



Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

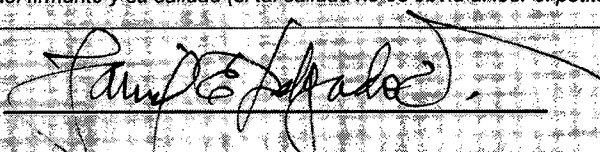


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Act. 457 PROCEDIPPH Folios: 17

DIRECCIÓN DE NUEVAS CREACIONES

PROCEDIMIENTO ACCELERADO DE EXÁMENES DE PATENTE (PPH)

T=F=17

1	NÚMERO DE SOLICITUD ANTE LA SIC	13 – 282.403
2	OFICINA DE PRIMERA PRESENTACIÓN (OPP)	ESTADOS UNIDOS DE AMERICA
3	NÚMERO(S) DE SOLICITUD O PUBLICACIÓN DE LA(S) SOLICITUD(ES) (OPP):	1. US 13/154,381 2. 3.
4	☐ REPRESENTANTE LEGAL ☒ APODERADO	
APELLIDOS DELGADO VILLEGAS		NOMBRES JAIME EDUARDO
		IDENTIFICACIÓN C.C. 19.270.955 T.P. 43.308 C.S.J.
DIRECCIÓN	Avda. Carrera 24 No. 37 - 31 Of. 202	
CIUDAD	BOGOTA D.C.	
PAÍS	COLOMBIA	
No. TELÉFONO	2444096	
CORREO ELECTRÓNICO	mde@mdelaw.com	
No. RADICACIÓN O PROTOCOLO DE PODER GENERAL		
5	CONDICIONES PARA ACCEDER A ESTE PROCESO:	
5.1.	DATOS DE PRIORIDAD ☒ SI ☐ NO	
(33) PAÍS DE ORIGEN		CÓDIGO PAÍS
1. ESTADOS UNIDOS DE AMERICA		US
2.		
		(31) NÚMERO
		2012/0309946 A1
		(32) FECHA (AAAA/MM/DD)
		06-12-2012
5.2.	DATOS SOLICITUD INTERNACIONAL PCT ☐ SI ☐ NO	
Solicitud Internacional No.		PCT/EP2012/055425
Fecha		27/03/2012
Publicación Internacional No.		WO2012/167963
Fecha		13/12/2012
El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO		
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS		
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:		
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:		
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO		
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO		
(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☒ SI ☐ NO		
(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad.		
7	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL	
Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el peticitorio)		
		

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TABLA DE CORRESPONDENCIA DE REIVINDICACIONES

REIVINDICACIONES RADICADAS EN FASE NACIONAL SOLICITUD EN COLOMBIA ORIGINALES	REIVINDICACIONES OTORGABLES EN LA SOLICITUD INTERNACIONAL US 8.592.575	REIVINDICACIONES MODIFICADAS PARA EXAMEN PATENTABILIDAD	EXPLICACIÓN SOBRE LA SUFICIENTE CORRESPONDENCIA Ó COMENTARIOS QUE EXPLICAN LA CORRESPONDENCIA
1. Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que comprende; Añadir ácido oxálico y/o oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II); calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; y separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, en el que la pectina extraída tiene un grado de esterificación (DE) de al menos 72 y un elevado grado de polimerización caracterizado por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.	1. Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que comprende: añadir ácido oxálico y/o oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel; calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; y separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 72 y un elevado grado de polimerización caracterizado por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.	1. Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que comprende: añadir ácido oxálico y/o oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel; calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; y separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 72 y un elevado grado de polimerización caracterizado por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.	La Reivindicación 1 original No incluye “en la piel” después de “molaridad total del calcio (II)”
2. El procedimiento de acuerdo con la reivindicación 2, en el que el pH de la mezcla antes de separar la pectina extraída de la mezcla está entre 3,1 y 3,4.	2. El procedimiento de acuerdo con la reivindicación 1, en el que el pH de la mezcla antes de separar la pectina extraída de la mezcla está entre 3,1 y 3,4.	2. El procedimiento de acuerdo con la reivindicación 1, en el que el pH de la mezcla antes de separar la pectina extraída de la mezcla está entre 3,1 y 3,4.	Reivindicación igual
3. El procedimiento de acuerdo con la reivindicación 2, en el que la mezcla se calienta hasta una temperatura de aproximadamente 70 a aproximadamente 80°C durante la separación de la pectina extraída.	3. El procedimiento de acuerdo con la reivindicación 2, en el que la mezcla se calienta hasta una temperatura de aproximadamente 70 a aproximadamente 80°C durante la separación de la pectina extraída.	3. El procedimiento de acuerdo con la reivindicación 2, en el que la mezcla se calienta hasta una temperatura de aproximadamente 70 a aproximadamente 80°C durante la separación de la pectina extraída.	Reivindicación igual
4. El procedimiento de acuerdo con la reivindicación 1, que comprende además filtrar la mezcla para eliminar los sólidos insolubles después de extraer la pectina y antes de separar la pectina extraída.	4. El procedimiento de acuerdo con la reivindicación 1, que comprende además filtrar la mezcla para eliminar los sólidos insolubles después de extraer la pectina y antes de separar la pectina extraída.	4. El procedimiento de acuerdo con la reivindicación 1, que comprende además filtrar la mezcla para eliminar los sólidos insolubles después de extraer la pectina y antes de separar la pectina extraída.	Reivindicación igual

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5. El procedimiento de acuerdo con la reivindicación 1, que comprende además poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio iónico antes de separar la pectina extraída.	5. El procedimiento de acuerdo con la reivindicación 1, que comprende además poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio iónico antes de separar la pectina extraída.	5. El procedimiento de acuerdo con la reivindicación 1, que comprende además poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio iónico antes de separar la pectina extraída.	Reivindicación igual
6. El procedimiento de acuerdo con la reivindicación 5, en el que la resina de intercambio iónico comprende una resina de intercambio catiónico seleccionada del grupo que consiste en que Lewatit® S1668, Lewatit® S1468, Purolite® C100E, y Amberlite® SR1L Na.	6. El procedimiento de acuerdo con la reivindicación 5, en el que la resina de intercambio iónico comprende una resina de intercambio catiónico eficaz para eliminar residuos de oxalato, residuos de calcio o combinaciones de los mismos de la pectina extraída.	6. El procedimiento de acuerdo con la reivindicación 5, en el que la resina de intercambio iónico comprende una resina de intercambio catiónico eficaz para eliminar residuos de oxalato, residuos de calcio o combinaciones de los mismos de la pectina extraída.	Reivindicación modificada
7. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.	7. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.	7. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.	Reivindicación igual
8. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.	8. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.	8. El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.	Reivindicación igual
9. El procedimiento de acuerdo con la reivindicación 1, que además comprende una o más de las etapas de lavado, prensado, desecación y molturación de la pectina extraída después de separar la pectina extraída para obtener un producto final de pectina.	9. El procedimiento de acuerdo con la reivindicación 1, que además comprende una o más de las etapas de lavado, prensado, desecación y molturación de la pectina extraída después de separar la pectina extraída para obtener un producto final de pectina.	9. El procedimiento de acuerdo con la reivindicación 1, que además comprende una o más de las etapas de lavado, prensado, desecación y molturación de la pectina extraída después de separar la pectina extraída para obtener un producto final de pectina.	Reivindicación igual
10. El procedimiento de acuerdo con la reivindicación 1, en el que el rendimiento por piel (Y_p) es superior a aproximadamente 23 %, en el que $Y_p = 0, \cdot y_s \cdot M / W_p$ e y_s es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y W_p es el peso de la piel usada en la suspensión acuosa.	10. El procedimiento de acuerdo con la reivindicación 1, en el que el rendimiento por piel (Y_p) es superior a aproximadamente 23 %, en el que $Y_p = 0,1 \cdot y_s \cdot M / W_p$ e y_s es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y W_p es el peso de la piel usada en la suspensión acuosa.	10. El procedimiento de acuerdo con la reivindicación 1, en el que el rendimiento por piel (Y_p) es superior a aproximadamente 23 %, en el que $Y_p = 0,1 \cdot y_s \cdot M / W_p$ e y_s es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y W_p es el peso de la piel usada en la suspensión acuosa.	Reivindicación modificada
11. El procedimiento de acuerdo con la reivindicación 10, en el que el producto del rendimiento por piel y la viscosidad intrínseca es superior a aproximadamente 165% dl/g.	11. El procedimiento de acuerdo con la reivindicación 10, en el que el producto del rendimiento por piel y la viscosidad intrínseca es superior a aproximadamente 165% dl/g.	11. El procedimiento de acuerdo con la reivindicación 10, en el que el producto del rendimiento por piel y la viscosidad intrínseca es superior a aproximadamente 165% dl/g.	Reivindicación igual

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12.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene un grado de esterificación (DE) de al menos 75.	12.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.	12.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.	Reivindicación igual
13.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.	13.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.	13.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.	Reivindicación igual
14.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 8,5 dl/g.	14.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 8,5 dl/g.	14.El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 8,5 dl/g.	Reivindicación igual
15. Una pectina producida por el procedimiento de la reivindicación 1, en la que la pectina tiene un grado de esterificación de al menos 75% y una viscosidad intrínseca de al menos 6,0 dl/g.	15. Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que consiste esencialmente en: añadir ácido oxálico y/o oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel; calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; opcionalmente filtrar la mezcla para eliminar los sólidos insolubles; poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio catiónico; y separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, para obtener un rendimiento por piel (Yp) es superior a aproximadamente 23 %, en el que $Yp = 0,1 \cdot yS \cdot M/WP$ e yS es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y WP es el peso de la piel usada en la suspensión acuosa; en el que la pectina extraída se tiene un grado de esterificación (GE) de al menos 72 y un grado de polimerización alto caracteriza por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.	15. Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que consiste esencialmente en: añadir ácido oxálico y/o oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel; calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; opcionalmente filtrar la mezcla para eliminar los sólidos insolubles; poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio catiónico; y separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, para obtener un rendimiento por piel (Yp) es superior a aproximadamente 23 %, en el que $Yp = 0,1 \cdot yS \cdot M/WP$ e yS es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y WP es el peso de la piel usada en la suspensión acuosa; en el que la pectina extraída se tiene un grado de esterificación (GE) de al menos 72 y un grado de polimerización alto caracteriza por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.	Reivindicación eliminada

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16. La pectina de la reivindicación 15, que tiene un grado de esterificación de al menos 76.	16. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.	16. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.	Reivindicación eliminada
17. La pectina de la reivindicación 15, que tiene un grado de esterificación de al menos 78.	17. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.	17. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.	Reivindicación eliminada
18. La pectina de la reivindicación 15, que tiene una viscosidad intrínseca de al menos 6,5 dl/g.	18. El procedimiento de la reivindicación 15, en el que el tiempo suficiente es de aproximadamente 1 hora a aproximadamente 3 horas.	18. El procedimiento de la reivindicación 15, en el que el tiempo suficiente es de aproximadamente 1 hora a aproximadamente 3 horas.	Reivindicación eliminada
19. La pectina de la reivindicación 15, que tiene una viscosidad intrínseca de al menos 7,5 dl/g.	19. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.	19. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.	Reivindicación eliminada
20. La pectina de la reivindicación 15, que tiene una viscosidad intrínseca de al menos 8,0 dl/g.	20. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.	20. El procedimiento de acuerdo con la reivindicación 15, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.	Reivindicación eliminada



OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas de los Estados Unidos
Dirección: COMISIONADO DE PATENTES
P.O Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

Nº SOLICITUD	FECHA DE EMISIÓN	PATENTE Nº	Nº DE EXPEDIENTE DEL MANDATARIO	Nº DE CONFIRMACIÓN
13/154.381	26/11/2013	8592575	10-501 (29776-0070)	1910

62488 7590 06/11/2013
CP Kelco US, INC
c/o Pete Pappas, Sutherland, Asbill & Brennan LLP
999 Peachtree Street, NE
SUITE 2300
ATLANTA, GA 30309-3996

NOTIFICACIÓN DE CONCESIÓN

El número previsto de patente y la fecha de concesión son las arriba indicadas.

Resolución sobre ajuste del plazo de protección de patente con arreglo a la U.S.C. 35 154 (b) (Solicitud presentada el día o con posterioridad al día 29 de mayo de 2000)

El ajuste del plazo de la patente es de 111 día(s). Cualquier patente que se haya de emitir a partir de la solicitud arriba identificada incluirá una indicación de dicho ajuste en su página de portada.

Si se ha presentado alguna solicitud de continuación (CPA, por sus siglas en inglés) en la solicitud arriba identificada, la fecha de presentación que determina el ajuste del plazo de la patente es la fecha de presentación del CPA más reciente.

El solicitante podrá obtener más información en detalle accediendo al sitio web PAIR de recuperación de información sobre solicitudes de patente (PAIR, *Patent Application Information Retrieval*) en <http://pair.uspto.gov>.

Cualquier pregunta relativa a la ampliación del plazo de la patente o a la resolución sobre el ajuste de plazo deberá dirigirse a la Oficina de Administración Legal de Patentes, disponible en el número: (571)-272-7702. Las preguntas relativas al abono de tarifas por la emisión y publicación de patentes deberán dirigirse a la Unidad de Ayuda a Solicitudes (AAU, *Application Assistance Unit*) de la Oficina de Gestión de Datos (ODM, *Office of Data Management*) disponible en el número: (571)-272-4200.

SOLICITANTE(S) (Por favor, consulte el sitio web PAIR <http://pair.uspto.gov> si desea añadir solicitantes adicionales):

Søren Vodstrup Jensen, Lille Skensved, DINAMARCA;
Susanne Oxenbøll Sørensen, Vallensbæk Strand, DINAMARCA;
Claus Rolin, Koege, DINAMARCA;

Los Estados Unidos representan el mercado más grande y dinámico del mundo y ofrecen una ubicación sin igual, ideal para la inversión, innovación y comercialización de nuevas tecnologías. EE.UU. ofrece impresionantes recursos y ventajas para quienes invierten y fabrican productos aquí. A través de SelectUSA, nuestra nación se esfuerza por atraer y fomentar la inversión empresarial. Si desea saber más acerca de por qué EE.UU. es el mejor país del mundo en el que desarrollar tecnología, fabricar productos y hacer crecer su negocio, visite SelectUSA.gov.

Índice de Reivindicaciones 	Nº de Solicitud/Control 13154381	Solicitante(s)/Patente sujeta a Re-examen JENSEN ET AL.
	Examinador LAYLA BLAND	Unidad de trabajo técnico 1623

✓	Rechazada
=	Autorizada

-	Cancelada
+	Restringida

N	No electa
I	Interferencia

A	Apelada
O	Objetada

☒ Las reivindicaciones reenumeradas en el mismo orden en que las presenta el solicitante ☐ CPA ☐ T. D. ☐ R.147										
REIVINDICACIÓN		FECHA								
Final	Original	19/01/2013	27/02/2013	20/08/2013						
	1	÷	✓	=						
	2	÷	✓	=						
	3	÷	✓	=						
	4	÷	✓	=						
	5	÷	✓	=						
	6	÷	✓	=						
	7	÷	✓	=						
	8	÷	✓	=						
	9	÷	✓	=						
	10	÷	✓	=						
	11	÷	✓	=						
	12	÷	✓	=						
	13	÷	✓	=						
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	15	÷	N	-						
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	20	÷	N	-						
	21			=						
	22			=						
	23			=						
	24			=						
	25			=						
	26			=						

Notificación de Autorización	Nº de Solicitud 13/154.381	Solicitante(s) JENSEN ET AL.	
	Examinador LAYLA BLAND	Unidad de trabajo técnico 1623	Status AIA (Primer inventor que registra) No

-- La FECHA DE ENVÍO de esta comunicación es la que figura en la hoja de portada junto a la dirección de correspondencia--

Siendo admisibles todas las reivindicaciones, EL EXAMEN DE MÉRITOS QUEDA (O PERMANECE) CERRADO para esta solicitud. Si no se incluyera en el presente documento (o no se hubiera remitido con anterioridad) se enviará una Notificación de autorización (PTOL-85) u otra comunicación apropiada a su debido tiempo. **LA PRESENTE NOTIFICACIÓN DE AUTORIZACIÓN NO CONSTITUYE UNA CONCESIÓN DE DERECHOS DE PATENTE.** La presente solicitud está sujeta a su retirada de la fase de emisión a criterio de la Oficina o a petición del solicitante. Véanse CFR 37, 1.313 y MPEP 1308.

- ☒ Esta comunicación responde a la enmienda efectuada por el Solicitante, remitida el 2 de agosto de 2013.
☐ Se presentaron una o más declaraciones/ declaraciones juradas, de conformidad con **CFR 37, 1.130(b)** el día _____;
- ☐ El solicitante ha elegido una opción en respuesta a un requisito de restricción fijado durante la entrevista el día _____; el requisito de restricción y su elección se incorporan a la presente instancia.
- ☒ Las reivindicaciones autorizadas son 1-14 y 21-26. Como resultado de las reivindicaciones aceptadas usted podrá optar al **Procedimiento Acelerado de Examen de Patente** en una de las oficinas de propiedad intelectual que participen en dicho programa en relación a la correspondiente solicitud. Si desea más información le remitimos a http://www.uspto.gov/patents/init_events/pph/index.jsp o envíe una consulta a PPHfeedback@uspto.gov.
- ☐ Se acusa recibo de una reivindicación de prioridad extranjera con arreglo a lo dispuesto en U.S.C. 35, apartados 119(a)-(d) o (f).

Copias certificadas:

a) ☐ Todas b) ☐ Algunas *c) ☐ Ninguna de las siguientes:

- Se han recibido ☐ copias certificadas de los documentos de prioridad.
- Se han recibido ☐ copias certificadas de los documentos de prioridad en la Solicitud n° ____.
- Se han recibido ☐ copias de las copias certificadas de los documentos de prioridad en esta solicitud de la fase nacional desde la Oficina Internacional (Norma 17.2 (a) del Tratado de Cooperación en materia de Patentes, PCT).

* Copias certificadas no recibidas: _____.

El Solicitante dispone de TRES MESES A PARTIR DE LA "FECHA DE ENVÍO" de esta comunicación para presentar una respuesta que cumpla los requisitos que figuran debajo a continuación. En caso de incumplimiento en el plazo oportuno supondrá el ABANDONO de la presente solicitud.

DICHO PERIODO DE TRES MESES NO ES PRORROGABLE.

- ☐ LOS DIBUJOS CORREGIDOS (si se necesita adjuntar hojas de sustitución) deben ser enviados.
☐ Incluye los cambios requeridos por el Examinador en la Enmienda/Comentario del Examinador adjunto o en la acción administrativa n°/de fecha de envío _____ de la Oficina.

Se deben hacer constar datos identificativos, como el número de la solicitud (véase CFR 37, 1.84 (c)) en la misma cara de los dibujos (no por detrás) de cada hoja. La(s) hoja(s) de sustitución deberán etiquetarse como tales en la cabecera con arreglo a lo dispuesto en CFR 37, 1.121 (d).

- ☐ DEPÓSITO DE y/o INFORMACIÓN acerca del depósito de MATERIAL BIOLÓGICO debe remitirse. Tenga en cuenta el comentario adjunto del Examinador en relación al requisito de DEPÓSITO DE MATERIAL BIOLÓGICO.

Adjunto(s)

- ☐ Notificación de citas de referencias (PTO-892).
- ☐ Declaraciones de divulgación de información (PTO/SB/08), N° de documento/Fecha de envío: _____.
- ☐ Comentario del examinador en relación al requisito de depósito de material biológico.
- ☐ Resumen de la entrevista (PTO-413), N° de documento/Fecha de envío: _____.
- ☐ Enmienda/comentario del examinador.
- ☒ Declaración del examinador con las razones para la autorización.
- ☐ Otros _____.

Nº de Solicitud/Control: 13/154.381

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INSTANCIA DETALLADA

Esta instancia administrativa se realiza en respuesta a las modificaciones efectuadas por el Solicitante y remitidas el 2 de agosto de 2013. Las reivindicaciones 1 y 6 se modifican; las reivindicaciones 15-20 se cancelan y las reivindicaciones 21-26 son de nuevo envío. Las reivindicaciones 1-14 y 21-26 están pendientes y se examinan conforme al examen de méritos aquí expuestos.

A la vista de la enmienda del Solicitante, el rechazo de las reivindicaciones 1-14 queda retirado.

El rechazo a las reivindicaciones 1-14 con arreglo a U.S.C. 35, 103 (a) por ser no patentable sobre la base de Joye a la vista de Christensen y Eschinasi queda retirado. Los argumentos del Solicitante son persuasivos.

De igual manera, la enmienda y los argumentos del Solicitante tal como se describen en la parte superior bastan para eliminar todas las objeciones efectuadas en la primera instancia administrativa y para situar a la solicitud en condiciones para autorizarla.

Cualquier comentario que el solicitante considere necesario deberá remitirse no más tarde de la fecha de abono de la tasa de emisión y, para evitar retrasos en la tramitación, preferentemente deberá acompañar a la tasa de emisión. Cualquiera de dichas remisiones deberá ir claramente etiquetada con la frase "Comentarios sobre la declaración de razones para la autorización."

Cualquier consulta relativa a la presente comunicación o a otras comunicaciones previas del examinador deberá remitirse a LAYLA BLAND cuyo número de teléfono es (571) 272-9572. El examinador está disponible con normalidad de lunes a viernes, de 10.00 a 18.30.

Si no logra contactar con el examinador por vía telefónica, el supervisor del examinador, Anna Jiang está disponible en el número (571) 272-0627. El número de fax asignado para este tipo de comunicaciones relativas a esta solicitud o procedimiento es el 571-273-8300.

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Unidad de trabajo técnico: 1623

Puede consultar la información relativa al estado de una solicitud en el sistema de Recuperación de Información de Solicitudes de Patente, PAIR (*Patent Application Information Retrieval*). La información acerca del estado de solicitudes publicadas puede obtenerse tanto del sistema PAIR Público como Privado. La información acerca del estado de solicitudes no publicadas únicamente está disponible en el sistema PAIR Privado. Si desea más información sobre el sistema PAIR le remitimos a <http://pair-direct.uspto.gov>. Si tiene alguna pregunta relativa al acceso al sistema PAIR Privado, contacte con el Centro de Negociado Electrónico ECB (*Electronic Business Center*) en el 866-217-9197 (gratuito). Si desea recibir ayuda de un Representante de Servicio al Cliente de la USPTO u obtener acceso al sistema de información automatizada, llame al 800-786-9199 (EN USA O CANADÁ) o al 571-272-1000.

/Layla Bland/

Examinador primario, Unidad de Trabajo Técnico 1623

N° de Serie: 13/154.381

Presentado el: 6 de junio de 2011

MODIFICACIÓN Y RESPUESTA A LA INSTANCIA ADMINISTRATIVA

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Modificaciones a las Reivindicaciones

Este listado de reivindicaciones reemplaza a todas las versiones y listados anteriores de reivindicaciones contenidas en la solicitud:

1. (Actualmente modificada) Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que comprende:

añadir ácido oxálico y/o un oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel;

calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina; y

separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla, en la que la pectina extraída tiene un grado de esterificación (GE) de al menos 72 y un elevado grado de polimerización caracterizado por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.

2. (Original) El procedimiento de acuerdo con la reivindicación 1, en el que el pH de la mezcla antes de separar la pectina extraída de la mezcla está entre 3,1 y 3,4.

3. (Original) El procedimiento de acuerdo con la reivindicación 2, en el que la mezcla se calienta hasta una temperatura de aproximadamente 70 a aproximadamente 80°C durante la separación de la pectina extraída.

4. (Original) El procedimiento de acuerdo con la reivindicación 1, que comprende además filtrar la mezcla para eliminar los sólidos insolubles después de extraer la pectina y antes de separar la pectina extraída.

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5. (Original) El procedimiento de acuerdo con la reivindicación 1, que comprende además poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio iónico antes de separar la pectina extraída.

6. (Actualmente modificada) El procedimiento de acuerdo con la reivindicación 5, en el que la resina de intercambio iónico comprende una resina de intercambio catiónico eficaz para eliminar residuos de oxalato, residuos de calcio o combinaciones de los mismos de la pectina extraída ~~seleccionado del grupo consistente en Lewatit® S1668, en Lewatit® S1468, Purolite® C100E y Amberlite® SR1L Na.~~

7. (Original) El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.

8. (Original) El procedimiento de acuerdo con la reivindicación 5, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.

9. (Original) El procedimiento de acuerdo con la reivindicación 1, que además comprende una o más de las etapas de lavado, prensado, desecación y molturación de la pectina extraída después de separar la pectina extraída para obtener un producto final de pectina.

10. (Original) El procedimiento de acuerdo con la reivindicación 1, en el que el rendimiento por piel (Y_p) es superior a aproximadamente 23 %, en el que $Y_p = 0,1 \cdot y_s \cdot M/W_p$ e y_s es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y W_p es el peso de la piel usada en la suspensión acuosa.

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MODIFICACIÓN Y RESPUESTA A LA INSTANCIA ADMINISTRATIVA

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11. (Original) El procedimiento de acuerdo con la reivindicación 10, en el que el producto del rendimiento por piel y la viscosidad intrínseca es superior a aproximadamente 165% dl/g.

12. (Original) El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.

13. (Original) El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.

14. (Original) El procedimiento de acuerdo con la reivindicación 1, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 8,5 dl/g.

15-20. (Canceladas)

21. (Nueva) Un procedimiento para extraer pectina que tiene un grado elevado de polimerización, que comprende principalmente:

añadir ácido oxálico y/o un oxalato hidrosoluble a una suspensión acuosa de una piel en una cantidad suficiente para proporcionar una mezcla que tenga un pH de entre 3,0 y 3,6 y una molaridad total de oxalato superior a la molaridad total del calcio (II) en la piel;

calentar la mezcla hasta una temperatura de aproximadamente 50 a aproximadamente 80°C durante un tiempo suficiente para extraer una pectina;

opcionalmente filtrar la mezcla para eliminar los sólidos insolubles;

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poner en contacto la mezcla que contiene la pectina extraída con una resina de intercambio catiónico; y

separar la pectina extraída de la mezcla precipitando la pectina extraída de la mezcla para obtener un rendimiento por piel (Y_p) superior a aproximadamente 23 %, en el que $Y_p = 0,1 \cdot y_s \cdot M/W_p$ e y_s es el rendimiento de la solución (extracto líquido) en g/kg, M es la masa total de la mezcla antes de separar la pectina extraída, y W_p es el peso de la piel usada en la suspensión acuosa.

en la que la pectina extraída tiene un grado de esterificación (GE) de al menos 72 y un elevado grado de polimerización caracterizado por una viscosidad intrínseca superior a aproximadamente 6,5 dl/g.

22. (Nueva) El procedimiento de acuerdo con la reivindicación 21, en el que la pectina extraída tiene un contenido en oxalato total inferior a 2,0 mg por 1 g de pectina.

23. (Nueva) El procedimiento de acuerdo con la reivindicación 21, en el que la pectina extraída tiene un contenido en calcio total inferior a 1 mg /g.

24. (Nueva) El procedimiento de acuerdo con la reivindicación 21, en el que el tiempo suficiente oscila entre aproximadamente 1 hora y aproximadamente 3 horas.

25. (Nueva) El procedimiento de acuerdo con la reivindicación 21, en el que la pectina extraída tiene un grado de esterificación (GE) de al menos 75.

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26. (Nueva) El procedimiento de acuerdo con la reivindicación 21, en el que la pectina extraída tiene una viscosidad intrínseca (VI) de al menos 7,8 dl/g.



US008592575B2

(12) **United States Patent**
Jensen et al.

(10) **Patent No.:** **US 8,592,575 B2**

(45) **Date of Patent:** **Nov. 26, 2013**

(54) **PROCESS FOR EXTRACTION OF PECTIN**

(75) **Inventors:** **Søren Vodstrup Jensen**, Lille Skensved (DK); **Susanne Oxenbøll Sørensen**, Vallensbæk Strand (DK); **Claus Rolin**, Koege (DK)

(73) **Assignee:** **CP Kelco ApS (DK)**

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 111 days.

(21) **Appl. No.:** **13/154,381**

(22) **Filed:** **Jun. 6, 2011**

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(51) **Int. Cl.**
C07H 1/06 (2006.01)
C07H 1/08 (2006.01)

(52) **U.S. Cl.**
USPC **536/128**

(58) **Field of Classification Search**
None

See application file for complete search history.

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Primary Examiner — Layla Bland

(74) *Attorney, Agent, or Firm* — Sutherland, Asbill & Brennan LLP

(57) **ABSTRACT**

Embodiments of the processes provided herein allow for extraction of high quality pectins from pectin-containing plant materials using oxalic acid for extraction of the pectin containing material. Generally described, the process for extracting pectin having a high degree of polymerization includes preparing an aqueous suspension of a pectin-containing plant material; adding oxalic acid and/or a water-soluble oxalate to the aqueous suspension in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium(II); heating the mixture to a temperature from about 50 to about 80° C. for a time sufficient to extract pectin from the pectin-containing plant material; and separating the extracted pectin from the mixture. The extracted pectin desirably is characterized as having a degree of esterification (DE) of at least 72 and a high degree of polymerization, the degree of polymerization being characterized by an intrinsic viscosity of greater than about 6.5 dL/g.

20 Claims, 7 Drawing Sheets

FC Industria y Comercio SUPERINTENDENCIA	PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA RELACIÓN DE ANEXOS		
Dependencia:	Fecha: <u>14</u> / <u>8</u> / <u>20</u> AAAA MM DD	Recorrido: <u>4</u>	
2020			
Tipo de Radicación: Entrada: ☒ Salida: ☐ Traslado: ☐ Convenio: ☐			
Radicador:		Planilla:	

ID	No. Radicación	Consecutivo	Item
1	13-282403	05	7
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Item	Unidad de Conservación
1	Bolsa
2	Caja
3	Libro
4	Sobre
5	Tomo
6	Otro
Item	Soporte Documental
7	CD ☒
8	Disquete
9	DVD
10	Folleto
11	Foto
12	Periódico
13	Plano
14	Publicación Periódica
15	USB
16	Otro

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Claudio

Process for selective extraction of pectins from plant material by differential pH

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Abstract

Extraction of natural hydrocolloid carbohydrate polymers, such as pectin, from plant matter is accomplished at somewhat elevated temperature and controlled conditions of acidity/alkalinity. In many cases the plant material contains a variety of different extractables, including non-polymeric carbohydrates (sugars) in addition to the pectins. Very recently two different kinds of pectin, a calcium-sensitive pectin (CSP) and a non-calcium-sensitive pectin (NCSP), have become interesting commercially. What is described in this work is a process to selectively extract NCSP and CSP by varying the pH of the extracting solution. In a first extraction with acidic aqueous solution, a pH between 3.0 and 3.3 without addition of polyvalent salt is sufficient to extract NCSP pectin. A second extraction under stronger acid conditions (pH of about 2.0) is sufficient to extract the remaining pectin, which is primarily CSP. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Selective extraction; Differential pH; Calcium-sensitive pectin extraction

1. Introduction

Hydrocolloids extractable from plant materials include pectin, carrageenan, guar and other less well known and less commercially important polysaccharides. These materials are generally considered foodstuffs and are controlled in the USA by Food and Drug Administration (FDA) regulations (Food Chemicals Codex, 1981; Code of Federal Regulations, 1983). The focus of this paper is on pectin, but the process described herein is not limited to pectin and might be applicable to other hydrocolloid extraction processes.

