1996           |
|                                                       |    |                      | DE                                   | 69517442 D1   | 13-07-2000           |
|                                                       |    |                      | DE                                   | 69517442 T2   | 26-10-2000           |
|                                                       |    |                      | WO                                   | 9613981 A1    | 17-05-1996           |
|                                                       |    |                      | EP                                   | 0790774 A1    | 27-08-1997           |
|                                                       |    |                      | ES                                   | 2146320 T3    | 01-08-2000           |
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|                                                       |    |                      | US                                   | 3773521 A     | 20-11-1973           |
| US 5176927                                            | A  | 05-01-1993           | FI                                   | 884668 A      | 12-04-1990           |
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|                                                       |    |                      | EP                                   | 0368015 A1    | 16-05-1990           |
| EP 0487122                                            | A  | 27-05-1992           | AU                                   | 8795591 A     | 28-05-1992           |
|                                                       |    |                      | CA                                   | 2055753 A1    | 22-05-1992           |
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| US 2002142069                                         | A1 | 03-10-2002           | WO                                   | 02060262 A2   | 08-08-2002           |

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

|                                                                |                                                                                                                                                         |                                                                |
|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Applicant's or agent's file reference<br><b>NO 7267/WO/PCT</b> | <b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 6 below. |                                                                |
| International application No.<br><b>PCT/EP 03/12053</b>        | International filing date (day/month/year)<br><b>30/10/2003</b>                                                                                         | (Earliest) Priority Date (day/month/year)<br><b>30/10/2002</b> |
| Applicant<br><b>NESTEC S.A.</b>                                |                                                                                                                                                         |                                                                |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/12053

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A21D8/04 A21D13/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A21D A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, FSTA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                | Relevant to claim No.    |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| X          | US 3 026 205 A (STONE IRWIN M)<br>20 March 1962 (1962-03-20)<br><br>column 2, line 17-37<br>example VIII<br>claims 1,4,7,8                                                                        | 1,2,4,<br>6-10,<br>13-15 |
| X          | US 2 615 810 A (IRWIN STONE)<br>28 October 1952 (1952-10-28)<br>cited in the application<br>column 1, line 28-31,39-50<br>column 3, line 9-13<br>examples I,II<br>column 4, line 52-56<br>claim 1 | 1,2,<br>6-10,<br>13-15   |
|            | -/-                                                                                                                                                                                               |                          |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "A" document member of the same patent family

Date of the actual completion of the international search

1 March 2004

Date of mailing of the international search report

11/03/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentplan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx 31 851 apo nl,  
Fax (+31-70) 340-3016

Authorized officer

Piret-Viprey, E

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/12053

15

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                             | Relevant to claim No.      |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| X          | <p>WO 99 53769 A (NOVONORDISK AS)<br/>28 October 1999 (1999-10-28)</p> <p>page 2, line 5-28<br/>page 4, line 28-32<br/>page 5, line 4-10,18,19,34<br/>example 1<br/>claims 1-4,11,16</p>                                                                                                                                                                                                                       | 1,2,4,<br>6-10,<br>12-15   |
| X          | <p>DD 128 201 A (DRECHSEL WOLFGANG;SCHLOBACH<br/>CARL RUDOLF; RUTTLOFF HEINZ; TAEUFEL<br/>ALFRE) 2 November 1977 (1977-11-02)<br/>example 2<br/>claim 4</p>                                                                                                                                                                                                                                                    | 1-3,5-7,<br>9-18           |
| X          | <p>TAÜFEL,RUTTLOFF,EMMER: "Enzymeinsatz zur<br/>Rationalisierung der Wfeelproduktion I.<br/>Zum Einsatz von Proteasen und Amylasen"<br/>BÄCKER UND KONDI TOR,<br/>vol. 32, no. 1, 1984, pages 22-24,<br/>XP002237629<br/>page 22, left-hand column, line 5-8<br/>page 22, right-hand column, paragraph 3<br/>table 2<br/>page 23, right-hand column, paragraph 2</p>                                           | 1-3,5-7,<br>9-18           |
| X          | <p>EP 0 445 585 A (LOTTE CO LTD)<br/>11 September 1991 (1991-09-11)</p> <p>page 3, line 2-7<br/>claims 1,2,5</p>                                                                                                                                                                                                                                                                                               | 1,2,4,<br>7-10,12,<br>14   |
| X          | <p>US 2002/004084 A1 (ENGELS, HASEBORG,<br/>HOOGLAND) 10 January 2002 (2002-01-10)</p> <p>page 3, left-hand column, paragraphs 2,4<br/>example 3<br/>claims 1,3,4,6,8,14</p>                                                                                                                                                                                                                                   | 1,2,6,7,<br>9,10,<br>12-15 |
| X          | <p>US 3 919 434 A (TSEN CHO C ET AL)<br/>11 November 1975 (1975-11-11)</p> <p>example II</p>                                                                                                                                                                                                                                                                                                                   | 1,2,6,7,<br>9,10,<br>12-15 |
| X          | <p>MANOHAR, RAO: "Effect of alpha-amylase<br/>and protease on the rheological<br/>characteristics of dough and quality of<br/>biscuits"<br/>ADVANCES IN FOOD SCIENCE,<br/>vol. 19, no. 1/2, 1997, pages 15-21,<br/>XP008015977<br/>page 16, left-hand column, paragraph 2<br/>page 16, right-hand column, last paragraph<br/>page 20, left-hand column, last paragraph<br/>-right-hand column, paragraph 1</p> | 1,2,4,<br>6-10,<br>12-15   |
|            | -/-                                                                                                                                                                                                                                                                                                                                                                                                            |                            |

## INTERNATIONAL SEARCH REPORT

**International Application No.**

**PCT/EP 03/12053**

16

**C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                    | Relevant to claim No.  |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| X          | US 5 176 927 A (HAARASILTA SAMPSA ET AL)<br>5 January 1993 (1993-01-05)<br><br>example 5<br>column 12, line 49-53<br>claims 1,7                                       | 1,2,<br>6-10,12,<br>13 |
| X          | EP 0 487 122 A (UNILEVER PLC ;UNILEVER NV<br>(NL)) 27 May 1992 (1992-05-27)<br>examples II,III<br>claims 1,2,4,6,10,13                                                | 1,6-10,<br>12,13       |
| X          | US 2002/142069 A1 (ELMUSA)<br>3 October 2002 (2002-10-03)<br>page 2, left-hand column, paragraphs 3,4<br>page 2; table 1<br>page 3; table 2<br>claims 1,9,15,17,25,26 | 1,6-10,<br>12,13       |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/12053

17

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date                                                                                                                                                                                                                                                                                                                              |
|----------------------------------------|------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| US 3026205                             | A                | 20-03-1962              | NONE                                                                                                                                                                                                                                                                                                                                          |
| US 2615810                             | A                | 28-10-1952              | NONE                                                                                                                                                                                                                                                                                                                                          |
| WO 9953769                             | A                | 28-10-1999              | AT 228307 T 15-12-2002<br>AU 754470 B2 14-11-2002<br>AU 3025399 A 08-11-1999<br>CA 2326412 A1 28-10-1999<br>DE 69904161 D1 09-01-2003<br>DE 69904161 T2 17-07-2003<br>WO 9953769 A1 28-10-1999<br>DK 1073339 T3 27-01-2003<br>EP 1073339 A1 07-02-2001<br>ES 2188143 T3 16-06-2003<br>US 6365204 B1 02-04-2002<br>US 2001055635 A1 27-12-2001 |
| DD 128201                              | A                | 02-11-1977              | DD 128201 A1 02-11-1977                                                                                                                                                                                                                                                                                                                       |
| EP 0445585                             | A                | 11-09-1991              | JP 1821569 C 10-02-1994<br>JP 3244340 A 31-10-1991<br>JP 5032008 B 14-05-1993<br>EP 0445585 A1 11-09-1991                                                                                                                                                                                                                                     |
| US 2002004084                          | A1               | 10-01-2002              | AU 696897 B2 24-09-1998<br>AU 3981395 A 31-05-1996<br>CA 2204634 A1 17-05-1996<br>DE 69517442 D1 13-07-2000<br>DE 69517442 T2 26-10-2000<br>WO 9613981 A1 17-05-1996<br>EP 0790774 A1 27-08-1997<br>ES 2146320 T3 01-08-2000<br>JP 10508477 T 25-08-1998                                                                                      |
| US 3919434                             | A                | 11-11-1975              | CA 944609 A1 02-04-1974<br>DE 2149280 A1 06-04-1972<br>FR 2110870 A5 02-06-1972<br>GB 1330933 A 19-09-1973<br>US 3773521 A 20-11-1973                                                                                                                                                                                                         |
| US 5176927                             | A                | 05-01-1993              | FI 884668 A 12-04-1990<br>AT 101001 T 15-02-1994<br>DE 68912883 D1 17-03-1994<br>DE 68912883 T2 30-06-1994<br>EP 0368015 A1 16-05-1990                                                                                                                                                                                                        |
| EP 0487122                             | A                | 27-05-1992              | AU 8795591 A 28-05-1992<br>CA 2055753 A1 22-05-1992<br>EP 0487122 A2 27-05-1992<br>JP 4311338 A 04-11-1992<br>ZA 9109214 A 21-05-1993                                                                                                                                                                                                         |
| US 2002142069                          | A1               | 03-10-2002              | WO 02060262 A2 08-08-2002                                                                                                                                                                                                                                                                                                                     |

## **ANEXOS DEL REPORTE DE BUSQUEDA INTERNACIONAL**

- 1. XP 002237629**
- 2. XP 008015977**
- 3. US 3 026 205 A**
- 4. US 2 615 810 A**
- 5. WO 99 53769 A**
- 6. DD 128 201 A**
- 7. EP 0 445 585 A**
- 8. US 2002/004084 A1**
- 9. US 3 919 434 A**
- 10. US 5 176 927 A**
- 11. EP 0 487 122 A**
- 12. US 2002/142069 A1**

# I. Mitteilung: Zum Einsatz von Proteasen und Amylasen

XP-002237629

Dr. A. Tütel, Prof. Dr. H. Rüttloff und I. Emmer, Akademie der Wissenschaften der DDR, Forschungszentrum für Molekularbiologie und Medizin, Zentralinstitut für Ernährung, Potsdam-Lehrbrücke  
Dipl.-Ing.-Ök. P. Dymov, Kossum-Waffelspezialbetrieb Brandenburg

Die Anwendung von mikrobiellen Enzympräparaten bei der Waffelherstellung erweist sich in quantitativer und qualitativer Hinsicht als sehr effektiv. Es werden ausgewählte Enzympräparate auf ihre Wirksamkeit gegenüber Weizenmehl und -mehl untersucht. Experimente über den Einfluß der Alpha-Amylase auf die Qualität des Waffelblisters haben ergeben, daß je nach der Mehlqualität ein Verhältnis von Protease und Alpha-Amylase im Bereich von 1:2 bis 1:7 optimal ist. Anhand rasterelektronenmikroskopischer Aufnahmen ist mit dem Proteaseeinsatz eine Auflockerung der Struktur des Waffelteiges verbunden. Ferner werden durch Proteasen aus dem Glutein Spaltprodukte niedrigerer Molmasse gebildet. Es werden nicht nur kostspielige Einsatzstoffe und Energie eingespart, sondern es wird auch der Produktionsprozeß rationalisiert und die Qualität des Waffelblisters hinsichtlich Rösche und vermindelter Hygrokrophie bedeutend verbessert.

Im Bereich der Lebensmittelindustrie sind Enzympräparate als wertvolle lebensmitteltechnologische Hilfsstoffe anzusehen (1). Sie werden bereits in großem Umfang bei der Traubenzuckerherstellung aus Stärke, zum Maischeaufschluß in der Brauerei und Brennerei, zur Klärung von Fruchtsäften, zur Mährensaftegewinnung sowie auf anderen Applikationsgebieten eingesetzt. Auch in der getreideverarbeitenden Industrie bestehen vielfältige Möglichkeiten, Enzympräparate mit hohem Nutzen zu verwenden. Vorrangiges Interesse verdienen Proteasen und Amylasen, die sind hydrolytisch wirkende Enzyme, welche hochmolekulare Proteine oder Stärke in Spaltprodukte zerlegen. Bekanntlich handelt es sich bei Enzymen um Proteine, die auf Grund ihrer Struktur, ihrer auf ein bestimmtes Substrat ausgerichteten Spezifität und ihrer Wirkungsweise befügt sind, die Stoffwechselprozesse in der belebten Natur mit hoher Effektivität und unter schonenden Bedingungen ablaufen zu lassen. Es ist ein Anliegen neuzeitlicher Lebensmittelproduktion, diese material- und energieökonomischen biologischen Prozesse mehr und mehr für den technologischen Bereich zu erschließen.

Durch Enzymeneinsatz kann auch die Waffelblistproduktion ganz erheblich rationalisiert werden. Infolge des hohen Wassergehalts des Teiges liegen für die Wirksamkeit der Enzyme günstige Bedingungen vor. Ferner läßt sich der Enzymeneinsatz ohne zusätzlichen Aufwand in die herkömmliche Verfahrensführung einordnen. Seit einiger Zeit werden im Kossum-Waffelspezialbetrieb Kosum Brandenburg Enzyme mit Erfolg eingesetzt. Über die dabei gesammelten Erfahrungen wird in diesem Beitrag berichtet.

Tabelle 1: Aktivität mikrobieller Proteasepräparate gegenüber Gluten und Weizenmehl

| Enzym               | Substrat   | Proteaseaktivität je ml bzw. g bei 50 °C in TE (Folte) |       |
|---------------------|------------|--------------------------------------------------------|-------|
|                     |            | in TE                                                  | in %  |
| Thermolysin         | Kasein     | 5000                                                   | 200   |
|                     | Gluten     | 1000                                                   | 50,4  |
|                     | Weizenmehl | 704                                                    | 35,2  |
| Bromelain           | Kasein     | 5000                                                   | 200   |
|                     | Gluten     | 900                                                    | 45,4  |
|                     | Weizenmehl | 2004                                                   | 100,2 |
| alkalische Protease | Kasein     | 5000                                                   | 200   |
|                     | Gluten     | 1200                                                   | 60,0  |
|                     | Weizenmehl | 675                                                    | 33,8  |
| Veron P             | Kasein     | 1000                                                   | 50,4  |
|                     | Gluten     | 877                                                    | 43,9  |
|                     | Weizenmehl | 487                                                    | 24,4  |

## 1. Material und Methoden

### 1.1. Substrat

— verschiedene Weizenmehle (Feuchtigkeitsgehalt 25...30,5 % mit Wassergehalt 13...14 Prozent, Proteingehalt 12...13 %)

### 1.2. Enzympräparate

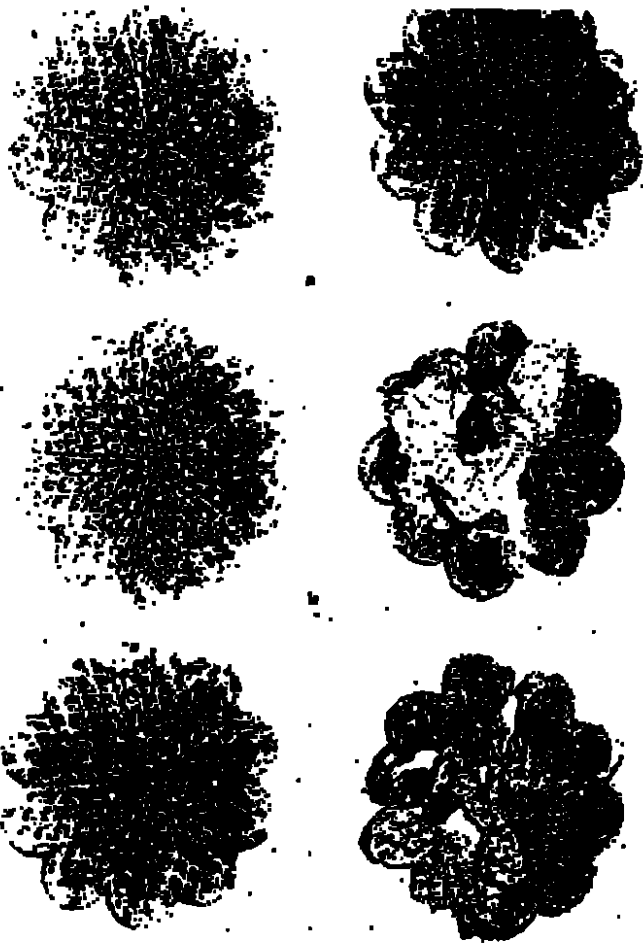
- Thermolysin (schwach alkalische Protease aus *Thermomyces vulgaris*, Konzentrat, Laborpräparat, Proteaseaktivität Tyrosineinheiten je g (TE/g))
- Bromelain (VEB Provisko Schönbeck, Proteaseaktivität 224 TE/ml, Alpha-Amylase-Aktivität 4386 E/ml, Glukonaseaktivität 1000 E/ml)
- Veron P (Röhm GmbH, Darmstadt, BRD, Proteaseaktivität 22 TE/g, Alpha-Amylase-Aktivität 204 E/g)
- alkalische Protease (Institut für Technische Mikrobiologie Berlin, Proteaseaktivität 514 TE/ml)
- Thermamyl (NOVO Industrie A/S, Kopenhagen, Dänemark)
- Alpha-Amylase aus *Bromelain* (Laborpräparat, Aktivität 15 000 E/g)

### 1.3. Bestimmung der Enzymaktivität

Die proteolytische Aktivität wird nach zwei Methoden ermittelt. Bei den verwendeten Enzympräparaten erfolgt ihre Bestimmung



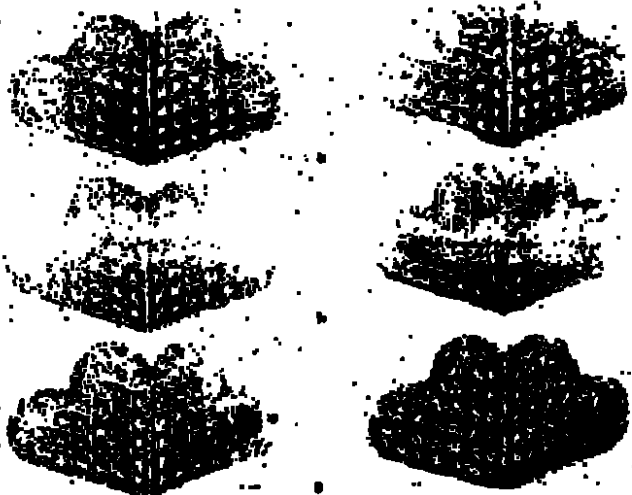
1 Rasterelektronenmikroskopische Aufnahmen des Weizenmehlteiges unter den Bedingungen der Herstellung von Waffelblistern. Frühere Vergrößerung 1000.  
a - Kontrolle des Thermolysin, b - Versuch mit Veron P



2 Abhängigkeit der Bräunung der Waffelblätter von zugesetzter Alpha-Amylase, die zugesetzt ist (a - 40 E für 10 min, b - 80 E für 10 min, c - 110 E für 10 min, d - 110 E für 10 min, e - 120 E für 2,5 min, f - 200 E für 2,5 min)

durch Messung der in Trichloroessigsäure löslichen Spaltprodukte bei 274 nm (2).

Bei den Applikationsversuchen hingegen wird die Methode für Zwecke der Enzymdosierung hinsichtlich Temperatur und pH-Wert den Anwendungsbedingungen angepasst. Die Bestimmung wird nach einem modifizierten Anion-Verfahren (3) mit Folin-Ciocalteu-Reagenz durchgeführt. (Substrate: Kasein 0,8 Prozent, Weizenmehl 8 Prozent, pH-Wert 8, Inkubationstemperatur 20 °C, Inkubationszeit 20 min.) Die Alpha-Amylase wird mit 3,5-Dinitrosalicylsäure bestimmt (4).



3 Backverhalten von Waffelbägen mit variiertem Wassergehalt bei Zusatz von Thermitase (a - Kontrollprobe, b - Wassergehaltsreduzierung um 7 Prozent, c - Wassergehaltsreduzierung um 10 Prozent, d - Wassergehaltsreduzierung um 15,5 Prozent, e - Wassergehaltsreduzierung um 18 Prozent, f - Wassergehaltsreduzierung um 20 Prozent)

| Enzympräparat                    | Verhältnis von Protein zu Alpha-Amylase | Backverhalten des Waffelbäges (Formstabilität, Bräunung, Rinde) |
|----------------------------------|-----------------------------------------|-----------------------------------------------------------------|
|                                  | g je ml bzw. g                          |                                                                 |
| Thermitase (Konzentrat)          | 1:1,4                                   | gut                                                             |
| Thermitase und Alpha-Amylase     | 1:2,8                                   | bessere gut                                                     |
| Thermitase und Alpha-Amylase     | 1:5,6                                   | sehr gut                                                        |
| Verm P (Trichloroacetat)         | 1:1,4                                   | gut                                                             |
| alkalische Protease (Konzentrat) | 1:1,4                                   | schlecht, eine hohe Bräunung erforderlich                       |
| Brauerenzyme 1 (Konzentrat)      | 1:1,4                                   | schlecht                                                        |
| Brauerenzyme 2 (Konzentrat)      | 1:1,4                                   | schlecht                                                        |

## 2. Technologischer Ablauf der Waffelblattherstellung

Bei der Waffelblattherstellung werden die Proteasen bei 18 ... 20 °C im Teigknetter mit der gemäß Versuchsplänen festgelegten für das jeweilige Weizenmehl optimalen Enzym- und Wassermenge vermischt. Ferner werden je Ansatz 0,3 kg Natriumhydrogencarbonat, 0,2 kg Magnesiumoxid und 1 l Pflanzenöl innerhalb von 10 ... 14 min dem Weizenmehl zugegeben, und der Teig wird 30 min lang geknetet. Die Temperatur der Masse beträgt 16 ... 20 °C. Nach 20 ... 30 min, sobald die Flüssigkeit des Teiges ankommt, wird dieser in Behälter zur Zwischenlagerung gepumpt. Die Viskosität der Suspension beträgt 0,65 ... 0,8 Pa · s. Vom Zwischenlagergefäß aus gelangt die Masse zum Waffelbäckautomaten. Bis zum Auftragen auf die Waffeleisen erwärmt sich auf 24 ... 28 °C. Die gebackenen Waffelblätter werden abgekühlt, gewogen und mittels Transportbandes bzw. Transporengabeln der Weiterverarbeitung zugeführt.

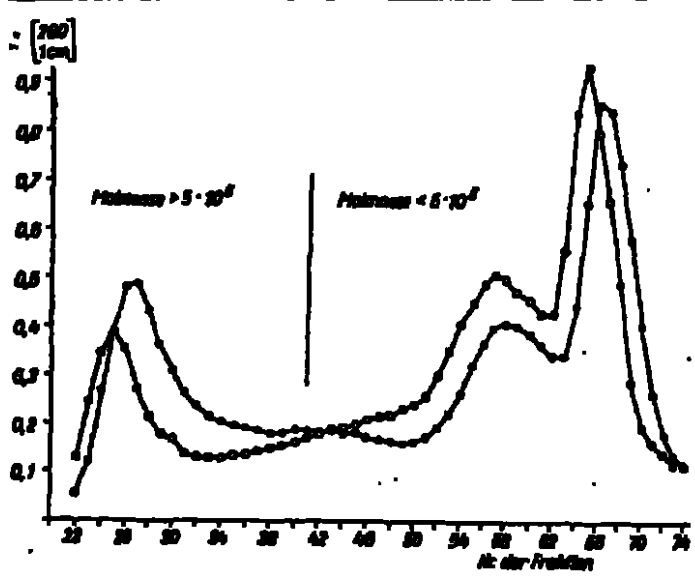
## 3. Ergebnisse und Diskussion

Die für das Backen in den Waffeleisen der Backautomaten geeignete Viskosität des Waffelteiges liegt zwischen 0,6 Pa · s und 0,8 Pa · s und ist vom Durchmesser der Öffnung der Auftragsdüse abhängig. Sie wird außerdem von der im jeweiligen Backautomaten erreichten Temperatur und der Saugkraft der Waffeleisen beeinflusst. Deshalb ist es für die Praxis wichtig, trotz der unterschiedlichen Viskosität ein gleichmäßiges qualitativ hochwertiges Produkt zu erhalten. Wie die Erfahrungen der Betriebsversuche und der seit nunmehr einigen Monaten laufenden Produktion gezeigt haben, ist dies mit Proteasenzusatz in wesentlich besserer Maße möglich als ohne Enzymbehandlung. Es wurden Enzympräparate in die Untersuchungen einbezogen, die sich entweder in der Produktion befinden (Brauerenzyme) oder deren Entwicklung vorgesehen ist (Thermitase, alkalische Protease).

Die Aktivität der getesteten Proteasepräparate gegenüber Weizenmehl ist unterschiedlich (5). Gemäß Tabelle 1 erreicht Thermitase die höchsten Umsätze, es folgen alkalische Protease, Verm P und Brauerenzyme. Andererseits ist die Wirksamkeit über Weizenmehl bei den verwendeten Proteasen auch gleich.

Das im Weizenmehl dominierende Protein Gluten bildet ein Netzwerk aus elastischen Fasern, das von großen und kleinen Stärkengranula umgeben ist. Bild 1 zeigt rasterelektronenmikroskopische Aufnahmen von Weizenmehlsuspensionen, die unter Berücksichtigung der Bedingungen der Waffelblatproduktion mit und ohne Proteasenzusatz verarbeitet worden sind. Die im Elektronenmikroskopischen Zentrum des Instituts für Getreideverarbeitung durchgeführten Aufnahmen lassen erkennen, daß es bei der Einwirkung des Enzympräparates zu einer Auflockerung der festen Bindung von Gluten und Stärke kommt. Es sind Ausbuchtungen und Vertiefungen sichtbar, die bei der Suspension ohne Enzymzusatz trotz der auch hier vorhandenen Wirkung der getreideeigenen Protease nicht in Erscheinung tritt.

Da Gluten und Stärke kompakt angeordnet sind, ist zu vermuten, daß für die erwünschte Viskositätsminderung der Masse neben der Protease auch die Alpha-Amylase Bedeutung hat. Das stärke-spaltende Enzym wirkt einerseits beschleunigend auf Proteolyse (6), führt andererseits aber bei hoher Alpha-Amylase-Aktivität zu einem unerwünschten weitgehenden Stärkabbau. Den Backprozeß sowie die Qualität des Endprodukts nachfolgend beeinflusst. Gemäß Bild 2 ist eine Korrelation zwischen Back-



4 Fraktionierung von Weizenmehlpoteinen an Sepharose 4 B; Zählrohrsystem: 1,5 · 50 cm, Kanalstrom AUC ●—● Kontrolle eines Enzyms, ○—○ Eluate von 2,5 TE Thermozyme je 10 g Mehl

des Waffelblattes und Aktivität der zugesetzten Alpha-Amylase vorhanden. Die Zunahme an Alpha-Amylase führt zur raschen Bräunung, aber auch zum Verbrennen der Waffeln. Im Inneren sind derartige Waffelblätter roh, unausgebacken und mit großen Hohlräumen versehen. Brauereienzym mit hohem Alpha-Amylase-Anteil verhält sich ebenso; das gleiche gilt wenn zu Thermozyme oder Veron P eine Alpha-Amylase höherer Aktivität zugesetzt wird.

Tabelle 2 gibt das Verhältnis von Protease und Alpha-Amylase in einigen Enzympräparaten im Vergleich zum Backverhalten des Waffelteiges an. Danach sind Präparate mit einem Verhältnis von Protease zu Alpha-Amylase zwischen 1:0,1 und 1:1,4 geeignet. Brauereienzym mit einem Verhältnis von 1:24 bis 1:38 hingegen nicht.

Für die Eignung eines Enzympräparates ist folglich derjenige Bereich des Verhältnisses von Protease zu Alpha-Amylase ausschlaggebend, der in Bezug auf Enzymeinsetz und angestrebte Wirkung optimal ist (1:2 bis 1:7). Enzympräparate mit einem darüberliegenden Quotienten, beispielsweise Brauereienzym, sind nicht anwendbar.

Man kann die Einsatzfähigkeit eines Enzympräparates auch dadurch ermitteln, daß der Grenzwert für die geeignete Alpha-Amylase-Aktivität bestimmt wird. Letzterer hängt von den Eigenschaften der Alpha-Amylase ab.

Alpha-Amylase mit einem hohen Temperaturoptimum führt wegen ihrer längeren Wirksamkeit während des Backprozesses zu einem weitergehenden Abbau. Bei Berücksichtigung der nicht überall einheitlichen Bedingungen am Waffelbackautomaten darf die Alpha-Amylase-Aktivität den Bereich von 80 bis 200 E je kg Weizenmehl nicht überschreiten.

Der Gehalt des Thermozympreparates an Alpha-Amylase ist niedrig, so daß beim Einsatz dieses Präparates nachteilige Wirkungen nicht auftreten. Ferner ist durch weitergehende Versuche festgestellt worden, daß im Gegensatz zur Alpha-Amylase-Aktivität eine Überdosierung an Protease keine negativen Veränderungen hinsichtlich Bräunung, Porenstruktur und Klebrigkeit der Waffelblätter verursacht. Bei der Prüfung der bei der Anwendung von Thermozympreparaten vorgesehenen Wasserreduzierung der Waffelteige werden die in Bild 3 veranschaulichten Ergebnisse erzielt. Danach ist eine Wasserreduzierung um 15 Prozent möglich. Eine weitere

Verminderung des Wassereinsatzes kann durch die enzymatische bedingten Veränderung des Wasserbindungsvermögens des Teiges zusammenhängt. Thermozyme liegt mit dem Verhältnis von Protease zu Alpha-Amylase von 1:1,4 nahe am Optimalbereich. Das Importpräparat Veron P ist vom Optimalbereich etwas weiter entfernt. Die funktionellen Eigenschaften und die sensorische Qualität des mit Proteasen hergestellten Waffelblattes sind besonders hervorzuheben. Die im Konsum-Waffelspezialbetrieb Brandenburg durchgeführten Betriebsversuche mit Einsatz von Thermozyme und Veron P haben eine merkliche Qualitätssteigerung des Waffelblattes (Rösche, gleichmäßige Bräunung und längere Erhaltung der Lagerfähigkeit) herbeigeführt, die von den durch die mikrobiellen Enzympräparate bewirkten Veränderungen der Glutenproteine und der Stärke bewirkt wird.

Die Verteilung der Proteine des Weizenmehls nach dem proteolytischen Abbau ist deshalb von Interesse. Bild 4 veranschaulicht die säulenchromatografische Auftrennung der Proteine im Gluten eines Weizenmehls. Im Vergleich zur Kontrollprobe wird eine deutliche Abnahme des Gehalts an hochmolekularem Glutenin und eine Zunahme von Komponenten, die in ihrer Molmasse der Gliadine und Albuminen entsprechen, nachweisbar (7). Dabei darf nicht übersehen werden, daß unter den gegebenen Bedingungen nur wenige Bindungen der Glutenkomplexes gelöst werden. Eine orientierend durchgeführte quantitative Auswertung ergibt eine Verringerung des Anteils der Proteine mit einer Molmasse  $5 \cdot 10^5$  um etwa 8 Prozent und einen dementsprechend größeren Anteil der Bestandteile mit niedrigerer Molmasse.

Die aus der Anwendung der Protease resultierenden Rationalisierungseffekte bestehen in einer besseren Materialökonomie, einer erheblichen Energieeinsparung, einer verstärkten Auslastung der Anlagen und einer Steigerung der Arbeitsproduktivität. Außerdem wird die Qualität der Waffelblätter (Farbe, Rösche, Hygroscopizität) verbessert. Bisher mußten die auf das Weizenmehl zurückzuführenden Qualitätsmängel des Waffelblattes durch einen Teil kostspielige Zusätze, wie Vollkornpulver, Sojamehl u. a., ausgeglichen werden.

Tabelle 3 veranschaulicht die Energie-, Material- und Produktionsvorteile vor und nach dem Beginn des Einsatzes von Proteasen unter Produktionsbedingungen. Dabei werden die ökonomischen und Qualitätsvorteile deutlich. Als Nachteil ist nur der höhere Bruchanteil, der aus der verbesserten Rösche des Waffelblattes resultiert, zu verzeichnen. Um diese Ergebnisse zu erreichen, sind die Verarbeitungsbedingungen an die Qualität des jeweils zu verarbeitenden Weizenmehls anzupassen. Die Charakterisierung der Weizenmehlqualität durch die mittels Rotationsviskosimeters gemessene Viskosität ermöglicht den jeweils günstigen Dosierungsbereich vor der Verarbeitung in kurzer Zeit zu ermitteln. Diese Methode, die auf der Grundlage einer mathematisch-statistischen Versuchsplanung erarbeitet wurde, wird in der 2. Mitteilung erörtert.

Der Einsatz von mikrobiellen Enzympräparaten (Konzentraten oder Trockenpräparaten) in der Dauerbackwarenindustrie macht keine zusätzlichen Vorkehrungen erforderlich. Man sollte jedoch beachten, daß es sich um biologisch aktive Substanzen handelt. Beim Dosieren sind deshalb das Einatmen von Staub oder die Berührung mit der Haut zu vermeiden.

Der Einsatz von Enzympräparaten in der Lebensmittelindustrie ist genehmigungspflichtig (Ministerium für Gesundheitswesen der DDR, Hauptabteilung Lebensmittel- und Ernährungshygiene). Wie in zahlreichen anderen Ländern gelten auch in der DDR Festlegungen hinsichtlich der Verwendung von mikrobiellen Enzympräparaten, die ihren risikolosen Einsatz sichern.

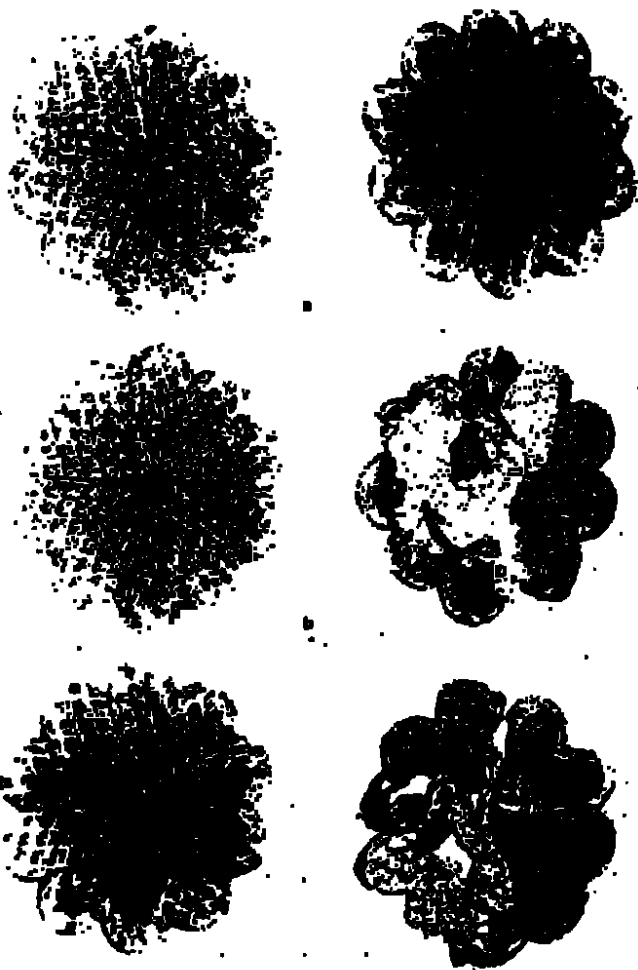
Die Ergebnisse zeigen, daß Enzympräparate als wirkungsvolle Rationalisierungsmittel auch bei der Dauerbackwarenherstellung mit Erfolg einsetzbar sind.

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(7) Huber, U., A. Thiel und H. Rothel; Z. Lebensmittel-Unters. -Forsch. 179 (1982) 295

Tabelle 2: Vollkornbrotbacker, Messen der Waffelbackproduktion im Konsum-Waffelback

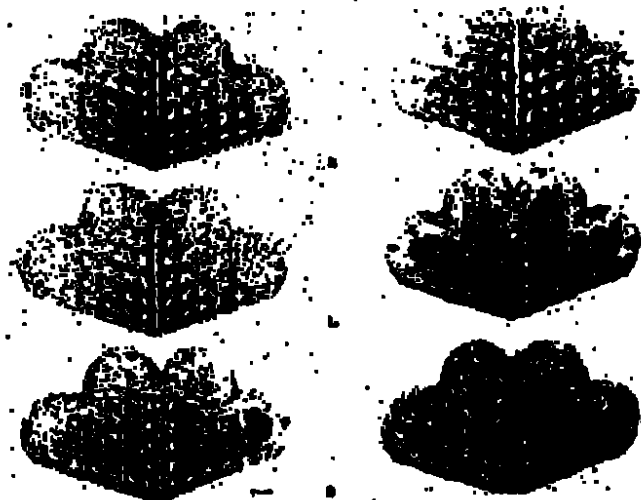
|                                                                          |     |
|--------------------------------------------------------------------------|-----|
| Steigerung der Waffelbackproduktion:                                     | 28% |
| Steigerung des Rohstoffverbrauchs:                                       | 12% |
| Steigerung der Rohstoffkosten:                                           | 18% |
| Steigerung der Lohnkosten:                                               | 19% |
| Steigerung an Energie:                                                   | 5%  |
| Steigerung der Qualität bezüglich Rösche, Konsistenz und Lagerfähigkeit: |     |



2 Abhängigkeit der Bräunung der Waffelblätter von separaten Alpha-Amylasen, die proteolytisch bei (a - 66 E für 10 min, b - 80 E für 10 min, c - 110 E für 10 min, d - 110 E für 10 min, e - 130 E für 2,5 min, f - 200 E für 2,5 min).

durch Messung der in Trichloressigsäure löslichen Spaltprodukte bei 274 nm (2).

Bei den Applikationsversuchen hingegen wird die Methode für Zwecke der Enzymdosierung hinsichtlich Temperatur und pH-Wert den Anwendungsbedingungen angepasst. Die Bestimmung wird nach einem modifizierten Anson-Verfahren (3) mit Folin-Ciocalteu-Reagens durchgeführt. (Substrate: Kasein 0,8 Prozent, Weizenmehl 8 Prozent, pH-Wert 8, Inkubationstemperatur 20 °C, Inkubationszeit 20 min.) Die Alpha-Amylase wird mit 2,5-Dinitro-salicylsäure bestimmt (4).



3 Backverhalten von Waffelteig mit veränderter Wassergehalt bei Einsatz von Thermilase (a - Kontrollprobe, b - Wassergehaltsreduzierung um 7 Prozent, c - Wassergehaltsreduzierung um 10 Prozent, d - Wassergehaltsreduzierung um 12,5 Prozent, e - Wassergehaltsreduzierung um 15 Prozent, f - Wassergehaltsreduzierung um 20 Prozent)

## Für die Waffelherstellung

| Enzympräparat                    | Vollständigkeit von Proteasen im Alpha-Amylase | Backverhalten des Waffelteigs (Porenstruktur, Bräunung, etc.)                                       |
|----------------------------------|------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Thermilase (Konzentrat)          | 1:1,4                                          | gut                                                                                                 |
| Thermilase und Alpha-Amylase     | 1:2,5                                          | besonders gut                                                                                       |
| Thermilase und Alpha-Amylase     | 1:1,5                                          | noch ausreichend                                                                                    |
| Verm P (Thermilasekonzentrat)    | 1:1,1                                          | gut                                                                                                 |
| alkalische Protease (Konzentrat) | 1:0,1                                          | unzureichend, der hohe Gehalt an alkalischen Substanzen führt zu einer nicht ausreichenden Bräunung |
| Bromelase 1 (Konzentrat)         | 1:0,5                                          | nicht ausreichend                                                                                   |
| Bromelase 2 (Konzentrat)         | 1:0,1                                          | nicht ausreichend                                                                                   |

## 2. Technologischer Ablauf der Waffelherstellung

Bei der Waffelherstellung werden die Proteasen bei 18 ... 20 °C im Teigkneten mit der gemäß Versuchsschritte festgelegten, für das jeweilige Weizenmehl optimalen Enzym- und Wassermenge vermischt. Ferner werden je Ansatz 0,3 kg Natriumhydrogencarbonat, 0,2 kg Magnesiumoxid und 1 l Pflanzöl innerhalb von 10 ... 14 min dem Weizenmehl zugesetzt, und der Teig wird 30 min lang geknetet. Die Temperatur der Masse beträgt 16 ... 20 °C. Nach 20 ... 30 min, sobald die Flüssigkeit des Teiges zunimmt, wird dieser in Behälter zur Zwischenlagerung gepumpt. Die Viskosität der Suspension beträgt 0,65 ... 0,8 Pa·s. Vom Zwischenlagergefäß aus gelangt die Masse zum Waffelbackautomaten. Bis zum Auftragen auf die Waffeleisen erwärmt sie sich auf 24 ... 28 °C. Die gebackenen Waffelblätter werden gekühlt, gewogen und mittels Transportbandes bzw. Transportwagens der Weiterverarbeitung zugeführt.

## 3. Ergebnisse und Diskussion

Die für das Backen in den Waffeleisen der Backautomaten geeignete Viskosität des Waffelteiges liegt zwischen 0,6 Pa·s und 0,8 Pa·s und ist vom Durchmesser der Öffnung der Auftragsdüse abhängig. Sie wird außerdem von der im jeweiligen Backautomaten erreichten Temperatur und der Porengüte der Waffelblättern beeinflusst. Deshalb ist es für die Praxis wichtig, trotz der unterschiedlichen Viskosität ein gleichmäßiges qualitativ hochwertiges Produkt zu erhalten. Wie die Erfahrungen der Betriebsverwaltungen und der seit nunmehr einigen Monaten laufenden Produktion gezeigt haben, ist dies mit Proteasenzusatz in wesentlich besserer Maße möglich als ohne Enzymbehandlung. Es wurden Enzympräparate in die Untersuchungen einbezogen, die sich entweder in der Produktion befinden (Bromelase) oder deren Entwicklung vorgesehen ist (Thermilase, alkalische Protease).

Die Aktivität der getesteten Proteasepräparate gegenüber Weizenmehl ist unterschiedlich (5). Gemäß Tabelle 1 erreicht Thermilase die höchsten Umsätze, es folgen alkalische Protease, Verm P und Bromelase. Andererseits ist die Wirksamkeit der Enzyme über Weizenmehl bei den verwendeten Proteasen auch gleich.

Das im Weizenmehl dominierende Protein Gluten bildet ein Netzwerk aus elastischen Fasern, das von großen und kleinen Stärkengranula umgeben ist. Bild 1 zeigt rasterelektronenmikroskopische Aufnahmen von Weizenmehlsuspensionen, die unter Berücksichtigung der Bedingungen der Waffelherstellung mit und ohne Proteasenzusatz verarbeitet wurden sind. Die im Elektronenmikroskopischen Zentrum des Instituts für Getreideverarbeitung gefertigten Aufnahmen lassen erkennen, daß es bei der Einwirkung des Enzympräparates zu einer Auflockerung der festen Bildung von Gluten und Stärke kommt. Es sind Ausbuchtungen in Vertiefungen sichtbar, die bei der Suspension ohne Enzymzusatz trotz der auch hier vorhandenen Wirkung der getreideeigenen Protease nicht in Erscheinung tritt.

Da Gluten und Stärke kompakt angeordnet sind, ist zu vermuten, daß für die erwünschte Viskositätsniedrigung der Mehlsuspension neben der Protease auch die Alpha-Amylase Bedeutung hat. Stärkespaltende Enzyme wirkt einerseits beschleunigend auf die Proteolyse (6), führt andererseits aber bei hoher Alpha-Amylase-Aktivität zu einem unerwünscht weitgehenden Stärkeabbau, der den Backprozeß sowie die Qualität des Endprodukts nachteilig beeinflusst. Gemäß Bild 2 ist eine Korrelation zwischen Bräunung

**EFFECT OF  $\alpha$ -AMYLASE AND PROTEASE ON THE RHEOLOGICAL CHARACTERISTICS OF DOUGH AND QUALITY OF BISCUITS.****R. Sai Manohar and P. Haridas Rao\****Flour Milling, Baking and Confectionery Technology Department, Central Food Technological Research Institute, Mysore 570 013, India.*

\* (To whom correspondence should be addressed.)

**Key words:** Biscuit, rheological characteristics, dough, texture

**SUMMARY:** The effects of  $\alpha$ -amylase and protease on the rheological characteristics of rotary moulded biscuit dough and quality of biscuit are determined. Use of either  $\alpha$ -amylase or protease at  $1.0 \text{ g kg}^{-1}$  or above reduced extrusion time, consistency as well as hardness. Use of any of the enzymes also affected the gluten development as indicated by the reduction of elastic recovery values from 0.46 to 0.325 mm and from 0.46 to 0.36 mm when  $2.0 \text{ g kg}^{-1}$  of  $\alpha$ -amylase and  $2.0 \text{ g kg}^{-1}$  of protease were incorporated, respectively. Farinograph consistency and elasticity were also reduced on incorporation of enzymes. These changes were observed at lower level of incorporation of  $0.5 \text{ g kg}^{-1}$  of the enzymes. Incorporation of enzymes increased the spread as well as the thickness of biscuits. Density reduced from 0.5420 to 0.5029 g/cc and from 0.5420 to 0.5036 g/cc with addition of  $2.0 \text{ g kg}^{-1}$  of  $\alpha$ -amylase and protease, respectively. Increase in crispness of biscuits was also reflected by reduction in breaking and compression strengths when enzymes were used. The studies clearly indicated the beneficial effect of using enzymes.

**INTRODUCTION**

Additives are used to alter the functional characteristics of flour to make it suitable for the preparation of different bakery products. Enzymes are generally employed to improve the machinability of the dough and quality of products. They are extensively used in the preparation of bread to improve the color, mellow the gluten, soften the texture, and improve the shelf life [1-3]. Among them, proteolytic enzymes are reported to be used in the preparation of crackers, to mellow the gluten and consequently improve the textural characteristics of the finished product [4].

Recently, Gaines and Finney reported the effect of using selected commercial enzymes on cookie dough consistency and cookie quality [5]. They observed that among the enzymes, cellulases were found to be effective in maintaining the stability of dough consistency and cookie size when doughs were rested for 2 hr before processing. They also found that the use of papain produced cookies with increased spread, and improved top grain from hard wheat flour; the product was comparable to control cookies made from soft wheat flour. On the other hand, Lee observed an insignificant effect of enzymes on the quality of dough due to its lower water activity [6].

