FZEA

FoRC
FOOD RESEARCH CENTER
Centro de Pesquisa em Alimentos

Des films à base de protéines ou d'amidon, avec ou sans incorporation de principes actifs

Paulo J. A. Sobral
University of São Paulo
Pirassununga (SP), Brazil

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USP

Starting by introducing the FZEA-USP in Brazil

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Ciência e inovação da fazenda à mesa

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Brazil

VENEZUELA
GUIANA
SURINAME
GUIANA FRANCESA
OCEANO ATLÂNTICO
COLÔMBIA
Boa Vista
RORAIMA
AMAPÁ
Macapá
Nota: Fernando de Noronha dista cerca de 700 km da costa e sua localização é definida pelas coordenadas 3° 50' S, 32° 25' W.
Pico da Neblina
Parque Nacional do Pico da Neblina
Parque Nacional do Jaú
Equador
Belém
Parque Nacional de Lençóis Maranhenses
Manaus
Parintins
Santarém
São Luís
Fortaleza
Tefé
Parque Nacional da Amazônia
AMAZONAS
MARANHÃO
Teresina
RIO GRANDE DO NORTE
CEARÁ
PARÁ
Marabá
Natal
João Pessoa
PARAÍBA
Boca do Acre
PIAUÍ
PERNAMBUCO
Recife
ACRE
Porto Velho
Rio Branco
Palmas
Parque Nacional do Araguaia
TOCANTINS
Maceió
ALAGOAS
RONDÔNIA
BAHIA
Aracaju
SERGIPE
PERU
MATO GROSSO
Salvador
Cuiabá
Brasília
GOIÁS
Goiânia
MINAS GERAIS
BOLÍVIA
MATO GROSSO DO SUL
Belo Horizonte
ESPÍRITO SANTO
Campo Grande
Vitória
SÃO PAULO
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RIO DE JANEIRO
CHILE
São Paulo
Rio de Janeiro
PARANÁ
Trópico de Capricórnio
Curitiba
OCEANO PACÍFICO
SANTA CATARINA
Florianópolis
ARGENTINA
OCEANO ATLÂNTICO
RIO GRANDE DO SUL
Porto Alegre
URUGUAI
0 100 200 300 mi
0 150 300 450 km

State of São Paulo

8 campi

- São Paulo
- Bauru
- Lorena
- Ribeirão Preto
- Piracicaba
- Pirassununga
- Santos
- São Carlos

instalações em mais outros **15 municípios**

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University of São Paulo in numbers

42 unidades
de ensino e pesquisa

333
cursos de **graduação**

264
cursos de **pós-graduação** (mestrado e doutorado)

4 museus
15 acervos
48 bibliotecas
4 hospitais
6 institutos especializados

Cerca de
97 mil
estudantes

60 mil
estudantes de **graduação**

37 mil
estudantes de **pós-graduação** (mestrado e doutorado)

+8 mil
alunos dos cursos de **especialização**

+43 mil
alunos dos programas de **extensão**

A USP realiza

22%

da **produção científica** brasileira no ***clarivate analytics***

Está entre as

10

instituições de pesquisa que **mais publicam no mundo**

1º

EM QUALIDADE DE PESQUISA

Ranking Universitário Folha 2023

95º

BEST OF RESEARCH

The World Ranking

Mais de

6.3

milhões

de citações no ***GOOGLE SCHOLAR***

Mais de

123 mil

publicações no ***ELSEVIER SCOPUS***

4

pesquisadores

ENTRE OS 10 BRASILEIROS **MAIS CITADOS** NO MUNDO NO ***CLARIVATE ANALYTICS***

Desde

2014

sua produção científica em todas as áreas tem **IMPACTO ACIMA DA MÉDIA MUNDIAL (1.0)**

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Faculty of Animal Science and Food Engineering

Faculty of Animal Science and Food Engineering

- Department of Basic Sciences (ZAB)
- Department of Animal Science (ZAZ)
- Department of Food Engineering (ZEA)
- Department of Biosystems Engineering (ZEB)
- Department of Veterinary Medicine (ZMV)

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Five Postgraduate Courses

- **Animal Science:** Master and Doctorate.
- **Food Engineering:** Master and Doctorate.
- **Material Engineering and Sciences:** Master and Doctorate.
- **Animal Biosciences:** Master and Doctorate.
- **Managing and Innovation in the Animal Industry:** Professional Master.

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USP

Now, introducing my self

FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

Now, introducing my self

Ilhéus

Now, introducing my self

- Food Engineer from the Federal University of Viçosa, Brazil (1983).

Brazil

Viçosa

Now, introducing my self

- Food Engineer from the Federal University of Viçosa, Brazil (1983).
- Master in Food Engineering from the State University of Campinas, Brazil (1987).

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Brazil

Campinas

Now, introducing my self

- Food Engineer from the Federal University of Viçosa, Brazil (1983).
- Master in Food Engineering from the State University of Campinas, Brazil (1987).
- "DEA" in "Génie des Procédés Option Génie Alimentaires" from the "Ecole Nationale Supérieure Des Industries Alimentaires", France (1988).
- PhD in "Génie des Procédés" by the "Institut National Polytechnique de Lorraine", France (1992).
- Assistant Professor at USP (1993).
- Associate Professor (by defending a 2nd thesis) (2000).
- Full Professor (2007).
- Researcher PQ1B of CNPq

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USP

- **Teaching:**
  - Mass transfer (UG)
  - Physical-chemistry of foods (UG and PG).
- **Research topics:**
  - Biopolymers-based Film Technology.
  - Nanoparticles and nanocomposites.
  - Biopolymers-based hydrogels.
  - Biomaterials: scaffolds.
  - Emulsions and nanoemulsions.
  - Food classifications.

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- **Team:**
  - 2 Technicians (PhD and Master)
  - 9 PhD Students
  - 2 Post-docs.

- **Laboratory:** SEM, AFM, FTIR, DSC, automatic film spreader, network analyzer, freeze-dryer, rheometer, texturometer, Aw Decagon, colorimeter, glossmeter, Microfluidizer, Lumisizer, RH/T controlled oven, among others.

And now, some results

On passive films based on biopolymers

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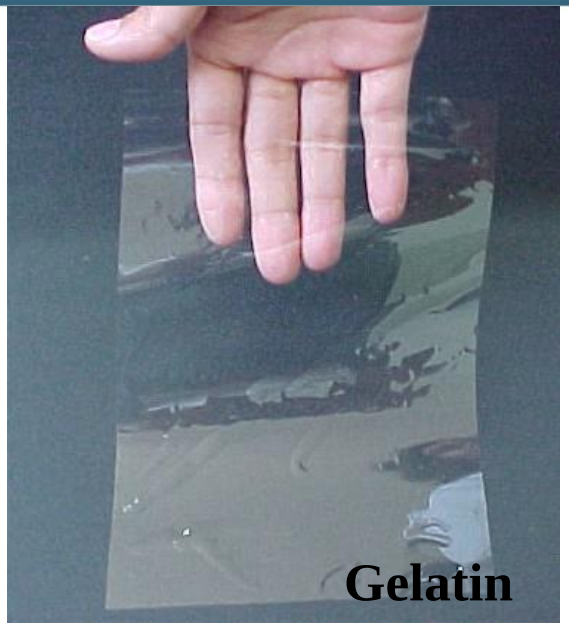

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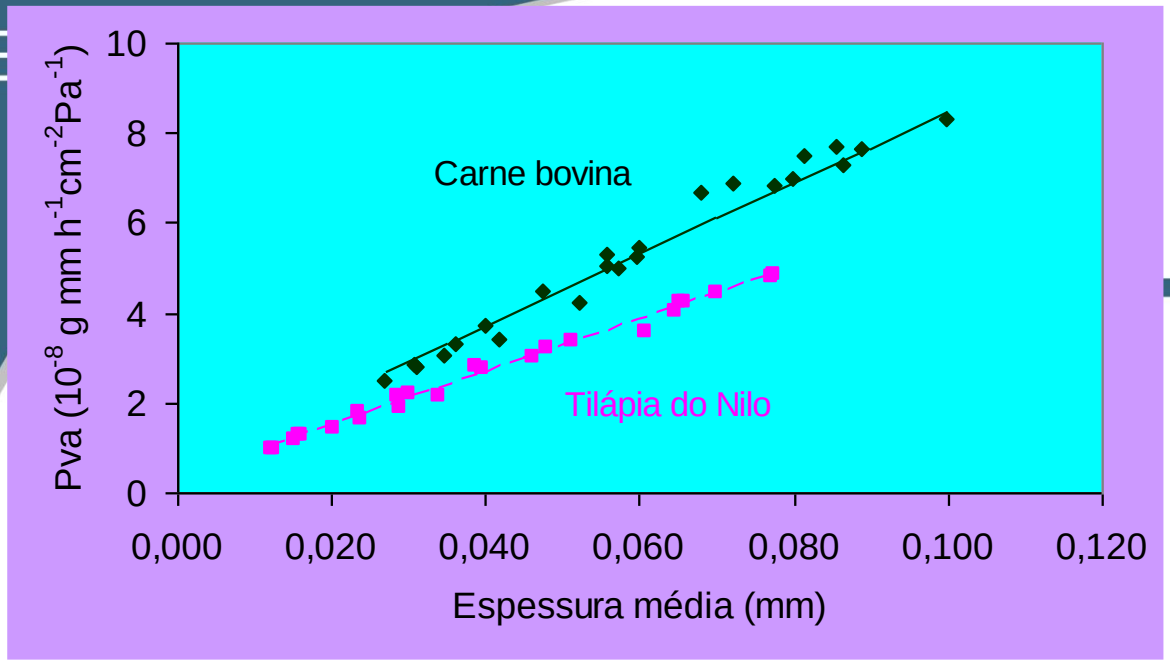
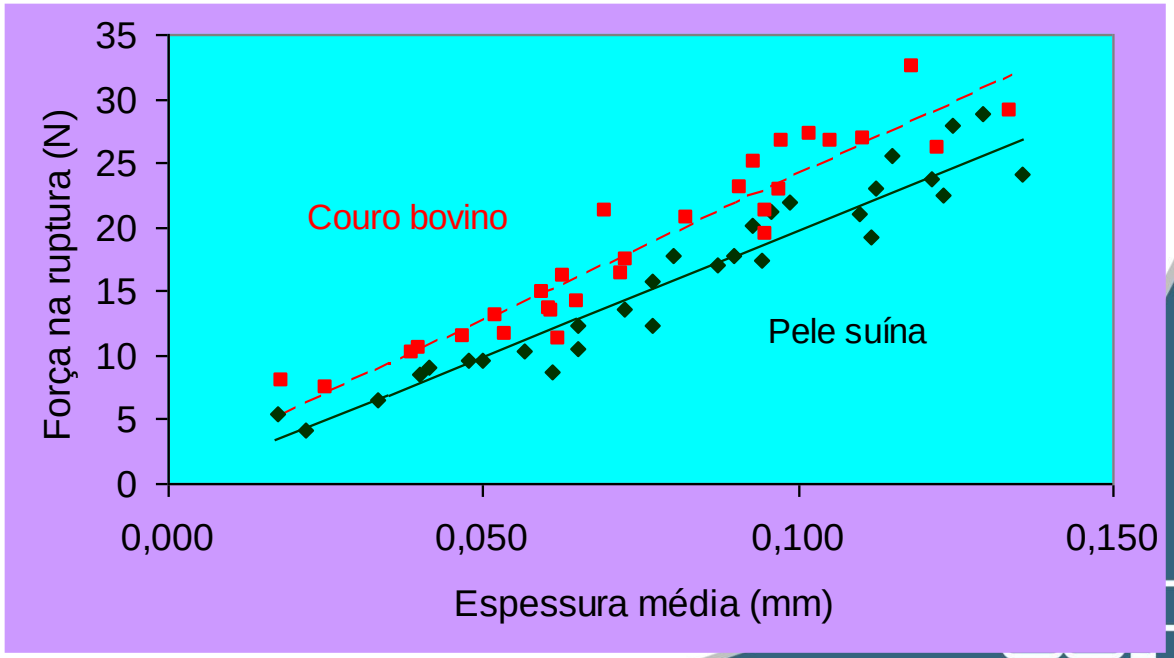
Thickness control



Source: Sobral (2000)

Myofibrillar proteins

gelatin



Source: Sobral (1999)