Pectin, which is chemically similar to cellulose, may be thought of as the “glue” that holds the cellulose together in the cell walls of plants. Pectin and cellulose are both polysaccharides, but pectin is primarily α -linked polygalacturonic acid (partly esterified with methyl ester) in which rhamnose may be found, and cellulose is essentially a polymer chain made up of β -1,4 linked sucrose units with no esterification. Not all plant materials are rich in pectin. The oldest pectin source is apple pomace left over from juicing. The most common source of pectin nowadays is from citrus peel, primarily lemon—but lime, orange and grapefruit may also be used. Novel sources of pectin include sugar beets

and sunflower heads, but these are not, at the moment, commercially significant. In order to qualify as pectin, the anhydro-galacturonic acid must make up at least 65% of the ash-free dry matter in pectin sold as a commercial product.

There are many processes for extraction of pectin, and there are many uses for these products (Aspinall, 1982; Whistler & BeMiller, 1973; Yalpani, 1988). Typically, a pectin process comprises

1. aqueous extraction from plant starting material;
2. purification of the liquid extract; and
3. isolation of the extracted pectin from the liquid.

Extraction technology is being studied continually (Hwang, Kim & Kim, 1998; Minkov, Minchev & Paev, 1996; Panchev, Kirtchev & Kratchanov, 1989), because pectin is a commercially important product.

Extraction of pectin may be by aqueous acid or base. The basic extraction process yields a pectin of low degree of esterification (low DE pectin) as a result of saponification of the ester groups, whereas the acid extraction process generally yields a pectin of high degree of esterification (high DE pectin), approximately equal to the naturally occurring DE. High DE pectin has a degree of esterification of 50% or greater. Low DE and high DE pectin generally have different uses in foodstuffs, because they gel by different mechanisms. Both are sold commercially. In addition,

* Corresponding author.

certain other modifications of pectin have found commercial utility, for example, amidated pectin in which the methoxyl groups are partly replaced by amine groups.

In the acid extraction process, plant material is treated with acid at temperatures between 70 and 90°C for a time sufficient to remove desired amounts and quality of pectin from the cellulose plant material. Extract juice from the extraction step is separated from the reaction mixture by filtration. Rotary drum vacuum filtration is common in the industry because the cake is very mushy and difficult to handle. The cake is neutralized and sold as cattle feed or put through a re-extraction step to extract more pectin before being filtered and disposed of. All kinds of equipment common to the extraction arts can be used for pectin extraction—from stirred tanks or pots to continuous counter-current extractors, for example, those described by Leach, Schols, Pyle and Niranjana (1993).

Pectin is precipitated from the extract juice by specified means—either alcohol precipitation (ethyl or isopropyl can be used) or by salting out with aluminum chloride. The precipitated pectin is separated from the precipitating solution by filtration or other means; it is then washed, dried and milled to the desired particle size. During processing the pectin may undergo an ion exchange step to put it in the sodium form for ease of use in foodstuffs applications.

The extract obtained by typical commercial acid extraction is composed of those molecules that are soluble under the conditions of pH, time and temperature used during the extraction. The extract is composed of a mixture of pectins of different molecular weight and degree of esterification. Molecular weights can range from 100,000 to 200,000, but weight-average molecular weights are more typically about 140,000. Control of specific pectin properties for desired end use is achieved by selection of raw material, blending, standardizing by adding sugar or dextrose, etc.

One of the main functional variations between high DE and low DE pectins is their sensitivity to the presence of polyvalent cations such as calcium. This property of the pectin is defined as calcium sensitivity. Calcium sensitive pectins can be gelled in the presence of calcium ions without sugar and are therefore useful for low-fat or sugarless, acidic foodstuff formulations—for which there is a large market, or fat hold-out applications—for which a strong market is developing. Low DE pectins are highly calcium sensitive and are used in low sugar applications where the mix must be gelled (diet jams and jellies, for example). These pectins tend to form very firm, hard gels and are highly pH sensitive. In most commercial applications, a blend of low DE pectins is formed in order to obtain a more broadly adaptable product. High DE pectins that are calcium sensitive tend to retain more water in the gel than low DE pectins (Glahn, 1995a,b) and therefore tend to be softer and more desirable for many end use applications. Thus, the development of efficient ways of producing high DE, calcium sensitive pectins is commercially desirable.

Pectins extracted by the acid process are normally high

DE pectins and contain a mixture of both calcium sensitive (CSP) and non-calcium sensitive pectins (NCSP). Analytical and chemical methods have yet to distinguish structurally between the two types, and many theories exist; one of which is that “hairy” regions (Ros, Schols & Voragen, 1998) formed by neutral sugars along the backbone of the chain may “trap” calcium ions or hold them in position, thereby contributing to the gel structure. Another theory is that the distribution of esterified groups along the chain backbone is the critical feature. A random distribution is thought to give relatively low calcium sensitivity, but a block distribution gives much higher calcium sensitivity. The block distribution gives a charge concentration, which may hold calcium ions in place in a gel structure.

Separation of NCSP and CSP from pectin extract juice by selective precipitation is described by Glahn (1995a,b). Selective precipitation is achieved by a precipitating solution of water and alcohol that contains, additionally, a dissolved polyvalent cation, such as calcium. The nitrate salt is generally used for minimization of corrosion in the process equipment and is compatible with the extracting acid (nitric). This solution causes the calcium sensitive pectin to gel, whereas the non-calcium sensitive pectin remains dissolved. By manipulating the concentrations of alcohol and calcium salt and temperature, gel particles can be formed and then separated by screen filtration methods. This approach for producing purified CSP involves the substantial additional use of a calcium salt (for the partial gelation step) and additional separation equipment not normally used for pectin production. The ion exchange step, which is used in typical pectin production processes, is significantly enlarged by this method, because a substantial amount of calcium needs to be added; the calcium concentration in the pectin is therefore high and must be reduced in the product, which is usually the acid or the sodium form of pectin (sometimes referred to as “sodium pectate”). This process also adds significantly to the solvent (alcohol) recovery system, since it uses additional alcohol.

A very recent process disclosed in International Publication WO 9703574, now issued as a European patent (Buchholt et al., 1998) uses a recombinant enzyme to block de-esterify pectin, thereby making it calcium sensitive and useful for the aforementioned commercial applications. The pectin is used to stabilize protein suspensions, e.g. acidified yogurt drinks, whey protein drinks, etc.—a typical application for CSP pectin. This is a sophisticated biotech process, and rather than separating CSP and NCSP, it creates CSP pectin. Because of the advanced technology of this process, it may not be commercially viable at the present time.

In this work we present a process by which naturally occurring CSP and NCSP can be separated directly from the peel, in-situ, by fractional extraction with differential pH and without the addition of polyvalent cation salt. This is done by a sequential, two-step process using different pH conditions in each extraction step. In the first step, mild pH

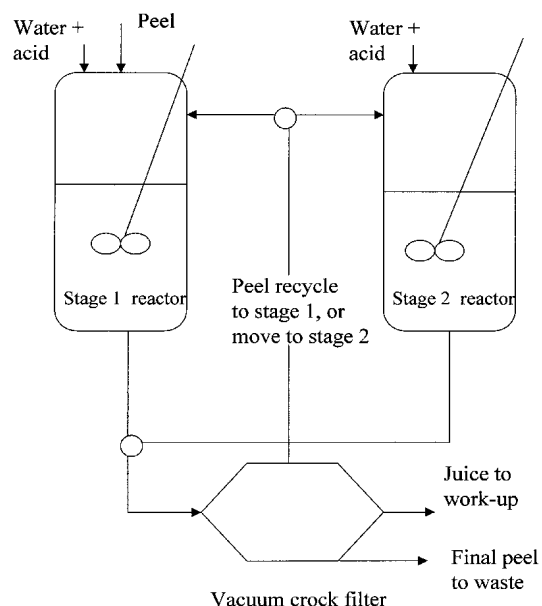


Fig. 1. Experimental apparatus.

conditions (pH of about 3.0 without addition of polyvalent salt) are used to extract pectin that turns out to be NCSP, as determined by analytical tests. In the second step, stronger acid conditions (pH of about 2.0, which is typical of normal pectin extractions) are used to extract pectin which turns out to be CSP, as determined by analytical tests. The general principal of fractional extraction is not new. Various solvents have been used in sequence to extract pectin fractions in the past (Fuchigami, 1990; Raynal, Mourges & Conte, 1991), but the novel feature of the present work is to use the same solvent at different concentration of acid to extract two kinds of pectin, NCSP and CSP.

2. Experimental

Experimental extractions were done by typical pot extraction methods using various sized glass vessels up to 12 l. Fig. 1 is a schematic of the experimental apparatus. Typical peel charges were in the range 100–400 g of dried peel (about 12% moisture) from various sources—mostly lemon peel from Argentina. This is the most popular source for peel, commercially. The extraction vessels were jacket-heated, and slow agitation was used. Temperature was measured by a temperature probe and was 70°C for all experiments. Extraction times lasted from 1 to 3 h, but after about 2 h not much additional extraction takes place. Water-to-peel ratios ranged from 20 to 30, and nitric acid was used for lowering the pH to desired extraction conditions. In some experiments calcium salt was added to inhibit extraction of CSP.

At the conclusion of the reaction step, the mixture was dumped into a large Buechner funnel or vacuum crock into which nylon filter cloth was placed. A rubber diaphragm

was used with the vacuum to press the cake. Generally, this simulated the action of a rotary drum vacuum filter typically used in pectin extractions. The filter cake typically contained about 20% solids after this treatment (4/1 liquid-to-solid ratio). In the cases where yield study was important, the cake was re-extracted in the same vessel with an appropriate amount of water and acid. In most cases, the liquid/solid ratio was between 20/1 and 30/1, as this is a typical optimal range in commercial practice. For NCSP extractions (in stage 1 of Fig. 1), the pH was in the range 3.0–3.3. The yields fell off significantly at higher pHs, and at lower pHs CSP started to be extracted. As an alternative, the pH could be lowered to about 2.5 for NCSP extraction, but addition of calcium salt in the range 1–50 mM was necessary under these conditions to prevent bleed through of CSP. In stage 2 of Fig. 1 CSP is extracted owing to a higher concentration of acid.

The extract juice was then evaporated to increase concentration to about 2% by weight. Then the liquid was ion exchanged with sodium ion exchange resin by stirring in a beaker for about 20 min. The liquid was then precipitated with isopropyl alcohol to about 50% by liquid weight. The resulting gummy mass was then dried overnight at about 50°C in a vacuum oven with nitrogen sweep. The product was then ground to size, typically 100% through an 80 US mesh screen. Samples were then analyzed for molecular weight, degree of esterification, galacturonic acid content, calcium sensitivity, and other chemical features. These tests are generally standard analytical procedures. Some further detail can be found in Joye, Luzio, Soederberg and Taytelbaum (1998).

For optimal yield, the peel could be extracted four times, two for NCSP and two for CSP. This usually results in over 90% theoretical pectin extracted. Following Fig. 1, the peel would be extracted for NCSP in the first stage, then separated in the vacuum crock filter. The juice from the filter would be worked up to recover the pectin: the peel would be returned to stage 1 for another extraction. The first procedure would be repeated, the juice going to a second work-up and the peel going to stage 2 for CSP extraction. The CSP extraction procedure would be done twice, each time the juice going to work-up. The stage-2 peel would be recycled the first time and sent to waste the second.

2.1. Calcium sensitivity test

The calcium sensitivity test has many variants. It is simply the measure of viscosity increase in a pectin solution when calcium ions are added. The specific test used here is as follows:

1. A pectin solution of about 0.6 wt% is prepared using distilled water. The pH is adjusted to 1.5 by using 1 M hydrochloric acid. The pectin must be in the acid or monovalent salt form.
2. 145 g aliquots of #1 are added to viscosity measurement vials.

Table 1
Results of fractional extraction experiments

Conditions and results	4a	5a	8a	11a	4b	4c
<i>STEP 1</i>						
Dilution/pH/time (h)	20/3.3/3	17/2.9/2	20/2.5/3	14/2.5/1	20/3.3/3	20/3.3/3
Calcium added (mM)	0	22.5	45	0	0	0
NCSP yield (g)	23.18	16.31	30.00	32.58	21.92	36.28
Molecular weight	122,000	124,000	158,000	184,000		
Calcium sensitivity (mPa s or cP)	3	5.5	2.5	200	6.5	0.5
GA	76.6	75.4	78.9	77.6	74.5	77.5
DE	74.6	76.9	76.2	74.5	77.9	78.5
Calcium content (ppm)	586	1500	4650	730		
<i>STEP 2</i>						
Dilution/pH/time (h)	17.5/2.0/0.75	17.5/1.75/1.5	17.5/1.5/0.75	17.5/1.5/0.75	17.5/2.0/0.75	17.5/2.0/0.75
CSP yield (g)	47.85	56.93	49.68	43.44	56.07	56.63
Molecular weight	149,000	174,000	177,000	195,000		
Calcium sensitivity (mPa s or cP)	871	634	542	456	556	355
GA	70.7	80.7	76.9	72.7	72.0	68.9
DE	64.8	67.4	66.3	65.9	69.7	68.1
Calcium content (ppm)	195	229	401	222		
YOG	215	202	172	169		
NCSP/CSP split (%)	33/67	22/78	38/62	43/57	22/78	39/61

3. 5 ml of a solution containing 250 mM calcium chloride is added to the 145 g pectin solution to give a final concentration of 8.3 mM calcium.
4. With stirring by a magnetic stirrer, 25 ml of an acetate buffer containing 1 M acetate ions and a pH of 4.75 is added to the pectin solution to bring the pH to 4.2. This also brings the final concentration of pectin to about 0.5 wt%.
5. The magnet is removed, and the glass is left at room temperature (18–25°C) until the next day, when the viscosity is measured at 25°C with a Brookfield viscometer.

Viscosities up to about 800 cP (or mPa s) can be measured in this way. Higher viscosities usually indicate gelification of the solution, and the results are less reliable. If the application demands this kind of performance, the concentration of pectin in the solutions is reduced. The method can be adapted to give a good relative indication of the calcium sensitivity of the samples.

When the viscosity of the same pectin samples is measured without the addition of calcium chloride—diluting with distilled water instead, a base line is established. This is termed CS[−], and the former test is CS⁺, and the difference between the two is the viscosity increase due to addition of calcium ions and is termed “delta CS.” For pectin samples of low calcium sensitivity, this difference is typically less than 20 cP. Viscosities of calcium sensitive pectins, on the other hand, are typically greater than 100 cP.

2.2. YOG test

This test measures the stabilizing power of pectin in acid

protein drinks. Solutions of pectin with different concentrations are mixed with yogurt, homogenized and heat treated at 70°C for 10 min. The amount of sediment and the viscosity are measured after cooling to 5°C. The minimum amount of pectin producing the lowest sediment and viscosity is desired. Why stabilization is at a lower viscosity and not a higher is a complex matter of colloid chemistry. Basically pectin will coat the particles up to a point, thereby stabilizing them and preventing coalescence and precipitation; any additional pectin will go to viscosify the solution and will not contribute to stabilization. Therefore, the minimum amount of pectin to stabilize is the desired end. The performance of an unknown batch is compared to that of a standard batch of pectin. This test is very complex and somewhat operator-sensitive, so the exact and lengthy details are not reported here. About 10 different concentrations of pectin are used. The milk solids content of the yogurt can vary, but 8.5% non-fat milk solids concentration was used in these tests. The sedimentation and viscosity of the solution as a function of pectin concentration are plotted for the standard and the unknown. A shift factor between the sedimentation curves and the viscosity curves is determined by a complex procedure, and the YOG grade calculated.

The standard YOG grade is 100. If all the stabilizing power of pectin comes from CSP, and if CSP is 50% of the total pectin, a pure CSP sample should give a YOG grade of 200. If the sample was not pure CSP, the YOG grade would be lower than 200. The parent pectin from which the CSP was separated can also be subjected to this test to give a measure of selectivity.

3. Results and discussion

Of the many results obtained, those given in Table 1 summarize our findings. In the original experiment there were 11 different conditions with one repeat run to make a total of 12 runs. This was done by a fractional statistical design, so that a response surface could be generated to find the significant variables for the extraction method. Time of extraction, peel source, dilution ratio in each step, and calcium level in the first extraction were thought to be significant variables as well as the pH in each step. Three different lemon peel sources from different growing sites in Argentina, designated a, b and c, were used for the data in Table 1. All runs included a short (15–20 min) re-extraction/wash step in each stage. This was done with hot water and additional acid just to maintain pH conditions. Subsequent experiments were then carried out with other peel sources, e.g. grapefruit, for comparison. We report here only the runs from the original experiments that gave significantly positive results for defining the best process conditions for the extraction. The number preceding the letter is a run number indicating type of conditions. All run 4s, for example 4a, 4b and 4c, have the same conditions. In runs 4b and 4c however, the molecular weight information was not available and the calcium content was not measured. In runs 4 and 11 no additional calcium was added to the reactor, so the calcium content of all run 4 materials should be about the same. Some calcium enters the reactor with the peel. This gives about 11 mM concentration at 25/1 dilution ratio. Increasing levels of calcium, added as calcium nitrate, are shown in runs 5 and 8. This is known to inhibit CSP extraction and may thereby increase selectivity as in Glahn (1995a,b) and Joye et al. (1998). We have only reported total weights in the table, not weight of pectin from each individual extraction step.

Runs 4 (without calcium, but at higher pH) and 5 (with calcium, but at lower pH) show similar results. Both give low calcium sensitivity numbers for the NCSP fraction, indicating pure NCSP, but give high calcium sensitivity numbers for the CSP, indicating good purity CSP and a clean split between the two. The overall yields and molecular weights of the two runs are similar, but the split is somewhat different. Run 4 gave a 1:2 NCSP/CSP split, whereas run 5 gave a 1:3 NCSP/CSP split. The CSP in run 5 is less pure than in run 4 as indicated by a lower calcium sensitivity number (871 vs. 634), thus it is likely that more NCSP is in this sample, and adding calcium may not be preferred. As a general guideline, calcium sensitivity numbers less than about 600 are less desirable as product.

Run 8a was done under more aggressive acid conditions (pH of 2.5), but increased calcium addition (45 mM added), and showed similar results to runs 4 and 5 with the “a” peel. However, the calcium sensitivity of the CSP fraction was significantly less, below 600, and the split was somewhat more evenly divided. The YOG grade was much lower than

200, indicating some impurity in the CSP. Therefore, these would not be the desired extraction conditions.

Run 11a also shows loss of YOG, though the split is good. There does not appear to be any CSP in the NCSP fraction, so the NCSP seems to be pure, but there could have been some bleed-through of the NCSP into the CSP fraction which would have lowered the YOG and the calcium sensitivity. The amount of NCSP in the extracted pectin varies with peel source (location) and other variables including time of harvest, but the expected maximum may be as high as 45% of the total pectin. Table 1 shows about 40% for the peels tested here.

The calcium sensitivity test can be used as a measure of selectivity. Run 11a done under aggressive acid conditions but no added calcium shows a split higher than 40%, but also shows a high calcium sensitivity for the NCSP fraction. This indicates bleed-through of CSP into the NCSP fraction and therefore a poor separation (low selectivity). Thus, runs 8a and 11a are not preferred conditions, whereas runs 4a and 5a are. If the extraction time for stage 1 is too long, or the pH is too low, CSP bleed-through will occur (see 11a in Table 1). Generally, stage 2 reaction times could be shorter than 2 h. We have no analytical measure to show that CSP produced in this way is “pure”. In this case, acceptable results are judged by performance in the calcium sensitivity test and by the YOG test described previously. Acceptable results are demonstrated by the data in Table 1, but the judicious use of additional calcium and its exact benefits, if any, await the results of future investigations.

Chemically, the galacturonic acid (GA) content of all extracted pectin samples in Table 1 are typical, between 70 and 80%. The degree of esterification (DE) is higher for the NCSP fraction (74–77%) than for the CSP fraction (64–68%). The reason for this is thought to be the higher acid concentration and the longer contact time of the CSP during the extraction process contributes to de-esterification. The dilution ratios are on a weight basis. The peel charge was 400 g in each experiment, thus the yields on a wt% basis can be computed, if desired. All experiments used a re-extract of about 10/1 liquid-to-wet peel ratio for each Step 1 and Step 2 reactions.

The calcium sensitivity numbers were obtained by the method discussed previously. In the test procedure the concentrations are such that the initial viscosity is very low for both NCSP and CSP samples. This can sometimes not be the case if the CSP sample already contains a significant amount of calcium. Therefore, the initial calcium level of CSP samples are rather important to the test. They should be less than 1500 ppm, preferably below about 500 ppm. This is accomplished by ion exchange before precipitation of the pectin. The calcium content of the NCSP is irrelevant, since this pectin is not calcium sensitive. The calcium levels for both are shown in the table.

The YOG test, as previously described, gives a quantitative measure of performance. A YOG grade of 100 is the standard. The parent pectin may or may not give this grade

(other YOG comparisons can be made with the parent pectin to get a measure of selectivity). If the CSP is pure, the YOG grade should be higher than 100. If the standard contained 50% CSP, the YOG grade of pure CSP should be 200. If the standard contained 60% CSP, then the YOG of pure CSP samples should be somewhat less than 200. If the standard contained 40% CSP, then the YOG grade of pure CSP should be greater than 200. The exact amount of CSP in the standard is not known precisely, but is thought to be about 55%. Then a YOG of 200 would indicate essentially pure CSP, and grades significantly lower than 200 would indicate some NCSP is mixed in with the CSP of the sample, and the extraction method needs improvement.

In the differential extraction process it is not essential to use the same type of equipment for each stage. For example, the NCSP extraction gives a firm peel, which can be separated from the mixture by screen filters, gravity belts, presses or the like. It does not require a rotary drum vacuum filter, as is otherwise common in the art. As a possible alternative, the whole NCSP extraction process could be done in a counter-current screw extractor, of the type common to sugar beet extraction, for example, or the one described in Leach et al. (1993). In this case, the exiting peel may be filtered only once, or not filtered at all—but the peel and juice can be gravity separated. In contrast, the CSP extraction is very messy; the peel is gummy and mushy, and continuous, countercurrent screw extractors do not work very well. Rotary vacuum filters work well separating the juice from this mushy peel, where other types of filters do not.

This work used batch or pot extractions, but the principle of differential pH could also be applied to continuous or other types of extraction technology. It is somewhat surprising that this process should work at all, but we theorize here that the NCSP pectin is the last laid down and has not had time to de-esterify (by enzymatic means, for example) to become CSP. Therefore, mildly acidic conditions are able to remove it from the biomass, whereas these are not strong enough to remove the CSP.

4. Conclusions

We have shown that a two-step process can be used to selectively extract a non-calcium sensitive pectin and a calcium sensitive pectin directly from the peel using a different pH in each step. The first step, extracting NCSP, should be done at a mild pH in the range 3.0–3.3. This can be done without additional calcium salt being added, but if it is desired, calcium salt can be added and the pH lowered somewhat to about the 2.7–3.0 range with similar results. However, a more aggressive acid extraction in stage 1

results in bleed-through of the CSP fraction and lowers the selectivity. The CSP extraction can be done conventionally.

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PECTIN SUBSTANCES OF SUGAR BEET PULP

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In the Republic of Uzbekistan at the present time a plant for the production of apple pectin is operating at the Gazalkent preserving factory. With the aim of broadening the sources of pectin from local raw material, we have studied the pectin substances of beet pulp.

In contrast to the pectin of apple and lemon residues, the pectin substances (PcSs) of beet pulp have strong bonds with proteins, cellulose, lignin, and other structural elements of the cell wall [1]. In view of this, after the extraction of sucrose, sugar beet was subjected to hydrolysis with comparatively strong solutions of mineral acids (HCl, HNO₃) and oxalic acid.

Below we give the results of the hydrolysis-extraction of beet pulp at a liquor ratio of 1:12 and the yields of PcSs:

Extractant	Time, h	Temperature, °C	Yield of PcSs, %	Ash content, %
0.2% HCl (A)	1.5	70	16.8	0.8
0.4% HCl (B)	3.0	85	23.6	2.1
2.0% HNO ₃ (C)	1.0	70	6.8	1.6
0.5% H ₂ C ₂ O ₄ (D)	2.0	70	5.49	3.0

A high yield of pectin with a low ash content was obtained on hydrolysis-extraction with 0.2-0.4% HCl. All the PcSs isolated consisted of light cream-colored powders.

The physicochemical parameters of the pectin substances obtained with different extractants differed to some degree:

Pectin substances	Degree of esterification	C _D , %	C _M , %	GalUA, %	OAc, %
A	55.2	9.6	7.8	63.94	3.44
B	67.0	10.3	6.6	64.66	1.61
C	42.8	7.5	10.6	63.70	2.4
D	97.0	8.2	4.8	52.3	3.86

The pectin substances of the beet extracted by solutions of hydrochloric and nitric acids were more highly methoxylated than those extracted by oxalic acid. The galacturonic acid contents of the PcSs scarcely differed, with the exception of the PcSs extracted by the H₂C₂O₄ solution. All the beet PcSs contained acetyl groups and a predominating amount of carboxy groups, in contrast to apple and citrus pectins. These factors were responsible for the low gelling power of the beet pectin and for its capacity for sorbing heavy-metal ions.

The monosaccharide compositions of all the PcSs were identical, as could be seen from an analysis of their hydrolysates (2 N H₂SO₄, 48 h). In all the hydrolysates, PC (butanol-pyridine-water (6:4:3)) revealed the presence of galacturonic acid, arabinose, xylose, rhamnose, and traces of glucose, which is characteristic for the class of pectin substances. After the extraction of the PcSs, we extracted hemicellulose (HMC) with a 10% caustic soda solution. The yield of polysaccharide was 2.21%. GalUA, Ara, Gal, Xyl, and Rha were detected in a hydrolysate by PC.

Thus, pectin substances and hemicellulose have been extracted from the pulp of local varieties of sugar beet. A physicochemical characterization of the PcSs has been made, and the monosaccharide compositions of the beet pectin and hemicellulose have been determined.

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Comparison of the Structural Features of Apple and Citrus Pectic Substances

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ABSTRACT

Pectic substances were extracted from Alcohol Insoluble Solids from lemon peel (albedo) and fractionated by ion exchange chromatography and gelfiltration. The pectin molecules contained rhamnose, arabinose, galactose, glucose and galacturonic acid residues; xylose residues were almost absent. Degradation with purified pectolytic enzymes and subsequent gelfiltration of the resulting pectin fragments showed that the neutral sugar side chains were present in 'hairy regions' (blocks of neutral sugar side chains). The distribution of the methoxyl groups was studied by HPLC analysis of enzyme-degraded pectins. Some influence of native pectinesterase on the distribution of the methoxyl groups was found. The results are compared with those of similarly extracted and purified apple pectic substances.

INTRODUCTION

In previous papers, investigations of the structure of apple pectic substances have been reported (de Vries *et al.*, 1981, 1982, 1983a, b, 1984). The extraction and purification of pectins from apple Alcohol Insoluble Solids (AIS) was described (de Vries *et al.*, 1981) and a model of apple pectin molecules was presented (de Vries *et al.*, 1982). In this model, the molecules consist of 'hairy regions' (rhamnogalacturonan segments carrying the neutral sugar side chains) and 'smooth regions' (homogalacturonan segments). The distribution of the methoxyl groups was

studied (de Vries *et al.*, 1983b) and it was found by high-pressure liquid chromatography (HPLC) of enzyme-degraded pectins that the distribution of the methoxyl groups could be a random one. The extractability and the molecular weight (MW) of the apple pectic substances were found to depend on storage time (de Vries *et al.*, 1983b).

In this paper, structural studies on lemon peel pectic substances are reported. The methods developed in the above mentioned studies were applied to lemon peel pectic substances; in this way, apple and citrus pectic substances extracted and purified according to the same methods could be compared.

METHODS

Analytical methods

The anhydro-uronic acid (AUA, MW 176) content of pectin fractions was determined by an automated *m*-hydroxydiphenyl/sulphuric acid assay (Thibault, 1979). The amount of AUA in the AIS-preparations was determined according to Ahmed & Labavitch (1977). The neutral sugars were analysed gas-chromatographically as their alditol acetates (Albersheim *et al.*, 1967; Darvill *et al.*, 1975) after hydrolysis in 2N TFA for 1 h. The methoxyl content was determined by gas chromatographic analysis of the methanol released on alkaline de-esterification (1 h at room temperature; 0.1M KOH). Methanol was converted to methyl nitrite and determined according to the method of Versteeg (1979).

Extractions

AIS (10 g) was extracted three times for 30 min (while being continuously stirred) with 300 ml of 0.05M Na-acetate buffer (pH = 5.2) at room temperature and the whole procedure was repeated at 70°C; extraction with 0.05M EDTA and 0.05M NH_4 -oxalate in 0.05M Na-acetate buffer followed (again three times, 70°C, 30 min). The extracts were filtered and the pectins were precipitated with ethanol at a concentration of 70%. These three extracts are referred to as the cold buffer, hot buffer and oxalate extracts.

Enzymic degradation

Highly purified pectin lyase ('type 2', van Houdenhoven, 1975) and pectate lyase (Rombouts *et al.*, 1978) were used. Enzyme reaction conditions were as follows.

Pectin lyase (EC 4.2.1.2) 0.2 mg ml⁻¹ substrate 3 units ml⁻¹ (extensive pH = 5.2, at 30°C for 4 min) determined spectrophotometrically for the esterified unsaturated product.

Pectate lyase (EC 4.2.1.2) 0.2 mg ml⁻¹ substrate and 15 units ml⁻¹ (extensive pH = 6.9, at 30°C for 4 min) determined spectrophotometrically for the esterified unsaturated product.

The extent of degradation was determined spectrophotometrically (McMillan & Vaughn, 1967) for the esterified unsaturated product.

