AGRO BIOMATERIALS BASED ON CHITOSAN AND STARCH FUNCTIONALIZED WITH POLYPHENOLS FROM MURTA (*UGNI MOLINAE* TURCZ) LEAF EXTRACT

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III Latin American Congress Biorefineries
Ideas for a sustainable world
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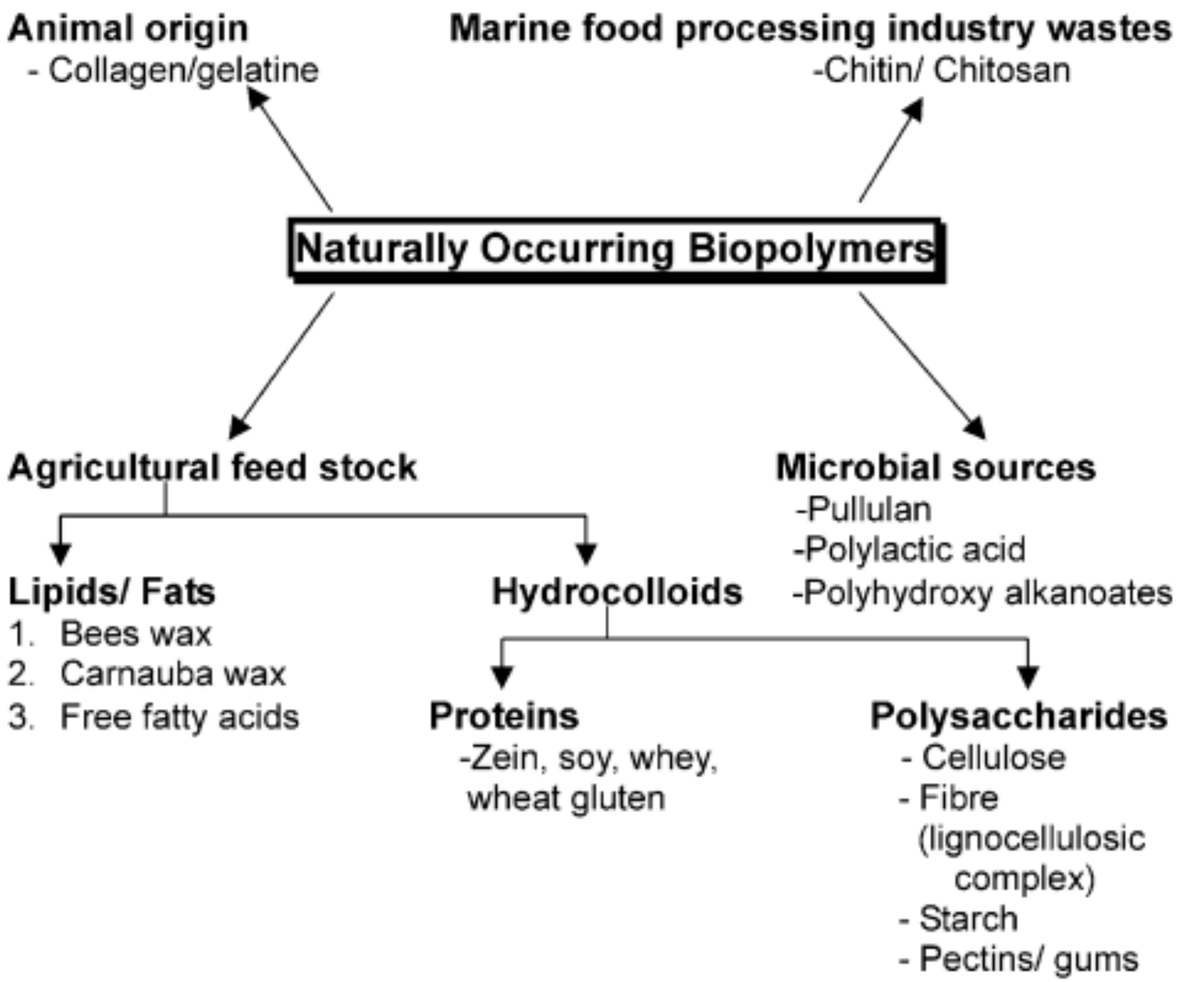
Naturally occurring biopolymers of use in biodegradable packaging films and composites

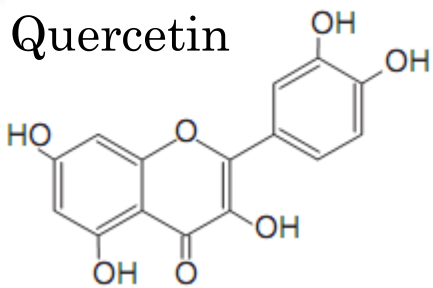


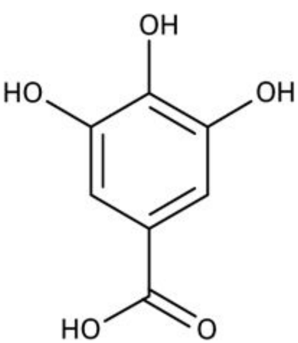
(Tharanathan, 2003)

Biomaterials based on chitosan functionalized with polyphenols are being broadly developed for pharmaceutical, cosmetic and food applications.

Chitosan

Quercetin



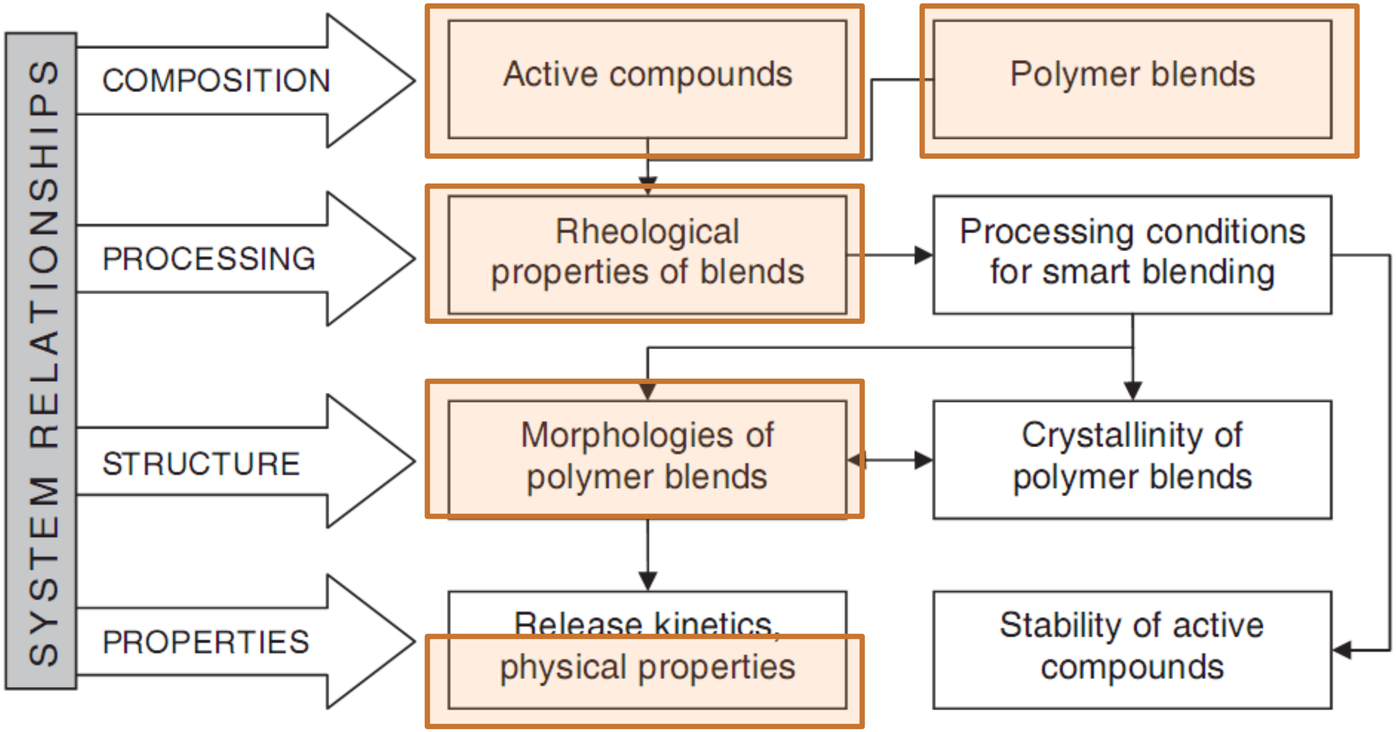


Gallic acid

- Green tea extract
- Indian gooseberry extract

Božič, Gorgieva, & Kokol. (2012)
Siripatrawan & Harte (2010))
Mayachiew, & Devahastin(2010).

Systematic approach for developing novel film using blends



(Lacoste *et al.*, 2005)

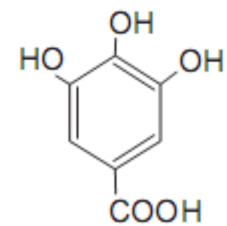
Objective

To investigate the effect of the polyphenol-rich aqueous extract from murta leaves (PEML) on the dynamic and steady-shear rheological behavior of film-forming solutions (FFS) based on chitosan and chitosan- cornstarch blend, and on chemical structure, morphology and physical properties of the resulting films.

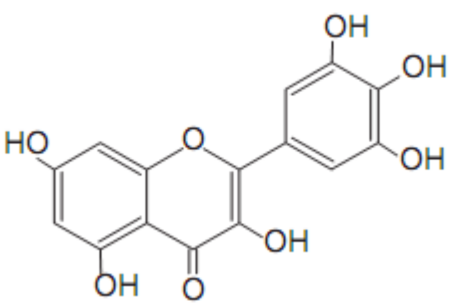
Plant material

Murta leaves (*Ugni molinae* Turcz) of ecotype 27-1

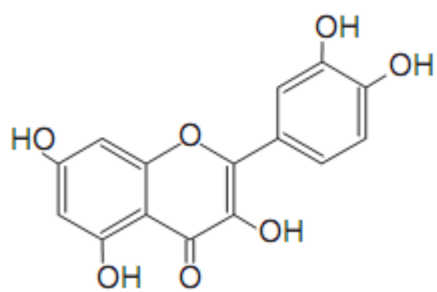
Phenol acids like gallic acid, as well as flavonoid aglycones and glycosides from quercetin, myricetin and kaempferol are among the main compounds found in those extracts are



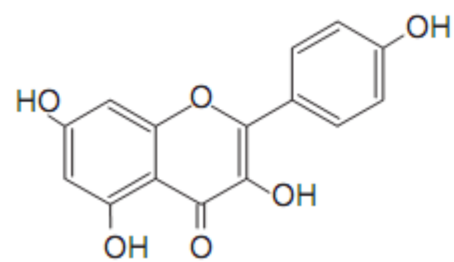
gallic acid



myricetin



quercetin



kaempferol

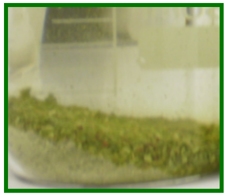
(Rubilar et al., 2005; Bifani et al., 2007).

Polyphenol-rich extract from murta leaves (PEML)

Dry
Air-dried leaves, 48 h at 35 ºC

7% moisture

Milled and sieved
1.00 to 2.38mm



Ratio = 1:10

Aqueous extraction
25 ºC for 90 min at 170 osc./min.

Filtered

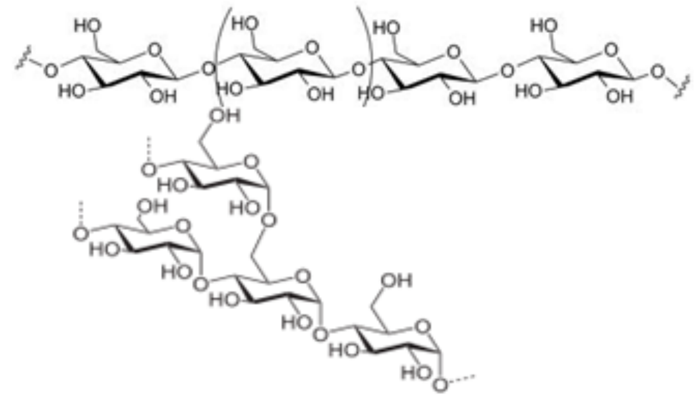
Sterilized
membrane PES of 0.22 μm

(Bifani et al., 2007)

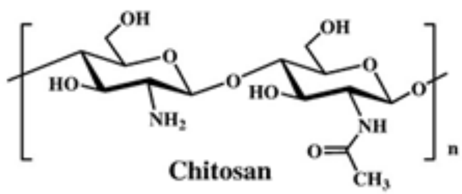
- IC $_{ABTS.+}$: 0.038 mg GAE/mL
- Total phenolic content :
40.67 mg GAE/g d.m. murta leaves.