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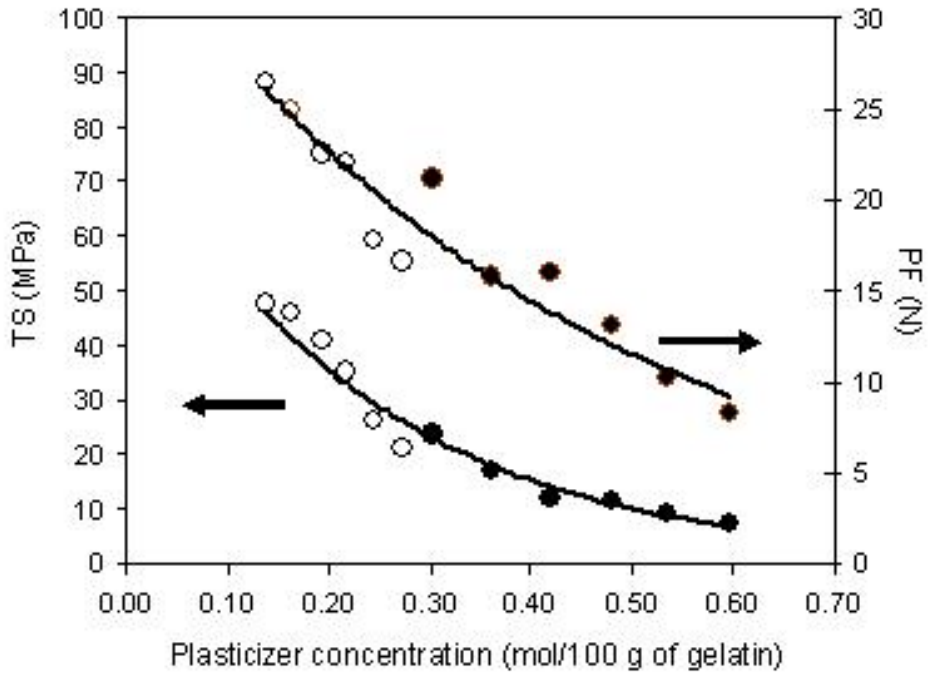
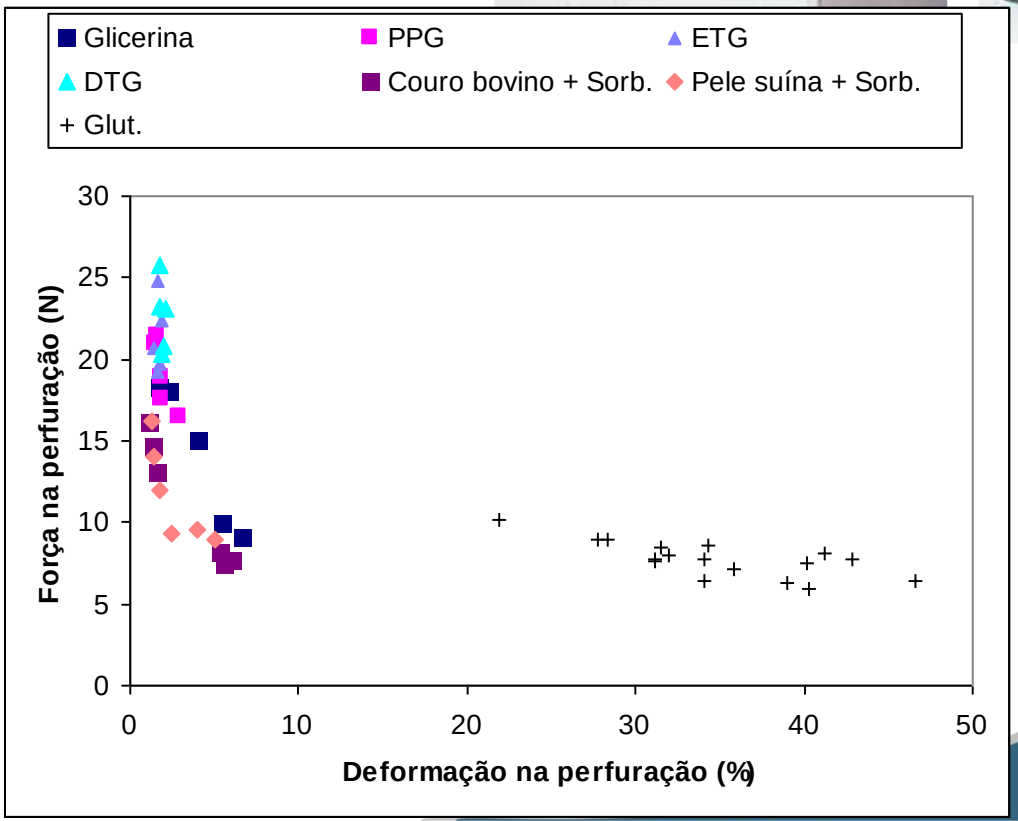
Mechanical properties



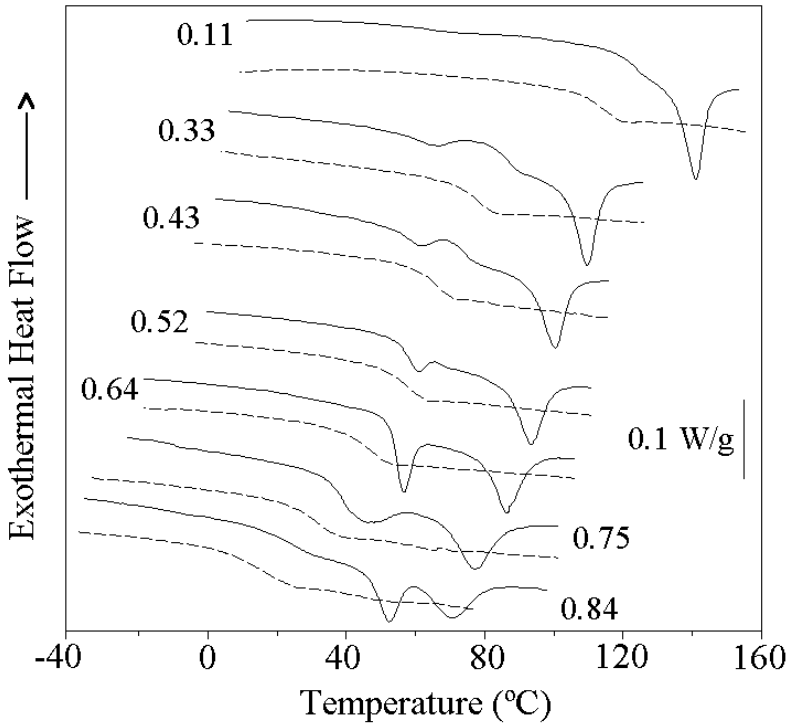
Source: Thomazine et al. (2005)

Mechanical properties



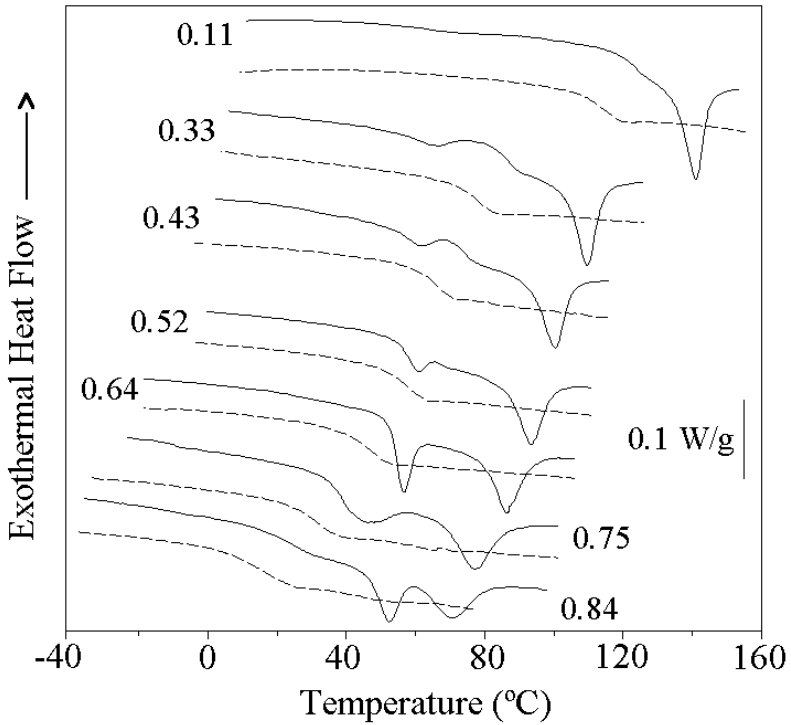
Source: Vanin et al. (2005)

Glass transition

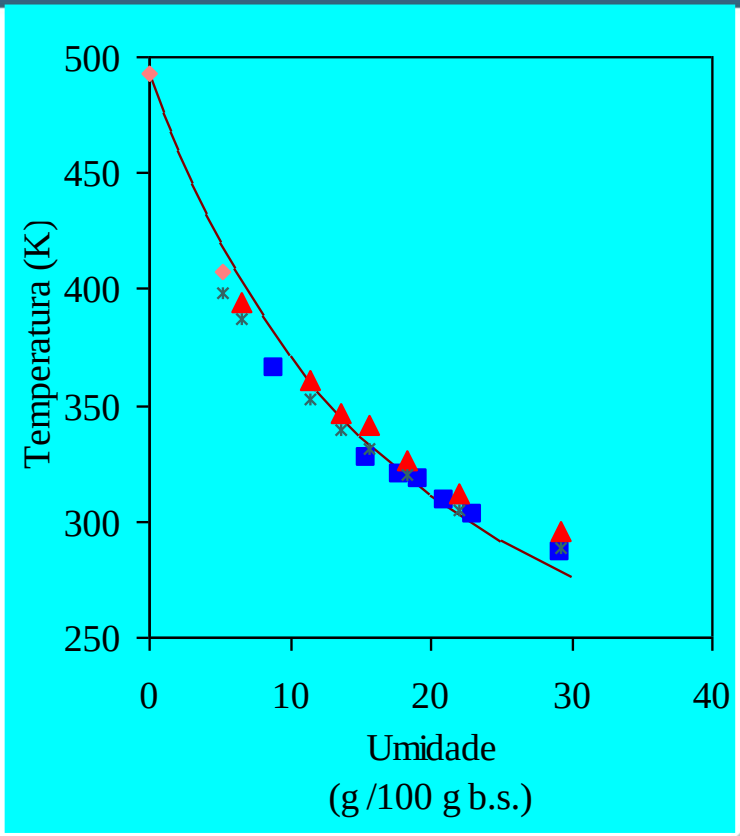


Pigskin gelatin

Glass transition



Pigskin gelatin



Glass transition temperature

Source: Sobral & Menegalli (2002)

On nanocomposite films based on biopolymers

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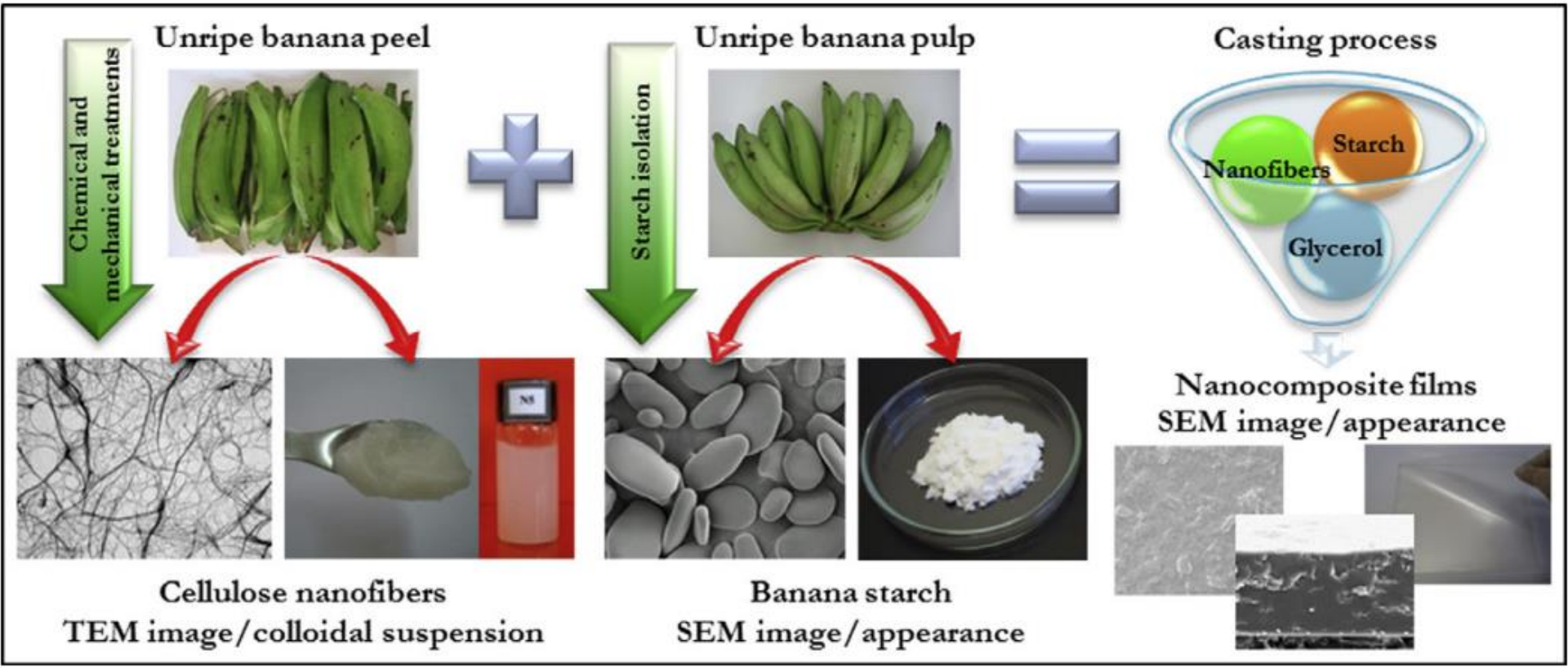

Source: Pelissari et al. (2017)

Source: Pelissari et al. (2014)