Gel filtration

An amount of 10–50 mg of pectin was dissolved in 2 ml water and applied to a Sepharose 4B column (Pharmacia). The void volume was determined by the included molecules, 300 ml. The eluent was water. The flow rate was 0.3 ml min⁻¹. Experiments were performed at room temperature.

High-pressure liquid chromatography

A Spectra Physics Liquid Chromatograph Model 770 variable wavelength detector was used. The fragments were separated on a Merck column with a 10 µm particle size, 37–44 µm, Chromapac concentration were used as eluent and the condition of the column was as follows.

Ion-exchange chromatography

About 100 mg of pectin was dissolved in 2 ml buffer, pH = 5.1, and a cellulose (Whatman DE 52) column was used. The pectins were separated on a column of 5–300 mNa-phosphate buffer. Experiments were performed at room temperature.

Pectin lyase (EC 4.2.2.10; poly(methoxylgalacturonide)lyase); 0.2 mg ml⁻¹ substrate and 0.002 units ml⁻¹ (limited degradation) or 3 units ml⁻¹ (extensive degradation) in 10 mM sodium citrate buffer, pH = 5.2, at 30°C for 4–10 h. The extent of degradation was determined spectrophotometrically at 235 nm, assuming $\epsilon_{235} = 5500 \text{ M}^{-1} \text{ cm}^{-1}$ for the esterified unsaturated product.

Pectate lyase (EC 4.2.2.2; poly(1,4- α -D-galacturonide)lyase); 1 mg ml⁻¹ substrate and 15 units ml⁻¹ enzyme in 25 mM sodium carbonate buffer, pH = 6.9, at 30°C for 4 h.

The extent of degradation (percentage of bonds broken) was determined spectrophotometrically at 235 nm, while $\epsilon_{235} = 4800 \text{ M}^{-1} \text{ cm}^{-1}$ (McMillan & Vaughn, 1964) was assumed for the de-esterified unsaturated product and $\epsilon_{235} = 5500 \text{ M}^{-1} \text{ cm}^{-1}$ (Edstrom & Phaff, 1964) for the esterified unsaturated product.

Gel filtration

An amount of 10–50 mg of (degraded) pectin was dissolved in about 2 ml water and applied to a 80 × 2.5 cm column of Sephacryl S-300 (Pharmacia). The void volume (Blue Dextran) of the column was 120 ml; the included molecules (glucose) appeared at an elution volume of 300 ml. The eluent was 0.1 M Na-phosphate, pH = 5.1, or water. The flow rate was 0.3 ml min⁻¹, controlled by an LKB peristaltic pump. Experiments were performed at room temperature.

High-pressure liquid chromatography (HPLC)

A Spectra Physics Liquid Chromatograph (SP 8000) equipped with a Schoeffel 770 variable wavelength detector was used. Oligomeric pectin fragments were separated on a 250 × 4.6 mm I.D. LiChrosorb 10NH₂ (Merck) column with a Vydac 501 SC guard column (100 × 2.1 mm I.D., 37–44 μ , Chrompack). Na-acetate buffers of varying pH and concentration were used as eluents. The exact conditions depended on the age and the condition of the column (Voragen *et al.*, 1982).

Ion-exchange chromatography

About 100 mg of pectin sample was dissolved in 5 mM Na-phosphate buffer, pH = 5.1, and applied to a 10 × 0.4 cm column of DEAE-cellulose (Whatman DE 52). After washing thoroughly with the starting buffer, the pectins were eluted from the column with a linear gradient of 5–300 mM Na-phosphate buffer of pH = 5.1 (200 ml). Experiments were performed at room temperature.

TABLE 1
Characteristics of Alcohol Insoluble Solids (AIS) from Lemon Albedo and Ripe
Apple Cortex

	AIS (% of fresh wt)	AUA (mg/g AIS)	AUA (mg/g fresh wt)	DE (%)
Lemon albedo	7.3	207	15.1	71
Ripe apple cortex	2.1	284	6.0	70

Note. AUA, anhydro-uronic acid; DE, degree of esterification. The preparation of the ripe apple AIS was described by de Vries *et al.* (1981).

Preparation of AIS

Lemons (*Citrus limon*) from Italy were purchased from a local green-grocer. The fruits were peeled (removal of the flavedo), after which the albedos were removed. The albedos were then mashed in a mincer and portions of 1 kg were extracted three times with 2.5 litres of 96% alcohol at 70°C. The AIS-preparation was air-dried overnight after solvent drying with acetone, ground in a hammer mill with a 10 µm sieve and stored at -40°C. Table 1 gives some characteristics of the AIS obtained by this procedure.

RESULTS AND DISCUSSION

Table 1 shows that there are differences between AIS from lemon peels and from apples. Lemon peels contain more pectin than apples, but the content in the AIS is lower. These differences can be explained by the difference in cell size: the cells of apple cortical tissue are large and the cell walls are relatively thin. The AUA content of lemon peel AIS (207 mg/g) is about the same as reported by Sinclair (1961) for orange albedo AIS (196 mg/g). The results of the pectin extractions are given in Table 2. Also in this table, a comparison can be made between apple AIS and lemon peel AIS.

The most important difference is that between the oxalate extracts in both cases: from lemon peel AIS more pectin can be extracted by oxalate. But also in this case, the degree of esterification (DE) is high

Pectin Fractions Obtained from
Lemon AIS

Extract	AUA (mg/l) apple cortex
Cold buffer	30
Hot buffer	30
Oxalate	31
Total	91

Note. AUA, anhydro-uronic acid in the text.

(70%). It must be said that the 'oxalate effect' may further be specific for oxalate and it is concluded that, in lemon peel AIS, the pectin is insoluble by Ca²⁺-binding. The pectin from the oxalate extract from cellulose is shown in Fig. 1.

The neutral sugar content of the pectin fractions extracted from apple and lemon peel AIS is given in Table 3.

The pectin molecules from apple AIS contain more glucose residues. Only the pectin from the oxalate extract of apple AIS contains xylose residues. This is the most striking difference between the oxalate extracts of apple and lemon peel AIS.

Aspinall *et al.* (1968) reported that the pectin from cellulose purified lemon peel AIS contains more glucose and less xylose. However, in the present study, the pectin from the oxalate extract of lemon peel AIS contains more xylose (1.3)

TABLE 2
Pectin Fractions Obtained from AIS from Ripe Apple Cortical Tissue and from
Lemon Albedo by Fractional Extraction

DE (%)	Extract	Amount extracted				Degree of esterification, %	
		AUA (mg)/AIS (g)		%		apple cortex	lemon albedo
		apple cortex	lemon albedo	apple cortex	lemon albedo		
71	Cold buffer	30	17	11	8	80	74
70	Hot buffer	30	37	11	18	76	70
	Oxalate	31	61	11	30	78	70
	Total	91	115	33	56		

Note. AUA, anhydro-uronic acid. Conditions during the extractions are described in the text.

(70%). It must be said, however, that we did not investigate this 'oxalate effect' any further. Therefore the question whether this effect is specific for oxalate or not, remains open. Hence, it cannot be concluded that, in lemon peel cell walls, more pectin has been rendered insoluble by Ca^{2+} -binding than in apple cell walls. The purification of the pectin from the oxalate extract of lemon albedo AIS on DEAE-cellulose is shown in Fig. 1 and Table 3.

The neutral sugar composition of the pectin pools is expressed as moles sugar residues/mole arabinose residues to make comparison with apple pectin fractions easier (Table 4).

The pectin molecules contained rhamnose, arabinose, galactose and glucose residues. Only trace amounts of xylose residues were present. This is the most striking difference between the pectins from the oxalate extracts of apple and citrus AIS (Table 4). In a crude pectin extract from grapefruits, Kawabata (1977) also reported the absence of xylose residues.

Aspinall *et al.* (1968) found only traces of xylose residues in DEAE-cellulose purified lemon peel pectin. In the crude cold water extract of lemon peels, however, they found (after partial hydrolysis) the disaccharide xylose-(1,3)-galacturonic acid. This suggests that in citrus

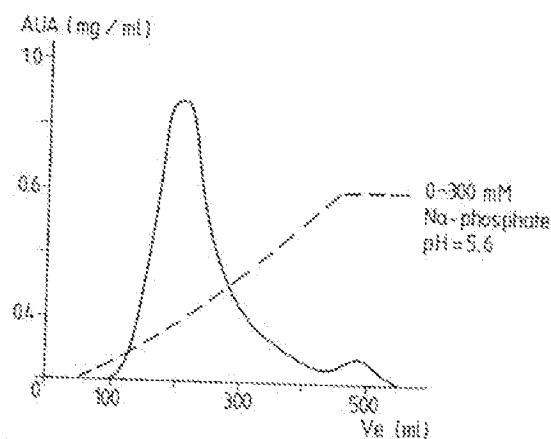


Fig. 1. Fractionation of the pectin from the oxalate extract of AIS from lemon peels on DEAE-cellulose. AUA, Anhydro-uronic acid; V_e , elution volume. The DEAE-cellulose column was eluted by a linear gradient of 5–300 mM Na-phosphate buffer, pH = 5.1 (400 ml).

TABLE 3
Neutral Sugar Composition of Citrus Pectin Pools Shown in Fig. 1

Sugar composition	Elution volume (ml)			
	100–150	150–250	250–400	400–500
Neutral sugar content (moles/mole of galacturonate residues)	0.09	0.12	0.15	0.0
Moles rhamnose/mole arabinose	0.09	0.05	0.09	0.1
Moles galactose/mole arabinose	0.55	0.59	0.26	0.3
Moles glucose/mole arabinose	0.10	0.05	0.25	0.2
Percentage of total anhydro-uronic acid	28	30	37	

pectin some xylogalacturonan regions are present, as has been shown to be the case for apple pectic substances (de Vries *et al.*, 1982). It may be that in citrus xylogalacturonans occur only in some parts of the cell wall. It is also possible that these pectin fragments containing xylose play a role as 'elicitor' in host-parasite relations of the plant (Albersheim *et al.*, 1981).

Neutral Sugar Composition Extracts of AIS

Sugar composition

Rhamnose/arabinose
Xylose/arabinose
Glucose/arabinose
Galactose/arabinose

* Moles of neutral sugar residue

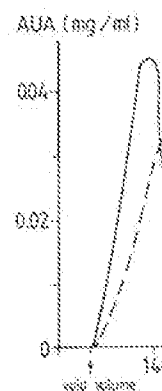


Fig. 2. Gel filtration of extracts of AIS from lemon peels. V_e , elution volume.

Both in citrus and in apple, the neutral sugar composition of the pectin is characterized by the presence of two types of galactans (Aspinall &

Figure 2 suggests that the molecular weight of the pectin fragments is relatively low.

TABLE 4
Neutral Sugar Composition of the DEAE-Cellulose Purified Pectin from the Oxalate
Extracts of AIS from Ripe Apple Cortex and Lemon Albedo

Sugar composition ^a	Ripe apple cortex	Lemon albedo
Rhamnose/arabinose	0.09	0.10
Xylose/arabinose	0.09	0.00
Glucose/arabinose	0.10	0.20
Galactose/arabinose	0.35	0.43

^a Moles of neutral sugar residues/moles of arabinose residues.

of AIS from lemon
elution volume. The
500 mM Na-phosphate

own in Fig. 1

elution (ml)

250-400 400-500

0.15 0.0

0.09 0.1

0.03 0.3

0.25 0.2

37

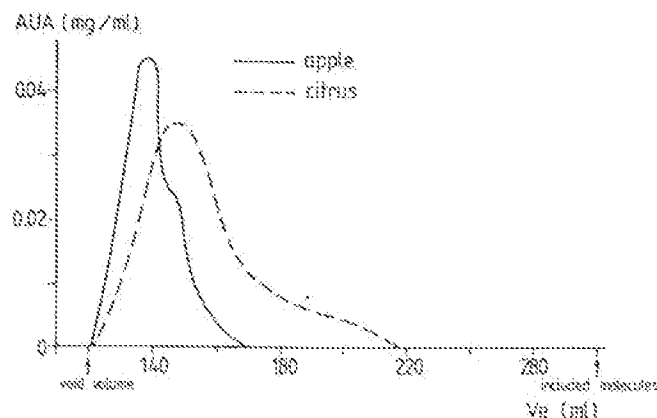


Fig. 2. Gel filtration of DEAE-cellulose purified pectin fractions from the oxalate
extracts of AIS from lemon peels and from ripe apples. AUA, Anhydro-uronic acid;
V_r, elution volume. The eluent was 0.1 M Na-phosphate buffer, pH = 5.1.

Both in citrus and apple pectic substances the ratio of galactose/
arabinose in the neutral sugar side chains is not constant (Table 3 and
de Vries *et al.*, 1981). In both cases this inconsistency is caused by the
presence of two types of arabinogalactans: 1,3/1,6-linked and 1,4-linked
galactans (Aspinall & Cottrell, 1970; de Vries *et al.*, 1982).

Figure 2 suggests that the molecular weight (the hydrodynamic
volumes) of citrus pectin is lower than that of apple pectin. The
molecular weight of apple pectic substances, however, decreases during

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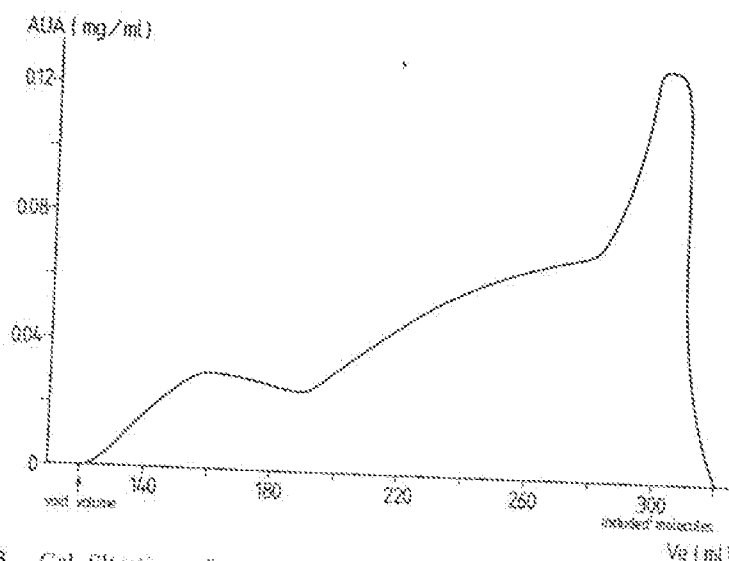


Fig. 3. Gel filtration of a pectin lyase degraded citrus pectin. AUA, anhydrouronic acid; V_e , elution volume. The substrate was a DEAE-cellulose purified pectin from the oxalate extract of AIS from lemon peels. Limited degradation with pectin lyase as described in the Results and Discussion section (extent of degradation was 7%); the eluent was water.

storage of the apples (de Vries *et al.*, 1983b). The interpretation of the data in Fig. 2 is also hampered by the fact that the oxalate fractions probably represent different sub-fractions of the pectic substances present (see Table 2).

Figure 3 shows the result of limited enzymic degradation and subsequent gel filtration of a citrus pectin fraction from the oxalate extract. Nearly all the neutral sugar residues appear in elution volumes, where high-molecular-weight fragments can be expected (Table 5). It can be concluded that in citrus pectin the neutral sugar side chains are concentrated in regions that cannot be degraded by pectin lyase, the so-called 'hairy regions', exactly as was shown for apple pectic substances (de Vries *et al.*, 1982). Aspinall & Cottrell (1970) found small amounts of molecules rich in neutral sugars in their lemon peel extracts. Zitko & Bishop (1965) reported the presence of two types of molecules in commercial citrus pectin: one type was rich in neutral sugars and the other was poor in neutral sugars. Our results show that these two types

Neutral

Percentage of total
Neutral sugar content
residues/mole of

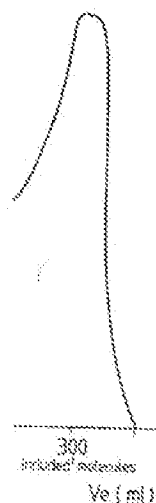
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Zitko & Bishop (1970) studied the
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molecular distribution
pectic substances

We studied the
by extensive degradation
fractionation of
as shown in Fig.
oligogalacturonide
esterified galacturonic
a, *b* and *c* indicate
galacturonate residues

In Table 6 a
pectin fractions
be small, which
the intramolecular
lemon peel pectin

It was shown
HPLC patterns of
'trans-esterified' pectins
de-esterified to 70%
pectins have a random

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TABLE 5
Neutral Sugar Content of Citrus Pectin Pools Shown in Fig. 3

Sugar	Elution volume (ml)		
	120-180	180-280	280-320
Percentage of total AUA	7	53	40
Neutral sugar content (moles neutral sugar residues/mole of galacturonate residues)	1.31	0.01	0.00

are fragments of the same molecules and that low degrees of degradation result in the appearance of the two types of molecule reported by Zitko & Bishop (1965). Aspinall *et al.* (1970) and Aspinall & Cottrell (1970) studied the distribution of the methoxyl groups of lemon peel pectic substances. It can be concluded from their work that an intermolecular distribution exists which may be similar to the one for apple pectic substances (de Vries *et al.*, 1983a).

We studied the intramolecular distribution of the methoxyl groups by extensive degradation of pectin with pectin lyase and subsequent fractionation of the partially esterified oligogalacturonides by HPLC as shown in Fig. 4. Using the HPLC system, the partially esterified oligogalacturonides are separated according to their number of non-esterified galacturonic acid residues (de Vries *et al.*, 1983a). In Fig. 4, *a*, *b* and *c* indicate pectin fragments with 0, 1 and 2 free (non-esterified) galacturonate residues.

In Table 6 a comparison is made between the HPLC patterns of pectin fractions from apple and lemon peel. The differences appear to be small, which may indicate that no substantial differences between the intramolecular distributions of the methoxyl groups of apple and lemon peel pectin exist.

It was shown in a previous paper (de Vries *et al.*, 1983b) that the HPLC patterns of apple pectins do not differ much from those of 'trans-esterified' pectins (pectins esterified to 95% and subsequently de-esterified to 70% in cold alkali). This suggests that apple and citrus pectins have a random distribution of methoxyl groups.

When the whole AIS from both apple and lemon peel is degraded by pectate lyase and the resultant pectin fragments are analysed by HPLC

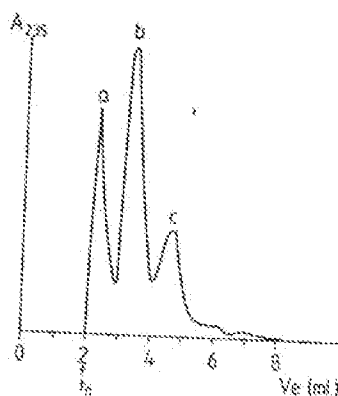


Fig. 4. High-pressure liquid chromatograms (HPLC) of citrus pectin degraded with pectin lyase. A_{225} , Absorbance at 225 nm; V_e , elution volume. HPLC conditions are as described in the text. The substrate was a pectin fraction from the oxalate extract of AIS from lemon peels. The extensive enzymic degradation is described in the Results and Discussion section.

TABLE 6
Percentage of Total AUA Present in the Peaks of the
High-Pressure Liquid Chromatograms of Pectin Lyase
Degraded Substrates

Pectin fragment	AUC, in extract, %	
	Apple	Lemon
a	13	17
b	48	40
c	30	28
d	9	15

Note. The substrates were DEAE-cellulose purified pectin fractions from the oxalate extracts of AIS from ripe apples (de Vries *et al.*, 1981) and from lemon albedo. Figure 4 gives the high-pressure liquid chromatogram for the pectin from lemon albedo; a, b and c are pectin fragments with 0, 1 or 2 non-esterified galacturonate residues (see Fig. 4 and de Vries *et al.*, 1983b) 'd' = $100 - (a + b + c)$.

Amounts
esterified
and from
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Digalacturonic
Trigalacturonic

Note. The
were alkali
oligomers
1983b).

(after de-esterification) be detected (Table 6). The di-galacturonic acid was detected on paper (de Vries *et al.*, 1981) and tri-galacturonic acid was detected on pectinesterase. It is clear that pectinesterase influences the overall DE is or

In conclusion our results are very similar. In the 'hairy regions' of the pectin, the same in both cases, pectin contains few

It must be realized that the results are influenced by factors such as pH (de Vries 1981), and conditions of commercial pectins

TABLE 7
Amounts of Di- and Trigalacturonic Acid Present in De-esterified Pectate Lyase Digests of AIS from Ripe Apples and from Lemon Albedo, Expressed as Percentage of the Total Amount of Uronic Acid Residues Present in the AIS

	Ripe apple AIS	Lemon albedo AIS
Digalacturonate	0.02	0.79
Trigalacturonate	0.00	0.26

Note. The pectate lyase digests (for conditions see text) were alkali-saponified and the degree of polymerization of oligomers present was determined by HPLC (de Vries *et al.*, 1983b).

(after de-esterification), differences between apple and lemon peel can be detected (Table 7). In the case of lemon peel AIS, small amounts of di-galacturonic acid and tri-galacturonic acid were found. In a previous paper (de Vries *et al.*, 1983a), it was shown that this occurrence of di- and tri-galacturonic acid is typical of pectins de-esterified by (citrus) pectinesterase. It can be concluded from Table 7 that citrus pectinesterase influences the distribution of the methoxyl groups in the citrus fruits. This influence, however, is very small: the resultant decrease of the overall DE is only 1 or 2% (Table 7).

CONCLUSIONS

In conclusion our results show that apple and citrus pectin molecules are very similar. In both cases the neutral sugar side chains are located in 'hairy regions'. The distribution of the methoxyl groups is probably the same in both cases, namely a random one. A difference is that citrus pectin contains few xylose residues.

It must be realised, however, that pectin structural parameters are influenced by factors like fruit storage conditions (O'Beirne *et al.*, 1981), and conditions during extraction. Differences among batches of commercial pectins may result from these factors.

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Physicochemical properties of pectins from ambarella peels (*Spondias cytherea*) obtained using different extraction conditions

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Abstract

Extraction and use of pectins from ambarella peels could add value to the waste products arising from processing of the fruit. Dried alcohol-insoluble residues (AIR) of ambarella peels were treated separately with HCl, deionised water and oxalic acid/ammonium oxalate solutions, and the resulting pectin extracts analysed for some biochemical and physicochemical parameters. The results show that pectin yield (9–30% dry AIR), uronic acid (557–727 mg/g dry weight), neutral sugars (125–158 mg/g), degree of methylation (50–58%) and acetylation (4–6%), molar mass (263,000–303,000 g/mol) and intrinsic viscosity (179–480 ml/g) varied significantly ($p < 0.05$) with the various extraction methods used. Extraction with oxalic acid/ammonium oxalate solution gave the highest pectin yield, with high molar mass and degree of methylation, making the extracts suitable for use as additives in the food industry. The results compared well to lime pectin extracted under the same conditions, indicating their commercial significance.

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Keywords: Ambarella peels; Pectins; Physicochemical properties; Extraction conditions

1. Introduction

Ambarella or golden apple (*Spondias cytherea*) is a tropical fruit belonging to the family of Anacardiaceae (Morton, 1987). In addition to its sweet and pleasant flavour (Graham, Wickham, & Mohammed, 2004; Jirovetz, Buchbauer, & Ngassoum, 1999), this fruit is a good source of minerals and vitamin C (Ishak, Ismail, Noor, & Ahmad, 2005). Ambarella fruit is quite delicious to eat when fresh and it is often used for making jelly, jams, pickles, relishes, soups, stews and juices (Morton, 1987) or may be canned in sucrose (Ramsundar, Comissiong, Badrie, Baccus-Taylor, & Spence, 2002). The peels represent about 19% of the total fruit weight and they constitute the main part of

byproducts. Citrus peel and apple pomace are the major raw materials used for the production of commercially acceptable pectins (May, 1990). Pectin preparations from ambarella peels could serve as an alternative.

Pectins are a family of complex polysaccharides mainly present in the primary cell wall and intercellular regions of dicotyledons (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Structurally, they are generally described as an alternation of “smooth” (made of homogalacturonans, HGs) and “hairy” (made of type I rhamnogalacturonans, RGs-I) regions. HG is composed of (1 → 4)-linked α -D-galacturonic acid (GalpA) residues that can be partly methylesterified at C₆ (Pilnik & Voragen, 1970) and possibly partly acetylated at O₂ or O₃ (Ralet, Bonnin, & Thibault, 2005).

Pectins have many applications in food science and nutrition, cosmetics and pharmacy. They are widely used

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as food additives for thickening, gelling and emulsifying jams, soft drinks, fish, meat products and milk products (Barrera, Ramírez, González-Cabriales, & Vázquez, 2002; May, 1997; Ralet et al., 2005). Commercially, pectins are extracted by treating the raw material with hot dilute mineral acids, mainly nitric acid (Rolin, 2002). The extraction conditions are in the range of pH 1.5–3, temperature 60–100 °C and time 0.5–6 h. Solid:liquid ratios of 1:17 for apple and 1:35 for citrus are often used (Ralet, Bonnin, & Thibault, 2002). These native pectins are generally highly methylated (DM > 50%) and slightly acetylated (Voragen et al., 1995). They gel at low pH (2.5–3.8) with a high sugar concentration (>55%) (Ralet, Crépeau, Bucholt, & Thibault, 2003; Rolin, Nielsen, & Glahn, 1998).

Relationships between pectin fine structure and functional properties do exist (Ralet & Thibault, 2002). Many studies have shown that the biochemical characteristics of pectins depend on the plant source and the extraction method used. Sugar beet pectins extracted by acid (HCl or HNO₃) at variable pH (1–3), temperatures (75–90 °C) and times (30–90 min) exhibited a galacturonic acid content and a degree of methylation varying from 295 to 528 mg/g (dry weight) and from 34 to 94%, respectively (Levigne, Ralet, & Thibault, 2002a). Pectins from *Cen*i mango peels extracted by deionised water exhibited a galacturonic acid content and a degree of methylation of 860 mg/g and 73%, respectively. When extraction was performed with HCl (pH 1.5/85 °C/ 1 h), *Cen*i mango pectins exhibited a lower galacturonic acid content and a similar high degree of methylation (660 mg/g and 76%, respectively) (Kratchanova, Benemou, & Kratchanova, 1991). Hence the source of pectin and the extraction conditions used have a significant impact on pectin biochemical characteristics and their suitability for application. Presently, there is hardly any information on the extraction of ambarella pectin.

The aim of this study was to evaluate the impact of different extraction conditions on the yield and some biochemical characteristics of ambarella peel pectins and their suitability as a source of industrial pectin.

2. Materials and methods

2.1. Sampling and sample treatment

Ripe ambarella (*S. cytherea*) and lime (*Citrus latifolia*) were harvested in an orchard near the city of Yaounde (Cameroon). The peels of the fruits were removed and chopped into pieces of 1 cm² using a stainless steel knife. The peels were then bleached in boiling water for 5 min and dried in an air convection oven at 50 °C. Alcohol-insoluble residue (AIR) was prepared by treating the dried peels four times with ethanol (85 vol.%) at 70 °C for 20 min.

2.2. Extraction of pectin

Three different extraction conditions were applied: hydrochloric acid (0.03 M; pH 1.5) at 85 °C for 1 h, deion-

ised water at 75 °C for 1 h and oxalic acid/ammonium oxalate (0.25%), pH 4.6, at 85 °C for 1 h. About 100 g of AIR were stirred with 4 l of each of the above extracting solutions. The extracts were separated from the AIR residues by filtering through a nylon cloth, and the pectin was coagulated with 3 volumes of ethanol (96%). Pectin coagulates were washed several times with 70% ethanol and finally with 96% ethanol and acetone before vacuum-drying at 50 °C overnight.

2.3. Chemical characterisation

The uronic acid contents of the peels, AIR and extracted pectins were determined colorimetrically by the automated *m*-phenylphenol method (Thibault, 1979).

Neutral sugars were analysed as their alditol acetate derivatives by gas liquid chromatography (GLC) after acid hydrolysis. The pectin was hydrolysed with 1 M H₂SO₄ (3 h, 100 °C). The peels and AIR were prehydrolysed with 13 M H₂SO₄ (30 min, 25 °C), diluted to 1 M and heated (2 h, 100 °C). The individual neutral sugars obtained were reduced with NaBH₄, acetylated and analysed by GLC according to (Blakeney, Harris, Henry, & Stone, 1983). Inositol was used as internal standard.

Pectin methoxyl and acetyl contents were determined by HPLC. Pectins were treated with 1 N NaOH in the presence of CuSO₄, and the methoxyl and acetic acid released were quantified by HPLC on a C18 column, as previously described (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002b). Isopropanol was used as internal standard. The degree of methylation (DM) and the degree of acetylation (DAc) were calculated as the molar ratio of methanol and acetic acid to galacturonic acid, respectively.

2.4. Determination of macromolecular parameters

Pectin average molar masses and intrinsic viscosities were determined using high-performance size-exclusion chromatography (HP-SEC), as described by Ralet, Bonnin, and Thibault (2001).

HP-SEC was performed at room temperature on a system constituted of one Shodex OH SB-G pre-column, followed by two columns in series (Shodex OH-Pack SB-804 HQ and OH-Pack SB-805 HQ, Shodex, Showa Denko KK, Miniato, Japan) eluted with 0.05 M NaNO₃ buffer, containing 0.02% NaN₃ as preservative, at a constant flow rate of 42 ml/h. Detectors used were a refractometer (RI) (ERC 7517 A), a differential viscosimeter (T-50A, Viscotek, Houston, TX) and a multiple angle laser light scattering device (MALLS) (Mini Dawn, Wyatt, Santa Barbara, CA) operating at three angles (41°, 90° and 138°). Pectins (~6 mg/ml) were solubilized in 0.05 M NaNO₃ containing 0.02% NaN₃, left overnight with tail-over-head continuous mixing, centrifuged and filtered (0.45 µm) before injection of 50 µl. Data for molar mass determinations were analyzed using Astra software (Wyatt, Santa Barbara, CA) taking a dn/dc of 0.146. Data for viscosimetry

determinations were analyzed using TriSEC software (Version 3.0, Viscotek, Houston, TX).

All analyses were performed in triplicate and the means of the results given on a dry weight basis. Results were compared using the Student's *t* test. Differences were determined by variance analysis ($p < 0.05$).