Structure of hydrocolloids studied for formulate blend FFS

Type	Source	Structure
Starch ⇒ Amylose + Amylopectin	Plant	α-(1→4)-D-glucose + α-(1→4) and α-(1→6)-D-glucose
Chitin ⇒ Chitosan	Animal	β-(1→4)-D-(N-acetyl)glucosamine ⇒ β-(1→4)-D-glusamine



Corn starch: 27% amylose



Chitosan

Deacetylation degree: 75%

Preparation of film-forming solutions (FFS)

Hidrocolloids

CH : Chitosan film
CH-PEML : Chitosan films with PEML
CH-CS : Chitosan-corn starch film
CH-CS-PEML : Chitosan-corn starch film with PEML

PEML was 40 % of the dissolution solvent of CH solution, according to Bifani et al., 2007

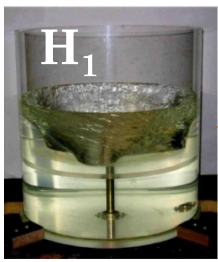
FFS	Hydrocolloid [% w/w]			Glycerol [g/g H_T]	PEML [b] [mL/g H_T]	
H_1-H_2	H_1	H_2	H_T [a]		Without	With [c]
CH	2.00	-	2.00	0.25	0	20
CH-CS	1.50	0.50	2.00	0.25	0	20

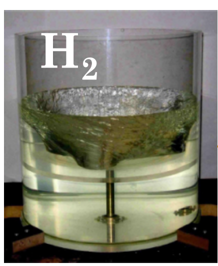
[a] H_T: Total concentration of hydrocolloids in solution,
[b] Polyphenol-rich extract from murta leaves,
[c] Total phenol content from PEML expressed as gallic acid equivalent (GAE): 81.33 mg GAE/ g H_T

Obtaining film-forming solutions

- Hydrocolloid (H)
- H_2O
- Glycerol
- Active agents: PEML

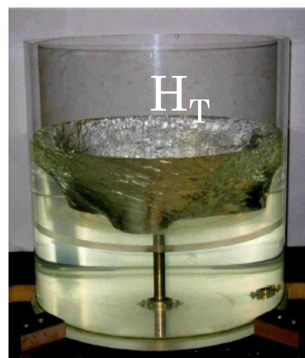
Film-forming solutions (FFS)





30 min at 45 ºC

Blend solution



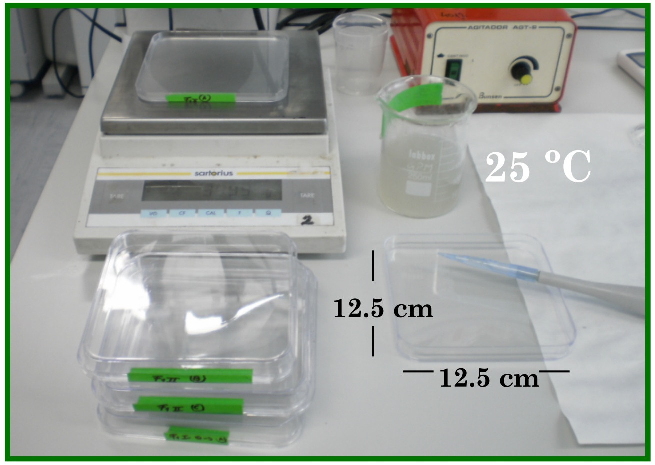
Rheologycal Properties

Dry

- Chemicals interaction
- Physical properties

Film formation

40 ± 0.01 g



Dry

40 °C for 24 h

Conditioned 50 ± 3% RH for 2 days at 22 °C.

Parameters for the Power law model, at shear rate above $0.4\ s^{-1}$ at 25 °C.

$$\boxed{\tau = K\,\dot{\gamma}^{\,n}}$$

Non-Newtonian behavior ($n \neq 1$).

Sample / PEML	*K* (Pa·s^n)			*n* (-)			r^2 (-)	
	Without	With		Without	With		Without	With
CH	4.23	164	**	0.70	0.32	**	0.993	0.984
CH-CS	3.15	150	**	0.63	0.17	**	0.990	0.915

** $P < 0.01$, NS: Not significant

Significance of difference between parameters *K* and *n* of FFS with and without PEML using orthogonal contrasts

Apparent viscosity

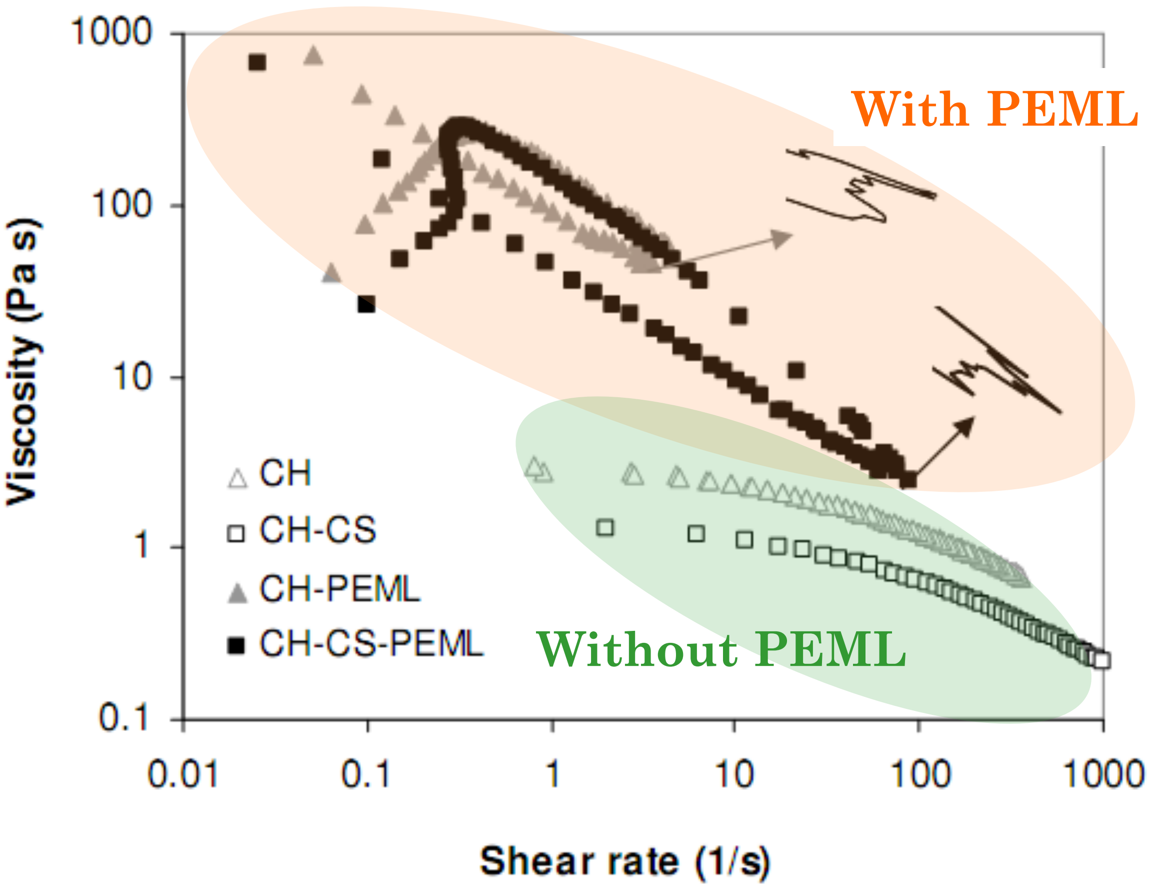
$$\eta_{(\dot{\gamma})} = K(\dot{\gamma})^{n-1}$$

Sample / PEML	$\eta_{(\dot{\gamma}=2)}$ (Pa·s) Without	With	
CH	3.45	102	**
CH-CS	2.44	84.5	**

** $P < 0.01$, NS: Not significant

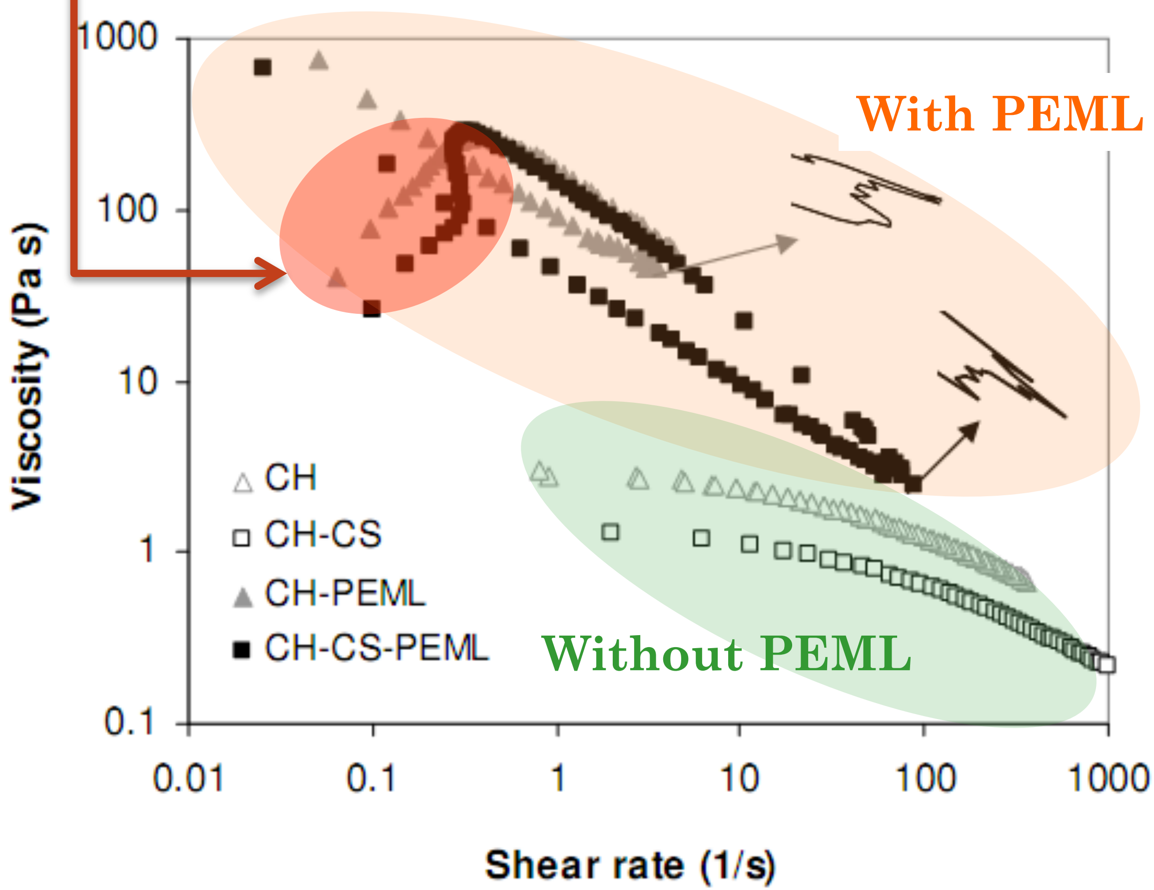
Effect of shear rate on viscosity of film-forming solutions (FFS)

Viscosity curves at 25 °C



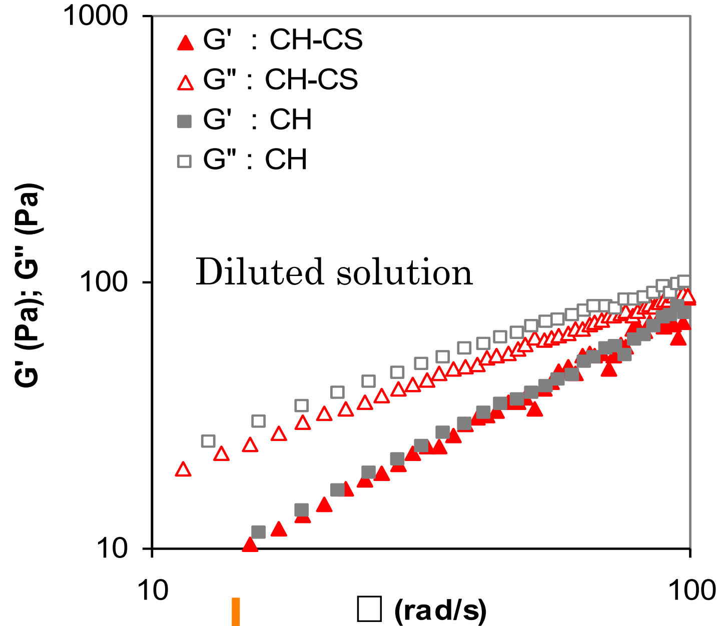
The dilatant behavior ($n > 1$) indicated that the formation of new linkages between hydrocolloids and PEML predominated over destroying the structure, with the result of a restructured network.