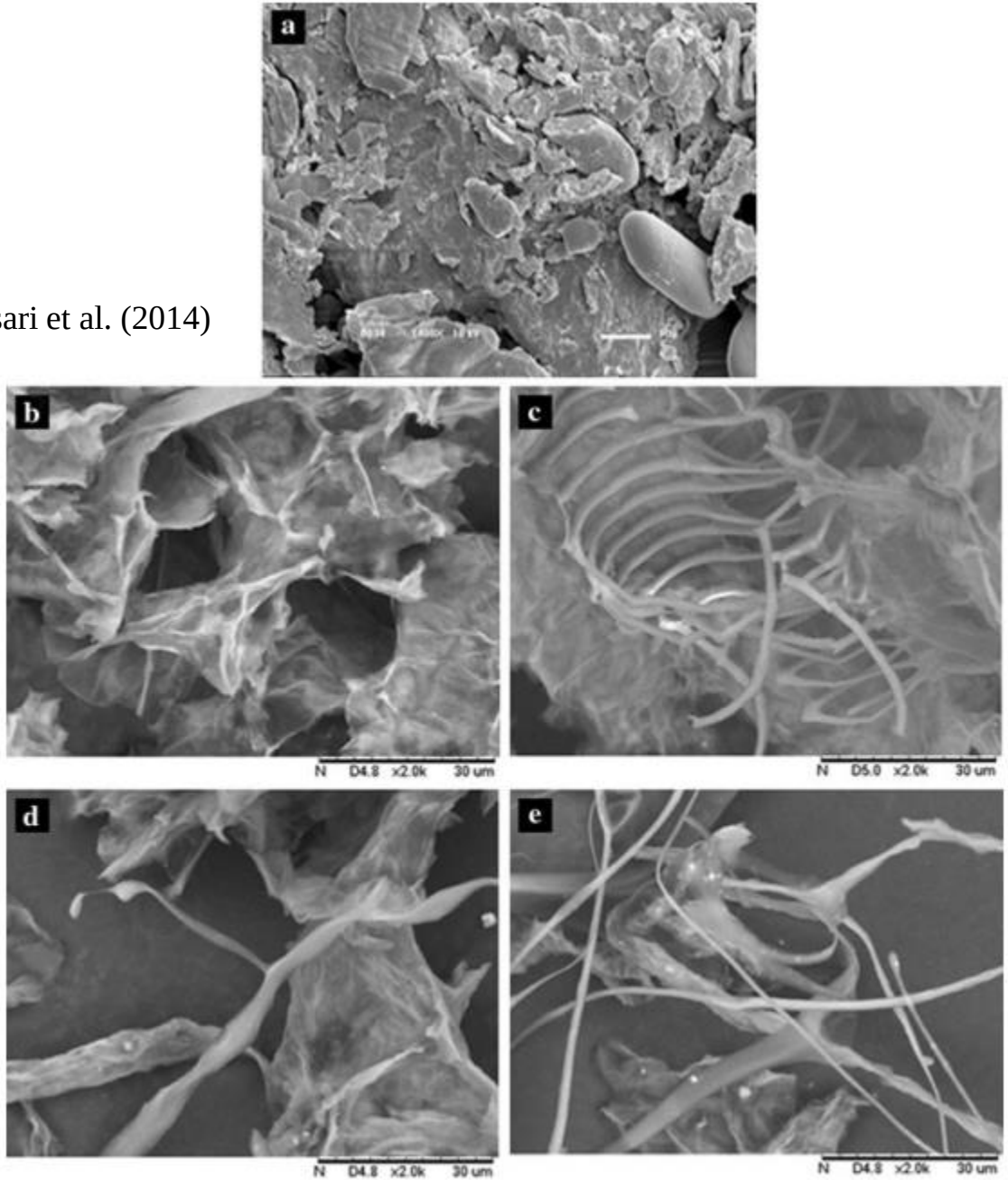

Fig. SEM images of the **a** banana peel bran (×1,000, *scale bar* 10 μm) and of the steps involved in the chemical treatment for the isolation of cellulose nanofibers: **b** first alkaline treatment, **c** first bleaching, **d** second bleaching, and **e** second alkaline treatment (×2,000, *scale bar* 30 μm)

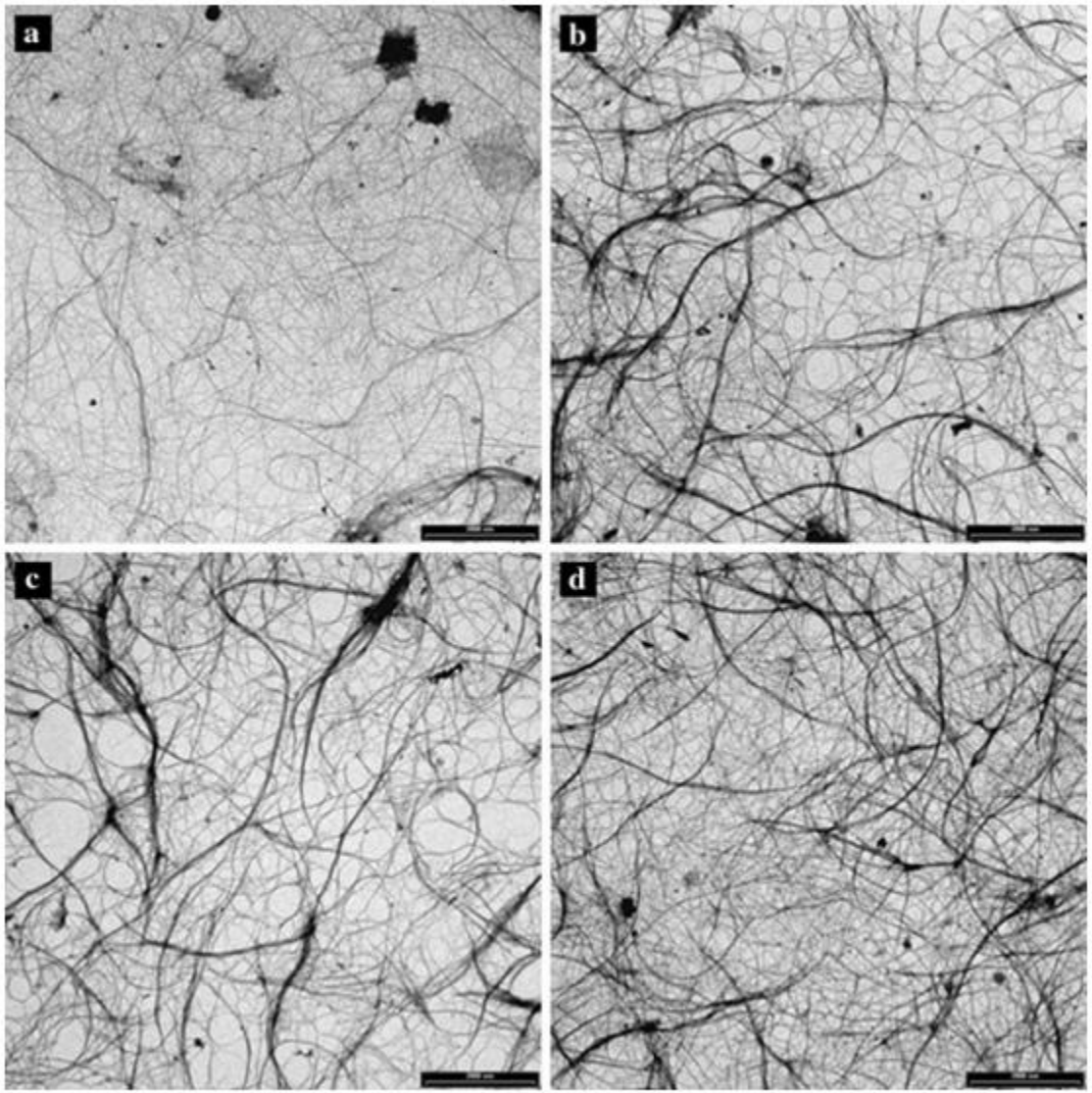

Fig. TEM images of cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer: **a** N0, **b** N3, **c** N5, and **d** N7 (×1,400, *scale bar* 2,000 nm)

Source: Pelissari et al., (2014)

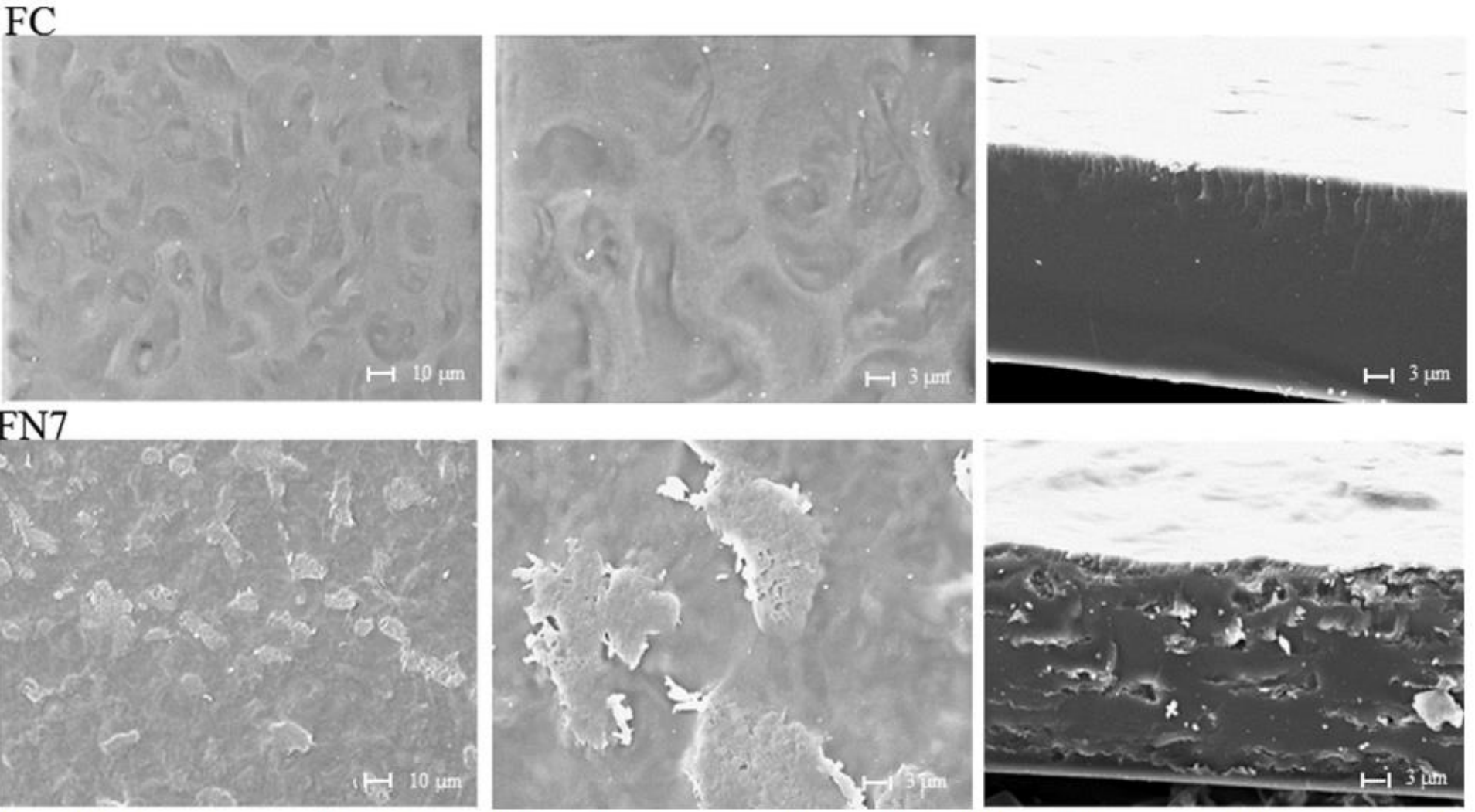

Fig. 1. SEM surfaces (500x, scale bar = 10 µm; 2000x, scale bar = 3 µm) and cross-sections (2000x, scale bar = 3 µm) of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer zero (FN0), three (FN3), five (FN5), and seven (FN7) times.

Source: Pelissari et al. (2017)

On active films based on biopolymers

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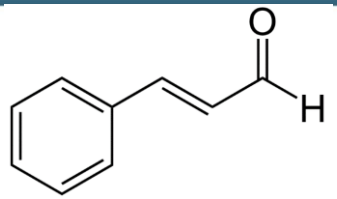
Active agents

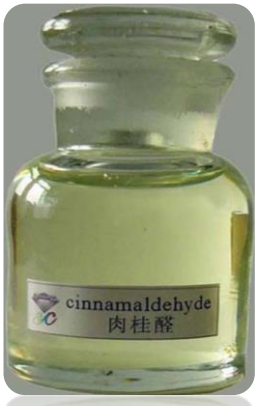
Examples of active agents used to produce active films:

i) Vegetable extracts: from herbs, spices, leaves, ...

ii) Purified compounds: carotenoids, phenolic compounds, α-tocopherol, essential oils, nisin, natamicin...