The information on the use of enzymes in short-dough, rotary moulded biscuits is scanty. However, sodium hydrogen sulphite is a reducing agent commonly used in hard semi-sweet dough to weaken the protein, and to

make the dough more extensible [7]. Hence, the effect of using two important, commercially available enzymes, namely  $\alpha$ -amylase and protease on the various rheological characteristics of short, rotary moulded dough, and on quality of biscuits was studied.

**MATERIALS AND METHODS****MATERIALS****Flour**

Two commercial wheat flour samples (A and B), procured from the local market were used in the studies.

**METHODS****Flour analysis**

Moisture, ash, gluten, color grade value, Hagberg's falling number, SDS sedimentation value, farinograph, and extensograph characteristics were determined using AACC methods [8]. Alkaline water retention capacity was determined according to the method of Yamazaki [9].

**Biscuit formula and ingredients**

Biscuit dough was prepared according to the following formula: flour 100 g, sugar 30 g, shortening 20 g, sodium

chloride 1 g, sodium hydrogen carbonate 0.5 g, ammonium hydrogen carbonate 1 g, baking powder 0.3 g, and water 16 and 19% for flour A and flour B, respectively. Commercially available bakery shortening (Marvo brand, Brooke Bond Lipton India Ltd., Calcutta) and powdered sucrose were used in the preparation. The various doughs were made using 300 g flour.

#### Preparation of the dough

Sugar and fat were creamed in a Hobart mixer (N-50) with a flat beater, for 2 min at 61 rpm. Dough water containing sodium hydrogen carbonate and ammonium hydrogen carbonate and sodium chloride was added to the above cream, and mixed for 5 min at 125 rpm to obtain a homogeneous cream. Finally, flour (sieved twice) with baking powder was added and mixed for 3 min at 61 rpm. Different doughs were prepared by incorporating 0, 0.5, 1.0 and 2.0 g kg<sup>-1</sup> of  $\alpha$ -amylase (39 units/mg solid, Sigma Chemicals, from fungal source), and protease (7-15 units/mg solid, Blocon India Ltd., Bangalore, from fungal source) in the biscuit formulation.

#### Measurement of rheological characteristics of dough

For obtaining samples for measuring rheological characteristics, the dough was sheeted using a rolling pin over a rectangular platform and frame of height 1.0 cm to get a sheet of 1.0 cm thickness [10]. The sheeted dough was cut into a circular shape using cutters of varying diameter (2.2 and 4.0 cm). The sheeted and cut cylindrical discs of 4.0 cm diameter and 1.0 cm height were used to assess the consistency, hardness, cohesiveness, adhesiveness, stickiness and apparent biaxial extensional viscosity, using an Instron Universal Testing Instrument (Model 4301). Compliance and elastic recovery of the dough were measured using penetrometer as per the procedure described by Manohar and Rao [11] using doughs of 1.0 cm thickness and 2.2 cm diameter.

Physical-textural characteristics of dough were evaluated by the two bite "texture profile analysis" (TPA) method of Peleg [12] and Bourne [13] using an Instron Universal Testing Instrument under the following conditions: plunger diameter, 8.5 cm; clearance, 1.5 cm; load cell, 500 kg; cross-head speed, 10.0 cm min<sup>-1</sup>. The cylindrical dough of 4.0 cm in diameter and 1.0 cm in height was used for the studies. Eighty percent compression was given for each sample. The real time plots were analyzed for the following:

(i) dough consistency (N sec): combined area of the two resistance peaks; (ii) dough hardness (N): maximum resistance to the first compression peak (height of peak 1); (iii) dough cohesiveness: ratio of area of the two resistance peaks ( $A_2/A_1$ ); (iv) dough adhesion (N sec): area of the first adhesion peak; (v) dough stickiness: as the com-

plementary angle (°) made by first adhesion peak and X axis.

Apparent biaxial extensional viscosity (ABEV) of the dough was measured using an Instron Universal Testing Instrument with the lubricated uniaxial compression method of Bagley and Christians [14] under the following conditions: plunger diameter, 8.5 cm; load cell, 100 kg; cross-head speed, 5.0 cm min<sup>-1</sup>; compression, 50%; and relaxation time, 200 sec. The cylindrical dough of 4.0 cm diameter and 1.0 cm height was used in the studies. Apparent biaxial extensional viscosity (ABEV) of the dough was computed by the formula:

$$\eta_{be} = Fh/\pi R_0^2 \dot{h}_0 e, \text{ Pa sec}$$

F = peak force (Newton); h = final height (m);  
R = initial radius (m);  $\dot{h}_0$  = initial height (m);  
 $e_t = [dh/dt][1/2h]$  (sec<sup>-1</sup>);  
 $dh/dt$  = cross head speed (m sec<sup>-1</sup>).

Dough consistency was measured as extrusion through using a 'Research' Water Absorption Meter (RWAM), per the procedure of Gaines [15] using 2 kg additional weight.

#### Farinograph characteristics

A Brabender Farinograph equipped with a 300-g bowl was used to determine dough consistency (peak height) and cohesion (band width). Olewnik and Kuip [16] used some additional amount of water (10%) while mixing the biscuit dough in the Farinograph to get the curve within the scale. Therefore, the method does not indicate the inherent characteristics of cookie dough. Hence, the method was modified for present studies and a 50-g bowl was used at lever position of 1:1. The Farinograph characteristics of biscuit dough prepared as described earlier were determined using a 40 g quantity of the dough. The curve was evaluated for various parameters as given below.

Consistency (BU): is the initial height of the curve after the dough is mixed in Farinograph for 1 min; Elasticity (BU): is the band width of the curve at 1 min; Mixing tolerance index (BU): (MTI) is the difference between initial and final consistencies. Increase in consistency is represented as negative MTI and decrease in consistency as positive MTI.

#### Preparation of biscuits

Dough was sheeted to a thickness of 3.5 mm with the help of an aluminium platform and a frame, and by rolling using a rolling pin. Biscuits were shaped with a cutter of 51 mm diameter and baked on an aluminium tray at 205 °C for 10 min. The biscuits were cooled for 30 min and stored in air tight tins for 24 hr, for further analysis. Biscuits were prepared from doughs containing enzymes at different levels.

## Evaluation of biscuits

### Physical characteristics

Diameter (D) and thickness (T) of six biscuits were measured by placing them edge to edge and by stacking one above the other, respectively. To obtain the average, measurements were made by rearranging and restacking. Average weight (W) of six biscuits was noted. Density of biscuits was expressed as g/cc.

### Breaking and compression strengths

The breaking strength of biscuits was measured by following triple beam snap (also called three-point break) technique of Gaines [17] using an Instron Universal Testing Instrument. Compression strength of biscuit was measured at 20% level of compression with a cross-head speed of 5.0 cm min<sup>-1</sup>. Peak force (Newton) was recorded.

### Color

The values of surface color of biscuit in terms of color difference ( $\Delta E$ ) and per cent whiteness (W) were measured using an UV-visible recording spectrophotometer (Model UV - 2100 Shimadzu Corporation, Japan) [18].

### Sensory analysis

The sensory analysis of biscuits was carried out by a panel of six experienced judges by assigning score for each quality attributes.

### Statistical analysis of data

The experiment was planned according to completely randomised design and the treatments were tested using Duncan's new multiple range test [19].

## RESULTS AND DISCUSSION

### Chemical and rheological characteristics of flour samples:

Flours A and B had gluten 8.8 and 10.8%; ash 0.38 and 0.40%; Falling Number 460 and 490, AWRC 56.0 and 64.0%, and SDS sedimentation value 29 and 38 ml, respectively. Flour A had a Farinograph water absorption capacity of 56.4 while flour B had 63.6%. Strength as indicated by area of the extensogram was 92 and 126 cm<sup>2</sup>, resistance to extension 380 and 520 BU, and extensibility 165 and 170 mm for flour A and flour B, respectively. The above data indicated that flour A is obtained from soft wheat and flour B from medium strong wheat.

### Effect of enzymes

#### Rheological characteristics

The dough from flour A, made using up to 1.0 g kg<sup>-1</sup> of  $\alpha$ -amylase, did not show any significant difference in extrusion time (Table 1). However, a slight reduction in

the value from 45 to 40 sec was observed at the higher enzyme level of 2.0 g kg<sup>-1</sup>. Incorporation of protease in both flours at increasing levels (0.5 to 2.0 g kg<sup>-1</sup>) reduced the extrusion time gradually (Table 2). Compliance values of dough followed similar pattern as extrusion time with use of any of the enzymes. Elastic recovery values also reduced increasing level of either  $\alpha$ -amylase or protease. The reduction when  $\alpha$ -amylase was used could be attributed to softening of the dough, due to hydrolysis of starch while the reduction due to protease could be attributed to breakage of peptide bonds [1]. Use of either of the enzymes reduced the apparent biaxial extensional viscosity of dough from flour A with increasing levels, except at lower levels of  $\alpha$ -amylase (0.5 g kg<sup>-1</sup>) (Tables 1-2). Incorporation of 2.0 g kg<sup>-1</sup> of  $\alpha$ -amylase and protease, the apparent biaxial extensional viscosity dropped from  $1.91 \times 10^5$  to  $1.54 \times 10^4$  and  $1.51 \times 10^4$  Pa sec, respectively, suggesting that the dough became less viscous when enzymes were incorporated.

Use of increasing levels of either of  $\alpha$ -amylase or protease gradually decreased consistency and hardness of the dough. Incorporation of 2.0 g kg<sup>-1</sup> of  $\alpha$ -amylase reduced consistency from 786 to 692 N sec and hardness from 662 to 542 N. Similar changes were found for protease (Table 2). Dough became more cohesive, adhesive and sticky on incorporation of either protease or  $\alpha$ -amylase. A similar observation has been made by several workers for bread [20, 1]. The flour B also responded similarly with different enzymes.

Insignificant changes in the various rheological characteristics observed on incorporation of either of the enzymes at lower level of 0.5 g kg<sup>-1</sup> could be attributed to the lower mobility, due to lower water activity, and the presence of higher amount of fat and sugar in the dough [6]. However, use of either of the enzymes at 2.0 g kg<sup>-1</sup> level had shown significant changes in the various rheological characteristics.

### Farinograph characteristics

Farinograph consistency of doughs from both flours reduced considerably, even at the lower incorporation level of 0.5 g kg<sup>-1</sup> of  $\alpha$ -amylase or protease (Figs. 1 and 2). A similar effect at lower level of enzyme incorporation was not observed in other rheological characteristics, possibly due to the difference in principles involved in the measurement. Elasticity of the dough was reduced with incorporation of any of the enzymes due to the softening of the dough. The increase in the consistency observed while mixing the biscuit dough containing any of the enzymes in the farinograph, indicated that the dough continued to develop. This increasing trend was observed even up to addition of 2.0 g kg<sup>-1</sup> of either  $\alpha$ -amylase or protease. However, the final consistency in the farinograph was lower for doughs containing enzymes, when compared to that of control biscuit dough.

Table 1: Effect of α-amylase on the rheological characteristics<sup>a</sup> of biscuit dough.

| α-amylase<br>(g kg <sup>-1</sup> ) | Extrusion<br>time<br>(sec) | Compliance<br>(%) | Elastic<br>recovery<br>X 10 (mm) | ABEV<br>X 10 <sup>-3</sup><br>(Pa sec) | Consistency<br>(N sec) | Hardness<br>(N) | Cohesiveness | Adhesiveness<br>(N sec) | Stickiness<br>(°) |
|------------------------------------|----------------------------|-------------------|----------------------------------|----------------------------------------|------------------------|-----------------|--------------|-------------------------|-------------------|
| Flour A                            |                            |                   |                                  |                                        |                        |                 |              |                         |                   |
| 0.0                                | 45a                        | 40.6b             | 4.60a                            | 1.91a                                  | 786a                   | 662a            | 0.194d       | 54.3c                   | 50.3d             |
| 0.5                                | 45a                        | 40.1b             | 4.05b                            | 1.89a                                  | 736b                   | 642b            | 0.216c       | 55.6c                   | 54.6c             |
| 1.0                                | 43a                        | 40.7b             | 3.80c                            | 1.65b                                  | 716c                   | 626b            | 0.228b       | 58.8a                   | 57.2b             |
| 2.0                                | 40b                        | 45.2a             | 3.25d                            | 1.54c                                  | 692d                   | 542c            | 0.241a       | 60.8a                   | 60.6a             |
| SEM <sup>b</sup> (±)               | 0.99                       | 0.36              | 0.039                            | 0.019                                  | 4.7                    | 4.9             | 0.0026       | 0.85                    | 0.66              |
| Flour B                            |                            |                   |                                  |                                        |                        |                 |              |                         |                   |
| 0.0                                | 45a                        | 39.5b             | 5.55a                            | 1.91a                                  | 848a                   | 664a            | 0.194c       | 48.3b                   | 48.6b             |
| 0.5                                | 45a                        | 40.5b             | 5.05b                            | 1.90a                                  | 789b                   | 642b            | 0.190c       | 47.5b                   | 48.2b             |
| 1.0                                | 44a                        | 40.1b             | 5.05b                            | 1.80b                                  | 760c                   | 615c            | 0.214b       | 54.5a                   | 50.2b             |
| 2.0                                | 38b                        | 45.5a             | 5.00b                            | 1.71c                                  | 739c                   | 600d            | 0.225a       | 55.6a                   | 54.2a             |
| SEM <sup>b</sup> (±)               | 0.96                       | 0.47              | 0.041                            | 0.016                                  | 6.3                    | 4.2             | 0.002        | 0.55                    | 0.78              |

ABEV-Apparent biaxial extensional viscosity

<sup>a</sup> Values for a particular column and for a particular flour followed by different letters differ significantly (P<0.05)

<sup>b</sup> Standard error of the mean at 12 degrees of freedom

Table 2: Effect of protease on the rheological characteristics<sup>a</sup> of biscuit dough.

| Protease<br>(g kg <sup>-1</sup> ) | Extrusion<br>time<br>(sec) | Compliance<br>(%) | Elastic<br>recovery<br>X 10 (mm) | ABEV<br>X 10 <sup>-3</sup><br>(Pa sec) | Consistency<br>(N sec) | Hardness<br>(N) | Cohesiveness | Adhesiveness<br>(N sec) | Stickiness<br>(°) |
|-----------------------------------|----------------------------|-------------------|----------------------------------|----------------------------------------|------------------------|-----------------|--------------|-------------------------|-------------------|
| Flour A                           |                            |                   |                                  |                                        |                        |                 |              |                         |                   |
| 0.0                               | 45a                        | 40.6b             | 4.60a                            | 1.91a                                  | 786a                   | 662a            | 0.194d       | 54.3c                   | 50.3b             |
| 0.5                               | 43ab                       | 41.2b             | 4.00b                            | 1.90a                                  | 742b                   | 639b            | 0.210c       | 56.8bc                  | 52.2b             |
| 1.0                               | 40b                        | 41.8ab            | 3.80c                            | 1.64b                                  | 696c                   | 72c             | 0.236b       | 58.2b                   | 58.8a             |
| 2.0                               | 38a                        | 42.8a             | 3.60d                            | 1.51c                                  | 646d                   | 486d            | 0.268a       | 62.4a                   | 60.9a             |
| SEM <sup>b</sup> (±)              | 1.2                        | 0.51              | 0.041                            | 0.024                                  | 6.7                    | 6.7             | 0.003        | 0.61                    | 0.78              |
| Flour B                           |                            |                   |                                  |                                        |                        |                 |              |                         |                   |
| .0                                | 45a                        | 39.5b             | 5.55a                            | 1.91a                                  | 848a                   | 664a            | 0.194d       | 48.3c                   | 48.6d             |
| 0.5                               | 45a                        | 40.6ab            | 5.25b                            | 1.89ab                                 | 786b                   | 642b            | 0.206c       | 48.6c                   | 52.4c             |
| 1.0                               | 40b                        | 41.2a             | 4.90c                            | 1.84b                                  | 732c                   | 592c            | 0.224b       | 54.8b                   | 54.9b             |
| 2.0                               | 38b                        | 42.0a             | 4.80c                            | 1.78c                                  | 712c                   | 582c            | 0.236a       | 58.6a                   | 59.1a             |
| SEM <sup>b</sup> (±)              | 1.26                       | 0.41              | 0.037                            | 0.017                                  | 8.2                    | 6.7             | 0.002        | 0.75                    | 0.85              |

ABEV-Apparent biaxial extensional viscosity

<sup>a</sup> Values for a particular column and for a particular flour followed by different-letters differ significantly (P<0.05)

<sup>b</sup> Standard error of the mean (SEM) at 12 degrees of freedom

Quality of biscuits

Physical characteristics

The spread of biscuits gradually increased from 54.0 to 54.6 and 55.2 mm with addition of 2.0 g kg<sup>-1</sup> of α-amylase and protease respectively (Tables 3-4). The extent of increase was greater with protease. There was a significant increase in thickness of biscuits particularly when

more than 1.0 g kg<sup>-1</sup> of either of the enzymes were used. Protease as well as α-amylase reduced the density from 0.5420 to 0.5036 g/cc and from 0.5420 to 0.5029 g/cc of biscuits, respectively.

Reduction in density was greater for biscuits from soft-wheat flour A, when enzymes were incorporated, as compared to that in biscuits made from medium-hard wheat flour B. On incorporation of 2.0 g kg<sup>-1</sup> of protease,

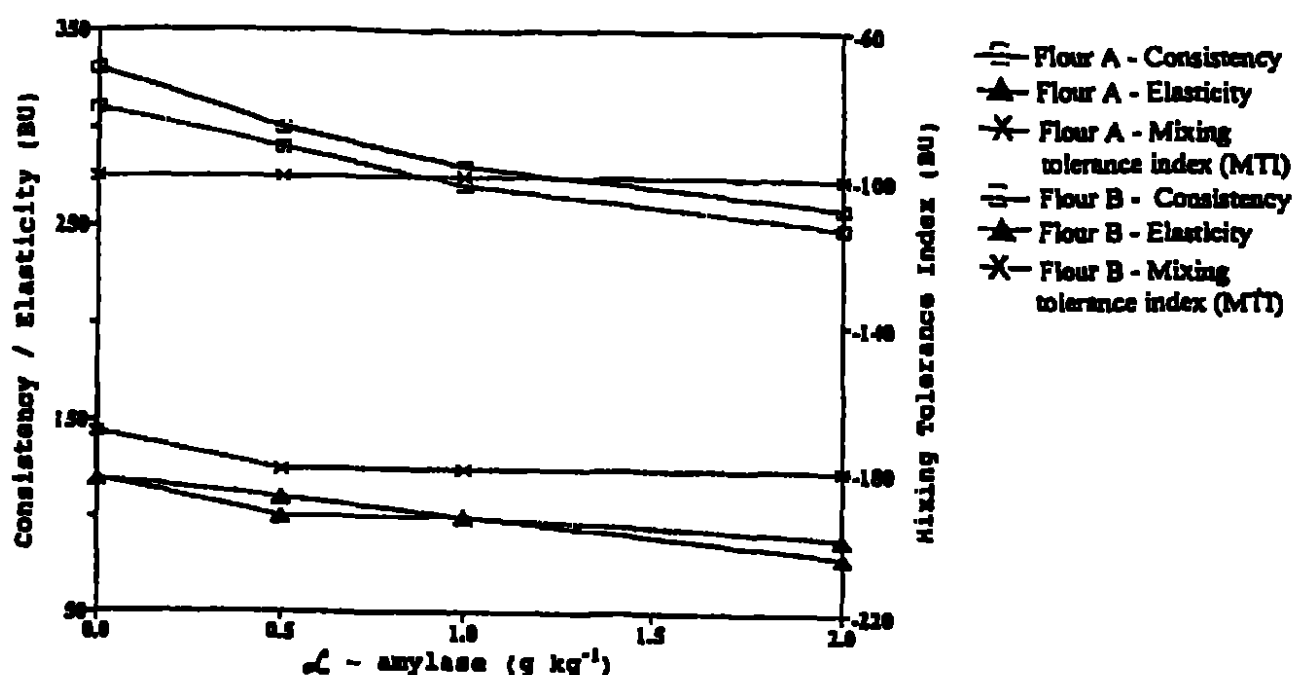


Figure 1: Effect of  $\alpha$ -amylase on the farinograph characteristics of dough biscuit

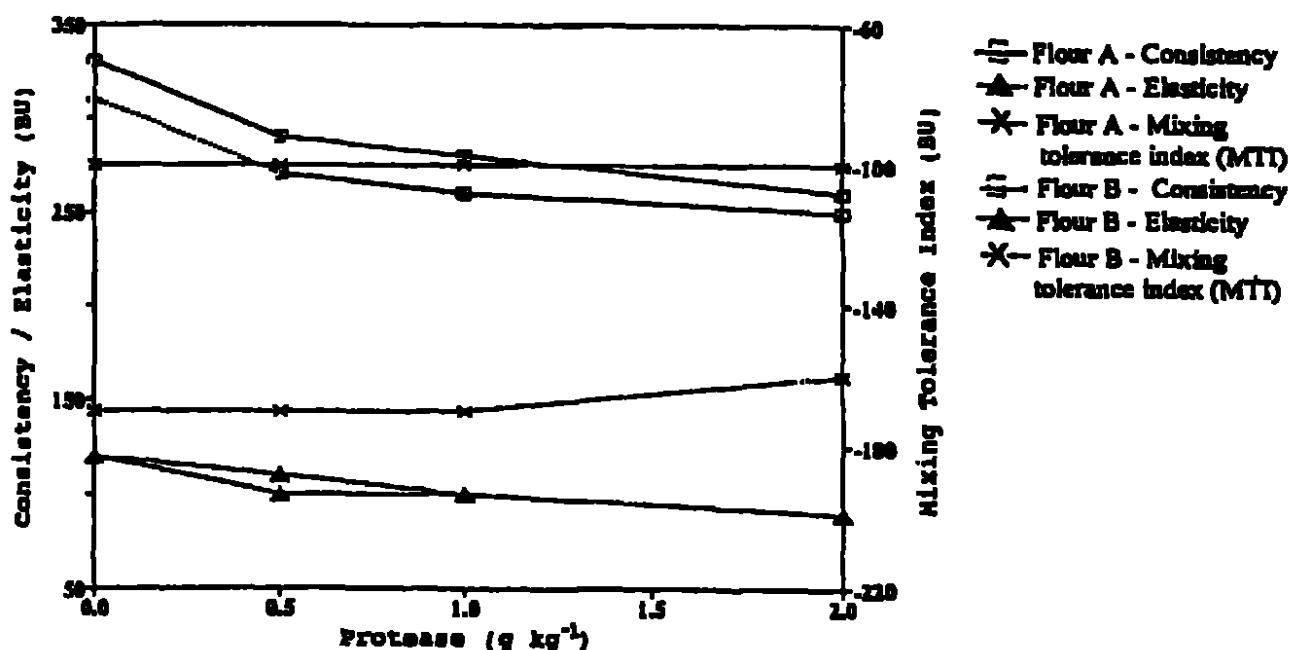


Figure 2: Effect of protease on the farinograph characteristics of dough biscuit

or  $\alpha$ -amylase, breaking strength significantly decreased from 2170 to 2045 g and from 2137 to 2060 g, respectively. The reduction in compression strength and breaking strength was greater for biscuits made from flour B with the incorporation of  $2.0 \text{ g kg}^{-1}$  of protease indicating a greater benefit of using protease in medium-hard wheat flour.

#### Sensory quality

Surface characteristics as well as color improved on incorporation of either of the enzymes at different levels, as indicated by sensory scores (Table 5). The improvement in color was also reflected in the color difference values (Tables 3 and 4). This could be attributed to the avail-

Table 3: Effect of  $\alpha$ -amylase on the physical characteristics<sup>a</sup> of biscuits.

| $\alpha$ -amylase<br>(g kg <sup>-1</sup> ) | Diameter<br>(mm) | Thickness<br>(mm) | Spread<br>ratio | Density<br>(g/cc) | Breaking<br>strength<br>(g) | Compression<br>strength<br>(kg) | Whiteness<br>(%) | Color difference<br>( $\Delta E$ ) |
|--------------------------------------------|------------------|-------------------|-----------------|-------------------|-----------------------------|---------------------------------|------------------|------------------------------------|
| Flour A                                    |                  |                   |                 |                   |                             |                                 |                  |                                    |
| 0.0                                        | 54.0b            | 5.7c              | 9.474a          | 0.5420a           | 1985b                       | 99.4a                           | 30.6a            | 34.7b                              |
| 0.5                                        | 54.2ab           | 5.8b              | 9.345b          | 0.5372b           | 2035a                       | 8.6a                            | 30.0ab           | 35.2a                              |
| 1.0                                        | 54.4ab           | 5.9ab             | 9.220c          | 0.5314c           | 1986b                       | 95.4b                           | 29.4ab           | 35.6a                              |
| 2.0                                        | 54.6a            | 5.95a             | 9.176d          | 0.5029d           | 1969b                       | 89.4c                           | 28.3c            | 36.3a                              |
| SEM <sup>b</sup> ( $\pm$ )                 | 0.13             | 0.03              | 0.025           | 0.001             | 9.8                         | 1.36                            | 0.41             | 0.39                               |
| Flour B                                    |                  |                   |                 |                   |                             |                                 |                  |                                    |
| 0.0                                        | 52.4d            | 5.65b             | 9.274b          | 0.5825a           | 2247a                       | 143.2a                          | 30.0a            | 35.2b                              |
| 0.5                                        | 52.8c            | 5.75a             | 9.183c          | 0.5793a           | 2213a                       | 119.2b                          | 29.9a            | 35.3b                              |
| 1.0                                        | 53.1b            | 5.80a             | 9.155c          | 0.5642b           | 2134b                       | 114.4b                          | 29.6ab           | 35.5b                              |
| 2.0                                        | 53.6a            | 5.75a             | 9.322a          | 0.5584c           | 2060b                       | 108.4c                          | 29.3b            | 36.1a                              |
| SEM <sup>b</sup> ( $\pm$ )                 | 0.1              | 0.03              | 0.023           | 0.002             | 10.4                        | 1.80                            | 0.32             | 0.41                               |

<sup>a</sup> Values for a particular column and for a particular flour followed by different letters differ significantly ( $P < 0.05$ )  
<sup>b</sup> Standard error of the mean (SEM) at 12 degrees of freedom

Table 4: Effect of protease on the physical characteristics<sup>a</sup> of biscuit.

| Protease<br>(g kg <sup>-1</sup> ) | Diameter<br>(mm) | Thickness<br>(mm) | Spread<br>ratio | Density<br>(g/cc) | Breaking<br>strength<br>(g) | Compression<br>strength<br>(kg) | Whiteness<br>(%) | Color difference<br>( $\Delta E$ ) |
|-----------------------------------|------------------|-------------------|-----------------|-------------------|-----------------------------|---------------------------------|------------------|------------------------------------|
| Flour A                           |                  |                   |                 |                   |                             |                                 |                  |                                    |
| 0.0                               | 54.0             | 5.70b             | 9.474a          | 0.5420a           | 1985a                       | 99.4a                           | 30.6a            | 34.7b                              |
| 0.5                               | 54.4             | 5.75b             | 9.461a          | 0.5354b           | 1971a                       | 96.5b                           | 30.0a            | 35.2b                              |
| 1.0                               | 54.8             | 5.85a             | 9.368b          | 0.5197c           | 1899b                       | 94.8b                           | 29.8ab           | 35.3b                              |
| 2.0                               | 55.2             | 5.90a             | 9.356b          | 0.5036d           | 1868b                       | 91.3c                           | 29.6b            | 36.2a                              |
| SEM <sup>b</sup> ( $\pm$ )        | 0.086            | 0.02              | 0.024           | 0.001             | 59.2                        | 1.26                            | 0.41             | 0.33                               |
| Flour B                           |                  |                   |                 |                   |                             |                                 |                  |                                    |
| 0.0                               | 52.4c            | 5.65b             | 9.274ab         | 0.5825a           | 2247a                       | 143.2a                          | 30.0a            | 35.2b                              |
| 0.5                               | 53.0b            | 5.70ab            | 9.298ab         | 0.5664b           | 2129b                       | 125.8b                          | 30.0a            | 35.2b                              |
| 1.0                               | 53.3ab           | 5.75a             | 9.270b          | 0.5645b           | 2094b                       | 113.3c                          | 29.8a            | 35.3b                              |
| 2.0                               | 53.5a            | 5.75a             | 9.304a          | 0.5585c           | 1861c                       | 97.6d                           | 29.1b            | 36.1a                              |
| SEM <sup>b</sup> ( $\pm$ )        | 0.11             | 0.02              | 0.021           | 0.001             | 11.5                        | 1.05                            | 0.39             | 0.35                               |

<sup>a</sup> Values for a particular column and for a particular flour followed by different letters differ significantly ( $P < 0.05$ )  
<sup>b</sup> Standard error of the mean (SEM) at 12 degrees of freedom

ability of greater amount of either free sugars or amino acids involved in the Maillard reaction [21]. The crispness of biscuits also improved on addition of enzymes to both the flours which was also reflected in the physical characteristics such as density, compression strength and breaking strength.

CONCLUSION

Use of either protease or  $\alpha$ -amylase showed significant

changes in the various rheological characteristics when used at a higher level of 2.0 g kg<sup>-1</sup>. The effect of  $\alpha$ -amylase or protease was found to be similar in both soft wheat flour (A) and medium hard wheat flour (B). Use either of the enzymes improved the crispness of biscuits, particularly in those made from medium-hard wheat flour. The above studies have clearly indicated the beneficial effect of using enzymes in the preparation of biscuits.

Table 5: Effect of  $\alpha$ -amylase and protease on the sensory characteristics of biscuits.

| (g kg <sup>-1</sup> ) | Flour A                 |               |                 | Flour B                 |               |                 |
|-----------------------|-------------------------|---------------|-----------------|-------------------------|---------------|-----------------|
|                       | Sensory characteristics |               |                 | Sensory characteristics |               |                 |
|                       | Surface<br>(10)         | Color<br>(10) | Texture<br>(20) | Surface<br>(10)         | Color<br>(10) | Texture<br>(20) |
| $\alpha$ -amylase     |                         |               |                 |                         |               |                 |
| 0.0                   | 9.2b                    | 6.5b          | 16.5c           | 8.9b                    | 6.3b          | 15.2c           |
| 0.5                   | 9.2b                    | 6.6b          | 17.0bc          | 9.0ab                   | 6.6ab         | 15.5c           |
| 1.0                   | 9.3ab                   | 6.8ab         | 17.5ab          | 9.2ab                   | 6.8ab         | 16.5b           |
| 2.0                   | 9.5a                    | 7.2a          | 18.0a           | 9.3a                    | 7.0a          | 17.5a           |
| Sem** ( $\pm$ )       | 0.12                    | 0.11          | 0.14            | 0.11                    | 0.13          | 0.14            |
| protease              |                         |               |                 |                         |               |                 |
| 0.0                   | 9.2b                    | 6.5c          | 16.5c           | 8.9b                    | 6.3c          | 15.2d           |
| 0.5                   | 9.3b                    | 6.7bc         | 17.0b           | 9.0ab                   | 6.5bc         | 16.0c           |
| 1.0                   | 9.5a                    | 7.0ab         | 18.0a           | 9.1ab                   | 6.9ab         | 17.0b           |
| 2.0                   | 9.6a                    | 7.4a          | 18.5a           | 9.2a                    | 7.2a          | 18.0a           |
| Sem** ( $\pm$ )       | 0.1                     | 0.12          | 0.14            | 0.1                     | 0.12          | 0.13            |

\* Values for a particular column and for a particular flour followed by different letters differ significantly ( $P < 0.05$ )

\*\* Standard error of the mean at 20 degrees of freedom

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3,026,205

## PROCESS OF PRODUCING BAKED CONFECTIONS AND THE PRODUCTS RESULTING THEREFROM BY ALPHA AMYLASE

Irwin M. Stone, Staten Island, N.Y., assignor to  
Baxter Laboratories, Inc.  
No Drawing. Filed Aug. 19, 1959, Ser. No. 834,679  
8 Claims. (Cl. 99-92)

This invention relates to an improved baking process and to the baked products resulting therefrom. More particularly, it relates to a simplified one-stage baking process for the preparation of pies, cakes with icings and other novel baked confections.

Pies are customarily prepared by what is essentially a three-stage process. In the first stage the crust or shell is prepared. This operation includes preparing and rolling out pie dough, cutting and inserting this sheeted pie dough into the baking pan, and, in some instances separately baking the crust or shell. The second stage involves the preparation of the filling material. In the third and final stage the filling material is introduced into the baking pan, and the combination of shell and filling material is subjected to a final baking operation to complete the baking of the shell and to cook or set the filling material.

In the preparation of frosted or iced cakes by the conventional methods, a four-stage process is employed. The cake batter is first prepared and then baked. Either concurrently with or subsequent to this baking operation, the icing or frosting is prepared. After the cake has cooled the icing is applied to and spread over the surface of the cake.

According to the practices of this invention pie-type baked confections are prepared by methods which eliminate the separate preparation and handling of the crust and filling material. Only a single batter is required. This batter is of the type conventionally employed for the preparation of cakes. When the methods of this invention are practiced, such a batter is prepared and baked by the conventional cake making methods; but the resulting product has the characteristics of a pie rather than a cake. The consequent elimination of the crust making operation permits a substantial reduction in the time and labor which are normally required for the commercial or household production of baked goods. Furthermore, the processes of this invention permit an unskilled housewife to turn out a uniform, well prepared pie-type confection without the risk of failure.

The methods of this invention make it possible to completely eliminate one of the conventionally employed steps required for the preparation of frosted or iced cakes. In the practice of this invention, as it relates to the preparation of such cakes, two batters are prepared, one the regular cake batter and the other a batter which is treated according to the methods described hereinafter. The batters are layered in the baking pan, the treated batter being the layer adjacent to the surface of the baking pan. On baking, the treated batter becomes the icing or frosting, thus eliminating the often tedious and time consuming step of the separate application of the icing to the cake.

The methods of this invention have further advantages in that they permit the preparation of new types of baked goods which have heretofore been unknown to the baking art. Pudding cakes, cakes with sweet confection-like fillings and other unusual variations are easily prepared with these methods.

Because they are more resistant to staling, the novel baked confections of this invention are superior to the conventional type of baked confections which become stale quite quickly. Even after long periods of storage

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these novel baked confections remain as fresh as they were immediately after baking.

In the practice of this invention relatively large quantities of heat-stable alpha amylase are added to the conventional type of cake batter. Upon baking, there result completely new and novel pie-like baked confections or other novel baked goods. The heat-stable alpha amylase during baking and cooling, attacks and digests a portion of the starch in the crumb, thereby changing the crumb structure into a pudding-like mass. This pudding-like mass contains the flavoring and sugary constituents of the pie and corresponds to the conventional type of pie filling material. The quantity of heat-stable alpha amylase which is employed is such that the digestion of the starch takes place in the interior crumb structure of the confection with relatively little digestive action on the crust structure.

This invention may also be applied to the production of new types of crackers, and it also makes possible the elimination of some of the steps which are normally employed in the present manufacture of crackers. For instance, an iced cracker can be produced in a single baking operation by layering a sweetened, flavored, alpha amylase treated dough on the regular cracker dough before it enters the baking oven, thus baking right on the cracker, the icing component. Normally, the icing or filling of a cracker sandwich requires a separate manufacturing operational step.

The heat-stable alpha amylase employed in the methods of this invention is obtained from a variety of sources. An enzyme may be obtained, for example, from certain species of *Bacillus mesentericus* and *Bacillus subtilis*. Such enzymes are quite heat-resistant, showing their maximum activity in the preparations of this invention at temperatures of 80° C. A certain degree of activity is retained even at a temperature of 100° C., the boiling point of water. At higher temperatures the enzyme is rapidly and completely inactivated.

The action of the heat-stable alpha amylases in the preparations of this invention during baking is believed to be as follows. When placed in a heated oven the batter in the baking pan gradually rises in temperature. That portion of the batter in contact with the surface of the baking pan and the open upper surface layer of the batter reaches a higher temperature than does the inside crumb portion of the batter. As a consequence, at normal baking temperatures, the enzyme in the outer batter layers is much more quickly inactivated than is the enzyme in the inner crumb portion of the batter. Since the internal temperature of the product never exceeds 100° C. during baking, the enzyme remains active in the crumb structure during baking and during the subsequent cooling period. This results in a product which has a normal pie-type dry crust and a moist, sweet, pie-type filling.

The quantity of enzyme necessary to produce a satisfactory product is affected by a number of variables, among which are the following: oven temperature, baking time, the size and type of pan, the thickness of the batter, the recipe and the amount and type of flour which is employed and the type of baked confection which is desired. The quantity and thermal stability of the enzyme employed is such that the crust is not materially affected, i.e., crust inactivation takes place rapidly to produce a normal dry crust, whereas there is retained in the crumb sufficient alpha amylase activity to attack and convert the fine, bubbly, solid, crumb structure to a moist, sweet pudding-type consistency. Within the normal range of enzyme concentration, considerable leeway is possible, depending upon the many variables which are of importance in obtaining the desired result.

Although empirically the amounts of alpha amylase employed in this invention are small, such amounts are

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much greater than the amounts which have heretofore been employed in the baking arts. For example, U.S. Patent No. 2,615,810 describes and claims a method for retarding the staling of bakery products in which there is employed from 0.1 to 4 SKB alpha amylase units per 100 grams of flour in the dough. Such SKB units are assayed by the method of Sandstedt, Kneen and Elish, published in *Cereal Chemistry*, volume XVI, page 712 (1939). In the methods of the present invention there is employed an amount of heat-stable alpha amylase enzymatic preparation equivalent to from 10 to 200 SKB alpha amylase units per 100 grams of flour in the dough. In the preferred methods, the heat-stable alpha amylase is present in amounts equivalent to from 15 to 50 SKB alpha amylase units per 100 grams of flour.

The examples set forth below will illustrate typical forms of this invention. In general, when a pie-type confection is baked according to the methods of this invention, less leavening material is employed than is customary, in order to avoid products with high sided crusts and depressed centers. The typical procedure for producing a pie-type confection by these methods is to employ the usual cake-making procedures for the preparation of the batter which contains the alpha amylase. The prepared batter is then placed in pans and baked according to conventional techniques. During the baking and cooling period the enzyme digests the internal crumb structure and produces the pudding or pie-type effect. No mechanical operations or extra handling are required, as this pie-type effect is obtained automatically, after the baked confection has cooled.

A more comprehensive understanding of this invention is obtained by reference to the following detailed examples:

## EXAMPLE I

*Chocolate Pie*

|                              |                                      |
|------------------------------|--------------------------------------|
| Flour.....                   | 2 1/4 cups.                          |
| Sugar.....                   | 2 3/4 cups.                          |
| Shortening.....              | 1/2 cup.                             |
| Eggs.....                    | 3.                                   |
| Cocoa.....                   | 1/2 cup.                             |
| Soda.....                    | 1 teaspoon.                          |
| Salt.....                    | Do.                                  |
| Vanilla.....                 | Do.                                  |
| Water.....                   | 1 1/4 cups.                          |
| Bacterial alpha amylase..... | 50 SKB units per 100 grams of flour. |

The batter is prepared by creaming, until fluffy, the shortening, sugar and eggs. This creamed mixture is beaten for five minutes with a high speed mixer. The sifted dry ingredients are then added alternately with the water and vanilla, the addition being accompanied by low speed mixing. The batter is then poured into the baking pan and baked at 350° F. for about 40 minutes. A novel chocolate pie results after the baked material cools.

## EXAMPLE II

*Nut and Fruit Baked Confection*

The following ingredients are sifted into a mixing bowl: two cups of cake flour, 1 1/4 cups of sugar, 1 1/2 teaspoons of soda, 1 1/2 teaspoons of salt, 2 tablespoons of cocoa, 1/2 teaspoon each of cinnamon, cloves, nutmeg and allspice. To this dry mixture are added 1/2 cup of shortening and 1 1/4 cups of applesauce. The resulting product is beaten for 2 minutes, 2 eggs are added, and the beating is continued until the batter is smooth. There are then stirred into the batter 3/4 cup each of cut-up dates, chopped raisins and chopped nuts and 1/2 cup diced citron. To the batter are added 25 SKB units of heat-stable alpha amylase per 100 grams of flour. The mixture is placed in pans and baked for 45 minutes at 350° F. This produces a moist, fruit and nut confection which retains its moist-freshness for long periods of time.

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## EXAMPLE III

*Pecan Pie*

A pecan pie-type baked confection is prepared as follows: A commercial dry cake mix, "Betty Crocker's Black Walnut Cake Mix" is obtainable in the open market. To 370 grams (1 commercially available package) there is added bacterial alpha amylase to the extent of 20 SKB alpha units per 100 grams of the dry mix. The batter is prepared in the normal manner, following the directions on the package. After preparation of the batter, 6 ozs. of shelled pecan halves are added to the batter which is then panned and baked for 28 minutes in an oven at 350° F. After cooling, a pecan pie results instead of the cake which would have been formed had the alpha amylase been omitted.

## EXAMPLE IV

*Marble Filled Cake*

## 20 WHITE CAKE PLUS MARBLING OF ALPHA TREATED CHOCOLATE CAKE

A batter is prepared from a conventional commercially obtainable white cake mix or recipe. A chocolate cake batter containing about 30 units of heat-stable alpha amylase per 100 grams of flour is then separately prepared. The recipe in Example I may be used for the preparation of this chocolate batter by increasing the sugar to 3 1/4 cups and decreasing the alpha amylase units to 30 SKB units per 100 grams of flour. The white cake batter is placed in a pan and the chocolate batter poured over the top surface. The batter is marbled by gently cutting and slightly mixing the chocolate batter with the white cake batter immediately prior to baking. After baking and cooling the white cake will be marbled by a sweet, moist chocolate baked confection.

## EXAMPLE V

*Pineapple Pie Cake*

This baked confection is prepared from a commercially available dry cake mix as follows: a package of Pillsbury Pineapple Cake Mix, as commonly available in grocery stores, is used. Bacterial alpha amylase to the extent of 15 SKB alpha units per 100 grams of dry mix is added during preparation of the batter. The batter is prepared according to the directions on the package and the baking directions are similarly used. After baking for 30 minutes in a 350° F. oven and subsequent cooling, a novel pineapple pie-like baked confection is obtained.

## EXAMPLE VI

*Cocunut Pie*

To about 1 lb. of a conventional commercially obtainable white cake mix there are added about 4 ounces of shredded coconut and 25 SKB units of heat-stable, alpha amylase per 100 grams of flour. After preparation of the batter, the batter is poured into pie tins and baked in the usual manner. After baking and cooling, a coconut pie results from the action of the enzyme on the crumb structure formed by the action of the heat on the batter.

## EXAMPLE VII

*Peach Pie*

Fruit pies may be prepared by using a conventional white cake mix batter in which the water content has been reduced in proportion to the amount of fresh fruit expected to be added. The amount of heat-stable bacterial amylase required for the preparation of these one-stage fruit pies is generally low, in the neighborhood of 10 SKB unit of heat-stable alpha amylase or less per 100 grams of flour. For instance, for the preparation of a peach pie the following recipe may be employed: a commercially available package of Betty Crocker's White Cake Mix, as normally sold to the housewife, is used to

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prepare the batter. Only about 40% of the water called for in the recipe is used. Six SKB units of heat-stable alpha amylase per 100 grams of flour are added during the preparation of the batter. To the batter there is added an equal weight of drained, sliced peaches. These ingredients are thoroughly incorporated in the batter which is then placed in a baking tin and baked for 22 minutes at 400-425° F.

EXAMPLE VIII

For the preparation of a unique sweet coated or iced cracker the following procedure may be used: two doughs are prepared; one the regular cracker base dough and two, the icing component. This is a regular type of cracker dough except that the sugar component is increased and the dough is flavored with a suitable flavoring ingredient, such as chocolate or other distinctively flavored components. Included in this dough is the heat-stable alpha amylase in a quantity sufficient to react during and after baking to produce a soft saccharin layer on the cracker base. Thus, through the use of this enzyme treatment, it is possible in a single baking operation to form filled or coated crackers, thereby completely eliminating the otherwise requisite conventional icing procedure.

The above examples are to be considered only as illustrative of the products and processes of this invention and are not to be construed as limiting the scope of the invention.

What is claimed is:

1. The process of making baked confections which comprises incorporating in a batter a heat-stable alpha amylase enzymatic preparation in amounts equivalent to from 10 to 200 SKB alpha amylase units per 100 grams of flour in the batter, said enzymatic preparation being present in such quantity and being of such thermal stability that effective amounts of alpha amylase are present and active in the crumb at temperatures above the starch digestion point to reduce the normal crumb structure to a pudding like mass and thereafter baking the batter.

2. The process of making baked confections which comprises incorporating in a batter a member of the group consisting of heat-stable bacterial alpha amylase enzymatic preparations derived from *Bacillus mesentericus* and *Bacillus subtilis* in amounts equivalent to from 10 to 200 SKB alpha amylase units per 100 grams of flour in the batter, said enzymatic preparation being present in such quantity and being of such thermal stability that effective amounts of alpha amylase are present and active in the crumb at temperatures above the starch digestion point to reduce the normal crumb structure to a pudding like mass and thereafter baking the batter.

3. The process of making baked confections which comprises incorporating in a batter a heat-stable alpha

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amylase enzymatic preparation in amounts equivalent to from 15 to 50 SKB alpha amylase units per 100 grams of flour in the batter, said enzymatic preparation being present in such quantity and being of such thermal stability that effective amounts of alpha amylase are present and active in the crumb at temperatures above the starch digestion point to reduce the normal crumb structure to a pudding like mass and thereafter baking the batter.

4. The process of making baked confections which comprises incorporating in a batter a member of the group consisting of heat-stable bacterial alpha amylase enzymatic preparations derived from *Bacillus mesentericus* and *Bacillus subtilis* in amounts equivalent to from 15 to 50 SKB alpha amylase units per 100 grams of flour in the batter, said enzymatic preparation being present in such quantity and being of such thermal stability that effective amounts of alpha amylase are present and active in the crumb at temperatures above the starch digestion point to reduce the normal crumb structure to a pudding like mass and thereafter baking the batter.

5. In the process of making baked confections which comprises incorporating into a batter an alpha amylase preparation in amounts equivalent to from 6 to about 200 SKB alpha amylase units per 100 grams of flour in the batter, said alpha amylase being of such thermal activity that effective amounts of alpha amylase are present and active in the crumb at temperatures above the starch digestion point to reduce the normal crumb structure to a pudding like mass and thereafter baking the batter.

6. A dry confection mix comprising flour and alpha amylase in an amount equivalent to from 6 to 200 SKB unit per 100 grams of flour, said alpha amylase being of such thermal stability that effective amounts are active above the starch gelatinization point to reduce the crumb structure to a pudding-like mass.