Viscosity curves at 25 °C

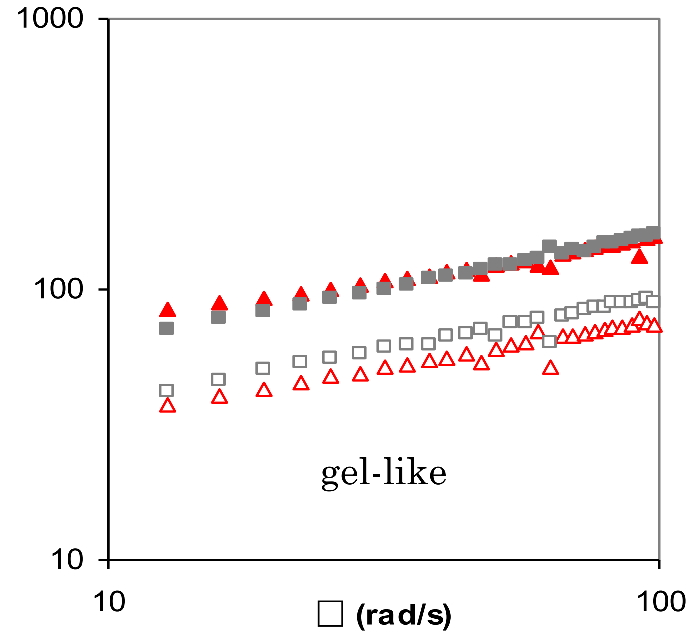


Effect of PEML on viscoelasticity of film-forming solutions at 25 °C

Without PEML



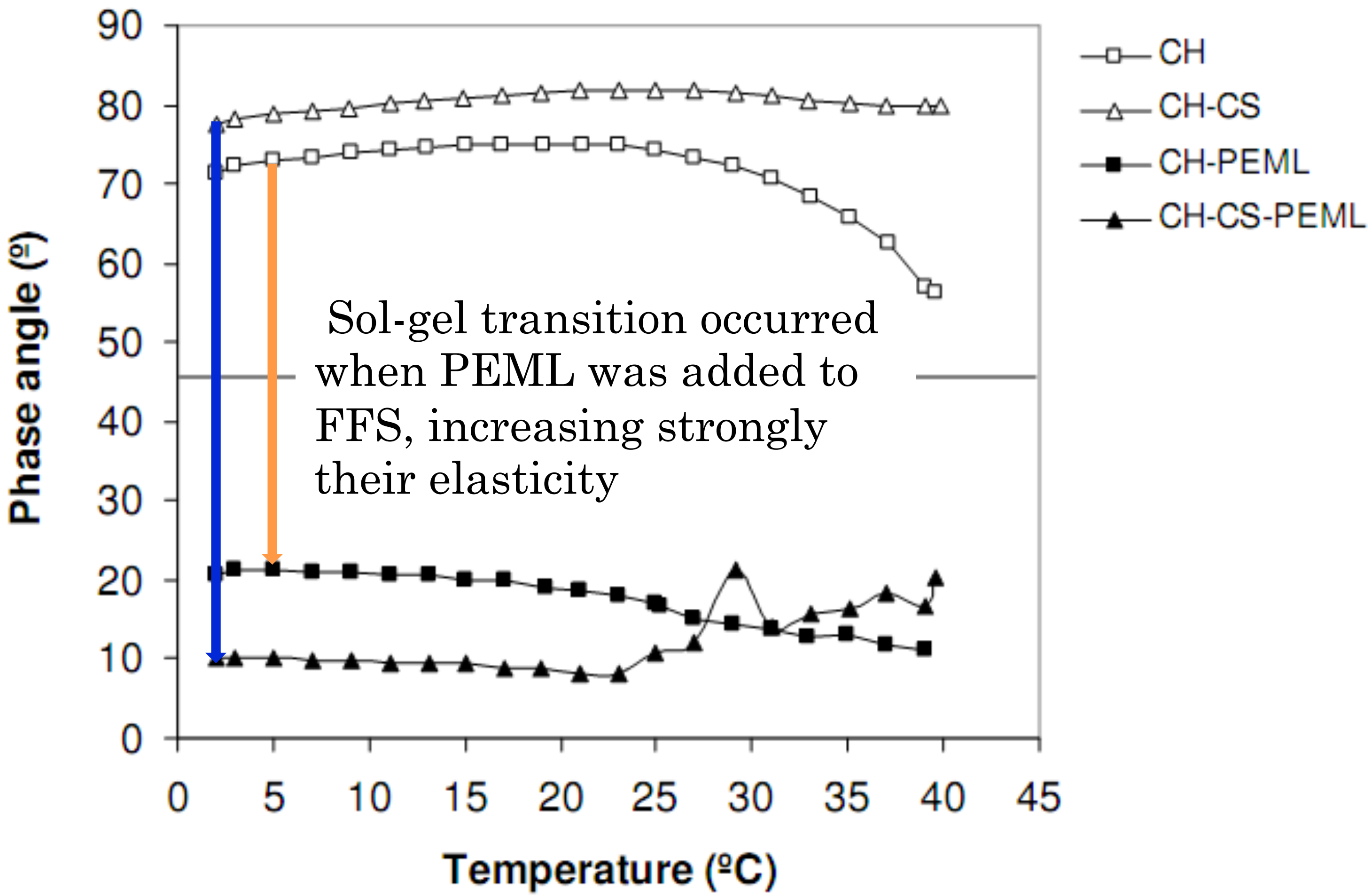
With PEML



Sample	Crossover point			
	G' = G" (Pa)	ω (rad/s)	η* (Pa·s)	tg (min)
CH	123	104	1.27	4.4
CH-CS	131	105	0.77	4.6

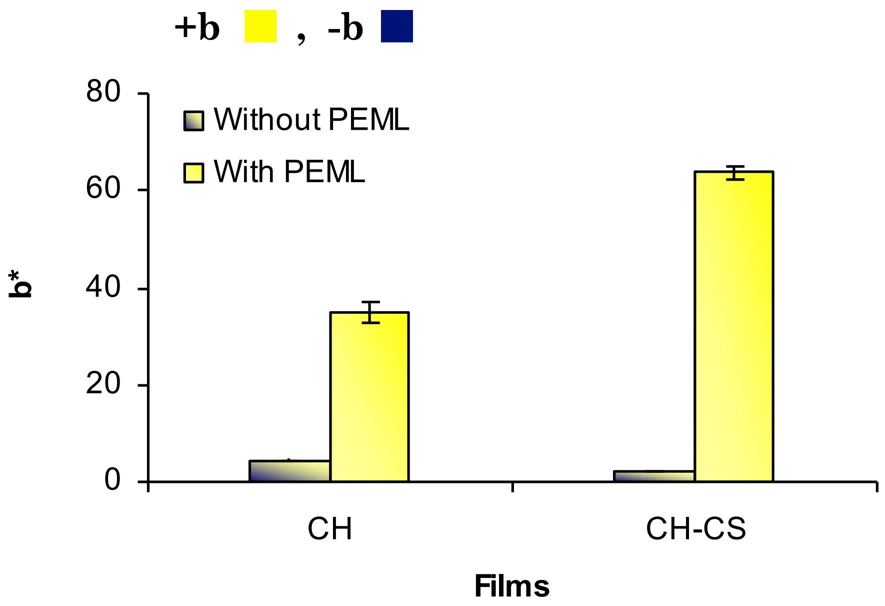
Mechanical spectra showing the angular frequency (w) dependence of storage modulus (G') and loss modulus (G") at 25 °C.

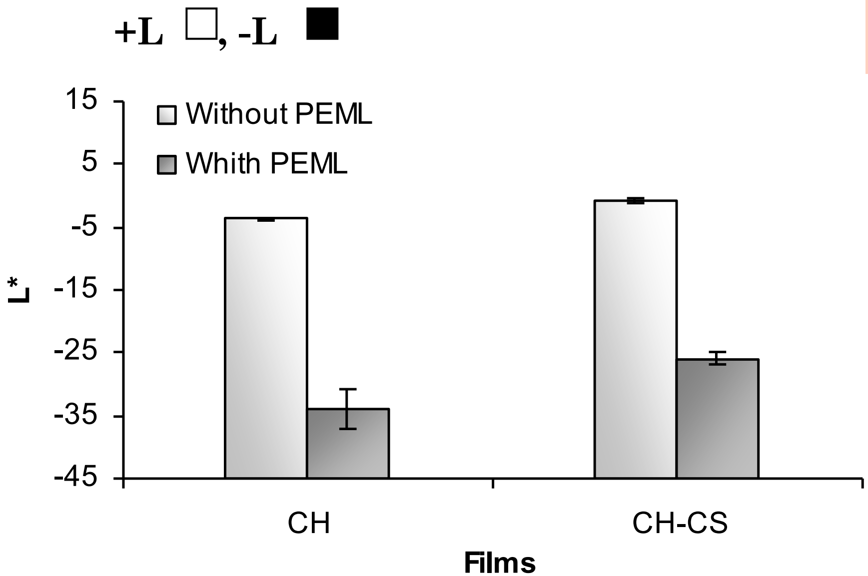
Effect of temperature on elasticity of film-forming solutions

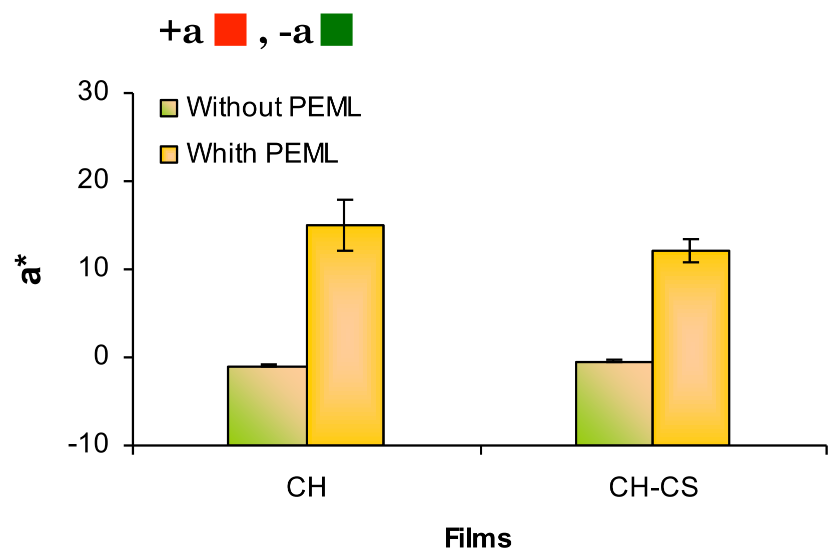


PEML increase elasticity of CH (71 %) and CH-CS (88%) film-forming solutions.

Effect of PEML on optical properties of films







Effect of PEML on mechanical properties of films

Film / Additive	Thickness (μm)	Mechanical properties		References
		TS (MPa)	EB (%)	
Chitosan-starch				
	72.0 ± 2.4 [c]	20.56 ± 1.78 [b]	13.52 ± 2.85 [b]	This work
PEML	126 ± 12 [a]	17.13 ± 1.22 [c]	6.57 ± 2.43 [c]	This work
	98.9 ± 5.1	37.50	27.95	Mathew et al. (2006)
	73.5 ± 3.9	~ 42	~ 57	Mathew and Abraham (2008)
Ferulic acid	78.9 ± 4.3	~ 49	~ 60	Mathew and Abraham (2008)

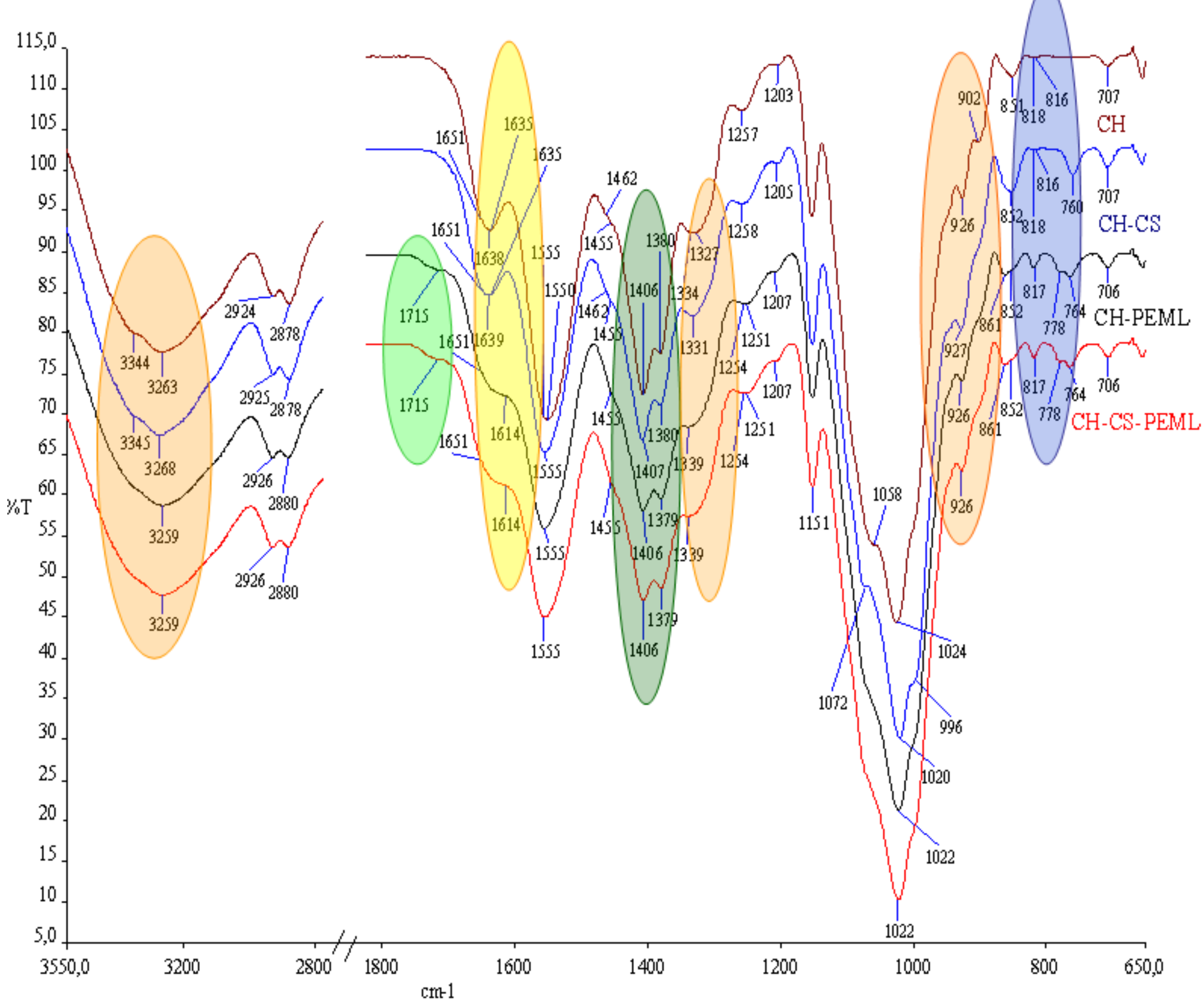
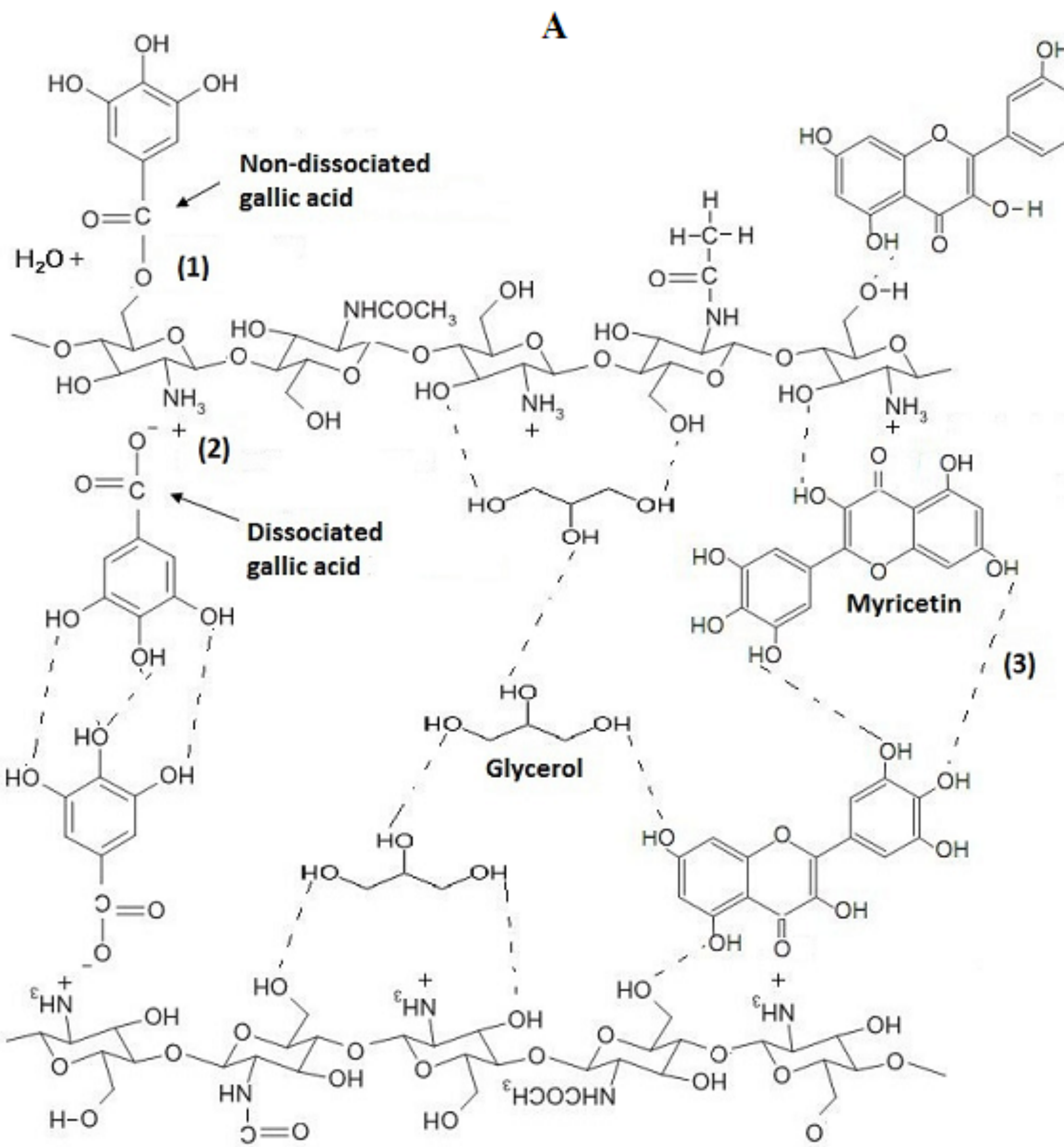