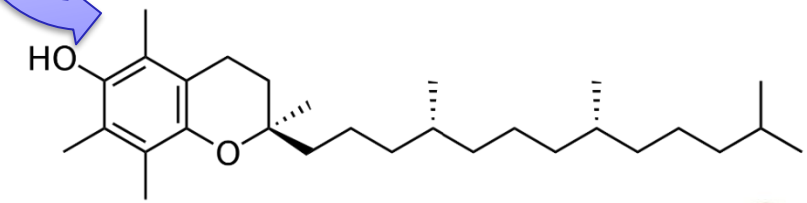
Purified compounds



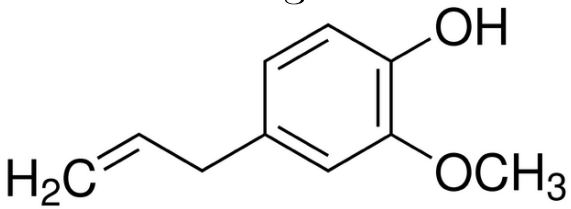


https://hypcl.tradeindia.com/cinnamic-aldehyde-1090836.html

https://en.wikipedia.org/wiki/Alpha-Tocopherol



Eugenol



http://www.natural-health-news.com/garlic/

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Vegetable extracts

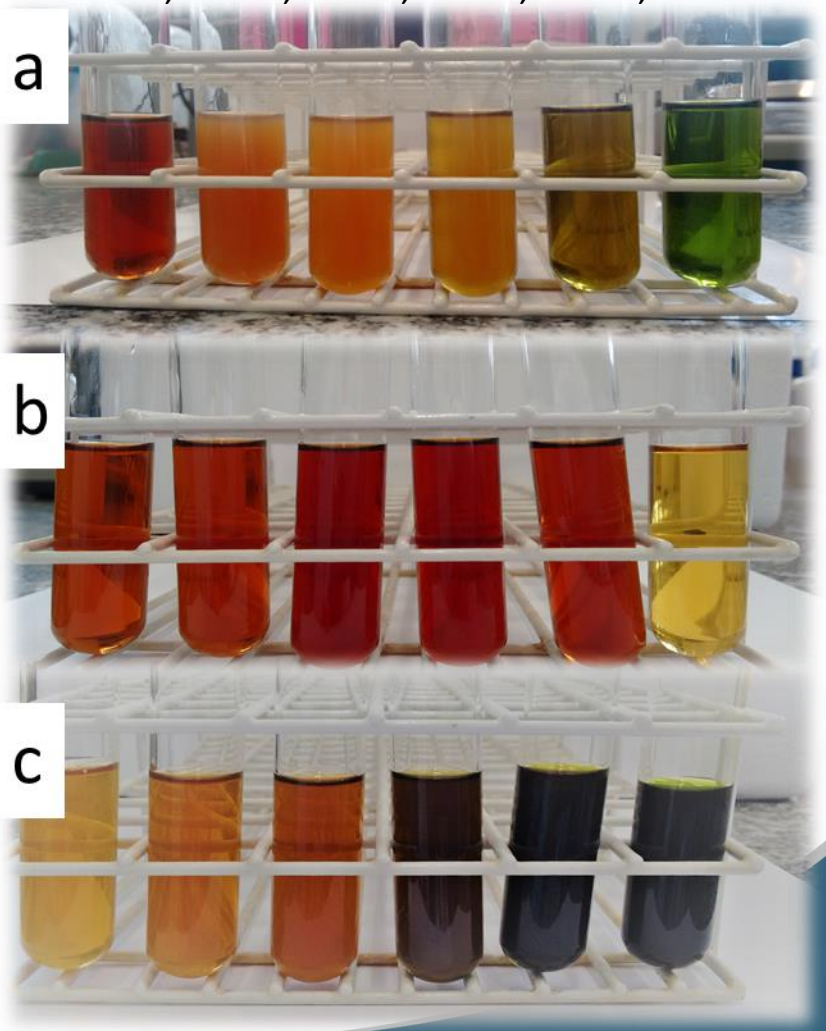
water:ethanol proportions in solvents: 100:0, 80:20, 60:40, 40:60, 20:80, 0:100.

Hydroethanolic extracts from plant materials:

a) Carqueja,

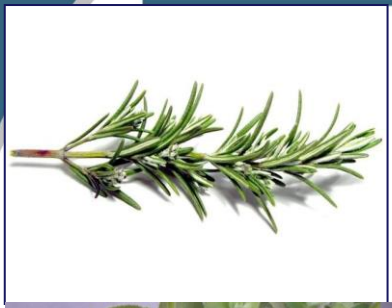
b) Guarana

c) Pitangueira leaves.



Source: Vargas (2015)

Vegetable extracts Compositions



Rosemary: eucalyptol, linalool, camphor, α-pinene, terpinen-4-ol, camphene, β-pinene, D-limonene, ρ-cymene, n-hexadecanoic acid, endo-borneol, L-α-terpineol, caryophyllene, bornyl acetate, tetradecanal.

Boldo: 1,8-cineol, ρ-cymene, phytol, eicosanoic acid, cis,cis,cis-7,10,13-hexadeca trienal and α-irone.

Cinnamon: cinnamaldehyde and α-copaene.

Guarana: caffeine,

as determined by GC-MS by Bonilla and Sobral (2017a).

Vegetable extracts Compositions

Table Individual abundances (as $g\,kg^{-1}$ equivalents) for each phenolic sub-class identified in guarana seed extracts (GSE).

Phenolic class equivalent	$g\,kg^{-1}$
Tyrosols	14.74 ± 2.64
Phenolic acids	7.38 ± 1.03
Anthocyanins	3.65 ± 0.67
Alkylphenols	1.33 ± 0.08
Flavonols	0.59 ± 0.10
Flavones	0.48 ± 0.08
Stilbenes	0.02 ± 0.00
Lignans	0.01 ± 0.00

Determined by UHPLC-MS by Goméz et al. (2018).

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Vegetable extracts Compositions

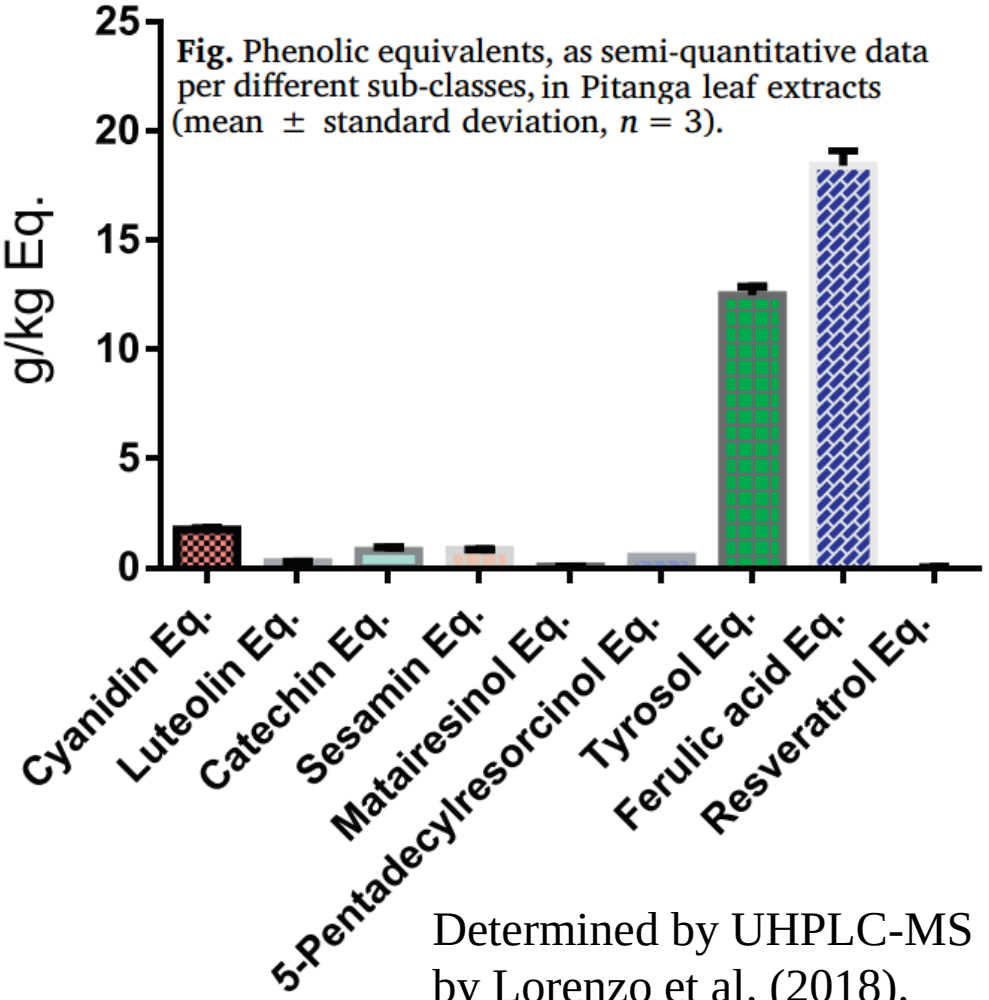

Fig. Phenolic equivalents, as semi-quantitative data per different sub-classes, in Pitanga leaf extracts (mean ± standard deviation, $n = 3$).

Determined by UHPLC-MS by Lorenzo et al. (2018).

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Antimicrobial activity

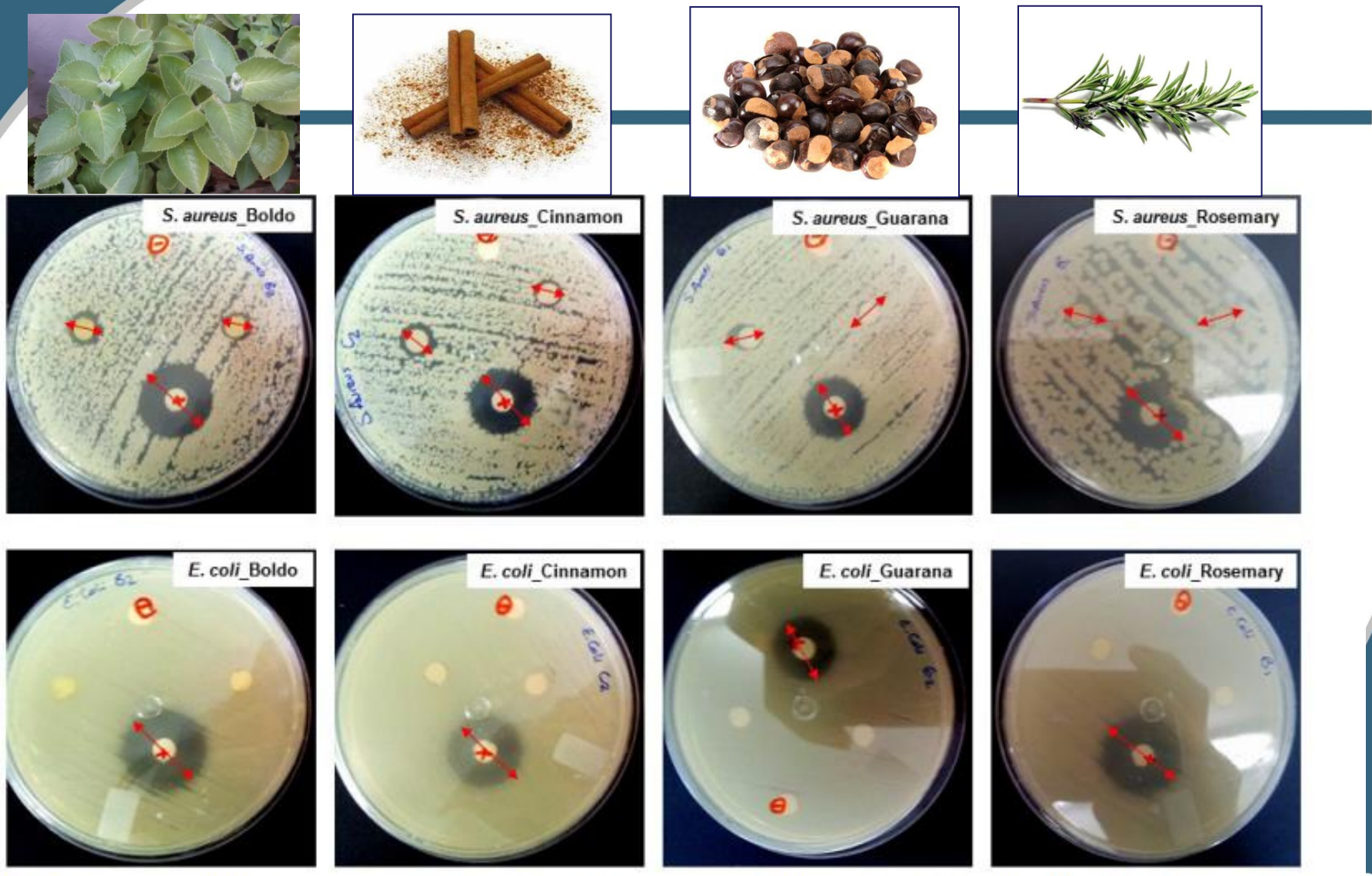


Figure Inhibition of bacterial growth *(E. Coli* and *S. Aureus)* by the boldo of Chile (*Peumus boldus molina*), cinnamon (*Cinnamomum sp.*), guarana (*Paullinia cupana) and* rosemary (*Rosmarinus officinalis*) extracts.

Source: Bonilla & Sobral (2017).