7. A dry baking mix comprising flour, sugar and shortening and alpha amylase enzyme in amounts equivalent to 6 to 200 SKB units per 100 grams of flour, said alpha amylase being of such thermal stability that effective amounts are active above the starch gelatinization point to reduce the crumb structure to a pudding-like mass.

8. A dry baking mix comprising flour, sugar and shortening and an alpha amylase enzyme preparation derived from a member selected from the class *Bacillus mesentericus* and *Bacillus subtilis* in an amount equivalent to 6 to 200 SKB units per 100 grams of flour, said alpha amylase being of such thermal stability that effective amounts are active above the starch gelatinization point to reduce the crumb structure to a pudding-like mass.

References Cited in the file of this patent

UNITED STATES PATENTS

2,615,810 Stone Oct. 28, 1952

## UNITED STATES PATENT OFFICE

2,615,810

## RETARDING THE STALING OF BAKERY PRODUCTS

Irwin Stone, New York, N. Y., assignor to Wallerstein Company, New York, N. Y., a corporation of New York

No Drawing. Application March 4, 1948,  
Serial No. 12,933

6 Claims. (Cl. 98-81)

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This invention relates to the inhibition, or prevention, of the staling of bread and other baked products.

The staling of bakery products incurs the loss of much money and causes the annual wastage of many millions of bushels of wheat. In normal times it has been estimated that the annual losses due to staling is 2.0 to 4.7% of total production.

Bread-staling processes can be divided into two parts: the staling of the crust and the staling of the crumb. The staling of the crust, which is relatively unimportant, changes the dry, crisp crust of fresh bread into a soft, leathery coating and is due mostly to transference of moisture from the center to the outside of the loaf. This staling is more noticeable in wrapped loaves. The staling of the crumb is of much more importance and is what is commonly recognized as staling. The texture of the crumb becomes harder, tougher, and more crumbly and increasingly opaque as the bread stales.

A principal object of the invention is to provide a process for the making of bakery products having improved resistance to staling.

Another object is to provide an anti-staling composition for treating baker's dough.

Further objects will appear hereinafter.

In the following discussion, when the term "bread" is used it is also implied that other bakery products such as rolls, muffins, biscuits, doughnuts, crackers or cake are also included.

Although the staling of the crumb is a complicated process not fully understood and as to the mechanism of which there are many conflicting theories, I have found that by the addition of small amounts of a heat-stable amylolytic enzyme to the dough before baking, that it is possible to prevent, or inhibit, or retard the rate of staling of bread. This heat-stable enzyme may be obtained from bacterial sources and is characterized by strong starch liquefying and dextrinizing power, alpha amylase activity, and proteolytic activity. The enzyme may be obtained, for example, from certain species of *Bacillus mesentericus* and *B. subtilis* and includes enzymes which are stable up to temperatures of 80° C. to 90° C. Such enzyme preparations of bacterial amylases of high heat stability and strong starch liquefying and dextrinizing power are commercially available.

Although the invention is not dependent on any theory, I believe that the action of this enzyme in baking is somewhat as follows: In the amounts employed, the enzyme produces substantially no effect at the low temperatures en-

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countered in the preparation and rising of the dough. At the temperatures at which the bread is baked, the starch gelatinizes and the enzyme partially degrades this gelatinized starch at the high temperatures of the bread during the baking process. The amounts of these heat-stable enzymes added to the dough are very small and have to be controlled within a narrow range because it is easily possible to over-treat the bread. In case of over-treating by using too much of the enzyme the crumb becomes soft, pasty, doughy, and sticky and the crumb character may even be completely destroyed. When used in the proper proportion, this enzyme acts upon the starch of the crumb at the high temperatures of baking after the starch is gelatinized and partly degrades it to a form which, if current theories are assumed correct, no longer retrogrades and stales.

I am aware that amylolytic enzymes are present normally in flour and that their supplementation in baking has been recommended. In fact, it is standard practice to add malt as a supplement either to a flour or to a bread formula in order to help produce the fermentable sugars used by the yeast in causing the bread to rise. However, all of the enzymes that have been used for this purpose are heat-labile and the added enzyme activity is destroyed by the heat of the baking before the starch gelatinizes and before the enzymes have had a chance to act upon the gelatinized starch. The action of these customary enzymes is to partially attack the raw starch granules at low temperatures, producing fermentable sugars. The heat-stable enzymes that I employ are used in amounts so small that they have practically no action on the raw starch in the bread dough and do not produce significant amounts of fermentable sugars. In fact, these heat-stable enzymes are poor producers of fermentable sugars, but are very active in the partial degradation and thinning of starches.

In the customary use of enzymes for the low temperature production of fermentable sugars in baking, only heat-labile enzymes can be used. If a heat-stable type of enzyme were employed for the production of fermentable sugars, the amount required for the fermentation would of necessity be so large that during the baking process the crumb structure of the bread would be completely destroyed and the interior of the bread would be liquefied.

I have found that only very small amounts of the specific heat-stable enzymes are needed to produce the desired effect. In the case of a bread

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formula only about 0.005%, on the weight of the flour, of a bacterial enzyme preparation having an alpha amylase activity of about 200 SKB units per gram of the enzyme preparation may be required. Alpha amylase activity may be conveniently determined by the method of Sandstedt, Kneen & Blah (S. K. B. units) as described in "Cereal Chemistry," vol. 16, page 712 (1939). This amount of enzyme is insufficient to produce any change in the dough before baking, but at the baking temperatures, the heat-stable enzyme is activated by the high temperatures and then produces its effect.

While the enzyme is normally quite stable, the addition of small amounts of calcium ion to a bread formula, which may be deficient in calcium ions, tends to increase this heat stability, thereby making it possible to use smaller amounts of enzyme to get the desired effect.

Example I

|                                                  | Parts    |
|--------------------------------------------------|----------|
| Flour                                            | 100      |
| Dry skim milk                                    | 4        |
| Shortening                                       | 3        |
| Sugar                                            | 6        |
| Salt                                             | 1.5      |
| Yeast                                            | 2        |
| Malt extract                                     | 0.25     |
| Potassium bromate                                | 0.003    |
| Bacterial alpha amylase preparation <sup>1</sup> | 0.005    |
| Water                                            | about 75 |

<sup>1</sup> A purified enzyme having an alpha amylase activity of 200 SKB units per gram. Less pure enzymes may also be employed if an amount of equivalent activity is employed.

The dry ingredients, flour, skim milk powder, salt, and sugar are mixed and the shortening worked in. The yeast, malt extract and bromate are dissolved in a portion of the water. The heat-stable bacterial amylase may either be added dry or dissolved in a portion of the water. All ingredients are then mixed in the usual manner to form a dough. The dough is allowed to rise in a warm place (about 30° C.) for an hour and three quarters. It is then given the first punch and placed back in the warm place. After 50 minutes it is given the second punch and placed back in the warm place. In 25 minutes the dough is divided, molded and placed into pans. The pans are put in the warm place to rise for 55 minutes. They are then baked for 25 minutes at 220-225° C.

This is an average straight dough bread baking procedure. My anti-staling method is also applicable to the sponge dough method of baking bread without any change in the customary procedure except to add the heat-stable amylolytic enzyme. This enzyme may be added either in the sponge or in the dough, or a portion may be added to both.

Example II

|                                                  | Parts    |
|--------------------------------------------------|----------|
| Flour                                            | 100      |
| Sugar                                            | 5        |
| Salt                                             | 1        |
| Yeast                                            | 2.5      |
| Malt extract                                     | 0.25     |
| Water                                            | about 75 |
| Bacterial alpha amylase preparation <sup>1</sup> | 0.001    |

<sup>1</sup> The bacterial alpha amylase preparation is a purified enzyme material having an alpha amylase activity of 200 SKB units per gram. A less pure bacterial amylase may be substituted if the amounts of equivalent activity are maintained.

The procedure for mixing and baking is the

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customary one and is similar to that given in Example I.

The optimum range of the enzyme concentration is that range which produces a slight partial degradation of the gelatinized starch without producing a gross visible change in the crumb structure of the bread. I have found that the addition of the heat-stable amylolytic enzyme in amounts in the range equivalent to about 1/4 to about 2 SKB alpha amylase units per 100 grams of flour is generally sufficient to retard and inhibit staling. This range would be equivalent to from about 0.00125% to about 0.010%, on the weight of flour, of a purified bacterial amylase having an activity of 200 units per gram. The optimum range of activity is not a fixed quantity but is dependent upon several factors among which, size of loaf, temperature of baking, concentration of calcium ions, bread formula employed, and type of flour are of importance.

While the optimum range of enzyme additive as given in the previous paragraph is preferred, it is possible in some cases to employ as little as 0.1 SKB alpha amylase units of heat-stable enzymatic preparation per 100 grams of flour to obtain the desired anti-staling effect. Also, in certain cases it is possible to use as much as 3 or 4 SKB units per 100 grams of flour without producing undesired changes in crumb structure.

My anti-staling process is also applicable to cakes and other bakery products. In cakes, due to the use of increased amounts of shortening the tendency toward staling is generally somewhat less. For this reason the amounts of heat-stable enzyme needed to give the desired anti-staling effect may be less than is required for bread. However, this varies considerably with the recipe employed, baking temperatures and other factors, but generally the range will not be far from that of bread.

Widespread use of the recent important development of canned bread is limited to a large extent by the staling of the bread in the cans. Although such canned bread may remain fresh for some time, it firms up eventually and becomes stale in the can. I have found that bread, baked by my process in which the dough contains heat-stable amylolytic enzymes, can be canned and still not undergo this crumb staling or firming which heretofore has prevented the widespread use of this canning process.

The term "dough" as used in the following claims has a broad meaning and includes not only conventional bread doughs but also cake batters, cracker mixes and other uncooked baker's products. The term "flour" is also used in a broad sense to include meal and other starchy ingredients of bread.

I claim:

1. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a heat-stable alpha amylase enzymatic preparation in amounts equivalent to from 0.1 to 4 SKB alpha amylase units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzyme are present and active in the crumb at temperatures above the starch gelatinization point, and thereafter baking the dough.

2. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a heat-stable bacterial alpha amylase enzymatic preparation in amounts equivalent to from 0.1 to 4 SKB alpha amylase

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units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzyme are present and active in the crumb at temperatures above the starch gelatinization point, and thereafter baking the dough.

3. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a member of the group consisting of heat-stable bacterial alpha amylase enzymatic preparations derived from *Bacillus mesentericus* and *Bacillus subtilis* in amounts equivalent to from 0.1 to 4 SKB alpha amylase units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzymes are present and active in the crumb at temperatures above the starch gelatinization point, and thereafter baking the dough.

4. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a heat-stable alpha amylase enzymatic preparation in amounts equivalent to from 1/4 to 2 SKB alpha amylase units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzymes are present and active in the crumb at temperatures above about 80° C. in the crumb and thereafter baking the dough.

5. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a heat-stable bacterial alpha amylase enzymatic preparation in amounts equivalent to from 1/4 to 2 SKB alpha amylase units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzyme are present and active in the crumb at

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temperatures above about 80° C. in the crumb and thereafter baking the dough.

6. The process of making bakery products having inhibited staling qualities which comprises incorporating in a dough a member of the group consisting of heat-stable bacterial alpha amylase enzymatic preparations derived from *Bacillus mesentericus* and *Bacillus subtilis* in amounts equivalent to from 1/4 to 2 SKB alpha amylase units per 100 grams of flour in the dough, said enzymatic preparation being of such thermal stability that effective amounts of alpha amylase enzyme are present and active in the crumb at temperatures above about 80° C. in the crumb and thereafter baking the dough.

IRWIN STONE.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
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| (54) Title: <b>PREPARATION OF DOUGH AND BAKED PRODUCTS</b><br>(57) Abstract<br><p>It is known that the addition of an anti-staling amylase reduces the rate of crumb firming during storage for 1-7 days after baking, but the inventors found a need to improve the softness in the initial period after baking, particularly the first 24 hours after baking. This can be overcome by using a phospholipase, so that bread made by the combined use of an anti-staling amylase and a phospholipase has improved softness, both when eaten on the same day and when stored for several days after baking. There is no significant change in the taste or smell of the baked product.</p> |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

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## PREPARATION OF DOUGH AND BAKED PRODUCTS

### FIELD OF THE INVENTION

The invention relates to process for preparing a dough or a baked product prepared from the dough. More particularly, it relates to such a process where the bread has an improved softness, both when eaten on the same day and when eaten after several days storage.

### BACKGROUND OF THE INVENTION

It is well known that the softness of bread deteriorates during storage from the time of baking to the time of consumption. The term staling is used to describe such undesirable changes in the properties of the bread. Staling results in an increase of the firmness of the crumb, a decrease of the elasticity of the crumb, and changes in the crust, which becomes tough and leathery.

Enzymatic retardation of staling by means of various amylases has been described. Thus, US 2,615,810; US 3,026,205 and O. Silberstein, "Heat-Stable Bacterial Alpha-Amylase in Baking", Baker's Digest 38(4), Aug. 1964, pp. 66-70 and 72, describe the use of alpha-amylase. WO 91/04669 (Novo Nordisk) describes the use of a maltogenic alpha-amylase from *Bacillus stearothermophilus*. It is also known to use  $\beta$ -amylase to retard staling.

It is also known to add a phospholipase to dough. Thus, US 4,567,046 and EP 171,995 (both to Kyowa Hakko) disclose that the addition of phospholipase A enhances the properties of dough and bread, including retardation of the staling.

M.R. Kweon et al., Journal of Food Science, 59 (5), 1072-1076 (1994) disclose the effect of 2-4 % by weight of phospholipid hydrolysate together with an anti-staling amylase on the retrogradation of starch in bread.

### SUMMARY OF THE INVENTION

The inventors confirmed that the addition of an anti-staling amylase reduces the rate of crumb firming during storage for 1-7 days after baking, but they found that there is a need to improve the softness in the initial period after baking, particularly the first 24 hours after baking. They further found that this can be achieved by using a phospholipase, so that bread made by the combined use of an anti-staling amylase and a phospholipase has improved softness, both when eaten on the same day and when stored for several days after baking. There is no significant change in the taste or smell of the baked product.

Accordingly, The invention provides a process for preparing a dough or a baked product prepared from the dough which comprises adding to the dough an anti-

staling amylase and a phospholipase. The invention also provides a dough and a pre-mix comprising these ingredients.

## DETAILED DESCRIPTION OF THE INVENTION

### 5 Anti-staling amylase

The anti-staling amylase used in the invention may be any amylase that is effective in retarding the staling (crumb firming) of baked products.

The amylase preferably has a temperature optimum in the presence of starch in the range of 30-90°C, preferably 50-80°C, particularly 55-75°C, e.g. 60-70°C. The  
10 temperature optimum may be measured in a 1 % solution of soluble starch at pH 5.5.

The anti-staling amylase may be an endo-amylase, preferably a bacterial endo-amylase, e.g. from *Bacillus*. A preferred example is a maltogenic alpha-amylase (EC 3.2.1.133), e.g. from *Bacillus*. A maltogenic alpha-amylase from *B. stearothermo-*  
15 *philus* strain NCIB 11837 is commercially available from Novo Nordisk A/S under the tradename Novamyl®. It is further described in US 4,598,048 and US 4,604,355 and in C. Christophersen et al., Starch, vol. 50, No. 1, 39-45 (1997).

Other examples of anti-staling endo-amylases are bacterial alpha-amylases, derived e.g. from *Bacillus*, particularly *B. licheniformis* or *B. amyloliquefaciens*.

The anti-staling amylase may be an exo-amylase such as  $\beta$ -amylase, e.g.  
20 from plant (e.g. soy bean) or from microbial sources (e.g. *Bacillus*).

The anti-staling amylase is added in an effective amount for retarding the staling (crumb firming) of the baked product. The amount of anti-staling amylase will typically be in the range of 0.01-10 mg of enzyme protein per kg of flour, e.g. 1-10 mg/kg. A maltogenic alpha-amylase is preferably added in an amount of 50-5000  
25 MANU/kg of flour, e.g. 100-1000 MANU/kg. One MANU (Maltogenic Amylase Novo Unit) may be defined as the amount of enzyme required to release one  $\mu$ mol of maltose per minute at a concentration of 10 mg of maltotriose (Sigma M 8378) substrate per ml of 0.1 M citrate buffer, pH 5.0 at 37 °C for 30 minutes.

### Phospholipase

30 The phospholipase may have A<sub>1</sub> or A<sub>2</sub> activity to remove fatty acid from the phospholipid and form a lyso-phospholipid. It may or may not have lipase activity, i.e. activity on triglycerides. The phospholipase preferably has a temperature optimum in the range of 30-90°C, e.g. 30-70°C.

The phospholipase may be of animal origin, e.g. from pancreas (e.g. bovine  
35 r porcine pancreas), snake venom or bee venom. Alternatively, the phospholipase may be of microbial origin, e.g. from filamentous fungi, yeast or bacteria, such as the

30  
genus or species *Aspergillus*, *A. niger*, *Dictyostelium*, *D. discoideum*, *Mucor*, *M. javanicus*, *M. mucedo*, *M. subtilissimus*, *Neurospora*, *N. crassa*, *Rhizomucor*, *R. pusillus*, *Rhizopus*, *R. arrizus*, *R. japonicus*, *R. stolonifer*, *Sclerotinia*, *S. libertiana*, *Trichophyton*, *T. rubrum*, *Whetzelinia*, *W. sclerotiorum*, *Bacillus*, *B. megaterium*, *B. subtilis*, *Citrobacter*, *C. freundii*, *Enterobacter*, *E. aerogenes*, *E. cloacae* *Edwardsiella*, *E. tarda*, *Erwinia*, *E. herbicola*, *Escherichia*, *E. coli*, *Klebsiella*, *K. pneumoniae*, *Proteus*, *P. vulgaris*, *Providencia*, *P. stuartii*, *Salmonella*, *S. typhimurium*, *Serratia*, *S. liquefasciens*, *S. marcescens*, *Shigella*, *S. flexneri*, *Streptomyces*, *S. violaceoruber*, *Yersinia*, or *Y. enterocolitica*. A preferred phospholipase is derived from a strain of  
10 *Fusarium*, particularly *F. oxysporum*, e.g. from strain DSM 2672, as described in co-pending PCT/DK 97/00557.

The phospholipase is added in an amount which improves the softness of the bread during the initial period after baking, particularly the first 24 hours. The amount of phospholipase will typically be in the range of 0.01-10 mg of enzyme protein per kg  
15 of flour (e.g. 0.1-5 mg/kg) or 200-5000 LEU/kg of flour (e.g. 500-2000 LEU/kg).

A phospholipase with lipase activity is preferably added in an amount corresponding to an lipase activity of 20-1000 LU/kg of flour, particularly 50-500 LU/kg. One LU (Lipase Unit) is defined as the amount of enzyme required to release 1  $\mu$ mol butyric acid per minute at 30.0°C; pH 7.0; with Gum Arabic as emulsifier and tributyrin  
20 as substrate.

#### Phospholipase activity (LEU)

In the LEU assay, the phospholipase activity is determined from the ability to hydrolyze lecithin at pH 8.0, 40°C. The hydrolysis reaction can be followed by titration with NaOH for a reaction time of 2 minutes. The phospholipase from porcine pancreas  
25 has an activity of 510 LEU/mg (taken as standard), and the phospholipase from *Fusarium oxysporum* has an activity of 1540 LEU/mg.

#### Phospholipid

The phospholipase may act on phospholipid provided by flour in the dough, so the separate addition of a phospholipid is not required. However, the softening effect may be increased by adding a phospholipid, preferably in an amount of 0.05-20  
30 g/kg of flour, e.g. 0.1-10 g/kg. The phospholipid may be a diacyl-glycero-phospholipid, such as lecithin or cephalin.

#### Dough

The dough of the invention generally comprises wheat meal or wheat flour  
35 and/or other types of meal, flour or starch such as corn flour, corn starch, rye meal,

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rye flour, oat flour, oat meal, soy flour, sorghum meal, sorghum flour, potato meal, potato flour or potato starch.

The dough of the invention may be fresh, frozen or par-baked.

The dough of the invention is normally a leavened dough or a dough to be  
5 subjected to leavening. The dough may be leavened in various ways, such as by adding chemical leavening agents, e.g., sodium bicarbonate or by adding a leaven (fermenting dough), but it is preferred to leaven the dough by adding a suitable yeast culture, such as a culture of *Saccharomyces cerevisiae* (baker's yeast), e.g. a commercially available strain of *S. cerevisiae*.

10 The dough may also comprise other conventional dough ingredients, e.g.: proteins, such as milk powder, gluten, and soy; eggs (either whole eggs, egg yolks or egg whites); an oxidant such as ascorbic acid, potassium bromate, potassium iodate, azodicarbonamide (ADA) or ammonium persulfate; an amino acid such as L-cysteine; a sugar; a salt such as sodium chloride, calcium acetate, sodium sulfate or calcium  
15 sulfate.

The dough may comprise fat (triglyceride) such as granulated fat or shortening, but the invention is particularly applicable to a dough where less than 1 % by weight of fat (triglyceride) is added, and particularly to a dough which is made without addition of fat.

20 The dough may further comprise an emulsifier such as mono- or diglycerides, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, polyoxyethylene stearates, or lysolecithin, but the invention is particularly applicable to a dough which is made without addition of emulsifiers (other  
25 than optionally phospholipid).

#### **Additional enzyme**

Optionally, an additional enzyme may be used together with the anti-staling amylase and the phospholipase. The additional enzyme may be a second amylase, such as an amyloglucosidase, a beta-amylase, a cyclodextrin glucanotransferase, or  
30 the additional enzyme may be a peptidase, in particular an exopeptidase, a transglutaminase, a lipase, a cellulase, a hemicellulase, in particular a pentosanase such as xylanase, a protease, a protein disulfide isomerase, e.g., a protein disulfide isomerase as disclosed in WO 95/00636, a glycosyltransferase, a branching enzyme (1,4- $\alpha$ -glucan branching enzyme), a 4- $\alpha$ -glucanotransferase (dextrin glycosyltransferase) or  
35 an oxidoreductase, e.g., a peroxidase, a laccase, a glucose oxidase, a pyranose oxidase, a lipoxygenase, an L-amino acid oxidase or a carbohydrate oxidase.

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The additional enzyme may be of any origin, including mammalian and plant, and preferably of microbial (bacterial, yeast or fungal) origin and may be obtained by techniques conventionally used in the art.

The xylanase is preferably of microbial origin, e.g. derived from a bacterium or 5 fungus, such as a strain of *Aspergillus*, in particular of *A. aculeatus*, *A. niger* (cf. WO 91/19782), *A. awamori* (WO 91/18977), or *A. tubigensis* (WO 92/01793), from a strain of *Trichoderma*, e.g. *T. reesei*, or from a strain of *Humicola*, e.g. *H. insolens* (WO 92/17573, the contents of which is hereby incorporated by reference). Pentopan® and Novozym 384® (both from Novo Nordisk A/S) are commercially available xylanase 10 preparations produced by *Trichoderma reesei*.

The amyloglucosidase may be an *A. niger* amyloglucosidase (such as AMG™, available from Novo Nordisk A/S, Denmark). Other useful amylase products include Grindamyl® A 1000 or A 5000 (available from Grindsted Products, Denmark) and Amylase® H or Amylase® P (available from Gist-Brocades, The Netherlands).

15 The glucose oxidase may be a fungal glucose oxidase, in particular an *Aspergillus niger* glucose oxidase (such as Gluzyme®, available from Novo Nordisk A/S, Denmark).

The protease may in particular be Neutrase® (available from Novo Nordisk A/S, Denmark).

20 The lipase may be derived from a strain of *Thermomyces* (*Humicola*), *Rhizomucor*, *Candida*, *Aspergillus*, *Rhizopus*, or *Pseudomonas*, in particular from *Thermomyces lanuginosus* (*Humicola lanuginosa*), *Rhizomucor miehei*, *Candida antarctica*, *Aspergillus niger*, *Rhizopus delemar* or *Rhizopus arrhizus* or *Pseudomonas cepacia*. In specific embodiments, the lipase may be Lipase A or Lipase B derived 25 from *Candida antarctica* as described in WO 88/02775, or the lipase may be derived from *Rhizomucor miehei* as described in EP 238,023, or *Humicola lanuginosa* described in EP 305,216, or *Pseudomonas cepacia* as described in EP 214,761 and WO 89/01032.

#### Baked product

30 The process of the invention may be used for any kind of baked product prepared from dough, either of a soft or a crisp character, either of a white, light or dark type. Examples are bread (in particular white, whole-meal or rye bread), typically in the form of loaves or rolls, French baguette-type bread, pita bread, tortillas, cakes, pancakes, biscuits, cookies, pie crusts, crisp bread, steamed bread, pizza and the 35 like.

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### Pre-mix

The present invention further relates to a pre-mix comprising flour together with an anti-staling amylase, a phospholipase and a phospholipid. The pre-mix may contain other dough-improving and/or bread-improving additives, e.g. any of the additives, including enzymes, mentioned above.

### Enzyme preparation

The invention provides an enzyme preparation comprising an anti-staling amylase and a phospholipase, for use as a baking additive in the process of the invention. The enzyme preparation is preferably in the form of a granulate or agglomerated powder. It preferably has a narrow particle size distribution with more than 95 % (by weight) of the particles in the range from 25 to 500  $\mu\text{m}$ .

Granulates and agglomerated powders may be prepared by conventional methods, e.g. by spraying the amylase onto a carrier in a fluid-bed granulator. The carrier may consist of particulate cores having a suitable particle size. The carrier may be soluble or insoluble, e.g. a salt (such as NaCl or sodium sulfate), a sugar (such as sucrose or lactose), a sugar alcohol (such as sorbitol), starch, rice, corn grits, or soy.

## EXAMPLES

### Example 1

Bread was baked with anti-staling amylase, phospholipase and phospholipid. As reference, bread was also baked without one or more of these ingredients.

The phospholipid was lecithin at a dosage of 10 g/kg. The phospholipase was from *Fusarium oxysporum* used at a dosage of 50, 250 or 500 LU/kg, corresponding to or 0.04, 0.19 or 0.38 mg/kg. The anti-staling amylase was a maltogenic  $\alpha$ -amylase from *B. stearothermophilus* (Novamyl) at a dosage of 750 MANU/kg (1 mg/kg). All dosages in the Examples were based on kg of flour.

Doughs were prepared according to a standard European straight dough procedure with 50 g yeast per kg of flour and 40 ppm of ascorbic acid. The doughs were scaled to 350 g and baked in lidded pans.

The crumb firmness was measured using a texture analyzer TA-XT2 from Stable Micro Systems. Texture was measured according to a modified ACCA method (American Cereal Chemists' Association). These measurements were made after 0 days (approximately 2 hours after baking) and again after 1, 2 and 7 days storage (wrapped in double plastic bags and stored at 22°C).

The results are shown as firmness versus additive and storage time:

| Additives                                                            | Phospholipase dosage (LU/kg) | 2 hours | 1 day | 2 days | 7 days |
|----------------------------------------------------------------------|------------------------------|---------|-------|--------|--------|
| Invention: Anti-staling<br>amylase + phospholipase +<br>phospholipid | 50                           | 316     | 417   | 517    | 868    |
|                                                                      | 250                          | 279     | 371   | 455    | 790    |
|                                                                      | 500                          | 248     | 324   | 410    | 752    |
| Reference:                                                           |                              |         |       |        |        |
| None (control)                                                       | 0                            | 296     | 875   | 1207   | 2162   |
| Antistaling<br>amylase                                               | 0                            | 469     | 563   | 801    | 1083   |
| Phospholipid +<br>phospholipase                                      | 50                           | 208     | 470   | 782    | 1560   |
|                                                                      | 250                          | 231     | 467   | 721    | 1424   |
|                                                                      | 500                          | 233     | 420   | 649    | 1303   |

**Example 2**

A baking test was made as in Example 1, but with dosages of 0.5 mg/kg of the phospholipase (770 LEU/kg) and 1 g/kg of the phospholipid. The results are given as firmness after storage, and for comparison the firmness is also expressed in % of the control.

| Additives                                                           | 2 hours       | 5 hours       | 12 hours      | 20 hours      | day 2         | day 3         |
|---------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Invention: Antistaling<br>amylase + phospholipase +<br>phospholipid | 181<br>(78%)  | 195<br>(65%)  | 223<br>(51%)  | 241<br>(46%)  | 277<br>(34%)  | 303<br>(32%)  |
| Reference:                                                          |               |               |               |               |               |               |
| None (control)                                                      | 233<br>(100%) | 302<br>(100%) | 434<br>(100%) | 526<br>(100%) | 824<br>(100%) | 959<br>(100%) |
| Antistaling<br>amylase                                              | 372<br>(160%) | 468<br>(155%) | 518<br>(119%) | 482<br>(92%)  | 547<br>(66%)  | 637<br>(66%)  |
| Phospholipid +<br>phospholipase                                     | 144<br>(62%)  | 144<br>(47%)  | 212<br>(49%)  | 258<br>(49%)  | 364<br>(44%)  | 482<br>(50%)  |

**Example 3**

A baking test was made as in Examples 1 and 2, using a different phospholipase. The phospholipase was from porcine pancreas at a dosage of 2 mg/kg (1020 LEU/mg). The dosages of the anti-staling amylase and the phospholipid were as in  
 5 Example 2, and the results are presented as in Example 2:

| Additives                                                           | 2 hours       | 5 hours       | 12 hours      | 20 hours      | day 2         | day 3          |
|---------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|----------------|
| Invention: Antistaling<br>amylase + phospholipase +<br>phospholipid | 342<br>(122%) | 411<br>(103%) | 420<br>(80%)  | 431<br>( 73%) | 485<br>(52%)  | 559<br>(48%)   |
| Reference:<br>None (control)                                        | 281<br>(100%) | 398<br>(100%) | 524<br>(100%) | 588<br>(100%) | 937<br>(100%) | 1157<br>(100%) |
| Antistaling<br>amylase                                              | 409<br>(146%) | 490<br>(123%) | 514<br>(98%)  | 528<br>(89%)  | 625<br>(67%)  | 673<br>(58%)   |
| Phospholipid +<br>phospholipase                                     | 218<br>(76%)  | 260<br>(65%)  | 367<br>(70%)  | 472<br>(80%)  | 668<br>(71%)  | 906<br>(78%)   |

The results of Examples 1-3 show that the addition of anti-staling amylase retards the crumb firming during storage, but increases the initial firmness compared  
 10 to the control without additives. The addition of phospholipid + phospholipase according to the invention is effective in avoiding the increased initial firmness and further reduces the rate of crumb firming during storage, compared to the anti-staling amylase alone.

**15 Example 4**

Bread loaves were baked with and without phospholipid (lecithin) as indicated below. The phospholipase was *F. oxysporum* used at a dosage of 1 mg/kg (1540 LEU/kg). The anti-staling amylase and the baking conditions were as described in Example 1. The results are given as firmness after storage:

|           |                                    |                        |                      | Firmness |       |        |
|-----------|------------------------------------|------------------------|----------------------|----------|-------|--------|
|           | Anti-staling<br>amylase<br>MANU/kg | Phospholipase<br>mg/kg | Phospholipid<br>g/kg | 2 hours  | 1 day | 3 days |
| Control   | 0                                  | 0                      | 0                    | 294      | 687   | 1179   |
| Invention | 750                                | 1                      | 10                   | 200      | 229   | 277    |
|           | 750                                | 1                      | 2                    | 167      | 218   | 287    |
|           | 750                                | 1                      | 1                    | 167      | 232   | 305    |
|           | 750                                | 1                      | 0.5                  | 189      | 269   | 333    |
|           | 750                                | 1                      | 0.1                  | 196      | 260   | 381    |
|           | 750                                | 1                      | 0                    | 199      | 264   | 372    |

The results show that addition of anti-staling amylase and phospholipase  
5 clearly improve the softness, both initial softness (2 hours) and softness after storage  
(3 days). The softening effect can be further improved by addition of phospholipid.  
The optimum dosage appears to be about 1 mg/kg of phospholipid.

**CLAIMS**

1. A process for preparing a dough or a baked product prepared from the dough, comprising incorporating into the dough an anti-staling amylase and a phospholipase.
2. The process of the preceding claim wherein the anti-staling amylase has optimum activity in bread at 70-90°C.
3. The process of either preceding claim wherein the anti-staling amylase is an endo-amylase from *Bacillus*.
4. The process of either preceding claim wherein the anti-staling amylase is a maltogenic alpha-amylase, preferably from *B. stearothermophilus*.
5. The process of any preceding claim wherein the phospholipase has a temperature optimum of 30-70°C.
6. The process of any preceding claim wherein the phospholipase is fungal, preferably from *Fusarium*, most preferably from *F. oxysporum*.
7. The process of any preceding claim which further comprises incorporating a phospholipid (preferably lecithin) into the dough.
8. The process of any preceding claim which does not comprise addition of fat.
9. The process of any preceding claim which does not comprise addition of lysophospholipid.
10. The process of any preceding claim which does not comprise addition of emulsifiers other than the phospholipid.
11. The process of any preceding claim wherein the dough consists essentially of flour, water, yeast, salt and sugar.

12. A dough which comprises an anti-staling amylase and a phospholipase.
13. A pre-mix for dough comprising flour, an anti-staling amylase and a phospholipase.
14. An enzyme preparation which comprises an anti-staling amylase and a phospholipase.
15. The preparation of the preceding claim which further comprises a phospholipid, preferably lecithin.
16. The preparation of the preceding claim which further comprises a hemicellulase, preferably a pentosanase, more preferably a xylanase.
- 10 17. The preparation of claim 13 or 14 which is a granulate or an agglomerated powder.
18. The additive of any of claims 13-15 wherein more than 95 % (by weight) has a particle size between 25 and 500  $\mu\text{m}$ .

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 99/00185

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A21D 8/04, A21D 2/26

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAPLUS, FSTA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| A         | --                                                                                                                                                                                                           | 4,6-11,14-18          |

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

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Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Eva Johansson/EB  
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 99/00185

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                         |                       |
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# INTERNATIONAL SEARCH REPORT

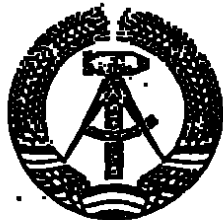
Information on patent family members

01/07/99

International application No.

PCT/DK 99/00185

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DEUTSCHE DEMOKRATISCHE REPUBLIK  
AMT FÜR ERFINDUNGS- UND PATENTWESEN

# PATENTCHRIFT 120201

Wirtschaftspatent

Erteilt gemäß § 5 Absatz 1 des Änderungsgesetzes zum Patentgesetz

In der vom Anmelder eingereichten Fassung veröffentlicht

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Int. Cl.<sup>2</sup>

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A 21 D 8/04

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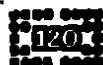
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- (1) Akademie der Wissenschaften der DDR, Berlin, DL  
(2) Drechsel, Wolfgang, Dr.; Täufel, Alfred, Dr. Dipl.-Lebensmittelchem.; Ruttloff, Heinz, Prof. Dr. Dipl.-Lebensmittelchem.; Schlobach, Carl-Rudolf, DL  
(3) siehe (72)  
(74) Akademie der Wissenschaften der DDR, Zentralinstitut für Ernährung, Büro für Schutzrechte und Neuererwesen, 1505 Bergholz-Rehbrücke, Arthur-Schaunert-Allee 114-116
- 

- (54) Verfahren zur Verringerung der Viskosität von Cerealienmehl-Suspensionen
- 

(57) Die Erfindung bezieht sich auf die Behandlung von Cerealienmehl-Suspensionen mit Enzymen zur Verringerung ihrer Viskosität bei der Herstellung aufgeschlossener bzw. gebackener Getreideprodukte, wie Semmelmehl, Instant-Getreideerzeugnisse, Waffeln, Kekse oder dergleichen. Die Erfindung hat das Ziel, die Wirtschaftlichkeit der Herstellung der genannten Getreideprodukte zu erhöhen. Es werden Verfahrensbedingungen aufgezeigt, die bei gleichzeitiger Verringerung der Viskosität eine Erhöhung des Feststoffanteils der Suspension zulassen. Erfindungsgemäß wird ein Gemisch zweier Enzyme, und zwar von Proteasen und von Alpha-Amylasen, eingesetzt. Die Enzyme werden nach der Einwirkung durch Erhitzen bei gleichzeitigem Feuchtigkeitsentzug inaktiviert. Das Mengenverhältnis von Cerealienmehl zu Wasser beträgt 1 : 0,5 bis 1 : 3 und die Einwirkungszeit 1 bis 60 min. Zur Verringerung der Viskosität auf etwa 1/4 der Viskosität einer unbehandelten Suspension werden 80 bis 1 000 TE Protease und 5 bis 30 E Alpha-Amylase pro kg Mehl zugesetzt.

7 Seiten



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Ist eine Zeitschrift erschienen.

(Teilweise aufgehoben gem. § 6 Abs. 1 d. Änd.-Ges. z. Pat. Ges.)

(74) siehe (74)

(74) Akademie der Wissenschaften der DDR, Zentralinstitut für Ernährung, Büro für Schutzrechte und Neuererwesen, 1505 Bergholz-Rehbrücke, Arthur-Scheunert-Allee 114-116

(54) Verfahren zur Verringerung der Viskosität von Cerealienmehl-Suspensionen

(57) Die Erfindung bezieht sich auf die Behandlung von Cerealienmehl-Suspensionen mit Enzymen zur Verringerung ihrer Viskosität bei der Herstellung aufgeschlossener bzw. gebackener Getreideprodukte, wie Semmelmehl, Instant-Getreideerzeugnisse, Waffeln, Kekse oder dergleichen. Die Erfindung hat das Ziel, die Wirtschaftlichkeit der Herstellung der genannten Getreideprodukte zu erhöhen. Es werden Verfahrensbedingungen aufgezeigt, die bei gleichzeitiger Verringerung der Viskosität eine Erhöhung des Feststoffanteils der Suspension zulassen. Erfindungsgemäß wird ein Gemisch zweier Enzyme, und zwar von Proteasen und von Alpha-Amylasen, eingesetzt. Die Enzyme werden nach der Einwirkung durch Erhitzen bei gleichzeitigem Feuchtigkeitsentzug inaktiviert. Das Mengenverhältnis von Cerealienmehl zu Wasser beträgt 1 : 0,5 bis 1 : 3 und die Einwirkungszeit 1 bis 60 min. Zur Verringerung der Viskosität auf etwa 1/4 der Viskosität einer unbehandelten Suspension werden 80 bis 1 000 TE Protease und 5 bis 30 E Alpha-Amylase pro kg Mehl zugesetzt.

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#### Anwendungsgebiet der Erfindung

Die Erfindung betrifft ein Verfahren zur Behandlung von Cerealienmehl-Suspensionen mit dem deren Viskosität bei gleichzeitiger Erhöhung des Feststoffanteils durch den vollständigen Abbau des in den Cerealien enthaltenen Glutens verringert werden kann. Eine derartige Behandlung ist von Bedeutung bei der Herstellung aufgeschlossener bzw. gebackener Getreideprodukte, wie Semmelmehl, Instant-Getreideerzeugnisse, Waffeln, Kekse und anderen Dauerbackwaren.

#### Charakteristik der bekannten technischen Lösungen

Es ist aus dem WP 106 130 bekannt, daß die Viskosität von Cerealienmehl-Suspensionen verringert werden kann, indem man diesen Suspensionen Proteasen zusetzt, die das in den Cerealien enthaltene Gluten zu Polypeptiden abbauen, so daß die viskositätserhöhenden plastisch-elastischen Eigenschaften des Glutens im erwünschten Sinne verändert werden. Zur Erzielung dieses Effektes ist eine relativ große Menge an Proteasen erforderlich.

Beispielsweise werden zur Herstellung einer freifließenden Suspension mit einer Viskosität von weniger als 20 000 cP bei der Herstellung von Semmelmehl und Instant-Getreiderzeugnissen mindestens 1000 TE Protease pro kg Weizenmehl benötigt, wenn man ein Weizenmehl-Wasser-Verhältnis von etwa 1 : 1 einhalten will. Dabei ist 1 TE diejenige Menge an Enzym, die aus einer 0,8 %igen Casein-Lösung  $1 \mu\text{Mol}$  Tyrosin-Äquivalent freisetzt.

Bei der Herstellung von Weizenmehl-Wasser-Suspensionen für die Waffelproduktion werden erfahrungsgemäß mindestens 800 TE Protease pro kg Weizenmehl benötigt, um eine gut verarbeitungsfähige Masse nach den in der Waffelindustrie üblichen Rezepturen zu erhalten.

Zur weiteren Verringerung der Viskosität von Cerealienmehl-Suspensionen gibt es gegenwärtig 3 Möglichkeiten:

- Erhöhung der Wasserzugabe.
- Erhöhung der einzusetzenden Proteasemenge
- Verlängerung der Einwirkungszeit der Proteasen.

Alle diese genannten Möglichkeiten sind mit Nachteilen verbunden. Eine Mehrzugabe von Wasser zur Suspension führt zu einem höheren Energieaufwand, da das zugesetzte Wasser beim anschließenden Verarbeitungsprozeß wieder verdampft werden muß. Ein höherer Einsatz an Proteasen führt aufgrund des relativ hohen Preises der Protease-Präparate zu einem unverhältnismäßig hohen Kostenanstieg. Eine Verlängerung der Einwirkzeit der Proteasen kann zu Störungen im technologischen Ablauf führen bzw. eine Kapazitätserweiterung ohne entsprechende Produktionserhöhung nach sich ziehen.

Des weiteren ist es auf der Grundlage des bekannten Verfahrens möglich, die Viskosität einer Cerealienmehl-Suspension unter Verringerung des Wassereinsatzes konstant zu halten, indem man den Proteaseeinsatz entsprechend steigert. Allerdings tritt dabei der Nachteil auf, daß die erforderliche

Proteasemenge nicht nur proportional, sondern progressiv ansteigt, so daß der Proteaseeinsatz und damit die Wassergabe aus wirtschaftlichen Gründen begrenzt ist.

Ziel der Erfindung

Ziel der Erfindung ist es, die Viskosität von Cerealienmehl-Suspension bei gleichzeitiger Erhöhung des Feststoffanteils weiter zu verringern, so daß aufgeschlossene bzw. gebackene Getreideprodukte, wie Semmelmehl, Instant-Getreideerzeugnisse, Waffeln, Kekse und andere Dauerbackwaren wirtschaftlicher als bisher hergestellt werden können.

Darlegung des Wesens der Erfindung

Der Erfindung liegt die Aufgabe zugrunde, Verfahrensbedingungen aufzuzeigen, die bei weiterer Verringerung der Viskosität von Cerealienmehl-Suspensionen eine Erhöhung des Feststoffanteils dieser Suspensionen zulassen.

Es wurde gefunden, daß dies erreicht wird, wenn man einer Cerealienmehl-Suspension nicht nur Protease, sondern ein Gemisch von Proteasen und Alpha-Amylasen zusetzt, diese mit der Cerealienmehl-Suspension reagieren läßt und danach die Enzyme in bekannter Weise durch Hitzeeinwirkung bei gleichzeitigem Feuchtigkeitsentzug inaktiviert. Dabei hat sich überraschenderweise gezeigt, daß bei Zusatz von Alpha-Amylase für den Abbau des Glutens eine wesentlich niedrigere Proteasemenge erforderlich ist als bei Abwesenheit von Alpha-Amylase unter analogen Bedingungen.

Von besonderer Bedeutung für das erfindungsgemäße Verfahren ist also ein bestimmtes Einsatzverhältnis zwischen Proteasen und Alpha-Amylasen. Zur Verringerung der Viskosität der Cerealienmehl-Suspensionen auf etwa 1/4 der Viskosität einer unbehandelten Suspension werden je nach dem herzustellenden Produkt 80 - 1000 TE Protease und 5-30 E Alpha-Amylase pro kg Mehl zugesetzt und die Enzyme bei Temperaturen im Bereich von 10 - 60 °C zur Einwirkung gebracht.

Es hat sich z. B. bei der Herstellung von Semmelmehl gezeigt, daß sich bei einer Zugabe von 9 - 27 E Alpha-Amylasen, vorzugsweise von 21 E Alpha-Amylasen pro kg Weizenmehl, die benötigte Menge an Proteasen von mindestens 1000 TE pro kg Weizenmehl auf etwa 250 TE verringern läßt, wobei gleichzeitig die der Suspension zuzusetzende Wassermenge um mindestens  $\frac{1}{3}$  reduziert werden kann, um dieselbe Wirkung zu erzielen, nämlich eine Senkung der Viskosität auf etwa  $\frac{1}{4}$ . Bei diesen Angaben ist zu berücksichtigen, daß 1 Alpha-Amylase-Einheit (E) diejenige Menge an Enzym ist, die aus einer 1 %igen Stärkelösung  $\frac{1}{100}$  Mol Glukose-Äquivalent freisetzt.

Als einzusetzende Enzympräparate kommen Mischungen, die Protease und Alpha-Amylase enthalten, in Betracht, z. B. eine Mischung von Thermitase und Brauerei-Enzym.

Eine verallgemeinerte Verfahrensweise der Erfindung besteht darin, daß Cerealienmehle mit Wasser von 10 - 60 °C in einem Rührwerksbehälter im Verhältnis 1 : 0,5 bis 1 : 3 eingemaischt werden, wobei das Wasser vor der Zugabe des Mehles und gegebenenfalls von weiteren Ingredientien-Proteasen und Alpha-Amylasen zugesetzt werden und die Suspension 1 - 60 Min. gerührt und anschließend das Reaktionsprodukt in bekannter Weise getrocknet bzw. gebacken wird.

Durch die Verringerung der einer Suspension zuzufügenden Wassermenge reduziert sich die aufgewendete Energie zur Verdampfung des Wassers beim anschließenden Back- und Trockenprozeß, wodurch sich eine beträchtliche Kostensenkung erzielen läßt.

#### Ausführungsbeispiele

Das erfindungsgemäße Verfahren soll nachstehend durch zwei Ausführungsbeispiele erläutert werden.

#### Beispiel 1

In einen Rührwerksbehälter werden 45 kg Wasser mit einer Temperatur von 30 - 60 °C, vorzugsweise 56 °C, eingefüllt.

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Dem Wasser werden 0,55 kg Kochsalz, 18 975 TE neutrale Protease und 1 570 g Alpha-Amylase zugesetzt. Unter ständigem Rühren werden nur 75 kg Weizenmehl, vorzugsweise Type W 630, zugegeben, so daß eine homogene Suspension entsteht. Die Viskosität dieser Suspension verringert sich durch Enzymwirkung nach etwa 10 Min. auf  $\frac{1}{4}$  des ursprünglichen Wertes.