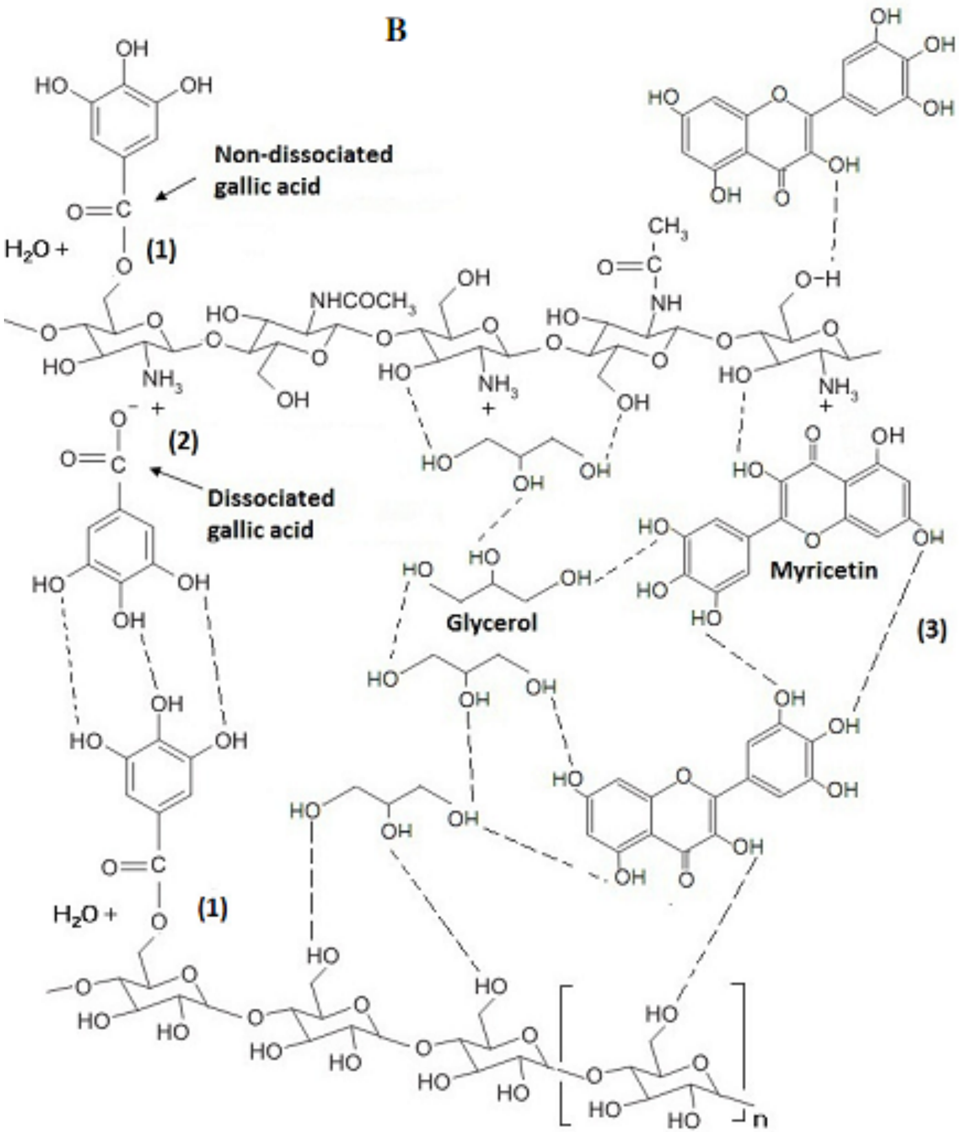
Figure 5. FTIR spectra of: chitosan films without (CH) and with PEML (CH-PEML), Chitosan-starch film without (CH-CS) and with PEML (CH-CS-PEML) after conditioning to 43% RH and 25ºC.

Interaction mechanisms at acid pH (4.5) of PEML on chitosan film



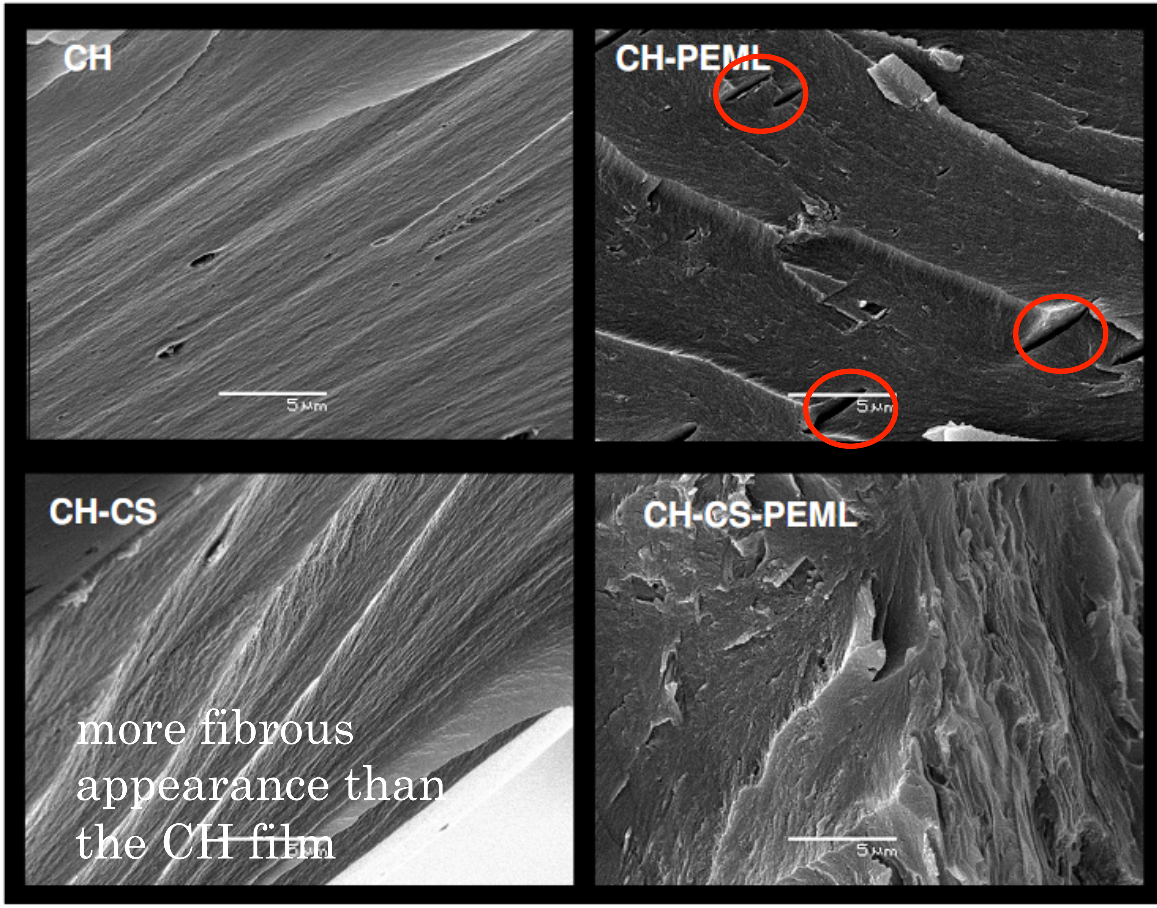
(1) ester linkage
(2) electrostatic interaction
(3) hydrogen bond.

Interaction mechanisms at acid pH (4.5) of PEML on chitosan film



(1) ester linkage
(2) electrostatic interaction
(3) hydrogen bond.

Effect of PEML on film cross-section



more fibrous appearance than the CH film

PEML creates several elongated horizontal pores of 1 - 4 µm.

the formation of horizontal layers and some zones with discontinuity or disorder, and pores, were observed

Scale bar 5 µm and 5000 X magnification

CONCLUSIONS

- Viscoelastic properties show that polyphenols from murta leaf extract turns CH and CH-CS solutions from a diluted solution into a gel-like structure, increasing their elasticity in 71 and 88% respectively, which is a clear indication of the improvement on the three-dimensional cross-linked networks stability.

- This restructuring ability of CH-PEML and CH-CS-PEML gels make them an important class of materials with applications such as in drug delivery or as natural-based wound dressings impregnated with bioactive compounds.

- PEML produced noticeable linkages in the presence of chitosan and starch, which reduced considerably polymer chains mobility and consequently, the film mechanical properties changes.

- Starch stabilized the interaction between CH and PEML, and then CH-CS-PEML was a more continuous and homogeneous film structure than CH-PEML.

ACKNOWLEDGEMENTS

We thank Mrs I. Seguel (M.Sc.) from Unidad de Recursos Genéticos, INIA Carillanca, Temuco, Chile, for the murta leaf ecotype.

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Thank you for your attention !!

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Ideas for a sustainable world

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Resultados y Discusiones

Fenoles totales y capacidad antioxidante de extractos de hojas

	Extracto	Nombre científico	Fenoles Totales [1]	I.O.A.AS[2] [%]	CI_{50} [3] ABTS$^{\cdot+}$	CI_{50} [4] DPPH$^{\cdot}$
	Murta	*Ugni molinae*	46,64 ± 0,85 ^{a}	84,60 ± 12,99 ^{a}	1,08 ± 0,05 ^{e}	2,55 ± 0,10 ^{f}
	Toronjil	*Melissa officinalis*	31,42 ± 2,21 ^{b}	90,74 ± 11,78 ^{a}	3,96 ± 0,57 ^{d}	10,95 ± 1,10 ^{e}
	Romero	*Rosmarinus officinalis*	19,56 ± 0,60 ^{c}	41,61 ± 8,27 ^{b}	8,03 ± 0,21 ^{c}	30,26 ± 1,38 ^{c}
	Laurel	*Laurus nobilis*	6,82 ± 0,81 ^{d}	0,01 ± 0,01 ^{d}	9,30 ± 0,10 ^{b}	48,28 ± 1,48 ^{b}
	Cedrón	*Lippia citriodora*	13,58 ± 0,61 ^{d}	23,57 ± 8,40 ^{c}	11,27 ±0,81 ^{a}	56,09 ± 3,61 ^{a}
	Matico	*Piper a ngustifolium*	13,31 ± 0,38 ^{d}	2,80 ± 1,12 ^{d}	11,40 ± 0,15 ^{a}	22,36 ± 0,78 ^{d}

Estadística Duncan ($p \leq 0,05$)
1 Fenoles Totales [mg EAG/g b.s.]
2 I.O.A.AS.: Inhibición de la oxidación de ácido ascórbico
3 CI_{50}: concentración de extracto que inhibe el 50% de los radicales ABTS$^{\cdot+}$. expresada como [mg hoja b.s/mL]
4 CI_{50}: concentración de extracto que inhibe el 50% de los radicales DPPH$^{\cdot}$ expresada como [mg hoja b.s/mL]

Aggregates

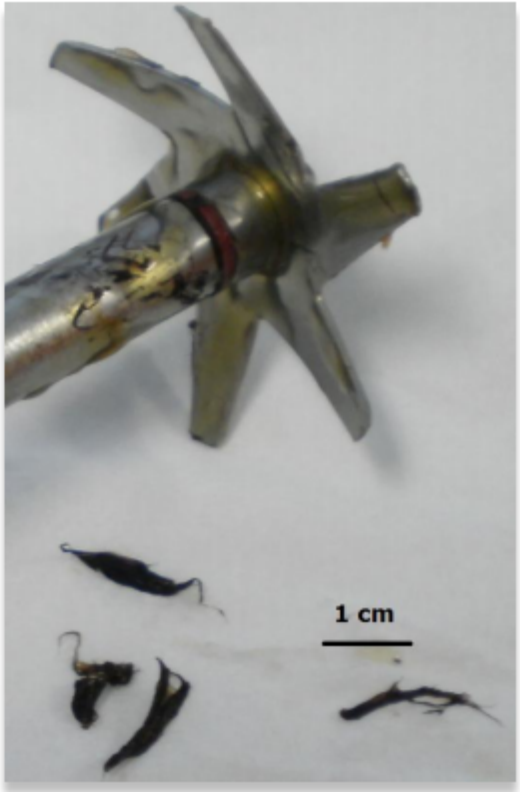


Figure. Aggregates obtained from CH-PEML and CH-CS-PEML during mechanical stirring.