3. Results and discussion

3.1. Peels and AIRs uronic acid and neutral sugars contents

The uronic acid and neutral sugars contents of ambarella and lime peels and their corresponding AIRs are summarised in Table 1. Uronic acid (108 mg/g dry weight) and total neutral sugar contents (347 mg/g) of ambarella peels were found to be lower than those of their corresponding AIR (140 and 403 mg/g, respectively). Glucose (probably of cellulosic origin) was the main neutral sugar; the other neutral sugars were galactose, arabinose, mannose, xylose and rhamnose. Ambarella AIRs exhibited an overall distribution of individual sugars similar to that of raw peels. Compared to ambarella peels and AIR, lime peels and AIR exhibited higher amounts of uronic acid (192–248 mg/g) and total neutral sugars (347–484 mg/g) (Table 1). Compared to the raw peels, AIRs were richer in uronic acids and neutral sugars, most likely because some ethanol-soluble compounds, e.g., lipids and pigments, were removed by the ethanolic treatment (Selvendran & Du Pont, 1980). Values of uronic acid found in the literature show that mango AIRs are richer in uronic acids (213 mg/g) than are ambarella AIR (Kratchanova et al., 1991).

3.2. Yield, extractability and biochemical characteristics of ambarella peels pectins

3.2.1. Extraction yields

The yield of ambarella pectin extraction varied from 16% to 22% dry weight of AIR, depending on the extrac-

tion condition used. The highest yields were obtained with oxalic acid/ammonium oxalate (OAAO) and the lowest with water (Table 2). In comparison, yields for lime (9–30%) were lower under water extraction conditions while similar results (17%) were found for *Tommy Atkins* mango pectins extracted with H_2SO_4 (pH 1.5) (Berardini et al., 2005). Other results showed high extraction yields using HCl (pH 1.5) for *Ceni* mango pectin (24.5 dry weight% AIR) (Kratchanova et al., 1991).

3.2.2. Uronic acid and neutral sugars contents

Ambarella pectins had uronic acid (557–727 mg/g dry weight) and total neutral sugars (125–158 mg/g) contents that varied widely, depending on the extraction method used. Water-extracted pectins were particularly rich in uronic acids while OAAO-extracted pectins were rich in total neutral sugars. HCl-extracted pectins were poorest in uronic acid. Lime pectins extracted with HCl and OAAO were rich in uronic acid and poor in neutral sugars content (Table 2). Previous results showed that *Ceni* mango pectins extracted using HCl (pH 1.5) had high levels of uronic acids (660 mg/g) and total neutral sugars (320 mg/g) (Kratchanova et al., 1991).

HCl-extracted ambarella pectins were low in arabinose and rhamnose, and high in galactose and glucose. However, with the other extraction conditions, both arabinose and galactose contents were high (Table 2). There were only small amounts of xylose and mannose. The distribution patterns of individual neutral sugars for water-extracted ambarella pectins were similar to those observed with water-extracted lime pectins, and significantly different ($p < 0.05$) from those obtained under the other extraction conditions (Fig. 1). Other authors have found that galactose, arabinose and glucose are the three main neutral sugars of pectins and their proportions depend on the source of pectin and the extraction process used (Iagher, Reicher, & Ganter, 2002; Ralet & Thibault, 1994).

3.2.3. Pectin extractability

Pectin extractability was expressed as the amount of uronic acid extracted from the AIR [uronic acid (g) of the extracted pectin $\times$ yield per 100 g of uronic acid initially present in the AIR] (Table 2). Pectin extractability from ambarella was very high with OAAO extraction (98% of the uronic acids initially present in the AIR). Fairly high values were observed with HCl and water extractions (77–81%). As for lime, we obtained similar values with HCl and OAAO, while a very low value of extractability (14%) was found under water-extraction conditions. The lowest value obtained for lime is explained by the fact that the extractability is based on the yield and uronic acid content of the extracted pectins. Uronic acids, which are the main components of pectin, are well extracted under OAAO conditions.

3.2.4. Degree of methylation (DM) and acetylation (DAc)

Results in Table 3 show that OAAO- and water-extracted ambarella pectins were more methylated (DM

Table 1
Individual neutral sugars and uronic acid contents (mg/g dry weight) of peels and their alcohol-insoluble residues (AIRs) from ambarella and lime

Characteristics		Samples			
		Ambarella		Lime	
		Peel	AIR	Peel	AIR
Individual neutral sugars (mg/g)	Rha	6.9 ± 0.2 ^c	7.2 ± 1.6 ^c	15.1 ± 0.1 ^b	19.6 ± 0.1 ^a
	Ara	55.4 ± 3.7 ^c	98.7 ± 1 ^b	97.2 ± 0.6 ^b	135.7 ± 0.5 ^a
	Xyl	18.2 ± 1.9 ^c	22 ± 3.3 ^c	26 ± 0.07 ^b	32.1 ± 0.1 ^a
	Man	21.8 ± 2.6 ^b	15.9 ± 1.3 ^c	22.1 ± 0.1 ^b	26.7 ± 0.1 ^a
	Gal	33.1 ± 2.8 ^d	75.2 ± 6.7 ^a	43.9 ± 0.1 ^c	55.7 ± 0.2 ^b
Total neutral sugars (mg/g)	Glc	208 ± 4 ^b	179 ± 13 ^c	207.3 ± 0.7 ^b	214.2 ± 0.8 ^a
		347 ± 16 ^c	403 ± 10 ^b	412 ± 6 ^b	484 ± 4 ^a
Uronic acids (mg/g)		108 ± 8 ^d	140 ± 6 ^c	192 ± 14 ^b	248 ± 10 ^a

Mean values from triplicate measurements $\pm$ standard deviation. Values in the same line followed by different superscripts are significantly different ($p < 0.05$).

Table 2

Neutral sugar contents, uronic acid contents, yields and extractabilities of pectins obtained under different extraction conditions from ambarella and lime

Pectin characteristics		Extraction process and raw material used					
		HCl (pH 1.5)		Water		Oxalic acid/ammonium oxalate	
		Ambarella	Lime	Ambarella	Lime	Ambarella	Lime
Uronic acids (mg/g)		557 ± 13 ^e	776 ± 3.7 ^b	727 ± 4.5 ^c	493 ± 14 ^f	631 ± 7 ^d	831 ± 16 ^a
Total neutral sugars (mg/g)		125 ± 3 ^c	88 ± 1 ^e	130 ± 7 ^{bc}	136 ± 0.4 ^b	158 ± 2.7 ^a	97 ± 0.6 ^d
Individual neutral sugars (mg/g)	Rha	17	7.8	13.8	13	19	6.9
	Ara	19	4.4	51	51	63	5.7
	Xyl	3.2	0.9	1.9	0.8	2	0.8
	Man	4.3	2.8	1.9	1.5	1.6	1.5
	Gal	35	29	31	26	41	25
	Glc	46	3	28	28	31	4
Yield (mg/g)	Dry	19.4 ± 0.6 ^d	26.9 ± 0.3 ^b	15.6 ± 0.5 ^e	8.8 ± 0.6 ^f	22 ± 0.3 ^c	29.7 ± 1.2 ^a
	AIR						
	Dry Peel	11.8 ± 0.4 ^d	19.8 ± 0.2 ^b	9.5 ± 0.3 ^e	6.7 ± 0.5 ^f	13.4 ± 0.2 ^c	22.6 ± 0.9 ^a
Extractability (%)		77 ± 4 ^b	82 ± 3 ^b	81 ± 2 ^b	14 ± 1 ^c	98 ± 2 ^a	97 ± 3 ^a

Mean values from triplicate measurements ± standard deviation. Values in the same line followed by different superscripts are significantly different ($p < 0.05$).

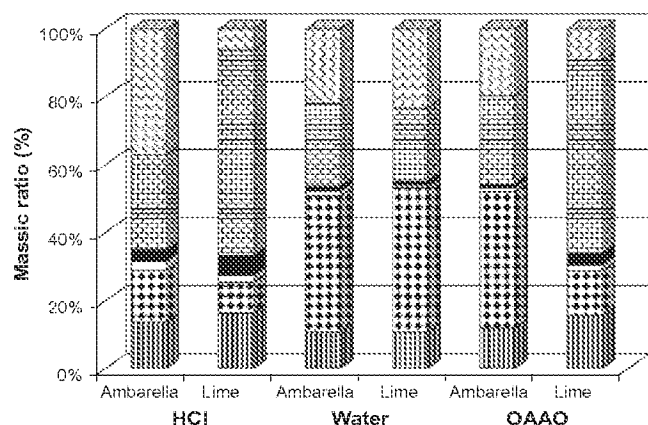


Fig. 1. Distribution patterns of individual neutral sugars of pectins extracted with HCl, water and oxalic acid/ammonium oxalate (OAAO) from ambarella and lime peels. (▨) Rhamnose; (▩) Arabinose; (▤) Xylose; (▥) Mannose; (▦) Galactose; (■) Glucose.

58%) than were HCl-extracted pectins (DM 50%). Compared to those of ambarella pectins, the DMs of lime pectins were significantly higher (58–82%) ($p < 0.05$). Previous studies on acid- (HCl or H_2SO_4 , pH 1.5) extracted pectins from *Cen*, *Alphonso* and *Tommy Atkins* mango peels showed that high degrees of methylation (66% and 76%)

could be obtained (Berardini et al., 2005; Kratchanova et al., 1991; Srirangarajan & Shrikhande, 1979). The enhancing influence of the HCl extraction on methylation degree was also shown on soy hull (Kalapathy & Proctor, 2001) and sugar beet pectins extracted at various HCl concentrations (Levigne et al., 2002a).

Ambarella pectins exhibited low degrees of acetylation (4.2–5.5%) for all three methods of extraction. Lowest values of DAc were found for lime pectins (1.2–1.6%) following HCl and OAAO extractions. The DAc values of ambarella extracted pectins were similar ($p < 0.05$) to that of lime pectin extracted with water (6.0%). Previous studies on acid-extracted apple and lemon pectins reported DAc values of 5.0 and 1.4%, respectively (Kravtchenko, Voragen, & Pilnik, 1992).

3.3. Macromolecular parameters

The weight average molar mass (M_w) of ambarella pectins varied from 263,000 to 303,000 g/mol and their corresponding intrinsic viscosities ($[\eta]$) from 179 to 480 ml/g. The molar masses were the same (at $p < 0.05$) using water and OAAO extraction processes. The same was true for intrinsic viscosities of OAAO- and water-extracted pectins which had the highest values. Compared to ambarella

Table 3

Degrees of methylation (DM) and acetylation (DAc), weight average molar mass (M_w) and intrinsic viscosity ($[\eta]$) of pectins extracted under different conditions from ambarella and lime peels AIRs

Extraction process	Raw material	DM (%)	DAc (%)	$\langle M_w \rangle$ (g/mol)	$[\eta]$ (ml/g)
HCl (pH 1.5)	Ambarella	50 ± 1.4 ^d	4.2 ± 0.1 ^b	303,000 ± 8 ^a	179 ± 7 ^e
	Lime	75 ± 1.9 ^b	1.6 ± 0.01 ^c	112,000 ± 5 ^e	337 ± 8 ^d
Water	Ambarella	58 ± 0.8 ^c	5.5 ± 0.3 ^a	263,000 ± 22 ^b	480 ± 7 ^b
	Lime	82 ± 2 ^a	6 ± 0.2 ^a	252,000 ± 18 ^d	414 ± 9 ^c
Oxalic acid/ammonium oxalate	Ambarella	58 ± 0.7 ^c	5.5 ± 0.3 ^a	263,000 ± 20 ^b	480 ± 4 ^b
	Lime	58 ± 1.4 ^c	1.2 ± 0.02 ^d	255,000 ± 10 ^c	757 ± 8 ^a

Mean values from triplicate measurements ± standard deviation. Values in the same column followed by different superscripts are significantly different ($p < 0.05$).

pectins, the molar masses of HCl-extracted lime pectins (112,000) were lower. HCl- and water-extracted lime pectins exhibited intermediate intrinsic viscosities (337–414 ml g⁻¹) while OAAO-extraction gave correspondingly higher values (Table 3). These results show the important variability of molar mass and intrinsic viscosity of pectins according to the extraction conditions used. Macromolecular parameter values depend, not only on the extraction process used, but also on the plant material used. *Cen* mango pectins extracted with the same extractant (HCl, pH 1.5) had low molar mass (83,000 g/mol) (Kratchanova et al., 1991) but sugar beet pectin extracted at pH 2 (HCl) had high molar mass (377,000) (Levigne et al., 2002a). These differences of molar masses could also be due to the type of method used for the determination of molecular parameter. According to Ralet et al. (2002), the water (in)-solubility and the aggregation of pectin could influence molar mass measurement.

4. Conclusion

The results showed that ambarella peels are a source of highly methylated pectins with high molecular weight. The extraction conditions had a significant impact on the biochemical characteristics of the extracted pectins. Oxalic acid/ammonium oxalate (OAAO) allowed high extraction yields, the recovered pectins being of high degree of methylation, high molar mass and high intrinsic viscosity. HCl extraction gave low yields of pectins with low uronic acids and DM. Water extraction gave the lowest yields of pectins with high degrees of methylation and high intrinsic viscosity. On the whole, OAAO-extraction of pectins from ambarella provides good yields, which are biochemically interesting due to their high molar mass. Since molar mass and degree of methylation are important parameters in gel-breaking strength (Voragen et al., 1995), OAAO ambarella pectins could be useful as food additives. Further studies are being conducted to investigate the rheological and gelling properties of ambarella peel pectins.

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Original article

Rheological and high gelling properties of mango (*Mangifera indica*) and ambarella (*Spondias cytherea*) peel pectins

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Summary Ambarella and mango peels are good sources of pectins (15–20%), with high degree of methylation (60–78%) and high molar masses. Ambarella and mango (*Améliorée* and *Mango* varieties) peel pectins were extracted using HCl or oxalic acid/ammonium oxalate (OAAO). Purified pectins were analysed for their flow behaviour and phase diagrams were established at pH 3 as sucrose vs. pectin concentration. The gelation kinetics and mechanical spectra of these pectin gels were studied and compared to those of commercial citrus (lime) pectins. At a concentration of 1% (w/v), all pectic solutions had a shear thinning behaviour but at 0.6% (w/v), only OAAO-extracted pectins exhibited such behaviour. Phase diagrams showed that at pH 3, gelation of OAAO mango extracted pectins was possible at low polymer concentration (0.2%; w/w) for a sucrose concentration of 60% (w/w). OAAO-extracted pectins exhibited a higher gelling ability than HCl-extracted ones. Sucrose (45–50%) and pectin (0.2–0.6%) concentration had a deep impact on the gel strength. Our results enable to conclude that the OAAO extraction from mango and ambarella peels allowed the recovery of pectins that exhibit high gelling properties.

Keywords Ambarella, extraction, gelation, mango, peel pectins, rheological properties.

Introduction

Mango (*Mangifera indica*) and ambarella (or golden apple) (*Spondias cytherea*) are tropical fruits, which belong to the family of Anacardiaceae (Morton, 1987a,b). In Cameroon, these fruits are widely eaten by the population between April and July where mangoes are produced (200 000–300 000 tons per year) with an important post-harvest loss (22% of the production) (Koubala *et al.*, 2008b). Processing of mango or ambarella into jam could constitute a way to reduce this loss. These sweet fruits are also used for (i) making juices and purees, (ii) canned in sucrose syrup (Ramsundar *et al.*, 2000), and (iii) sun or osmotic dehydration dried (Jiokap *et al.*, 2001; Kameni *et al.*, 2003). Peels and stones, which are the by-products of these fruits, can be used for animal feeding. It has been shown that these peels are good sources of pectins (Berardini *et al.*, 2005; Koubala *et al.*, 2008a) as are citrus peels and apple pomaces, which are the two main sources of commercial pectins (May, 1990).

Pectins are a family of complex polysaccharides mainly present in the primary cell wall and intercellular regions of dicotyledons (Voragen *et al.*, 1995). Structurally, they are generally described as an alternation of smooth (made of homogalacturonans, HGs) and hairy (made of type I rhamnogalacturonans, RGs-I) regions. HG is composed of (1 → 4)-linked α -D-galacturonic acid (GalpA) residues that can be partly methylesterified at C₆ (Pilnik & Voragen, 1970) and possibly partly acetylated at O₂ or O₃ (Ralet *et al.*, 2002). RG-I's backbone is composed of the repeating disaccharide [→ 4)- α -D-GalpA-(1 → 2) α -L-Rhap-(1 →] and about 20–80% of the rhamnosyl (Rhap) residues are substituted at C₄ with neutral sugars, mainly arabinose and galactose, which constitute the side chains of RG-I (Ridley *et al.*, 2001; Schols & Voragen, 2002). The degree of methylation (DM) and degree of acetylation (DAc) are defined as the percentage of GalA units esterified with methanol or acetic acid respectively. Pectins have many applications in food science and nutrition, cosmetics and pharmacy. They are widely used as food additives for their thickening, gelling and emulsifying properties in jams (May, 1997), soft drinks,

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fish (Barrera *et al.*, 2002), meat and milk products (Ralet *et al.*, 2002). Highly methylated (DM > 50%) pectins gel at low pH (2.5–3.8) with a high sugar concentration (> 55%) (Rolin *et al.*, 2002). Rheological properties of pectin gels are influenced by intrinsic (molar mass, rhamnose content, DM, DAc) and extrinsic (concentrations in polymer and sucrose, pH and temperature) parameters (Rolin *et al.*, 1998). It has been reported that extracting conditions have an effect on the biochemical and rheological properties of pectins (Ralet *et al.*, 1994; Koubala *et al.*, 2008b). Studies on the rheological properties of polysaccharides from mango pulp showed a shear thinning behaviour up to a concentration of 3 g L⁻¹ (Iagher *et al.*, 2002). Buttercup squash fruit pectins extracted with HNO₃ formed only weak gels compared to citric acid-extracted pectins which formed strong gels (O'Donoghue & Somerfield, 2007). It has been shown that *Ceni* and *Springfield* mango peels pectins extracted using HCl (pH 1.5) had good gelling properties (gel strength of 162–232° Sag) compared with water-extracted pectins (Kratchanova *et al.*, 1991).

Recent studies on mango (*Améliorée* and *Mango* varieties) and ambarella peels pectins highlighted the deep impact of extraction conditions on the yield and the biochemical characteristics (uronic acids and neutral sugars content, DM and molar mass) (Koubala *et al.*, 2008a,b). In this work, we present the rheological and gelling properties of mango and ambarella peels pectins extracted with different conditions [HCl and oxalic acid/ammonium oxalate (OAAO)]. The effect of sucrose and pectin concentration on the gelling capacity is also presented. The opportunity of using these pectins as food additives is assessed.

Materials and methods

Sampling

Ambarella and three mango varieties (*Améliorée*, *MangoNord* and *MangoSud*) pectins were extracted from peels using HCl or OAAO as previously described (Koubala *et al.*, 2008a,b). The considered extraction conditions were: hydrochloric acid 0.03 M (pH 1.5) at 85 °C for 1 h, and OAAO 0.25% (pH 4.6) at 85 °C for 1 h. Lime pectins, which were extracted in the same conditions and commercial citrus HM pectin (Danisco, Pectin Cultor, Denmark, DM 70%) were used for comparison. Raw pectins were solubilised overnight in deionised water (1% w/w) under magnetic stirring at 4 °C. The resulting extracts were centrifuged (9000 × g/15 min) (Sigma 3K30; Sigma Laboratory Centrifuges, Osterode am Harz, Germany). Recovered supernatants were filtered on a membrane (3 µm) and were then added to three volumes of ethanol (96%) to precipitate pectins. Pectins coagulum was washed (six

times) with ethanol (72%) and with pure acetone (two times) before drying in a ventilated oven at 40 °C overnight.

Experimental

Chemical characterisation of purified pectin

The uronic acid contents of the extracted pectins were determined colorimetrically by the automated m-phenylphenol method (Thibault, 1979). Neutral sugars were analysed as their alditol acetate derivatives by gas liquid chromatography (GLC) after acid hydrolysis (Blakeney *et al.*, 1983). Pectin methoxyl contents were determined by HPLC. Pectins were treated with 1 N NaOH and the methoxyl released were quantified by HPLC on a C18 column, as previously described by Levigne *et al.* (2002). Pectin average molar masses and intrinsic viscosities were determined using high-performance size-exclusion chromatography (HP-SEC), as described by Ralet *et al.* (2001).

The importance of HG zone and the number and/or length of the neutral sugars (side chains) were assessed using 'AU/Rha' (HG zone) and 'Ara + Gal/Rha' (side chains) ratios, respectively. AU, Rha, Gal and Ara are respectively the uronic acid, rhamnose, galactose and arabinose content of the purified pectin.

Preparation and rheological characterisation of pectins solutions

Pectins were dissolved in deionised water by magnetic stirring overnight at 4 °C. The pH of the solution was adjusted to three with few drops of HCl (0.1 M).

The rheological behaviour of the pectic solutions at two concentrations (0.6% and 1% w/v) was investigated with a controlled-strain rheometer (RFS II, BD022; Rheometrics, Inc., Piscataway, NJ, USA), using a cone/plane geometry (diameter 50 mm, angle 2.29°, gap 50 µm) at 20 °C. Flow measurements were performed between 0.01 and 100 s⁻¹. Dynamic measurements were performed in the range of 0.01–100 rad s⁻¹ at a percentage strain between 10% and 30%, depending on the pectin concentration, so as to remain in the viscoelastic linear domain. The variation of storage (*G'*) and loss (*G''*) modulus (mechanical spectra) was recorded over these pulsation range.

Preparation of the gels and phase diagrams

Aqueous solutions containing different concentrations of mango (*MangoNord*, *MangoSud* extracted with OAAO) peels pectins were prepared. Various quantities of sucrose (40–70% w/w) were added and each mixture was heated at 100 °C for 30 min while stirring. Citric acid (0.5 M) was then added slowly to reach 0.05 M (pH 3) in the final slurry. The slurry was poured into a previously oiled glass tube (5 × 1.1 cm). The surface of the slurry was covered with paraffin oil and the mixture

was left for 16 h at room temperature. After this delay the system was considered to be a sol when it flowed easily. Sol/gel transition was defined at the onset of the meniscus deformation when the tube was held horizontal. Determination of the sol-gel transition was reproducible within less than 5% marginal error.

Rheological characterisation of pectin gels

Rheological properties of pectin/sucrose gels were determined with a Carri-med CSL 100 (CSL100, 105847; Carri-Med Ltd, Dorking, Surrey, UK) controlled stress rheometer, using a temperature-regulated cone-plate device (radius 2 cm; angle $4^{\circ}01'02''$, gap 98 μm). The pectin/sucrose solution was prepared as described above and acidified. The final concentration of sucrose in the slurry was 60% and that of pectins was 0.6% (w/w) at pH 3. The pectin concentration was increased to 1% when the system did not gel. The effect of sucrose and pectin concentrations on the gelling properties of two mango peel pectins was also studied. The slurry was poured onto the rheometer plate heated at 90 °C and the surface of the sample was covered with paraffin oil. The system was cooled at 20 °C before starting rheological measurements.

Kinetics of gel formation at 20 °C were monitored by measuring the storage (G') and the loss modulus (G'') at 1 Hz at 1% deformation for 22–24 h. At equilibrium, mechanical spectra of the set gels were recorded by subjecting the gels to a frequency sweep (0.01–10 Hz) at a constant strain of 1%, well within the viscoelastic linear range.

Statistical treatment

Measurements were performed at least twice. Results were compared using Student's *t*-test, and the differences were determined by variance analysis ($P < 0.05$). Correlation between biochemical and rheological properties of pectins was studied by Pearson correlations ($P < 0.05$).

Results and discussion

Biochemical characteristics of purified mango and ambarella peels pectins

Biochemical characteristics of purified ambarella and mango peels pectins were significantly ($P < 0.05$) different according to the extraction conditions and the source of raw material used (Table 1). HCl extracted pectins have the highest value of neutral sugars (214–259 mg g^{-1}) meanwhile those extracted using OAAO are highly methylated (69–77%) with high molar mass and intrinsic viscosity. Mango peels pectins show good biochemical characteristics whatever the extraction condition used. Compared to unpurified mango and ambarella peels pectins (Koubala *et al.*, 2008a; b),

purification increased the neutral sugar and uronic acid contents, as well as the DM of pectins. Table 1 shows that OAAO extracted pectins have more HG zone than HCl-extracted ones. Compared to ambarella and lime pectins, mango pectins exhibit a particularly high intrinsic viscosity values which means that they have higher DP or molecular weight (Morris, 1995).

Flow behaviour of pectin solutions

The flow behaviour of mango and ambarella peel pectin solutions (1% and 0.6%; w/v) is summarised in Table 2. At 1% concentration for all extracted pectins, viscosities decreased when the shear rate increased evidencing the shear thinning behaviour of the samples, as already noticed by Marcotte *et al.* (2001). The shear thinning behaviour observed is due to the disruption of entanglement of pectin chains at high enough concentration when shearing is applied (Rolin *et al.*, 1998). At 0.6% (w/v), only OAAO-extracted pectin solutions exhibited a shear thinning behaviour whereas HCl-extracted ones showed a newtonian behaviour (not shown). At a given shear rate, highest viscosities were recorded for OAAO-extracted pectins. Commercial and extracted lime pectins exhibited the same flow behaviour but the recorded viscosity values (11 mPa s) at a constant shear rate (10 s^{-1}) are lower than that of *MangoNord* extracted pectin (17 mPa s). These high viscosity values obtained for OAAO-extracted mango pectins could be explained by their high intrinsic viscosity, which is a reflect of their molar mass. Significant correlation ($p < 0.05$) were established between viscosity (at 100 s^{-1}) and the molar mass ($r = 0.77$) (Table 3). According to Rolin *et al.* (1998), at low concentrations ($< 0.5\%$), pectin solutions exhibit generally a newtonian behaviour. It has been reported that viscosity usually increases with polymer concentration (Ganter *et al.*, 1992).

Frequency sweeps performed over a range of 0.01–100 rad s^{-1} at 1% concentration showed that whatever the pulsation, the loss modulus (G'') was higher than the storage modulus (G') (Fig. 1). Both the slopes of the curves and the crossover of G' and G'' values observed at highest pulsation (around 100 rad s^{-1}) demonstrated a macromolecular solution behaviour. Mechanical spectrum obtained with lime pectin was similar to that of mango and ambarella peels pectin. For a given pulsation, values of G' and G'' are well correlated with viscosities. The highest values of G' and G'' were recorded for OAAO mango extracted pectins, which exhibited the highest values of intrinsic viscosity (Table 1).

Phase diagrams of sucrose/pectins systems

The sol/gel transition lines of two pectins/sucrose systems are plotted in Fig. 2. The transition lines for

Table 1 Physico-chemical characteristics (uronic acid and neutral sugar content, degree of methylation (DM), molar mass (M_w), intrinsic viscosity ($[\eta]$) and (Ara + Gal)/Rha and AU/Rha ratios) of some mango and ambarella peel pectins

Plant material	Extractant	Uronic acids (mg g ⁻¹)	Neutral Sugars (mg g ⁻¹)	(Ara + Gal)/Rha (w/w)	AU/Rha (w/w)	DM (%)	M_w (kDa)	$[\eta]$ (mL g ⁻¹)
<i>Améliorée</i>	HCl (pH 1.5)	686 ± 5.5 ^a	259 ± 10 ^a	21	72	65	140	289 ± 4 ⁱ
<i>Mang Nord</i>		713 ± 2.1 ⁱ	207 ± 9 ^b	22	86	75	327	876 ± 64 ^d
<i>MangoSud</i>		748 ± 0.2 ^d	214 ± 12 ^b	26	101	72	297	778 ± 43 ^{de}
<i>Améliorée</i>	OAAO	677 ± 1.1 ^b	230 ± 15 ^b	25	82	69	636	1100 ± 34 ^c
<i>MangoNord</i>		745 ± 8.1 ^d	178 ± 6.6 ^c	23	105	75	656	1680 ± 4 ^a
<i>MangoSud</i>		797 ± 11 ^b	106 ± 1.2 ^f	15	135	77	630	1537 ± 33 ^b
<i>Ambarella</i>	HCl	557 ± 13 ^j	125 ± 3 ^c	3	33	50	110	179 ± 7 ^j
	OAAO	631 ± 7 ⁱ	158 ± 2.7 ^d	5	33	60	370	783 ± 4 ^e
<i>Lime</i>	HCl	776 ± 3.7 ^c	88 ± 1 ^h	9	99	75	112	337 ± 8 ^h
	OAAO	831 ± 16 ^a	97 ± 0.6 ^g	12	120	58	255	757 ± 8 ^f
	Commercial	825 ± 1.1 ^a	79 ± 2 ⁱ	6	79	65	205	614 ± 4 ^g

Mean values from triplicate measurements ± standard deviation. Values in the same column followed by different superscripts are significantly different ($P < 0.05$).

Pectins were extracted using HCl (pH 1.5) or oxalic acid/ammonium oxalate (OAAO) and purified. They are compared to lime extracted pectin and commercial pectin.

Table 2 Flow behaviour of mango and ambarella peels pectins suspensions (0.6 and 1% w/v)

Plant source	Extraction condition	Pectins concentration (w/v)	
		0.6%	1%
<i>Améliorée</i>	HCl (pH 1.5)	N	ST
<i>Mang Nord</i>		N	ST
<i>MangoSud</i>		N	ST
<i>Améliorée</i>	OAAO	ST	ST
<i>MangoNord</i>		ST	ST
<i>MangoSud</i>		ST	ST
<i>Ambarella</i>	HCl	N	ST
	OAAO	ST	ST
<i>Lime</i>	HCl	N	ST
	OAAO	ST	ST
	Commercial	N	ST

N, Newtonian behaviour; ST, shear thinning behaviour.

Pectins were extracted using HCl (pH 1.5) or oxalic acid/ammonium oxalate (OAAO) and compared with commercial (*Danisco*) and laboratory extracted (HCl and OAAO) lime pectins.

these two systems have the same shape. For both samples (OAAO extracted *MangoNord* and *MangoSud* pectins), and above 65% (w/w), the sucrose concentration affects only in a few extend the quantity of pectin needed. No gels can be obtained under 2% (w/w) of pectins for sucrose below 40% (w/w). The mango pectin concentration used to obtain gel is lower than that of Citrus (lime and *Danisco*) pectin. The gel-formation ability of mango peels pectins in the presence of only 45% (w/w) sucrose is probably a result of their high molar mass, their DM and the high 'uronic acid/rhamnose' ratio. *MangoNord* and *MangoSud* mango peels

pectins exhibit the same gelling capacity. This could be related to their similar biochemical characteristics (Table 1). Ralet *et al.* (1994) showed using HCl-extracted lemon pectins, that at pH 3 and 60% (w/w) sucrose, 0.15% (w/w) pectins was necessary to obtain a gel. For mango peels pectins, a concentration of 0.25% (w/w) is necessary to have gelation. Decreasing the pH of the sucrose/pectin system from 3 to 2.5 considerably increased the gelling capacity of pectins (results not shown). The influence of pH on the gelation of high-ester citrus pectin was studied by El-Nawawi & Heikel (1997) who observed that the setting time (reverse gelling capacity) of HM pectins increased (threefold) when the pH changed from 2.1 to 2.9.