Anschließend kann die entstandene Weizenmehl-Wasser-Suspension ohne Schwierigkeiten auf allen zur Verwendung gelangenden Anlagen, wie Walzentrocknern, Zerstäubungstrocknern, Rillenwalzentrocknern und Aufschlußmaschinen zu Semmelmehl bzw. Instant-Getreideprodukten weiterverarbeitet werden.

#### Beispiel 2

In einen Rührwerksbehälter werden 120 kg Wasser mit einer Temperatur von 10 - 60 °C, vorzugsweise 42 °C, eingefüllt. Dem Wasser werden 39 600 TE neutrale Protease und 800 E Alpha-Amylase zugesetzt. Unter ständigem Rühren werden nun

75,0 kg Weizenmehl, vorzugsweise Type W 630,

0,6 kg Kochsalz

0,1 kg Natriumhydrogenkarbonat

0,1 kg Hirschhornsalz

0,5 kg Magnesiumoxyd

2,0 kg Vollsoja

1,5 kg Sonnenblumenöl

zugefügt, und es wird so lange gerührt, bis eine homogene dünnflüssige Suspension entstanden ist. Das ist normalerweise nach etwa 7 Min. der Fall. Anschließend kann die Suspension in die Vorratsbehälter der Waffelbackautomaten gepumpt und weiterverarbeitet werden. Die Backzeit für Waffelblätter bzw. Formwaffeln wird verkürzt, so daß die Energiezufuhr entsprechend um etwa  $\frac{1}{3}$  gedrosselt werden kann. Die Qualität der Waffeln, die unter Einsatz von Enzymen hergestellt werden, ist besser als bei Verarbeitung der herkömmlichen Waffelmassen.

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Erfindungsansprüche

1. Verfahren zur Verringerung der Viskosität von Cerealienmehl-Suspensionen durch Behandlung mit Enzymen und nachfolgender Hitzeinaktivierung der Enzyme, dadurch gekennzeichnet, daß auf die Cerealienmehl-Suspension, vorzugsweise auf eine Weizenmehl-Wasser-Suspension, gleichzeitig Proteasen und Alpha-Amylasen zur Einwirkung gebracht werden.
2. Verfahren nach Punkt 1, dadurch gekennzeichnet, daß der Cerealienmehl-Suspension zur Verringerung der Viskosität auf etwa  $\frac{1}{4}$  der Viskosität einer unbehandelten Suspension je nach dem herzustellenden Produkt 80 bis 1000 TE Protease und 5 bis 30 E Alpha-Amylase pro kg Mehl zugesetzt werden und die Einwirkung der Enzyme bei Temperaturen im Bereich von 10 bis 60 °C erfolgt.
3. Verfahren nach Punkt 1 und 2, dadurch gekennzeichnet, daß als Enzym-Präparat, das Protease und Alpha-Amylase enthält, ein Gemisch von Thermitase und Brauerei-Enzym eingesetzt wird.
4. Verfahren nach Punkt 1 bis 3, dadurch gekennzeichnet, daß Cerealienmehl mit Wasser von 10 bis 60 °C in einem Rührwerksbehälter im Verhältnis 1 : 0,5 bis 1:3 eingemischt werden, wobei dem Wasser vor der Zugabe des Mehles und gegebenenfalls von weiteren Ingredientien Proteasen und Alpha-Amylasen zugesetzt werden und die Suspension 1 bis 60 Min. gerührt und anschließend das Reaktionsprodukt in bekannter Weise gebacken bzw. getrocknet wird.



Publication number: **0 445 585 A1**

**EUROPEAN PATENT APPLICATION**

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**Biscuit by enzyme treatment and production method thereof.**

There is disclosed a biscuit by enzyme treatment comprising a material selected from the group consisting of cereals such as potato, corn, soybean or the like added to a dough as partial substitution of wheat flour, having been subjected to enzyme treatment with amylase alone or with combination of amylase and protease, followed by molding and baking. There is also disclosed a method for producing a biscuit wherein a material selected from the group consisting of cereals such as potato, corn, soybean or the like is added to a dough as partial substitution of wheat flour, and enzyme treatment is performed with amylase alone or with combination of amylase and protease, followed by molding and baking.

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The present invention relates to a biscuit and a method for producing a biscuit by performing enzyme treatment, in particular relates to a biscuit and a method in which, for example, potato, corn, soybean or the like is used as partial substitution of wheat flour, and an adding amount of these materials to a biscuit can be increased by the enzyme treatment, thereby it is realized to provide a biscuit having a taste, flavor and mouth-feel lacking for conventional ones.

Generally, biscuits are produced by adding only wheat flour as cereal flour to a dough in many cases. Corn starch, corn flour, or the like is sometimes used as a starch component other than wheat flour, however, its adding amount usually does not exceed several %. Various biscuits are produced at present, however, it is considered that these show such diversity included in a range of dough in which wheat flour is used as almost all of the materials at most, so that from this meaning, it is also considered that a biscuit in which a large amount of cereal flour or cereals other than wheat flour are added to a biscuit dough exceeds the range of diversity of the conventional biscuits.

Conventionally, if a material other than wheat flour is added to a biscuit dough, such problems take place due to difference in starch, protein and the like that the consistency of the dough is lost, and processing by baking is deteriorated and the like, therefore due to these causes, only such a product could be obtained that provided no leavening having a very hard mouth-feel. In addition, powder feeling, stickiness, rough feeling and the like peculiar to the material other than wheat flour made the mouth-feel of the product deteriorated.

On account of these reasons, the adding amount of the material other than wheat flour to the biscuit has been restricted, and a product could not be provided which made the best of the taste and flavor and the mouth-feel of the material.

The inconvenient factors which deteriorate the mouth-feel of the product such as the powder feeling, the stickiness, the rough feeling and the like peculiar to the components of the material other than wheat flour such as potato, corn, soybean and the like are caused by physicochemical characteristics peculiar to these materials. If these inconvenient factors are intended to be decreased, and the physicochemical characteristics of the materials are improved to control and remove the inconvenient factors, then there causes such a possibility that the taste and flavor and the mouth-feel lacking for the conventional ones which are advantages of these materials are deteriorated at the same time. Therefore, when production of a biscuit is planned in which the material other than wheat flour is utilized to make the best of the unique taste and flavor and the mouth-feel of the original material, it is necessary to specifically control and remove only the factors inconvenient for the biscuit production.

The object of the present invention is to use a relatively large amount of cereals such as potato, corn, soybean, or the like as partial substitution of wheat flour, and only the factors inconvenient for the biscuit production given by these materials are specifically controlled and removed, so as to control the leavening (processing by baking) by the adjustment of water retentivity of starch and the like and control the powder feeling, the stickiness, the rough feeling and the like given by the components of the materials to be used themselves, thereby the adding amount of these materials to a biscuit is increased to produce a biscuit having a taste and flavor and a mouth-feel lacking for the conventional ones.

According to the present invention, there is provided a biscuit by enzyme treatment comprising a material selected from the group consisting of cereals such as potato, corn, soybean or the like added to a dough as partial substitution of wheat flour, having been subjected to enzyme treatment with amylase alone or with combination of amylase and protease, followed by molding and baking.

According to the present invention, there is also provided a method for producing a biscuit wherein a material selected from the group consisting of cereals such as potato, corn, soybean or the like is added to a dough as partial substitution of wheat flour, and enzyme treatment is performed with amylase alone or with combination of amylase and protease, followed by molding and baking.

As the material other than wheat flour, without limiting to those exemplified above, all cereal powder or cereals subjected to the enzyme action by amylase alone or the combination of amylase and protease can be used. The cereal powder or cereals are preferably added as granule materials or powder materials.

As the enzyme, amylase and protease can be used.

It is preferable that to the biscuit dough containing potato not less than 15% by dry weight are added 0.001 to 1.0% of amylase or amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.

The adding amount of potato to the dough is preferably 15 to 40%, most preferably 25 to 35% by dry weight, thereby a product having a stable quality can be obtained. If it is not more than 15%, the potato taste and flavor is deficient, and if it exceeds 40%, there is given such a tendency that sufficient leavening cannot be obtained even after the amylase treatment. Conventionally, when it is not less than 15%, "floating" (leavening) is not provided, thus the mouth-feel has been very hard. This is considered to be due

to the influence of the water retentivity of starch.

The enzyme to be used for the treatment of the potato-containing dough is amylase alone or the combination of amylase and protease, and the adding amount is in a range of 0.001 to 1.0% respectively, however, it is preferable as activity that amylase is 3500 to 35000 units and protease is 3000 to 30000 units (determined by the FCC method for both of them) for one kg of potato (dry weight). If amylase is less than 0.001%, the effect on improvement of leavening is not provided, and if it exceeds 1.0%, the control of the treatment time becomes difficult, sometimes resulting in deterioration of the taste and flavor.

It is preferable that to the biscuit dough containing corn not less than 15%, preferably not less than 20 to 45% by dry weight is added the combination of 0.001 to 1.0% of amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.

When the biscuit is produced using corn as the material, conventionally it has been found that the hardness of protein expresses in the mouth-feel by addition not less than 15% of it.

It is preferable that to the biscuit dough containing soybean not less than 15% by dry weight is added the combination of 0.001 to 1.0% of amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.

When the biscuit is produced using soybean as the material, conventionally due to the protein of soybean, a hard and tight dough having a little consistency is given, resulting in a hard mouth-feel.

In the present invention, various materials other than wheat flour can be used, and especially for the cereals such as potato, corn, and soybean, the use of amylase and protease is especially effective.

When the material other than wheat flour is added to the dough as the partial substitution of wheat flour to perform the enzyme treatment, the treatment condition should be optionally determined depending on the type of the material, the adding amount, the type of the enzyme and its adding amount, however, in general the treatment temperature can be 30 to 50° C, and the treatment time can be 30 to 300 minutes. Evaluation of the treatment can be carried out, for example, by the mouth-feel of the product and the specific gravity of the product.

Incidentally, the present invention can be applied to other confectionery such as cookies, crackers and the like.

As described above, the material other than wheat flour such as cereals of potato, corn, soybean and the like has the inconvenient factors to deteriorate the mouth-feel of the product such as the powder feeling, the stickiness, the rough feeling and the like peculiar to it, however, when the production of a biscuit is planned in which the original and unique taste and flavor and the mouth-feel of the material are sufficiently utilized, it is necessary to specifically control and remove only the factors inconvenient for the biscuit production with maintaining the unique taste and flavor and the mouth-feel.

In the present invention, these materials other than wheat flour are used as the partial substitution of wheat flour, and it is made possible to increase the adding amount of these materials to the biscuit by the enzyme treatment, thereby it is realized that the biscuit having the taste and flavor and the mouth-feel lacking for the conventional ones is provided.

Namely, in the present invention, only the factors of these materials inconvenient for the biscuit production are specifically controlled and removed by the enzyme treatment, and the reduction of the water retentivity of starch, the improvement in processing by baking and leavening, and the reduction of the stickiness are designed, so as to increase the adding amount of these materials to the biscuit, so that there can be provided the biscuit having the taste and flavor and the mouth-feel lacking for the conventional ones.

For example, the adding amount of potato can be greatly increased by the treatment of the present invention notwithstanding the conventional limit of 5 to 10%, and the potato feeling can be increased. This makes it possible to produce a hard dough biscuit which has the potato feeling and is light and crisp having little powder feeling.

According to the present invention, the enzyme treatment production method of the material other than wheat flour makes it possible to increase the adding amount of the material which could be added in a little amount in the conventional ones, and the biscuit having a novel taste, flavor and mouth-feel can be obtained which could not be obtained only by wheat flour.

In addition, according to the present invention, the treatment of amylase alone or of the combination of amylase and protease can improve the processing by baking and leavening by the adjustment of the water retentivity and reduce the powder feeling, the stickiness, the rough feeling and the like given by the components of the material themselves, so that the adding amount to the biscuit can be increased.

Further, according to the present invention, in accordance with the increase in the adding amount of these materials, it becomes possible to produce the biscuit having a blending ratio which could not been achieved only by wheat flour (for example, a hard dough biscuit having a large amount of oils and fats and the like), and the biscuit having the mouth-feel lacking for the conventional ones can be obtained in which

the original mouth-feel of the material itself is sufficiently utilized.

According to the present invention, the material other than wheat flour such as for example potato, corn, soybean and the like is contained as the partial substitution of wheat flour by not less than 15%, and by means of the enzyme treatment, only the inconvenient factors for biscuit production given by these materials are specifically controlled and removed, and the leavening (processing by baking) is controlled by the adjustment of the water retentivity of starch, protein or the like, and the powder feeling, the stickiness, the rough feeling and the like given by the components of the material to be used themselves are controlled, thereby the limit of the adding amount of these materials to the biscuit is increased, and the biscuit having the taste and flavor and the mouth-feel lacking for the conventional ones can be produced in which the taste and flavor of the material is sufficiently utilized.

The present invention will be explained in detail hereinafter according to examples, however, the present invention is not limited only to the following examples.

Incidentally, all of the numerals described here (the addition %) are shown by dry weight % for a product.

#### Example 1, Comparative tests 1 and 2 (Comparison of the specific gravity of the product)

To a dough containing 40% of potato was added 0.05% of Denazyme SA (Nagase Biochemicals, Ltd.), and the enzyme treatment was performed at 40 ° C for 30 minutes, after which molding and baking were carried out to produce a hard dough biscuit to be a group of amylase addition and 30 minutes treatment (Example 1).

A hard dough biscuit was produced in the same manner with the enzyme treatment time of 0 minute to be a group of amylase addition and 0 minutes treatment (Comparative test 1).

In addition, a dough without addition of amylase was used to be kept static for 30 minutes, after which a hard dough biscuit was produced in the same manner to be a group without treatment of amylase (Denazyme SA) that is a group without amylase addition and 30 minutes treatment (Comparative test 2).

The results of comparison of the product specific gravity are shown in Table 1 with respect to these biscuits.

Table 1

|                    | Product specific gravity (g/cm <sup>3</sup> ) | Product moisture content (%) |
|--------------------|-----------------------------------------------|------------------------------|
| Example 1          | 0.362                                         | 4.3                          |
| Comparative test 1 | 0.501                                         | 4.1                          |
| Comparative test 2 | 0.519                                         | 4.5                          |

#### Example 2

To a biscuit dough containing 35% of potato and having an oils and fats content of 10% was added 0.01% of Dlasmen (Daiwa Chemical Co., Ltd.) as amylase, which was subjected to the treatment at 40 ° C for 30 minutes, followed by rolling, laminating, demolding, and baking to try to produce a hard dough biscuit.

This biscuit had the potato feeling, which was a light and crisp hard dough biscuit having little powder feeling.

#### Example 3

A biscuit dough containing 30% of corn flour in which 0.1% of Protease P (Amano Pharmaceutical Co., Ltd.) and 0.03% of Kokulase (Sankyo Co., Ltd.) as amylase were added was prepared, which was treated at 40 ° C for 45 minutes, followed by rolling, laminating, demolding, and baking to obtain a hard dough biscuit.

In this biscuit, the deterioration in the mouth-feel due to the use of corn flour (a tendency of being hard and difficult to float) was improved, and it was a biscuit having a soft mouth-feel having rich taste and flavor

of corn.

Example 4

A biscuit dough containing 25% of roasted soybean powder in which 0.02% of Amylase AD (Amano Pharmaceutical Co., Ltd.) as amylase and IP-Protease (Seirin Pharmaceutical Co., Ltd.) as protease were added was treated at 37 ° C for 60 minutes, followed by molding and baking to obtain a biscuit.

In this biscuit, the hardness of the mouth-feel due to the addition of soybean powder was restricted, and it was a biscuit in which the good taste and flavor from soybean were sufficiently utilized.

Claims

1. A biscuit by enzyme treatment comprising a material selected from the group consisting of cereals such as potato, corn, soybean or the like added to a dough as partial substitution of wheat flour, having been subjected to enzyme treatment with amylase alone or with combination of amylase and protease, followed by molding and baking.
2. A method for producing a biscuit wherein a material selected from the group consisting of cereals such as potato, corn, soybean or the like is added to a dough as partial substitution of wheat flour, and enzyme treatment is performed with amylase alone or with combination of amylase and protease, followed by molding and baking.
3. The method for producing a biscuit according to claim 2, wherein to the biscuit dough containing potato as the cereals other than wheat flour not less than 15% by dry weight are added 0.001 to 1.0% of amylase or amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.
4. The method for producing a biscuit according to claim 2, wherein to the biscuit dough containing corn as the cereals other than wheat flour not less than 15% by dry weight is added combination of 0.001 to 1.0% of amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.
5. The method for producing a biscuit according to claim 2, wherein to the biscuit dough containing soybean as the cereals other than wheat flour not less than 15% by dry weight is added combination of 0.001 to 1.0% of amylase and protease respectively, and the enzyme treatment is performed, followed by molding and baking.



European  
Patent Office

# EUROPEAN SEARCH REPORT

Application Number

EP 91 10 2458

| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                          |                                                         |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| Category                                                                                                                                                                                                                                                                                             | Citation of document with indication, where appropriate, of relevant passages                                                                                                                                                                                                                      | Relevant to claims                                                                                                                                                                                                       | CLASSIFICATION OF THE APPLICATION (Int. Cl.)            |
| X                                                                                                                                                                                                                                                                                                    | FR-A-2 327 731 (GIST BROCADES N.V.)<br>" Claims 1-4,9-14,18; page 2, lines 28-34; example 4 "<br>- - - -                                                                                                                                                                                           | 1-5                                                                                                                                                                                                                      | A 21 D 2/36                                             |
| X                                                                                                                                                                                                                                                                                                    | CHEMICAL ABSTRACTS, vol 93, 1980, page 736, abstract no. 44293x, Columbus, Ohio, US; K. CHAKRABORTI et al.:<br>"High-protein bread from wheat and nondefatted soybean flours",<br>& NAUCHNI TR., VISSH INST. KHRANIT. VKUSOVA PROM-ST., PLOVDIV 1977, 24. Pl. 1, 117-28<br>" Abstract "<br>- - - - | 1,2,5                                                                                                                                                                                                                    |                                                         |
| P,X                                                                                                                                                                                                                                                                                                  | FR-A-2 636 504 (MINOTERIE DU COUTELET et al.)<br>" Claims "<br>- - - -                                                                                                                                                                                                                             | 1,2                                                                                                                                                                                                                      |                                                         |
| A                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                    | 4                                                                                                                                                                                                                        |                                                         |
| X                                                                                                                                                                                                                                                                                                    | GB-A-1 787 52 (R. GRAHAM)<br>" Claims; page 2, lines 106-125 "<br>- - - -                                                                                                                                                                                                                          | 1,2,5                                                                                                                                                                                                                    |                                                         |
| X                                                                                                                                                                                                                                                                                                    | GB-A-2 228 19 (R. OSMUNDSEN)<br>" Claims 1-3; page 1, lines 18-66; example X "<br>- - - -                                                                                                                                                                                                          | 1-4                                                                                                                                                                                                                      |                                                         |
| X                                                                                                                                                                                                                                                                                                    | FR-A-2 674 628 (SOCIETE AUXILIAIRE DE MEUNERIE)<br>" Summary and claims "<br>- - - - -                                                                                                                                                                                                             | 1,2,4                                                                                                                                                                                                                    | TECHNICAL FIELDS<br>(SEARCHED (Int. Cl.))<br><br>A 21 D |
| The present search report has been drawn up for all claims                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                          |                                                         |
| Place of search<br><br>The Hague                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                    | Date of completion of search<br><br>05 June 91                                                                                                                                                                           | Examiner<br><br>COUCKE A.O.M.                           |
| CATEGORY OF CITED DOCUMENTS<br>X: particularly relevant if taken alone<br>Y: particularly relevant if combined with another document of the same category<br>A: technological background<br>O: non-written disclosure<br>P: intermediate document<br>T: theory or principle underlying the invention |                                                                                                                                                                                                                                                                                                    | E: earlier patent document, but published on, or after the filing date<br>D: document cited in the application<br>L: document cited for other reasons<br><br>A: member of the same patent family, corresponding document |                                                         |



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(54) **DRY BAKERY PRODUCTS AND A PROCESS FOR THEIR PREPARATION**

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(76) **Inventors:** AALDRIK ENGELS, HX  
AALSMEER (NL); EVERWIEN TER  
HASEBORG, MT HUIZEN (NL);  
MARTIN HOOGLAND, PH LAREN  
(NL)

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**Correspondence Address:**  
**CUSHMAN DARBY & CUSHMAN**  
**PILLSBURY MADISON & SUTRO**  
**1100 NEW YORK AVENUE N W**  
**NINTH FLOOR EAST TOWER**  
**WASHINGTON, DC 200053918**

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(86) **PCT No.:** PCT/EP95/04340

(57) **ABSTRACT**

The invention concerns dough as well as a process for the preparation of dry bakery products, particularly crackers, comprising the addition to the dough of a bakery ingredient prepared by fermentation of cereal germs with lactic acid producing bacteria. The fermentation is preferably carried out with Lactobacilli, such as *Lactobacillus plantarum*. During the fermentation yeast and/or enzymes such as proteases and carbohydrases may be added to the fermentation mixture. After the fermentation the mixture is preferably concentrated or converted into a dry product which can be easily shipped stored and handled.

The use of the bakery ingredient leads to a simplified and shorter process for preparing dry cereal products and improved product properties. For cracker manufacture the traditional sponge step can be omitted.

Figure 1

**Classical:  
"Sponge & Dough" Method**

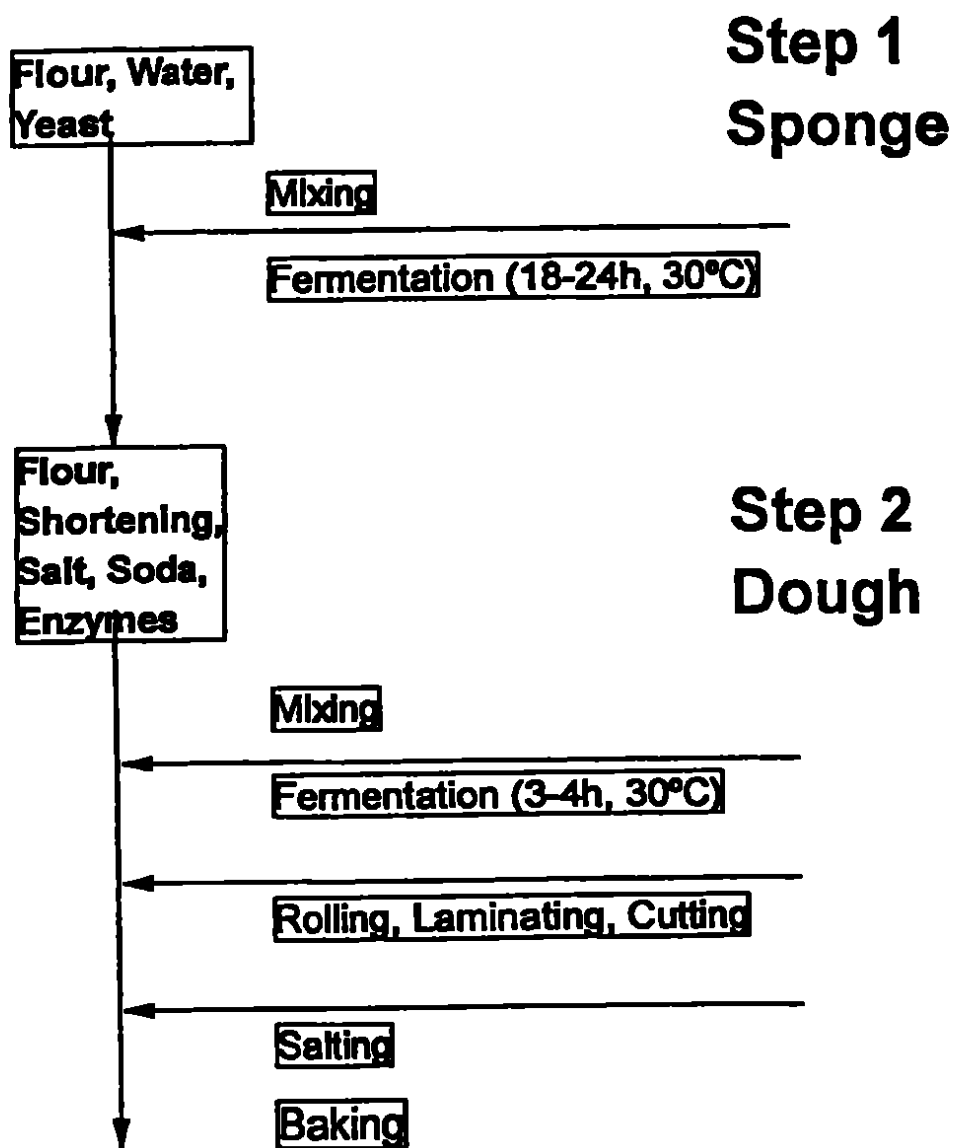


Figure 2

Continuous Flour Fermentation Process

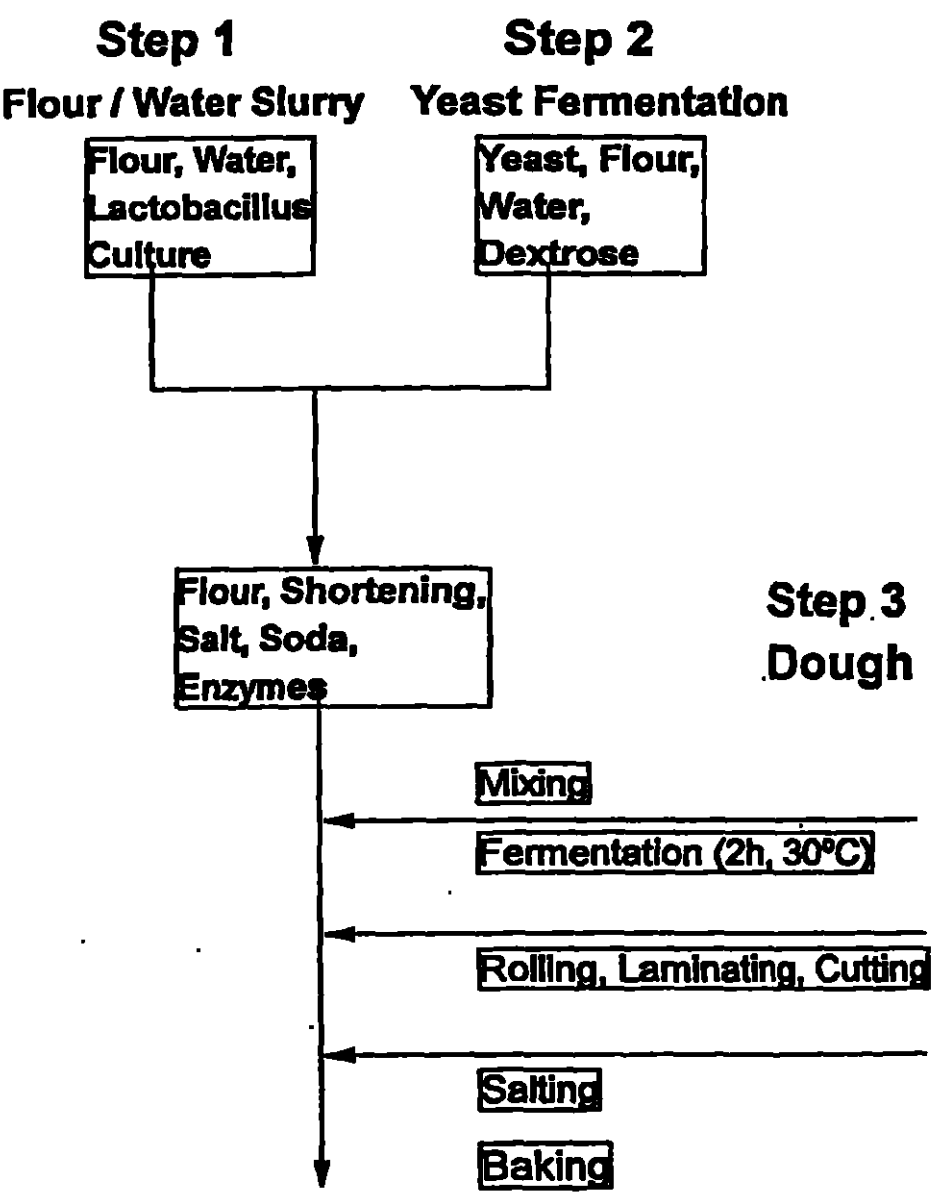
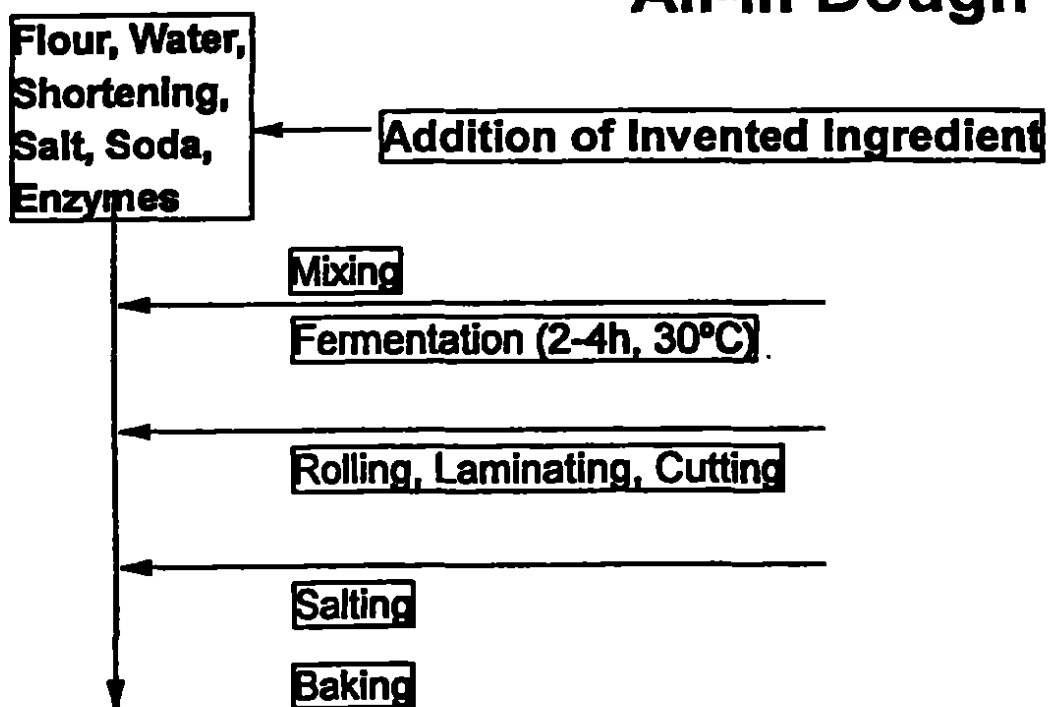


Figure 3

**Invention:  
One - Step  
"All-in Dough" Process**

**Step 1  
All-in Dough**



## DRY BAKERY PRODUCTS AND A PROCESS FOR THEIR PREPARATION

[0001] The present invention relates to dry bakery product, a process for preparing them and a novel bakery ingredient employed in that preparation. more particularly the invention is concerned with crackers and a process for their preparation.

[0002] Conventionally, so-called premium crackers (also known as cream crackers or fermented crackers) are generally manufactured by preparing a mixture of flour, water, yeast and sugar, followed by a conventional sponge fermentation involving low speed mixing of dough ingredients (flour, water, yeast and often salt) on a dough mixer for 20-25 minutes or using a combination of low and high speed mixing. The mixture is fermented in bulk for the time stated in the recipe, commonly 18-24 hours (sponge fermentation). During this fermentation the yeast ferments out the sugars while the lactic acid bacteria that are always present in commercial yeast products assimilate some of the products of yeast and lactic acid bacteria. The mixture provides components that contribute to taste, texture and appeal of premium crackers. Such a mixture is then added to the rest of the ingredients which make up the premium cracker dough and processing proceeds in a conventional way.

[0003] On a laboratory scale two main conventional ways of processing can be distinguished: classical premium cracker manufacture and starter culture based premium cracker manufacture, see R. C. Hosensay and various collaborators in *Cereal Chemistry*, 57(3) 185-8 1980, 57(4) 249-52 1980, 62(3) 158-62 1985, 66(1) 7-10 1989. Both involve the preparation of a pre-fermentation slurry from flour and water, fermented with a lactobacilli containing yeast or with yeast and a separately added lactobacilli starter culture. This prefermented slurry is then added to the sponge fermentation mixture and the sponge fermentation step is performed which typically takes 18 hours at 30° C. In practice, these pre-fermentation steps are not carried out but a sponge fermentation is done as the first step. It is followed by the dough fermentation step which takes typically 4 hours at 30° C. Dough handling is completed in the usual way by rolling, laminating and cutting. Total production time before baking is generally well over 20 hours. This is schematically set out in FIG. 1.

[0004] The process can be shortened by using the continuous or semi-continuous pre-culture process involving a yeast based fermentation and a lactic acid based fermentation. Quantities of these fermentation broths are then added to the final dough mixture as required. This process is used in industry and is schematically set out in FIG. 2. A problem likely to occur with this approach is the difficulty of maintaining adequate process control so as to ensure a consistent addition of the fermented ingredients to the main dough ingredients. An incorrectly matured fermented ingredient will produce a substandard final product. The pre-fermentation procedures can reduce the manufacturing time per batch to a few hours. However, the pre-fermentation processes require continuous maintenance. The fermentations periodically require to be re-set with resulting considerable production inconvenience. Additionally, the total number of processing steps is not greatly reduced. In EP-B-0 153 057 a process is described in which flour is continuously fermented in a 10% aqueous slurry with lactobacilli and

optionally yeast. This slurry is then added to the cracker dough. The total process is described by D. Fox et al in *Australian Journal of Biotechnology*, 3(2) 139-43 1989. This process in practice requires the fermented flour slurry to be produced on the same location where the cracker dough is produced. Also, the continuous fermentation is difficult to adapt to required variations in production volume of the crackers

[0005] Thus, all methods as described above have various disadvantages, mainly being long process times (fermentation and proofing) and a large number of process steps which makes processing complicated and involves extensive and complicated equipment. For example, a cracker manufacturer who wishes to operate the process using the pre-fermentation method instead of the more classical methods will need to invest in new, different and complicated equipment.

[0006] Hence, there is a need for an improved process for manufacturing dry bakery products such as rusks, crackers (including fermented and cream crackers, chemically leavened crackers, soda crackers, etc), dry biscuits and the like. Such process should involve fewer process steps, and/or lead to shorter and simplified processing and/or less investment in expensive machinery on the part of the cracker manufacturer. The process may also lead to dry baked goods with improved properties such as flavour, taste, texture or appearance.

[0007] It has now been found that dry baked goods can be produced, with a simple process for preparing the dough, which does not involve the use of diluted aqueous slurries of fermented flour and/or sponges by the manufacturer, by using a new bakery ingredient as hereinafter described.

[0008] The new bakery ingredient comprises fermented cereal germs, prepared by subjecting cereal germs to fermentation with lactic acid bacteria and optionally yeast. The fermentation may be carried out in solid state or in an aqueous medium. If carried out in an aqueous medium, the fermented cereal germs according to the invention are generally obtained as a wet slurry. This slurry can be used as such as a baking improver, but it may advantageously be concentrated by partial removal of water. Furthermore, by substantially complete removal of water it may be converted into a dry form, which has the additional benefit of improved storage stability and easy handling and shipping. Additionally, it is also possible to use liquid extracts of the fermented cereal germs which may in turn be converted to concentrated or a dry form. The bakery ingredient comprising the fermented cereal germs in any of the forms described above constitutes one aspect of the invention.

[0009] The cereal germs may be derived from wheat, barley, rye, corn, rice and other edible cereals. Germs from wheat or barley are preferred, particularly wheat.

[0010] The fermentation for obtaining the fermented cereal germs is carried out using standard fermentation techniques, preferably using starter cultures comprising lactic acid producing bacteria. More preferred lactic acid bacteria are lactobacilli such as *Lactobacillus plantarum* or *Lactobacillus brevis*. Yeast may be added to the starter cultures used, in particular *Saccharomyces cerevisiae*.

[0011] The fermentation of the cereal germs can be carried out using a level of solids of between 5 and 95% by weight, preferably between 5 and 40%, more preferably between

20-40%, and can be carried out in any hygienic fermentation vessel with provisions for good mixing and temperature control. The temperature is generally kept at 15-50° C., preferably 25-40° C. Prior to fermentation the fermentation mixture may be pasteurized or sterilized e.g. by using HTST equipment, whereafter the lactic acid producing bacteria and optionally the yeast are added to the mixture. The fermentation process is carried out for 15 minutes to 72 hours, preferably 8-72 hours, more preferably 30-72 hours, most preferably 48-72 hours, and the matrix may be pasteurized or sterilized again in order to inactivate viable organisms and/or enzymes.

[0012] Before or during the fermentation the cereal germs may be treated with various enzymes, preferably those having protease and/or carbohydrase (particularly amylase, amylo-glucosidase, hemicellulase or cellulase) activity. Such treatment is preferably carried out simultaneously with the fermentation. The treatment leads to improved properties of the fermented cereal germs which in turn leads to improved processing of the dough as well as flavour/taste and odour of the cracker in which the bakery ingredient is used.

[0013] In addition to the cereal germs, the starter culture, and optionally the yeast and/or the enzymes, the fermentation mixtures may also include cereal (particularly wheat) flour and other ingredients such as salt, malt, sugars such as dextrose, sucrose or molasses, and proteins (particularly gluten) which may be (partly) chemically or enzymatically degraded.

[0014] The resulting fermentation broth can be processed in various ways, including stabilising/preserving the final product. Such processing may include chilling (e.g. to refrigerator temperatures), concentration by evaporation of water leading to a concentrated slurry or paste, or processing leading to a solid product such as deep freezing or drying (e.g. by use of spray-dryer, freeze dryer, ring-dryer, fluid-bed dryer or vacuum-band dryer equipment). If before such further processing solids are removed from the fermentation broth an extract is obtained which can be further processed as described above. Before or after drying the fermentation product may be combined with carrier materials (e.g. modified starch or maltodextrin) or with other dry ingredients commonly used for bakery applications, such as dry yeast, dry flour or dry sugars. The resulting mixture can be subjected to further drying if desired. The final dry product should preferably have a moisture content of 10% or less, more preferably below 6%. During or after fermentation of the cereal germs other parts of cereals (e.g. bran or endosperm) may also be added.

[0015] The bakery ingredient according to the invention may be used as a baking improver in the preparation of dry bakery products, particularly crackers. The term "baking improver" is herein to be understood to be a composition comprising at least one component which facilitates or improves the processing of dough or improves the properties of dough and/or of the bakery products prepared therefrom. Such improvements may be in: processing time, fermentation time, proofing time, dough rheology, dough handling, specific volume, dough stability, dough flexibility, dough tolerance, appearance of the bakery products prepared from the dough (e.g. colour), organoleptic properties of the bakery products (flavour, taste, odour), structure or texture of the

bakery products, staling properties, shelf life, etc. The bakery ingredient according to the invention may be added as such to flour or dough or may be first combined with other bakery ingredients, including bakery ingredients which are known in the art as baking improvers according to the definition above, such as enzymes ( $\alpha$ -amylase, xylanase, glucose oxidase, peroxidase, etc.), emulsifiers (mono- and diglycerides), salts (e.g. NaCl and  $\text{NaHCO}_3$ ), chemical oxidizers (e.g. ascorbic acid and  $\text{KBrO}_3$ ) and proteins (particularly gluten) which may be (partly) chemically or enzymatically degraded.

[0016] Thus, a further aspect of the invention is a new process for the preparation of premium crackers in which the bakery ingredient according to the invention is incorporated in the dough prior to baking, whereafter the dough is further processed and baked as known in the art. The dough comprising the bakery ingredient according to the invention is another aspect of the invention. The preparation of the dough according to the invention can be carried out as a one-step process, in which all ingredients, including the bakery ingredient according to the invention, are mixed at the same time and the resulting dough processed in the usual way.

[0017] Therefore, an aspect of the invention is the use of the bakery ingredient prepared from fermented cereal germs as set out above, which permits shortening and simplifying of the cracker production process. This new process can best be compared with both the classical process as well as the starter culture based process as set out herein before, the major difference being that through the use of the bakery ingredient the first processing steps of both prior art processes can be avoided, thus resulting in a much shorter total processing time and avoidance of the use of large quantities of fermented slurries. The preparation and processing of the final dough stage is the only remaining step. In the new process a mixture is prepared from flour, water, yeast, sugar (and if desired additional ingredients such as prior art baking improvers as mentioned above, flavours, herbs etcetera). The new bakery ingredient is incorporated in this mixture, which can be done after mixing together the conventional ingredients, but this can also be done in any earlier stage by mixing the bakery ingredient with one or more of the conventional ingredients before incorporation in the bulk of the dough. After mixing all the dough ingredients, including the new bakery ingredient, the dough is preferably allowed to ferment for a few hours (0.5-8 hours, preferably 1-4 hours) at a moderate temperature (between 15 and 50° C., preferably between 25 and 35° C.). Following this, the dough is processed in conventional ways: rolling, laminating, cutting, and baking. An example of such a process is schematically given in FIG. 3. In addition, since the process requires only the extra addition of a single (low volume) ingredient, there are no technical requirements involved and thus no major capital cost implications for production procedures or maintenance. When the process according to the present invention is compared with the conventional methods of preparing premium crackers, it can be concluded that the total number of production operations can significantly be reduced from four to one step, and as a consequence the process for the preparation of the dough for crackers can be reduced from well over 20 hours for the conventional "sponge and dough" process to below 8 hours (or even below 4 hours) for the process according to the present invention. The bakery ingredient can be produced in a

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different location than the production of the dry bakery products and can in concentrated or dry form easily be shipped and stored and used as and when required.

[0018] In preparing the dough for dry cereal products such as premium crackers with the new bakery ingredient according to the invention the use of various other conventional baking improvers as mentioned above is recommended, such as enzyme preparations comprising  $\alpha$ -amylase, protease and/or hemicellulase (e.g. xylanase), stabilizers, emulsifiers, hydrocolloids, dough relaxing compounds (such as proteinase or wheat gluten, which may be dried and/or modified by chemical, physical or enzymatical action).

[0019] The bakery ingredient according to the invention may be added to the dough in an amount so as to provide between 0.1 and 10%, preferably between 0.2 and 4% and more preferably between 0.5-2.5% by weight of fermented cereal germs in the dough, based on the total amount of flour. It may also be added directly to the flour in the amounts specified above, or to another ingredient for the dough in such an amount that in the dough the amount is within the limits specified above calculated on the amount of flour used.

[0020] As outlined above, the bakery ingredient according to the invention may be used in the manufacture of premium crackers (including fermented- and cream crackers) for processing reasons. However, the bakery ingredient may also contribute to flavour, taste, texture and appearance which is also applicable to other forms of dry bakery products, including chemically leavened crackers, soda crackers, etc.

[0021] The cereal germs used for the preparation of the bakery ingredient are a side-product obtained when preparing flour from cereal.

[0022] In table 1 below the new process is compared with both the conventional "sponge and dough" process as well as with the process according to EP 153 057 on a number of parameters.

| TABLE 1                                                       |                  |                                |                                    |
|---------------------------------------------------------------|------------------|--------------------------------|------------------------------------|
| comparison of methods for the preparation of premium crackers |                  |                                |                                    |
| Issue                                                         | Sponge and Dough | Process according to EP 153057 | Process according to the invention |
| Capital investment requirements for sponge process            | Significant      | Very High                      | None                               |
| Management of sponge process                                  | Yes              | Yes                            | None                               |
| Labour costs for sponge process                               | High             | Moderate                       | None                               |
| Working capital charge for sponge process                     | Moderate         | High                           | None                               |
| Energy costs for sponge process                               | High             | Reduced                        | None                               |

| TABLE 1-continued                                             |                           |                                |                                    |
|---------------------------------------------------------------|---------------------------|--------------------------------|------------------------------------|
| comparison of methods for the preparation of premium crackers |                           |                                |                                    |
| Issue                                                         | Sponge and Dough          | Process according to EP 153057 | Process according to the invention |
| Space requirement for sponge process                          | High                      | Reduced                        | None                               |
| Consequences of failure of sponge process                     | Considerable              | Huge                           | Not relevant                       |
| Reproducibility of "sponge" product                           | Batch to batch variation  | Variable                       | Specifiable                        |
| On-line control of sponge process                             | Required                  | Required                       | None required                      |
| Acid value of "sponge" product                                | On-line check             | On-line check                  | Done controllably                  |
| Failed sponge batches                                         | Risk                      | Risk                           | None                               |
| Consequences of plant failure down the line                   | High                      | Moderate                       | Low                                |
| Recipe stability                                              | Batch to batch adjustment | Continual adjustment           | Minor adjustment                   |
| Process duration                                              | Long                      | Medium                         | Short                              |
| New product development                                       | Long development          | Requires process development   | Simple recipe variations           |

[0023] As is immediately evident, the new process using the bakery ingredient according to the invention has many benefits for the preparation of premium crackers.

[0024] The invention is further exemplified by the following examples, but it is in no way limited thereto.

EXAMPLE 1

[0025] Preparation of the Bakery Ingredient.

[0026] Fermentations for the production of the bakery ingredient were carried out on a 2 liter scale using double walled glass fermenters equipped with pH measurement/logging facilities using Ingold pH electrodes in combination with Consort R601 (Consort B. V., Antwerpen, Belgium) pH transmitters. During fermentation the temperature was maintained at 30° C. through the jacket using an external water circulation bath.

[0027] The fermenter was filled with 800 ml tap water and 300 grams of commercially available wheat germ flakes (Spillers). During filling the slurry was gently agitated at 50-100 rpm using a 10 cm diameter stirring blade in combination with a stirring motor. Fermentation was started, using the non-sterilised substrate, by the inoculation of 20 ml of a washed cell suspension (109 cfu/ml) of the homo fermentative lactic acid bacterium *Lactobacillus plantarum* NRRL B18 368 and 0.33 ml of each of the following enzyme preparations viz. protease L30 and  $\alpha$ -amylase amylo 300 L35 (trade names of Quest International, Naarden).

[0028] The fermentation was stopped after 48 hours by in-situ pasteurisation of the whole broth (30 min at 80° C.), which was subsequently freeze dried in a Uniopt 800 L

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Freeze Mobile 24 (The Virtis Company Inc., Gardiner, N.Y. 12525) laboratory freeze dryer using the following drying conditions: condenser temperature  $-60$  to  $-80^{\circ}\text{C}$ ., product temperature  $-30^{\circ}\text{C}$ ., vacuum 10 Pa. Finally the freeze dried product was made into a fine powder using a Tefal, Quick Foodmaster horizontal knife mixer.