Resultados

Microestructura

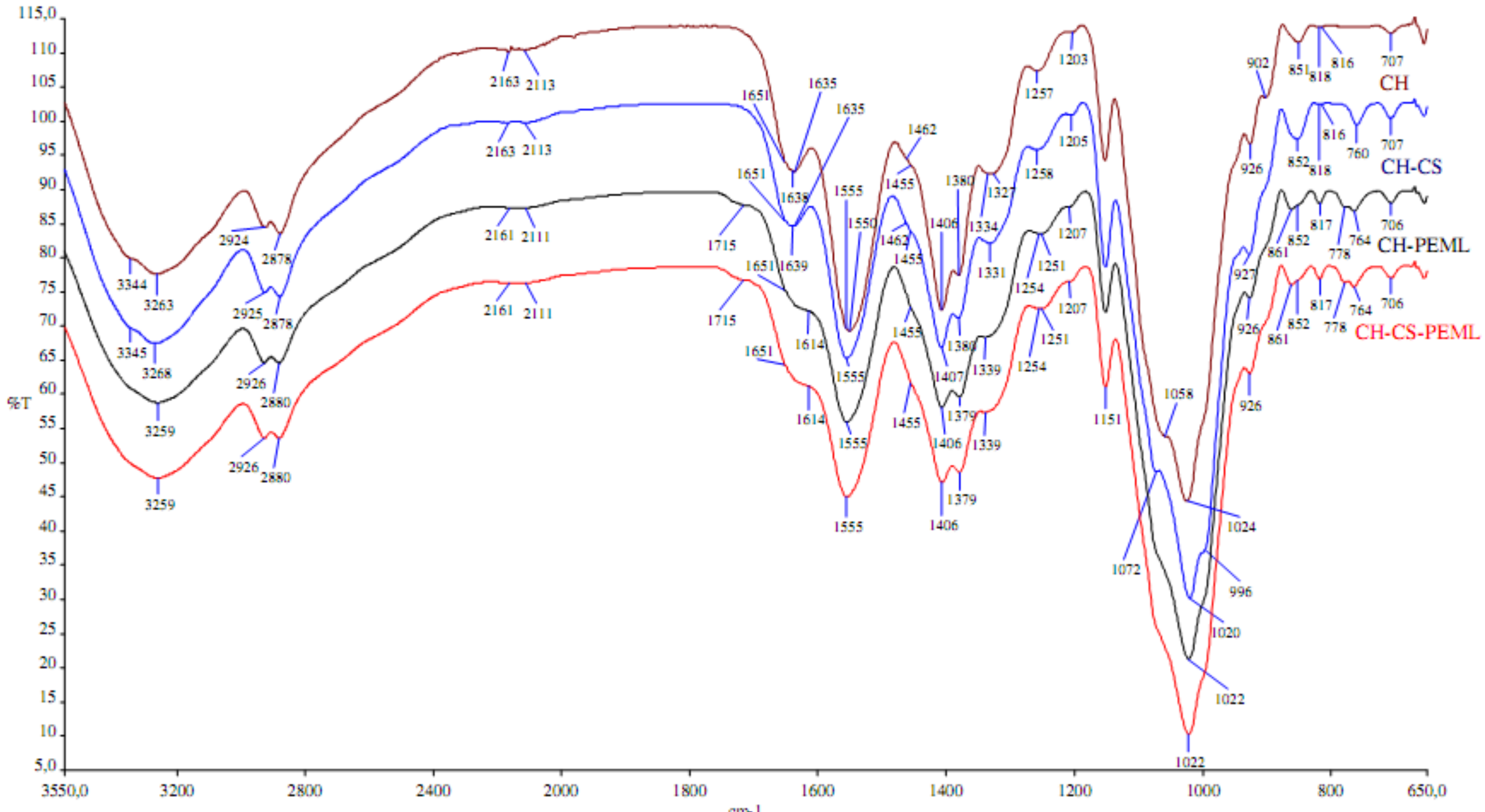


Figure 5. FTIR spectra of: chitosan films with PEML (CH-PEML) and without (CH), Chitosan-starch film with PEML (CH-CS-PEML) and without (CH-CS) after conditioning to 43% RH and 25 ºC.

Trends

To reduce residues produced by packaging

Eco-friendly packaging for food preservation

Edible films and coatings based on biodegradable polymers

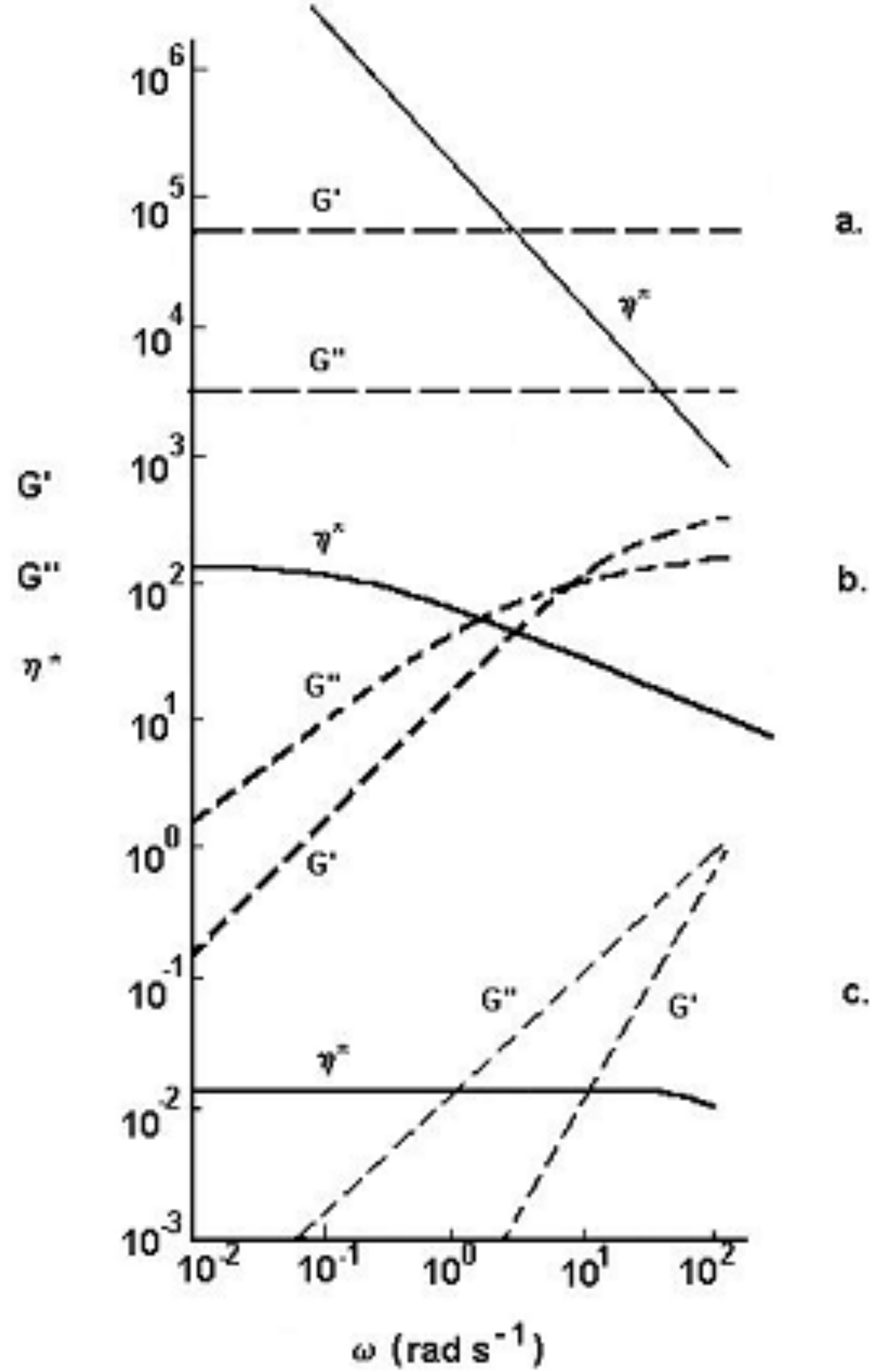
(Kester y Fenema, 1986; Gennadios y Weller, 1990; Guilbert *et al.,* 1997; Rocha & Morai, 2007).

Frequency sweep

0.01–50 Hz at 25ºC

Mechanical spectra

Viscoelastic behavior



Physical properties of chitosan and chitosan-starch films formulated with polyphenol-rich extract from murta leaves (PEML), and other literature cited films

Film / Additive	Thickness (μm)	Color		Mechanical properties		References
		a*	b*	TS (MPa)	EB (%)	
Chitosan						
	70.7 ± 5.0 [c]	-1.01 ± 0.11 [b]	4.52 ± 0.39 [c]	29.69 ± 1.89 [a]	45.10 ± 1.40 [a]	This work
PEML	77.5 ± 3.5 [b]	14.99 ± 2.98 [a]	35.11 ± 2.14 [b]			This work
	97.0 ± 8.0	-0.01 ± 1.19	44.67 ± 2.39	17.34 ± 2.43	44.20 ± 7.90	Pereda et al. (2011)
	62.1 ± 6.3	1.11 ± 0.16	1.37 ± 0.10	23.66 ± 2.63	54.62 ± 3.12	Siripatrawan & Harte (2010)
Green tea extract	62.1 ± 6.3	3.32 ± 0.66	28.87 ± 0.83	25.13 ± 1.91	58.14 ± 4.24	Siripatrawan & Harte (2010)
	80	0.70 ± 1.00	29.00 ± 2.00	24.00 ± 3.00	29.00 ± 2.00	Moradi et al. (2012)
Grape seed extract	80	21.0 ± 0.90	16.00 ± 0.20	16.00 ± 0.60	21.00 ± 3.00	Moradi et al. (2012)
Chitosan-starch						
	72.0 ± 2.4 [c]	-0.41 ± 0.07 [c]	2.08 ± 0.19 [d]	20.56 ± 1.78 [b]	13.52 ± 2.85 [b]	This work
PEML	126 ± 12 [a]	12.15 ± 1.23 [a]	63.63 ± 1.12 [a]	17.13 ± 1.22 [c]	6.57 ± 2.43 [c]	This work
	98.9 ± 5.1			37.50	27.95	Mathew et al. (2006)
	73.5 ± 3.9			~ 42	~ 57	Mathew and Abraham (2008)
Ferulic acid	78.9 ± 4.3			~ 49	~ 60	Mathew and Abraham (2008)

Values of this work in the same column with different superscripts mean that the values are significantly different ($P < 0.05$).

PEML: Polyphenol-rich extract from murta leaves, TS: Tensile strength (MPa) and EB: Elongation at break (%).

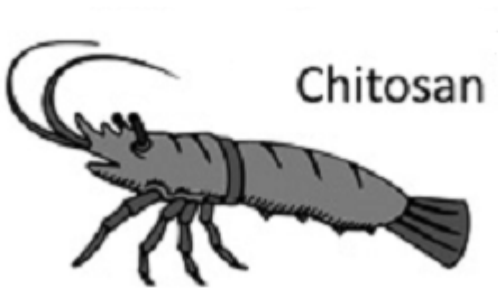
Resultados

Mecanismo de interacción modelo

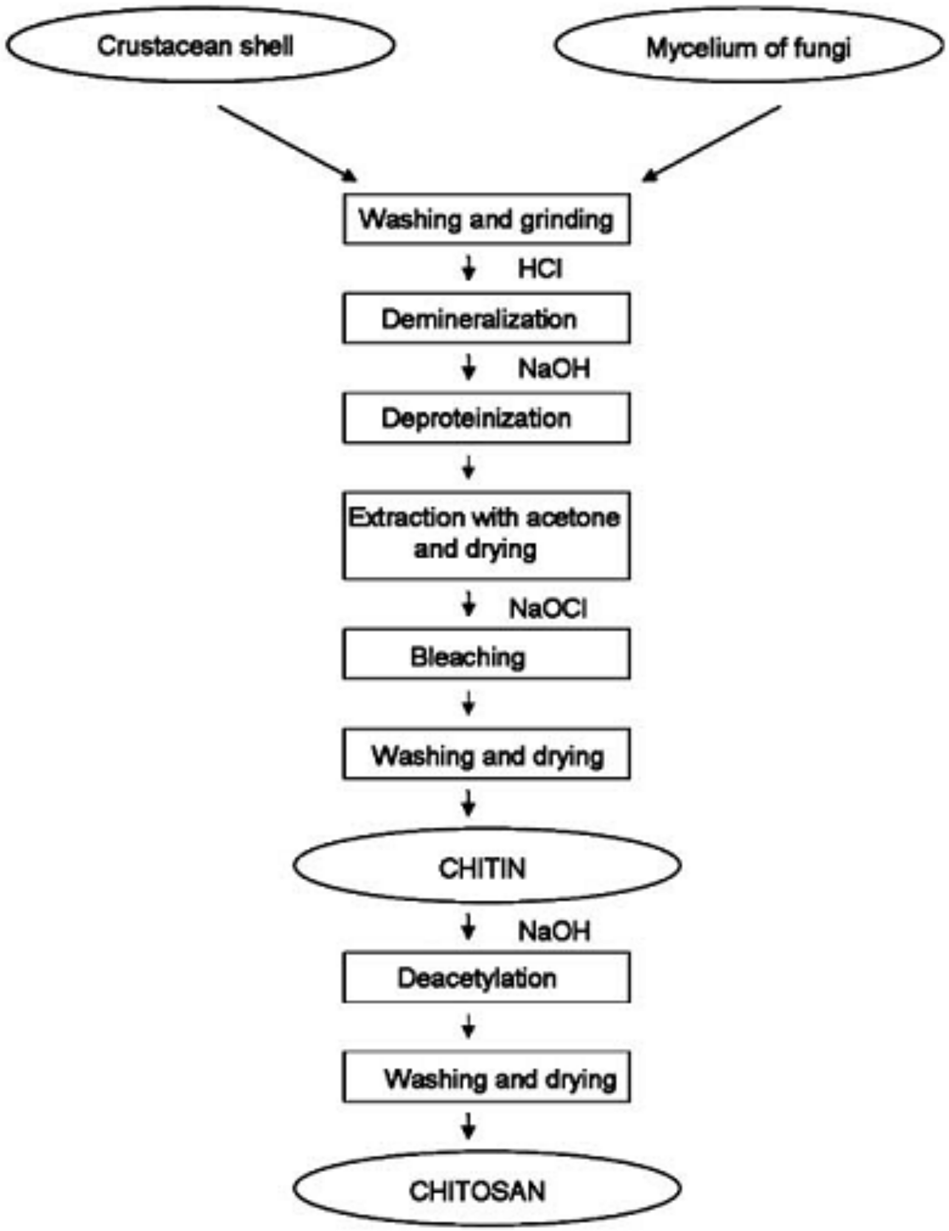
Figure 6. Interaction mechanisms at acid pH (4.5) of chitosan-starch film with PEML (CH-CS-PEML): chitosan chain (above), corn starch chain (below). As models of phenolic acids and flavonoids from PEML, gallic acid and myricetin are presented.