Active films with vegetable extracts

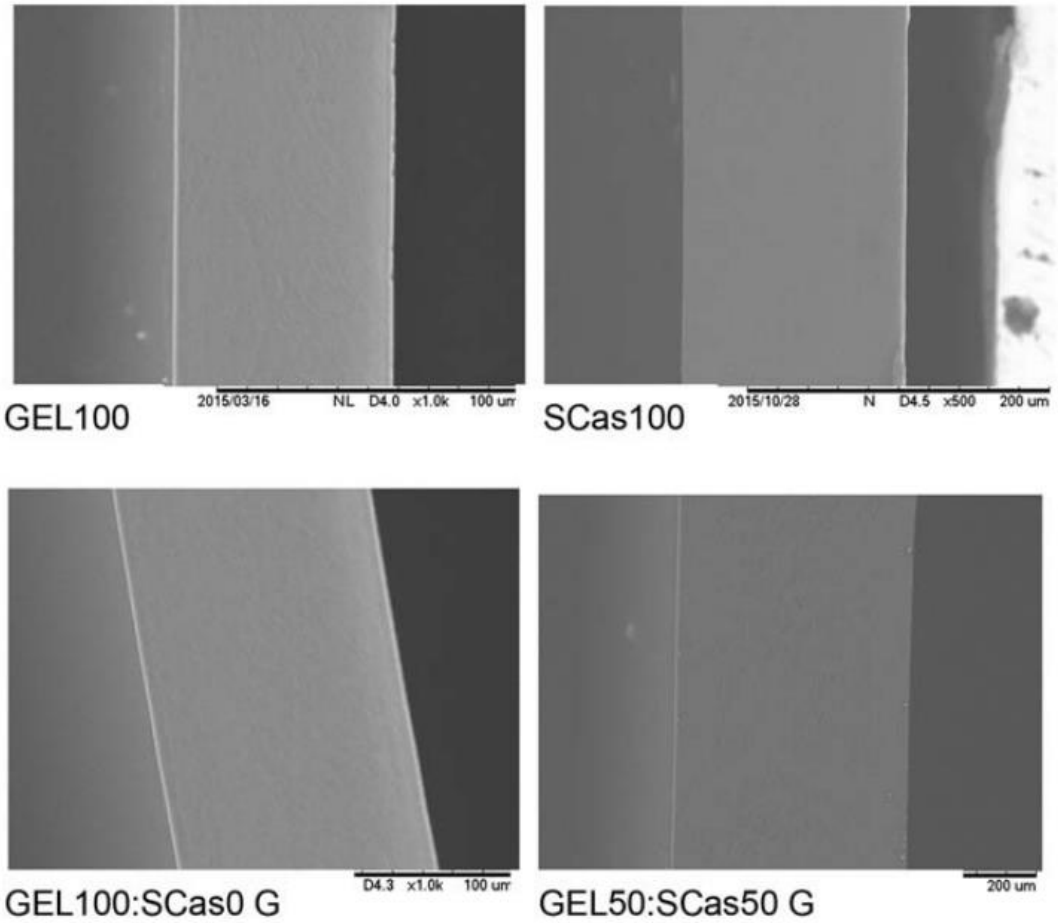


Figure Examples of scanning electron microscopy (SEM) images of cross-sections of biopolymer pure films (GEL100 or SCas100) and blend films (GEL100:SCas0 or GEL50:SCas50) with guarana (G) extract.

Films based on blends of gelatin/sodium caseinate, loaded with extracts of cinnamon, guarana, rosemary and boldo.

(Bonilla et al., 2017b).

Active films with vegetable extracts

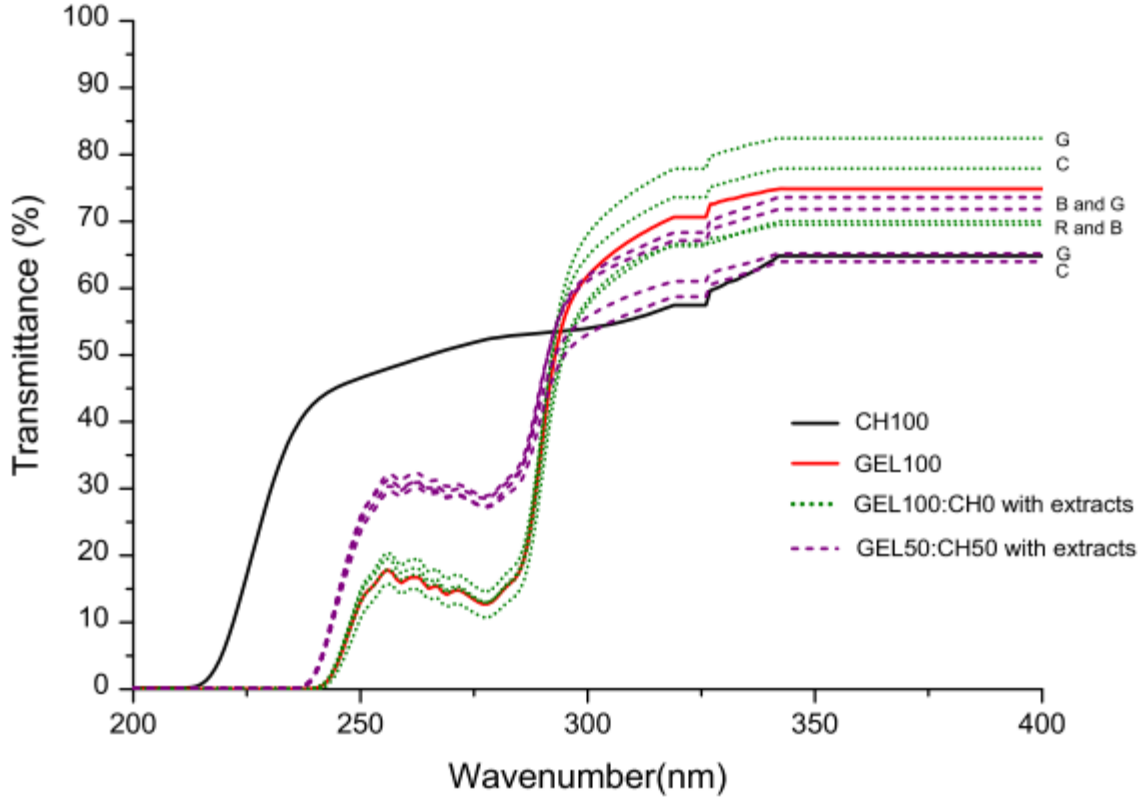


Fig. . UV-Vis spectra for pure chitosan films (CH100), pure gelatin films (GEL100) and GEL100:CH0 and GEL50:CH50 blend films with guarana (G), boldo (B), cinnamon (C) and rosemary (R) extracts.

Source: Bonilla et al. (2016)

Active films with vegetable extracts

Table Physical properties and antioxidant characteristics of films.

Film Properties *Physical properties*	T0	T1 (2.25g PE/100g FFS)	T2 (1g NA/100g FFS)	T3 (2.25g PE + 1g NA/100g FFS)
ΔE*	5.53 ± 0.39^{a}	48.48 ± 2.48^{c}	18.65 ± 5.80^{b}	49.74 ± 1.17^{c}
Opacity	2.2 ± 0.4^{a}	3.2 ± 0.6^{a}	36.0 ± 5.3^{b}	47.7 ± 1.4^{c}
Average roughness, R_a (nm)	73.4 ± 1.8^{a}	70.2 ± 5.7^{a}	239.2 ± 39.3^{b}	224.4 ± 6.9^{b}
Tensile strength, σ^B (MPa)	9.4 ± 1.9^{a}	10.9 ± 1.4^{a}	13.3 ± 1.4^{b}	10.6 ± 1.6^{a}
Elongation at break, ε^B (%)	24.3 ± 3.9^{c}	18.4 ± 2.3^{b}	2.8 ± 0.7^{a}	3.7 ± 0.7^{a}
Elastic modulus, E (MPa/%)	2.7 ± 0.5^{a}	2.7 ± 0.9^{a}	6.0 ± 1.2^{c}	4.3 ± 1.1^{b}
Puncture Force, PF (N)	14.0 ± 1.3^{c}	11.5 ± 1.2^{b}	6.8 ± 1.5^{a}	6.6 ± 0.5^{a}
Puncture Deformation, PD (%)	4.2 ± 1.5^{c}	1.7 ± 0.6^{b}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}
ABTS•+ (% Scavenging activity)	7.68 ± 1.57^{a}	59.88 ± 2.33^{d}	14.33 ± 0.55^{b}	37.22 ± 1.62^{c}
DPPH• (% Scavenging activity)	3.38 ± 0.11^{a}	86.20 ± 1.37^{d}	25.79 ± 3.14^{b}	71.56 ± 2.99^{c}
FRAP (μmol $FeSO_4$/g film) -4 min	215.25 ± 27.76^{a}	4565.34 ± 90.38^{c}	325.41 ± 39.04^{a}	2918.52 ± 222.37^{b}
FRAP (μmol $FeSO_4$/g film) -30 min	253.85 ± 24.26^{a}	5670.98 ± 266.63^{c}	508.84 ± 84.23^{a}	3594.93 ± 297.30^{b}

* Values (means ± SD) in a row with different superscripts indicate significant differences ($p < 0.05$)

FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

Films of cassava starch/chitosan with pitanga leaf ethanolic extract and/or natamycin (Chakravartula et al., 2020).

Active films with vegetable extracts

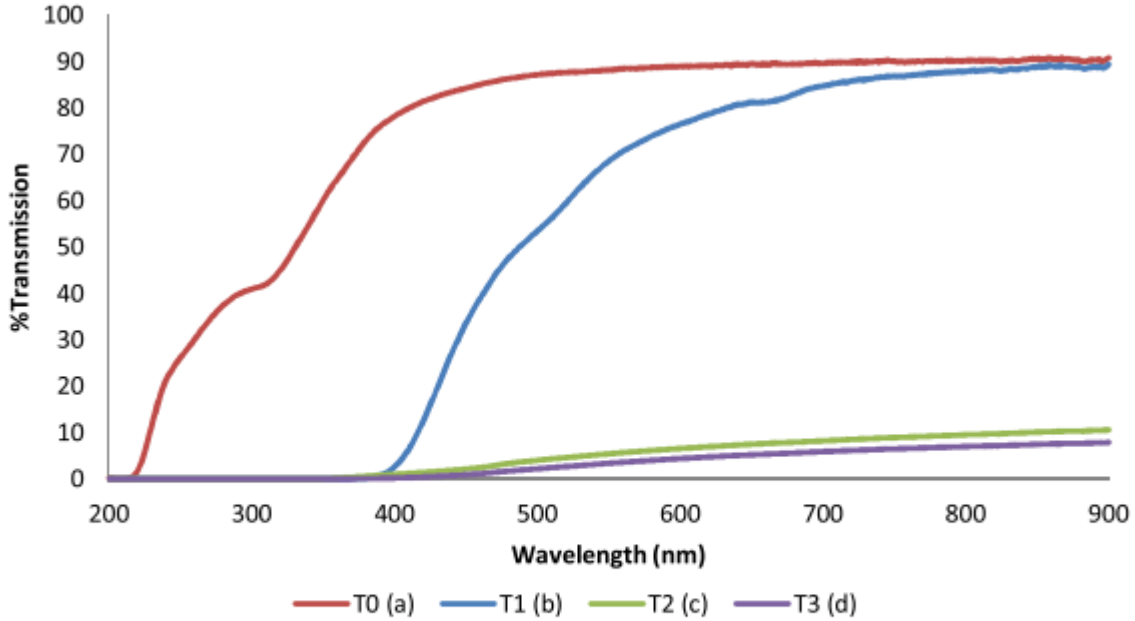


Fig. UV–vis light barrier properties (200-900 nm) of CS/CH active films: T0: without additives, T1: with 2.25 g PE/100 g FFS, T2: with 1 g NA/100 g FFS, and T3: with 2.25 g PE + 1 g NA/100 g FFS.

Films of cassava starch/chitosan with pitanga leaf ethanolic extract and/or natamycin (Chakravartula et al., 2020).

Active bi-layers films with vegetable extracts

Fig. Photographs of bi-layer gelatin films. G: bi-layer gelatin film without additives in the second layer; GN: bi-layer film with nisin in the second layer; GE: bi-layer film with PLHE in the second layer; GNE: bi-layer film with nisin and PLHE in the second layer

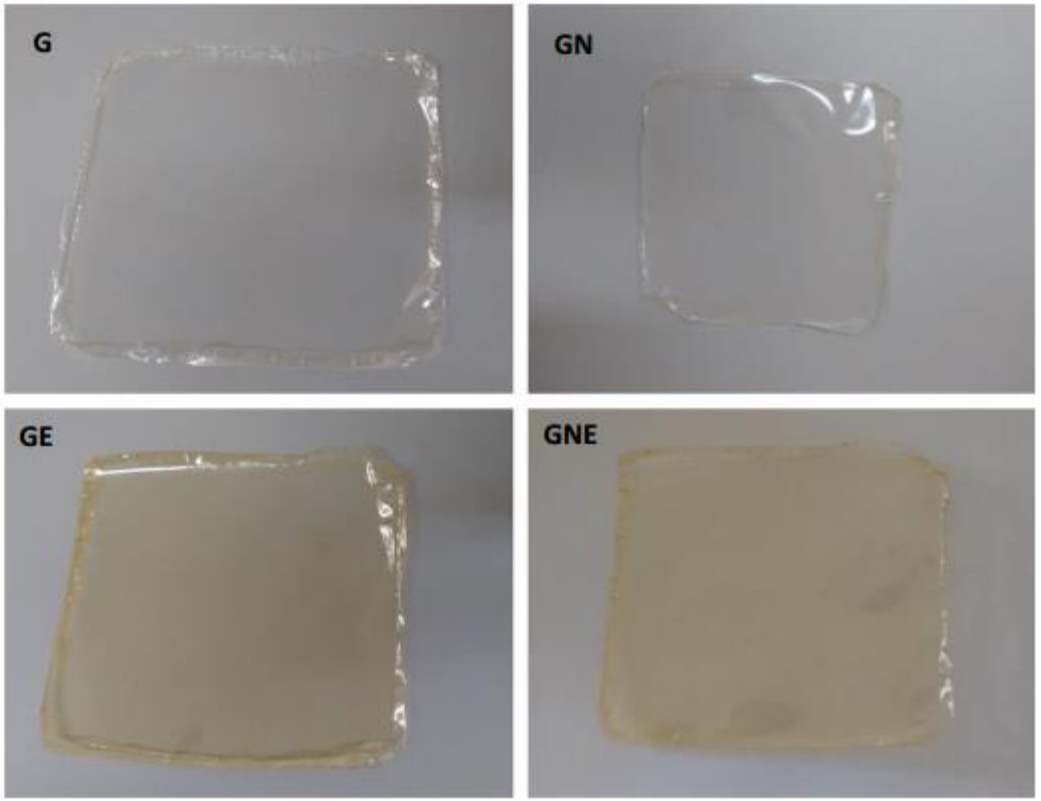
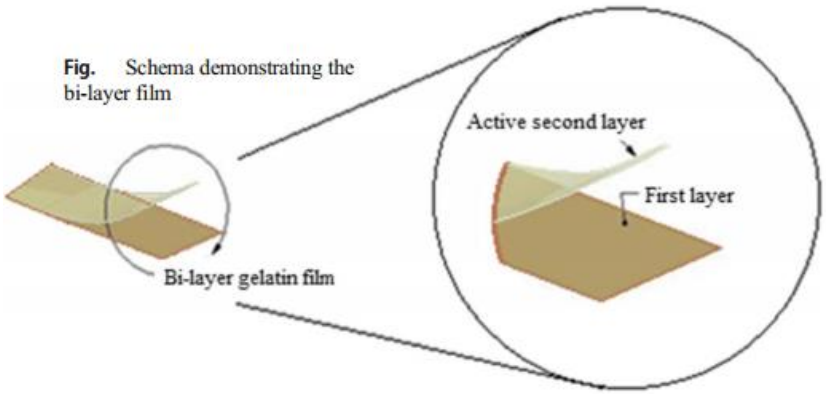


Fig. Schema demonstrating the bi-layer film



Films of gelatin with pitanga leaf ethanolic extract and/or nisin (Luciano al., 2021).