### EXAMPLE 2

#### [0029] Preparation of the Bakery Ingredient.

[0030] In a suitable double walled fermentor 10 l of a mixture consisting of 27% w/w of wheat germ flour (Roland Industries Inc, St Louis, Mo.), 72.9% w/w of tap water and 0.1% w/w of Promalt 295 (enzyme system having  $\alpha$ -amylase,  $\beta$ -glucanase and protease activities\*) is kept at a temperature of  $55$ – $60^{\circ}\text{C}$ . for 30 minutes and thereafter at  $85^{\circ}\text{C}$ . for 30 minutes. The mixture was cooled to  $35^{\circ}\text{C}$ . and to it was added: 0.033% v/w of Amylo 300 (liquid amyloglucosidase preparation\*), 0.033% v/w of Bioprotease FL (liquid fungal protease preparation\*) and 0.001% w/w of Sardo SC (frozen starter culture containing 45.109 vcc/ml. of *Lactobacillus plantarum*\*). Fermentation was continued for 48 hours during which the pH dropped to 3.5. The fermentation broth was pasteurized (30 minutes at  $80^{\circ}\text{C}$ .), 2.5% w/w of Paselli MD10 (maltodextrin marketed by Avebe, Veendam, The Netherlands) was added and the mixture was spray dried using an inlet temp. of  $180^{\circ}\text{C}$ . and an outlet air temp of  $90^{\circ}\text{C}$ .

[0031] The bakery ingredient was obtained as a slightly yellow coloured powdered product.

[0032] trade names of Quest International, Naarden, The Netherlands.

### EXAMPLE 3

#### [0033] Application of the Bakery Ingredient in the Manufacture of Premium Crackers.

[0034] Premium crackers were prepared using the conventional sponge and dough process, as well as the process according to the invention. The following recipes were adhered to:

| Recipes:            |                         |                                    |                                            |
|---------------------|-------------------------|------------------------------------|--------------------------------------------|
| Sponge and Dough    |                         | Process according to the invention |                                            |
| <u>Sponge:</u>      |                         | <u>All-in:</u>                     |                                            |
| 650 g               | Wheat Flour             | 1000 g                             | Wheat Flour                                |
| 310 g               | Water                   | 330 g                              | Water                                      |
| 4 g                 | Yeast (concentrated)    | 110 g                              | Shortening                                 |
|                     |                         | 18 g                               | Salt                                       |
|                     |                         | 4 g                                | Yeast                                      |
|                     |                         | 4.5 g                              | Soda                                       |
| <u>Dough:</u>       |                         |                                    |                                            |
| <u>Sponge plus:</u> |                         |                                    |                                            |
| 350 g               | Wheat Flour             | 0.05 g                             | Bicake <sup>®</sup> BSC*                   |
| 110 g               | Shortening              | 30 g                               | Fermented wheat germ (invented ingredient) |
| 18 g                | Salt                    |                                    |                                            |
| 4.5 g               | Soda                    |                                    |                                            |
| 0.05 g              | Bicake <sup>®</sup> BSC |                                    |                                            |

\*A mixture of protease and  $\alpha$ -Amylase marketed by Quest International, Naarden, the Netherlands.

[0035] Processing was done along the following lines:

|                    |                                      | Sponge & Dough process | Process according to the invention |
|--------------------|--------------------------------------|------------------------|------------------------------------|
| Sponge:            | Mixing time (min):                   | 5                      | —                                  |
|                    | Sponge temp. ( $^{\circ}\text{C}$ ): | 30                     | —                                  |
|                    | Fermentation time (h):               | 18                     | —                                  |
| Dough:             | Mixing time (min):                   | 5 at speed I           | 5 at speed I                       |
|                    | Dough temp. ( $^{\circ}\text{C}$ ):  | 30                     | 30                                 |
|                    | Fermentation time (h):               | 4                      | 4                                  |
| Processing:        | Layers:                              | 8                      | 8                                  |
|                    | Dough height (mm):                   | 1.0–1.25               | 1.0–1.25                           |
|                    | Dough weight per piece (g):          | 4.5                    | 4.5                                |
|                    | Cutter dimension (mm):               | 55 $\times$ 47         | 55 $\times$ 47                     |
|                    | Trappature ( $^{\circ}\text{C}$ ):   | 265                    | 265                                |
| Baking conditions: | Baking time (min):                   | 4.5–5                  | 4.5–5                              |

[0036] The following equipment was used:

[0037] Mixer: Morton double-Z-mixer with 2 speeds and double jacketed walls plus thermostat. Connected to the mixer was a recording device for measuring dough temperature and mixing energy.

[0038] Proofer: set on  $26^{\circ}\text{C}$ . and 75% relative humidity.

[0039] Sheeter: Seewer Rondo.

[0040] Oven: deck-oven, with adjustable top- and bottom heat.

[0041] The resulting crackers were tested and evaluated with the following result:

[0042] Evaluation of Crackers:

|                             | Sponge and Dough | All-in produced Cracker |
|-----------------------------|------------------|-------------------------|
| Appearance:                 | good, uniform    | good, uniform           |
| Bottom:                     | even             | even                    |
| Structure, internal:        | opened, even     | opened, even            |
| Height of 10 crackers (mm): | 53               | 54                      |
| pH of final cracker:        | 7.7              | 7.6                     |
| Eating properties:          | tender, crisp    | tender, crisp           |

1. Dough for the preparation of dry baked goods, characterized in that it comprises a bakery ingredient prepared from cereal germs fermented by lactic acid forming bacteria.

2. Dough according to claim 1 characterized in that it is rolled and laminated.

3. Dough according to claims 1 or 2, characterized in that the baked goods are crackers.

4. Dough according to claim 3, characterized in that the crackers are premium crackers.

5. Dough according to claims 1-4, characterized in that the bakery ingredient comprises fermented wheat germs.

6. Dough according to claim 1-5, characterized in that the lactic acid forming bacteria are lactobacilli.

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7. Dough according to claim 6, characterized in that the lacticacilli are *Lactobacillus plantarum* and/or *Lactobacillus brevis*.

8. Dough according to any one of claims 1-7, characterized in that cereal germs were fermented in the presence of enzymes having protease and/or carbohydrase activity.

9. Dough according to claims 1-8, characterized in that it comprises the bakery ingredient in an amount so as to provide 01-10% by weight of fermented cereal germs, calculated on the amount of flour.

10. Dough according to claims 1-9, characterized in that the bakery ingredient is concentrated by partial removal of water.

11. Dough according to claims 1-9, characterized in that the bakery ingredient is in a dry form.

12. Dough according to claim 11 characterized in that the bakery ingredient is prepared by spray-drying

13. Dough according to claims 1-12 characterized in that it comprises an extract of the fermented cereal germs

14. Dough according to claim 1-13, characterized in that it comprises an enzyme composition having  $\alpha$ -amylase, (hemi-)cellulase and/or protease activity.

15. Dough according to claims 1-14, characterized in that it comprises gluten which- is at least partly modified.

16. Process for the preparation of dry baked goods characterized in that a dough according to any one of claims 1-15 is baked.

17. Dry baked goods obtained by the process of claim 16.

\* \* \* \* \*

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United States Patent [19]

[11] 3,919,434

Tsen et al.

Best Available Copy

[45]\*Nov. 11, 1975

[54] SHORTENING SPARING PROCESS FOR CHEMICALLY LEAVENED BAKED AND FRIED PRODUCTS AND COMPOSITIONS FOR PREPARING THE SAME

[75] Inventors: Cho C. Tsen; William J. Hoover, both of Manhattan, Kans.

[73] Assignee: The Kansas University Research Foundation, Manhattan, Kans.

[\*] Notice: The portion of the term of this patent subsequent to Oct. 20, 1990, has been disclaimed.

[22] Filed: Nov. 9, 1973

[21] Appl. No.: 414,234

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 78,237, Oct. 5, 1970, Pat. No. 3,773,521.

[52] U.S. CL..... 426/553; 426/551

[51] Int. CL<sup>2</sup>..... A21D 10/04; A21D 10/00; A21D 2/16; A21D 2/26

[58] Field of Search ..... 426/153, 155, 156, 345, 426/551, 553

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Primary Examiner—James R. Hoffman  
Attorney, Agent, or Firm—Schmidt, Johnson, Hovey & Williams

[57] ABSTRACT

A method of substantially decreasing the shortening content normally required in chemically leavened wheat flour based doughs or batters which comprises incorporating into the dough or batter in lieu of the shortening omitted, from about 0.1 to 3% by weight, based upon the flour content therein, of an additive selected from the group consisting of the sodium and calcium salts of acyl lactylates of C<sub>12</sub>-C<sub>22</sub> fatty acids. The preferred additives are sodium stearoyl lactylate and calcium stearoyl lactylate, each being utilized at a concentration of about 0.5% by weight. In optional procedures, a protein supplement such as soy flour can be added to the dough or batter products without noticeably affecting the appearance and taste characteristics of the finished product.

12 Claims, No Drawings

# **SHORTENING SPARING PROCESS FOR CHEMICALLY LEAVENED BAKED AND FRIED PRODUCTS AND COMPOSITIONS FOR PREPARING THE SAME**

This application is a continuation-in-part of application Ser. No. 78,237, filed Oct. 5, 1970, now U.S. Pat. No. 3,773,521 and entitled SHORTENING SPARING PROCESS FOR WHEAT FLOUR BASED BAKED OR FRIED DOUGH PRODUCTS AND DOUGHS PRODUCED THEREBY. The subject matter of the parent application is expressly incorporated herein by reference.

This invention relates to a process for substantially decreasing the amount of shortening normally required in chemically leavened, wheat flour based doughs or batters without adversely affecting the appearance and taste characteristics of the finished products. More particularly, it is concerned with such a process as well as premixes and compositions useful in connection therewith for the production of protein fortified or unfortified baked or fried goods which employ a constituent selected from the group of the sodium or calcium salts of acyl lactylates of  $C_{14}$ - $C_{22}$  fatty acids as an additive in lieu of the shortening content omitted or at reduced levels from the dough or batter formulation. In preferred forms, sodium or calcium stearoyl-2-lactylate is utilized as the shortening sparing agent, and a protein supplement such as soy flour can optionally be added to the dough or batters to increase the nutritional content thereof.

Wheat flour based goods such as breads, cookies, crackers, biscuits and cakes are staple foods in many countries of the world because of their relatively high caloric value, ready availability of wheat flour at an economical price, and the attractive organoleptic and appearance properties of the food products. Wheat flour based products of this type typically contain water, salt, sugar, shortening and dough conditioning agents in addition to the wheat flour base, with shortening conventionally being added to improve the volume, organoleptic and taste characteristics of the final baked or fried product.

As is well understood in the art, wheat flour based products of the class described are deemed to comprise two separate categories, namely yeast leavened or chemically leavened doughs or batters. Conventional wheat flour based breads which contain yeast as a leavening agent therein are a prime example of products of the first mentioned class, and these are generally characterized by a semi-rigid, but pliable dough. Semi-rigid doughs of this type are generally necessary in order to provide a matrix of protein which entraps small cells of carbon dioxide produced during fermentation by the yeast. When such a dough is placed in an oven for baking the  $CO_2$  trapped therein expands and the protein and starch matrix of the dough becomes progressively denatured and gelatinized and more rigid. At the end of the cooking thereof such as baking or frying, the entrapped gas has escaped and a self-sustaining, finished raised bread product remains.

On the other hand, chemically leavened products are generally of two types, batters and underdeveloped doughs, and are characterized by the lack of a semi-rigid protein matrix. For example, a conventional cake batter, which is a high moisture, semi-fluid, pourable suspension containing 70 to 80% water, cannot effectively be leavened by yeast, because during fermenta-

tion thereof the  $CO_2$  developed quickly escapes to the atmosphere and thus cannot serve to raise the product during baking. Accordingly, it is necessary with such batter products to thoroughly admix the ingredients thereof and rely upon relatively quick raising in the oven during baking. Hence, chemically leavened systems employ additives such as baking powder which produce the requisite raising gas at a time substantially coincident with the heating, drying out and denaturalization and gelatinization of the protein and starch components of the batter. As can be appreciated, timing of the gas-producing action of the chemical leavening agent is exceedingly critical in systems of this type by virtue of the lack of a semi-rigid matrix in the batter.

Similarly, cookie or cracker doughs do not have the requisite matrix characteristics which allow yeast to be an effective leavening agent therein. This stems from the fact that these doughs are relatively dry and cannot be mixed and kneaded as extensively as in the case of bread doughs thus resulting in stiff dough systems which are generally referred to in the art as "underdeveloped." In other words, underdeveloped doughs are not subjected to adequate mixing to cause the proteins and starches therein to become pliable and stretchable, but rather these doughs are of a stiff, dry, non-pliable consistency. Accordingly, it is necessary to employ chemical leavening agents in such cookie or cracker doughs in order to provide a degree of spreading rather than raising which aids in the formulation of desirable thin, flat, end products.

As alluded to previously, shortening is usually present in both yeast and chemically leavened food products. However, a special problem is posed with respect to the latter formulations because of the relatively large quantities of shortening normally employed therein. For example, many conventional cake batters contain up to 50% by weight (baker's weight) shortening, while on the same basis yeast leavened breads in many instances contain 3% or less by weight shortening. Similarly, cookies can contain 5 to 98% shortening by weight, and crackers are in many instances approximately 10% by weight shortening, all calculated as baker's weight. Therefore, in view of the fact that shortening is several times as expensive as the base wheat flour material, any reduction in shortening content of chemically leavened doughs or batters represents a significant commercial breakthrough. This is particularly true if it is possible to substantially lower the shortening content of such products while nevertheless maintaining substantially identical or improved taste and appearance qualities, and if the additive employed for this purpose is not itself prohibitively expensive.

Moreover, it is desirable in many instances to increase the nutritional content of the wheat flour based chemically leavened products outlined, particularly from a protein standpoint. However, attempts to incorporate protein supplements into wheat flour based food products, and especially those of the chemically leavened variety, have heretofore met with little success, particularly in conjunction with an attempt to simultaneously decrease the shortening level of the dough or batter formulations. Soy flour for example is an especially attractive protein supplement for chemically leavened products such as crackers, biscuits and cakes because of the high content of good nutritional quality protein in soy flour. Also, soy flour is especially valuable as an additive by virtue of the fact that it contains

from about 3.2 to 3.8% lysine as compared with 0.375% in wheat flour. Thus, with the addition of 12 grams of soy flour to 100 grams of wheat flour, the lysine content will be more than doubled to a value of from 0.76 to 0.83%. The resulting product thus provides an exceptional vehicle for nutritional improvement of the human diet.

Supplementation of the protein content of wheat flour based, chemically leavened batters or doughs to a level to significantly improve nutrition has not heretofore been successful because of the adverse effect on the quality of the food product attributable to the supplement added, not only from the organoleptic standpoint, but also from the undesirable appearance, physical quality and decreased shelf-life of the resulting goods. These problems are further compounded when efforts are made to limit the shortening content of the dough or batter formulation. In order for shortening sparing and protein supplementation to be commercially practical, the additive permitting these objectives to be carried out must be economical, uniform in quality and of properties which do not result in a significant change in the physical and nutritional properties of the dough or batter.

It has now been discovered that by incorporation of from 0.1 to 3% by weight, based upon the weight of wheat flour, of either the sodium or calcium salts of acyl lactylates of  $C_{14}$ - $C_{22}$  fatty acids (preferably sodium stearyl-2-lactylate or to a slightly lesser degree calcium stearyl-2-lactylate) in chemically leavened, wheat flour based dough or batter formulations, not only may the shortening required be substantially decreased, but at the same time a protein supplement may be added to the composition in an amount to improve the nutritive quality of the resultant products without a consequent loss in final product appearance or organoleptic characteristics. In particular, the shortening content of such batters and doughs can be decreased by an amount of up to about 35% of that normally present in the formulations, which as can be appreciated represents a substantial saving to commercial scale producers. This result is enhanced by virtue of the fact that the defined additives are effective at a level (preferably about 0.5% by weight) where the cost thereof is substantially less than the price of shortening omitted in a given dough or batter formulation. Moreover, a number of highly advantageous protein supplements can be added to the formulations including soy flour, soy isolates, nonfat milk solids, whey products, fish protein concentrates, cotton seed flour, chick-pea flour, sesame seed flour, corn-soy milk blend flour, wheat protein concentrate, wheat gluten, defatted wheat germ, Torula yeast, wheat soy blend flour, edible single cell proteins compatible with wheat flour for baking or frying purposes, and mixtures thereof.

It is therefore the primary object of this invention to provide a novel process for drastically lowering the amount of shortening required in wheat flour based, chemically leavened doughs or batters by addition of a sodium or calcium salt of the acyl lactylates of  $C_{14}$ - $C_{22}$  fatty acids which in the quantities employed costs less than the shortening omitted and serves the added function of allowing a protein supplement to be introduced into the formulation without changing the desirable properties of the food product.

It is a further important object of the invention to provide a method of substantially lowering the shortening requirements of chemically leavened, wheat flour

based doughs or batters which may be carried out without altering in any significant way the processing conditions required for preparation of the food products therefrom, in the baking or frying processes themselves, or in the equipment needed for mixing and processing of the dough or batter.

Yet another object of the invention is to provide chemically leavened, wheat flour based dough or batter premixes or compositions which contain abnormally low amounts of shortening therein by conventional standards and can be supplemented by the addition of protein-containing additives without in any way detracting from the taste and organoleptic properties of the final product.

The following examples will illustrate the broad applicability of the invention disclosed herein. It is to be understood that the specific formulas and tests referred to hereunder are not to be construed as limiting the applicability of the lactylate additives to any particular types of chemically leavened, wheat flour based doughs or batters, but instead should be interpreted as exemplary of the large number of such goods which can be produced in accordance with the concepts hereof.

### EXAMPLE I

A series of baking tests employing wheat flour based, chemically leavened cake batters were conducted according to the Official Method of the American Association of Cereal Chemists (AACC Method 10-90, Cereal Laboratory Methods 7th Ed. AACC, St. Paul, 1962). The following basic formula was employed in each of these tests:

TABLE I

| Ingredient        | Basic Cake Formula |                        |
|-------------------|--------------------|------------------------|
|                   | g.                 | Amount % (flour basis) |
| Flour             | 200.0              | 100.0                  |
| Sugar             | 200.0              | 100.0                  |
| Shortening        | 100.0              | 50.0                   |
| Nonfat dry milk   | 24.0               | 12.0                   |
| Frozen egg whites | 18.0               | 9.0                    |
| Salt              | 6.0                | 3.0                    |
| Baking powder     | 12.0               | 6.0                    |

The above listed ingredients were mixed to form a batter with varying amounts of shortening and additive therein in the conventional manner in an appropriate mixer and cooked in an oven until done. The volume of all of the resulting cakes was then measured by the rape seed displacement method.

The results of each of the individual tests wherein varying amounts of shortening and sodium stearyl-2-lactylate (SSL) were employed is given below in Table II. Moreover, a number of test results are shown wherein varying amounts of soy flour were added to the basic formulation of Table I as a protein supplement.

TABLE II

| Effects of Soy Flour, Shortening, and SSL Levels on Cakes' Volume* |              |       |                     |
|--------------------------------------------------------------------|--------------|-------|---------------------|
| Soy Flour %                                                        | Shortening % | SSL % | Average Volume C.C. |
| 0                                                                  | 35           | 0     | 1150                |
| 0                                                                  | 35           | 0.5   | 1275                |
| 0                                                                  | 30           | 0     | 1075                |
| 0                                                                  | 30           | 0.5   | 1235                |
| 0                                                                  | 25           | 0     | 1045                |
| 0                                                                  | 25           | 0.5   | 1240                |
| 12                                                                 | 35           | 0     | 1175                |
| 12                                                                 | 35           | 0.5   | 1125                |
| 12                                                                 | 30           | 0     | 1140                |

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TABLE II-continued

| Effects of Soy Flour, Shortening, and SSL Levels on Cakes' Volume* |              |       |                     |
|--------------------------------------------------------------------|--------------|-------|---------------------|
| Soy Flour %                                                        | Shortening % | SSL % | Average Volume C.C. |
| 12                                                                 | 30           | 0.5   | 1125                |
| 12                                                                 | 25           | 0     | 1115                |
| 12                                                                 | 25           | 0.5   | 1115                |

\*All 12 given on flour weight basis (baker's weight)

As can be appreciated from a study of Table II, all cakes, regular and soy fortified, with 0.5% SSL were found to have a larger volume than those without SSL at a comparative shortening level. In fact, the volume of regular and soy fortified cakes containing 25% shortening and 0.5% SSL were larger than that of cakes containing 35% shortening. This means that the addition of 0.5% SSL can significantly reduce the shortening requirement of cakes.

## EXAMPLE II

A number of soda crackers were prepared according to the average formula outlined in *Cookie and Cracker Technology* by Metz (AVI Publishing Co., Westport, Conn., 1968), with slight modifications as set forth below in Table III.

TABLE III

| SODA CRACKER FORMULA |                       |
|----------------------|-----------------------|
| Sponge Portion       |                       |
| Flour                | 60 g.                 |
| Shortening           | Variable              |
| Protease             | 2 ml. (1 tablet/1 L.) |
| $\alpha$ -amylase    | 2 ml. (1 tablet/1 L.) |
| Water                | Variable              |
| Yeast                | 20 ml. (0.2%)         |
| Mixing               | 4 min.                |
| Dough Portion        |                       |
| Flour                | 40 g.                 |
| Malt syrup           | 1.0 g.                |
| Soda                 | 0.7 g.                |
| Salt                 | 1.2 g.                |
| Mixing               | 7.0 min.              |

The sponge portion containing variable amounts of shortening and water were admixed in a conventional Hobart mixer and allowed to ferment for a period of 18 hours and 30 minutes at approximately 85° F and 85% relative humidity. Following this fermentation, the dough portion containing the soda chemical leavening agent was admixed and incorporated into the yeast leavened sponge portion. After thorough admixing of these two portions to insure a uniform consistency, the resultant dough was allowed to sit for a period of 5 hours and 30 minutes at approximately 85° F and 85% relative humidity.

Following the above described treatment, the dough was sheeted on a conventional Aneta sheeter a number of times, and pressed into a cracker form. The cracker dough was thereafter baked in an oven at about 500° F for about 1 1/4 minutes or until the crackers were lightly browned.

In general, it is recognized in the art that the quality of crackers may be relatively uniformly evaluated by their weight. The lighter the weight, the thinner the cracker layers will be; and eating quality is generally much improved when it is composed of thin layers. Table IV documents the results obtained from weighing 12 pieces of the crackers produced in each run. As can

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be seen, in a number of cases 6 or 12% by weight soy flour was incorporated into the composition as a protein additive, and these results are likewise set forth in Table IV.

TABLE IV

| EFFECTS OF SOY FLOUR, SHORTENING AND SSL LEVELS ON CRACKER'S WEIGHT (QUALITY)* |              |       |                            |
|--------------------------------------------------------------------------------|--------------|-------|----------------------------|
| Soy Flour %                                                                    | Shortening % | SSL % | Cracker Wt. (12 pieces) g. |
| 0                                                                              | 6            | 0     | 20.5                       |
| 0                                                                              | 6            | 0.5   | 18.8                       |
| 0                                                                              | 9            | 0     | 19.5                       |
| 0                                                                              | 9            | 0.5   | 17.9                       |
| 6                                                                              | 6            | 0     | 25.6                       |
| 6                                                                              | 6            | 0.5   | 21.4                       |
| 6                                                                              | 9            | 0     | 26.2                       |
| 6                                                                              | 9            | 0.5   | 22.3                       |
| 12                                                                             | 6            | 0     | 24.1                       |
| 12                                                                             | 6            | 0.5   | 22.2                       |
| 12                                                                             | 9            | 0     | 24.4                       |
| 12                                                                             | 9            | 0.5   | 19.9                       |

\*All 12 given on flour weight basis (baker's weight)

The test results tabulated in Table IV show that both shortening and SSL can reduce the weight and therefore improve the quality of crackers, but SSL is in general more effective in exerting the reducing action. The weight data also indicates that crackers with 6% shortening and 0.5% SSL are better than those with 9% shortening. This observation is confirmed by results on high-protein crackers made from wheat flour fortified with 6 or 12% defatted soy flour. Furthermore, organoleptic tests confirm that crackers containing SSL taste much better (i.e., are crispier) than those without SSL. In short, the test results indicate that SSL can spare shortening normally required in crackers and simultaneously improve the eating quality thereof, even those of the high protein variety.

## EXAMPLE III

In order to confirm the advantages of using additives in accordance with the present invention in chemically leavened cookies, the following studies were made.

A series of baking tests were conducted according to the Standard AACC Method 10-56 for testing the baking quality of cookie flour (spread-factor test) using wheat flours and blends thereof fortified with various levels of soy flour. The following formula was followed, unless otherwise indicated:

TABLE V

| COOKIE FORMULA                             |           |                                 |
|--------------------------------------------|-----------|---------------------------------|
| Ingredients                                | Weight g. | Percentage (flour weight basis) |
| Wheat flour or a blend of wheat-soy flours | 225.0     | 100.00                          |
| Sugar                                      | 130.0     | 58.04                           |
| Hydrogenated vegetable shortening          | 62.7      | 28.00                           |
| Salt                                       | 2.1       | 0.93                            |
| Sodium bicarbonate                         | 2.5       | 1.12                            |
| Water                                      | Var       | Var                             |
| Suspension of nondairy milk*               | 40.0      | 17.85                           |

\*20.2 g. of spray-dried, nondairy milk was dissolved in 19.8 ml. water. 40 ml. of water was used instead of the milk suspension for making soy-fortified cookies.

Following mixing of the above listed ingredients in the conventional manner to form a relatively stiff dough, cookies were cut therefrom using a 70 mm. cookie cutter. The cookies were then oven baked until done and allowed to cool. The spread ratio (W/T.

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where W is the average diameter and T the average thickness) was then calculated for the various cookies by determining the width, thickness and weight of six cookies on an average basis. In each case six cookies were laid end to end for width measurement and stacked for thickness measurements. In addition, the baking tests were repeated at least once on a different day to substantiate results.

In order to further test the lactylate additives of the present invention, four different wheat flour were employed in the cookie tests. The chemical characteristics of these wheat flours are given below in Table VI.

TABLE VI

| WHEAT FLOUR ANALYSIS |            |           |       |
|----------------------|------------|-----------|-------|
|                      | Moisture % | Protein % | Ash % |
| Flour I              | 12.5       | 10.1      | 0.42  |
| Flour II             | 12.5       | 8.6       | 0.41  |
| Arthur flour         | 12.7       | 10.4      | 0.37  |
| Logan flour          | 12.6       | 10.7      | 0.37  |

Analysis of the wheat flours was determined as described in AACC Method except for the fat which was determined by AOCS Method (Aa 4-38) using petroleum ether as the extracting solvent.

Soft wheat flour I was a commercially available cookie flour, as was wheat flour II. Arthur and Logan wheats (two soft wheat varieties) were supplied by the Soft Wheat Quality Laboratory, USDA, and milled on a Ming Muhomat mill.

In the first test, the effects of various shortening levels and SSL on the spread ratio of cookies prepared from wheat flour I fortified with 12% defatted soy flour was determined in accordance with the above outlined procedures. The results of these tests were collected in the following Table VII.

TABLE VII

| SPREAD RATIOS OF COOKIES AS AFFECTED BY SHORTENING VARIATIONS AND SSL ADDITIONS TO FLOUR I, II, LOGAN AND ARTHUR FLOURS |                     |          |                           |          |                      |          |                          |          |                         |          |
|-------------------------------------------------------------------------------------------------------------------------|---------------------|----------|---------------------------|----------|----------------------|----------|--------------------------|----------|-------------------------|----------|
| Shortening %                                                                                                            | Regular Cookies (I) |          | Soy-Fortified Cookies (I) |          | Regular Cookies (II) |          | Regular Cookies (Arthur) |          | Regular Cookies (Logan) |          |
|                                                                                                                         | No SSL              | 0.5% SSL | No SSL                    | 0.5% SSL | No SSL               | 0.5% SSL | No SSL                   | 0.5% SSL | No SSL                  | 0.5% SSL |
| 12                                                                                                                      | —                   | —        | —                         | —        | 5.2                  | 6.6      | 5.6                      | 6.9      | 5.0                     | 6.8      |
| 16                                                                                                                      | 6.18                | 6.93     | 5.19                      | 5.31     | 6.7                  | 7.8      | 7.0                      | 8.1      | 6.8                     | 8.4      |
| 20                                                                                                                      | 6.47                | 7.55     | 5.64                      | 5.68     | 7.0                  | 8.4      | 8.0                      | 8.4      | 8.4                     | 9.3      |
| 24                                                                                                                      | 6.97                | 7.84     | 6.11                      | 6.29     | 9.0                  | 8.9      | 8.9                      | 10.2     | 9.5                     | 9.9      |
| 28                                                                                                                      | 7.46                | 8.46     | 6.54                      | 6.87     | 8.5                  | 10.0     | 10.4                     | 10.7     | 10.0                    | 10.3     |
| 32                                                                                                                      | 8.07                | 8.63     | 7.11                      | 7.56     | —                    | —        | —                        | —        | —                       | —        |

\*All % given on flour weight basis (baker's weight)

The above results demonstrated that SSL can increase the spread ratios of cookies of the regular and soy-fortified variety and can also reduce or spare the shortening required in such cookies. The sparing action is more pronounced with regular cookies than with soy-fortified cookies and with cookies containing less shortening than those containing more shortening.

It is well known in the art that the spread ratio of cookies can be increased with larger additions of shortening, but the finding that SSL could increase spread ratio and simultaneously spare shortening was highly unexpected. Therefore, additional baking tests were carried out to determine if the same results would be obtained using SSL in cookies prepared from three other flours (II, Arthur and Logan). These results are set forth in the right hand section of Table VII which

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confirm the improving effect of SSL with different kinds of wheat flour.

Inclusion of SSL in cookies not only permits preparation thereof with significantly lower amounts of shortening therein but also improves the cookies' quality, reduces the caloric value thereof, and in some instances lowers production costs.

In order to determine the various levels of effectiveness of SSL, a series of cookie tests identical to those previously described were performed wherein various levels of SSL were employed in cookies containing two different amounts of shortening (22 and 28% by weight respectively). Moreover, the effect of SSL on cookies supplemented with defatted soy flour was also determined. The results of these tests are documented in Table VIII.

TABLE VIII

| SPREAD RATIO (W/T) OF COOKIES PREPARED FROM FLOUR II AND FLOUR II FORTIFIED WITH 12% DEFATTED SOY FLOUR USING INDICATED LEVELS OF SSL AND TWO LEVELS (22 and 28%) OF SHORTENING* |              |          |                                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|----------|-----------------------------------|
| Additive %                                                                                                                                                                       | Shortening % | Flour II | Flour II + 12% defatted soy flour |
| 0                                                                                                                                                                                | 22           | 7.4      | 6.4                               |
| 0.25% SSL                                                                                                                                                                        | 22           | 8.5      | 6.7                               |
| 0.50% SSL                                                                                                                                                                        | 22           | 8.7      | 6.7                               |
| 1.00% SSL                                                                                                                                                                        | 22           | 9.3      | 6.9                               |
| 1.50% SSL                                                                                                                                                                        | 22           | 9.4      | 7.1                               |
| 0                                                                                                                                                                                | 28           | 8.8      | 7.0                               |
| 0.25% SSL                                                                                                                                                                        | 28           | 9.4      | 7.3                               |
| 0.50% SSL                                                                                                                                                                        | 28           | 10.0     | 7.4                               |
| 1.00% SSL                                                                                                                                                                        | 28           | 10.3     | 7.6                               |
| 1.50% SSL                                                                                                                                                                        | 28           | 10.6     | 7.6                               |

\*all % given on flour weight basis (baker's weight)

EXAMPLE IV

The comparative effectiveness of calcium stearoyl-2-lactylate (CSL) as a shortening sparing additive was likewise tested in cookies in a manner identical with that described in Example III, using soft flour II. The spread ratios of the finished cookies are listed in Table IX and it can be seen that both CSL and SSL improve cookie quality, as shown by the spread ratios; however SSL is generally more effective for this purpose. Note also that the quality of cookies with 22% shortening and 0.5% SSL or CSL is better than that of cookies with 28% shortening and no additives.

TABLE IX\*

| Shortening % | Additive % | Spread Ratio W/T |
|--------------|------------|------------------|
| 22           | 0          | 8.30             |
| 22           | 0.25% SSL  | 9.36             |
| 22           | 0.50% SSL  | 10.24            |

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TABLE IX<sup>a</sup>-continued

| Shortening<br>% | Additive<br>% | Spread Ratio<br>W/T |
|-----------------|---------------|---------------------|
| 32              | 0.25% CSL     | 9.12                |
| 32              | 0.50% CSL     | 9.67                |
| 32              | 0             | 9.45                |

<sup>a</sup>All % given on flour weight basis (baker's weight)

Results tabulated in Table VIII substantiate the finding that SSL is effective in increasing cookie spread ratio with lesser amounts of shortening than with the amounts normally employed. However, while cookies fortified with 12% defatted soy flour exhibit increased spread ratios and a shortening sparing effect with addition of SSL, greater increases were observed in the unfortified cookies.

Unlike other parameters utilized to estimate cookie quality, cookie spread and thickness can be conveniently and accurately measured, and is therefore a standard of the art. However, the fundamental reasons for variations in spread and thickness are not fully understood. Variables include changes in quality and quantity of ingredients (flour, sugar, water, shortening, and baking powder) and in processing (mixing, sheeting, cutting, or molding).

Sodium stearyl-2-lactylate is generally prepared by admixing lactic acid in an aqueous medium with commercial grade stearic acid (an admixture of myristic, palmitic and stearic fatty acids) at a sufficiently elevated temperature to remain in the molten condition. In general, 1.0 equivalent of fatty acids is used for each 1.2 equivalence of lactic acid as monomer for each lactyl group desired. In this case therefore, 2.4 equivalence of lactic acid are provided for each 1.0 equivalent of fatty acids. The mixture is stirred with heating whereupon about 1 equivalent of sodium hydroxide is added. The mixture is then heated to bring the temperature thereof up to about 200° C to complete the reaction. The reaction is carried out under an atmosphere of an inert gas to remove water vapor and prevent oxidation of the stearic acid. A solid, slightly cream colored material is produced upon cooling of the reaction products and it is then ground to a fine powder for use. The powder is a mixture of sodium salts of a homologous series of stearyl lactylic acids, in which the number of lactyl groups in the molecules in a function of the relative ratios of the constituents brought into the admixture. Calcium stearyl-2-lactylate is prepared in a similar manner except that calcium carbonate is used as the neutralization agent.

The respective reaction mixture will contain unreactive lactic acid and lactylates, unreacted stearic acids, polylactylates and their salts. A specific procedure for producing sodium stearyl-2-lactylate of which the numeral 2 indicates the average number of lactyl groups in the molecule, is set forth in detail in U.S. Pat. No. 2,789,922 insofar as stearyl-2-lactylic acid ester is concerned and U.S. Pat. No. 2,733,252 which describes not only the preparation of sodium stearyl-2-lactylate from stearic acid, sodium bicarbonate and lactic acid, but also sets forth the parameters for production of the calcium lactylate composition. It is to be understood in this respect that although best results have been obtained using sodium stearyl-2-lactylate, and to a slightly lesser degree calcium stearyl-2-lactylate, other equivalent lactylates may be employed in this invention including those stearyl lactylates

(and particularly the sodium and calcium lactylates) having a different average number of lactyl groups in the molecule than is the case in the preferred lactylate additives specified herein. For example, the average number of lactyl groups can be less than 1.0 although a larger quantity of the additive must be employed for equivalent effectiveness. The specifications for the preferred acyl lactylate, sodium stearyl-2-lactylate are set forth in Food Additive Regulation 21 CFR, Section 121.121).

Having thus described the invention, what is claimed as new and desired to be secured by Letters Patent is:

1. In a method of preparing a wheat flour based, chemically leavened dough or batter wherein an amount of shortening is incorporated into the dough or batter to impart particular organoleptic properties to baked or fried goods derived therefrom, the improvement which comprises the steps of:

admixing the ingredients making up the chemically leavened dough or batter using a quantity of shortening therein which is from 12½ to about 35% less than the amount of shortening employed to give the baked or fried products derived from the dough or batter said particular organoleptic properties; and

incorporating into the dough or batter ingredients in lieu of the shortening omitted, from about 0.1 to 3% (baker's weight) of an additive selected from the group consisting of the sodium and calcium salts of the acyl lactylates of C<sub>14</sub>-C<sub>22</sub> fatty acids, the amount of said additive incorporated into said dough or batter being sufficient to maintain said particular organoleptic properties of said derived baked or fried goods at levels substantially equal to or exceeding those of baked or fried goods derived from an otherwise identical dough or batter containing said amount of shortening and being essentially free of said additive.

2. A method as set forth in claim 1 wherein said additive is sodium stearyl-2-lactylate.

3. A method as set forth in claim 1, wherein said additive is calcium stearyl-2-lactylate.

4. A method as set forth in claim 1 wherein said additive is incorporated into said ingredients in an amount of about 0.5% (baker's weight).

5. A method as set forth in claim 1 wherein a supplement which increases the protein content of said dough or batter is admixed with said ingredients.

6. A method as set forth in claim 5 wherein said protein supplement is selected from the group consisting of soy flour, soy isolates, nonfat milk solids, whey products, fish protein concentrate, cottonseed flour, chickpea flour, sesame seed flour, corn-soy-milk blend flour, wheat protein concentrate, wheat gluten, defatted wheat germ, Torula yeast, wheat-soy blend flour, edible single cell proteins, and mixtures thereof.

7. In a wheat flour based, chemically leavened dough or batter composition wherein an amount of shortening is present in the composition to impart particular organoleptic properties to baked or fried goods derived therefrom, the improved dough or batter composition which comprises:

an admixture of wheat flour and shortening and other ingredients necessary for said chemically leavened dough or batter composition, the quantity of shortening present in the latter being from 12½ to about 35% less than the amount of shortening employed to give said derived baked or fried products said

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particular organoleptic properties; and from about 0.1 to 3% (baker's weight) of an additive selected from the group consisting of the sodium and calcium salts of the acyl lactylates of  $C_{12}$ - $C_{24}$  fatty acids.

the amount of said additive present in said dough or batter being sufficient to maintain said particular organoleptic properties of said derived baked or fried goods at levels substantially equal to or exceeding those of baked or fried goods derived from an otherwise identical dough or batter containing said amount of shortening and being essentially free of said additive.

8. The composition as set forth in claim 7 wherein said additive is sodium stearyl-2-lactylate.

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9. The composition as set forth in claim 7 wherein said additive is calcium stearyl-2-lactylate.

10. The composition as set forth in claim 7 wherein said additive is present in an amount of about 0.5% (baker's weight).

11. The composition as set forth in claim 7 wherein a supplement which increases the protein content of said end products is present in said composition.

12. The composition as set forth in claim 11 wherein said supplement is selected from the group consisting of soy flour, soy isolates, nonfat milk solids, whey products, fish protein concentrate, cottonseed flour, chick-pea flour, sesame seed flour, corn-soy-milk blend flour, wheat protein concentrate, wheat gluten, defatted wheat germ, Torula yeast, wheat-soy-blend flour, edible single cell proteins, and mixtures thereof.

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United States Patent [19]  
Haarasilta et al.

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[45] Date of Patent: \* Jan. 5, 1993

[54] METHOD OF IMPROVING THE PRODUCTION PROCESS OF DRY CEREAL PRODUCTS BY ENZYME ADDITION

[75] Inventors: Sampsa Haarasilta, Kerava; Timo Pullinen, Vantaa; Ina Tammersalo-Karsten, Espoo; Seppo Vaisanen, Kerava; Harri Frantl, Vantaa, all of Finland

[73] Assignee: Caltor Ltd., Helsinki, Finland

[\*] Notice: The portion of the term of this patent subsequent to Feb. 5, 2008 has been disclaimed.

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[52] U.S. Cl. .... 426/30; 426/18; 426/61

[58] Field of Search ..... 426/7, 18, 20, 61, 549

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Primary Examiner—Joseph Golian  
Assistant Examiner—Leslie Wong  
Attorney, Agent, or Firm—Baker & McKenzie

[57] ABSTRACT

The invention relates to a method of improving the production process of dry cereal products, such as crispbread and biscuits, by adding hemicellulose and cellulose degrading enzymes to the dough. The enzyme addition improves the properties of the dough and increases the process capacity by decreasing the need for dough liquid.

22 Claims, No Drawings

# METHOD OF IMPROVING THE PRODUCTION PROCESS OF DRY CEREAL PRODUCTS BY ENZYME ADDITION

This application is a continuation of application Ser. No. 419,877, filed Oct. 11, 1989 now abandoned.

## FIELD OF THE INVENTION

This invention relates to a method of improving the production process of dry cereal products by adding cellulolytic and/or hemicellulolytic enzymes to the dough used to make these products. The invention improves the properties of the dough (for example, dough transferability) and decreases the amount of water which needs to be added to the dough to achieve an acceptable final product. The decrease in water needed has profound effects on the efficiency and capacity of the baking process. The invention permits a wider choice of flour and results in a final product with improved crispness and other properties.

## BACKGROUND OF THE INVENTION

One of the most popular forms of baked goods are so-called "dry cereal products": namely, baked products which have low water concentrations and are, therefore, crispier than soft products such as bread or cake. As used herein, the term "dry cereal products" refers to baked products made from grain, such as crispbread, biscuits, rusk, brendcrumba, sour crispbread, crackers, baked snacks, waffles, cookies, pretzels, ice cream cones and dark bread.

Dry cereal products have a longer shelf life and can be distributed over wide geographic areas in contrast to soft products which are highly perishable and are typically distributed on a more local basis. Dry product processes tend to be larger and more intricate—and often more automated—as a result.

A bottleneck in the production process of dry cereal products, such as crispbread, biscuits, sour crispbread, and dark bread, etc. is often the limited production capacity of ovens used, but increasing oven size requires high capital investment. Attempts have also been made to improve the process efficiency by using flour with a low water-binding capacity and by other apparatus improvements. Such expedients, however, have generally been inadequate.

In an industrial production process of dry cereal products, such as crispbread and biscuits, the ingredients (flour, liquid, requisite additives, such as salt, sugar, dough raising agents and possible spices) are mixed into a dough. A thin dough cake is baked rapidly at a high, accurately programmed temperature, and dried. The moisture content of the final product is extremely important and will vary depending on the product involved. In many instances, the crispness and preservation properties are maximized at a moisture content of between about 7% and about 9% by weight. On an average, the crispness of biscuits is at best at a moisture content of less than 4% by weight. In addition to water content, the crispness and preservation properties of biscuits and other products are affected by other factors, such as fat content. For example, the protein content of flour used in baking semi-sweet biscuits is of great importance for the crispness of the final product.

The quality of flour to be used in a dry bread product depends on the product to be prepared. In crispbread production, whole rye flour is used in most cases; how-

ever, the popularity of wheat crispbread is on the increase. Most biscuits are made of wheat, while the demand and consumption of oatmeal biscuits and especially of rye biscuits are insignificant.

The most important flour properties are those affecting the crumb structure, crispness and volume. With doughs raised with yeast, the "falling" number required for rye flour ranges from 120 to 200. (The "falling" number is measured by procedures well known and defined in the prior art.) The falling number of rye flour to be used in a dough raised by whipping gas into the dough (so called "ice bread") should also be relatively high. Sour, thick crispbread can be made from rye flour having a lower falling number, because the acidity prevents the inherent enzymes of flour from decomposing starch too far. The falling number of flour used in the production of biscuits should be high (more than 300) due to, e.g., their long production process and the rather high temperature of dough water in the production of semi-sweet biscuits, for instance.

Flour used in the production of crispbread usually has a relatively low protein content, generally varying between 8 and 12% by weight. The water-binding capacity of such flour is not as high as that of flour rich in protein, so less energy is required for drying during baking. In biscuit baking, protein content is of vital importance to the quality of flour and that of the product. The protein content of the flour should range from 7.5% to 10.5% by weight on dry substance. Flour having a protein content less than 10% by weight is best suited for baking semi-sweet biscuits.

Yeast or a chemical raising agent can be used for proofing. Proofing can also be effected by mixing gaseous substances into the dough.

In the production of both crispbread and biscuits, water or milk is used as dough liquid. In the production of crispbread, the amount of liquid needed is nearly equal to that of flour, that is, the dough is considerably softer than in the production of normal soft bread. In biscuit production, less liquid is usually needed than in the production of crispbread. The use of large amounts of liquid requires long baking times and large amounts of energy at the baking stage to reduce the water concentration of the final product to an acceptable level.

In a dry cereal product production process, the amount of dough liquid is in most cases determined by the technical qualities of the process and the apparatus. For example, the softness of a dough suitable for use in a crispbread production process and, as a consequence, the amount of liquid needed is determined on one hand by the mechanical strength of the blades of the dough mixer and on the other hand the operation of the prick-ing device. In addition, the webforming ability sets certain requirements on the softness of dough. Dough softness is described by a consistency value. (Consistency is measured by a penetrometer by allowing the measuring cone of the penetrometer to fall into the dough for 10 seconds, whereby the penetration of the cone in millimeters gives the consistency of the dough; the greater the value, the softer the dough. A penetrometer is a standard piece of equipment well known in the baking art.)

The crispness, taste, and machinability of crispbreads and biscuits can be increased by the use of fat and sugar.

In crispbread production, the dough is formed into a weblike mat after suitable proofing. The mat can be baked as such, or it can be cut or chopped into separate cakes, or the mat is formed into separate cakes by means

of moulds, rolls, and the like. The cakes can be patterned before baking, e.g., by pricking. Pricking provides the product with a pattern typical of it, and binds the surface layers of the cake together. If necessary, the final proofing of the products is carried out after the patterning, whereafter the products are baked. So called "ice bread" is not proofed at this stage; it is baked immediately after the pricking.

Biscuits, such as semi-sweet biscuits, are often made by so-called "all-in" (or "straight") dough making process, in which the dough is prepared by mixing all the dry ingredients simultaneously with the dough liquid and other liquid ingredients. The way in which the dough is prepared is of great importance in the production of semi-sweet biscuits. As semi-sweet biscuits should possess an extensible gluten network, relatively high dough temperatures, about +40° C., are desirable in the dough preparation phase. An example of a mixer suitable for the production of biscuits is a horizontal two-blade mixer standard in the baking industry. After preparation, the dough should be allowed to stand so that the dough liquid is absorbed in the dry ingredients (floor time). After the floor time the dough is moulded, e.g., by means of a four-roll feeder and then folded by passing it through a pair of rolls. After folding the dough is passed through sheeting rolls. Sheeted dough is pricked and cut with a roll cutter (drum cutter).