Effect of extracting condition on the gelling ability of mango and ambarella peels pectins

Optimum gelling conditions of mango and ambarella peels pectins were determined using results previously obtained by phase diagrams (Fig. 2).

Gelation kinetics of mango and ambarella peels pectins

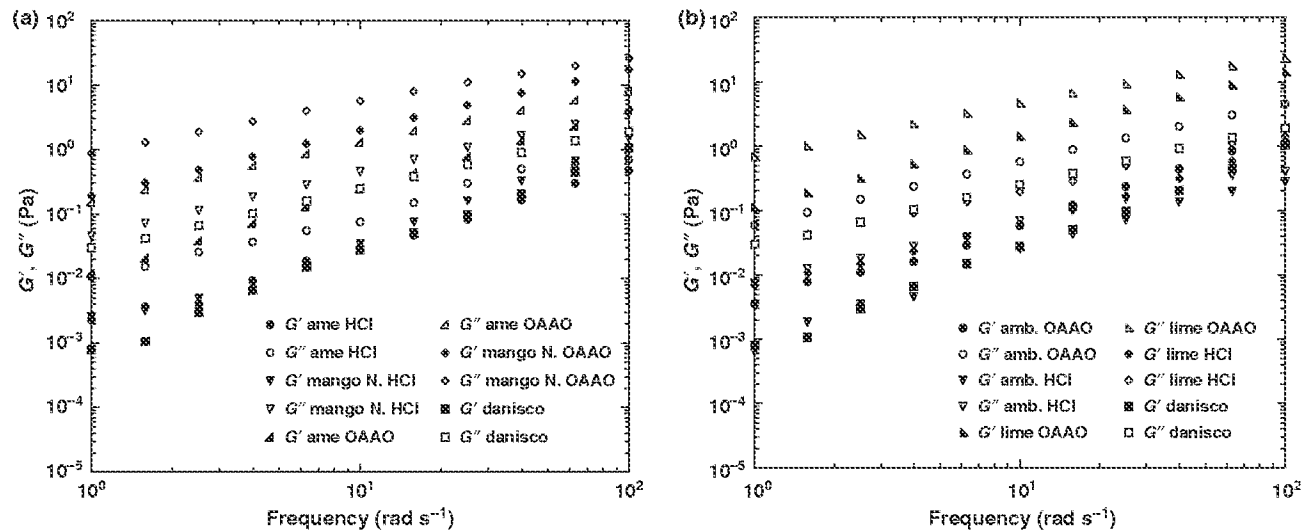
The gelation kinetics of mango and ambarella peels pectins are very different according to the extraction condition and the plant material used (Table 4). Gel systems were formed with 60% (w/w) sucrose and 0.6% (w/w) pectins at pH 3. In all cases, a rapid evolution of storage modulus (G') was recorded during the first minutes followed by a slow evolution before a constant value of G' was reached after 10 or 15 h (not shown). In these conditions, the highest values of G' were recorded for OAAO-extracted pectins (900–1100 Pa), especially for *MangoNord* mango peel pectins (Table 4). Pectin concentration was increased at 1% (w/w) when no

Table 3 Correlation between biochemical (DM, M_w , $[\eta]$, (Ara + Gal)/Rha and AU/Rha ratio) and rheological (flow behaviour and gel strength described as recorded value of G') properties of mango and ambarella peels pectins

Rheological parameters	Biochemical properties				
	DM	M_w	$[\eta]$	(Ara + Gal)/Rha	AU/Rha
Viscosity (Pa.s) of pectins solution at 100 s ⁻¹	0.47*	0.77	0.89	0.52*	0.60
Gel strength G' (Pa) at 0.01 Hz	0.59	0.74	0.88	0.57*	0.74

* No significant correlation. r values are given at $P < 0.05$.

Gel were formed with 0.6% (w/w) pectins and 60% (w/w) sucrose at pH 3.

**Figure 1** Frequency sweep of mango (Ame = *Améliorée*, *MangoNord*) (a) and ambarella (Amb) (b) peels pectins at a concentration of 1% (w/v) compared to lime and commercial (*Danisco*) pectins. Pectins were extracted using HCl or OAAO.

gelation occurred at 0.6% (w/w). At a pectin concentration of 1% (w/w), commercial citrus pectin exhibited high gelling properties ($G' = 1300$ Pa). No gelation was observed with HCl-extracted ambarella pectin, even at 1% (w/w) concentration. Since the intrinsic viscosity of pectin is tightly correlated with its gelling capacity (Table 3), one of the reasons of the weak gelation of HCl-ambarella pectin could be its low intrinsic viscosity (179 mL g⁻¹). This pectin shows also a low content of galacturonic acid (557 mg g⁻¹) coupled with a low DM value (50%), all these factors being known to badly affect the gelation process in presence of sugar and in acidic conditions. The highest gelling ability of OAAO-extracted mango peels pectins could be explained by their high molar masses, DM and probably their extended side chains characterised by their high intrinsic viscosity (Table 1). In agreement with Voragen *et al.* (1995) and Biton (2002), these highly methylated (71–77%) OAAO mango pectin are said to be rapid or ultra-rapid set (3–7 min). Löfgren & Hemansson (2007)

noticed that to have the best gelling ability, HM (DM: 72%) lime pectins should be used at high concentrations ($\geq 0.8\%$) (w/w). With *Mango* mango peel pectins extracted using OAAO, a good gelling ability was obtained at 0.6% (w/w). Considering the same source of pectin, the gelling ability of pectins is different according to the extractant used. This was also the case of HCl-extracted lime pectins which formed stronger gels compared to water-extracted lime pectins after extrusion cooking (Ralet *et al.*, 1994).

Study of the gels strength

Figure 3 presents the evolution of storage (G') and loss (G'') moduli at various frequencies (0.01–10 Hz) for mango and ambarella peel pectin systems compare with lime and commercial pectins. Nearly all sucrose/pectins mixtures evidenced gel formation (except for Ambarella-HCl), with G' higher than G'' at all frequencies and a slight dependence of G' with frequencies. The higher the value of G' , the stronger are the formed gels formed with

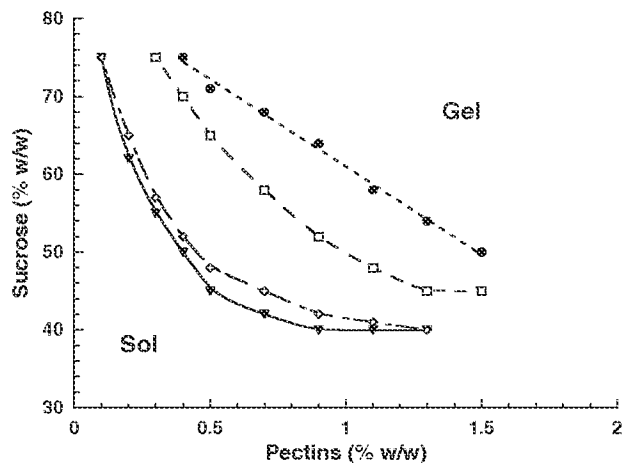


Figure 2 Phase diagrams at pH 3 of *MangoNord* (▼) and *MangoSud* (◇) peels pectins compared to lime pectin (□), extracted with OAAO, and to commercial citrus pectin (●). The critical pectin concentration for gelation is determined as a function of sucrose concentration (w/w %).

mango and ambarella peel pectin. The strongest gels ($G'/G'' = 13\text{--}21$) were obtained with OAAO-extracted mango pectins (0.6%). Figure 3a shows that mechanical spectrum of *MangoNord* pectin (0.6% w/w) gel presents the same characteristic (evolution of G' and G'') as that of gel obtained with 1% of commercial lime pectins. HCl-extracted mango peels pectins formed, at the same concentration, stronger gels than commercial citrus pectin and citrus pectins extracted in the same condition. No gel was formed using 1% (w/w) HCl ambarella peel pectins ($G' < G''$). Whatever the extractant used, *Améliorée* mango peel pectins formed weak gels compared to *MangoNord* mango peel pectins. Compared to mango peels pectins, ambarella peels

pectins exhibited lower gelling properties. The slight increase in G' and the high G'/G'' ratio, observed for the mechanical spectra in the frequency range explored, showed that most of these systems are viscoelastic solids referred to as true gels (Clark & Ross-Murphy, 1987). Statistical analyses showed a significant correlation ($r = 0.59\text{--}0.74$) at $P < 0.05$ between some biochemical characteristics (DM, M_w and AU/Rha) of pectins and gels strength (recorded G') (Table 3). This confirms that HG proportion and molar mass are important parameters for the gelation of mango and ambarella peels pectins.

Effect of pectin and sucrose concentration on the gelling properties of ambarella and mango peel pectins

Effect of pectin concentration
Pectin concentration affected considerably the gelling ability of mango peel pectins as shown in Fig. 4a. Gels could be formed at pH 3 for 60% (w/w) of sucrose and at pectin concentrations ranging from 0.2% to 0.6% (w/w). With 0.6% of pectins, the gels were formed within few minutes after cooling the system at 20 °C. When using 0.2% pectins, there was a slow evolution of storage modulus (G') that reached a constant value 5 h after gelation began (figure not shown). At high pectin concentrations (0.6%), *MangoNord* pectins exhibited the best gelling properties. Since the number and length of neutral sugars side chains are also important in pectins gelation, this could be due by the fact that *MangoNord* pectins (178 mg g^{-1} ; 1680 mL g^{-1}) have high neutral sugar contents compared to those of *MangoSud* (106 mg g^{-1} ; 1537 mL g^{-1}) and also higher intrinsic viscosities. For both pectins, at equilibrium, values of G' decreased 5-fold when using 0.2% of pectins instead of 0.6% (w/w).

Plant source	Extractant	G' (Pa)		
		At 10 min	At 1 h	At 20 h
<i>Améliorée</i>	HCl	0.3 ± 0.01^i	0.4 ± 0.01^i	1 ± 0.01^i
<i>MangoNord</i>		100 ± 8^d	207 ± 9^d	558 ± 10^d
<i>MangoSud</i>		150 ± 7^c	292 ± 10^c	584 ± 5^c
Ambarella		0.2 ± 0.01^k	0.25 ± 0.0^j	0.8 ± 0.01^i
Lime		4 ± 0.2^g	52 ± 7^f	228 ± 10^f
Lime	Commercial	2 ± 0.01^i	2.5 ± 0.2^h	4 ± 0.1^h
<i>Améliorée</i>	OAAO	9 ± 1^i	44 ± 4^i	252 ± 7^c
<i>MangoNord</i>		300 ± 11^a	674 ± 15^a	1185 ± 13^a
<i>MangoSud</i>		170 ± 7^b	403 ± 6^b	873 ± 25^b
Ambarella		3 ± 0.1^h	3.5 ± 0.2^g	5 ± 0.01^g
Lime		73 ± 3^c	135 ± 7^c	573 ± 15^{cd}

Mean values from triplicate measurements $\pm$ standard deviation. Values in the same column followed by different superscripts are significantly different ($P < 0.05$). The gels were formed at pH 3, 60% (w/w) of sucrose for 0.6% (w/w) of pectins from mango (*Améliorée*, *MangoNord*, *MangoSud*) or ambarella peels. They are compared with commercial (Danisco) pectins and laboratory extracted lime pectins. Pectins were extracted using HCl or OAAO

Table 4 Gelling capacity of mango and ambarella peels pectins expressed by values of storage modulus (G') obtained at 10 min, 1 h and 20 h after the beginning of gelation kinetics (20 °C).

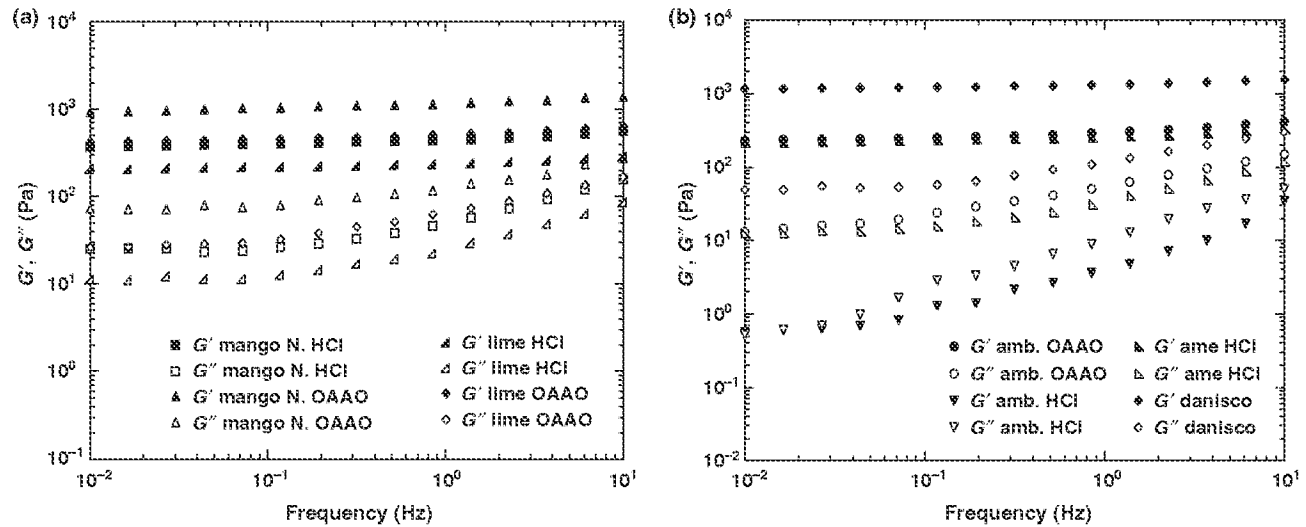


Figure 3 Frequency sweep of mango (Ame = Anéliorée, MangoNord) and ambarella (Amb.) peels pectins gel compared to lime and commercial (Danisco) pectin. Extracted pectins were used at 0.6% (w/w) (a) and 1% (b) against 60% (w/w) of sucrose concentration at pH 3.

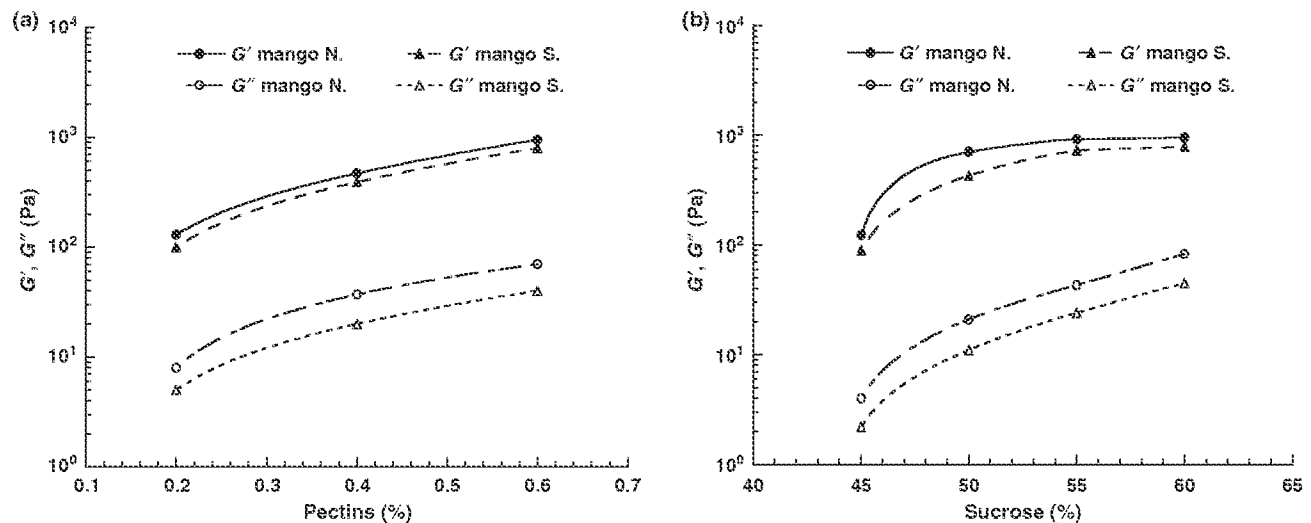


Figure 4 Evolution of storage modulus (G') with OAAO pectins (from Mango N. = MangoNord; Mango S. = MangoSud) or sucrose concentration at pH 3. Pectins concentration varied from 0.2% to 0.6% (w/w) when sucrose was fixed at 60% (w/w) (a). Sucrose concentration varied from 45% to 60% (w/w) while pectins was constant at 0.6% (w/w) (b).

Effect of sucrose concentration

The gelation kinetics of 0.6% mango (MangoNord and MangoSud) peels pectins also varied according to the sucrose concentration used. At high sucrose concentration (60–50%; w/w), MangoNord pectins exhibited the best gelling ability. At lower concentration of sucrose ($\leq 45\%$), no difference in gelling ability was observed between the two mango peels pectins. Results suggest that to form gels, OAAO mango peels need lower

($\leq 55\%$) quantities of sucrose. This is well demonstrated in Fig. 4b where values of G' varied slightly at higher sucrose concentration. This observation is in agreement with Ralet *et al.* (1994) who noticed that for water and HCl-extracted lime pectins, G' increased markedly from 45% to 55% (w/w) of sucrose and remained constant at high sucrose concentration (55–60%). If sugar concentration has an effect on the gelling properties of pectins, the type of sugar also influences the gelling ability of

pectins: G' values of glucose gels are higher than those of fructose gels (Rao & Cooley, 1994). Evageliou *et al.* (2000) also observed that replacement of sucrose by glucose or fructose caused large changes in gelation of HM pectin at acidic pH, in the order fructose < sucrose < glucose.

Conclusion

Our study allows concluding that HM mango and ambarella peel pectins exhibited a shear thinning behaviour at 1% (w/v) concentration. With respect to plant source and extraction conditions, OAAO-extracted *Mango Nord* peels pectins showed the best viscoelastic properties under various shear rates even compared to the citrus commercial pectins (*Danisco*). This is correlated to the higher values of intrinsic viscosity of mango pectins. Only 0.2% (w/w) of OAAO mango pectins in the presence of 60% (w/w) of sucrose at pH 3 was needed to form strong gels. OAAO-extracted pectins exhibited better gelling properties ($G' = 900\text{--}1100$ Pa) than HCl-extracted pectins ($G' = 600$ Pa). Whatever the extraction condition used, gels formed using mango peels pectins were stronger than those formed with ambarella peels pectins. The variation of pectin concentration markedly affected the gels strength. OAAO extraction conditions could be used in food industries to recover high yields (Koubala *et al.*, 2008a,b) ultra rapid set pectins.

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Microwave-assisted extraction of lime pectin[☆]

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Abstract

Pectin was extracted from lime flavedo, albedo and pulp by employing microwave-assisted extraction (MAE) under pressure. Heating times ranged from 1 to 10 min. Optimal time of heating was 3 min. Molar mass, viscosity, radius of gyration and hydrated radius were found to decrease with heating time. At 3 min heating time, depending on the lime fraction which was extracted, weight average molar mass ranged from about 310,000 to 515,000 Da, and weight average intrinsic viscosities ranged from about 9.5 to 13 dL/g. Pectins dissolved in 0.05 sodium nitrate were characterized by HPSEC with online light scattering, dynamic light scattering and viscosity detection. Molar mass polydispersity passed through maximum at 4 min. Molecules of pectin became less compact with increasing heating time. The results obtained here are consistent with previous evidence that extracted pectins may exist in solution as networks, partially formed networks, i.e. branched molecules or linear molecules depending on extraction conditions and the solvent in which they are dissolved.

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Keywords: Lime pectin; Microwave; HPSEC; Light scattering; Viscosity; Molar mass; Radius of gyration; Hydrated radius

1. Introduction

Pectin is a polysaccharide consisting mostly of two moieties (Carpita & McCann, 2000). These are homogalacturonan, (1→4) linked, α -D-galacturonic acid and its methyl ester; and rhamnogalacturonan I, (1→2) repeating linked, α -L-rhamnose-(1→4) α -D-galacturonic acid disaccharide. Rhamnogalacturonan I contains arabinan, galactan and arabinogalactan side chains. These monosaccharide units comprise most of sugar units found in pectin.

Pectin is found ubiquitously in the epicarp (flavedo), mesocarp (albedo) and endocarp (edible portion) of the lime. After the juice is squeezed out of the lime, the insoluble material which remains is called pulp. Previously, we have determined the weight average molar mass (M_w) and intrinsic viscosity [η_w] and the z-average radius of gyration (R_{gz}) as a function of heating time for the

microwave-assisted extraction (MAE) of pectin from lime albedo (Fishman, Chau, Coffin, & Hotchkiss, 2003). Changes in M_w , η_w and molecular shape with heating time could be rationalized by the hypothesis that continued heating of pectin caused the disaggregation of pectin networks into its component parts.

Typically, in pectin manufacture by the food industry, the whole peel is extracted (Braddock, 1999). In this study we fractionated limes into flavedo, albedo and pulp with the juice and seeds removed. The pectin molecular properties from each fraction were characterized by HPSEC with online static and dynamic light scattering (DLS) and viscosity detection. Also galacturonate, degree of methyl esterification and neutral sugar content were determined for each fraction. In addition, pectin from the albedo was characterized by offline multiangle DLS.

2. Methods

2.1. Materials

Fresh flavedo, albedo and pulp were obtained from tropical seedless limes which were grown in Florida. Upon

[☆] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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arrival, the flavedo was stripped from the skin with a potato peeler, followed by removal of the albedo with a paring knife. The juice was separated from the pulp by placing the endocarp in an electric juicer. The particle sizes of the flavedo, albedo and pulp were reduced by grinding in a Cuisinart mini prep plus processor. Prior to extraction, the pulp was washed three times with 1 L of HPLC-grade water per 100 g of pulp to remove extraneous material which might be present. Excess water was removed by squeezing it out through miracloth. The washed pulp was stored in sealed polyethylene bags at -20°C until extraction.

2.2. Extraction

Lime pectin was extracted by a patented MAE procedure (Fishman & Chau, 2000; Fishman, Chau, Hoagland, & Ayyad, 2000). Samples were heated in a CEM, model MDS-2000 microwave oven. Flavedo, albedo or pulp (F, A or P) was irradiated at a frequency of 2450 MHz with 630 W of power. The slurried sample (1 g) was dispersed in 25 mL of pH 2, HCl and placed in each of six microwave transparent pressure vessels. One vessel was equipped with devices which sensed and controlled pressure and temperature. Heating times ranged from 1 to 10 min. Pressures were not allowed to exceed $50 \pm 2 \text{ lbs/in}^2$. The temperature at that pressure was about 140°C . The change of temperature and pressure with heating time has been published previously (Fishman, Chau et al., 2003). After heating, the samples were allowed to cool for half an hour at room temperature and filtered through miracloth. The filtrate was precipitated with 70% isopropanol (IPA), separated from the mother liquor, washed first with 70% IPA followed by 100% IPA. Then, the sample was dried under vacuum without heating and stored in a refrigerator until analyzed.

2.3. Chromatography

Dry sample (2 mg/mL) was dissolved in mobile phase (0.05 M NaNO_3), centrifuged at 50,000 g for 10 min and filtered through a 0.22 or 0.45 μm Millex HV filter (Millipore Corp., Bedford, MA). The flow rate for the solvent delivery system, model 1100 series degasser, auto sampler and pump (Agilent), was 0.7 mL/min. The injection volume was 200 μL . Samples were run in triplicate. The column set consisted of two PL Aquagel OH-60 and one OH-40 size exclusion columns (Polymer Laboratories, Amherst, MA) in series. The columns were in a water bath set at 45°C . Two HPSEC chromatographs were employed. These differed only in the column effluent detectors used. One chromatograph was fitted with a Dawn DSP multi-angle laser light scattering photometer (MALLS) (Wyatt Technology, Santa Barbara, CA), model 100 differential pressure viscometer (DPV) (Viscotek Corp., Houston TX) and an Optilab DSP interferometer (RI) (Wyatt Technology). Electronic outputs from the 90° light scattering angle,

DPV and RI were sent to one directory of a computer for processing with TRISEC software (Viscotek Corp.). Electronic outputs from all the scattering angles measured by the MALLS, DPV and RI were sent to a second directory for processing with ASTRATM software (Wyatt Technology). The other chromatograph was fitted with a model PD2020/DLS two-angle static light scattering photometer (TALLS) (Precision Detectors, Franklin, MA) installed in a model 2410 differential refractometer (RI) (Waters Inc., Milford, MA). The PD2020 was also fitted with add on, 90° DLS module permitting simultaneous measurement of static and DLS. The electronic outputs from the RI, static and DLS modules were sent to a computer and processed by PrecisionAcquire32 and PrecisionAnalyze software.

2.4. Multi-angle DLS

Offline, batch-mode multiangle DLS measurements have been described previously (Farrell et al., 1996). Briefly, measurements were with a Malvern System Model 4700c equipped with a 256-channel correlator. Light at 488 nm was generated by a Spectra Physics Model 2020, 5-W laser. DLS measurements were made at 30° , 60° , 90° , 120° and 150° . The data were processed by Malvern Automeasure, V. 4.12 software. Multi-angle analysis was performed with Malvern software (Cummings & Staples, 1987). System performance and analysis software were tested on 91 and 455 nm diameter beads and found to be accurate within $\pm 2\%$.

2.5. Compositional analysis

Anhydrogalacturonate content (%G) was determined by the Sulfamate/3-Phenylphenol Colorimetric Method (Fillisetti-Cozzi & Carpita, 1991) as modified by Yoo, Fishman, Savary, and Hotchkiss Jr. (2003). Degree of methyl esterification (%DE) was determined by an HPLC method developed by Voragen, Schols, and Pilnik (1986). Neutral sugar content (%NS) was determined with the Phenol-Sulfuric Acid Colorimetric Method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

3. Results and discussion

3.1. Basis of MAE

In general, advantages of MAE of pectin in a closed vessel include rapid heating time (localized super heating), control of temperature and pressure, higher temperatures, higher pressures and negligible volatility of solvents.

The kinetics of heating for samples extracted by microwave energy is determined by the $\tan \delta$ of the sample. $\tan \delta$ is the ratio of the dielectric loss, ϵ'' , to dielectric constant, ϵ' , of the sample (Ness & Collins, 1988).

$$\tan \delta = \epsilon''/\epsilon' \quad (1)$$

The value of κ'' is a measure of a sample's ability to obstruct microwave energy as it passes through the sample whereas κ'' is a measure of a sample's ability to dissipate that energy (i.e. convert electromagnetic energy into heat energy). Ionic conduction and dipole rotation are the most important mechanisms for producing dielectric loss. Thus, the speed of heating increases with increasing rapidity of dipole rotation and ionic conduction.

Preliminary experiments on albedo dispersed in pure water, revealed that no pectin could be extracted by MAE under pressure (data not shown). These experiments appear to indicate that rapid MAE of pectin occurs by conventional acid extraction. Heated acid solubilizes pectin and other components held in the cell wall. First, relatively weak forces such as ionic or hydrogen bonds are broken, followed by covalent bonds such as glycosidic linkages. Acid also hydrolyzes pendent ester groups attached to pectin. Rapid rotation of water dipoles coupled with the ionic conductance of H^+ and Cl^- ions provides a solvent with high dielectric loss capable of being rapidly heated. The advantage of rapid heating and extraction (minutes) over conventional slow extraction (i.e. half an hour or longer) is that fewer co-valent bonds are broken. This in turn produces pectin with higher molar mass and viscosity (Fishman, Chau et al., 2000; Fishman, Chau, Kolpak, & Brady, 2001). As further evidence for support of the rapid heating hypothesis, high molar mass and viscosity pectin can be obtained by extracting pectin under conditions of rapid heating under pressure by steam injection (Fishman, Walker, Chau, & Hotchkiss, 2003).

We also conducted preliminary studies (data not shown) on the effect of pH at the start of heating on the molar mass and intrinsic viscosity of the pectin extracted. The heating time was 3 min. We found that yield was inversely related to increasing the pH from 1 to 3 whereas molar mass and intrinsic viscosity was increased more or less proportionately with increasing pH. Thus we chose pH 2 to obtain optimal yield with high molar mass and intrinsic viscosity.

3.2. Dry matter and pectin distribution of fractions

Table 1 contains the wet and dry weight of the three lime fractions. About 71% of the lime is volatile under vacuum. The albedo contains about 40% dry matter whereas the flavedo contains about 36% and the pulp about 21%. Table 2 contains pectin recovered as percentage of the dry weight of the starting material. The relatively low percentages of dry weight and pectin in the flavedo are probably due to a significant amount of volatile organics in that tissue.

3.3. Anhydro-galacturonate and neutral sugar composition of albedo, flavedo and pulp

The compositional data for albedo, pulp and flavedo are in Table 3. There appears to be no consistent trends with

Table 1
Weight distribution of fractions

Fraction	Wet weight (g)	Dry weight (g)	% Dry weight
Albedo	66.65	26.52	39.78
Pulp	134.2	28.28	21.07
Flavedo	60.35	21.73	36.01
Total	261.2	76.53	96.86

Table 2
Weight percentage of pectin recovered

Time (min)	Albedo	Pulp	Flavedo	Total
2.5	6.2 (0.1) ^a	6.0 (0.1)	1.1 (0.5)	13.1(1.0)
3.0	5.1 (0.8)	8.2	1.3 (0.2)	14.6
4.0	4.6 (0.8)	10.0	3.8 (3.3)	17.9
6	5.2	3.4 (1.0)	3.2 (0.2)	11.8
10	2.3 (0.4)	3.4 (0.9)	2.1 (0.3)	8.6(1.0)

^aStandard deviation of triplicate analysis.

time of heating or fraction for the variables measured. Thus, over the time range of 2.5–10 min, compositional values appear to be independent of extraction time. The mean percentages are: neutral sugar (%NS), 7–10, anhydrogalacturonic acid (%G), 84–89 and degree of methylesterification %DE, 59–75.

3.4. Molecular properties of albedo, flavedo and pulp

Previously, Fishman et al.(2001) found that pectins tended to be somewhat less aggregated in lithium acetate/ acetic acid buffer than in $NaNO_3$ solution. In $NaNO_3$, the molar mass of pectin is about 10–20% larger for high methoxyl pectin than for pectin in the lithium buffer. Unfortunately, over a period of time, the lithium buffer, unlike $NaNO_3$, was found to corrode the stainless steel capillaries of the on-line viscometer detector. Since the simultaneous measurement of intrinsic viscosity and molar mass was considered to be essential in studying changes in aggregation, we chose to abandon the lithium buffer in favor of $NaNO_3$. Furthermore, as is shown in this study and in two others (Fishman, Chau et al., 2003; Fishman, Chau et al., 2000), the molar mass of less aggregated high methoxyl pectin is appreciably lower than 280,000 found with use of the lithium buffer system. Thus, the somewhat higher value obtained in $NaNO_3$ over lithium buffer should not affect conclusions concerning the structure of less aggregated pectin.