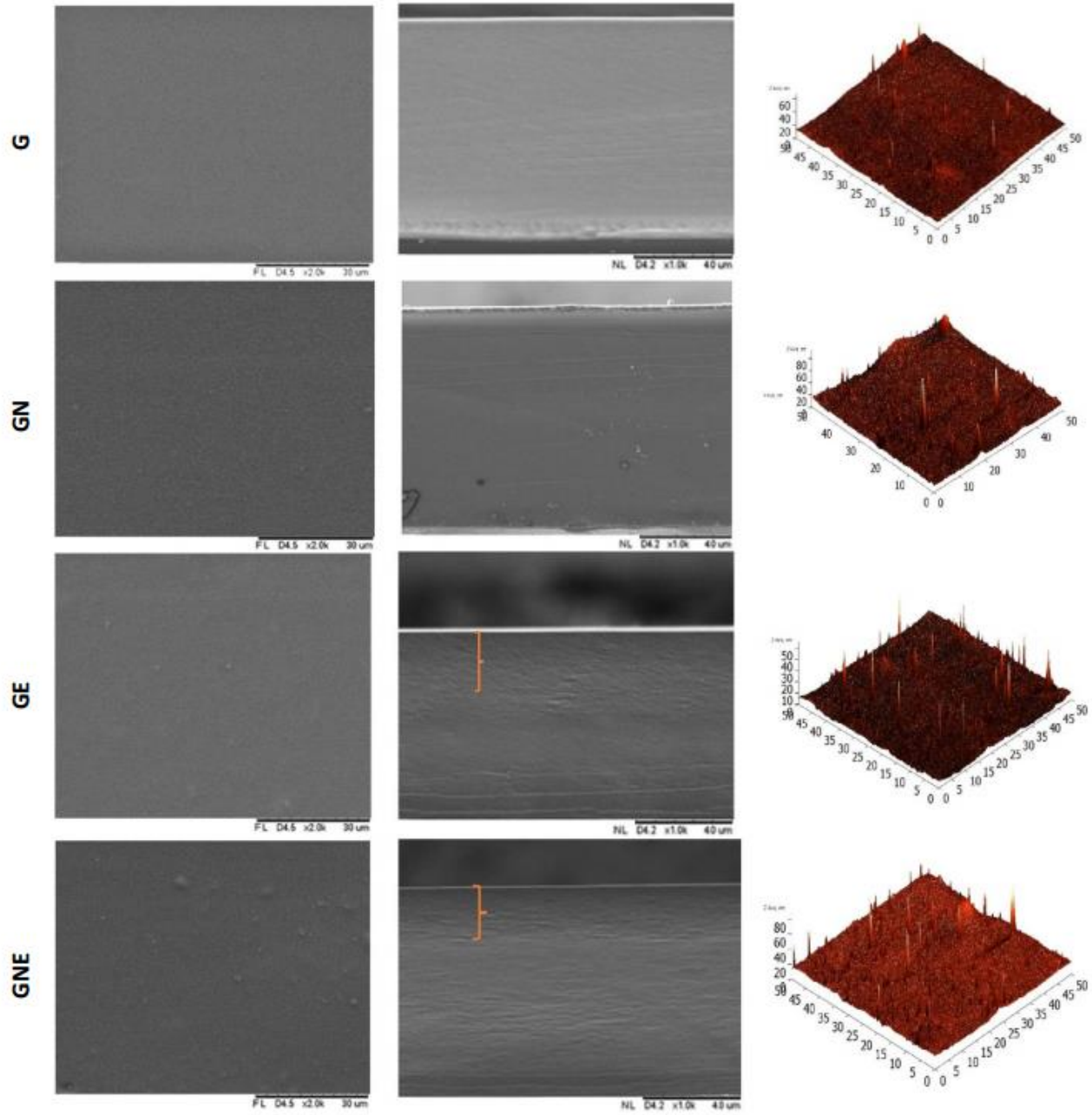


Fig. 3 Surface (left) and cross-section (center) micrographs by scanning electron microscopy (MEV), and surface (right) micrographs by atomic force microscopy (AFM) of bi-layer gelatin-based films. G: bi-layer gelatin film without additives in the second layer; GN: bi-layer film with nisin in the second layer; GE: bi-layer film with PLHE in the second layer; GNE: bi-layer film with nisin and PHLE in the second layer. The brackets in cross section micrographs of GE and GNE films show the second layer

Source: Luciano et al. (2021)

Active bi-layers films with vegetable extracts

Table Total phenolic compounds (TPC), ABTS• + radical inhibition, and DPPH• radical inhibition of active bi-layer gelatin-based films*

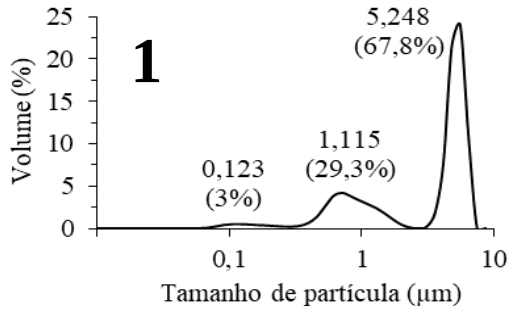
Activities	G	GN	GE	GNE
TPC (mg gallic acid/g film)	0.5 ± 0.3^{c}	1.8 ± 0.1^{b}	7.6 ± 0.9^{a}	7.4 ± 1.1^{a}
ABTS• + (mg trolox equivalent/100 g film)	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}	0.75 ± 0.03^{a}	0.79 ± 0.03^{a}
DPPH• (%)	2.3 ± 0.9^{c}	4.7 ± 0.1^{b}	58.3 ± 1.5^{a}	55.7 ± 1.3^{a}

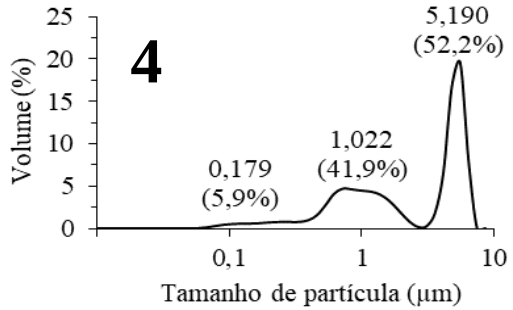
*G: bi-layer gelatin film without additives in the second layer; GN: bi-layer film with nisin in the second layer; GE: bi-layer film with PLHE in the second layer; GNE: bi-layer film with nisin and PHLE in the second layer. Different letters in the same line indicate significant differences between the mean values according to the Tukey test ($p < 0.05$)

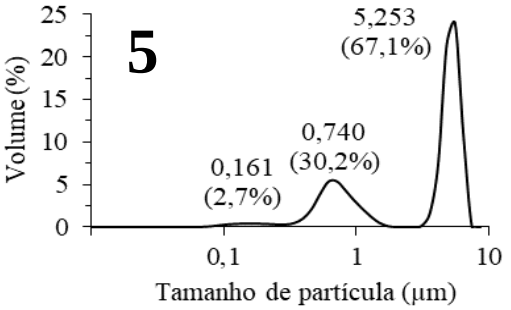
Films of gelatin with pitanga leaf ethanolic extract and/or nisin (Luciano al., 2021).

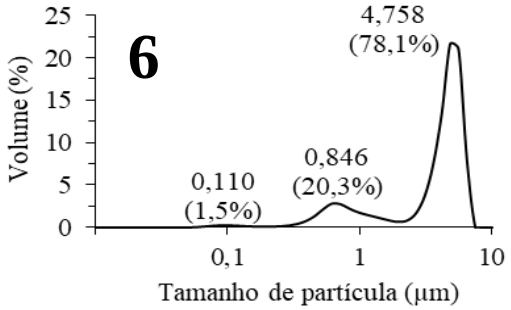
Active films with double emulsions (W/O/W) loaded with vegetable extracts