The products lose moisture concentration during baking. The moisture content of a conventional finished crispbread product is about 7% to about 12% by weight. The moisture content of product raised with gas may be as low as about 2% by weight. On an average, the moisture content of biscuits does not exceed about 4% by weight.

To achieve the desired final moisture content, the baked product usually has to be dried further. The drying can be effected by using a convection oven, basket conveyors transported above the oven in the waste heat, "after-baking", that is, drying in a tunnel oven, or dielectric drying. If necessary, the baked product can be chopped, e.g., by sawing.

It is known that cellulolytic/hemicellulolytic enzymes cleave non-starch polysaccharides present in flour. Addition of cellulolytic and/or hemicellulolytic enzymes has been used to improve the properties of the baking process and the finished baking product.

Japanese Patent Application No. 5701/1968 describes a method of improving the quality of white sour bread by adding to the dough an enzyme composition having cellulolytic and/or hemicellulolytic activity. It is emphasized in the patent application that the addition of the enzyme composition causes decomposition of fibrous components present in flour, such as cellulose and pentosans, the presence of which as such would considerably deteriorate the quality of bread by rendering the dough inhomogeneous and by preventing the formation of gluten. It is recited that the addition of cellulolytic/hemicellulolytic enzymes provides soft bread with increased volume, more uniform grain structure and slower ageing than bread prepared with prior art methods.

Finnish Patent Application No. 881905 (U.S. application Ser. No. 341,389 filed Apr. 21, 1989, now U.S. Pat. No. 4,990,343 describes the use of cellulolytic/hemicellulolytic enzymes in combination with glucose oxidase or glucose oxidase and sulphydryl oxidase in baking soft bread from wheat and rye flour. These enzymes are used for optimizing the baking process. Moreover, it has

been found that the enzyme combinations improve the rheological properties, gluten, processability, and tolerance of a flour dough. It has also been found to improve the volume, grain structure and anti-staling properties of a bakery product.

However, because of the significant differences in the process for baking dry cereal products (such as crispbread, etc.) and the increased moisture content of dough used to bake these products, enzyme treatment of dough for dry cereal products has heretofore not been utilized. It has now been found according to the present invention that an addition of cellulolytic and/or hemicellulolytic enzymes increases the process capacity in the production of dry cereal products. Unexpectedly and surprisingly, the enzyme addition of the invention makes the dough softer, so less dough liquid is required as compared with conventional techniques. The reduced concentration of water requires less energy than prior art methods for baking the product and the need for after-drying is decreased or eliminated. In large scale baking processes, this improved efficiency will result in substantial cost savings.

In addition, the final product obtained by the method of the invention has a lower shrinkage and increased crispness.

#### SUMMARY OF THE INVENTION

One of the objects of the invention is to improve the baking process of dry cereal products by decreasing the necessary moisture concentration. The present invention contemplates a method for improving the production process of dry cereal products and the quality of the baked product produced therefrom which comprises combining flour, yeast and water with an amount of an enzyme preparation comprising hemicellulose and/or cellulose degrading enzymes effective to reduce the amount of moisture necessary to form a suitable baking dough. The resulting dough requires shorter baking time and the need for post-baking drying in order to reduce the moisture content of the final product to an acceptable level is decreased or eliminated.

Preferably, the enzyme preparation contains between about 0 to about 50,000 Units of hemicellulose degrading enzymes and about 0 to about 20,000 Units of cellulose degrading enzymes per kilogram of flour. An enzyme preparation which contains about 10 to about 20,000 Units of hemicellulose degrading enzymes and about 10 to about 10,000 Units of cellulose degrading enzymes per kilogram of flour is particularly preferred.

The method of the present invention can be used to produce dry cereal products such as crispbread, made of rye or wheat flour, as well as other dry cereal products such as biscuits, pretzels, ice cream cones, dark bread and the like.

When the final product is a rye crispbread, the method of the present invention wherein the enzyme preparation comprises about 1,000 to about 3,000 Units of hemicellulose degrading enzymes and/or about 400 to about 1,200 Units of cellulose degrading enzymes per kilogram of flour is preferred. When the final baked product is a wheat crispbread, an enzyme preparation which contains about 500 Units to about 2,000 Units of hemicellulose degrading enzymes and/or about 200 Units to about 800 Units of cellulose degrading enzymes per kilogram of flour is preferred. When the final baked product is a semi-sweet biscuit, an enzyme preparation which contains about 2,500 Units to about 10,000 Units

of a hemicellulose-degrading enzyme per kilogram of flour is preferred.

The instant invention contemplates a method which reduces the necessary moisture concentration of the baking dough up to approximately 15% by weight.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

##### A. General

The method of the invention is characterized by adding an enzyme preparation containing hemicellulose and/or cellulose degrading enzymes to the dough.

The method of the invention provides a baking process with a greater capacity than prior art methods. The baking process of the invention has a lower energy consumption than prior art methods. The proof time is shorter and the baking shrinkage is lesser when the method of the invention is applied. The choice of flour is easier. The obtained product has advantageous properties over products prepared with prior art methods, such as increased crispness.

The enzyme preparation of the invention contains enzymes degrading hemicellulose (xylan, mannan, galactomannan, araban) and its cleavage products or ester-bonds contained therein and/or enzymes degrading cellulose.

For example, an enzyme preparation (BAKEZYME 1201, manufacturer Finnsugar Ltd.) having the following hemicellulolytic and cellulolytic activity is suitable for use in the present invention:

|                               |                |
|-------------------------------|----------------|
| Xylanase                      | 25,000 U/min/g |
| Carboxymethyl cellulase (CMC) | 9,300 U/min/g  |
| Filter paper activity (FP)    | 300 U/min/g    |

Xylanase activity represents hemicellulolytic activity while carboxymethyl cellulase activity and filter paper activity represent cellulolytic activity.

A suitable enzyme dosage level is, e.g., about 0 to about 50,000 Units, preferably about 10 to about 20,000 Units of hemicellulase (xylanase) and about 0 to about 20,000 Units, preferably about 10 to about 10,000 Units of cellulase (calculated as carboxymethyl cellulase activity), or correspondingly about 0 to about 1,000 Units, preferably about 0.5 to about 500 Units of cellulase (calculated as filter paper activity) per kg flour.

The definitions of the different enzyme activities and the methods of determining the enzyme activities are set forth below:

**Xylanase activity** (Khan A. W. et al, Enzyme Microb. Technol. 8 (1986) 373-377):

1 ml of a suitably diluted enzyme solution in acetate buffer (0.05M NaAc, pH 5.3) is tempered at 50° C. 1 ml of xylan substrate (1% xylan, 0.05M NaAc, pH 5.3) is added. The sample is incubated for 30 min at 50° C. The reaction is stopped by adding 3 ml of DNS reagent (3,5-dinitrosalicylate), and the color is developed by boiling the same mixture for 5 min. The absorbance is measured at 540 nm. One enzyme Unit liberates 1 micromole of reducing sugars per one minute calculated as glucose.

**Carboxymethyl cellulase activity** (Mandels M., Weber J., Adv. Chem. Ser. 95 (1969) 391-413):

1 ml of suitably diluted enzyme solution in acetate buffer (0.05M NaAc, pH 4.8) and 1 ml of CMC substrate (1% CMC, 0.05M NaAc, pH 4.8) are mixed together. The solution is incubated for 10 min. at 50° C.

The reaction is stopped by adding 3 ml of DNS reagent. One enzyme Unit liberates 1 micromole of reducing sugars per one minute.

**Filter paper activity** (Ghose, T. K. et al., Symposium of Enzymatic Hydrolysis of Cellulose, Bailey M., Enari T. M., Linko M., Eds. (SITRA, Aulanko, Finland, 1975), p. 111-136:

A piece of filter paper (Whatman 1, 50 mg) is added to 1 ml of acetate buffer (0.05M NaAc, pH 4.8). 1 ml of suitably diluted enzyme solution is added. The solution is incubated for 1 h at 50° C. The reaction is stopped by adding 3 ml of DNS reagent, and the color is developed and measured similarly as in the xylanase determination. One activity Unit liberates 1 micromole of reducing sugars per one minute calculated as glucose.

##### B. Experimental

##### EXAMPLE 1

##### Crispbread Baking Trials

The use of the enzyme preparation disclosed herein results in the decrease of the moisture content for suitable baking doughs for dry cereal products. To establish the unexpected decrease in the amount of water using the present invention, the following test was carried out, in which BAKEZYME 1201 was added to the dough in the amount of 50 mg/kg flour.

Rye meal with a relatively high falling number was used in this crispbread baking test. The flour was measured for the following properties:

|                                   |             |
|-----------------------------------|-------------|
| Moisture content (% by weight)    | 13.3        |
| Enzyme activity (U/kg flour d.b.) |             |
| alpha-amylase                     | 675 U/min   |
| peramase                          | 4,700 U/min |
| xylanase                          | 2,100 U/min |
| Protein (Kjeldahl) (% d.b.)       | 10.0        |
| Starch (Brechtner-Mandelin kit)   | 64.1        |
| Protein content (% by weight)     | 12          |
| Falling number (% g)              | 270         |

The composition of the dough was as follows (amounts given as percentages on the amount of flour; the amount of flour used was 4 kg):

|                 |     |
|-----------------|-----|
| Flour           | 100 |
| Yeast           | 2.5 |
| Salt            | 1.4 |
| Water (control) | 90  |

In the trials set forth in Table 1, the enzyme dosage level was kept constant (50 mg/kg flour) with the exception of the control, and the amount of water was decreased (90%, 87.5% and 85% of the original amount).

The baking was carried out using the following method. First, room temperature flour, to which enzyme weighed with an analytical balance had been added, was introduced into the mixing bowl. Salt and yeast suspended in water were then added in solution form. Dough temperature was adjusted to 28° C. by means of the water temperature. The ingredients were mixed using a Hobart A 200 mixer for 12 minutes at speed 1. Mixing was followed by first proofing of 240 minutes, whereafter the dough was braked (homogenized) in the Hobart bowl. Sugar solution was also added for yeast food.

The dough was then rolled into a mat, pricked (embossed), proofed for 28 minutes (37° C., 90% relative humidity), and baked at a high temperature for 9 minutes. Changes in the consistency of the different doughs are shown in Table 1.

-continued

|                                                                       |                                                 |
|-----------------------------------------------------------------------|-------------------------------------------------|
| alpha-amylase<br>pectinase<br>xylanase<br>Protein (Kjeldahl) (% d.w.) | 500 Units<br>7,200 Units<br>2,400 Units<br>11.1 |
|-----------------------------------------------------------------------|-------------------------------------------------|

TABLE 1

| Measurement Time      | Consistency of Dough (as measured by penetrometer) |         |                                                                                                            |                          |                        |
|-----------------------|----------------------------------------------------|---------|------------------------------------------------------------------------------------------------------------|--------------------------|------------------------|
|                       | control                                            | control | Addition of enzyme preparation:<br>Hemicellulose (xyl.) 1,250 U/kg flour<br>Cellulase (CMC) 470 U/kg flour |                          |                        |
|                       |                                                    |         | - 8.5% H <sub>2</sub> O                                                                                    | - 12.5% H <sub>2</sub> O | - 15% H <sub>2</sub> O |
| After mixing of dough | 16.0                                               | 16.0    | 15.5                                                                                                       | 15.5                     | 15.5                   |
| 1 h                   | 16.5                                               | 17.2    | 18.0                                                                                                       | 18.0                     | 16.0                   |
| 2 h                   | 19.0                                               | 18.0    | 21.0                                                                                                       | 21.0                     | 19.5                   |
| 3 h                   | 21.0                                               | 20.0    | 24.0                                                                                                       | 23.0                     | 21.0                   |
| After first proofing  | 24.0                                               | 22.0    | 24.0                                                                                                       | 25.5                     | 24.0                   |
| After baking          | 29.0                                               | 28.5    | 33.0                                                                                                       | 34.0                     | 30.5                   |

The results establish that even though the amount of water was decreased up to 15% from the control, the dough was still processable. The same final dough consistency of the control was achieved with a 15% decrease in the amount of water.

The finished, cooled product was further measured for its height, crispness, humidity and volume weight. There were no differences in these properties between the control product and products baked with dough treated with the enzyme preparation of the instant invention with up to a 15% reduction in water concentration.

|                                  |      |
|----------------------------------|------|
| Starch (Boehringer-Mannheim kit) | 63.1 |
| Penicillin content (% by weight) | 16.4 |
| Falling number (7 g)             | 165  |

The composition of the dough was as follows (amounts given as percentages on the amount of flour: the amount of flour used was 4 kg):

|                 |     |
|-----------------|-----|
| Flour           | 100 |
| Yeast           | 3.8 |
| Salt            | 2.1 |
| Water (control) | 90  |

EXAMPLE 2

Baking Trials With Varying Enzyme Dosages

Baking trials with various enzyme dosages and water concentrations were utilized. Flour with a lower falling number (165) than in the tests of Example 1 was used for these trials. Otherwise the baking was carried out as described in Example 1, adding BAKEZYME 1201 preparation in the amount of 90-150 mg/kg flour. In terms of enzyme activities, this enzyme dosage level is equivalent to about 2,250 to about 3,750 Units of hemicellulase (xylanase) and about 840 to about 1,400 Units of cellulase (calculated as carboxymethyl cellulase activity) or correspondingly about 35 to about 60 Units of cellulase (calculated as filter paper activity). The flour exhibited the following properties:

Water amounts in this series of baking trials with respect to the control (in which the amount of water was 90% on the weight of flour=100%) were 112%, 107%, 100% and 85%. BAKEZYME 1201 dosage levels were 90, 100, 105, 120, 150 mg/kg flour.

The dough was mixed similarly as in Example 1 except that the first proof time was only 120 minutes. At the braking stage, a glucose solution was added for yeast food. The dough moulding steps were the same as in Example 1 except that the proof time was only 21 minutes (at 35° C., 85% relative humidity). The products were baked at a high temperature for 8 minutes and allowed to cool before measurement of properties. Dough consistencies as a function of time are shown in Table 2.

TABLE 2

| Trial                          | Consistency of Dough (as measured by penetrometer) |      |       |       |      |      |      |      |      |      |      |
|--------------------------------|----------------------------------------------------|------|-------|-------|------|------|------|------|------|------|------|
|                                | 1                                                  | 2    | 3     | 4     | 5    | 6    | 7    | 8    | 9    | 10   | 11   |
|                                |                                                    |      | cont. | cont. |      |      |      |      |      |      |      |
| Hemicellulase (xyl.) U/kg      | —                                                  | —    | —     | —     | —    | 2250 | 2300 | 2500 | 2630 | 3000 | 3750 |
| Cellulase (CMC) U/kg           | —                                                  | —    | —     | —     | —    | 840  | 930  | 930  | 980  | 1120 | 1400 |
| Water (ml)                     | 4030                                               | 3850 | 3600  | 3060  | 3060 | 3060 | 3060 | 3060 | 3060 | 3060 | 3060 |
| Water amount % (on zero level) | 112                                                | 107  | 100   | 100   | 85   | 85   | 85   | 85   | 85   | 85   | 85   |
| After mixing of dough          | 19.0                                               | 17.8 | 16.0  | 16.0  | 12.5 | 14.5 | 17.5 | 17.5 | 17.0 | 17.0 | 18.5 |
| 40'                            | 22.0                                               | 20.5 | 18.8  | 18.0  | 14.0 | 17.5 | 18.0 | 18.5 | 18.0 | 20.0 | 21.0 |
| 80'                            | 24.5                                               | 24.0 | 20.5  | 21.5  | 17.0 | 19.0 | 21.5 | 21.5 | 19.5 | 21.0 | 22.0 |
| After first proofing           | 26.5                                               | 25.0 | 21.0  | 22.0  | 17.5 | 20.0 | 22.0 | 22.0 | 22.5 | 22.5 | 23.0 |
| After braking                  | 28.5                                               | 27.5 | 24.5  | 26.5  | 19.0 | 24.0 | 26.5 | 25.0 | 25.0 | 25.5 | 26.0 |

|                                   |      |
|-----------------------------------|------|
| Moisture content (% by weight)    | 11.2 |
| Enzyme activity (U/kg flour d.w.) |      |

Doughs having a "normal" consistency after braking (that is, the two controls (trials 3 and 4) and doughs prepared with a water amount of 85% and enzyme dosage level 100-120 mg/kg (2,500-3,000 U/kg)) (trials

7-10) gave similar final products. Samples baked from a softer dough (enzyme dosage level 150 mg/kg (3.750 U/kg) (trial 11) or water amounts of 112% and 107%, respectively) (trials 1 and 2) yielded non-homogeneous texture. Product prepared from too hard a dough (enzyme dosage level 90 mg/kg (2.250 U/kg) + 85% water without enzyme) (trial 5) gave bread with reduced height.

### EXAMPLE 3

#### Crispbread Baking Trials

A baking test was carried out on a continuously operated bakery-scale crispbread baking line. The ratios and quality of the dough ingredients were the same as those used in Example 2. Enzyme preparation (BAKEZYME 1210) in the amount of 90 mg/kg flour was added: (enzyme activity: 2,250 Units of hemicellulase (xylanase) and 840 Units of cellulase (CMC) per kg flour); the amounts of dough water were reduced to about 85% of the "normal" percentage, as in Example 2. Since the mass flow contained more dry substance than usual, the weight of an unbaked cake was dropped 6.25% from the normal value.

The width of the unbaked cake could also be reduced by about 1.0% since the dough prepared with the enzyme addition had a better rise than a product prepared without enzyme addition.

The oven time utilized was conventional, whereby a 7.7% increase in capacity was obtained with the enzyme addition (decrease in energy demand).

With the addition of the enzyme preparation, the dough rose more rapidly than normal and the properties of the finished product were good. The crispness of the product was observed to be slightly greater than that of a control product prepared without enzyme addition.

A similar test was carried out by keeping the amount of energy at a conventional value and shortening the oven time by 7.7%, corresponding to the decrease in energy demand. The properties of this product were good and similar to the properties noted above, suggesting that less energy is required (when the present invention is utilized) to bake the final product than normally required.

### EXAMPLE 4

#### Wheat Crispbread Baking Trials

Wheat crispbread was baked using the invention on a pilotscale test basis.

Flour with the following properties was used in the wheat crispbread baking tests:

|                                                         |        |
|---------------------------------------------------------|--------|
| Moisture content (%)                                    | 13.4   |
| Ash content (% d.a.)                                    | 0.6    |
| Amount of moist gluten (%)                              | 26.0   |
| Water binding ability (farinograph method) <sup>1</sup> | 58.1%  |
| Maximum temperature (amylograph method) <sup>1</sup>    | 74° C. |
| Maximum height of amylogram                             | 330 AU |
| Enzyme activities (U/kg flour d.a.)                     |        |
| amylase                                                 | 430    |
| protease                                                | 3030   |
| xylanase                                                | 1980   |

<sup>1</sup>The farinograph and amylograph methods used by bakers worldwide are to evaluate the rheological and mechanical properties of dough. The farinograph method is described by American Association of Cereal Chemists method AACCI-51, the amylograph by International Association of Cereal Chemists, Method PL 15a.

Flour and the other dry ingredients were first mixed into a powdery mixture. Yeast and water were subsequently added to the mixture.

Composition of the powder mixture was as follows:

|       |                                   |
|-------|-----------------------------------|
| Flour | 67.3%                             |
| Sugar | 5.0%                              |
| Fat   | 5.0%                              |
| Salt  | 1.8%                              |
| Yeast | 5% on weight of powder mixture    |
| Water | 57.5% on weight of powder mixture |

In the test series, the enzyme dosage level and the water decrease were determined in such a manner that the dough consistency remained constant.

The baking (2 kg of dough) was carried out similarly as in Example 1 except that the first proof time was 1 hour and the final proof time 45 min. Enzyme dosage levels used (BAKEZYME 1201) and water amounts are shown in Table 3 as set forth below.

TABLE 3

Consistency of Crispbread Dough (measured by penetrometer) Over Time

| Trial <sup>a</sup>                   | 1    | 2    | 3    | 4    |
|--------------------------------------|------|------|------|------|
| Hemicellulase (xyl. (U/kg)           | —    | 625  | 1250 | 1875 |
| Cellulase (CMC) (U/kg)               | —    | 230  | 470  | 705  |
| Amount of water (ml)                 | 1160 | 1070 | 960  | 850  |
| Amount of water, % on powder mixture | 57.5 | 53.5 | 49.0 | 42.5 |
| Consistency after mixing the dough   | 30.0 | 30.5 | 30.5 | 30.0 |
| Consistency after first proof        | 21.0 | 21.5 | 21.0 | 21.5 |
| Consistency after baking             | 30.0 | 31.0 | 31.0 | 31.5 |

<sup>a</sup>Trial 1 functioned as the control.

There were no differences in the measured properties of products prepared from the dough with constant consistency. The measured properties of the final product were the same as in Example 1.

### EXAMPLE 5

#### Semi-Sweet Biscuit Trials

Semi-sweet biscuits were baked with wheat flour having the following properties:

|                                   |             |
|-----------------------------------|-------------|
| Moisture content (%)              | 12.7        |
| Enzyme activity (U/kg flour d.a.) |             |
| alpha-amylase                     | 233 Units   |
| protease                          | 1,450 Units |
| xylanase                          | 1,809 Units |
| Protein (% d.a.) (Kjeldahl × 5.7) | 10.8        |
| Falling number                    | 363         |
| Ash (% d.a.)                      | 0.66        |
| Swelling number                   | 5           |
| Water binding (ml/100 g flour)    | 172         |

Composition of the basic dough:

| Ingredient          | (g) | (% on flour) |
|---------------------|-----|--------------|
| Wheat flour         | 493 | 100          |
| Vegetable fat       | 82  | 16.6         |
| Icing sugar         | 90  | 18.2         |
| Glucose             | 17  | 3.4          |
| Fructose            | 17  | 3.4          |
| Sucrose syrup T5 40 | 21  | 4.3          |
| Cornstarch          | 70  | 14.2         |
| Dry skim milk       | 21  | 4.2          |
| Table salt          | 5   | 1            |

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-continued

| Ingredient           | (g)   | (% on flour) |
|----------------------|-------|--------------|
| Volatile salt        | 0.9   | 1.3          |
| Tartaric acid        | 0.3   | 0.2          |
| Baking soda          | 1.9   | 0.4          |
| Sodium disulfite     | 3.8   | 0.5          |
| 10% aqueous solution |       |              |
| Water                | 160   | 32.5         |
| Total                | 175.2 |              |

The enzyme preparation used was **BAKEZYME 1201** having a xylanase activity of 25,000 U/g. (manufacturer Finnsugar Ltd.) was added to the dough as follows:

| Sample      | Xylanase U/g flour |
|-------------|--------------------|
| A (control) | 0                  |
| B           | 500                |
| C           | 1,000              |
| D           | 1,500              |
| E           | 2,500              |
| F           | 5,000              |
| G           | 10,000             |

Baking was carried out under pilot kitchen conditions by the so-called "all-in" baking process as follows:

Water (+42° C.) was introduced into the bowl of a Kenwood Chef mixer. Sodium disulfite solution and starch syrup were added to the water as well as the mixed dry ingredients of the formula. Fat was added on the dry ingredients.

The dough was kneaded (kneading hook) for one minute at the lowest speed of the mixer and for four minutes at speed IV. After kneading the temperature of the doughs varied between about +29° C. and +31° C. and pH between about 8.0 and about 8.3.

The softness of the doughs was measured immediately after kneading with a penetrometer (SUR, PNR 6, cone probe, penetration time 10 s). The softness and stickiness of corresponding doughs containing more water, for the reasons of the measuring technique used, were also measured with a farinograph (Brabender) by introducing 300 g of dough into the trough of the farinograph and by kneading it at constant temperature +30° C. at farinograph speed 2 for 10 minutes. The softness value was obtained after 5 minute kneading as farinogram height (Brabender unit, BU), stickiness was measured from the corresponding graph point as farinogram width (BU).

After 10 minutes of floor time the doughs were first rolled manually into 3×3 mm thick sheets and then on a grease-proof paper into a 1 mm thick sheet which was cut manually with a round mould (diameter 61 mm). The whole cut dough sheet was transferred to a baking sheet covered with a 1 mm hole network. On the baking sheet the dough sheet was pricked manually by means of a pricking wheel and the dough remaining outside the cut portions was removed.

The biscuits were baked in a convection oven for 7 minutes at 180°-200° C., whereafter they were transferred onto a tray and allowed to cool at room temperature.

After having cooled for half an hour, the finished biscuits were measured for their moisture content (at 130° C., 1 h). The moisture content varied from about 3.0% to about 6.5% by weight. The pH of the biscuits varied from about 7.0 to about 7.7.

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The effect of the enzyme treatment on the softness and stickiness of the biscuit dough is set forth in Tables 4 and 5 below.

TABLE 4

Effect of Enzyme Treatment on the Softness of Biscuit Dough as Measured with a Penetrometer

| Sample | Xylanase (U/kg flour) | Penetration (1/10 mm) (%) | Penetration delta A |
|--------|-----------------------|---------------------------|---------------------|
| A      | 0                     | 252                       | 0                   |
| B      | 500                   | 256                       | -2                  |
| C      | 1,000                 | 247                       | -2                  |
| D      | 1,500                 | 255                       | -1                  |
| E      | 2,500                 | 256                       | -2                  |
| F      | 5,000                 | 263                       | -4                  |
| G      | 10,000                | 269                       | -6                  |

TABLE 5

Effect of Enzyme Treatment on the Softness and Stickiness of a Biscuit Dough as Measured with a Farinograph

| Sample | Xylanase (U/kg flour) | Softness (BU) | Softness delta A (%) | Stickiness (BU) | Stickiness delta A (%) |
|--------|-----------------------|---------------|----------------------|-----------------|------------------------|
| A      | 0                     | 675           | 0                    | 161             | 0                      |
| B      | 500                   | 735           | -3                   | 170             | -10                    |
| C      | 1,000                 | 681           | -2                   | 160             | 0                      |
| D      | 1,500                 | 770           | -12                  | 220             | -27                    |
| E      | 2,500                 | 751           | -10                  | 170             | -6                     |
| F      | 5,000                 | 670           | -0.7                 | 131             | -19                    |
| G      | 10,000                | 161           | -76                  | 81              | -50                    |

The enzyme treatment improved the processability and rollability of the biscuit dough. This effect was most marked with the xylanase activity of 2,500-10,000 U/kg flour.

The enzyme treatment increased the softness of the dough, especially with the xylanase activity of 2,500-10,000 U/kg flour, with the best results from the xylanase activity of 5,000-10,000 U/kg flour.

The use of an enzyme preparation also decreased the stickiness of the biscuit dough, particularly with the samples with xylanase activity of 5,000-10,000 U/kg flour.

By means of the enzyme treatment, the amount of dough water could be decreased about 9% on the total amount of water without affecting adversely the processability and the quality of the final product, when the enzyme preparation was added in the dough so that the xylanase activity was about 2,500 to about 10,000 U/kg flour; preferably about 10,000 U/kg flour.

The enzyme treatment described herein is likewise applicable to the production of other dry cereal products, such as rusks, breadcrumbs, sour crispbread, crackers, baked snacks, waffles, pretzels, ice cream cones and dark bread.

The foregoing general discussion and experimental examples are intended to be illustrative of the present invention, and are not to be considered limiting. Other variations within the spirit and scope of this invention are possible and will present themselves to those skilled in the art.

We claim:

1. A method for the production of dry cereal products having a final moisture content of less than about 12% by weight, which provides for a reduction in the amount of initial water added to create processable dough, which comprises:

combining flour, water, yeast and at least 10 units of an enzyme preparation per kilogram of flour, said enzyme preparation comprising cellulose and hem-

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icellulose degrading enzymes in an amount effective to form a mass with suitable consistency for processing and baking:

processing said dough:

baking said processed dough at a suitable temperature:

drying said dry cereal product, if necessary, to reduce the final moisture content of said product to less than about 12% by weight.

2. The method of claim 1 wherein the amount of water added is up to about 15% less than the amount of water necessary to form a mass of suitable consistency in the absence of the enzyme addition.

3. The method of claim 2 wherein the baking time of said processed dough is about 15% less than the baking time required in the absence of the enzyme addition.

4. The method of claim 1 wherein said dry cereal product is crispbread.

5. The method of claim 4 wherein said crispbread is rye crispbread.

6. The method of claim 4 wherein said crispbread is wheat crispbread.

7. The method of claim 1 wherein said dry cereal product is a semi-sweet biscuit.

8. The method of claim 6 wherein said enzyme preparation is added in an amount of about 500 to about 2,000 Units of hemicellulose degrading enzymes and about 200 to about 800 Units of cellulose degrading enzymes per kilogram of flour.

9. The method of claim 7 wherein said enzyme preparation is added in an amount of about 2,500 to about 10,000 Units of hemicellulose degrading enzymes per kilogram of flour.

10. The method of claim 1 wherein said dry cereal product has a final moisture content of between about 7% to about 9% by weight.

11. The method of claim 2 wherein said dry cereal product has a final moisture content of less than about 4% by weight.

12. The method of claim 1 wherein said hemicellulose degrading enzyme is xylanase.

13. The method of claim 1.

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wherein said enzyme preparation is added in the amount of about 10 to about 50,000 Units of hemicellulose degrading enzymes per kilogram of flour.

14. The method of claim 1.

wherein said enzyme preparation is added in the amount of about 10 to about 20,000 units of cellulose degrading enzymes per kilogram of flour.

15. The method of claim 1.

wherein said enzyme preparation is added in the amount of about 10 to about 50,000 of hemicellulose degrading enzymes and about 10 to about 20,000 units of cellulose degrading enzymes per kilogram of flour.

16. The method of claim 5,

wherein said enzyme preparation is added in the amount of about 1,000 to about 3,000 Units of hemicellulose degrading enzymes per kilogram of flour.

17. The method of claim 5,

wherein said enzyme preparation is added in the amount of about 400 to about 1,200 Units of cellulose degrading enzymes per kilogram of flour.

18. The method of claim 5,

wherein said enzyme preparation is added in the amount of about 1,000 to about 3,000 Units of hemicellulose degrading enzymes and about 400 to about 1,200 Units of cellulose degrading enzymes per kilogram of flour.

19. The method of claim 6,

wherein said enzyme preparation is added in the amount of about 500 to about 2,000 Units of hemicellulose degrading enzymes per kilogram of flour.

20. The method of claim 6,

wherein said enzyme preparation is added in the amount of about 200 to about 800 Units of cellulose degrading enzymes per kilogram of flour.

21. The method of claim 6,

wherein said enzyme preparation is added in the amount of about 500 to about 2,000 Units of hemicellulose degrading enzymes and about 200 to about 800 Units of cellulose degrading enzymes per kilogram of flour.

22. The method of claim 15 wherein said enzyme preparation is added in an amount of about 10 to about 20,000 Units of hemicellulose degrading enzymes and about 10 to about 10,000 Units of cellulose degrading enzymes per kilogram of flour.

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Applicant: UNILEVER NV  
Burgemeester s'Jacobplein 1 P.O. Box 700  
NL-3000 DK Rotterdam(NL)  
BE CH DE DK ES FR GR IT LI NL SE AT

Applicant: UNILEVER PLC  
Unilever House Blackfriars P.O. Box 68  
London EC4P 4BQ(GB)  
GB

Inventor: Oola, Johannes Cornelia, UNILEVER  
RESEARCH LAB.  
Olivier van Noortlaan 120  
NL-3133 AT Vlaardingen(NL)  
Inventor: Steenhoek, Arle, UNILEVER  
RESEARCH LAB.  
Olivier van Noortlaan 120  
NL-3133 AT Vlaardingen(NL)

Representative: Hartong, Richard Leroy et al  
Unilever N.V. Patent Division P.O. Box 137  
NL-3130 AC Vlaardingen(NL)

**Fat-free pastry mix.**

The invention is concerned with dry fat-free pastry mixtures; these mixtures comprise separately packed components, being

- i) an egg white/sugar mix,
- ii) a pregelatinizable starch;
- iii) flour,
- iv) optionally improvers like baking powder, enzymes or aerating agents.

Also the batters (chilled, frozen and ambient) are part of the invention.

A process for the preparation of the batters is disclosed. Baked products like pastries, cookies, sponge cakes and cakes can be made from these batters.

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Until now, all pastry mixes known comprise a fatty component in order to achieve a pastry having the desired structure after baking.

However, for several years there has been a great demand for healthier products, by which is meant products containing less fat or no fat at all. A solution in order to come to compositions that are fat-free and simultaneously lead to the desired structure of the baked product and still have a good taste, has not been found so far.

We have now succeeded in preparing a dry fat-free pastry mixture with the desired properties.

Therefore, our invention is concerned in the first place with dry fat-free pastry mixtures that are characterized by separately packed components comprising:

- I) an egg white/sugar mix, wherein the weight ratio of egg white (as fresh egg white) : sugar = 1:2-2:1;
- II) a mix of one or more pregelatinizable starches;
- III) flour,

wherein the amount of pregelatinizable starch in the total mix is  $\frac{1}{3}$ - $\frac{1}{2}$  of the amount of sugar present and the amount of flour is  $\frac{1}{2}$ -2 times the amount of sugar.

The egg white is preferably a dried egg white that is mixed with water before it is used in our process for the preparation of a batter from our mixes.

The components are packed separately. When a batter is required, the egg white is aerated in the presence of the sugar, the aerated egg white/sugar mix is mixed with a starch that has previously been gelatinized, optionally in the presence of flour, and the components are mixed together. During this mixing, the amount of shear should be such that good mixing is obtained while the air that is encapsulated in the components, is not liberated from the system.

The starch or flour component of our mix preferably contains some baking powder (up to 5 wt.%).

The starch that is used, can be derived from any source of starch, such as wheat, maize, rice, potato, tapioca. The starch can be unhydrolyzed, but also hydrolyzed starches (having DE values as low as 0.5) can be used.

Therefore, it is preferred that the starch component of the mixture contains a multiple purpose enzyme mixture. Suitable enzymes are enzymes capable of hydrolyzing carbohydrates. Examples of these enzymes are amylases, xylanases, pentosanases and arabinoxylases. However, also proteases can be used successfully. The best results are obtained when the enzyme mixtures display simultaneously amylase and xylanase activity. The amount of starch-based enzyme is 10-5,000 dpm for commercially available enzyme compositions. This amount is, of course, strongly dependent on the enzyme activity of the enzymic mixture.

In the mixes, preferably in the starch or flour component, also monoglyceride, monoglyceride hydrates, aerated monoglyceride hydrates, complexes of monoglyceride and starch or other aerating agents can be present. It is, however, also possible to add these components to the egg white/sugar mix. The monoglyceride part hereof is derived from saturated and/or unsaturated fatty acids having 12-20 C atoms.

Other aerating compounds that can be used are the well-known emulsifiers, such as calcium- or sodiumstearoyllactylate (CSL, SSL), DATE, Polyglycerolesters (PGE), Polysorbates (Tween, Span), Propylene glycolmonostearate (PGM) etc. and mixtures of those.

The aeration of the egg white/sugar component is preferably performed to an overrun of 20-150%.

Part of the invention is also the batter, either at frozen, chilled or ambient temperature, which can be obtained from our mixes, while also the baked products which are obtainable by baking of our mixes, are part of the invention. Examples of baked products are short pastries, pastries, cookies, sponge cakes and cakes.

The process for the preparation of fat-free pastry batters is another part of our invention. This process comprises the following steps:

- 1) Whipping of a mixture of fresh egg white and sugar until an overrun of 20-150% is achieved;
- 2) Pregelatinizing starch in water, optionally in the presence of an enzyme mixture with hydrolyzing activity, in particular carbohydrate hydrolyzing activity;
- 3) Combining the aerated mixture of 1) with the pregelatinized starch of 2);
- 4) Adding flour to either the pregelatinized starch of 2) or to the mixture of 3);
- 5) Stirring of the mixture until a batter with the desired consistency has formed.

In this process also monoglyceride hydrates, aerated monoglyceride hydrates or other aerating agents or complexes of starch and monoglyceride hydrates can be added to the products of steps 1) and/or 2) and/or 3) and/or 4).

In these processes, the amounts of components used correspond, of course, with the amounts mentioned for the mixes per se.

The pregelatinization of the starches is performed by mixing 1 part by weight of starch with 7-15 parts by weight of water. The mixing is done at ambient temperature, although temperatures of 10-50°C can be

used. If an enzyme is used, the enzyme can be mixed with the starches prior to the addition of water or the enzyme can be added to a freshly prepared mix of starch and water.

EXAMPLE I

300 g sugar and 300 g fresh egg white were aerated for 5 minutes in a Hobart mixer. 100 g starch is pregelatinized in 500 g water at ambient temperature. The aerated egg white/sugar mix is combined with the gelled starch and the mixture is aerated for another 5 minutes (Hobart). 300 g flour and 3 g baking powder were added to the mixture thus obtained, with mixing.

In this way, a batter was obtained. A cake was baked from this batter by baking at 180°C for 45 minutes.

A cake having a good cake structure and an acceptable taste was obtained.

EXAMPLE II

The basic recipe is:  
400 g sugar  
400 g eggwhite (fresh)  
400 g flour  
150 g gelatinised starch (100 g starch + 1000 g water)  
4 g baking powder.

Rice starch.

Rice starch (100 g) was stirred into 70°C water (1000 g) to gelatinise. If enzymes are applied, it is done in this stage. This mass was cooled down to 20°C and mixed with the aerated sugar eggwhite mixture. Then the flour is carefully mixed in.

|                    | A      | B     | C     | D          |
|--------------------|--------|-------|-------|------------|
| Amylase (Fungamyl) | -      | 0.05g | -     | 0.05g      |
| Xylanase           | -      | -     | 0.05g | -          |
| Protease           | -      | -     | -     | 0.07g      |
| S.V. Batter ml/g   | 1.40   | 1.51  | 1.51  | 1.50       |
| S.V. Cake ml/g     | 2.0    | 2.0   | 2.0   | 2.1        |
| Texture            | long   | short | short | very short |
| Moistness          | medium | high  | high  | med./high  |

EXAMPLE III

Potato starch.

Procedure as with rice starch (ex II).

|                    | A      | B              | C              | D          |
|--------------------|--------|----------------|----------------|------------|
| Amylase (Fungamyl) | -      | 0.05g          | -              | -          |
| Xylanase           | -      | -              | 0.05g          | -          |
| Protease           | -      | -              | -              | 0.07g      |
| S.V. Batter ml/g   | 1.40   | 1.40           | 1.40           | 1.40       |
| S.V. Cake ml/g     | 2.0    | 2.2            | 2.2            | 2.0        |
| Texture            | long   | short/crumblly | short/crumblly | very short |
| Moistness          | medium | high           | high           | high       |

EXAMPLE IV

**Wheat starch.**

Procedure as with rice starch (ex II).

|                                                                                                                                                                                               | A      | B     | C         | D      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------|-----------|--------|
| Amylase (Fungamyl)                                                                                                                                                                            | -      | 0.05g | -         | -      |
| Xylanase                                                                                                                                                                                      | -      | -     | 0.05g     | -      |
| Protease                                                                                                                                                                                      | -      | -     | -         | 0.07g  |
| S.V. Batter ml/g                                                                                                                                                                              | 1.42   | 1.46  | 1.82      | 1.24   |
| S.V. Cake ml/g                                                                                                                                                                                | 2.0    | 2.2   | 2.4       | 1.8    |
| Texture                                                                                                                                                                                       | long   | short | short     | short  |
| Moistness                                                                                                                                                                                     | medium | high  | med./high | medium |
| N.B. It will be clear that the amount of enzyme applied is related to its activity. The sample of amylase had an activity of 2500 SKB/g, the xylanase 200.000 U/g and the protease 4500 GU/g. |        |       |           |        |

**Claims**

1. Dry fat-free pastry mixture, characterized by separately packed components comprising
  - i) an egg white/sugar mix, wherein the weight ratio of egg white : sugar = 1:2-2:1;
  - ii) a mix of one or more pregelatinizable starches;
  - iii) flour,
 in such amounts that the amount of pregelatinizable starch in the total mix is  $\frac{1}{3}$ - $\frac{1}{2}$  of the amount of sugar, and the amount of flour in the total mix is  $\frac{1}{3}$ -2 times the amount of sugar.
2. Dry fat-free pastry mixture according to Claim 1, wherein the starch component also contains a multiple purpose enzyme mixture.
3. Dry fat-free pastry mixture according to Claim 2, wherein the enzyme mixture displays carbohydrate hydrolyzing activity.
4. Dry fat-free pastry mixture according to Claim 2 or 3, wherein the enzyme mixture displays at least one of the following activities : amylase activity, protease activity, xylanase activity, pentosanase activity and/or arabinoxylanase activity.
5. Dry fat-free pastry mixture according to Claims 2-4, wherein the amount of enzyme is 10-5,000 dpm based on starch.
6. Dry fat-free pastry mixture according to Claim 1, wherein the starch or flour component also contains baking powder.
7. Dry fat-free pastry mixture according to Claims 1-6, wherein the pregelatinizable starch is derived from wheat, maize, rice, potato or tapioca.
8. Dry fat-free pastry mixture according to Claims 1-7, wherein the starch or flour component also contains monoglycerides, monoglyceride hydrates, aerated monoglyceride hydrates or complexes of monoglyceride and starch or other aerating agents.
9. Dry fat-free pastry mixture according to Claim 8, wherein the monoglyceride hydrate is derived from saturated and/or unsaturated fatty acids having 12-20 C atoms.
10. Fat-free pastry batter, characterized by the presence of the components of the mixture of Claims 1-9.
11. Fat-free pastry batter according to Claim 10, wherein the egg white/sugar component is aerated to an overrun of 20-150%.

12. Fat-free pastry batter according to Claim 10 or 11, wherein the batter is frozen or chilled.
13. Baked products, characterized in that these products are obtained after baking of the batters of Claims 10-12.
14. Baked products according to Claim 13, wherein these products comprise short pastries, pastries, cookies, sponge cakes or cakes.
15. Process for the preparation of a fat-free pastry batter, characterized by :
- 1) Whipping of a mixture of fresh egg whites and sugar until an overrun of 20-150% is achieved;
  - 2) Pregelatinizing starch in water, optionally in the presence of an enzyme mixture with hydrolyzing activity;
  - 3) Combining the aerated mixture obtained in 1) with the pregelatinized starch of 2);
  - 4) Adding flour to either the pregelatinized starch of 2) or to the mixture obtained in 3);
  - 5) Stirring of the mixture until a batter with the desired consistency has formed.
16. Process according to Claim 15, wherein a monoglyceride, monoglyceride hydrate, an aerated monoglyceride hydrate or a complex of monoglyceride and starch or other aerating agent are added to either the product of 1) and/or 2) and/or 3) and/or 4.



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㉔ Applicant: UNILEVER N.V.  
Burgemeester s'Jacobplein 1 P.O. Box 700  
NL-3000 DK Rotterdam(NL)

㉕ BE CH DE DK ES FR GR IT LI NL SE AT

㉖ Applicant: UNILEVER PLC  
Unilever House Blackfriars P.O. Box 68  
London EC4P 4BQ(GB)

㉗ GB

㉘ Inventor: Oole, Johannes Cornelia, UNILEVER  
RESEARCH LAB.

Olivier van Noortlaan 120  
NL-3133 AT Vlaardingen(NL)

Inventor: Steenhoek, Arie, UNILEVER  
RESEARCH LAB.

Olivier van Noortlaan 120  
NL-3133 AT Vlaardingen(NL)

㉙ Representative: Hartong, Richard Leroy et al  
Unilever N.V. Patent Division P.O. Box 137  
NL-3130 AC Vlaardingen(NL)

㉚ **Fat-free pastry mix.**

㉛ The invention is concerned with dry fat-free pas-  
try mixtures; these mixtures comprise separately  
packed components, being

- i) an egg white/sugar mix,
- ii) a pregelatinizable starch;
- iii) flour,
- iv) optionally improvers like baking powder, en-  
zymes or aerating agents.

Also the batters (chilled, frozen and ambient) are  
part of the invention.

A process for the preparation of the batters is  
disclosed. Baked products like pastries, cookies,  
sponge cakes and cakes can be made from these  
batters.

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EUROPEAN SEARCH REPORT

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| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                       |                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                  |                                                      |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Category                                                                                                                                                                                                                  | Citation of document with indication, where appropriate, of relevant passages                                                                                                                             | Relevant to claim                                                                                                                                                                                                                                                                | CLASSIFICATION OF THE APPLICATION (Int. Cl.6)        |
| A                                                                                                                                                                                                                         | GB-A-1 008 665 (BAXTER LABORATORIES INC.)<br>* page 2, column 2, line 75 - line 109; claims *                                                                                                             | 1-6                                                                                                                                                                                                                                                                              | A21D10/00<br>A21D8/04                                |
| A                                                                                                                                                                                                                         | US-A-4 361 862 (RILE, C.E. ET AL.)<br><br>* column 4, line 18 - column 5, line 6 *<br>* column 6, line 20 - column 10, line 2; claims *                                                                   | 1,6,<br>8-10,<br>12-14,16                                                                                                                                                                                                                                                        |                                                      |
| A                                                                                                                                                                                                                         | US-A-4 396 635 (ROUDEBUSH, R.M. & PALMISTO, P.D.)<br><br>* abstract *<br>* column 2, line 16 - line 58 *<br>* column 3, line 58 - column 4, line 60 *<br>* column 6, line 6 - column 7, line 54; claims * | 1,6-10,<br>12-14,16                                                                                                                                                                                                                                                              |                                                      |
| A                                                                                                                                                                                                                         | EP-A-0 020 170 (THE AMERICAN INSTITUTE OF BAKING)<br>* abstract *<br>* page 10, line 1 - page 12, line 26; claims *                                                                                       | 1,6,7,<br>11,16                                                                                                                                                                                                                                                                  |                                                      |
| A                                                                                                                                                                                                                         | GETREIDE, MEHL UND BROT,<br>vol. 6, 1976, BOCHUM, GERMANY<br>pages 112 - 116;<br>GANS, T.C.; 'Der Einsatz von mikrobiellen Enzymen in der Bäckerei.'<br>* the whole document *                            | 2-6                                                                                                                                                                                                                                                                              | TECHNICAL FIELDS<br>SEARCHED (Int. Cl.6)<br><br>A21D |
| The present search report has been drawn up for all claims                                                                                                                                                                |                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                  |                                                      |
| Place of search<br>THE HAGUE                                                                                                                                                                                              |                                                                                                                                                                                                           | Date of completion of the search<br>06 AUGUST 1992                                                                                                                                                                                                                               | Examiner<br>BEVAN S. R.                              |
| CATEGORY OF CITED DOCUMENTS                                                                                                                                                                                               |                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                  |                                                      |
| X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another document of the same category<br>A : technological background<br>O : non-written disclosure<br>F : interpretation document |                                                                                                                                                                                                           | T : theory or principle underlying the invention<br>E : earlier patent document, but published on, or after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br><br>a : member of the same patent family, corresponding document |                                                      |

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(54) CONDITIONER FOR BREAD

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(76) Inventor: Ali A. Elmusa, Leawood, KS (US)

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Correspondence Address:

STEVEN R. LUDWIG

STERNE, KESSLER, GOLDSTEIN & FOX

P.L.L.C.