Molar masses (M) and radii of gyration (R_g) were determined by three different methods involving online static light scattering in conjunction with HPSEC. The LSV method combines light scattered at one angle (90°) and online viscometry to obtain M and R_g (Fishman, Doner, Chau, & Hoagland, 2000). TALLS uses light scattered at two angles (90° and 15°) (Mourey & Coll,

Table 3
Composition of pectin

Time (min)	Albedo			Pulp			Flavedo		
	%DE ^a	%G ^b	%NS ^c	%DE	%G	%NS	%DE	%G	%NS
2.5	66 (1) ^d	88 (2)	6 (1)	88 (1)	72 (2)	9 (1)	70 (1)	81 (1)	11 (1)
3	64 (1)	89 (2)	11 (1)	58 (1)	92 (4)	5 (1)	59 (1)	88 (4)	13 (2)
4	66 (1)	86 (2)	8 (1)	79 (1)	80 (6)	10 (1)	53 (2)	92 (3)	5 (1)
6	73 (1)	85 (4)	10 (1)	87 (1)	81 (4)	8 (1)	56 (2)	89 (6)	12 (1)
10	72 (1)	91 (3)	9 (1)	65 (1)	93 (11)	5 (1)	56 (1)	94 (2)	10 (1)
Mean	68 (4)	88 (2)	9 (2)	75 (13)	84 (9)	7 (2)	59 (7)	89 (5)	10 (3)

^aPercentage degree of methylesterification.

^bPercentage anhydrogalacturonate.

^cPercentage neutral sugars.

^dStandard deviation of triplicate analysis.

Table 4
Molar masses of lime fractions^a

Time (min.)	Albedo		Pulp		Flavedo	
	$M_w \times 10^{-3}$	M_w/M_n	$M_w \times 10^{-3}$	M_w/M_n	$M_w \times 10^{-3}$	M_w/M_n
2.5	335 (8) ^b	1.31 (0.01)	539 (7)	1.13 (0.01)	354 (4)	1.47 (0.03)
3	311 (1)	1.38 (0.03)	515 (3)	1.15 (0.01)	310 (1)	1.61 (0.05)
4	96.9 (1)	1.90 (0.05)	163 (4)	1.42 (0.01)	188 (2)	1.86 (0.04)
6	28.1 (0.2)	1.41 (0.02)	34.7 (0.7)	1.40 (0.05)	42.8 (3)	1.82 (0.3)
10	16.5 (0.3)	1.27 (0.01)	13.0 (0.7)	1.10 (0.01)	17.1 (0.4)	1.38 (0.06)

^aMeasured by MALLS.

^bStandard deviation of triplicate analysis.

1995), and MALLS uses light scattered at 16 angles (14.4°, 25.9°, 34.8°, 42.8°, 51.5°, 60.0°, 69.3°, 79.7°, 90.0°, 100.3°, 110.7°, 121.2°, 132.2°, 142.5°, 152.6° and 163.3°) (Wyatt, 1993).

All three methods showed that molar mass decreased with time of heating. M_w values measured by the MALLS method are in Table 4. When the weight average molar mass (M_w) was below 10^5 , all three methods gave about the same values for M_w . In the range $1-2 \times 10^5$, values from MALLS and LSV were in good agreement but values from TALLS were problematical. In that range TALLS values were found to be higher, lower or about the same as MALLS values. Above 2×10^5 , MW values from LSV were found to be higher than MALLS values whereas TALLS values were found to be lower except in one case where there was good agreement between TALLS and MALLS values.

In Fig. 1, M_w is plotted against heating time during extraction. As mentioned above, molar mass decreased with increasing heating time, for all three fractions. Pulp had a substantially higher molar mass than the other two fractions at heating times of 2.5 and 3 min. At heating times of 4 min or more, molar masses for all three fractions were fairly close. As shown by the data in Table 4 and Fig. 2, the pulp had the lowest polydispersity (M_w/M_n) in the heating range of 2.5–4 min and the lowest molar mass

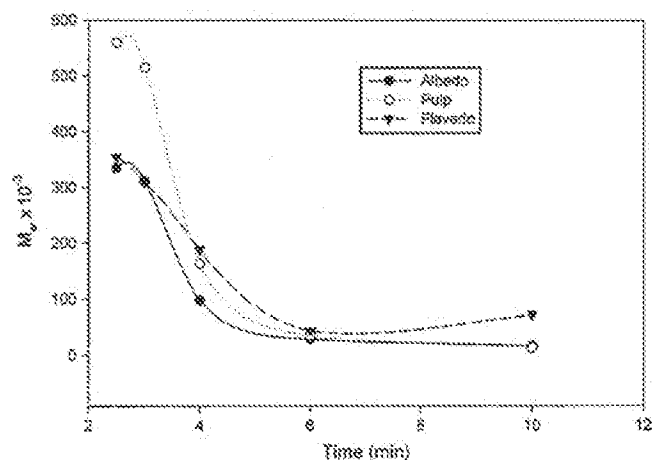


Fig. 1. Dependence of molar mass on heating time for pectin from the albedo, pulp and flavedo.

at 10 min of heating. Furthermore, the polydispersity passes through a maximum with heating time for the three fractions. These results are consistent with a previous extraction study (Fishman, Chau et al., 2003) which indicates that at heating times of less than 3 min, extracted pectin is high in molar mass, aggregated and narrow in polydispersity. As heating time increases beyond 2.5 min,

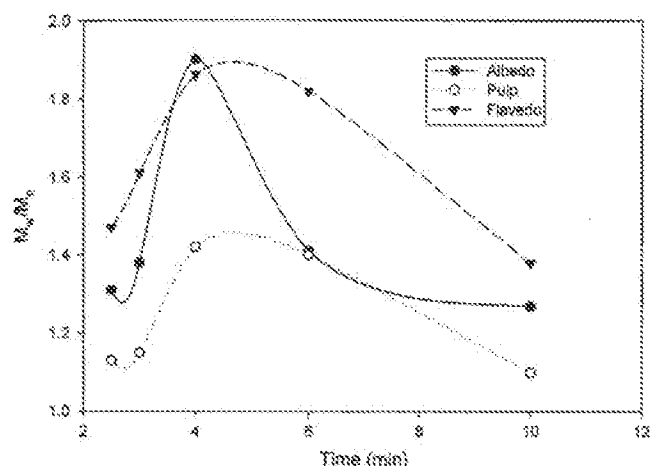


Fig. 2. Dependence of polydispersity of molar mass on heating time for pectin from the albedo, pulp and flavedo.

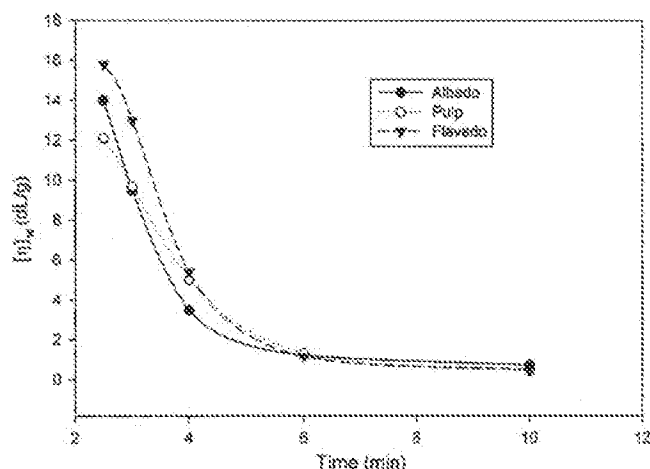


Fig. 3. Dependence of intrinsic viscosity on heating time for pectin from the albedo, pulp and flavedo.

pectin which is in solution is dissociated. The polydispersity broadens as mixtures of different sized aggregates form and reach a maximum between 4 and 5 min. Upon further heating, the aggregates are broken down into their component parts which are of comparable size. After 10 min of heating, mostly component parts are present and distributions are once again narrow.

It is of interest to note that unlike conventional methods of extraction, microwave extraction under pressure, produces high methoxyl pectin with a wide range of molar masses (see Tables 3 and 4).

Intrinsic viscosities, $[\eta]_w$, are plotted against time of heating for the three lime fractions (Fig. 3). At 3 min heating time, the order of $[\eta]_w$ values was flavedo > pulp = albedo. As was the case with M_w , there is a large decline in $[\eta]_w$ between 2.5 and 4 min.

The radii of gyration (R_{gz}) for the three lime fractions measured by MALLS are plotted against time in Fig. 4. R_{gz} follows a first-order linear regression with heating time. All

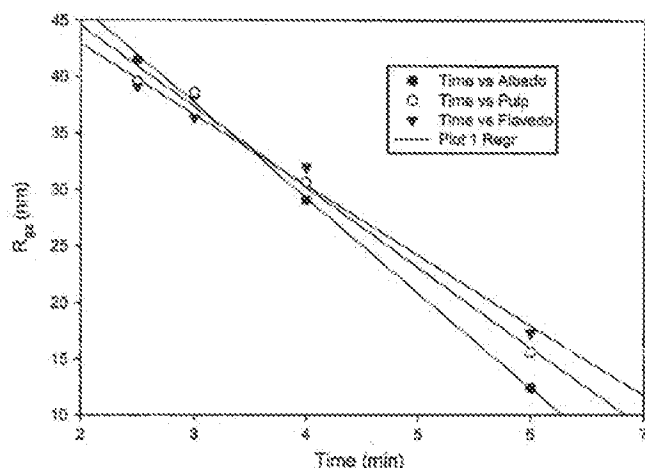


Fig. 4. Dependence of radius of gyration on heating time for pectin from the albedo, pulp and flavedo.

three lines have correlation coefficients of 0.99 or greater. At 3 min heating time, the order of R_{gz} values is pulp = albedo > flavedo. At 10 min R_{gz} values were too small to be measured by MALLS.

Fig. 5A-C are Mark-Houwink (M-H) plots at heating times ranging from 2.5 to 10 min for albedo, pulp and flavedo. These data were obtained by the LSV method. Previously we have shown for orange pectin obtained by steam injection, that there was a linear correlation between M_w values obtained by the LSV method and the MALLS method (Fishman, Walker et al., 2003). Furthermore, the LSV method gave higher M_w values than the MALLS method. Nevertheless, because of the linear correlation between the two sets of M_w values, we expect that the slope of the M-H plots would be fairly independent of the set of molar mass values which were used. Only the intercepts might differ significantly. In Fig. 6, we have plotted M_w from the LSV method against M_w from the MALLS method. Again, we have found a high linear correlation between the two M values (i.e. $r^2 = 0.96$). The only difference between this study and the previous one was that the slope in Fig. 6 was 1.57 rather than 3 which was found previously. A possible explanation for this difference in slopes is that there is a higher heterogeneity in molecular shapes for extracted pectins from orange than there is for extracted lime pectins. Previously, we have found that orange pectin is more highly branched than lime pectin (Fishman, Chau et al., 2003) and that the M_w values from the LSV method tend to diverge from M_w values from the MALLS method for samples which are mixtures of heterogeneous shapes (Fishman, Walker et al., 2003). In Fig. 5, the aggregate plots for each fraction show a downward curvature over the entire heating range indicating the presence of more than one shape. Also, as the heating time increases, the molar masses of the distribution cover a lower range of M_w values. In Fig. 7, the M-H exponents against M_w obtained from MALLS for the pectin in the three plant fractions. The data in Fig. 7

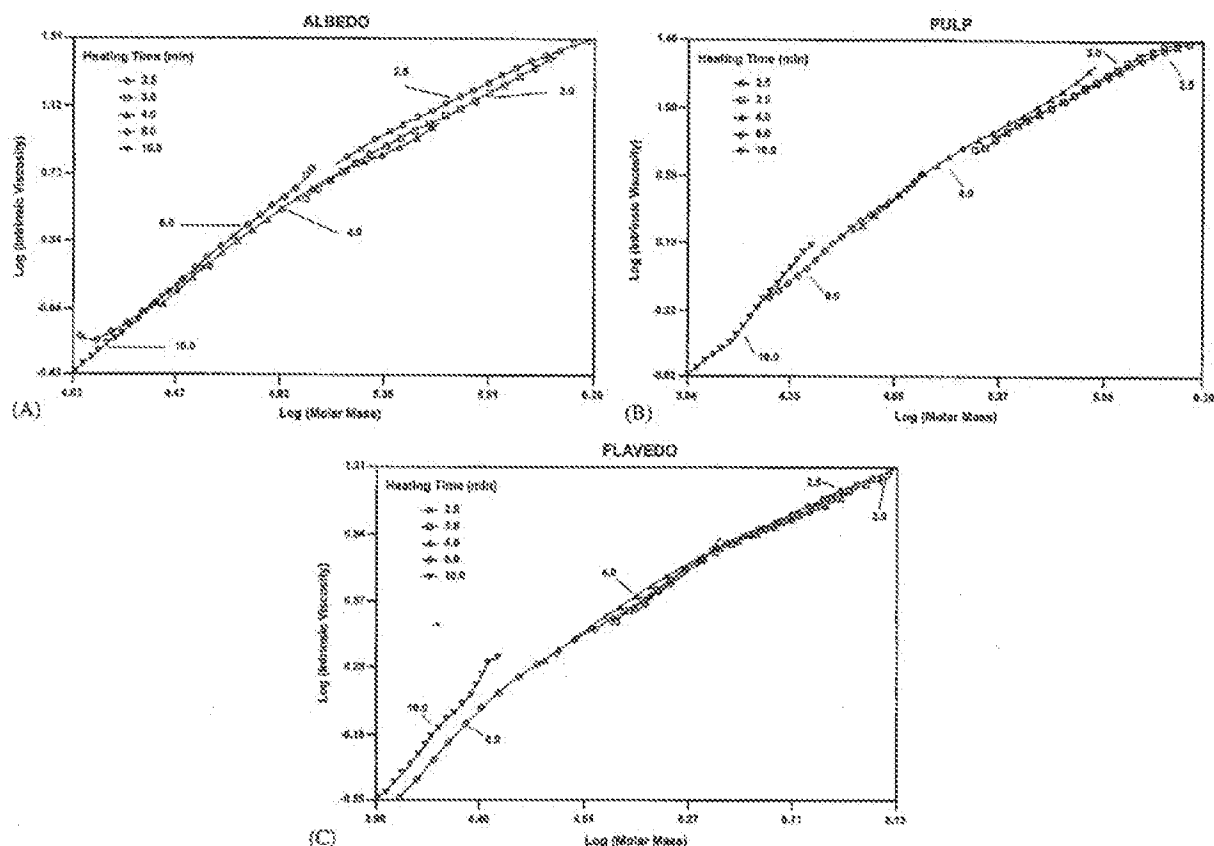


Fig. 5. (A) Dependence of Mark-Houwink (M-H) plots on heating time for pectin from the albedo, (B) dependence of M-H plots on heating time for pectin from the pulp and (C) dependence of M-H plots on heating time for pectin from the flavedo.

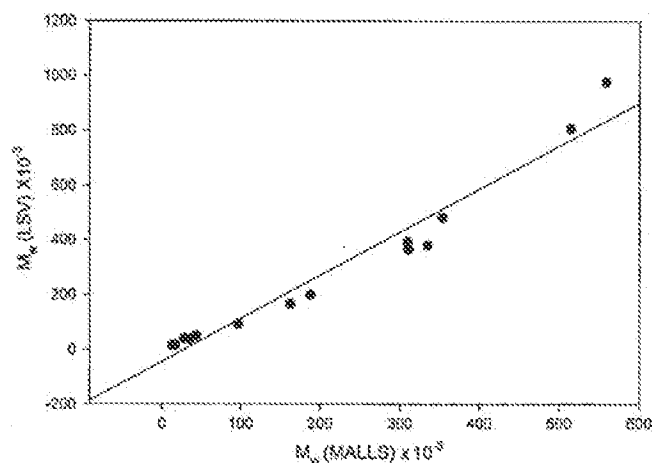


Fig. 6. Correlation of molar mass between MALLS and LSV methods for obtaining molar mass.

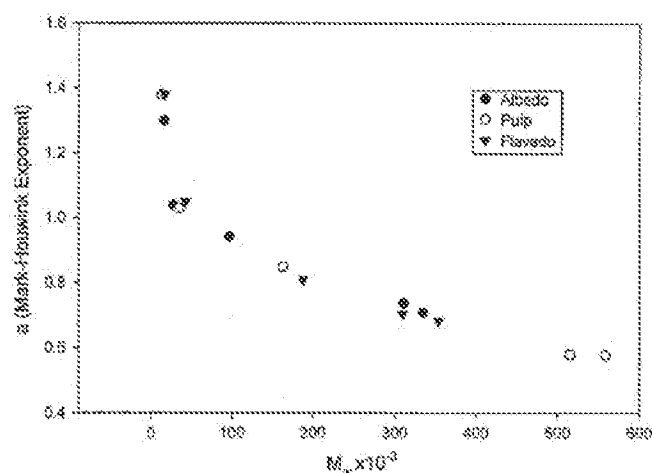


Fig. 7. Dependence of Mark-Houwink exponents on molar mass for pectins from the albedo, pulp and flavedo.

revealed that the " α " values decrease with increasing M_w . This trend is consistent with the concept that the aggregates tend to occupy less space per unit of molar mass than the partially or fully dissociated components of the aggregates. Furthermore, the data are consistent with concepts obtained from electron microscope images (Fishman, Cooke, Hotchkiss, & Damert, 1993; Fishman, Cooke, Levaj, & Gillespie, 1992). Namely, that pectin can exist in

solution as networks, partially formed networks (i.e. branched molecules) and linear molecules (i.e. rods, segmented rods and kinked rods). In fact, this progression of states is the reverse of network formation as described for polymer gels (deGennes, 1979).

In Fig. 8, we have constructed a dynamic Zimm plot, by plotting the z -average hydrated molecular diameter obtained from DLS measurements of albedo pectin heated

for 2.5 min ($2 \times$ hydrated radius, R_{hz}) against the sum of the angular dependence of scattering and the pectin concentration dependence (Chapman, Morris, Selvendran, & O'Neill, 1987). The concentrations in mg/mL are given at the bottom of the lattice and the scattering angles to the right of the lattice.

The value of the hydrated radius of a macromolecule can be obtained from changes in light scattered from dilute solution with time because Brownian motion causes local concentration fluctuations of dissolved molecules. When visible light impinges on these solutions, under certain conditions, the light is absorbed and reemitted (scattered) by the dissolved molecules. If the intensity of light due to these fluctuations is measured as a function of time, the translational diffusion constant, D , for that molecule can be obtained (Burchard, 1996). The Stokes-Einstein equation relates D and R_h , according to Eq. (2)

$$D = kT / (6\pi\eta_0 R_h) \quad (2)$$

Here, k is the Boltzman constant, T is absolute temperature and η_0 is viscosity of the solvent.

The weight average molar mass of the pectin heated for 2.5 min is 3.35×10^5 . For this same sample, the polydispersity ratios M_w/M_n and M_z/M_n are 1.31 and 1.75, respectively; and the R_{gz} value is 41.5 nm. Simultaneous

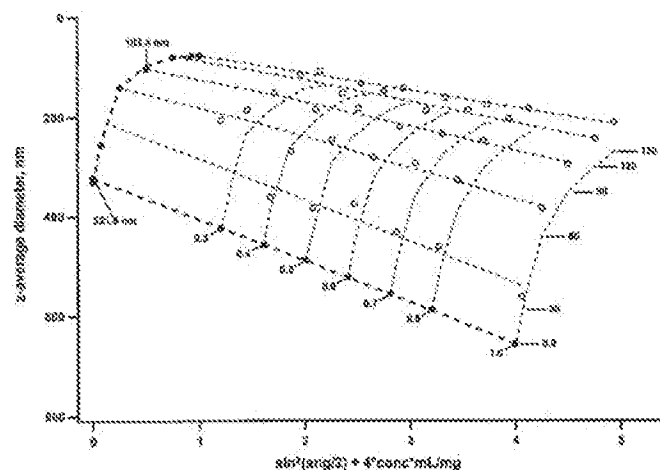


Fig. 8. Dynamic Zimm plot of pectin from albedo heated for 2.5 min.

extrapolation of scattering angle and concentration to zero, gives a value of 321.8 nm for the hydrated diameter or a value of 160.9 for the value of R_{hz} . The ratio of R_{gz}/R_{hz} is symbolized by ρ . This value is 0.26 for the pectin which was heated for 2.5 min. The value of ρ for a cross-linked microgel with branches is 0.6, a homogeneous sphere is 0.778, a random coil is 1.5–2.05 depending on polydispersity and goodness of solvent, a rigid rod is >2 (Burchard, 1996). The rather low value of ρ , may be due to the presence of a very small quantity aggregated pectic fragments which would raise the value of R_{hz} . The value of the hydrated diameter obtained by extrapolating the data obtained from light scattered at 90° is 103.4 nm and R_{hz} is 51.7 nm. A ρ value of 0.87 is obtained using 41.5 as R_{gz} . This value of ρ is consistent with earlier findings that pectin obtained by rapid MAE is in the form of a partially formed network, i.e. branched. The rationale for taking the R_{hz} value at 90° rather than the value extrapolated to 0° is that the 90° value should be less sensitive to trace amounts of large aggregates which may be present. Table 5 contains values of R_{hz} obtained at 90° from the online DLS detector described in Section 2.3 and ρ values using R_{gz} values in that table. The R_{hz} value, 51 nm, for the albedo pectin in Table 5 is about the same as the 90° value obtained from the data shown in Fig. 8. These data were obtained from the stand-alone multiangle DLS detector described in Section 2.3. Based on the M-H exponents obtained (see Fig. 7), it would appear that the ρ values obtained for pectin at 4 and 6 min were too low. Nevertheless, R_{gz} and R_{hz} decreased with M_w as expected.

4. Conclusions

With few exceptions, lime albedo, pulp and flavedo gave similar trends when changes in molar mass, intrinsic viscosity, radius of gyration and hydrated radius were monitored with time of heating during extraction. Molar mass, intrinsic viscosity, radius of gyration and hydrated radius, all decreased with time of heating during extraction. Also, for all three fractions, polydispersity of molar mass exhibited a maximum at 4 min of heating and shapes became less compact as heating times increased. Nevertheless, there were some differences in properties among

Table 5
Radii of lime fractions

Time (min)	Albedo			Pulp			Flavedo		
	R_{gz}^a	R_{hz}^b	ρ	R_{gz}	R_{hz}	ρ	R_{gz}	R_{hz}	ρ
2.5	41.5 (1.0) ^c	51 (1)	0.82	39.6 (0.2)	51 (1)	0.78	39.1 (0.1)	49 (1)	0.80
3	38.4 (0.5)	55 (3)	0.69	38.6 (0.1)	49 (1)	0.80	35.4 (1)	44 (1)	0.82
4	29.1 (0.8)	44 (1)	0.65	30.7 (0.2)	35 (1)	0.88	32.0 (1)	32 (3)	1.0
6	12.4 (2)	12 (2)	1.0	15.6 (2)	23 (1)	0.70	17.3 (3)	28 (7)	0.6

^a R_{gz} (MALLS).

^b R_{hz} (90°).

^c Standard deviation of triplicate analysis.

fractions at specific times of heating. All these observations can be rationalized by the hypothesis that lime pectin is extracted as a mixture of networks, partially formed networks and components thereof. The relative amounts of these various moieties depend upon the time of heating.

Acknowledgement

We thank André White and Tung Nguyen for their technical assistance in obtaining the pectin composition data.

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/154,381	11/26/2013	8592575	10-501 (29776-0070)	1910

62488 7590 11/06/2013
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c/o Pete Pappas, Sutherland, Asbill & Brennan LLP
999 Peachtree Street, NE
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ATLANTA, GA 30309-3996

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 111 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Søren Vodstrup Jensen, Lille Skensved, DENMARK;
Susanne Oxenbøll Sørensen, Vallensbak Strand, DENMARK;
Claus Rolin, Koege, DENMARK;

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<i>Index of Claims</i> 	Application/Control No. 13154381	Applicant(s)/Patent Under Reexamination JENSEN ET AL.
	Examiner LAYLA BLAND	Art Unit 1623

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant					☐ CPA		☐ T.D.		☐ R.1.47	
CLAIM		DATE								
Final	Original	01/19/2013	02/27/2013	08/20/2013						
	1	÷	✓	=						
	2	÷	✓	=						
	3	÷	✓	=						
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Notice of Allowability	Application No. 13/154,381	Applicant(s) JENSEN ET AL.	
	Examiner LAYLA BLAND	Art Unit 1623	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to Applicant's amendment submitted August 2, 2013.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 1-14 and 21-26. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|--|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☐ Examiner's Amendment/Comment |
| 2. ☐ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____ | 6. ☒ Examiner's Statement of Reasons for Allowance |
| 3. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material | 7. ☐ Other _____. |
| 4. ☐ Interview Summary (PTO-413),
Paper No./Mail Date _____. | |

Art Unit: 1623

DETAILED ACTION

This office action is in response to Applicant's amendment submitted August 2, 2013.

Claims 1 and 6 are amended, claims 15-20 are cancelled, and claims 21-26 are newly submitted.

Claims 1-14 and 21-26 are pending and are examined on the merits herein.

In view of Applicant's amendment, the rejection of claims 1-14 under 35 U.S.C. 112, second paragraph is withdrawn.

The rejection of claims 1-14 under 35 U.S.C. 103(a) as being unpatentable over Joye in view of Christensen and Eschinasi is withdrawn. Applicant's arguments are persuasive.

Accordingly, Applicant's amendment and arguments as discussed above are sufficient to remove all rejections made in the prior office action and to place the application in condition for allowance.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LAYLA BLAND whose telephone number is (571)272-9572.

The examiner can normally be reached on Monday - Friday, 10:00 - 6:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anna Jiang can be reached on (571) 272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Art Unit: 1623

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Layla Bland/
Primary Examiner, Art Unit 1623

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A process for extracting pectin having a high degree of polymerization comprising:

adding oxalic acid and/or a water-soluble oxalate to an aqueous suspension of a peel in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium₂(II) in the peel;

heating the mixture to a temperature from about 50 to about 80°C for a time sufficient to extract a pectin; and

separating the extracted pectin from the mixture by precipitating the extracted pectin from the mixture, wherein the extracted pectin has a degree of esterification (DE) of at least 72 and a high degree of polymerization as characterized by an intrinsic viscosity of greater than about 6.5 dL/g.

2. (Original) The process according to claim 2, wherein the pH of the mixture prior to separating the extracted pectin from the mixture is between 3.1 and 3.4.

3. (Original) The process according to claim 2, wherein the mixture is heated to a temperature from about 70 to about 80°C during the separating of the extracted pectin.

4. (Original) The process according to claim 1, further comprising filtering the mixture to remove insoluble solids after extracting the pectin and prior to separating the extracted pectin.

5. (Original) The process according to claim 1, further comprising contacting the mixture containing the extracted pectin with a ion-exchanging resin prior to separating the extracted pectin.
6. (Currently Amended) The process according to claim 5, wherein the ion-exchanging resin comprises a cation-exchanging resin effective to remove oxalate residues, calcium residues, or combinations thereof from the extracted pectin ~~selected from the group consisting of Lewatit® S1668, Lewatit® S1468, Purolite® C100E, and Amberlite® SR1L Na.~~
7. (Original) The process according to claim 5, wherein the extracted pectin has a total oxalate content less than 2.0 mg per 1 g of pectin.
8. (Original) The process according to claim 5, wherein the extracted pectin has a total calcium content less than 1 mg/g.
9. (Original) The process according to claim 1, further comprising one or more of the steps of washing, pressing, drying, and milling the extracted pectin after separating the extracted pectin to obtain a final pectin product.
10. (Original) The process according to claim 1, wherein the yield per peel (Y_p) is greater than about 23%, wherein $Y_p = 0.1 \cdot y_s \cdot M/W_p$ and y_s is the yield of solution (liquid extract) in g/kg, M is the total mass of the mixture before separating the extracted pectin, and W_p is the weight of the peel used in the aqueous suspension.

11. (Original) The process according to claim 10, wherein the product of the yield per peel and intrinsic viscosity is greater than about $165 \% \cdot \text{dL/g}$.
12. (Original) The process according to claim 1, wherein the extracted pectin has a degree of esterification (DE) of at least 75.
13. (Original) The process according to claim 1, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 7.8 dL/g.
14. (Original) The process according to claim 1, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 8.5 dL/g.
- 15- 20. (Cancelled)
21. (New) A process for extracting pectin having a high degree of polymerization consisting essentially of:
- adding oxalic acid and/or a water-soluble oxalate to an aqueous suspension of a peel in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium (II) in the peel;
 - heating the mixture to a temperature from about 50 to about 80°C for a time sufficient to extract a pectin;
 - optionally filtering the mixture to remove insoluble solids;

contacting the mixture containing the extracted pectin with a cation-exchanging resin; and

separating the extracted pectin from the mixture by precipitating the extracted pectin from the mixture to obtain a yield per peel (Y_p) greater than about 23%, wherein $Y_p = 0.1 \cdot y_s \cdot M/W_p$ and y_s is the yield of solution (liquid extract) in g/kg, M is the total mass of the mixture before separating the extracted pectin, and W_p is the weight of the peel used in the aqueous suspension;

wherein the extracted pectin has a degree of esterification (DE) of at least 72, a high degree of polymerization as characterized by an intrinsic viscosity of greater than about 6.5 dL/g.

22. (New) The process according to claim 21, wherein the extracted pectin has a total oxalate content less than 2.0 mg per 1 g of pectin.

23. (New) The process according to claim 21, wherein the extracted pectin has a total calcium content less than 1 mg/g.

24. (New) The process according to claim 21, wherein the time sufficient is from about 1 hour to about 3 hours.

25. (New) The process according to claim 21, wherein the extracted pectin has a degree of esterification (DE) of at least 75.

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26. (New) The process according to claim 21, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 7.8 dL/g.



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(12) **United States Patent**
Jensen et al.(10) **Patent No.:** **US 8,592,575 B2**(45) **Date of Patent:** **Nov. 26, 2013**(54) **PROCESS FOR EXTRACTION OF PECTIN**(75) Inventors: **Søren Vodstrup Jensen**, Lille Skensved
(DK); **Susanne Oxenbøll Sørensen**,
Vallensbæk Strand (DK); **Claus Rolin**,
Koege (DK)(73) Assignee: **CP Kelco ApS** (DK)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 111 days.(21) Appl. No.: **13/154,381**(22) Filed: **Jun. 6, 2011**(65) **Prior Publication Data**

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(51) **Int. Cl.****C07H 1/06** (2006.01)**C07H 1/08** (2006.01)(52) **U.S. Cl.**USPC **536/128**(58) **Field of Classification Search**

None

See application file for complete search history.