Example of interactions:

(1) ester linkage,
(2) electrostatic interaction, and
(3) hydrogen bond.



Aranaz et al 2009. Functional Characterization of Chitin and Chitosan

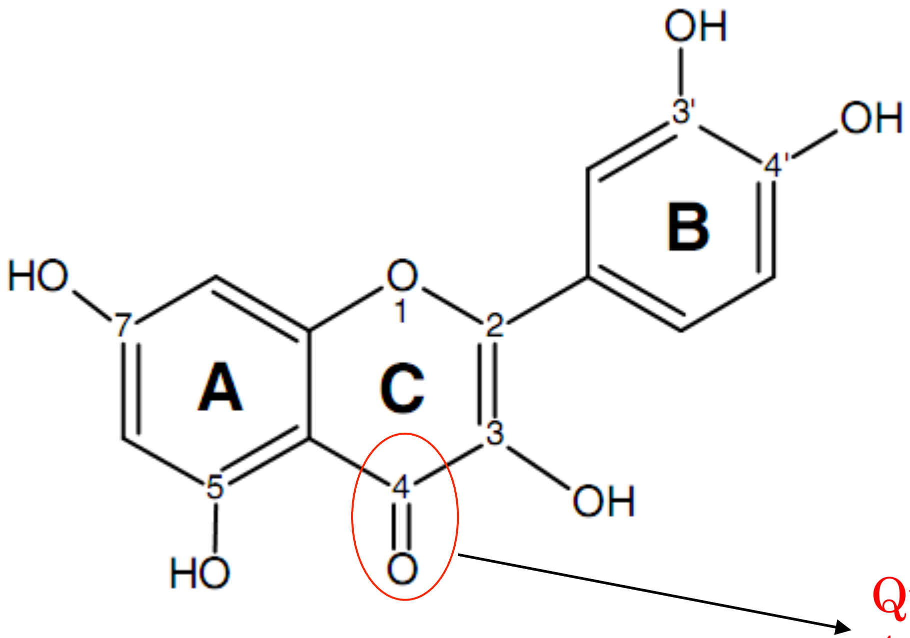


Scheme 1. Preparation of chitin and chitosan from raw material.

Blending two different hydrocolloids can strongly change both the physical and rheological properties of the composite film-forming solution (FFS), affecting the functionality of the resulting coatings and films.

INTERACCIÓN CHITOSAN Y QUERCETINA

On the contrary, and since CBC contains the –$NH(CH_2)_4COOH$ groups and still some unmodified –NH_2 groups (from chitosan) and –$NHCOCH_3$ groups (from chitin), which are capable of interacting and binding to **quercetin hydroxyl** and **carbonyl groups**, the mass transport **(Dias 2011)**



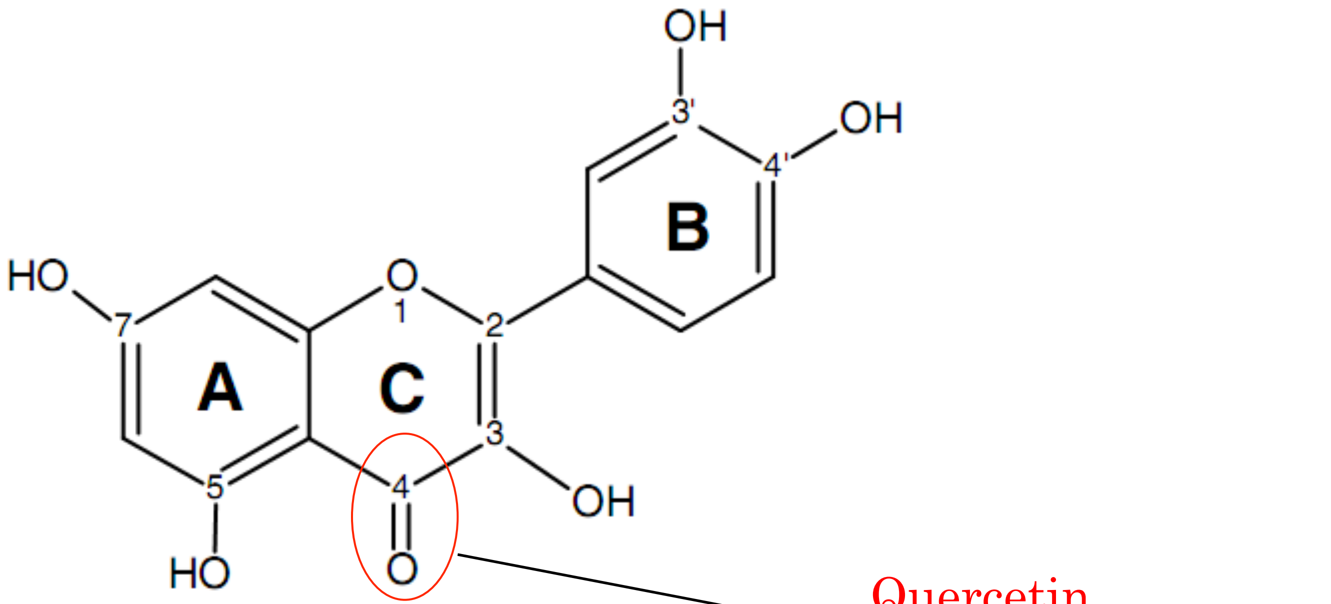
Quercetin
4-***carbonyl groups.***

Quercetin

Fuente figura: Trouillas 2006. A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: The specificity of the 3-OH site

INTERACCIÓN CHITOSAN Y QUERCETINA
En Dias et al 2011

Previous studies have reported the occurrence of relevant specific interactions (namely hydrogen bonding) and even the ionic complexation in acidic conditions between several phenolic compounds (including quercetin) and chitosanwhich permitted phenolics isolation from aqueous extraction media as well as the development of potential controlled release systems (Popa et al., 2000; Yamada et al., 2000; Alexandrova et al., 2006; Xia et al., 2007; Zhang et al., 2008; Pasanphan and Chirachanchai, 2008).



Quercetin

Fuente figura: **Trouillas 2006.** A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: The specificity of the 3-OH site

PEML was obtained by extraction (25 ºC, 90 min and 170 rpm) of leaf powder, ecotype 27-1

Espesor

The incorporation of starch in the chitosan film did not change significantly the thickness (CH film: 71 μm and CH-CS film: 72 μm). PEML strongly increases thickness of CH-CS-PEML film (126 μm) and slightly increases thickness of CH-PEML film (78 μm).

MECHANICAL PROPERTIES

filmsTensile strength (TS) and elongation at break (EB) of CH films with 75% DD (TS = 29.69 ± 1.89 MPa and EB = 45.1 ± 1.4%) was close to TS and EB of chitosan films with 90 % DD (6) and (7), which

Calculo % extracto PEML en la solución finmogénica

Si 2 g H_T/ 100 mL solvente ⇒ 1 g H_T/ 50 mL solvente

⇒ 50 mL solvente → 100%

20 mL extracto → X

∴ **Porcentaje de extracto incorporado en la solución filmogénica es: 40% PEML**

Table 2. Composition of film-forming solutions, with or without polyphenol-rich extract from murta leaves (PEML).

FFS	Hydrocolloid [% w/w]			Glycerol [g/g H_T]	PEML [b] [mL/g H_T]	
H_1-H_2	H_1	H_2	H_T [a]		Without	With [c]
CH	2.00	-	2.00	0.25	0	20
CH-CS	1.50	0.50	2.00	0.25	0	20

[a] HT: Total concentration of hydrocolloids in solution, [b] Polyphenol-rich extract from Murta leaves, [c] Total phenol content from PEML expressed as gallic acid equivalent (GAE): 81.33 mg GAE/ g HT

The incorporation of PEML was 40 % of the dissolution solvent of CH solution.

Considering the results obtained before (Bifani et al., 2007) in relation to the reduction in the oxygen permeability of films obtained, after incorporation of 40 mL of aqueous extract of murta leaves in 100 mL of film-forming solution of carboxymethylcellulose.

Antecedentes

- An increase of the antioxidant activity of the chitosan is observed when flavonoids are added (Sousa, Guebitz & Kokol, 2009) .

-The increase can depends on the type of the flavonoid used as well as on the quantity of the flavonoid grafted (Sousa, Guebitz & Kokol (2009) .

Introduccion (Cortado de la introducción de paper)

There have been observed interactions between chitosan and polyphenolic compounds from green tea (Siripatrawan & Harte, 2010) and Indian gooseberry extract (Mayachiew & Devahastin, 2010), as well as tannic acid (Rivero et al., 2010), catechin and gallic acid (Curcio et al., 2009, Božič, Gorgieva & Kokol, 2012a). When ferulic acid was incorporated into starch–chitosan blend film (Mathew & Abraham, 2008), interactions between the amide groups of chitosan and hydroxyl groups of starch and ferulic acid improved the barrier properties and tensile strength of the resulting films.

Antioxidant activity of quercetin functionalized chitosan Bozic et al 2012b

All tested samples showed strong inhibitory capacity to the ABTS•+ cation radicals; however the highe was for quercetin control, its oligomers/polymers and its functionalized chitosans (Bozic et al 2012b)

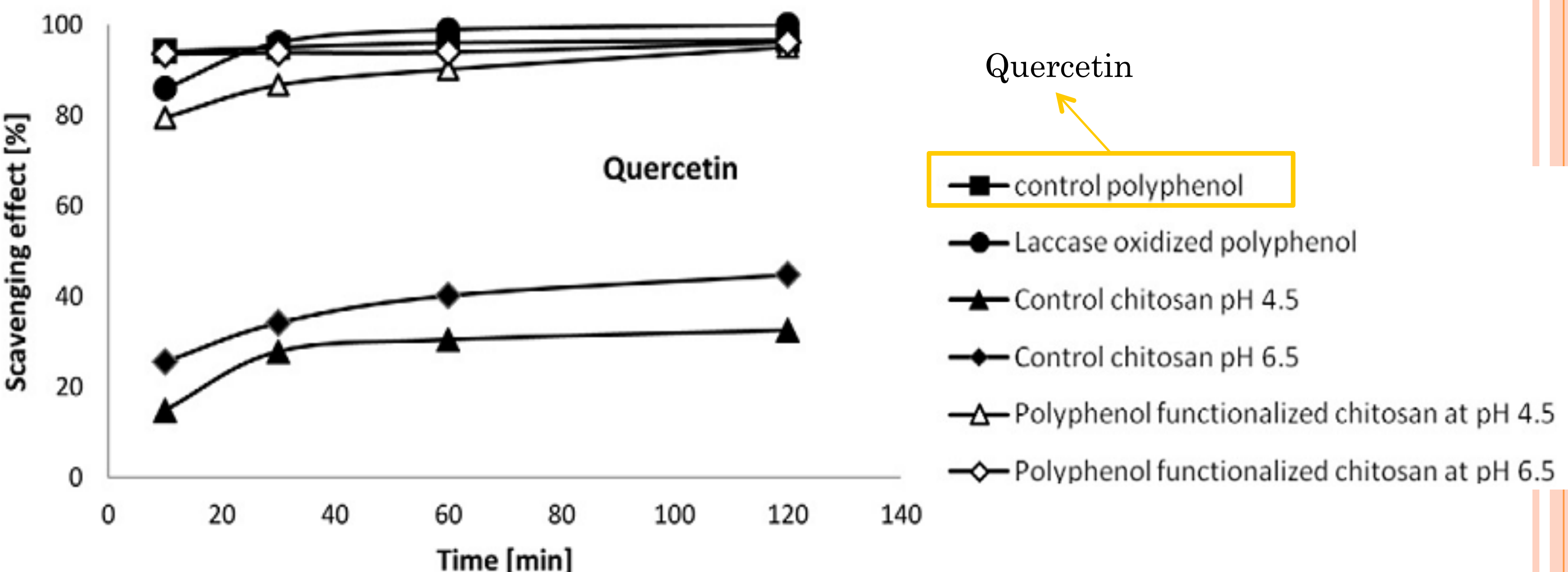


Fig. 7. Time dependent radical inhibition effects of control polyphenolic compounds and their laccase reaction products (reaction conducted at 10 mM polyphenols concentration in 100 ml phosphate buffer (100 mM) pH 6.5 with 153 U of laccase for 24 h at 30 ◦C under constant stirring), and by the functionalized chitosans at pH 4.5 and 6.5.

Nomenclatura:

PEML: polyphenol extract from murta leaves

CH: chitosan

CS: Corn Starch

Scavenging activity of chitosan

One is free-radical scavenging activity, in that chitosan may eliminate various free-radicals by the action of nitrogen on the C-2 position.