1100 NEW YORK AVENUE, N.W.

SUITE 600

WASHINGTON, DC 20005-3934 (US)

(57)

ABSTRACT

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(60) Provisional application No. 60/265,122, filed on Jan. 31, 2001.

The present invention relates to a bread conditioner comprising an isolated soy protein. The bread conditioner can further comprise xylanase, sodium stearoyl lactylate, ascorbic acid, fungal alpha amylase, cellulase, and azodicarbonamide. Another aspect of the present invention relates to a dough composition comprising a bread conditioner, which results in a loaf of bread containing at least 6.25 grams of soy protein per 50 gram serving of bread.

## CONDITIONER FOR BREAD

### BACKGROUND OF THE INVENTION

#### [0001] 1. Field of the Invention

[0002] This invention generally relates to a bread conditioner comprising an isolated soy protein and various enzymes. This invention further relates to a dough comprising a bread conditioner that provides at least 6.25 grams of soy protein per 50 gram serving of bread.

#### [0003] 2. Background Art

[0004] The nutritional benefits of soy have long been recognized, as soy is rich in various antioxidants, dietary fiber, and certain minerals and vitamins. There is a particular interest in soy protein because of its ability to lower cholesterol and reduce the risk of cardiovascular health risks.

[0005] On Oct. 30, 1999, the FDA announced its approval of a health claim based on the association between consumption of soy protein and a reduced risk of coronary heart disease. To qualify for the health claim, a food must contain at least 6.25 grams of soy protein per "reference amount customarily consumed" (RACC). For bread, the RACC is a 50 gram serving. Thus, in the baking industry, it is desired to provide a bread containing at least 6.25 grams of soy protein per 50 gram serving.

[0006] For many years, soy protein products have been added in significantly lower quantities to grain-based foods, including many bakery product, specifically bread. Such additions of soy protein, however, have not resulted in the required 6.25 grams of soy protein per 50 gram serving. For example, U.S. Pat. No. 4,759,934 to Ferrara discloses a bread which is high in protein, however, contains less than two grams of soy protein per 50 gram serving.

[0007] Making a bread that will qualify for the soy protein health claim is not an easy task. Adding sufficient soy protein to qualify for the health claim, while continuing to maintain product quality, has been difficult because of the substantial amount of soy protein required. One way to increase the level of soy protein in bread is by incorporating soy protein isolates. However, one of the problems with using soy protein as a protein source, particularly when using soy protein isolates, is that soy tends to have a bitter taste. Additionally, soy products do not add significant strength to bread products. Previous attempts to make edible, saleable bread with 6.25 grams of soy protein per 50 gram serving have been unsuccessful. Thus, what is needed is a bread conditioner that will provide at least 6.25 grams of soy protein in a satisfactory loaf of bread while maintaining product quality, and providing a satisfactory and edible loaf of bread.

[0008] In the bread industry, it is known to add additives and/or conditioners to a bread dough that result in a bread having improved flavor, texture, volume and freshness. Enzymes have been particularly useful as bread conditioners, such as those described in U.S. Pat. No. 6,110,508 to Olesen et al., U.S. Pat. No. 6,039,982 to Wagner et al., U.S. Pat. No. 5,318,785 to DeStefanis and U.S. Pat. No. 5,232,719 to Lad et al.

[0009] However, using such known bread conditioners and additives, the desirable level of 6.25 grams of soy protein has not been attained in the production of an edible,

satisfactory loaf of bread. Although, it is possible to reach the 6.25 gram soy protein objective by adding soy grits, soy isolate and soy concentrate to bread, the resulting bread has been inedible and unsaleable. Thus, a bread conditioner is desired that, through the use of a synergistic combination of soy protein, various enzyme strains, and other ingredients, will contribute strength and conditioning to a bread dough and achieve at least 6.25 grams of soy protein per 50 gram serving of bread in a satisfactory loaf of bread meeting size, weight, volume, grain and appearance criteria.

### BRIEF SUMMARY OF THE INVENTION

[0010] The present invention relates to a bread conditioner comprising an isolated soy protein and other ingredients, which can include xylanase, sodium stearyl lactylate, ascorbic acid, fungal alpha amylase, cellulase, and an oxidant. This invention further relates to a bread product comprising a bread conditioner that contains at least 6.25 grams of soy protein per 50 gram serving of a satisfactory loaf of bread.

### DETAILED DESCRIPTION OF THE INVENTION

[0011] A preferred embodiment of the present invention is now described. While specific configurations and arrangements are discussed, it should be understood that this is done for illustrative purposes only. A person skilled in the relevant art will recognize that other configurations and arrangements can be used without departing from the spirit and scope of the invention. It will be apparent to a person skilled in the relevant art that this invention can also be employed in a variety of other devices and applications.

[0012] One embodiment of the present invention is directed to a bread conditioner comprising an isolated soy protein. Isolated soy proteins allow a bread conditioner to supply significant amounts of soy protein to a bread dough. In a preferred embodiment, the isolated soy protein has a protein content higher than about 70 percent. In a more preferred embodiment, the isolated soy protein has a protein content between about 85 and 95 percent. In a most preferred embodiment, the isolated soy protein has a protein content of between about 87 and 92 percent. The isolated soy protein can also comprise fat, ash, moisture and carbohydrates. A preferred isolated soy protein is ARDEX F Dispensible, which is a commercially available isolated soy protein manufactured by Archer Daniels Midland Company, and has a protein content of about 90 percent. However, alternate isolated soy proteins having varying protein contents can be used, as would be apparent to one skilled in the relevant art.

[0013] In a preferred embodiment, the bread conditioner further comprises various enzyme strains and other ingredients which contribute strength, conditioning, texture, flavor, volume, and/or freshness when added to a bread dough. Preferably, the bread conditioner includes xylanase, which adds strength to the bread and increases the extensibility of the dough.

[0014] Conventionally, low concentrations of xylanase have been used in bread conditioners. However, using low concentrations of xylanase often creates an undesirable stickiness of the dough. In a preferred embodiment of the present invention, xylanase is added to the bread conditioner

at a concentration level of about 750-2000 parts per million (ppm) based on the weight of wheat flour in a dough. More preferably, the xylanase has a concentration level of about 900-1300 ppm. In a most preferred embodiment, the xylanase has a concentration level of about 1050-1150 ppm. The use of xylanase in the bread conditioner of the present invention adds necessary strength and extensibility, while minimizing the stickiness of the dough. A preferred xylanase enzyme is VERON SPECIAL, which is a commercially available xylanase enzyme manufactured by Rohm GmbH. However, alternate xylanase enzymes can be used at varying concentrations, as would be apparent to one skilled in the relevant art.

[0015] In a preferred embodiment, the bread conditioner of the present invention further comprises a sodium stearoyl lactylate (SSL). SSL acts as a strengthener. Preferably, SSL is added to the bread conditioner at a concentration level of about 2500-5000 ppm based on wheat flour. In a more preferred embodiment, SSL is added to the bread conditioner at a concentration level of about 3500-5000 ppm, and in a most preferred embodiment, SSL is added to the bread conditioner at a concentration level of about 4500-5000 ppm. A preferred SSL is PANIPLEX SK, which is a commercially available sodium stearoyl lactylate manufactured by Archer Daniels Midland Company. However, alternate sodium stearoyl lactylates can be used at varying concentrations, as would be apparent to one skilled in the relevant art.

[0016] In a preferred embodiment, the bread conditioner of the present invention further comprises a fungal alpha amylase. Alpha amylases are useful for providing sugars fermentable by yeast, and can attack long starch chains at random at their interior and dextrinize and liquefy starch. Starch dextrinization during early stages of baking results in an improved grain and a softer texture. Alpha amylases induce changes in dough characteristics, such as decreasing water absorption capacity, slackening the dough, and developing stickiness.

[0017] In a preferred embodiment, the fungal alpha amylase has a level of about 200-750 skb. More preferably, the fungal alpha amylase has a level of about 300-600 skb. In a most preferred embodiment, the fungal alpha amylase has a level of about 400-500 skb. The skb unit expresses the alpha-amylase activity as measured by the method of Sandstedt, Kneen and Blich described in Association of American Cereal Chemists, 8th Edition, reprinted 1990, Official method 22-01, and also described in Technical Bulletin No. 1024, U.S. Department of Agriculture. One skb unit is the amount of enzyme which at 30 degrees Celsius and under the other specified reaction conditions is capable of dextrinizing one gram of soluble starch in one hour. In a preferred embodiment, the fungal alpha amylase is CLARASE 40000, which is a commercially available fungal alpha amylase manufactured by Genencor International, Inc. However, alternate fungal alpha amylases can be used at varying skb levels, as would be apparent to one skilled in the relevant art.

[0018] In a preferred embodiment, the bread conditioner of the present invention further comprises cellulase. In a preferred embodiment, cellulase can be added to the bread conditioner at a concentration of about 50-200 ppm based on wheat flour. More preferably, cellulase can be added at a concentration of about 75-150 ppm. In a most preferred

embodiment, cellulase can be added at a concentration of about 90-110 ppm. A preferred cellulase is VERON CP, which is a commercially available cellulase manufactured by Rohm GmbH. However, alternate cellulases can be used at varying concentrations, as would be apparent to one skilled in the relevant art.

[0019] In a preferred embodiment, the bread conditioner of the present invention further comprises an oxidant, such as azodicarbonamide, calcium peroxide, potassium iodate, calcium iodate, potassium bromate, or calcium bromate. Oxidants help to relax the dough. In a preferred embodiment, azodicarbonamide is added to the bread conditioner at a concentration of less than about 45 ppm based on wheat flour. In a more preferred embodiment, azodicarbonamide is added to the bread conditioner at a concentration of about 20-45 ppm. In a most preferred embodiment, azodicarbonamide is added at a concentration of about 35-45 ppm. However, various oxidants can be provided at varying concentrations, as would be apparent to one skilled in the relevant art.

[0020] In a preferred embodiment, the bread conditioner of the present invention further comprises ascorbic acid. Ascorbic acid acts as a toughener and a strengthener. In a preferred embodiment, ascorbic acid is added to the bread conditioner at a concentration of about 175-300 ppm based on wheat flour. More preferably, ascorbic acid can be added at a concentration of about 250-300 ppm. In a most preferred embodiment, ascorbic acid can be added at a concentration of about 270-290 ppm. In alternate embodiments, ascorbic acid can be provided at varying concentrations, as would be apparent to one skilled in the relevant art.

[0021] Thus, in a preferred embodiment, a bread conditioner of the present invention, which is to be added to a dough containing 250 pounds of wheat flour, can have the following composition:

| BREAD CONDITIONER                                             |                                         |
|---------------------------------------------------------------|-----------------------------------------|
| Ingredients                                                   | Percent weight of the bread conditioner |
| Isolated soy protein (dispensable ARDEX F, about 90% protein) | 96.514%                                 |
| Sodium Stearoyl lactylate (PANIPLEX SK)                       | 2.630%                                  |
| Xylanase (VERON SPECIAL)                                      | 0.980%                                  |
| Ascorbic Acid                                                 | 0.147%                                  |
| Fungal alpha amylase (CLARASE 40000)                          | 0.080%                                  |
| Cellulase (VERON CP)                                          | 0.048%                                  |
| Azodicarbonamide                                              | 0.021%                                  |

[0022] The bread conditioner of the present invention is preferably added to a dough composition which will result in a bread that contains at least 6.25 grams of soy protein per 50 gram serving of bread. This preferred bread conditioner adds about two grams of soy protein to a 50 gram serving of bread, while providing a carrier for the other ingredients in the bread dough, and further providing strength, conditioning, texture, flavor, volume, and/or freshness to the bread dough. The bread conditioner can be prepared by mixing or blending the ingredients. In alternate embodiments, the bread conditioner of the present invention can comprise further enzymes and ingredients, as would be apparent to one skilled in the relevant art.

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[0023] Another aspect of the present invention relates to a dough for making bread which provides at least 6.25 grams of soy protein per 50 gram serving of bread. In a preferred embodiment, the dough comprises the bread conditioner of the present invention. The bread conditioner of the present invention allows the dough to provide an edible and satisfactory loaf of bread while attaining 6.25 grams of soy protein per serving. In addition to the bread conditioner, the dough composition of the present invention can further comprise one or more of the following ingredients: gluten, wheat flour, soy flour, grits, soy grits, water, oil, salt, honey, calcium propionate, ELASDO (a dough improver which is commercially available from Archer Daniels Midland Company), and yeast.

[0024] As discussed above, the bread conditioner of the present invention preferably provides about two grams of soy protein per fifty gram serving of bread. In a preferred embodiment, soy flour and soy grits can be added to the dough to enable the resulting loaf of bread to attain at least 6.25 grams of soy protein per 50 gram serving.

[0025] Preferably, the bread conditioner is pre-mixed, then added to the dough composition in order to produce a loaf of bread. The handling of the dough and baking of the bread can be done in any suitable manner, as would be apparent to one skilled in the relevant art. For example, the dough can be kneaded, then subjected to one or more proofing treatments, and then baked at a suitable temperature for a sufficient period of time.

[0026] In a preferred embodiment, the bread conditioner is added to the following dough composition, which results in a loaf bread containing at least 6.25 grams of soy protein per 50 gram serving:

| BREAD COMPOSITION  |                                                                                  |
|--------------------|----------------------------------------------------------------------------------|
| Ingredients        | Weight in pounds                                                                 |
| Flour              | 250                                                                              |
| Soy Flour          | 41                                                                               |
| Bread Conditioner  | 47.5                                                                             |
| Gluten             | 30                                                                               |
| Soy grits          | 75                                                                               |
| Water              | 400                                                                              |
|                    | (300 lbs added initially, 25 lbs added later, plus 75 lbs soaked with the grits) |
| Oil                | 8                                                                                |
| Salt               | 8                                                                                |
| Honey              | 30                                                                               |
| Calcium Propionate | 2                                                                                |
| ELASDO             | 2                                                                                |
| Yeast              | 14                                                                               |

[0027] In a preferred embodiment, the soy grits are soaked for 10-15 minutes prior to being added to the mixer, and should not be oversoaked. In the above composition, 75 pounds of water are used to soak the 75 pounds of soy grits. The ingredients, without the soy grits, can be mixed at a temperature of 78-80 degrees Fahrenheit for 30 seconds on low and 11 minutes on high. In a preferred embodiment, 25 pounds of the water is held back, and added after 5 minutes of mixing. Next, the soy grits can be added, and the bread conditioner can be mixed for an additional 2 minutes on high. In a preferred embodiment, the proof time can be about 50 minutes. The proof time can be longer if less yeast is to

be used in the dough composition. The bread should be baked for a suitable time. For example, the bread can be baked at about 400 degrees Fahrenheit for about thirty minutes. In a preferred embodiment, the soy bread composition can be baked in a 3"x4"x10" pan. Alternate proof times, baking times, baking temperatures, and pan sizes can be used, as would be apparent to one skilled in the relevant art.

[0028] Further, the conditioner of the present invention can be used with other baked products such as rolls, taco shells, tortillas, cakes, breakfast rolls, muffins, pancakes, cereal, health bars, soft pretzels, and pasta.

[0029] While the invention has been particularly shown and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that they have been presented by way of example only, and not limitation, and various changes in form and details can be made therein without departing from the spirit and scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents. Additionally, all references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are each entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references.

[0030] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

What is claimed is:

1. A conditioner for bread, comprising an isolated soy protein and xylanase.
2. The conditioner of claim 1, wherein said isolated soy protein has a protein content greater than about 70 percent.
3. The conditioner of claim 1, wherein said isolated soy protein has a protein content of about 85 to about 95 percent.
4. The conditioner of claim 1, wherein said xylanase has a concentration level of about 750 ppm to about 2000 ppm.
5. The conditioner of claim 1, wherein said isolated soy protein is about 90 percent to about 98 percent by weight of the conditioner.
6. The conditioner of claim 1, wherein said isolated soy protein is about 96 percent by weight of the conditioner.
7. The conditioner of claim 1, further comprising sodium stearoyl lactylate.

8. The conditioner of claim 7, wherein said sodium stearoyl lactylate has a concentration level of about 2500 ppm to about 5000 ppm.

9. The conditioner of claim 7, further comprising fungal alpha amylase.

10. The conditioner of claim 9, wherein said fungal alpha amylase is about 200 skb to about 750 skb.

11. The conditioner of claim 9, further comprising cellulase.

12. The conditioner of claim 11, wherein said cellulase has a concentration level of about 50 ppm to about 200 ppm.

13. The conditioner of claim 11, further comprising an oxidant selected from the group consisting of azodicarbonamide, calcium peroxide, potassium iodate, calcium iodate, potassium bromate, and calcium bromate.

14. The conditioner of claim 13, wherein said oxidant is azodicarbonamide having a concentration level of about 20 to 45 ppm.

15. The conditioner of claim 13, further comprising ascorbic acid.

16. The conditioner of claim 15, wherein said ascorbic acid has a concentration level of about 175 ppm to about 300 ppm.

17. A dough for producing bread which provides at least 6.25 grams of soy protein per 50 gram serving, comprising:

wheat flour;

water; and

a bread conditioner comprising isolated soy protein.

18. The dough of claim 17, further comprising soy flour.

19. The dough of claim 18, further comprising soy grits.

20. The dough of claim 19, further comprising gluten, grits, calcium propionate, and yeast.

21. The dough of claim 17, wherein said isolated soy protein has a protein content of about 85 to about 95 percent.

22. The dough of claim 21, wherein said isolated soy protein is about 90 percent to about 98 percent by weight of the conditioner.

23. The dough of claim 21, wherein said isolated soy protein is about 96 percent by weight of the conditioner.

24. The dough of claim 21, wherein said bread conditioner is about 19 percent by weight based upon the weight of said wheat flour.

25. The dough of claim 17, wherein said bread conditioner further comprises sodium stearoyl lactylate, xylanase, ascorbic acid, fungal alpha amylase, cellulase, and azodicarbonamide.

26. The dough of claim 25, wherein said bread conditioner further comprises:

about 96 percent isolated soy protein by weight of said conditioner;

about 2500 ppm to about 5000 ppm sodium stearoyl lactylate;

about 750 ppm to about 2000 ppm xylanase;

about 175 ppm to about 300 ppm ascorbic acid;

about 200 skb to about 750 skb fungal alpha amylase;

about 50 ppm to about 200 ppm cellulase; and

about 20 ppm to about 45 ppm azodicarbonamide.

\* \* \* \* \*

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(71) Solicitante: (para todos los estados designados, excepto Estados Unidos de América): NESTEC S.A. [CH/CH]; Avenue Nestlé 55, CH-1800 Vevey, (CH).

(72) Inventores: e

(75) Inventores / Solicitantes (para los Estados Unidos de América únicamente): NICOLAS, Pierre [BE/CH]; 18, Route du Tirage, CH-1806 Saint-Légier (CH). HANSEN, Carl, Erik [NO/CH]; Ch. du Pré-d'Yverdon 5E, CH-1066 Epalinges (CH).

(74) Agente: RAULINE, Mathilde; Nestec S.A., Av. Nestlé 55, CH-1800 Vevey, (CH).

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(54) TÍTULO: "PRODUCTO ALIMENTICIO A BASE DE HARINA QUE COMPRENDE ALFA-AMILASA TERMOESTABLE"

(57) Resumen: La invención se refiere a un producto alimenticio a base de harina que comprende una  $\alpha$ -amilasa termoestable y almidón modificado in situ, así como a un proceso que comprende los pasos de elaborar una mezcla pastosa mezclando por lo menos harina, agua y una  $\alpha$ -amilasa termoestable y cocinar la mezcla pastosa sobre por lo menos una superficie caliente, al uso de  $\alpha$ -amilasa termoestable para manipular los atributos de textura de los productos alimenticios a base de harina, y a un método para modificar el almidón en un barquillo sin incrementar la viscosidad de la mezcla pastosa. Los productos alimenticios a base de harina preferidos son barquillos, galletas dulces y galletas saladas.

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(71) Applicant (for all designated States except US): NESTEC  
S.A. [CH/CH]; Av. Nestlé 55, CH-1800 Vevey (CH).

(72) Inventors; and

(73) Inventors/Applicants (for US only): NICOLAS,  
Pierre [BE/CH]; 18, Route du Tirage, CH-1806  
Saint-Légier (CH). HANSEN, Carl, Erik [NO/CH];  
Ch. du Pré-d'Yverdon 5B, CH-1066 Epalinges (CH).

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(54) Title: FLOUR BASED FOOD PRODUCT COMPRISING THERMOSTABLE ALPHA-AMYLASE

(57) Abstract: The invention relates to a flour based food product comprising a thermostable  $\alpha$ -amylase and in-situ modified starch, as well as to a process comprising the steps of making a batter by mixing at least flour, water and a thermo-resistant  $\alpha$ -amylase and cooking the batter on at least one hot surface, to the use of thermostable  $\alpha$ -amylase to manipulate textural attributes of flour based food products, and to a method for modifying starch in a wafer without increasing batter viscosity. Preferred flour based food products are wafers, biscuits and crackers.

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**CAPITULO REIVINDICATORIO COMO FUE  
ORIGINALMENTE PRESENTADO EN FASE  
INTERNACIONAL JUNTO CON SU  
TRADUCCION**

**REIVINDICACIONES**

1. Un producto alimenticio a base de harina, CARACTERIZADO porque comprende una  $\alpha$ -amilasa termoestable y almidón modificado in situ.

2. El producto alimenticio a base de harina de acuerdo con la reivindicación 1, CARACTERIZADO porque el producto alimenticio a base de harina es un barquillo, una galleta dulce o una galleta salada.

3. El producto alimenticio a base de harina de acuerdo con la reivindicación 2, CARACTERIZADO porque el barquillo es un barquillo plano, un barquillo de azúcar o un barquillo de forma tridimensional.

4. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 3, CARACTERIZADO porque la  $\alpha$ -amilasa está presente en una cantidad de 3 a 2500 unidades por gramo de la masa o mezcla pastosa final, preferiblemente 10 a 1000 unidades por gramo de mezcla pastosa.

5. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 4, CARACTERIZADO porque también comprende proteinasas y/o xilanasas.

6. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 5, CARACTERIZADO porque comprende unos agentes de desprendimiento de gas y/o unos microorganismos generadores de gas.

7. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 6, CARACTERIZADO porque el peso molecular del almidón ha sido reducido o unas dextrinas solubles han sido producidas.

8. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 7, CARACTERIZADO porque la  $\alpha$ -amilasa es de origen bacteriano, de hongos o vegetal.

9. Un proceso para elaborar un producto alimenticio a base de harina, CARACTERIZADO porque comprende los pasos de elaborar una mezcla pastosa o una masa mezclando por lo menos harina, agua y una  $\alpha$ -amilasa termoestable y cociéndola sobre por lo menos una superficie caliente.

10. El proceso de acuerdo con la reivindicación 9, CARACTERIZADO porque la  $\alpha$ -amilasa no pre-trata la mezcla pastosa o la masa.

11. El proceso de acuerdo con la reivindicación 9 o la reivindicación 10, CARACTERIZADO porque el producto alimenticio a base de harina es un barquillo.

12. El proceso de acuerdo con una de las reivindicaciones 9 a 11, CARACTERIZADO porque la mezcla pastosa o masa del producto alimenticio a base de harina comprende adicionalmente por lo menos una proteasa y/o por lo menos una xilanasa.

13. El proceso de acuerdo con una de las reivindicaciones 9 a 12, CARACTERIZADO porque la mezcla pastosa o masa comprende unos agentes de desprendimiento de gas y/o unos microorganismos generadores de gas.

14. Uso de una  $\alpha$ -amilasa termoestable para manipular los atributos de textura de productos alimenticios a base de harina seleccionados del grupo consistente en barquillos, galletas dulces y galletas saladas, CARACTERIZADO porque la  $\alpha$ -amilasa no pre-trata el producto alimenticio a base de harina.

15. El uso de una  $\alpha$ -amilasa termoestable de acuerdo con la reivindicación 14, CARACTERIZADO porque está junto con por lo menos un agente de desprendimiento de gas.

16. Un método CARACTERIZADO porque sirve para modificar el almidón en un barquillo sin aumentar la viscosidad de la mezcla pastosa.

17. El método de acuerdo con la reivindicación 16, CARACTERIZADO porque la mezcla pastosa no se pega a las placas de cocción.

18. El método de acuerdo con la reivindicación 16 o la reivindicación 17, CARACTERIZADO porque la mezcla pastosa es tratada con  $\alpha$ -amilasa termoestable.

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**Claims**

1. Flour based food product comprising a thermostable  $\alpha$ -amylase and in-situ modified starch.
- 5 2. Flour based food product according to claim 1 wherein the flour based food product is a wafer, a biscuit or a cracker.
3. Flour based food product according to claim 2 wherein the wafer is a flat wafer, a  
10 sugar wafer, or a three dimension shaped wafer.
4. Flour based food product according to one of claims 1 to 3 wherein the  $\alpha$ -amylase is present in an amount of 3 to 2500 units per gram of the final dough or batter, preferably 10 to 1000 units per gram of batter  
15 5. Wafer according to one of claims 1 to 4 also comprising proteinases and/or xylanases.
6. Flour based food product according to one of claims 1 to 5 comprising gassing  
20 agents and/or gas generating microorganisms.
7. Flour based food product according to one of claims 1 to 6 wherein the molecular weight of starch has been reduced or soluble dextrins have been produced.
- 25 8. Flour based food product according to one of claims 1 to 7 wherein the  $\alpha$ -amylase is of bacterial, fungal or plants origin.
9. Process for making flour based food product comprising the steps of making a batter or a dough by mixing at least flour, water and a thermostable  $\alpha$ -amylase and  
30 baking it on at least one hot surface.
10. Process according to claim 9, wherein the  $\alpha$ -amylase does not pre-treat the batter or the dough.
- 35 11. Process according to claim 9 or claim 10, wherein the flour based food product is a wafer.

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12. Process according to one of claims 9 to 11 wherein the flour based food product batter or dough further comprises at least one protease and/or at least one xylanase.
13. Process according to one of one of claims 9 to 12 wherein the batter or dough  
5 comprises gassing agents and/or gas generating microorganisms.
14. Use of thermostable  $\alpha$ -amylase to manipulate textural attributes of flour based food products from the group consisting of wafers, biscuits and crackers, wherein the alpha-amylase does not pre-treat the flour-based food product..  
10
15. Use of a thermostable  $\alpha$ -amylase according to claim 14 together with at least a gassing agent.
16. Method for modifying starch in a wafer without increasing batter viscosity.  
15
17. Method according to claim 16 wherein the batter is not sticking to the baking plates.
18. Method according to claim 16 or claim 17 wherein the batter is treated with  
20 thermostable  $\alpha$ -amylase.

## PATENT COOPERATION TREATY

## PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT  
(PCT Article 36 and Rule 70)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                          |                                                                                                                                                                  |  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| Applicant's or agent's file reference<br>NO 7257/WOPCT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                          | <b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEAA16)                                      |  |
| International application No.<br>PCT/EP 03/12053                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | International filing date (day/month/year)<br>30.10.2003 | Priority date (day/month/year)<br>30.10.2002                                                                                                                     |  |
| International Patent Classification (IPC) or both national classification and IPC<br>A21D804                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                          |                                                                                                                                                                  |  |
| Applicant<br>NESTEC S.A. et al.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                          |                                                                                                                                                                  |  |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 807 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>                                                                                                                                                               |                                                          |                                                                                                                                                                  |  |
| <p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p> |                                                          |                                                                                                                                                                  |  |
| Date of submission of the demand<br>02.04.2004                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                          | Date of completion of this report<br>09.02.2005                                                                                                                  |  |
| Name and mailing address of the international preliminary examining authority:<br> European Patent Office - P.B. 5816 Patentplan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tlx 31 651 epo nl<br>Fax: +31 70 340 - 3016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                          | Authorized Officer<br>Pirist-Viprey, E<br>Telephone No. +31 70 340-1039<br> |  |

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/EP 03/12053**

**I. Basis of the report**

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-12 as originally filed

**Claims, Numbers**

1-18 as originally filed

**Drawings, Sheets**

1-3 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

|                               |             |                |
|-------------------------------|-------------|----------------|
| Novelty (N)                   | Yes: Claims | 3,11,18        |
|                               | No: Claims  | 1,2,4-10,12-17 |
| Inventive step (IS)           | Yes: Claims |                |
|                               | No: Claims  | 1-18           |
| Industrial applicability (IA) | Yes: Claims | 1-18           |
|                               | No: Claims  |                |

2. Citations and explanations

see separate sheet

**Re Item V**

**Reasoned statement with regard to novelty, Inventive step or Industrial applicability; citations and explanations supporting such statement**

The following documents (D1-D5) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US-A-3026205  
D2: US-A-2615810  
D3: WO-A-9953769  
D4: DD-A-128201  
D5: Bäcker Und Konditor

1 - The application does not meet the requirements of Article 6 PCT, because claims 16 is not clear.

It is clear from the description on page that the following feature is essential to the definition of the invention: "thermostable alpha-amylase".

Since independent claim 16 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

2 - Remarks:

2.1 - Taking into account the comments of the applicant, the terms "thermostable" and "the alpha-amylase does not pre-treat the batter or the dough" are interpreted respectively as "active at a temperature of about 70 to 105°C" and "the alpha-amylase does not act before the baking step".

2.2 - D1 can be used for assessing the novelty of the claims 1-18 because none of these claims exclude an enzyme active during and after the baking step

2.3 - D2 and D3 can be used for assessing the novelty of claims 1-18 because none of these claims exclude an enzyme presenting an anti-staling effect.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP 03/12053

2.4 - Claim 2 of D3 indicates that the anti-staling amylase has optimum activity in bread at 70-90°C, this falls in the thermostable activity, see paragraph 2.1.

3 - Furthermore, the above-mentioned lack of clarity notwithstanding, the subject-matter of claims 1,2,4-10,12-17 is not new in the sense of Article 33(2) PCT, and therefore the criteria of Article 33(1) PCT are not met.

- D1 describes in col.2, l.17-37 and example VIII the production of crackers which comprises the incorporation of heat-stable alpha-amylase showing a maximum activity at temperatures of 80°C. A certain degree of activity is retained even at a temperature of 100°C. At higher temperature the enzyme is rapidly and completely inactivated.

The subject-matter of claims 1,2,4,6-10,13-15 is therefore not new.

- D2 describes in col.1, l.28-31, l.39-50, ex. I,II and cl. 1 a process of making bakery products (e.g. biscuit or cracker) which comprises incorporating in a dough a heat-stable alpha amylase. The enzyme is stable up to temperatures of 80°C to 90°C (above the starch gelatinization point)..

The subject-matter of claims 1,2,6-10,13-15 is therefore not new.

- D3 describes on p.2, l.5-28, on p.4, l.28-32 and cl. 1-4,11,16 the preparation of dough and baked products (e.g. biscuit) comprising incorporating an anti-staling amylase (heat-stable), a phospholipase and eventually additional enzymes (e.g. xylanase or protease).

The subject-matter of claims 1,2,4-10,12-15 is therefore not new.

- D4 describes in ex. 2 the preparation of wafers from a batter comprising water, alpha-amylase, protease, wheat flour, salt, sodium hydrogenocarbonate.

The subject-matter of claims 16 and 17 is therefore not new.

- D5 describes in Tab. 2 and p.23, right-hand col., par.2 the influence of addition of enzymes (alpha-amylase and protease) on the production of wafers.

The subject-matter of claims 16 and 17 is therefore not new.

4 - Dependent claims 3, 11 and 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

- In claim 3 a slight constructional change in the product of claim 1 is defined which comes within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

- In claim 11 a slight constructional change in the process of claim 9 is defined which comes within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

- In claim 18 a slight constructional change in the method of claim 16 is defined which comes within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

Consequently, the subject-matter of claims 3, 11 and 18 lacks an inventive step.

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**TRATADO DE COOPERACION DE PATENTES**  
**PCT**  
**REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL**

(PCT Artículo 36 y Regla 70)

|                                                                                               |                                                                                                                                  |                                                |
|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Registro de referencia del solicitante o agente<br>NO 7267/WO/PCT                             | <b>PARA FUTURAS ACCIONES</b> Ver Notificación o Transmisión del Reporte de Examen Preliminar Internacional (Formato PCT/IPEA416) |                                                |
| Solicitud Internacional No.<br>PCT/EP03/12053                                                 | Fecha de registro internacional (día/mes/año)<br>30.10.2003                                                                      | Fecha de prioridad (día/mes/año)<br>30.10.2002 |
| Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC<br><br>A21D 8/04 |                                                                                                                                  |                                                |
| Solicitante<br><br>NESTEC S.A.                                                                |                                                                                                                                  |                                                |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>1. Este reporte de examen preliminar internacional ha sido preparado por esta Autoridad Examinadora Preliminar Internacional y se transmite al solicitante de conformidad con el Artículo 36.</p> <p>2. Este REPORTE consta de un total de 6 páginas, incluyendo la carátula.</p> <p><input type="checkbox"/> Este reporte también está acompañado de ANEXOS, es decir, páginas de descripción, reivindicaciones y/o dibujos que han sido modificados y son la base de este informe y/o páginas que contienen rectificaciones hechas ante esta Autoridad (ver Regla 70.16 y la Sección 607 de las Instrucciones Administrativas de conformidad con el PCT).</p> <p>Estos anexos constan de un total de páginas.</p>                                                                                                                                                              |
| <p>3. Este informe contiene indicaciones relacionadas con los siguientes ítems:</p> <p>I <input checked="" type="checkbox"/> Bases del Reporte</p> <p>II <input type="checkbox"/> Prioridad</p> <p>III <input type="checkbox"/> No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial.</p> <p>IV <input type="checkbox"/> Falta de unidad de invención</p> <p>V <input checked="" type="checkbox"/> Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación.</p> <p>VI <input type="checkbox"/> Ciertos documentos citados</p> <p>VII <input type="checkbox"/> Ciertos defectos en la solicitud internacional</p> <p>VIII <input type="checkbox"/> Ciertas observaciones sobre la solicitud internacional</p> |

|                                                                                                                                                                                                                                |                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Fecha de presentación de la demanda<br>02.04.2004                                                                                                                                                                              | Fecha de terminación de este reporte<br>09.02.2005                                |
| Nombre y dirección de la autoridad internacional de examen preliminar:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk- Pays Bas<br>Tel. +31 70 340 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Funcionario autorizado<br><br>Piret-Viprey, E<br><br>Teléfono No. +31 70 340-1039 |

Forma PCT/IPEA/409 (carátula) (Enero 2004)

**REPORTE DE EXAMEN PRELIMINAR  
INTERNACIONAL**

Solicitud Internacional No. PCT/EP03/12053

**I. Bases del Reporte**

1. Respecto a los elementos de la solicitud internacional: (Páginas de remplazo que han sido suministradas a la oficina receptora en respuesta a una invitación bajo el artículo 14 están referenciados en este reporte como "presentados originalmente" y no están anexos a este reporte dado que no contienen modificaciones (Reglas 70.16 y 70.17):

Descripción, Páginas

1-12 como se presentaron originalmente

Reivindicaciones, Números

1-18 como se presentaron originalmente

Dibujos, Hojas

1-3 como se presentaron originalmente

2. Respecto al idioma, todos los elementos marcados anteriormente estuvieron disponibles o fueron suministrados a esta Autoridad en el idioma en el cual la solicitud internacional fue presentada, a menos que se indique lo contrario bajo este ítem.

Estos elementos estuvieron disponibles o fueron suministrados a esta Autoridad en el idioma, el cual es:

- ☐ el idioma de una traducción suministrada para una búsqueda internacional (según la Regla 23.1 (b)).  
☐ el idioma de una publicación de la solicitud internacional (según la Regla 48.3 (b)).  
☐ el idioma de la traducción suministrada para el examen preliminar internacional (según las Reglas 55.2 y/o 55.3).

3. Respecto al nucleótido y/o secuencia amino ácida revelado en la solicitud internacional, se llevó a cabo el examen preliminar internacional según el listado de secuencia:

- ☐ contenido en la solicitud internacional en forma escrita.  
☐ registrado junto con la solicitud internacional en forma legible por computador.  
☐ proporcionado subsiguientemente a esta Autoridad en forma escrita.  
☐ proporcionado subsiguientemente a esta Autoridad en forma legible por computador.  
☐ Se ha proporcionado la afirmación respecto a la cual el listado de secuencia escrito proporcionado subsiguientemente no va más allá de la revelación en la solicitud internacional según se presentó.  
☐ Se ha proporcionado la afirmación respecto a la cual la información presentada en forma legible por computador es idéntica al listado de secuencia escrita.

4. Las modificaciones han resultado en la cancelación de:

- ☐ la descripción, páginas  
☐ las reivindicaciones, Nos.  
☐ los dibujos, páginas

**REPORTE DE EXAMEN PRELIMINAR**  
**INTERNACIONAL**

Solicitud internacional No. PCT/EP 03/12053

5. ☐ Este reporte ha sido establecido como si (algunas) de las modificaciones no se hubiesen hecho, ya que se ha considerado que van más allá de la revelación según se presentó, como se indicó en Casilla Suplementaria (Regla 70.2 (c)):

(Cualquier hoja de remplazo que contenga tales modificaciones debe ser referida bajo el ítem 1 y anexada a este reporte.)

6. Observaciones adicionales, si son necesarias:

**V. Afirmación razonada bajo el Artículo 35(2) respecto a novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan dicha afirmación**

1. Afirmación

|                               |     |                                   |
|-------------------------------|-----|-----------------------------------|
| Novedad (N)                   | Sí: | Reivindicaciones 3, 11, 18        |
|                               | No: | Reivindicaciones 1,2, 4-10, 12-17 |
| Nivel inventivo (IS)          | Sí: | Reivindicaciones                  |
|                               | No: | Reivindicaciones 1-18             |
| Aplicabilidad industrial (IA) | Sí: | Reivindicaciones 1-18             |
|                               | No: | Reivindicaciones                  |

2. Citas y explicaciones  
 Ver hoja separada

**REPORTE DE EXAMEN PRELIMINAR  
INTERNACIONAL-HOJA SEPARADA**

Solicitud internacional No. PCT/EP 03/12053

**Re ítem V**

**Afirmación razonada respecto a novedad, nivel inventivo o aplicabilidad Industrial; menciones y explicaciones que sustentan dicha afirmación**

Se hace referencia a los siguientes documentos (D1-D5) en esta comunicación; se adherirá la numeración en el resto del procedimiento :

D1: US-A-3026205

D2: US-A-2615810

D3: WO-A-9953769

D4: DD-A-128201

D5: Backer Und Konditor

1 - La solicitud no cumple con los requerimientos del Artículo 6 PCT porque la reivindicación 16 no es clara.

Es claro de la descripción en la página        que la siguiente característica es esencial para la definición de la invención: "alfa-amilasa termoestable".

Puesto que la reivindicación Independiente 16 no contiene esta característica no cumple con el requerimiento establecido en el Artículo 6 PCT tomado en combinación con la Regla 6.3(b) PCT de que cualquier reivindicación Independiente debe contener todas las características técnicas esenciales para la definición de la invención.

**2 – Comentarios:**

2.1 – Teniendo en cuenta los comentarios del solicitante, los términos "termoestable" y "la alfa-amilasa no pre-trata la mezcla o masa" son interpretados respectivamente como "activos a una temperatura de cerca de 70 a 105°C" y "la alfa-amilasa no actúa antes del paso de hornear".

2.2 – D1 puede usarse para evaluar la novedad de las reivindicaciones 1-18 porque ninguna de estas reivindicaciones excluye una enzima activa durante y después del paso de hornear.

2.3 – D2 y D3 pueden usarse para evaluar la novedad de las reivindicaciones 1-18 porque ninguna de estas reivindicaciones excluye una enzima que presenta un efecto anti-afteamiento.

2.4 – La reivindicación 2 de D3 indica que la amilasa anti-afiejamiento tiene una actividad óptima en el pan a 70-90°; esto cae dentro de la actividad termoestable, ver el párrafo 2.1.

3 – Además, no obstante la falta de claridad anteriormente mencionada, la materia objeto de las reivindicaciones 1,2,4-10, 12-17 no es novedosa en el sentido del Artículo 33(2) PCT, y por lo tanto los criterios del Artículo 33(1) PCT no se cumplen.

- D1 describe en la col. 2, l.17-37 y en el ejemplo VIII la producción de galletas que comprende la incorporación de la alfa-amilasa estable al calor que muestra una actividad máxima a una temperatura de 80°C. Un cierto grado de actividad es retenido aún a una temperatura de 100°C. A una temperatura superior la enzima se inactiva rápida y completamente.

La materia objeto de las reivindicaciones 1, 2, 4, 6-10, 13-15 por lo tanto no es novedosa.

- D2 describe en la col. 1, l.28-31, l.39-50, ej. I,II y cl. 1 un proceso para hacer productos de panadería/pastelería (v.g. bizcochos o galletas) que comprende incorporar en una masa una alfa-amilasa estable al calor. La enzima es estable hasta temperaturas de 80°C a 90°C (por encima del punto de gelatinización del almidón).

La materia objeto de las reivindicaciones 1, 2, 6-10, 13-15 por lo tanto no es novedosa.

- D3 describe en la p. 2, l.5-28, en la p. 4, l.28-32 y cl. 1-4, 11, 16 la preparación de la masa y productos horneados (v.g. bizcochos) que comprenden la incorporación de una amilasa anti-afiejamiento (estable al calor), una fosfolipasa y eventualmente enzimas adicionales (v.g. xilanasa o proteasa).

La materia objeto de las reivindicaciones 1, 2, 4-10, 12-15 por lo tanto no es novedosa.

- D4 describe en el ej. 2 la preparación de obleas de una masa que comprende agua, alfa-amilasa, proteasa, harina de trigo, sal, hidrogenocarbonato de sodio.

La materia objeto de las reivindicaciones 16 y 17 por lo tanto no es novedosa.

- D5 describe en la Tabla 2 y en la p. 23, columna de la derecha, par. 2 la influencia de la adición de enzimas (alfa-amilasa y proteasa) en la producción de obleas.

La materia objeto de las reivindicaciones 16 y 17 por lo tanto no es novedosa.

4 – Las reivindicaciones dependientes 3, 11 y 18 no contienen ninguna característica que, en combinación con las características de cualquier reivindicación a la que se refieren, cumpla con los requerimientos del PCT respecto a paso inventivo, siendo las razones las siguientes:

- En la reivindicación 3 se define un sutil cambio estructural en el producto de la reivindicación 1 el cual está dentro del alcance de la práctica acostumbrada seguida por personas expertas en la técnica, especialmente puesto que las ventajas así logradas pueden predecirse fácilmente.
- En la reivindicación 11 se define un sutil cambio estructural en el proceso de la reivindicación 9 el cual está dentro del alcance de la práctica acostumbrada seguida por personas expertas en la técnica, especialmente puesto que las ventajas así logradas pueden predecirse fácilmente.
- En la reivindicación 18 se define un sutil cambio estructural en el método de la reivindicación 16 el cual está dentro del alcance de la práctica acostumbrada seguida por personas expertas en la técnica, especialmente puesto que las ventajas así logradas pueden predecirse fácilmente.

Por consiguiente, la materia objeto de las reivindicaciones 3, 11 y 18 carece de nivel inventivo.

## **ANEXO EXPLICATIVO DE MODIFICACIONES A LAS REIVINDICACIONES**

En ejercicio del derecho conferido por el Artículo 28 numeral 1 del Tratado de Cooperación en materia de Patentes PCT, atentamente me permito informar a ese Despacho que el capítulo reivindicatorio tal como fue presentado originalmente en Fase Internacional ha sido modificado.

Las modificaciones realizadas se encuentran plenamente sustentadas en la descripción de la presente Invención y en este sentido, no configuran una ampliación del invento o de la divulgación contenida en la solicitud presentada.

**PRODUCTO ALIMENTICIO A BASE DE HARINA QUE COMPRENDE ALFA-  
AMILASA TERMOESTABLE**

**RESUMEN-OBJETO Y FINALIDAD DE LA INVENCION**

La invención se refiere a un producto alimenticio a base de harina que comprende una  $\alpha$ -amilasa termoestable y almidón modificado in situ, así como a un proceso que comprende los pasos de elaborar una mezcla pastosa mezclando por lo menos harina, agua y una  $\alpha$ -amilasa termoestable y cocinar la mezcla pastosa sobre por lo menos una superficie caliente, al uso de  $\alpha$ -amilasa termoestable para manipular los atributos de textura de los productos alimenticios a base de harina, y a un método para modificar el almidón en un barquillo sin incrementar la viscosidad de la mezcla pastosa. Los productos alimenticios a base de harina preferidos son barquillos, galletas dulces y galletas saladas.

## MEMORIA DESCRIPTIVA

La presente invención se refiere a un barquillo producido usando una  $\alpha$ -amilasa termoestable en el paso de cocción al horno, así como a un proceso para manipular los atributos de textura de los barquillos, usando tales  $\alpha$ -amilasas durante el paso de cocción, y al uso de tal  $\alpha$ -amilasa para manipular las propiedades de textura de los barquillos.

## ANTECEDENTES DE LA INVENCION

Las  $\alpha$ -amilasas son muy conocidas en el arte y son usadas en muchos campos, especialmente en la cocción al horno de pan pero también en repostería. Algunas patentes se refieren al uso de  $\alpha$ -amilasa para retardar el envejecimiento de panes y productos de pastelería. Ése es el caso, por ejemplo, de la Patente de EE.UU. 2.615.810 de Irwin Stone, la cual describe que, con la adición de pequeñas cantidades de enzima amilolítica estable al calor a la masa antes de la cocción al horno, es posible prevenir, inhibir o retardar la tasa de envejecimiento del pan. Esta enzima estable al calor puede ser obtenida a partir de fuentes bacterianas y se caracteriza por un fuerte poder de licuación y dextrinización de almidón, actividad de  $\alpha$ -amilasa y actividad proteolítica, y es estable a temperaturas de 80°C a 90°C. El proceso contra el envejecimiento también es

aplicable, de acuerdo con esa patente, a las tortas y otros productos de pastelería.