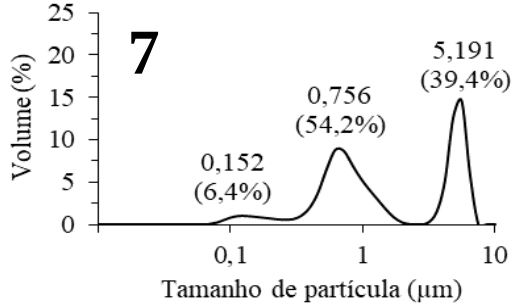
D = 0.3 – 0.7 µm

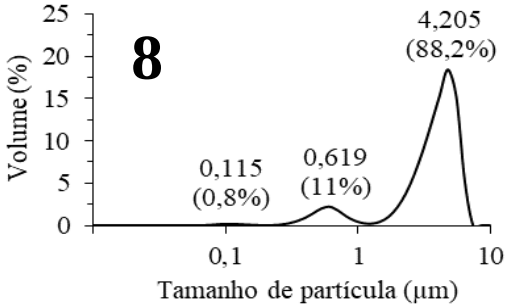


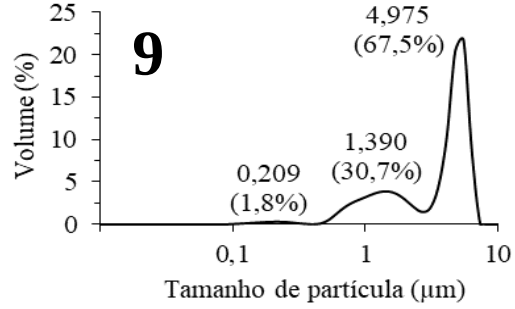




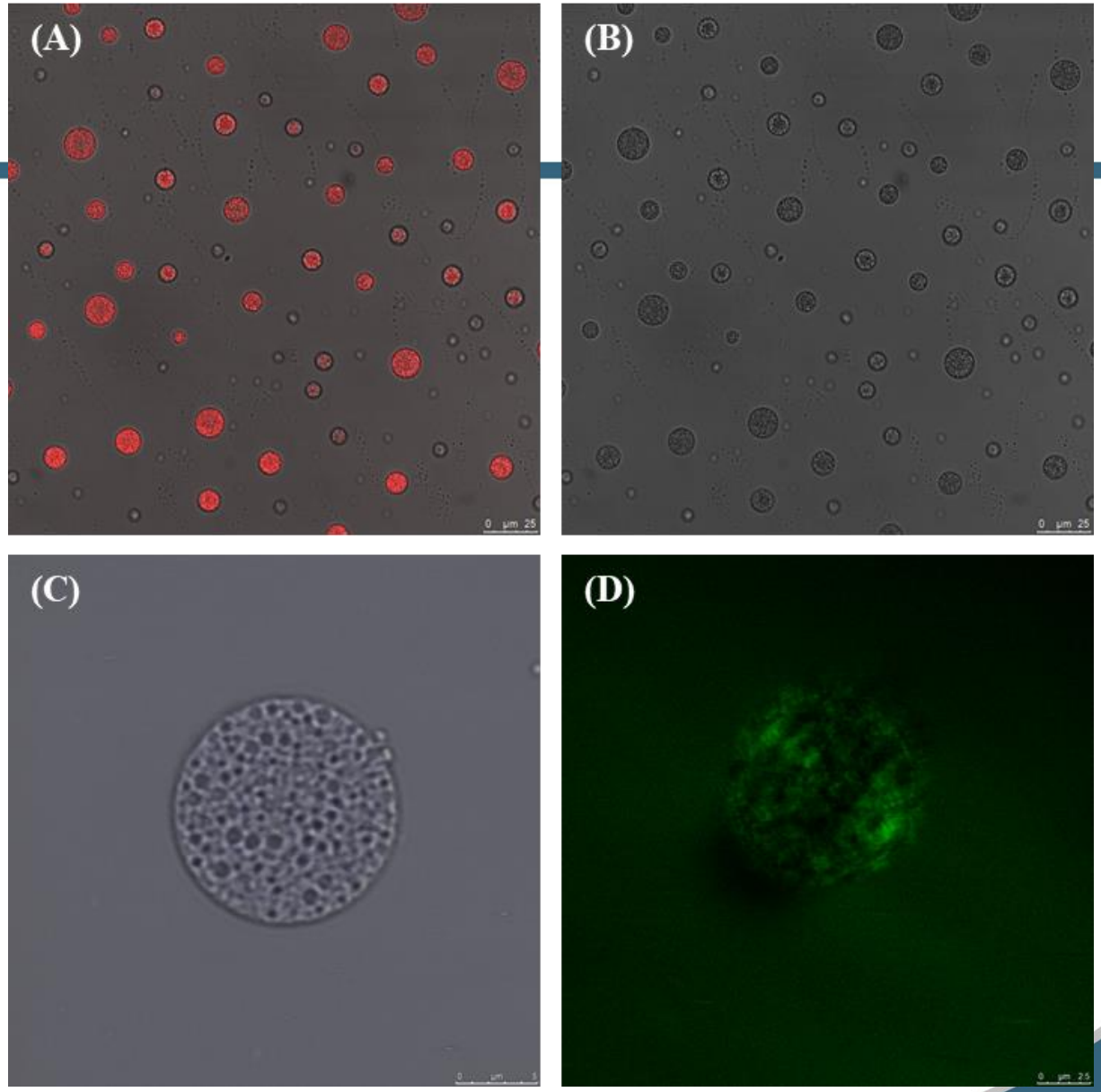




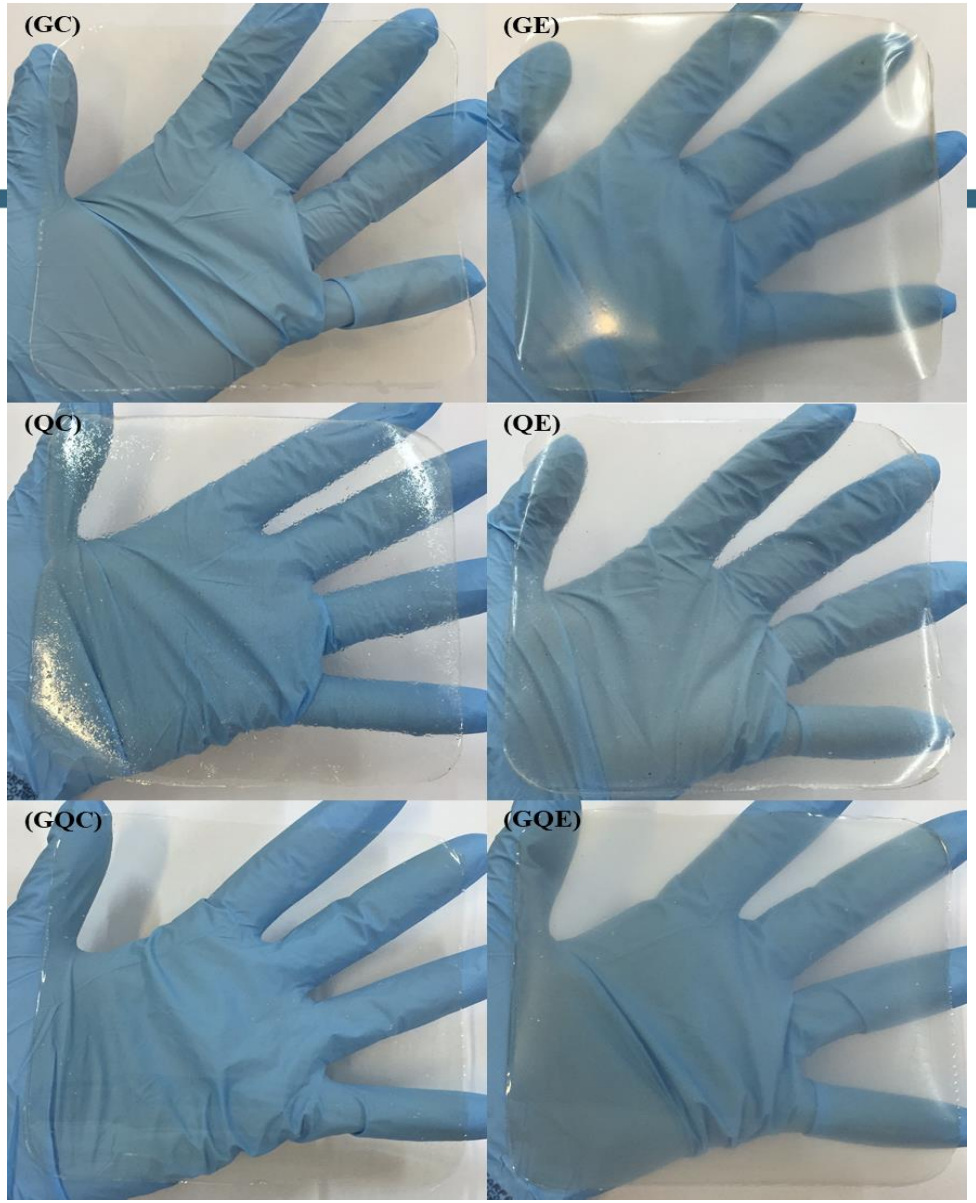




Source: Tessaro et al. (2021)

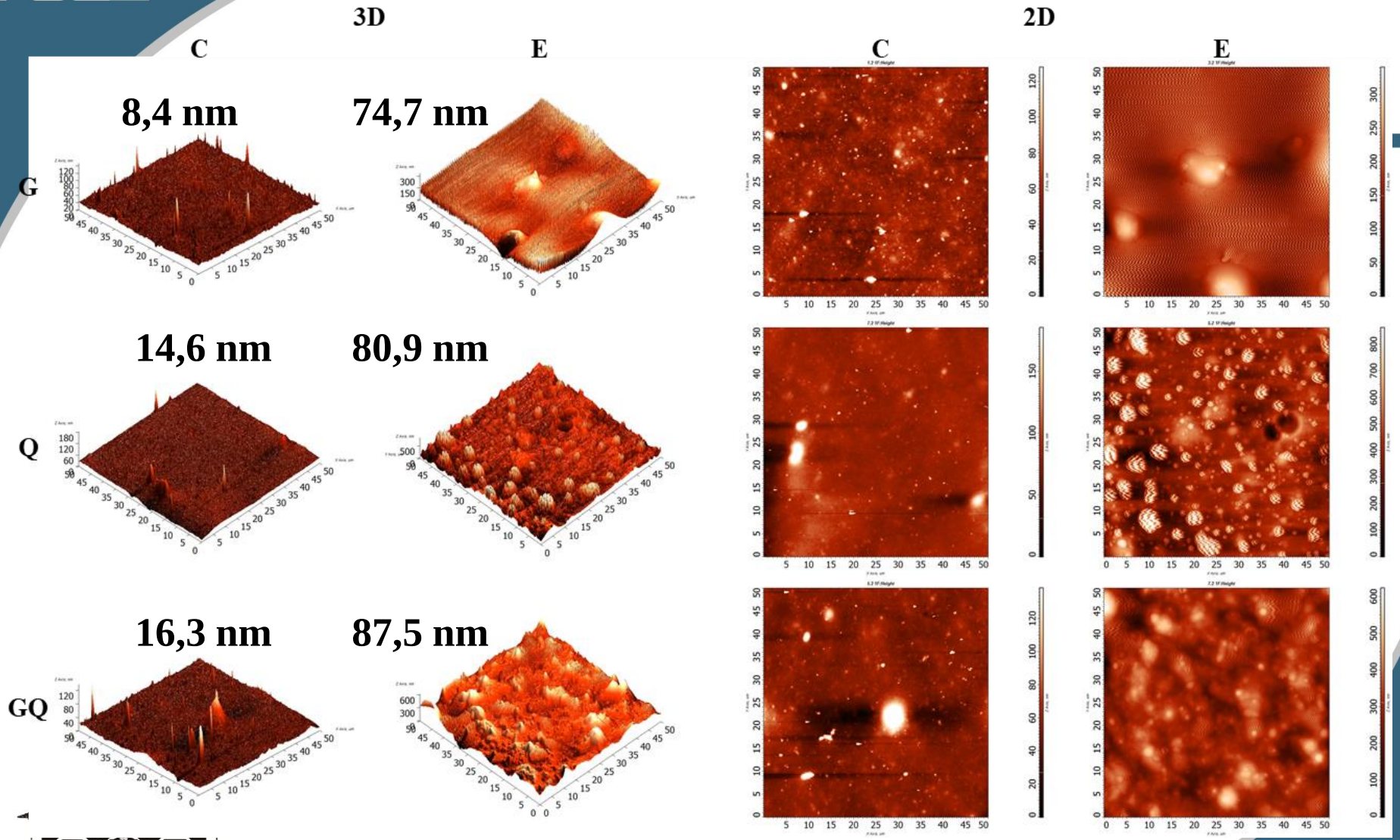


Source: Luciano et al. (2021)



Source: Luciano et al. (2021)

AFM



Source: Luciano et al. (2021)

Active films with emulsified active compound

Eugenol nanoemulsions stabilized with soy lecithin or sodium caseinate (Dammak & Sobral., 2019).

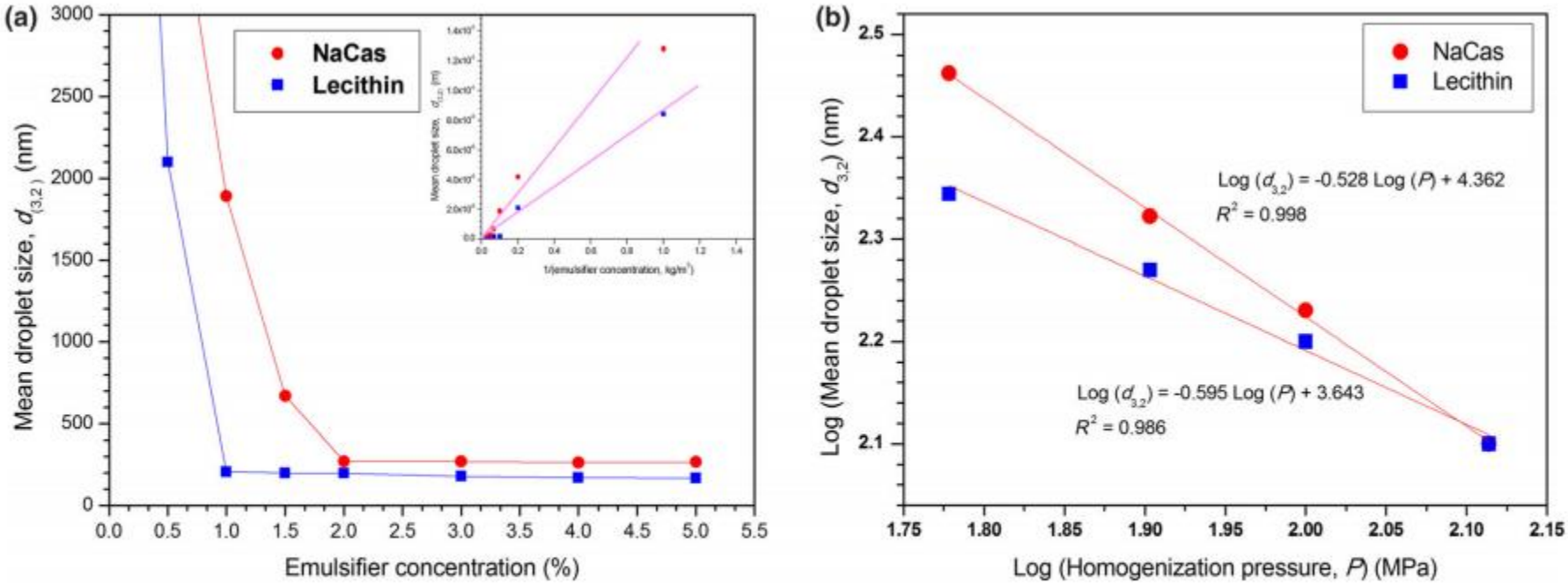


Figure (a) Mean droplet size ($d_{3,2}$) of eugenol-loaded nanoemulsions as a function of soy lecithin and sodium caseinate concentrations; the inset graph was plotted using eqn 1. All of these nanoemulsions were produced under standardised homogenisation conditions (100 MPa, three cycles). (b) Influence of homogenisation pressure (three cycles) on the mean droplet size ($d_{3,2}$) of eugenol-loaded nanoemulsions stabilised with 1% (w/w) of soy lecithin and 2% (w/w) of sodium caseinate. [Colour figure can be viewed at wileyonlinelibrary.com]

Active films with emulsified active compound

Eugenol nanoemulsions stabilized with soy lecithin or sodium caseinate (Dammak & Sobral., 2019).