It was reported [29] that the scavenging activity of chitosan is related to the fact that the free radicals can react with the hydrogen ions from the ammonium ions (NH_3^+) to form stable molecules (Sousa, Guebitz & Kokol, 2009)

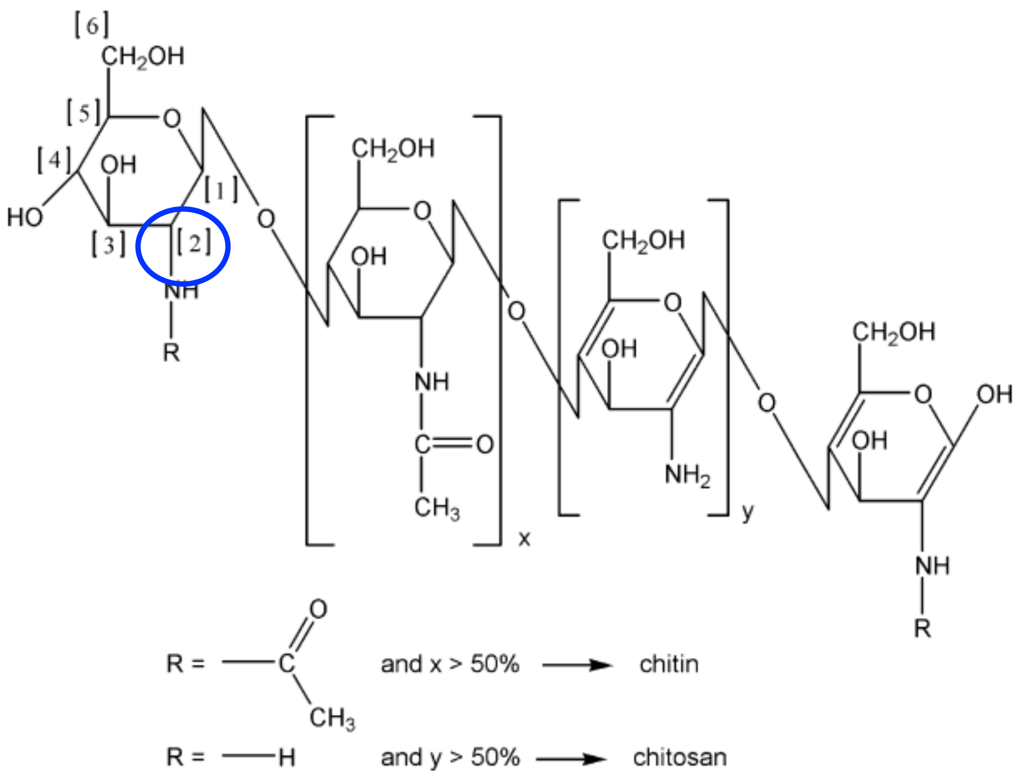


Fig. 2. Structure of chitin and chitosan (reproduced from Ref. [51] by permission of Elsevier Science, Amsterdam).

pH IDEAL 4.5

- Acoording to Bozic 2012a the highest antioxidant activity was found to be for chitosan modified with phenolic acids at pH 4.5, exhibit also an increased activity against Escherichia coli and Listeria monocytogenes compared to untreated chitosan

- **La mayor actividad antioxidante se encontró que era de quitosano modificado con ácidos fenólicos a pH 4,5,** exhiben también un aumento de la actividad frente a Escherichia coli y Listeria monocytogenes en comparación con quitosano no tratada

- Božič, M., Gorgieva, S., & Kokol, V. (2012a). Laccase-mediated functionalization of chitosan by caffeic and gallic acids for modulating antioxidant and antimicrobial properties. *Carbohydrate Polymers*, 87(4), 2388-2398.

2012_05 31: Cambio redacción preparación CH FFS

Chitosan-based FFS: Chitosan was dispersed in acetic acid (1% v/v) to prepare solutions of 1.0, 1.5 and 2% w/w. This dispersion was stirred using an Ultra-Turrax T25 with four-blade propellers at **500 rpm and 65 ºC for 2 h.** After the chitosan was completely dissolved, the solutions were filtered through cheese-cloth. The incorporation of PEML was performed as part of the dissolution solvent in the individual preparation of CH solution.

Cambié la redacción ya que aplicar 65ºC al extracto seguramente pudo afectarlo. Use esta temperatura porque la viscosidad era muy alta y a baja Tº pensé que no se homogeneizaría bien, pero no pensé en el extracto

Chitosan-based FFS: Chitosan was dispersed in acetic acid (1% v/v) to prepare solutions of 1.0, 1.5 and 2% w/w. This dispersion was stirred for **2 h** using an Ultra-Turrax T25 with four-blade propellers**.** After the chitosan was completely dissolved, the solutions were filtered through cheese-cloth. The incorporation of PEML was performed as part of the dissolution solvent in the individual preparation of CH solution.

Rheology

Rheological measurements: Fundamentals and methods

Dynamic viscoelasticity and steady state flow measurements were carried out in a controlled-stress rheometer Bohlin CVO (Bohlin Instruments, Inc. Grandbury, NJ) with a cone-plate geometry (cone angle 4º, diameter = 40 mm, gap = 150 μm). Before analysis, the sample was placed into the rheometer, which was equilibrated at 25 ºC (Gómez-Guillén et al., 2007).

RHEOLOGY

Steady shear measurements

Experimental data were fitted to Ostwald–de Waele model or rheological Power law model (Eq. (1)).

$$\tau = K\,\dot{\gamma}^{\,n} \qquad \textbf{(1)}$$

Where **τ** is shear stress (Pa), ***K*** is consistency index (Pa·s), ***n*** is flow index (-) and is shear rate (s^{-1}).

For n = 1, the Power law model shows a Newtonian fluid, this is a fluid which exhibits a viscosity that is shear rate-independent.

For non-Newtonian material ($n \neq 1$), when $n < 1$ material has shear-thinning or dilatant behavior and viscosity increases with shear rate, whilst that when $n > 1$ material has shear-thickening or pseudoplastic behavior and viscosity decreases with shear rate.

Rheology

Dynamic measurements of viscoelastic properties

The viscous and elastic properties of the FFS can be evaluated through dynamic rheological studies. Frequency sweeps assess the effect of additives on changes in microstructure and stability of the FFS during storage and transportation, whereas a temperature sweep can show phase transitions and elasticity, allowing the appropriate temperature ranges for the formulation and application of the coating film on the product (Moraes, Carvalho, Habitante, Solorza-Feria & Sobral, 2009).

Three dynamic studies were performed:

(1) An oscillatory stress sweep test from 0.03 to 400 Pa, at a constant frequency of 0.1 Hz and 25 ºC was made to set the upper limit of the linear viscoelastic region (LVR).

(2) Frequency sweep over a range of 0.01–50 Hz at 25 °C was performed at an oscillatory stress within LVR for each solution. Viscoelastic parameters, storage or elastic modulus (G', Pa), loss or viscous modulus (G", Pa), complex modulus (G*, Pa) (Eq. (3)), complex viscosity (η*, Pa·s) (Eq. (4)) and tangent of the phase angle (Tan δ) (Eq. (5)) as a function of angular frequency (w, rad/s) were measured, obtaining the typical mechanical spectra. FFS behavior with respect to frequency was classified as predominantly viscous (G' < G") or predominantly elastic (G' > G"), as well as by the presence of crossover point (G' = G"), indicating the frequency at which the FFS behavior shifts from viscous to elastic. Classification of the sample structure as gel-like (strong or weak gel), concentrated solution (entanglement network) or dilute solution according to **Ross-Murphy (1984)** and **Clark & Ross-Murphy (1987)** were also applied.

$$\left|G^*\right| = \sqrt{G'^2 + G''^2} \quad \text{(Eq. (3))}$$

$$\left|\eta^*\right| = \frac{\left|G^*\right|}{\omega} \quad \text{(Eq. (4))}$$

$$\mathrm{Tan}\delta = \frac{G''}{G'} \quad \text{(Eq. (5))}$$

Where G' is a measure of the energy stored and recovered in a cyclic deformation whereas G" is a measure of the dissipated energy, Tan δ is the tangent of the phase angle (δ), it represents the ratio of viscous modulus to elastic modulus and is a useful quantifier of the presence and extent of elasticity in a fluid, η* is the frequency-dependent viscosity function determined during forced harmonic oscillation of shear stress; it contains both real and imaginary parts.

(3) Temperature ramps were performed at a scan rate of 1 ºC/min and 0.1 Hz from 40 to 2 ºC and back to 40 ºC. Here, the phase transitions with temperature and elasticity of the FFS were evaluated through the phase angle (δº). Elasticity is the reversible behavior of stress/strain, which is measured as the reciprocal of the phase angle, where purely elastic solids have a phase angle of 0° and purely viscous fluids have a phase angle of 90°.

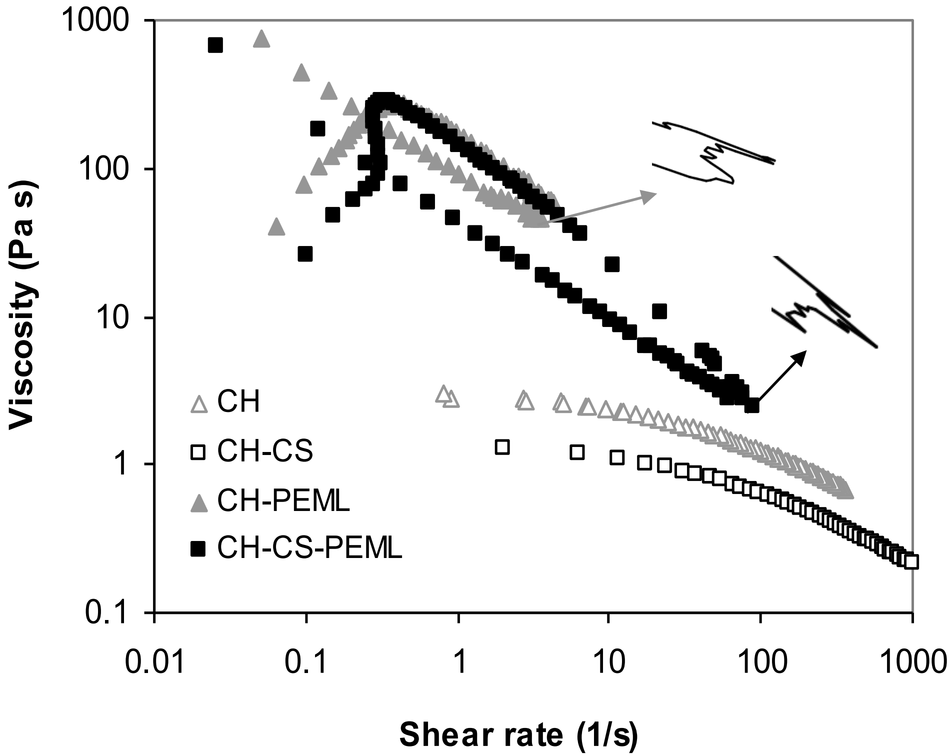
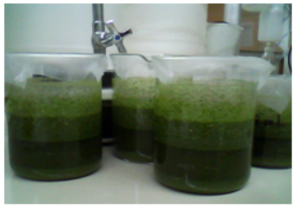
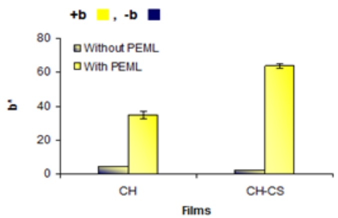
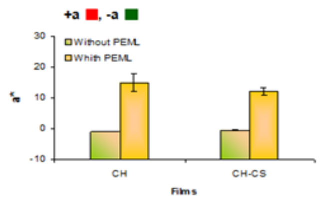


Figure 1. Viscosity curves of film-forming solutions with PEML (filled symbols) and without (open symbols) at 25 °C.




+b , -b
80
60
40
20
0
b*
Without PEML
With PEML
CH
CH-CS
Films


+a , -a
30
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-10
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Without PEML
Whith PEML
CH
CH-CS
Films

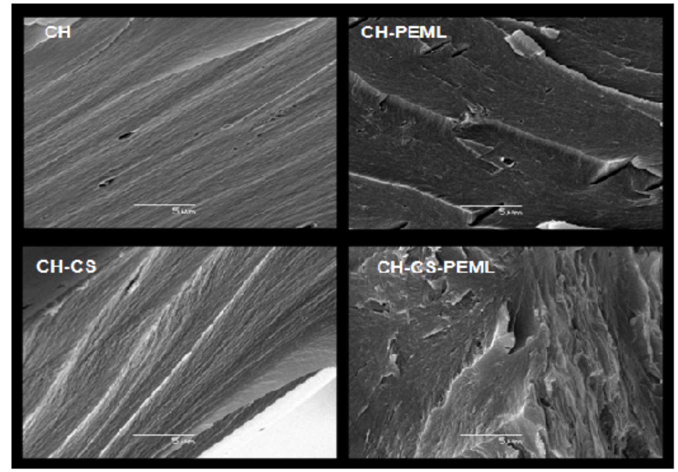

CH
CH-PEML
CH-CS
CH-CS-PEML

Figure 2. a) Fibers obtained from CH-PEML and CH-CS-PEML during mechanical stirring and b) film from CH-CS-PEML

FT-IR

•Absorptions due to stretching and bending of **covalent bonds** in molecules

•Visible region – 400 to 800 nm

•Visible wavelengths typically expressed in nanometers (1 nm = 1 x 10-9 m)

Effect of PEML on film optical properties

Film / Additive	Mechanical properties		References
	TS (MPa)	EB (%)	
Chitosan			
	29.69 ± 1.89 [a]	45.10 ± 1.40 [a]	This work
PEML			This work
	17.34 ± 2.43	44.20 ± 7.90	Pereda et al. (2011)
	23.66 ± 2.63	54.62 ± 3.12	Siripatrawan & Harte (2010)
Green tea extract	25.13 ± 1.91	58.14 ± 4.24	Siripatrawan & Harte (2010)
	24.00 ± 3.00	29.00 ± 2.00	Moradi et al. (2012)
Grape seed extract	16.00 ± 0.60	21.00 ± 3.00	Moradi et al. (2012)