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Brennan LLP(57) **ABSTRACT**

Embodiments of the processes provided herein allow for
 extraction of high quality pectins from pectin-containing
 plant materials using oxalic acid for extraction of the pectin
 containing material. Generally described, the process for
 extracting pectin having a high degree of polymerization
 includes preparing an aqueous suspension of a pectin-con-
 taining plant material; adding oxalic acid and/or a water-
 soluble oxalate to the aqueous suspension in an amount suf-
 ficient to provide a mixture having a pH of between 3.0 and
 3.6 and a total molarity of oxalate greater than a total molarity
 of calcium(II); heating the mixture to a temperature from
 about 50 to about 80° C. for a time sufficient to extract pectin
 from the pectin-containing plant material; and separating the
 extracted pectin from the mixture. The extracted pectin desir-
 ably is characterized as having a degree of esterification (DE)
 of at least 72 and a high degree of polymerization, the degree
 of polymerization being characterized by an intrinsic viscos-
 ity of greater than about 6.5 dL/g.

20 Claims, 7 Drawing Sheets

FIG. 1

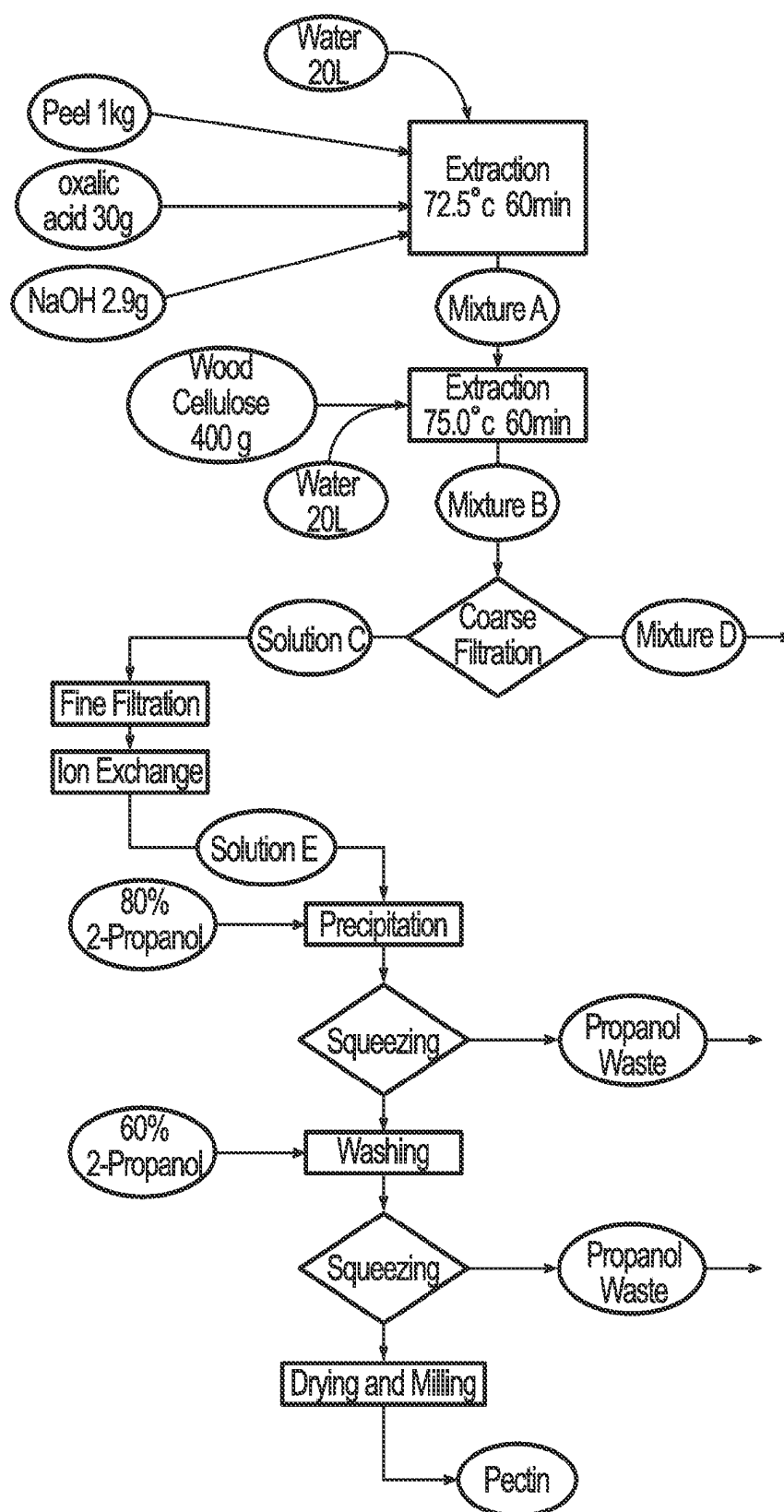
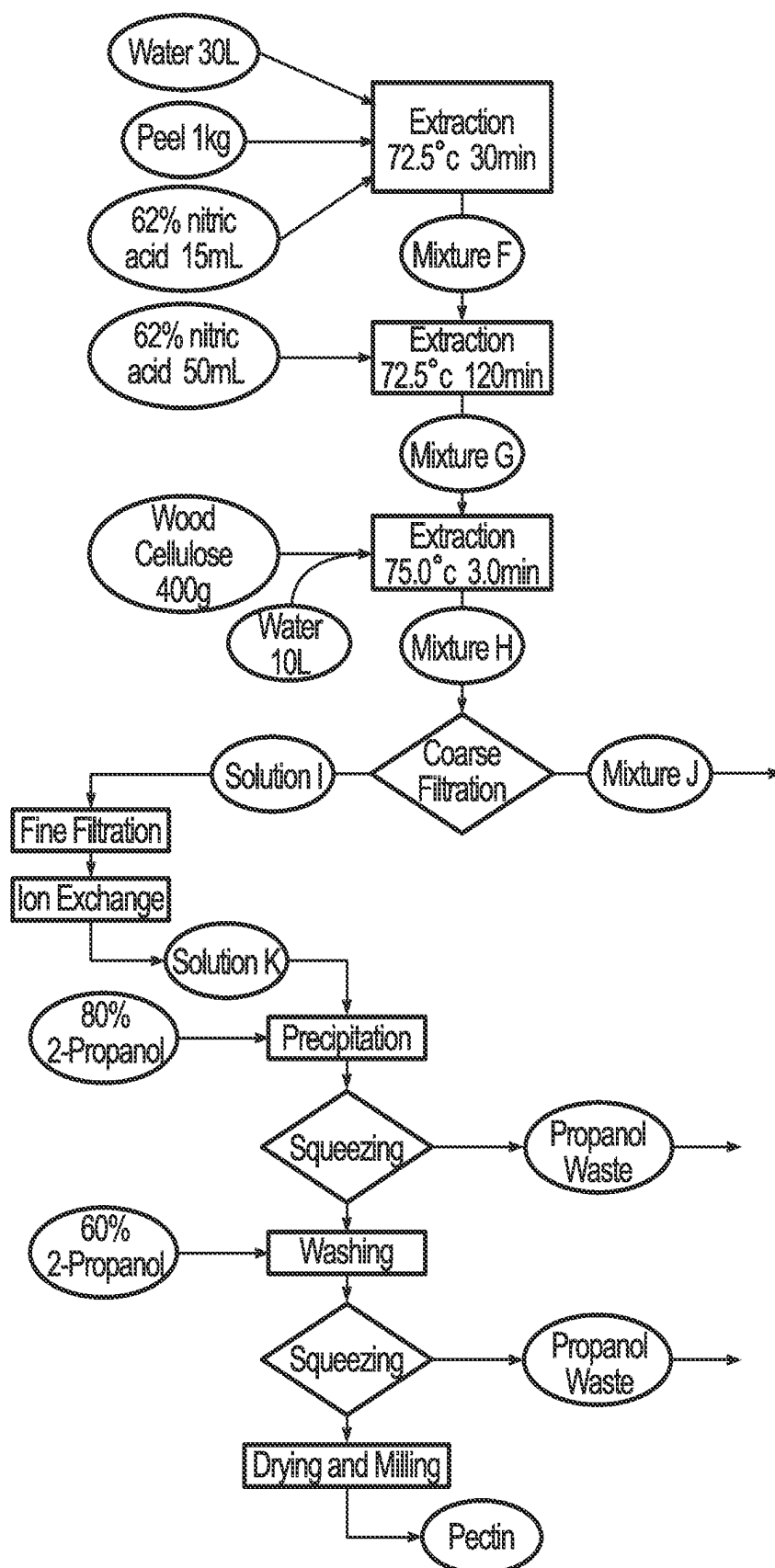


FIG. 2



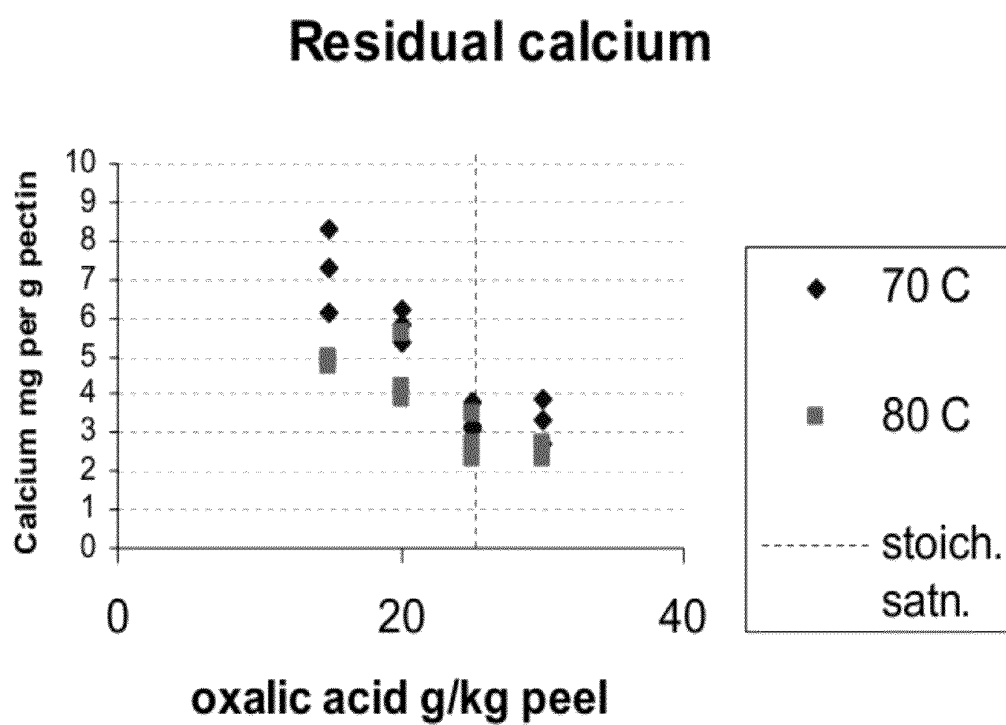


FIG. 3

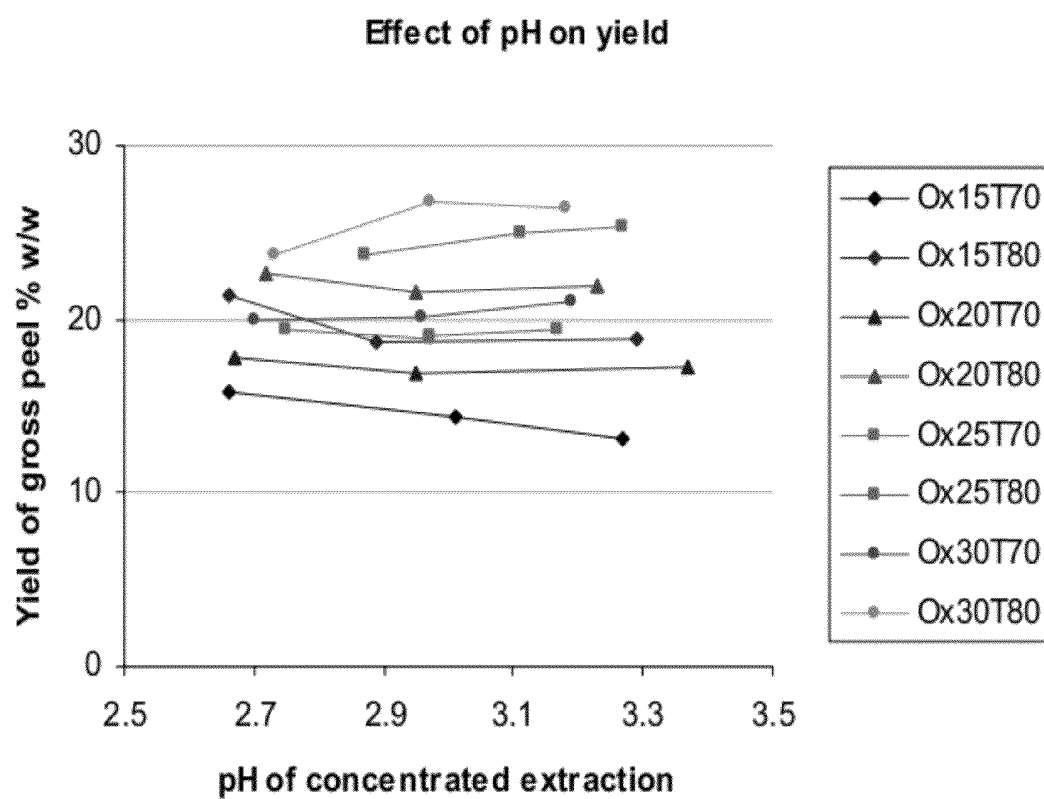


FIG. 4

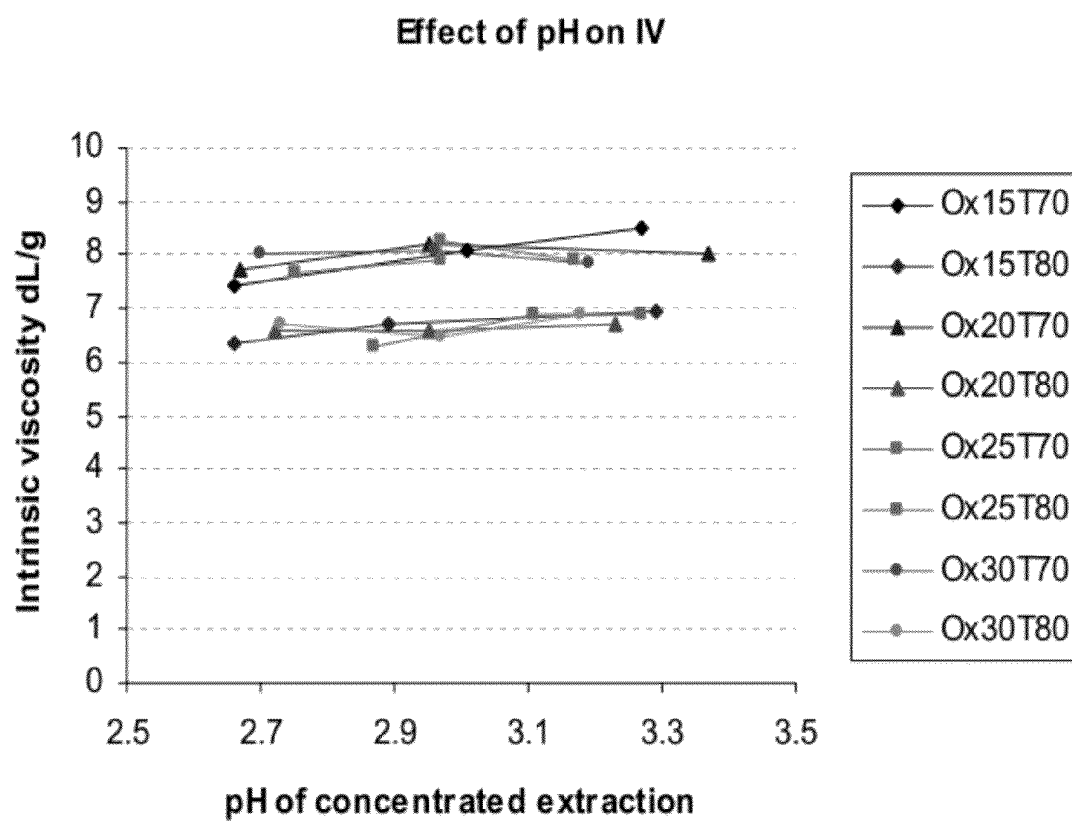


FIG. 5

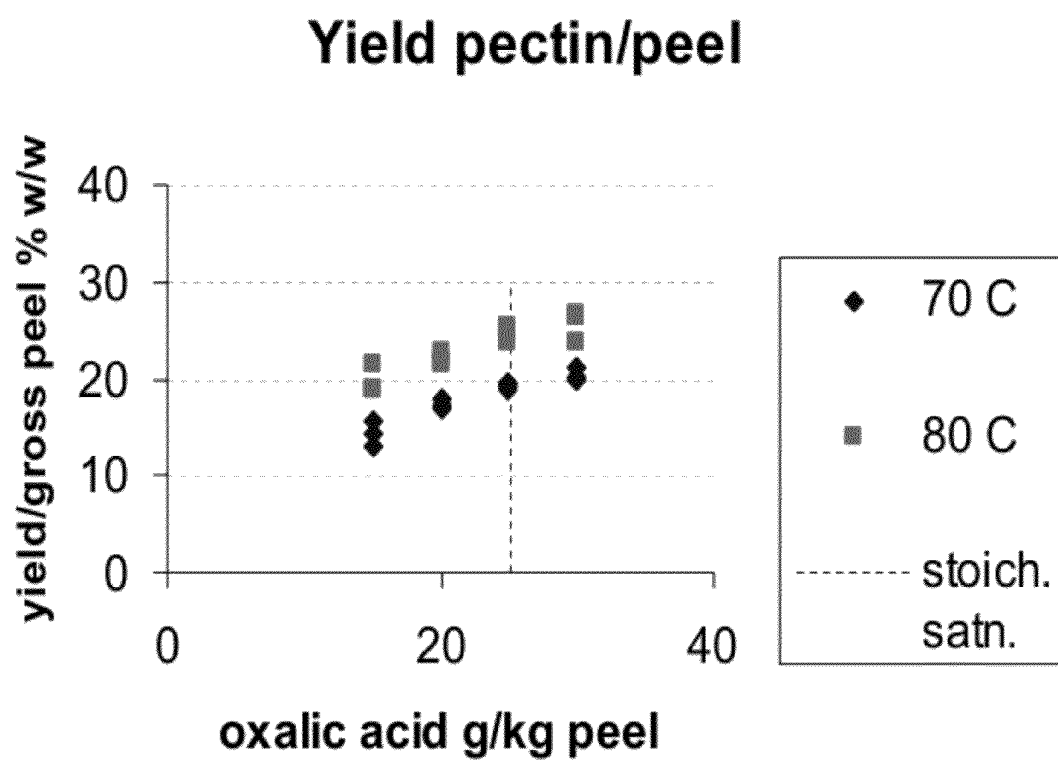


FIG. 6

Product of yield times IV

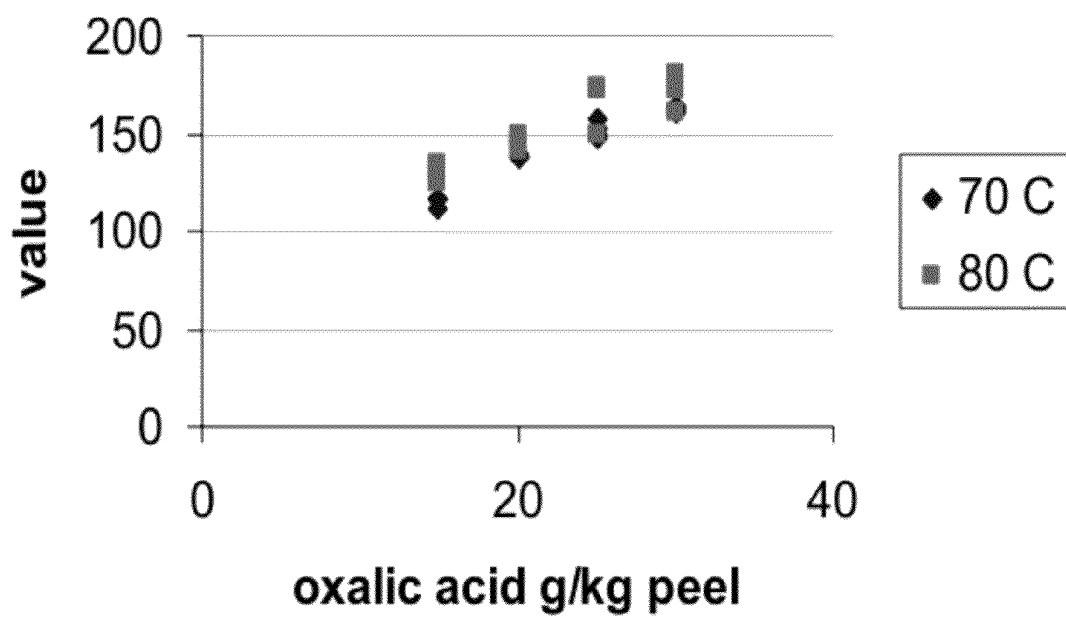


FIG. 7

PROCESS FOR EXTRACTION OF PECTIN

TECHNICAL FIELD

Embodiments of the present description relate to methods for extraction of high quality pectins. In particular, the present description relates to low-temperature processes for extraction of pectin from citrus peels that avoids significant degradation of pectin during the extraction process.

BACKGROUND

Pectin is a complex polysaccharide associated with plant cell walls. It consists of an alpha 1-4 linked polygalacturonic acid backbone intervened by rhamnose residues and modified with neutral sugar side chains and non-sugar components such as acetyl, methyl, and ferulic acid groups. The neutral sugar side chains, which include arabinan and arabinogalactans, are attached to the rhamnose residues in the backbone.

A significant amount of research on pectins has been carried out due to its importance as a food product, a dietary fiber and a component of cell walls in higher plants, and to the growing awareness of a number of pharmacological activities. Current methods of extracting pectin, however, do not produce pectins of sufficient quality for all such uses.

Conventional methods of pectin extraction require extended heating of the pectin-containing plant materials at sub-boiling temperatures (approximately 65-85° C.) in an acidic media (pH below 2.2) for 1 to 10 hours. These processes, however, have high residence times and energy requirements and may provide only a moderate yield (20-30%) of pectin using a counter-current process (i.e., a process using a number, n , of extraction stages with which the originating raw material goes to extraction stage #1, the originating pure water goes the stage # n , and for any stage # i , assuming $1 < i < n$, the liquid comes from stage $i+1$ while the solids come from stage $i-1$). Thus, since the acidic condition is aggressive, and since a considerable part of the pectin becomes exposed to the acidic condition repeatedly, the pectins often are degraded during the conventional extraction processes.

As will be appreciated by those skilled in the art, it is desirable to extract a pectin having as high a degree of polymerization (DP) as possible for as low a cost as possible. The selection of production conditions like solid/liquid ratio, acidity, temperature, etc. all effect these conflicting considerations. For example, in order to accomplish an even moderately high yield using conventional processes, extraction conditions must be aggressive. In addition, following the extraction, the solids-liquid separation should be as complete as possible because the liquid that is retained by the solids after the separation will be exposed to the aggressive extraction conditions for a second time (due to the counter-current extraction setup). For the separation to be as complete as possible, however, the viscosity of the liquid preferably should be low, which is accomplished either by separating at high temperature, separating with a low solids/liquid ratio, or a combination of both. Use of a high temperature in combination with a low pH, however, is detrimental to the pectin quality (e.g., its degree of polymerization). Conversely, a low solids/liquid ratio becomes costly because larger amounts of water need to be removed from the pectin either by evaporation or by distillation of alcohol. Thus, there remains a need for a cost-effective process for extracting pectins without compromising the quality of the extracted pectins.

In addition, it is also important that the final pectin is free from unwanted contaminants, including any residual pres-

ence of the acid (and its salts) that was used for extracting the pectin. Thus, there also is a need in the pectin manufacturing industry for cost-effective ways of reducing the residual presence of the acids that were added for the extraction.

Additional aspects will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustration of a system for extracting pectin according to a particular embodiment.

FIG. 2 is a schematic illustration of a conventional system for extracting pectin.

FIG. 3 is a graph illustrating the calcium content of pectin as a function of the dosage of oxalic acid.

FIG. 4 is a graph illustrating the effect of pH upon pectin extraction yield.

FIG. 5 is a graph illustrating the intrinsic viscosity of extracted pectin as a function of pH.

FIG. 6 is a graph illustrating the dosage of oxalic acid and the yield of pectin in percent of the peel.

FIG. 7 is a graph illustrating the product of (pectin yield times pectin intrinsic viscosity) as a function of the oxalic acid dosage.

DETAILED DESCRIPTION

Embodiments of the present invention relate to methods for extraction of high quality pectins from pectin-containing plant materials using oxalic acid for extraction of the pectin containing material. Generally described, a process for extracting pectin having a high degree of polymerization in a single-stage extraction comprises preparing an aqueous suspension of a pectin-containing plant material; adding oxalic acid and/or a water-soluble oxalate to the aqueous suspension in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium(II); heating the mixture to a temperature from about 50 to about 80° C. for a time sufficient to extract pectin from the pectin-containing plant material; and separating the extracted pectin from the mixture. The extracted pectin desirably is characterized as having a degree of esterification (DE) of at least 72 and a high degree of polymerization, the degree of polymerization being characterized by an intrinsic viscosity of greater than about 6 dL/g.

Pectin-containing plant materials are well-known in the art and include fresh and processed materials as well as plant residues. Desirably, the pectin-containing plant material is a fruit, non-limiting examples of which include stone fruits such as peaches, pome fruits such as apples, and citrus fruits such as limes, lemons, oranges and grapefruit. The material may be prepared for extraction using any suitable means and any suitable portion of the pectin-containing plant material, for example, by juicing, peeling, coarsely chopping, milling or grinding the pectin-containing plant material or portions thereof. In particular embodiments, the pectin-containing plant material is the fruit peel. The fruit peel of a citrus fruit, as used herein, means the flavedo, albedo, and juice sacks (pulp sacks) which remain after juicing the fruit. In alternative embodiments, the pectin-containing plant material is one or

more portions of the fruit peel (e.g., the albedo, fractions of albedo, juice sacks, or combinations thereof).

In embodiments, oxalic acid and/or a water-soluble oxalate are added to an aqueous solution of the pectin-containing plant materials. The pectin-containing plant materials generally are present in an amount from about 2 to about 5% by weight of the mixture. The oxalic acid and/or water-soluble oxalate are present in an amount from about 20 to about 50 g per kg dry material of the pectin-containing plant material. Desirably, the mixture has a pH between 3.0 and 3.6, a pH between 3.1 and 3.4, or a pH between 3.2 and 3.3. The mixture also desirably has a total molarity of oxalate greater than a total molarity of calcium(II). Those skilled in the art will appreciate that along with the oxalic acid and/or water-soluble oxalate, the pH of the mixture can be modified with the addition of minor amounts of suitable bases (e.g., NaOH) or acids (e.g., nitric acid) using methods known in the art.

The pectin then is extracted from the pectin-containing plant materials by heating the mixture to a temperature from about 50 to about 80° C. for a time sufficient to extract the pectin. During the pectin extraction, the mixture desirably is gently stirred and/or agitated using methods known to those skilled in the art. In particular embodiments, the mixture is heated to a temperature from about 60 to about 80° C. or to a temperature from about 70 to about 80° C. Although the yield generally increases with the time of extraction, the time preferably is minimized to reduce any loss in the degree of polymerization in the pectin being extracted. Accordingly, in particular embodiments a time sufficient to extract the pectin is from about 0.5 hours to about 5 hours, from about 1 hour to about 3 hours, or from about 1.5 hours to about 2.5 hours.

Following extraction of the pectin from the pectin-containing plant materials, insoluble solids (i.e., the residue of the pectin-containing plant material) may be removed from the mixture of the extracted pectin using any suitable means, for example, by one or more filtering steps (e.g., coarse and/or fine filtration). The extracted pectin then may be precipitated from the mixture, for example by pouring the extract into an effective amount of alcohol. Useful alcohols include, but are not limited to, any alcohol which is compatible with food applications and which effectively precipitates pectins and dissolves alcohol-soluble materials. In particular embodiments, the alcohols are isopropyl alcohol, propanol, ethanol, or methanol. Following precipitation, the extracted pectin may be separated from the solution and washed with alcohol to remove as many alcohol-soluble impurities as possible. The pectin then may be dried and processed into a powder or further modified (e.g., de-esterified and/or amidated) using methods known in the art.

In still other particular embodiments, residues may be removed from the extracted pectin either prior to or after its separation from the alcohol-water mixture. Such residues may include, for example, small amounts of the oxalic acid and oxalate residues that were used for the extraction. Although a moderate presence of conventional acids (e.g., nitric acid) used in extraction processes generally is accepted, oxalic acid residues likely would only be permissible in very low amounts due to a lack of prior regulatory use and evaluation in commercial pectin production. Thus, it is desirable to remove the oxalic acid and calcium oxalate residues from the extracted pectin. Accordingly, in particular embodiments the process further comprises exposing the solution of extracted pectin to a cation-exchanging resin before separating the extracted pectin from solution, thereby significantly reducing the oxalate and calcium residues in the final extracted pectin. Non-limiting examples of suitable cation-exchanging resins include Lewatit® S1668 and Lewatit® S1468 (Lanxess Deut-

schland GmbH, Leverkusen, Germany), Purolite® C100E (Purolite International Ltd., Llantrisant, United Kingdom), and Amberlite® SR1L Na (Rohm and Haas, Philadelphia, Pa.). The solution of extracted pectin may be at any suitable temperature and may be heated or cooled as required. For example, in embodiments the solution of extracted pectin is at a temperature from about 55 to about 70° C.

The pectins obtained using the processes provided herein have a higher degree of esterification (DE) and a higher degree of polymerization (DP) while being extracted in a higher yield than in conventional processes. Desirably, the extracted pectin has a degree of esterification of greater than or equal to 75 and a degree of polymerization that is characterized by an intrinsic viscosity of about 6.5 to about 9 dL/g. In particular embodiments, the extracted pectin has a degree of esterification of at least 76, at least 77, or at least 78, and a degree of polymerization that is characterized by an intrinsic viscosity of at least 7.0 dL/g, at least 7.5 dL/g, at least 7.8 dL/g, or at least 8.0 dL/g.