En una referencia bastante similar, WO 2001/011975 se refiere a la producción de productos horneados con rancidez retardada incorporando a la masa unas partículas de  $\alpha$ -amilasa recubiertas con lipasa para protección y liberación prolongada, y la patente de EE.UU. 5.059.430 de Enzyme Bio Systems Ltd. protege una composición para retardar la rancidez de productos horneados, que comprende una mezcla de  $\alpha$ -amilasas microbianas y bacterianas.

En una referencia diferente, la patente de EE.UU. 4.299.848 de Deutt Itt describe la desactivación selectiva de proteasa en la  $\alpha$ -amilasa comercial, controlando el calentamiento de una solución acuosa con amortiguador; ésta es usada como un preservativo del pan.

También se conoce el uso de enzimas en la elaboración de barquillos, el uso de amilasas en la industria del horneado y el uso de  $\alpha$ -amilasa bacteriana termoestable en la industria del almidón y en la industria alimenticia.

La elaboración de barquillos usa enzimas, preferiblemente endo-proteinasas (tales como proteinasa bacteriana neutra de *Bacillus subtilis* o papaína de *Carica papaya*), para hidrolizar los enlaces de péptido en el gluten de trigo, que tienen el efecto de prevenir la formación de terrones de gluten, y también xilanasa (pentosanasa) para hidrolizar el esqueleto de xilano en arabinoxilano (pentosan), que tiene el efecto de dismi-

nuir la capacidad de adhesión de agua de los pentosanos del trigo y redistribuir el agua entre otros componentes de trigo. Unas combinaciones de estas enzimas son también usadas, principalmente para disminuir la viscosidad de la mezcla pastosa, hacer que las mezclas pastosas sean más homogéneas, aumentar la capacidad de trabajado, permitir que unos grados de harina estándar sean empleados y/o aumentar el nivel de harina en la mezcla pastosa. Estas preparaciones han sido ampliamente aceptadas (*Food Marketing & Technology*, Abril de 1994, p. 14).

La industria de la cocción al horno también usa enzimas, en particular  $\alpha$ -amilasas de hongos en la elaboración del pan y en la industria de las galletas. Las  $\alpha$ -amilasas de hongos en la elaboración del pan convierten el almidón dañado en maltodextrinas (convertidas subsiguientemente en maltosa por la beta-amilasa y dióxido de carbono por la levadura), liberan agua de los gránulos de almidón dañado y degradan una parte del almidón gelatinizado durante las fases tempranas del horneado. Los efectos de estas acciones consisten en un aumento del volumen de la hogaza mejorando la capacidad de retención de gas, una textura mejorada, una estructura de miga más uniforme, una corteza más oscura, una suavidad mejorada de la miga y un retraso en el envejecimiento. Usualmente se prefieren las  $\alpha$ -amilasas debido a que presentan una baja termoestabilidad y por lo tanto son desactivadas por el calor al final del proceso de horneado.

La  $\alpha$ -amilasa en la industria de las galletas actúa liberando agua de los gránulos de almidón dañado y también proporcionando un sustrato para las levaduras para generar dióxido de carbono (por ejemplo galletas de primera calidad). Los principales efectos son una prevención del efecto de cuadrículado (grietas durante el secado) así como un efecto de fermentación y desarrollo del sabor (galletas de primera calidad).

WO 0239820 de The Pillsbury Company está dirigida al uso de hidrolizados de almidón de alto peso molecular o formadores de hidratos cristalinos (tales como maltosa, isomaltosa, trehalosa, lactosa y raffinosa) en productos alimenticios, tales como productos de pastelería, en donde se desea una consistencia crujiente en un ambiente de alta humedad.

Las  $\alpha$ -amilasas bacterianas termoestables son usadas en la industria del almidón; de hecho, las endoamilasas hidrolizan los enlaces 1,4- $\alpha$ -glucosídicos en amilosa y amilopectina; en consecuencia, el almidón es desintegrado a dextrinas solubles. Esto es mayormente usado en la licuación continua de almidón a altas temperaturas (cocinas a chorro de vapor o equipos similares, por ejemplo).

Las  $\alpha$ -amilasas termoestables son también usadas en la industria alimenticia, usualmente para la remoción de neblinas de almidón desde jarabes, la licuación de almidón para fabricación de cerveza y fermentaciones de alcohol o la producción de cereales precocidos.

## DESCRIPCION DE LA INVENCION

Se ha descubierto ahora que las  $\alpha$ -amilasas termoestables tienen la capacidad de proporcionar a los barquillos nuevas texturas diferentes mediante modificación en situ del almidón de harina. Por consiguiente, la presente invención también se refiere a un proceso para manipular los atributos de textura de barquillos usando  $\alpha$ -amilasas termoestables, y al uso de las  $\alpha$ -amilasas termoestables para manipular los atributos de textura de los barquillos.

## BREVE DESCRIPCION DE LAS FIGURAS

La Fig. 1 muestra unas secciones transversales de los barquillos obtenidas con cantidades variables de  $\alpha$ -amilasa termoestable en la preparación de la mezcla pastosa ( $\alpha$ -amilasa: % en mezcla pastosa).

La Fig. 2 presenta un cromatograma de las maltodextrinas producidas en barquillos de acuerdo con el nivel de  $\alpha$ -amilasa añadido a la mezcla pastosa. El número de residuos de glucosa en cada oligosacárido es indicado a la derecha.

La Fig. 3 muestra unos gráficos de tiempos de siseo medidos versus las concentraciones de enzima.

## DESCRIPCION DETALLADA DE LA INVENCION

En la presente descripción, la frase "producto alimenticio a base de harina" debe ser entendida como un producto tal como un barquillo, un galleta dulce o una galleta salada,

entre otros, que tiene una humedad al final del paso de horneado que no excede el 6%. El producto alimenticio a base de harina preferido de acuerdo con la invención es un barquillo.

La frase "producto alimenticio" está destinada a abarcar cualquier materia consumible. Por lo tanto, puede ser un producto destinado al consumo por seres humanos, pero la frase también abarca productos a ser consumidos por animales, por ejemplo mascotas, tales como perros, gatos, conejos, cobayos, ratones, ratas, aves (por ejemplo loros), reptiles y peces (por ejemplo carpas doradas). Sin embargo, el término también incluye alimentos a ser consumidos por otros animales domésticos, tales como ganado, por ejemplo vacas, caballos, cochinos, ovejas, cabras, búfalos, camellos y similares.

La elaboración de barquillos consiste en preparar una mezcla pastosa que contiene principalmente harina y agua, a la cual otros ingredientes menores pueden ser añadidos. Típicamente se usa 40 a 50% de harina en la mezcla pastosa para la elaboración de barquillos planos comerciales. Las formulaciones comunes pueden comprender también por lo menos uno de los siguientes ingredientes: grasa y/o aceite, lecitina y/o emulsores, azúcar, huevos enteros, sal, bicarbonato de sodio, bicarbonato de amonio, polvo de leche descremada, harina de soya, levadura, y/o enzimas tales como xilanasas o proteasas, por ejemplo. En la elaboración del barquillo, después de la preparación la mezcla pastosa es usualmente cocida entre dos placas metálicas grabadas calentadas, por un tiempo determinado a una

cierta temperatura, por ejemplo 2 minutos a 160°C, para producir grandes láminas de barquillos planos con un bajo nivel de humedad. Después del enfriamiento los barquillos son procesados de acuerdo con los requerimientos del producto final.

La textura del barquillo resulta de la generación de celdas de gas en una estructura de gel principalmente compuesta por almidón gelatinizado. La alta temperatura de las placas de horneado induce una rápida gelatinización de los gránulos de almidón presentes en la harina y producción y expansión de las burbujas de gas en el interior de la matriz gelatinosa. Estas celdas de gas son, en la práctica común, principalmente generadas a partir de agentes de desprendimiento de gas tales como bicarbonato añadido o dióxido de carbono producido por la levadura durante la fermentación de la mezcla pastosa y a partir del vapor producido por el calentamiento. Por lo tanto el barquillo puede ser visto como una espuma sólida de celdas de gas contenidas por paredes de celdas de almidón gelatinizado y secado.

La presente invención describe un proceso enzimático capaz de producir barquillos de diferentes texturas y propiedades, adecuados para ser usados tal cual o para su incorporación en productos alimenticios, productos de repostería y otros productos a base de azúcar. Tal como se describió anteriormente, una mezcla pastosa comprende usualmente alrededor de 40-50% de harina, por ejemplo harina de trigo, la cual en sí misma contiene aproximadamente 70% de almidón que ocurre principalmente

en forma de gránulos. Este almidón no puede ser modificado por enzimas amilolíticas antes de la gelatinización, un proceso que involucra la disolución de gránulos de almidón por calentamiento. Sin embargo, la gelatinización del almidón de harina en el tanque de mezcla pastosa por tratamiento térmico e hidrólisis enzimática subsiguiente no es factible ya que causa un aumento dramático en la viscosidad y hace que la mezcla pastosa ya no pueda ser bombeada y trabajada.

En consecuencia, un aspecto de la invención se refiere a la modificación enzimática de almidón gelatinizado en el paso de cocción al horno para crear nuevas texturas de barquillos "a petición". Por consiguiente, se usan  $\alpha$ -amilasas termoestables para reducir el peso molecular del almidón. La enzima usada es preferiblemente de origen bacteriano, es mayormente activa a un pH de 5 a 7 y a una temperatura de aproximadamente 70 a 105°C. Por ejemplo, la enzima puede ser producida a partir de las especies *Bacillus* o cualquier otra bacteria que tenga una actividad de  $\alpha$ -amilasa. Eventualmente, la enzima podría ser una  $\alpha$ -amilasa de hongo, una  $\alpha$ -amilasa de origen vegetal, especialmente de cereal. De una manera general, las  $\alpha$ -amilasas existentes que exhiben una estabilidad térmica relativamente baja, siendo desactivadas rápidamente a más de 65°C, no son contempladas para llevar a cabo esta invención.

Aunque no se desea la limitación de la teoría, se cree que la adición de  $\alpha$ -amilasas a los barquillos y el uso de las mismas en el procesamiento de los barquillos induce dos

efectos principales que afectarán la textura del barquillo. En primer lugar, la enzima inducirá una disminución en la viscosidad del almidón en el paso de cocción al horno, conduciendo a una modificación en la expansión y el tamaño de las burbujas de gas. En segundo lugar, la enzima modificará la estructura macromolecular del almidón conduciendo a una modificación de las propiedades físicas en las paredes de celda sólidas en el barquillo secado.

Las enzimas amilolíticas pueden solamente atacar el almidón de manera eficiente si los gránulos de almidón han comenzado un proceso de gelatinización que ocurre a unas temperaturas superiores a 50-60°C. De acuerdo con esta invención, la hidrólisis enzimática es llevada a cabo justo después de la gelatinización del almidón entre las placas calientes de cocción usando una  $\alpha$ -amilasa termoestable.

Debe quedar entendido que la presente invención no se refiere en absoluto a la anti-rancidez. El proceso anti-rancidez puede ser explicado como sigue: cuando los gránulos de almidón son dispersados en agua y son totalmente gelatinizados por la cocción, la estructura cristalina y los polímeros del almidón son solubilizados. A medida que esta solución se enfría (por ejemplo, después del horneado del pan), los polímeros formarán una estructura parcialmente cristalina (retrogradación = recristalización). Las amilasas son a menudo usadas para prevenir la rancidez (retrogradación) en sistemas de alta humedad, tales como el pan.

En un barquillo horneado, la movilidad del barquillo no es suficiente para causar retrogradación. Así, la invención no se refiere a la anti-rancidez.

La  $\alpha$ -amilasa es preferiblemente añadida a la mezcla pastosa al mismo tiempo que los otros ingredientes, y se deja que hidrolice el almidón en el horno a una temperatura de aproximadamente 100°C durante el período de tiempo correspondiente a la evaporación del agua. La enzima es entonces desactivada progresivamente a las temperaturas superiores alcanzadas en la fase de secado. La  $\alpha$ -amilasa puede ser también añadida a la mezcla pastosa justo antes de la etapa de cocción al horno, ya que la enzima hidrolizará el almidón solamente en el horno. Este enfoque elimina el riesgo de posible desactivación de la  $\alpha$ -amilasa por ataque proteolítico en las mezclas pastosas que contienen proteasas.

Al ajustar el nivel de  $\alpha$ -amilasa así como la cantidad de agentes de desprendimiento de gas, es posible crear diferentes texturas de barquillo adaptadas a varios productos alimenticios. Además la descomposición enzimática del almidón, al aumentar el nivel de azúcares de reducción, facilita las reacciones de tostado y tiene un impacto positivo sobre el sabor de los productos finales (reacciones de Maillard).

El barquillo obtenido puede ser presentado al consumidor como un barquillo propiamente dicho, pero también puede ser procesado adicionalmente. Por ejemplo, es posible elaborar un emparedado compuesto por capas externas de barquillos que

enmarcan diferentes rellenos, por ejemplo chocolate, mermelada, chocolate compuesto, helado, sorbete, pasta de nueces, producto a base de crema, torta, mousse, caramelo blando, chocolate praliné, jalea, reelaborado de barquillo o una combinación de estos ingredientes, con o sin inclusiones del mismo ingrediente en un estado diferente, o de un ingrediente diferente. El emparedado también puede ser una sucesión de pares de barquillo y relleno, la primera y la última capa siendo barquillo, que comprende desde 2 hasta 15 capas de barquillo.

También es posible usar el barquillo como el centro o una parte del centro de un producto recubierto. El recubrimiento puede ser cualquiera de los recubrimientos usuales, por ejemplo un recubrimiento de chocolate, un recubrimiento de mezcla, un recubrimiento de caramelo blando o un recubrimiento de migajas.

Si el producto alimenticio a base de harina es un barquillo, puede ser un barquillo plano que tiene formas geométricas o formas con personajes de dibujos animados, así como letras del alfabeto o números, por ejemplo. También puede ser un barquillo de forma tridimensional tal como un cono, un vaso, un plato o un barquillo de azúcar, por ejemplo. Si el producto alimenticio a base de harina es una galleta dulce, puede comprender inclusiones de azúcar, chocolate, caramelo blando, frutas, nueces o similares. Si el producto alimenticio a base de harina es una galleta salada, dicha galleta puede comprender queso, pasta de tomate, jamón, hierbas y/o sabores de especias,

por ejemplo, y puede ser conformada como un cuadrado, un triángulo, un disco, un personaje o una cara de dibujos animados, o cualquier otra forma adecuada.

El proceso de acuerdo con la invención comprende los pasos de preparar una mezcla de por lo menos harina, agua y una  $\alpha$ -amilasa termoestable, y cocer el producto en un horno o sobre o entre placas de cocción. En una realización, cuando el producto alimenticio a base de harina es un barquillo, el tiempo de cocción puede estar comprendido entre 60 y 180 segundos, preferiblemente 90 a 120 segundos. La temperatura de las placas está preferiblemente comprendida entre 140 y 180°C al comienzo y al final del proceso, preferiblemente 160°C, y la variación de temperaturas, de acuerdo con las realizaciones preferidas de la invención, no debe ser inferior a 25°C ni superior a 50°C.

También se ha encontrado de manera sorprendente que la descomposición enzimática de almidón operada por la  $\alpha$ -amilasa aumenta el nivel de azúcares de reducción y así facilita las reacciones de tostado junto con un impacto positivo sobre el sabor de los productos finales. Mientras más  $\alpha$ -amilasa esté presente en la mezcla pastosa, más rápidamente se logrará el tostado. En consecuencia, el proceso de acuerdo con la invención también permite un tostado más rápido del barquillo.

También se ha encontrado que hay una relación entre el nivel de  $\alpha$ -amilasa incorporada a la mezcla pastosa y el tiempo de siseo durante la cocción al horno. El tiempo de si-

seo es definido como el período de tiempo correspondiente al ruido audible producido por liberación de gas y vapor de agua al comienzo de la fase de cocción. Por consiguiente, una mezcla pastosa que contiene  $\alpha$ -amilasa termoestable tendrá un tiempo de siseo reducido, indicando que la liberación de gas y vapor de agua desde el barquillo durante la cocción es facilitada. En consecuencia, se logra más rápidamente un tostado preciso.

El producto de acuerdo con la invención es un barquillo que contiene por lo menos harina, agua y es tratado con una  $\alpha$ -amilasa termoestable. Preferiblemente, es preparado usando agentes de desprendimiento de gas tales como dióxido de carbono, bicarbonato de sodio, bicarbonato de amonio o componentes de bajo punto de ebullición capaces de crear estructuras de celda de gas. Los agentes de desprendimiento de gas pueden ser reemplazados por, o añadidos junto con, microorganismos generadores de gas, tales como bacterias o levaduras. La  $\alpha$ -amilasa termoestable puede ser incorporada a la mezcla pastosa en niveles de 3 a 2500 unidades por gramo de mezcla pastosa, preferiblemente 10 a 1000 unidades por gramo de mezcla pastosa de acuerdo con la estructura deseada en el producto final.

Las unidades usadas en la presente son Unidades Wohlgemuth Modificadas (MWU por sus siglas en inglés), definidas como la cantidad de enzima que dextriniza un miligramo de almidón soluble a un valor de azul definido en 30 minutos bajo las

condiciones de ensayo establecidas por Valley Research Inc., South Bend, EE.UU.

En otra realización, el barquillo de acuerdo con la invención es también preparado a partir de una mezcla pastosa tratada con proteasas, tales como endo-proteinasas, o xilanasas (pentosanasas), o una combinación de estos tipos de enzimas. Las proteinasas son usadas para prevenir la formación de terrores, y cualquier otro tipo de enzima conocido en la industria de la cocción al horno que tenga el mismo propósito puede ser usado indiferentemente en combinación con las enzimas antes citadas o como reemplazo de las mismas. Preferiblemente, la  $\alpha$ -amilasa es incorporada a la mezcla pastosa justo antes de la cocción, por ejemplo 60 segundos antes de la cocción, con el fin de prevenir que la  $\alpha$ -amilasa sea dañada por la proteinasa.

El barquillo de acuerdo con la invención presenta un perfil de cromatografía particular (véase la Fig. 2). De hecho, la  $\alpha$ -amilasa reduce el peso molecular del almidón liberando dextrinas solubles. Por consiguiente, el barquillo de acuerdo con la invención puede ser caracterizado por su perfil de maltodextrina, ninguna maltodextrina libre siendo añadida a la preparación. Estos oligosacáridos pueden ser analizados midiendo el nivel de azúcares de reducción en el producto final o por técnicas cromatográficas (TLC, HPLC) o electroforesis (FACE). La degradación del almidón puede ser estimada por filtración de gel.

El proceso de acuerdo con la invención consiste en mezclar conjuntamente por lo menos harina, agua y una  $\alpha$ -amilasa termoestable para elaborar una mezcla pastosa que será entonces sometida a cocción. En una realización preferida, la mezcla pastosa es cocida entre dos placas de cocción por aproximadamente 120 segundos pero, en otras realizaciones consideradas, una masa puede ser preparada y cocida parcial o totalmente sobre una superficie caliente o en un horno, por ejemplo.

Aunque no se desea la limitación de la teoría, se cree que una explicación de la invención puede ser la siguiente: cuando la mezcla pastosa se pone en contacto con una o más superficies de cocción calientes, forma una capa muy delgada de almidón gelatinizado (piel) no degradado por la  $\alpha$ -amilasa ya que la enzima en contacto íntimo con las placas de cocción calientes es inmediatamente desactivada. El almidón gelatinizado en la parte interna del barquillo permanece por mayor tiempo a una menor temperatura, lo cual favorece la hidrólisis enzimática. El almidón es modificado en el interior de la mezcla pastosa durante la cocción, es decir, es modificado in situ. El hecho de que las maltodextrinas, que son conocidas por sus propiedades pegajosas, no estén directamente en contacto con las superficies de cocción, explica por qué los barquillos o galletas de acuerdo con la invención pueden ser cocidos sin pegarse a pesar de un alto contenido de maltodextrinas. Al final del tiempo de cocción, casi toda, o toda, la  $\alpha$ -amilasa presente en el barquillo ha sido desactivada. Dicha desactivación es lo-

grada por la alta temperatura en el interior de la mezcla pastosa, que puede llegar hasta 160°C, por ejemplo.

El proceso de acuerdo con la invención puede comprender el paso de añadir proteasas, tales como endo-proteinasas, o xilanasas (pentosanasas) o una combinación de estos tipos de enzimas, a la mezcla pastosa. Las proteinasas son usadas para prevenir la formación de terrones de gluten, y cualquier otro tipo de enzima conocido en la industria de la cocción al horno que tenga el mismo propósito puede ser usado indiferentemente en combinación con las enzimas antes citadas o como reemplazo de las mismas.

También puede comprender los pasos de añadir agentes de desprendimiento de gas tales como dióxido de carbono, bicarbonato de sodio, bicarbonato de amonio y/o microorganismos generadores de gas con el fin de tener un efecto sinérgico sobre la textura del barquillo.

Se ha encontrado de manera sorprendente una relación entre el contenido de  $\alpha$ -amilasas y el tostado del barquillo. Por consiguiente, mientras más  $\alpha$ -amilasa esté contenida en la mezcla pastosa, más rápidamente se obtendrá el tostado del barquillo.

El último aspecto de la presente invención se relaciona con el uso de  $\alpha$ -amilasa para manipular los atributos de textura de los productos a base de harina. Preferiblemente, tales productos a base de harina son productos delgados. Por consiguiente, la cantidad de  $\alpha$ -amilasa usada permite variacio-

nes en la textura del barquillo (véase la Fig. 1). Tal como se puede observar, cuando el nivel de  $\alpha$ -amilasa usada aumenta, crece el tamaño de las burbujas de aire en el barquillo. Otra modificación inducida por el tratamiento con amilasa es el cambio de textura de la pared propiamente dicha de la celda de gas debido a la reducción del peso molecular del almidón.

El uso de  $\alpha$ -amilasas de acuerdo con la invención puede ser combinado con el uso de agentes de desprendimiento de gas; de hecho, el uso combinado de estos dos ingredientes posibilita la generación de varias estructuras adaptadas a los productos deseados.

El uso de  $\alpha$ -amilasa termoestable en barquillos presenta muchos beneficios. Por consiguiente, unas texturas nuevas y específicas pueden ser procesadas a petición, variando la cantidad de  $\alpha$ -amilasa termoestable en la mezcla pastosa. Otro aspecto positivo de la invención reside en el hecho de que, de manera sorprendente y contrariamente a las expectativas de aquéllos versados en el arte, la viscosidad de la mezcla pastosa que comprende  $\alpha$ -amilasa termoestable no es aumentada, y la mezcla pastosa sigue siendo fácilmente trabajable. Adicionalmente, la pegajosidad de la mezcla pastosa no cambia y la mezcla pastosa de acuerdo con la invención no se pega a las placas de cocción.

De acuerdo con el último aspecto de la invención, se proporciona un método para modificar el almidón en un barquillo sin aumentar la viscosidad de la mezcla pastosa y en el cual la

mezcla pastosa no se pega a las placas de cocción, por tratamiento de dicha mezcla pastosa con una  $\alpha$ -amilasa termoestable.

### Ejemplos

Los siguientes ejemplos son ilustrativos de algunos de los productos y métodos para su elaboración, que caen dentro del alcance de la presente invención. No deben ser considerados en modo alguno como limitativos de la invención. Unos cambios y modificaciones pueden ser efectuados con respecto a la invención. Es decir, aquéllos versados en el arte reconocerán muchas variaciones en estos ejemplos para cubrir un amplio grupo de fórmulas, ingredientes, procesamientos y mezclas para ajustar de manera racional los niveles naturales de los compuestos de la invención para una variedad de aplicaciones.

Ejemplo 1: Ilustración de las modificaciones en una estructura de barquillo de acuerdo con la cantidad de enzima usada en la preparación de la mezcla pastosa

Una mezcla pastosa que comprende 780 g de harina de trigo, 720 g de agua e ingredientes menores es tratada por 30 minutos a 35°C con una mezcla comercial de enzimas que contiene proteasa y xilanasa para obtener la viscosidad deseada. 2 g de bicarbonato de sodio son añadidos a la mezcla. Unas cantidades variables de  $\alpha$ -amilasa termoestable, Validase H 340L de Valley Research que contiene 340.000 Unidades Wohlgemuth Modificadas (MWU), son añadidas a las muestras de mezcla pastosa 1 minuto

antes de la cocción. Las concentraciones finales de enzima en las mezclas pastosas son 0,0016, 0,00625, 0,025, 0,05 y 0,1%. Las mezclas pastosas son cocidas por 2 minutos en un horno con placas calentadas hasta 160°C.

Las microfotografías en la Fig. 1 muestran unas secciones transversales de los barquillos obtenidas en consecuencia. La expansión de las celdas de gas es bien visible, principalmente en el caso de altos niveles de  $\alpha$ -amilasa, indicando que unos cambios estructurales suaves o extensos pueden ser generados aplicando los conceptos descritos en la presente invención.

Ejemplo 2: Modificaciones del almidón en los barquillos, inducidas por el tratamiento con  $\alpha$ -amilasa

Una mezcla pastosa fue preparada mezclando 720 g de harina de trigo, 780 g de agua, ingredientes menores y 2 g de bicarbonato de sodio. Varias cantidades de la  $\alpha$ -amilasa descrita en el ejemplo 1 son añadidas a unas alícuotas para obtener unas mezclas pastosas que contienen 0,00078, 0,0016, 0,0031, 0,00625, 0,0125, 0,025, 0,05, 0,1, 0,2, 0,4% de enzima. Un minuto después de la adición, las mezclas pastosas son cocidas por 2 minutos en un horno con placas calentadas hasta 157°C. Los barquillos son molidos y extraídos en 7 mM de carbonato de sodio a 40°C por 10 minutos. Los extractos son centrifugados y los sobrenadantes son analizados por cromatografía de capa delgada.

Los resultados pueden ser observados en la Fig. 2, que muestra que el almidón presente en la harina puede ser modificado de maneras diferentes de acuerdo con el nivel de enzima, una alta cantidad de maltodextrinas correspondiendo a un alto grado de hidrólisis del almidón.

Por consiguiente, es posible producir un amplio grupo de perfiles de degradación de almidón variando la concentración de la enzima, y por lo tanto es posible obtener diferentes texturas de barquillo.

#### Ejemplo 3: Tiempo de siseo

Otra forma de detectar la modificación del almidón durante la cocción al horno es la medición de los tiempos de siseo. Un tiempo de siseo largo corresponde a un almidón gelatinizado de alta viscosidad, que ofrece una resistencia relativamente alta al desprendimiento de gas. En cambio la hidrólisis enzimática, al disminuir el peso molecular del almidón y por lo tanto la viscosidad, facilita el escape del gas. En consecuencia se observan unos tiempo de siseo más cortos.

La Fig. 3 muestra los resultados de este experimento. Los datos fueron recogidos durante la cocción de las mezclas pastosas mencionadas en el ejemplo 2.

#### Ejemplo 4: Análisis sensorial de los barquillos

Una mezcla pastosa que comprende 780 g de harina de trigo, 730 g de agua e ingredientes menores es tratada por 30

minutos a 35°C con una mezcla comercial de enzimas que contiene proteasa y xilanasa para obtener una viscosidad adecuada. 2 g de bicarbonato de sodio son añadidos a la mezcla. La  $\alpha$ -amilasa termoestable descrita en el ejemplo 1 es añadida en el primer experimento en un nivel de 0,25 g/kg de mezcla pastosa, en el segundo experimento en un nivel de 0,5 g/kg de mezcla pastosa y las mezclas pastosas son cocidas por 2 minutos en un horno con placas calentadas hasta 160°. También se preparan unos barquillos de referencia sin  $\alpha$ -amilasa.

Unos datos sensoriales descriptivos sobre la textura fueron recopilados a partir de un grupo de 8 evaluadores entrenados.

*Resultados del primer experimento:* Una dureza y una condición quebradiza aumentadas fueron percibidas por la mayoría de los evaluadores. El derretimiento en la boca también fue mencionado como un cambio principal, resultando en un barquillo con propiedades de textura nuevas y agradables.

*Resultados del segundo experimento:* Un aumento significativo en la dureza y la condición crujiente fue reportado por los evaluadores, mientras que las puntuaciones para el atributo de fragilidad fueron similares a aquéllas obtenidas con los barquillos de referencia.

#### Ejemplo 5: Galleta laminada

Una masa es preparada añadiendo 319,5 g de harina a una mezcla que comprende 110 g de agua, 40 g de aceite de pal-

ma, 25 g de sucrosa, 3,75 g de cloruro de sodio, 2,85 mg de bicarbonato de sodio y 250 mg de Validase BAA (Valley Research, Inc., EE.UU., 1.200.000 MWU/g). Una masa de referencia es preparada usando la misma formulación pero sin adición de enzima. Las masas son mezcladas por 120 segundos en una mezcladora Hobart N-50 con la velocidad puesta en la posición 1 y entonces se dejan reposar por 2 horas.

Las masas son laminadas en un laminador vertical con la brecha entre rodillos ajustada en la posición más corta (0,098 pulgada). Las láminas resultantes son cortadas en pedazos rectangulares (6,0 x 3,5 cm) y las galletas son cocidas por 7'30" a 230°C en un horno giratorio.

Unos datos sensoriales fueron recopilados de un grupo de 8 evaluadores entrenados. Todos los evaluadores pudieron distinguir las diferencias físicas y sensoriales entre las galletas. Las galletas modificadas con enzima fueron clasificadas como más duras y más crujientes que las galletas estándar. También se encontró que estaban más tostadas que las galletas estándar y también se mencionó un aumento en la condición dulce. Además los evaluadores también indicaron que la sensación harinosa y pegajosa desagradable en las galletas estándar desapareció en las galletas tratadas con  $\alpha$ -amilasa termoestable.

## REIVINDICACIONES

1. Un producto alimenticio a base de harina, CARACTERIZADO porque comprende una  $\alpha$ -amilasa termoestable y almidón modificado in situ.

2. El producto alimenticio a base de harina de acuerdo con la reivindicación 1, CARACTERIZADO porque el producto alimenticio a base de harina es un barquillo, una galleta dulce o una galleta salada.

3. El producto alimenticio a base de harina de acuerdo con la reivindicación 2, CARACTERIZADO porque el barquillo es un barquillo plano, un barquillo de azúcar o un barquillo de forma tridimensional.

4. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 3, CARACTERIZADO porque la  $\alpha$ -amilasa está presente en una cantidad de 3 a 2500 unidades

por gramo de la masa o mezcla pastosa final, preferiblemente 10 a 1000 unidades por gramo de mezcla pastosa.

5. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 4, CARACTERIZADO porque también comprende proteinasas y/o xilanasas.

6. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 5, CARACTERIZADO porque comprende unos agentes de desprendimiento de gas y/o unos microorganismos generadores de gas.

7. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 6, CARACTERIZADO porque el peso molecular del almidón ha sido reducido o unas dextrinas solubles han sido producidas.

8. El producto alimenticio a base de harina de acuerdo con una de las reivindicaciones 1 a 7, CARACTERIZADO porque la  $\alpha$ -amilasa es de origen bacteriano, de hongos o vegetal.

9. Un proceso para elaborar un producto alimenticio a base de harina, CARACTERIZADO porque comprende los pasos de elaborar una mezcla pastosa o una masa mezclando por lo menos harina, agua y una  $\alpha$ -amilasa termoestable y cociéndola sobre por lo menos una superficie caliente.

10. El proceso de acuerdo con la reivindicación 9, CARACTERIZADO porque la  $\alpha$ -amilasa no pre-trata la mezcla pastosa o la masa.

11. El proceso de acuerdo con la reivindicación 9 o la reivindicación 10, CARACTERIZADO porque el producto alimenticio a base de harina es un barquillo.

12. El proceso de acuerdo con una de las reivindicaciones 9 a 11, CARACTERIZADO porque la mezcla pastosa o masa del producto alimenticio a base de harina comprende adicionalmente por lo menos una proteasa y/o por lo menos una xilanasas.

13. El proceso de acuerdo con una de las reivindicaciones 9 a 12, CARACTERIZADO porque la mezcla pastosa o masa

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comprende unos agentes de desprendimiento de gas y/o unos microorganismos generadores de gas.

14. Un método CARACTERIZADO porque sirve para modificar el almidón en un barquillo sin aumentar la viscosidad de la mezcla pastosa.

15. El método de acuerdo con la reivindicación 14, CARACTERIZADO porque la mezcla pastosa no se pega a las placas de cocción.

16. El método de acuerdo con la reivindicación 14 o la reivindicación 15, CARACTERIZADO porque la mezcla pastosa es tratada con  $\alpha$ -amilasa termoestable.

Fig. 1

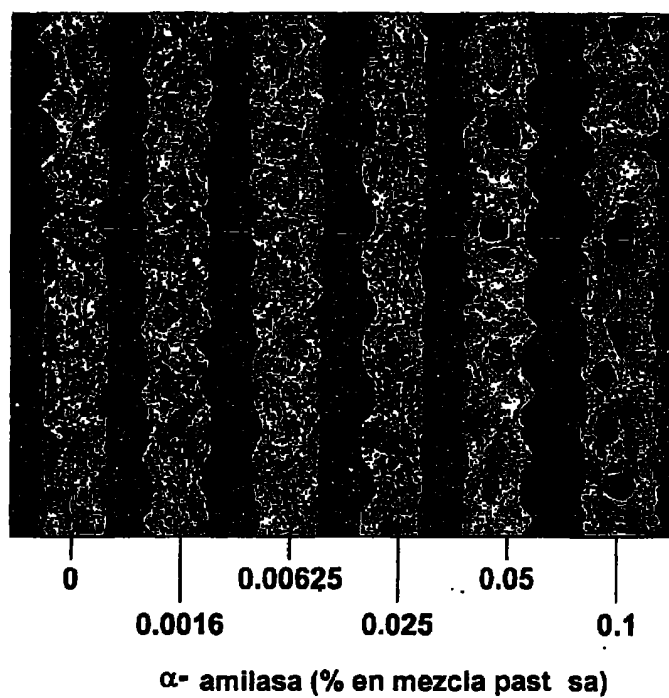
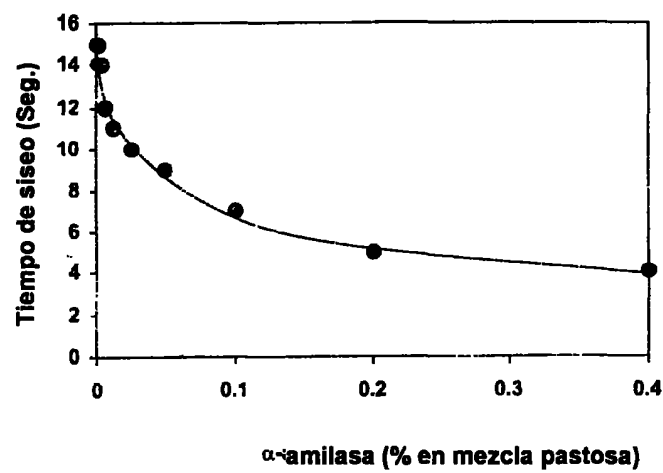




Fig. 3



TRADUCCIÓN OFICIAL No. 089/05 DE UN DOCUMENTO ESCRITO EN FRANCÉS, EL CUAL PARA SU IDENTIFICACIÓN SE SELLA CON EL SELLO DEL TRADUCTOR. ESTA ES UNA TRADUCCIÓN OFICIAL EFECTUADA EL DIA 15 DE MARZO DE 2005 POR JEAN-JACQUES TURPIN, C.E.: 193849 DE BOGOTÁ, TRADUCTOR E INTÉRPRETE OFICIAL SEGÚN RESOLUCIÓN No. 2680/91 DEL 19 DE DICIEMBRE DE 1991, EXPEDIDA POR EL MINISTERIO DE JUSTICIA DE COLOMBIA.

Autenticaciones que se hallan a continuación de un documento de traspaso conferido en inglés y español por (1) NICOLAS Pierre y (2) HANSEN Carl Erik, domiciliados respectivamente en (1) 18, route du Tirage, CH-1806 SAINT-LEGIER, de nacionalidad belga, de profesión ingeniero químico y (2) Chemin du Pré-d'Yverdon 5E, CH-1066 EPALINGES, de nacionalidad noruega, de profesión bioquímico, únicos y verdaderos inventores de **"PRODUCTO ALIMENTICIO A BASE DE HARINA QUE COMPRENDE UNA ALFA-AMILASA TERMOESTABLE"**. Así mismo, declaran ceder y transferir sin reserva ni limitación dicha invención a favor de Nestec S.A., una compañía organizada y existente de conformidad con las leyes de Suiza, domiciliada en Avenue Nestlé 55, CH-1800 Vevey, Suiza.

Firmas ilegibles de NICOLAS Pierre y HANSEN Carl Eric.

Legalización No. 4610, por parte de la Señora Brigitte FAHRNI CHIUSANO, Notaria en Vevey para el Cantón de Vaud, Suiza, de las firmas del Señor NICOLAS Pierre domiciliado en St-Légier, Cantón de Vaud y del Señor HANSEN Carl Erik domiciliado en Épalinges, Cantón de Vaud, quienes son personalmente conocidos del suscrito Notario.

Dado en Vevey, el primero de marzo de dos mil cinco. Firma ilegible del Notario.

Sello: Brigitte FAHRNI CHIUSANO, Notaria - Estampilla por valor de 2 francos.

**APOSTILLA**

**(Convención de La Haya del 5 de octubre de 1961)**

1. - País: Suiza

El presente documento público

2. - ha sido firmado por Brigitte Fahmi Chiusano,

3. - obrando en calidad de Notario en Vevey

4. - con sello de Brigitte Fahmi Chiusano, Notario

**CERTIFICADO**

5. - En Lausana

6.- El 02 de marzo de 2005

7. - Por la Cancillería del Estado del Cantón de Vaud

8. - Bajo el número 12387

9. - Sello: Cancillería del Consejo de Estado

10. - Firma por el Canciller de Estado de P. Equey

Es traducción fiel y completa de la cual se deja copia en los archivos de esta oficina para confrontación y a la cual me remito.

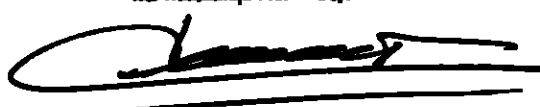
Traducción oficial No. 089/05.

Traductor: Jean-Jacques Turpin.

Bogotá, D.C., 15 de marzo de 2005.

Traducción Oficial No.  
JEAN-JACQUES H. TURPIN  
Traductor e Interpreté Oficial  
FRANCO : ESPANOL  
Res. Ministerio No. 2480/91

089/05



MINISTERIO DE  
RELACIONES EXTERIORES

338220

*Jean Turpin*  
COTA FIRMA APARECE EN EL  
DOCUMENTO DESEMPENA  
LAS FUNCIONES INDICADAS

NO SE ASUME  
RESPONSABILIDAD DEL TEXTO

2005 MAR 16 A 10:58

*mas*  
JEFE DE LEGALIZACIONES  
PROBIA LLC

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## ASSIGNMENT

The undersign, (Inventors' details and signature :  
bottom of page)

hereby state under oath to be sole and true  
inventor of

**FLOUR BASED FOOD PRODUCT  
COMPRISING THERMOSTABLE ALPHA-  
AMYLASE**

state as well that assign, without  
reservations or limitation, in favor of

**Nestec S.A., a Swiss Body Corporate of  
Avenue Nestlé 55  
1800 Vevey, Switzerland**

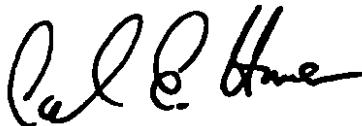
all rights inherent to said invention, and that the  
above mentioned corporation hereby is enabled  
to apply, in its name, for the corresponding  
patent in the Republic of Colombia.

### Inventors' details and signature(s)

**NICOLAS Pierre**  
18, Route du Tirage  
CH-1808 SAINT-LEGER  
Citizen of BELGIUM  
Chemical Engineer



**HANSEN Carl Erik**  
Ch. du Pré-d'Yverdon 5E  
CH-1068 EPALINGES  
Citizen of NORWAY  
Biochemist



## TRASPASO

abajo firmado

(Detalles y firma de los inventores al final de la  
página)

declarar bajo juramento ser único  
y verdadero inventor de

**PRODUCTO ALIMENTICIO A BASE DE  
HARINA QUE COMPRENDE ALFA-AMILASA  
TERMOESTABLE**

Declarar asimismo que ced y transf  
sin reserva ni limitación, a favor de la sociedad

**Nestec S.A.,**  
organizada y existente de conformidad con las  
leyes de **Suiza**

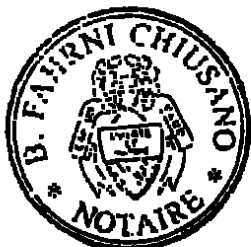
domiciliada en **Avenue Nestlé 55 1800 Vevey, Suiza**  
todos los derechos inherentes a dicha invención  
y que la citada sociedad queda facultada para  
solicitar en su nombre la patente correspondiente  
en la República de Colombia.

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Légalisation No 4'610.-

Sur la base d'une comparaison de signature, la soussignée Brigitte FAHRNI CHIUSANO, Notaire à Vevey pour le Canton de Vaud, Suisse, -- atteste l'authenticité de la signature apposée d'autre part par -- M. PierresNPCORAS, domicilié à St-Légier, Canton de Vaud et par -- M. Carl Erik HANSEN, domicilié à Epalinges, Canton de Vaud, les--- quels sont personnellement connus du notaire soussigné. -----

VEVEY, LE PREMIER MARS DEUX MILLE CINQ. -----



*Bahli -t.*

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Pays: SUISSE

Le présent acte public

2. a été signé par Brigitte Fahrni Chiusano

3. agissant en qualité de notaire à Vevey

4. est revêtu du sceau/timbre de B. Fahrni Chiusano  
notaire

Attesté

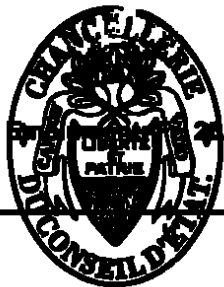
5. à Lausanne

6. le 2 mars 2005

7. par la Chancellerie d'Etat du Canton de Vaud

8. sous No 12387

9. Sceau/timbre:



10. Signature

pr le Chancelier d'Etat:

*P. Equey*  
P.Equey



**REQUISITOS MINIMOS PARA ADM  
PCT**

SUPERINDUSTRIA Y COMERCIO  
Radicacion : 05039405 00000000 Folios: 118  
Fecha (ANO): 2005-04-26 16:04:21  
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTA  
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

PATENTE DE INVENCION [ X ]

MODELO DE UTILIDAD [ ]

- ♦ Indicación de que se solicita la concesión de una patente [ X ] 1
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud [ X ] 1
- ♦ Descripción de la invención [ X ]
- ♦ Dibujos de ser estos pertinentes [ ]
- ♦ Comprobante de pago de las tasas establecidas [ X ] 2

COMPLETA [ X ]

INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- ♦ Indicación de que se solicita el registro de Diseño Industrial [ ]
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- ♦ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- ♦ Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ]

INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- ♦ Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- ♦ Representación gráfica del esquema trazado [ ]
- ♦ Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ]

INCOMPLETA [ ]

  
Firma Responsable

Fecha \_\_\_\_\_

Carrera 13 No. 27-00 Piso 5, 7 y 10  
Conmutador: 3 82 08 40  
Fax: 350 52 20  
E-mail: [info@sic.gov.co](mailto:info@sic.gov.co)  
[www.sic.gov.co](http://www.sic.gov.co)  
Bogotá, D.C., Colombia

2020  
Bogotá DC

Doctora  
**ALICIA LLOREDA RICAURTE**  
Apoderado  
**NESTEC S.A.**

|                    |                            |                   |
|--------------------|----------------------------|-------------------|
| <b>REFERENCIA:</b> | Radicación No.             | 05-39485          |
|                    | Solicitud Internacional    | PCT/EP2003/012053 |
|                    | Fecha inicio fase nacional | 31-05-2005        |
|                    | Trámite                    | 011               |
|                    | Evento                     | 379               |
|                    | Actuación                  | 416               |
|                    | Oficio No.                 | 490               |
|                    | Folios                     | 001               |

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en la Decisión 486 de la Comisión de la Comunidad Andina, el Tratado de Cooperación en Materia de Patentes (PCT), el Reglamento y la Circular Única de la Superintendencia de Industria y Comercio, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial.

Cúmplase  
Dado en Bogotá DC

19 JAN. 2005

  
**ALIX CARMENZA CÉSPEDES DE VERGEL**  
Jefe de la División de Nuevas Creaciones

202030



**Industria y Comercio**  
**SUPERINTENDENCIA**

## DIVISION DE NUEVAS CREACIONES

**RADICACIÓN EXPEDIENTE No. 05-039485**

**EL EXTRACTO DE LA PRESENTE SOLICITUD DE PATENTE DE INVENCION**

**PCT FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No 555**

**DE FECHA 31 DE AGOSTO DEL 2005, EL TERMINO HABIL SEÑALADO POR**

**LA LEY EXPIRA EL 28 DE NOVIEMBRE DEL 2005**

**NOTA:** Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

**SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.**

SI

NO      **X**     

**Bogotá D.C., Noviembre de 2008**

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# SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: S-39485

EDICTO No. 20472

## LA SECRETARÍA GENERAL AD-HOC HACE SABER:

Que esta Superintendencia profirió Resolución No. 33870 de 11-DIC-2006. Que el contenido de la parte resolutive es como sigue: El Superintendente de Industria y Comercio.

### RESUELVE

**ARTICULO PRIMERO:** Declarar abandonada la solicitud de privilegio de patente de invención que entró en fase nacional en virtud del Tratado de Cooperación en Materia de Patentes (PCT), de acuerdo con las razones expuestas en la parte considerativa de la presente resolución.

**ARTICULO SEGUNDO:** Notificar personalmente el contenido de la presente resolución a la

Rad. Exp. N° 05-39485

doctora Alicia Lloreda Ricaurte, en su calidad de representante legal de Natzac S.A., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

### NOTIFÍQUESE Y CUMPLASE

Dada en Bogotá, D.C., a los 11 DIC. 2006

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,

JAMES RIBERO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:00 a.m. de hoy.

Secretaría General Ad-Hoc 22-DIC-2006 Este edicto se desfiló hoy 09-ENE-2007 a las 4:30 p.m.  
Secretaría General Ad-Hoc  
EDICTO No. 20472