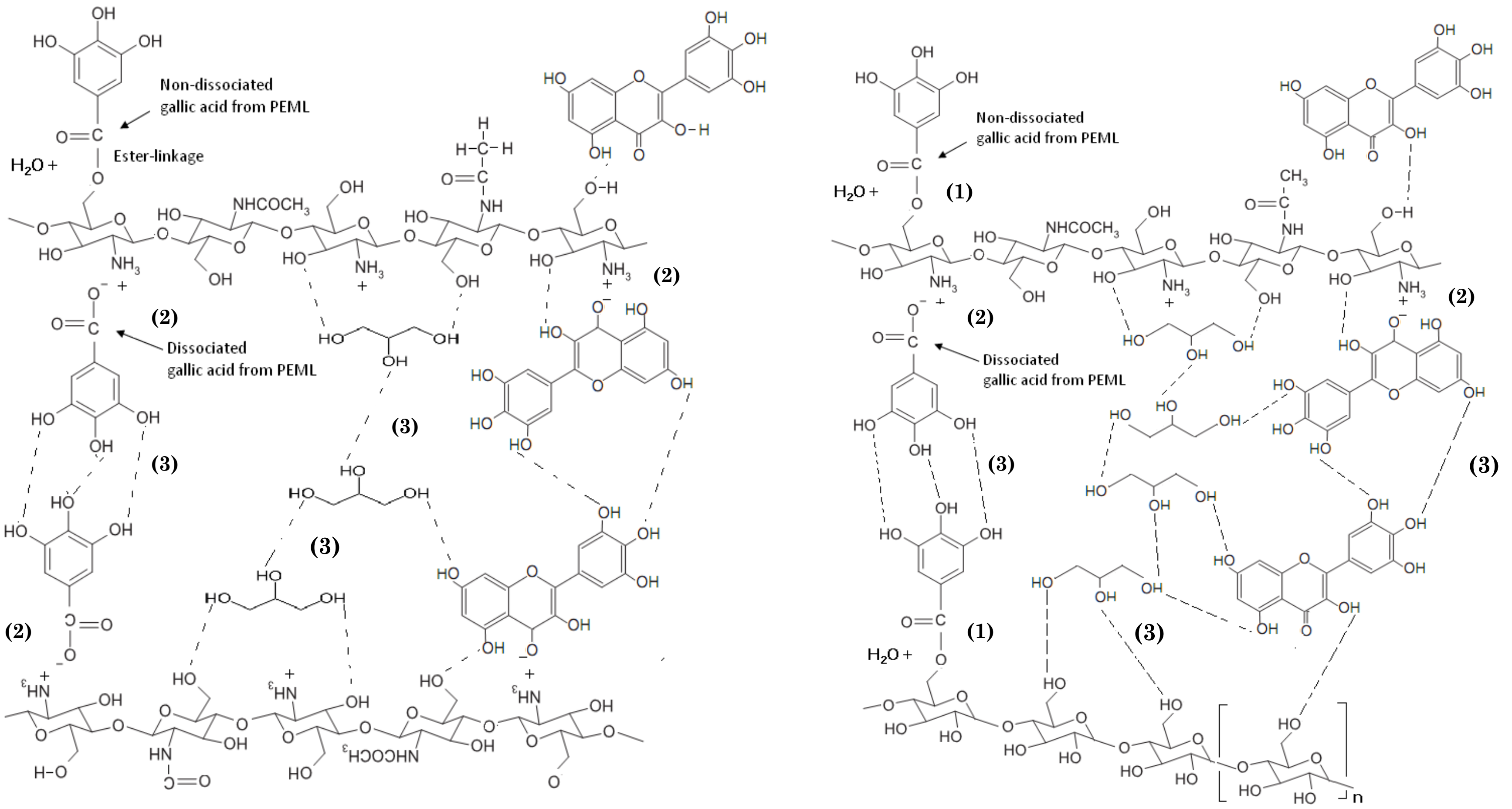


Figure 6. Interacction mechanisms at acid pH (4.5 ± 2) of: A) Chitosan film with PEML (CH-PEML) and B) Chitosan-starch film with PEML (CH-CS-PEML). (1) Ester linkage, (2) Electrostatic interaction, (3) Hydrogen bonds.

In both film with PEML, new band at 1614 cm^{-1} correspond to C=C stretching of aromatic rings (Yan et al., 2010) and new bands at 861 and 778 cm^{-1} correspond to vibrations of H-atoms in aromatic rings. Both phenolic acids (as gallic acid) and flavonols (quercetin, kaempferol and myricetin) present in PEML contain aromatic rings, which indicate that they are in the films .

Andrea: Ver si se entiende mejor ahora.

La idea es que dado que aparecieron 3 nuevos peak, que representan a anillos aromáticos, estos están reflejando tanto la presencia de ácidos fenólicos (como acido gálico) y flavonoles (como quercetina, miricetina kaempferol) que están presentes en PEML (Rubilar et al., 2005; Bifani et al., 2007)

Three new peaks appeared, representing aromatic rings and reflect the presence of both phenolic acids (as gallic acid) and flavonols (myricetin quercetin, kaempferol), present in PEML and therefore, in the films (Rubilar et al., 2005; Bifani et al., 2007).

Nueva banda a 1710 cm-1 (tensión C=O del grupo carboxilo)

Resultados y Discusiones

- New peak near 1715 cm−1 that was found in PEML-loaded CH and CH-CS films, also was observed in chitosan film loaded with polyphenolic compounds from olive-leaf extract (1700 cm-1) (Kosaraju, D'Ath & Lawrence, 2006), which indicates interaction between the **hydroxyl/ carboxyl/aldehyde groups** of the olive-leaf extract and the amine functionality of the chitosan molecule.

- Peak at 1720 cm-1 have been attributed to ester linkage between chitosan and Indian gooseberry extract, which was stronger with an increase in the extract concentration (Mayachiew & Devahastin, 2010). Ester linkages were also reported between chitosan (pH ~ 4.5, pKa ~ 6.3) loaded with caffec acid (pKa COOH = 4.0), gallic acid (pKa COOH = 4.4, pKa OH=10) and quercetin laccase around 1715 cm-1 (Božič, Gorgieva & Kokol, 2012a, 2012b), tannic acid (pKa COOH = 4.4) at 1730 cm-1 (Rivero, García & Pinotti, 2010) and 4-hydroxycinnamic acid at 1700 cm-1 (Monier et al., 2010). Where the highest antioxidant activity was found to be for chitosan modified with phenolic acids at pH 4.5, exhibit also an increased activity against Escherichia coli and Listeria monocytogenes compared to untreated chitosan (Božič, Gorgieva & Kokol, 2012a).

- Ester linkages have also been reported between polymerized products of phenolic acid (Božič *et al.*, 2012a).

Conclusiones anteriores

- The viscosity of chitosan and chitosan-starch film-forming solutions is strongly increases using PEML. This viscosity restrict the application of these solutions as a coating at 25 ºC.
- Viscoelastic properties show that PEML turns CH and CH-CS film-forming solutions from a sol or diluted solution into a gel-like structure, increasing their elasticity in 88 and 71% respectively, which is a clear indication of the improvement on the three-dimensional cross-linked networks stability. This restructuring ability of CH-PEML and CH-CS-PEML physical gels makes them an important class of materials with many applications, such as in drug delivery.
- The PEML interact with chitosan and starch chains into the films, resulting in additional linkages between the polymer chains. Starch stabilizes the interaction of chitosan and PEML, generating CH-CS-PEML good film-forming solution and film.

Sacado de la introducción paper

- A systematic approach for developing new edible films based on hydrocolloid blends and active additives is using the following relationship of systems: composition of active compound and polymer blends, procesing conditions evaluated by rheological properties for film-forming solutions (FFS), and physical properties, stability and structure of films (Lacoste, Schaich, Zumbrunnen & Yam, 2005).

- Lacoste, A., Schaich, K. M., Zumbrunnen, D., & Yam, K. L. (2005). Advancing controlled release packaging through smart blending. *Packaging Technology and Science*, 18, 77-87.

Sacado de la introducción paper

- High viscosity, low surface tension and high flocculation rate of the film-forming solution favour the increase in water vapour resistance of the films (Villalobos-Carvajal, Hernández-Muñoz, Albors & Chiralt, 2009).

- Villalobos-Carvajal, R., Hernández-Muñoz, P., Albors, A., & Chiralt, A. (2009). Barrier and optical properties of edible hydroxypropyl methylcellulose coatings containing surfactants applied to fresh cut carrot slices. *Food Hydrocolloids*, 23(2), 526-535.

Sacado de la introducción paper

- Further to antioxidant activity, chitosan has radical scavenging effects, which depends on their concentrations and deacetylation grade (Park, Je & Kim, 2004).

Sacado de la introducción paper

- These changes occur due to the compatibility/incompatibility between two macromolecules, which depend on their molecular weight, chemical structure, conformation and hydration behavior. As on the addition in minor amounununts of various chemicals or additives (Greener-Donhowe & Fennema, 1994; Maria, Carvalho, **Sobral,** Habitante & Solorza-Feria, 2008; Phan The, Debeaufort, Voilley & Luu, 2009).

- Greener-Donhowe, I. K., & Fennema, O. R. (1994) Edible films and coatings: characteristics, formation, definitions, and testing methods. In Edible coatings and films to improve food quality; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M. O., Eds.; Technomic Publishing Co.: Lancaster, PA, pp. 1-24.
- Phan The, D., Debeaufort, F., Voilley, A., & Luu, D. (2009). Biopolymer interactions affect the functional properties of edible films based on agar, cassava starch and arabinoxylan blends. *Journal of Food Engineering*, 90(4), 548-558
- Maria, T. M. C.; Carvalho, R. A.; **Sobral**, P.J.A.; Habitante, A. M. B. Q.; Solorza-Feria, J. (2008). **The effect of the degree of hydrolysis of the PVA and the plasticizer concentration on the color, opacity, and thermal and mechanical properties of films based on PVA and gelatin blends**. *Journal of Food Engineering*, 87, 191-199.

Sacado de la introducción paper

- Films and coatings antioxidant power (reduction ability and free radical-scavenging capacity) can be increased with the addition of some flavonoids (Sousa, Guebitz & Kokol, 2009) and polyphenol-rich aqueous extracts, such as murta leaves (Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007), oregano or rosemary (Gómez-Estaca, Bravo, Gómez-Guillén, Alemán & Montero, 2009) and ginseng (Norajit, Kim & Ryu, 2010).

- **Gómez-Estaca**, J., Bravo, L., Gómez-Guillén, M. C., Alemán, A., & Montero, P. (2009). Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry*, 112(1), 18-25.
- **Norajit**, K., Kim, K. M., & Ryu, G. H. (2010). Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract. *Journal of Food Engineering*, 98(3), 377-384.

Sacado de la introducción paper

- Siripatrawan & Harte (2010) developed an active film from chitosan incorporated with aqueous green tea extract. Their polyphenols formed hydrogen bonding and covalent bonding occuping functional group of chitosan matrix (2%) and decreasing free hydrogen group that can form hydrophilic bonding with water. As a result, mechanical and water barrier properties of film are improved and its antioxidant activity is enhanced.

Sacado de la introducción paper

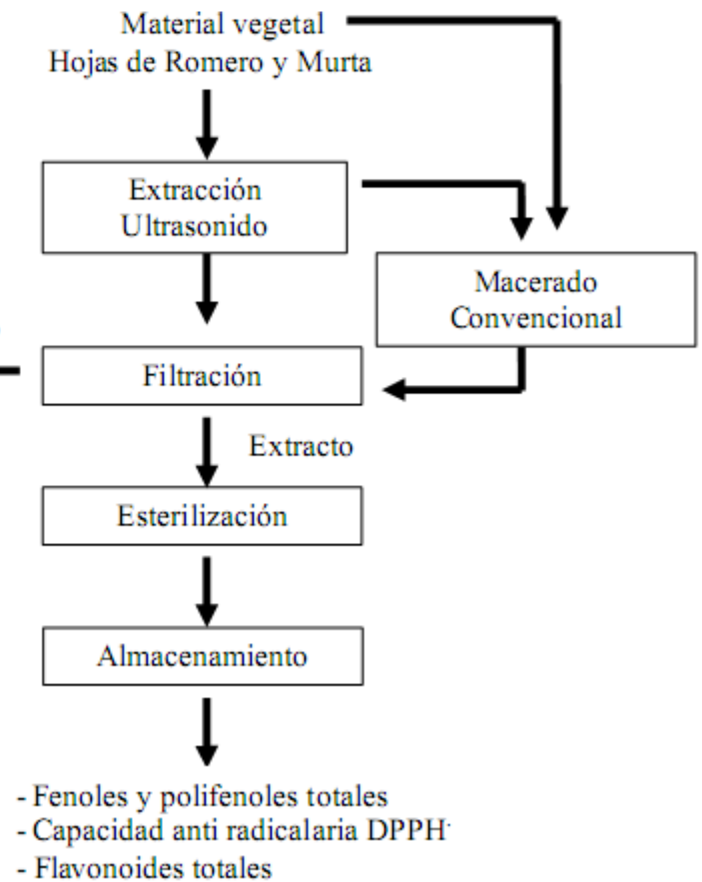
- Beside, flavonoid-functionalized chitosan polymer not only introduces a wide range of antioxidant activity but also improves its antimicrobial activity (Sousa *et al*, 2009).

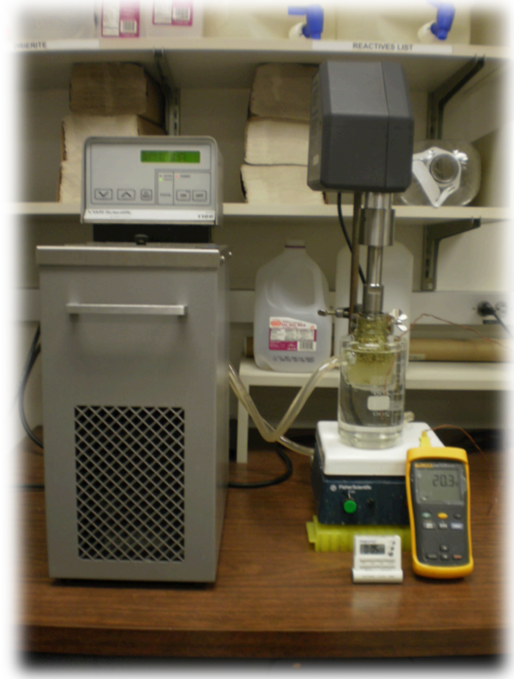
- Therefore, natural compounds from plants such as flavonoids and phenolic acids, incorporated into edible films and coatings, could act not only as antioxidant, but also as antimicrobial, anti-browning and/or cross-linking agents, being crosslinking an important step in the preparation of hydrocolloid films to ensure their stability and mechanical resistance (Mathew & Abraham, 2008).

Método

Comparar

- **US**
- **M**
- **US+M**





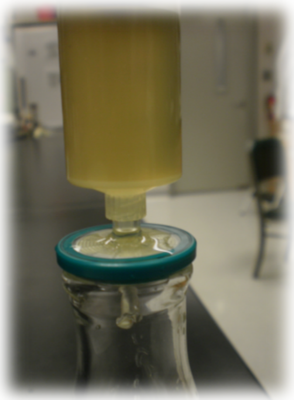


Fig. Diagrama de flujo: Etapas experimentales para evaluar el efecto del sistema de extración en la concentración de fenoles y flavonoides, capacidad antioxidantes y estructura de hojas de romero y murta