Those skilled in the art will appreciate, however, that the pectin properties depend on the species of plant material, maturity of the plant material, and delay before processing of the plant material. For example, in embodiment the pectin extracted from a high quality lemon peel has a degree of esterification of about 75-78 and an intrinsic viscosity of about 9 dL/g (as compared to a degree of esterification of 74-76 and an intrinsic viscosity of about 7.5 dL/g when extracted using conventional methods). In another embodiment, however, the pectin extracted from a low quality plant material has a degree of esterification of greater than 73 and an intrinsic viscosity of about 6.5 dL/g.

The processes provided herein desirably also provide a higher yield per peel than conventional pectin extraction methods. In particular embodiments, the yield per peel is greater than about 23, greater than about 25, or greater than about 27. Those skilled in the art will appreciate, however, that the expected yield is dependent upon the quality of raw material and the raw material's natural variations.

The yield per peel may be approximated from the following formula:

$$Y_p \% = 0.1 y_s M / W_p$$

in which Y_p is the yield per peel in %, Y_s is the yield of solution (liquid extract) in g/kg, M is the total mass of the extraction mixture just before filtration, and W_p is the weight of peel that was used for the extraction mixture. Those skilled in the art will appreciate that this calculation of yield per peel is only an approximation because it ignores that a minor part of the total mass of the extraction mixture is not dissolved and, therefore, represents a volume within which the dissolved pectin does not distribute.

In particular embodiments, the value of the processes provided herein are characterized by the product of the yield per peel and intrinsic viscosity. Desirably, the product of the yield per peel and intrinsic viscosity of pectins obtained by the processes provided herein is greater than the product of the yield per peel and intrinsic viscosity of pectins obtained by conventional processes. For example, in particular embodiments the product of the yield per peel and intrinsic viscosity of pectins is greater than about 160, greater than about 165, greater than about 175, or greater than about 185.

The foregoing process improves upon prior art methods of pectin extraction by providing a method that (1) achieves a low viscosity during filtration while minimising pectin loss of degree of polymerization; (2) eliminates Ca^{++} while reducing the need for regenerating ion-exchanging resin; (3) increases the yield of pectin as compared to the amount of spent raw

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material while minimising pectin loss of degree of polymerization; (4) reduces the amount of water that needs to be removed from the extracted pectin; and (5) reduces the residuals in the final product of the acid that was added for the extraction.

Not wishing to be bound by any theory, it is believed that the process provided herein achieves these desirable results in part by a specific balance of the extraction media selection (oxalic acid), pH, and temperature. In addition, it is believed that precipitation of a major portion of the Ca^{++} ions as calcium oxalate reduces the tendency of the dissolved pectin to become viscous in the presence of Ca^{++} ions, thereby reducing the viscosity of mixture during filtration and reducing the residual oxalic acid in the final product. The combination of good yield and low viscosity further makes it possible to operate with a relatively high pectin concentration in the filtrated liquid, thereby reducing the amount of water that must be removed from the pectin. Finally, the presence of residual oxalic acid in the final pectin is further reduced by exposing the liquid pectin extract to a cation-exchanging resin before precipitating the pectin from solution.

Embodiments of the present description are further illustrated by the following examples, which are not to be construed in any way as imparting limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description therein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims. Unless otherwise specified, quantities referred to by percentages (%) are by weight (wt %).

EXAMPLES

The following protocols were used to analyze the degree of esterification (DE), intrinsic viscosity (IV), the strength, residual calcium content, and residual oxalate content in the Examples below.

Protocol 1: Determination of Degree of Esterification

The degree of esterification was measured using the method set forth in FAO JECFA Monographs 4 (2007). 100 mL of the acid alcohol (100 mL 60% IPA+5 mL HCl fuming 37%) was added to 2.00 g of pectin while stirring with a magnetic stirrer for 10 min. The mixture was passed through a Büchner funnel with filter paper and the beaker was rinsed with 90 mL acid alcohol and also passed through the Büchner funnel with filter paper. The filtrate was then washed first with 1000 mL 60% IPA and thereafter with approximately 30 mL 100% IPA. The sample then was dried for approximately 15 min. on the Buchner funnel with vacuum suction.

Pectin samples weighing approximately 0.40 g were measured for duplicate determination (deviation between duplicate determinations must not exceed 1.5% absolute, otherwise the test was repeated). The pectin samples were first moistened with approximately 2 mL 100% IPA. Approximately 100 mL deionized water then was added to the moistened samples while stirring with a magnetic stirrer. The samples were then evaluated by titration, either by means of an indicator or by using a pHmeter/autoburette.

Titration Using Indicator.

5 drops of phenolphthalein indicator was added to the sample and it was titrated with 0.1 M NaOH until a change of colour was observed (record it as V1 titer). 20.0 mL 0.5 M NaOH was added while stirring and covered with foil for exactly 15 min. 20.0 mL 0.5 M HCl was added while stirring until the colour disappeared. 3 drops of phenolphthalein indi-

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cator then was added and it was titrated with 0.1 M NaOH until a change of colour was observed (record it as V2 titer). In order to compensate for possible inaccuracies of balancing the two portions of 20 mL of 0.5 M NaOH and HCl respectively, a so-called "blind measurement" was performed (i.e., 100 mL of o deionized water was treated in the same way as the sample solution, including the titrations). The last titration result was then recorded as B1 titer. The degree of esterification was then characterized by the following calculations.

$$Vt=V1+(V2-B1)$$

$$\% \text{ DE (Degree of esterification)}=[(V2-B1)/Vt]*100$$

Protocol 2: Determination of Intrinsic Viscosity

Samples were prepared using a Viscotek GPC max VE 2001 GPC solvent/sample module or AS 3500 auto sampler unless the samples were known to contain non-soluble material (in which case the samples were manually prepared). The molecular weight, intrinsic viscosity, and molecular weight distribution of pectin in the samples was then determined using size exclusion chromatography (SEC). The molecules were separated according to their size by gel permeation chromatography with the effluent from the chromatography column passing three detectors (RI, RALLS, and a viscosity detector). The Viscotek software converted the detector signals to molecular weight and intrinsic viscosity and calculated the weighted averages for the entire population.

Equipment:

Viscotek TDA 302 triple detector or TDA 305; Viscotek GPC max VE 2001 GPC solvent/sample module or AS 3500 auto sampler; Sample preparation module; Mettler Toledo balance and MicroLab. 500 dispenser; Pierce Reacti-Therm III heating/stirring module; Columns: BioBasis SEC60 (300×7.8), SEC120 (300×7.8), SEC300 (300×7.8) SEC1000 (300×7.8), all from Thermo, or Shodex guard SB400 G-8B, SB401-8F, SB402,5-8F, SB403-8F, SB404-8F; or in FIPA setup with either CPKelco Special (SuperDex Peptid from GE Health Care) or BioBasis SEC60 (150×7.8) from Thermo; RALLS detector (Right Angle Laser Light Scattering Detector); LALLS detector (Low Angle Laser Light Scattering Detector); RI Detector (Refractive Index); Viscometer Detector; Computer software: OmniSEC.

Manual Sample Preparation:

Samples known to contain non-soluble material were manually dissolved and centrifuged (10,000 G for 10 min.) and the supernatant was transferred to a new vial prior to injection.

Sample Preparation Using Sample Preparation Module, Mettler Toledo Balance and Mikrolab. 500 Dispenser and Pierce Reacti-Therm III Heating/Stirring Module:

In the omniSEC software, the SASP program was used to prepare the samples before analysis. The amount/capacity ratio was approx 30 mg powder to 15 ml eluent (2 mg per ml) in 20 ml vials. After preparing the sample, the vials were placed for 30 minutes at 70° C. in the heating stirring module.

Sample Preparation Using Balance and Heating/Stirring Module when Using AS 3500 Auto Sampler:

Approximately 2.0 mg pectin was weighed in an auto sampler vial and placed in the auto sampler rack. Using template 4 from the AS3500 auto sampler, the following units were used: Dilution cycles: 3; Heater: ON Temp: 70° C.; Load 20 µl solvent S-1 (S-1=96% ethanol); Add 10 µl to sample; Load 1500 µl solvent S-2 (S-2=0.3 M Li-acetate buffer); Add 1300 µl to sample (0.1% pectin solution—1 mg/ml); Mix for 9.9 minutes; Mix for 9.9 minutes; Wait for 10.0 minutes; Enable Overlay: YES (starts the next sample preparation before end of analysis for running sample). The run time at the auto

sampler was set at 50 minutes and a 100 μ L full loop injection was used. When the auto sampler was used, the sample was automatically filtrated by a 0.5 μ m in-line filter placed after the auto sampler loop.

Protocol 3: Determination of Pectin Strength (YogSimBuf method)

YOG SIM BUF pH 3.75 Buffer (2000 mL).

A buffer was prepared by dissolving 13.03 g calcium lactate ($C_6H_{10}O_6Ca$, $5 \cdot H_2O$), 2.79 g di-potassium hydrogen phosphate (K_2HPO_4), 2.00 g sodium benzoate, 2.03 g potassium-di-hydroxide-phosphate (KH_2PO_4), 40.00 g lactose ($C_{12}H_{22}O_{11}$), 300.00 g sucrose ($C_{12}H_{22}O_{11}$), 7.23 g lactic acid (90%) was weighed out and added to the solution and the beaker was rinsed with ion-exchanged water. Finally, 0.43 g sodium hydroxide (NaOH) was dissolved in the flask and the solution was diluted to 2000 mL with deionized water. The buffer solution pH was adjusted by adding citric acid until a pH reading of 3.68 ± 0.02 was obtained, which resulted in a pH of 3.75 ± 0.05 in the final pectin-buffer system. This buffer solution was stable until precipitation.

Pectin Stock Solution (1.2%) (100 mL).

A pectin stock solution was prepared by moistening 1.20 g pectin with 5.00 ml 100% IPA in a flask. While stirring, 95 ml boiling deionized water ($>85^\circ C$.) was added to the flask and the mixture was placed on a water bath/block heater at $75^\circ C$. for 30 min. while stirring. The solution was cooled to room temperature and deionized water was added to dilute the solution to 100.0 g.

The buffer solution, pectin stock solution and deionized water were maintained at a temperature of $23 \pm 2^\circ C$. The pectin stock solution was diluted with deionized water according to the table below.

Pectin conc. In final system %	Deionized water (g)	Pectin stock solution (1.2%) (g)
0	15.0	0
0.02	14.5	0.5
0.04	14.0	1.0
0.06	13.5	1.5
0.08	13.0	2.0
0.10	12.5	2.5
0.12	12.0	3.0
0.14	11.5	3.5
0.16	11.0	4.0
0.18	10.5	4.5
0.20	10.0	5.0
0.22	9.5	5.5
0.24	9.0	6.0
0.26	8.5	6.5
0.28	8.0	7.0
0.30	7.5	7.5
0.32	7.0	8.0
0.34	6.5	8.5
0.36	6.0	9.0
0.38	5.5	9.5
0.40	5.0	10.0
0.42	4.5	10.5
0.44	4.0	11.0
0.46	3.5	11.5
0.48	3.0	12.0
0.50	2.5	12.5
0.52	2.0	13.0
0.54	1.5	13.5
0.56	1.0	14.0
0.58	0.5	14.5
0.60	0	15.0

15 ml of the buffer was added to the diluted pectin solution while stirring at a speed sufficient to provide a vortex of

approximately 1 cm for a total of 25 seconds. After 1 minute, the solution was analyzed using a Brookfield LVT with adaptor at 6 rpm (factor 1). The pectin-buffer solution also was visually evaluated to characterize its appearance (i.e., presence or absence of any gel lumps, inhomogeneous appearance, etc.). The process is repeated at various dilutions until the following data points are identified: (1) a concentration with a viscosity between 18-25 mPa·s, and (2) a concentration between 25 and 35 mPa·s. The reading was valid if the pH on the solutions between 25 and 35 mPa·s was between 3.70 and 3.80.

The raw result after following the above procedure consisted of two datapoints (c_1, η_1) and (c_2, η_2) with which 18 mPa·s $\leq \eta_1 \leq 25$ mPa·s and 25 mPa·s $\leq \eta_2 \leq 35$ mPa·s. The method result, c_T , was calculated as follows, provided that the viscosities were entered in the unit mPa·s:

$$c_T = c_1 + (c_2 - c_1) \cdot (\ln(25/\eta_1)) / (\ln(\eta_2/\eta_1))$$

Protocol 4: Determination of Residual Calcium Content

Approximately 0.5 g extracted pectin was used to evaluate the presence of residual calcium. The samples were digested with nitric acid (65%) and hydrogen peroxide (30) in a microwave oven digester as prescribed by the manual. After digestion, the reaction vessels were left to cool in a fume hood. The samples were then transferred to a 50-mL measuring flask. When measuring residual calcium, it was necessary to add cesium chloride to avoid interference (1 mL 2.5% CsCl-solution per 10 mL solution to make the solution approx. 2000 ppm in Cs). The acid concentration (HNO₃) in the measuring solutions was from 3% to 10%. The emission intensity of the samples was then measured. If it was not within the emission intensity of the standards, the samples were appropriately diluted.

Protocol 5: Determination of Residual Oxalate Content

Approximately 0.2 g extracted pectin was used to evaluate the presence of residual oxalate. 2-propanol (1 mL) was added to the extracted pectin and the mixture was stirred until the pectin was moistened. Milli-Q deionized water (40 mL) was subsequently added while agitating. The samples were heated to $90^\circ C$. in a closed bottle for between 30 and 60 minutes and then cooled to room temperature. The samples were then evaluated using HPLC chromatography (Apparatus: Waters TM600 with refractive index detector and conductivity detector; Flow: 1.4 mL/minute; Temperature: $40^\circ C$.; Injection: 100 or 200 mL full loop; Eluent: 0.85 mM NaHCO₃+0.9 mM Na₂CO₃ in deionized water).

The quantitative response of the conductivity detector to oxalic acid passing by the detector was quantified as the area (the "integrated detector response") in a diagram (x-axis=time, y-axis=detector response). The integrated detector response was delimited by a straight baseline before and after the peak in accordance with usual practices and conventions in the art. The integrated detector responses from the unknown samples were then compared to a linear calibration curve of the integrated detector responses from known samples of oxalic acid dihydrate in deionized water (x-axis=concentration, y-axis=integrated detector response), and the corresponding concentration of oxalic acid was inferred from the linear calibration curve.

Example 1

Lemon pectin was extracted from a dried lemon peel from the Tucuman province of Argentina. The fruit was harvested in 2009, after which the fruit was cut open and utilized for production of citrus juice and citrus peel oil. The fruit left-overs were then chopped and washed several times in water

for leaching away a large part of the natural soluble materials, like sucrose, and subsequently dried. These routine operations were performed by the peel supplier.

The lemon peel was subsequently used in an exemplary extraction process (illustrated in FIG. 1) to extract pectin from the lemon peel. The dried lemon peel (1000 g) was suspended in a mixture of 20 L of water, 30.0 g oxalic acid di-hydrate, and 2.90 g NaOH. This mixture was incubated with very gentle stirring for 60 minutes at 72.5° C. ("Mixture A"). Mixture A then was diluted with a suspension of 400 g wood cellulose (an insoluble filtration aid) in 20 L of hot water, and the incubation and gentle agitation was continued for 60 minutes at 75.0° C. ("Mixture B"). Mixture B then was poured onto a filter-cloth in a Büchner funnel in order to separate the solution ("Solution C") from the materials that did not dissolve (which together with the solution that could not be separated is referred to as "Mixture D").

Solution C then was further filtered through a bed of Filtercel 450 (a filter-aid used for removing haze) and stirred with Lewatit ionic exchanging resin. The solution obtained after draining away the resin ("Solution E") was poured into about three parts (i.e., three times its own volume) of 80% 2-propanol. The precipitate was squeezed for removing as much of the spent alcohol as possible, and was then washed in a mixture of 60% 2-propanol and 40% water before being squeezed again. The volume of the mixture used for washing the precipitate was about the same as the volume of Solution E. Following the washings, the precipitate was dried, milled and sieved through a 250µ pore-size powder-sieve.

The pH of an aliquot of Mixture B was 3.52 after cooling to 25° C. The amount of dried pectin that could be isolated per kg of Solution E was 5.71 g. In the present example, the total mass of the extraction mixture just before filtration, weight of peel used for the extraction mixture, and yield per peel were calculated as follows:

$$M=1+20+0.03+0.0029+0.400+20 \text{ kg}=41.4 \text{ kg.}$$

$$W_P=1 \text{ kg.}$$

$$Y_P\%=0.1\cdot5.71\cdot41.4/1=23.6$$

The pectin also was analyzed in the laboratory to determine the degree of esterification (DE) and the intrinsic viscosity (IV) (Protocols 1 and 2 described above, respectively). The pectin had a DE=76.4 and IV=7.83 dL/g. The product of yield times intrinsic viscosity, which provides a more relevant metric for value, was 186.

In addition, the strength of the pectin was measured using the YogSimBuf method, which expresses the concentration of pectin at which the solution attains a viscosity of 25 mPa·s. (Protocol 3 described above). The YogSimBuf (YSB) for the lemon peel extracted pectin was 2.51 g/L.

Example 2

Comparative

The same lemon peel described in Example 1 was used in a conventional pectin extraction process (illustrated in FIG. 2). The dried lemon peel (1000 g) was suspended in a mixture of 30 L of water and 15 mL of 62% nitric acid, and incubated with very gentle stirring for 30 minutes at 72.5° C. ("Mixture F"). A further 50 mL of 62% nitric acid was added to Mixture F and the incubation at 72.5° C. was continued for 120 minutes ("Mixture G"). Mixture G was diluted with 10 L hot water plus 400 g wood cellulose and incubated for 30 minutes at 75° C. ("Mixture H"). Mixture H was then poured onto a

filter-cloth in a Büchner funnel in order to separate the solution ("Solution I") from the materials that did not dissolve (which together with the solution that could not be squeezed away from the materials is referred to as "Mixture J").

Solution I was then further filtered through a bed of Filtercel 450 (a filter-aid, used for removing haze) and stirred with Lewatit ionic exchanging resin. The solution obtained after draining away the resin ("Solution K") was poured into about three parts (i.e., three times its own volume) of 80% 2-propanol. The precipitate was squeezed for removing as much of the spent alcohol as possible, and was then washed in a mixture of 60% 2-propanol and 40% water before being squeezed again. The volume of the mixture used for washing was about the same as the volume of Solution K. Following the washings, the precipitate was dried, milled and sieved through a 250µ pore-size powder-sieve.

The pH of an aliquot of mixture H was 2.05 after cooling to 25° C. The amount of dried pectin that could be isolated per kg of solution K was 5.95 g. Using the same symbols and formula as Example 1, the total mass of the extraction mixture just before filtration and yield per peel were calculated as follows:

$$M=30+1+0.015+0.050+0.4+10=41.5$$

$$Y_P=24.7.$$

Both the DE and IV were measured using the same protocols described above. The pectin had a DE=74.6 and IV=6.59 dL/g. The product of yield times intrinsic viscosity was 163 (only 88% of that of Example 1). The YogSimBuf was calculated as 3.24 g/L, which suggests that the strength of the pectin extracted using the conventional process was only 77% of that of Example 1 (keeping in mind that "low" means "strong").

Example 3

The same method described in Example 1 was used to evaluate the effect of the dosage of oxalic acid, pH, and temperature on extraction of lemon peel. A series of 24 similar experiments were conducted in which the dosing of oxalic acid dihydrate was varied at four levels (15, 20, 25, and 30 g per kg of dried peel) and the intended pH was varied at three levels (2.70, 3.00, and 3.30) by either adding nitric acid or sodium hydroxide alongside with the oxalic acid. The pH values that were actually achieved, however, were slightly different from the intended values (see below). In addition, the temperature was varied at two levels (70 and 80° C.).

In all experiments, 1000 g dried peel obtained from the same source as Examples 1 and 2 was suspended with the specified amounts of other ingredients in 20 L water for 60 minutes at the stipulated temperature. The mixture was diluted with 20 L more water and incubated for 60 minutes at the same temperature, and finally filtered and analyzed like described in Example 1, except that the extract was not contacted with an ion-exchanging resin (i.e., meaning that the pectin produced in Example 3 contained more residual calcium than that of Examples 1 and 2). The total calcium mg/g in the pectin was determined using Protocol 4, described above.

The experimental conditions and results are summarized below:

	Experiment number											
	1	2	3	4	5	6	7	8	9	10	11	12
oxalic acid (g)	30	30	30	15	15	15	20	20	20	25	25	25
nitric acid (ml 62%)	6	0	0	16	10	4	12	6	1	9	3	0
1N NaOH (ml)	0	0	73	0	0	0	0	0	0	0	0	24
temperature (° C.)	70	70	70	70	70	70	70	70	70	70	70	70
Extn. mix. pH at 25° C.	2.70	2.96	3.19	2.66	3.01	3.27	2.67	2.95	3.37	2.75	2.97	3.17
Yield %	20.00	20.12	20.99	15.76	14.36	13.04	17.76	16.96	17.17	19.37	18.88	19.40
pectin/peel DE	77.90	77.50	77.50	78.50	78.70	79.30	78.20	78.60	78.70	78.20	78.30	78.20
IV (dL/g)	8.0	8.1	7.8	7.4	8.1	8.5	7.8	8.2	8.0	7.6	7.9	7.9
YogSimBuf (g/L)	2.65	2.54	2.45	3.39	3.41	3.26	3.12	3.05	2.77	2.85	3.07	2.88
Calcium (mg/g)	3.9	3.3	2.7	8.3	7.3	6.1	6.2	5.8	5.4	3.8	3.1	3.1

	Experiment number											
	12	14	15	16	17	18	19	20	21	22	23	24
oxalic acid (g)	30	30	30	15	15	15	20	20	20	25	25	25
nitric acid (ml 62%)	6	0	0	16	10	4	12	6	1.2	9	3	0
1N NaOH (ml)	0	0	72	0	0	0	0	0	0	0	0	24
temperature (° C.)	80	80	80	80	80	80	80	80	80	80	80	80
Extn. mix. pH at 25° C.	2.73	2.97	3.18	2.66	2.89	3.29	2.72	2.95	3.23	2.87	3.11	3.27
Yield %	23.76	26.72	26.35	21.36	18.72	18.88	22.65	21.54	21.83	23.66	25.04	25.24
pectin/peel DE	77.50	77.10	76.60	78.20	78.70	78.80	77.60	77.80	78.10	77.50	77.40	77.40
IV (dL/g)	6.7	6.4	6.9	6.3	6.7	7.0	6.6	6.6	6.7	6.3	6.9	6.9
YogSimBuf (g/L)	3.52	3.49	3.07	4.53	4.30	4.42	3.96	4.03	3.73	3.50	3.36	3.32
Calcium (mg/g)	2.4	2.7	2.3	5.0	4.9	4.7	5.6	3.9	4.2	3.5	2.7	2.3

These results may be better understood in graphical form, as set forth in FIGS. 3-7 and described below.

FIG. 3 shows the calcium content of the experimental pectin samples as a function of the dosage of oxalic acid in g of $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$, $M=126.1$ per kg peel. Not wishing to be bound by any theory, it is believed that because the peel contained 8.0 mg Ca^{++} per g peel, the stoichiometric balance between oxalate acid and calcium should occur at the dosage 25.2 g oxalic acid di-hydrate. What was observed was that the more oxalic acid used for the extraction, the less calcium residual was found in the ensuing pectin samples until leveling off after exceeding a stoichiometric balance. In particular, less calcium was found in pectin that was extracted at 80° C., likely because the yield of pectin was larger at this temperature as compared to 70° C. and the calcium residual was therefore mixed with a larger relative amount of pectin at the higher temperature.

FIG. 4 shows the effect of pH upon extraction yield with individual curves for the eight possible combinations of the four oxalic acid dosages and two temperatures. As can be seen from the graphical illustration, the yield was not strongly dependant upon the extraction pH within the ranges of pH and oxalic acid dosing that were tested in this series of experiments. However, there was a pronounced tendency for the yield to be higher at 80° C. than at 70° C. The yield also tended to be higher with the high oxalic acid dosages as compared to the lower ones. For example, the yield for extraction with 30 mg/g oxalic acid at 70° C. was higher than the yield for 15 mg/g at 80° C.

FIG. 5 shows the intrinsic viscosity as a function of pH with individual curves for each of the eight combinations of the four dosages and two temperatures. As can be seen from the

graphical illustration, the highest intrinsic viscosity results were obtained at the lowest extraction temperature. In addition,

on average, the samples extracted at the lowest pH had a lower intrinsic viscosity than those extracted at the highest pH. When this data is interpreted without considering data from other investigations, however, the relationship between pH and intrinsic viscosity is uncertain and may be interpreted in more than one way. Not wishing to be bound by any theory, it is believed that the intrinsic viscosity can be influenced by a gradual thermal decay of dissolved pectin as well as by possible differences between the pectin that is released willingly and the pectin that releases only in the extractions with high yield, respectively.

FIG. 6 shows as independent parameter the dosage of oxalic acid and as dependent parameter the yield of pectin in percent of the peel. The highest yield was obtained at the highest oxalic acid dosage. The data of FIG. 4, however, seems to suggest that the yield is more an effect of the extraction media (i.e., oxalic acid) rather than an acidity (pH) effect.

FIG. 7 shows the product of yield times intrinsic viscosity as a function of the oxalic acid dosage. The value of the yield increased with the dosage of oxalic acid and became less dependent upon the extraction temperature.

Example 4

Further experiments were conducted to evaluate the reduction of oxalate in the final pectin by contacting the aqueous extract with cationic-exchanging resin. The same process was used as described in Example 1, except the Filtercel-filtrated Solution C was divided into two about equally large portions, one portion being treated with a Lewaitit ion-exchanging resin as described in Example 1 and one portion with which the Lewaitit treatment was omitted. Both of these solutions

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were subsequently treated as described for Solution E in Example 1. The calcium content and oxalate content of the final pectin were subsequently analyzed using Protocols 4 and 5, respectively, each described above.

	Residual Oxalate Content		Residual Calcium Content	
	With ion-exchange (mg oxalate/g pectin)	Without ion-exchange (mg oxalate/g pectin)	With ion-exchange (mg calcium/g pectin)	Without ion-exchange (mg calcium/g pectin)
Experiment A	below detection limit	2.29	0.17	2.08
Experiment B	below detection limit	2.17	0.09	1.71
Average	below detection limit	2.23	0.13	1.90

As can be seen from the foregoing, use of the ion-exchanging resin effectively eliminated oxalate residue from the final pectin.

While the invention has been described in detail with respect to specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereof.

The invention claimed is:

1. A process for extracting pectin having a high degree of polymerization comprising:

adding oxalic acid and/or a water-soluble oxalate to an aqueous suspension of a peel in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium (II) in the peel;

heating the mixture to a temperature from about 50 to about 80° C. for a time sufficient to extract a pectin; and

separating the extracted pectin from the mixture by precipitating the extracted pectin from the mixture, wherein the extracted pectin has a degree of esterification (DE) of at least 72 and a high degree of polymerization as characterized by an intrinsic viscosity of greater than about 6.5 dL/g.

2. The process according to claim 1, wherein the pH of the mixture prior to separating the extracted pectin from the mixture is between 3.1 and 3.4.

3. The process according to claim 2, wherein the mixture is heated to a temperature from about 70 to about 80° C. during the separating of the extracted pectin.

4. The process according to claim 1, further comprising filtering the mixture to remove insoluble solids after extracting the pectin and prior to separating the extracted pectin.

5. The process according to claim 1, further comprising contacting the mixture containing the extracted pectin with a ion-exchanging resin prior to separating the extracted pectin.

6. The process according to claim 5, wherein the ion-exchanging resin comprises a cation-exchanging resin effective to remove oxalate residues, calcium residues, or combinations thereof from the extracted pectin.

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7. The process according to claim 5, wherein the extracted pectin has a total oxalate content less than 2.0 mg per 1 g of pectin.

8. The process according to claim 5, wherein the extracted pectin has a total calcium content less than 1 mg/g.

9. The process according to claim 1, further comprising one or more of the steps of washing, pressing, drying, and milling the extracted pectin after separating the extracted pectin to obtain a final pectin product.

10. The process according to claim 1, wherein the yield per peel (Yp) is greater than about 23%, wherein $Yp = 0.1 \cdot y_s \cdot M / W_p$ and y_s is the yield of solution (liquid extract) in g/kg, M is the total mass of the mixture before separating the extracted pectin, and W_p is the weight of the peel used in the aqueous suspension.

11. The process according to claim 10, wherein the product of the yield per peel and intrinsic viscosity is greater than about 165%·dL/g.

12. The process according to claim 1, wherein the extracted pectin has a degree of esterification (DE) of at least 75.

13. The process according to claim 1, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 7.8 dL/g.

14. The process according to claim 1, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 8.5 dL/g.

15. A process for extracting pectin having a high degree of polymerization consisting essentially of:

adding oxalic acid and/or a water-soluble oxalate to an aqueous suspension of a peel in an amount sufficient to provide a mixture having a pH of between 3.0 and 3.6 and a total molarity of oxalate greater than a total molarity of calcium (II) in the peel;

heating the mixture to a temperature from about 50 to about 80° C. for a time sufficient to extract a pectin;

optionally filtering the mixture to remove insoluble solids; contacting the mixture containing the extracted pectin with a cation-exchanging resin; and

separating the extracted pectin from the mixture by precipitating the extracted pectin from the mixture to obtain a yield per peel (Yp) greater than about 23%, wherein $Yp = 0.1 \cdot y_s \cdot M / W_p$ and y_s is the yield of solution (liquid extract) in g/kg, M is the total mass of the mixture before separating the extracted pectin, and W_p is the weight of the peel used in the aqueous suspension;

wherein the extracted pectin has a degree of esterification (DE) of at least 72, a high degree of polymerization as characterized by an intrinsic viscosity of greater than about 6.5 dL/g.

16. The process according to claim 15, wherein the extracted pectin has a total oxalate content less than 2.0 mg per 1 g of pectin.

17. The process according to claim 15, wherein the extracted pectin has a total calcium content less than 1 mg/g.

18. The process according to claim 15, wherein the time sufficient is from about 1 hour to about 3 hours.

19. The process according to claim 15, wherein the extracted pectin has a degree of esterification (DE) of at least 75.

20. The process according to claim 15, wherein the extracted pectin has an intrinsic viscosity (IV) of at least 7.8 dL/g.

* * * * *