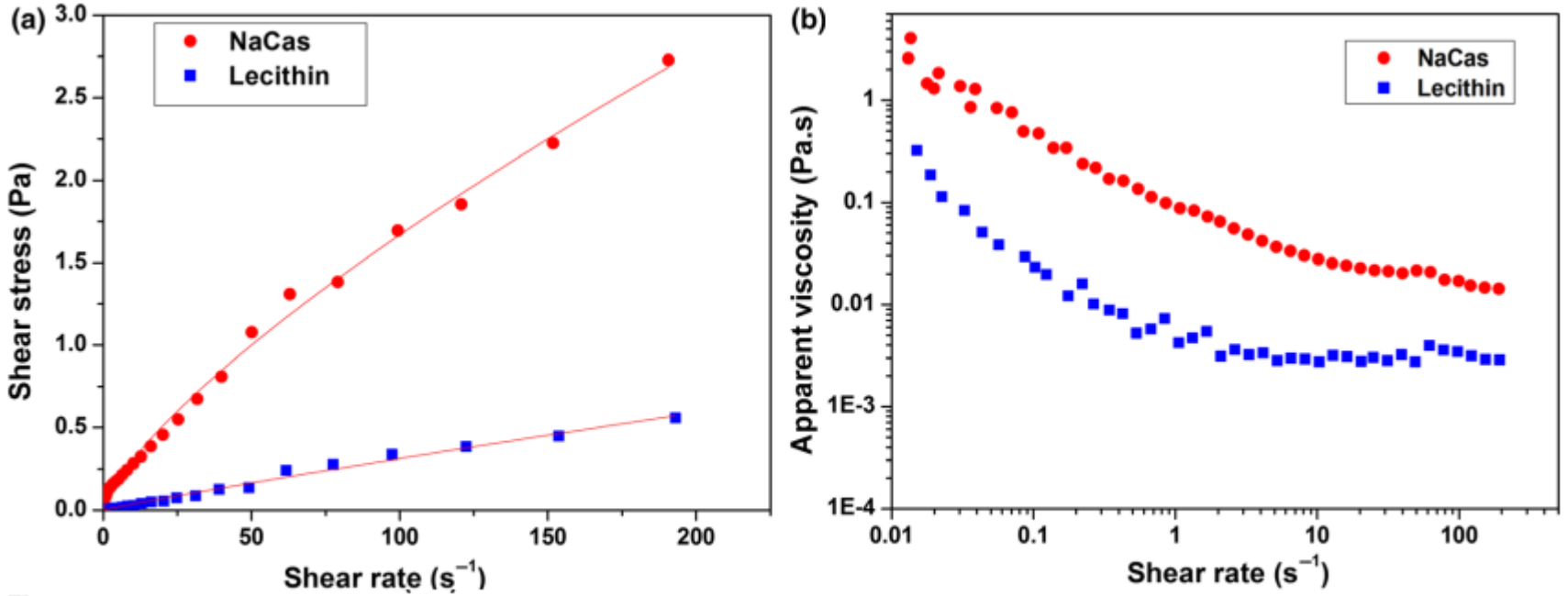


Figure (a) Flow behaviour of sodium caseinate and soy lecithin-stabilised nanoemulsions encapsulating eugenol. The lines were plotted using eqn 2. (b) Apparent viscosity as a function of the shear rate.

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Active films with emulsified active compound

Films of gelatin with eugenol nanoemulsions stabilized with soy lecithin or sodium caseinate (Dammak & Sobral., 2019).

Table Thermal properties and Trolox-equivalent antioxidant capacity (TEAC) of the gelatin films

Gelatin films added with NE	1st Scan			2nd Scan	TEAC (μmol TE per g of dry weight film)
	T_g (°C)	ΔH_m (J g^{-1})	T_m (°C)	T_g (°C)	
Control	48.1 ± 0.8^a	25.3 ± 0.5^a	66.8 ± 0.2^a	34.4 ± 1.4^a	0.0 ± 0.0^c
NaCas-stabilised NE	48.8 ± 0.4^a	22.4 ± 0.2^{ab}	66.5 ± 0.8^a	29.2 ± 2.7^b	4.8 ± 0.1^a
SL-stabilised NE	48.5 ± 0.6^a	23.1 ± 0.8^b	66.0 ± 0.4^a	32.6 ± 1.1^{ab}	3.6 ± 0.1^b

Mean ± standard deviations from triplicate determinations. Different letters in the same column indicate significant differences at $P < 0.05$. NaCas, sodium caseinate; NE, Nanoemulsion; SL, Soy lecithin; TE, Trolox-equivalent; T_g, Glass transition temperature; T_m, Melting temperature; ΔH_m, Melting enthalpy.

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Active films with emulsified active compound

Characteristics of α-tocopherol, garlic essential oil and cinnamaldehyde nanoemulsified in water (Córdoba & Sobral, 2017).

Table 1
Mean particle size, polydispersity index and zeta potential of O/W nanoemulsion containing cinnamaldehyde, garlic essential oil and a-tocopherol stored at 25 °C by 30 days*.

Time (Days)	Droplet size (nm)	Polydispersity index	Zeta potential (mV)
0	79.34 ± 1.17[a]	0.148 ± 0.012[a]	−12.43 ± 0.68[a]
30	79.87 ± 0.45[a]	0.132 ± 0.002[a]	−13.17 ± 0.97[a]

*Mean values ± standard deviation. n = 3. No significant differences ($p > 0.05$) among the average values was obtained according to the Duncan's test.

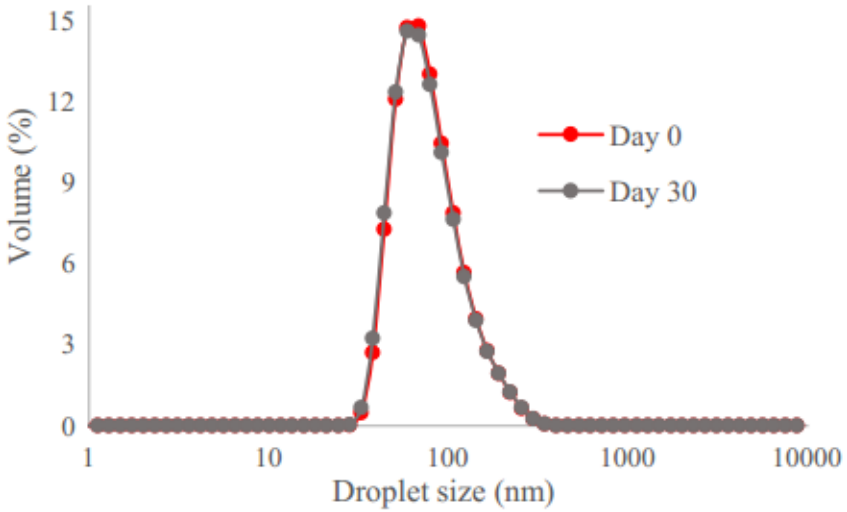

Fig. 1. Particle size distribution of O/W nanoemulsion containing cinnamaldehyde, garlic essential oil and α-tocopherol during storage at 0 and 30 days.

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Active films with emulsified active compound

Films of gelatin, gelatin-chitosan or gelatin-sodium caseinate blends, with α-tocopherol, garlic essential oil and cinnamaldehyde nanoemulsified in water (Córdoba & Sobral, 2017).

Table
Antioxidant activity of gelatin-based films loaded with NACs expressed like TEAC and $ABTS^{\cdot+}$ radical scavenging*.

Films**	TEAC (mg/L)	$ABTS^{\cdot+}$ radical scavenging effect (%)
Control	0.00 ± 0.00^{d}	0.4 ± 0.2^{d}
G-NACs	4.24 ± 0.27^{c}	14.5 ± 0.4^{c}
G-Cs-NACs	13.5 ± 0.41^{a}	29.0 ± 0.5^{a}
G-Ch-NACs	8.68 ± 1.61^{b}	21.5 ± 2.1^{b}

*Mean values ± standard deviation. n = 3. Different letters in the same column indicate significant differences ($p < 0.05$) among the average values obtained by application of the Duncan's test. **G-NACs = gelatin plus nanoemulsified active compounds; G-Cs-NACs = gelatin-sodium caseinate blend plus nanoemulsified active compounds; G-Ch-NACs = gelatin-chitosan blend plus nanoemulsified active compounds.

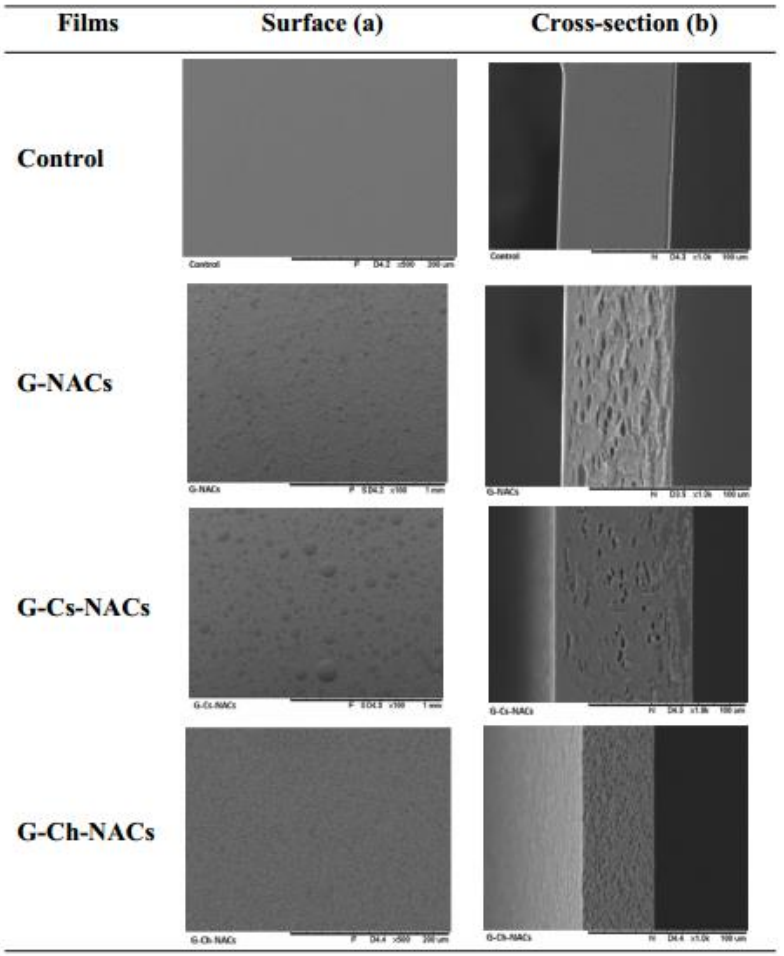

Fig. Scanning electron micrographs of control film, and of films based on G-NACs = gelatin plus nanoemulsified active compounds, G-Cs-NACs = gelatin-sodium caseinate blend plus nanoemulsified active compounds, and G-Ch-NACs = gelatin-chitosan blend plus nanoemulsified active compounds.

Active films with emulsified active compound

Films of gelatin with rutin emulsified in water (Dammak et al., 2017).

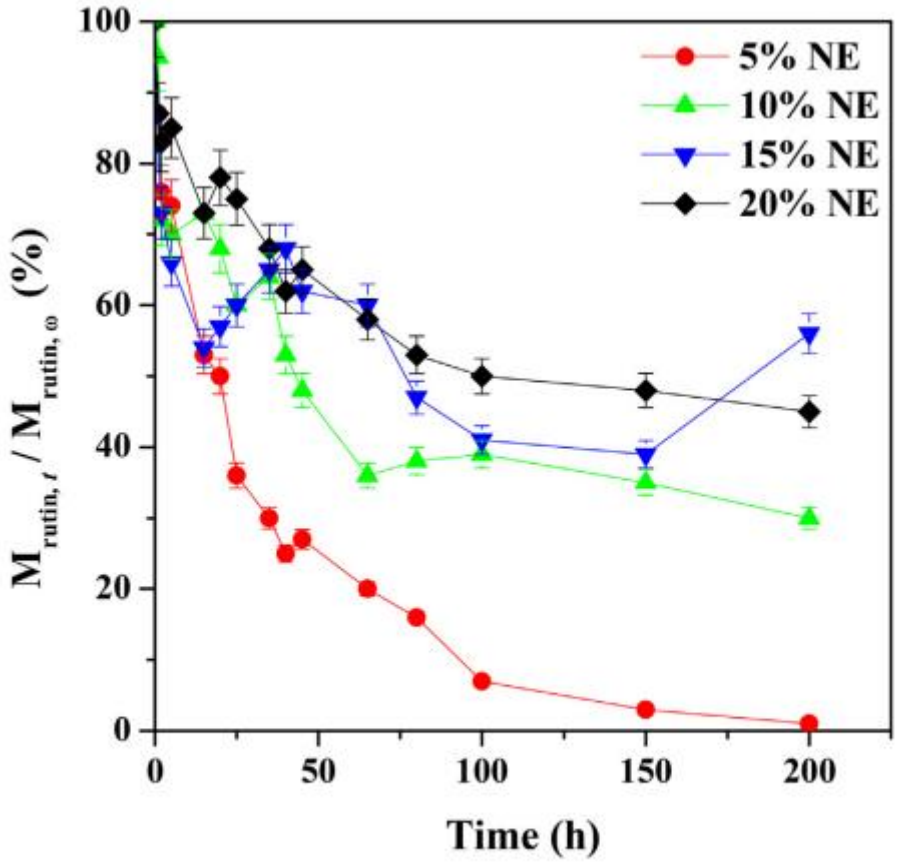

Fig. Rutin release from gelatine-films incorporated with rutin-loaded nanoemulsions at various concentrations as a function of time.

Active films with emulsified active compound

Films of gelatin with rutin emulsified in water (Dammak et al., 2017).

Table Color parameters and transparency values of gelatin films loaded with rutin oil-in-water nanoemulsion (NE) at various levels.

	Film color				
Film type	L^*	a^*	b^*	ΔE^*	Transparency values
Control	90.41 ± 1.53^{a}	1.98 ± 0.29^{a}	3.85 ± 0.46^{abcd}	2.69 ± 1.62^{a}	3.08 ± 0.00^{a}
5% NE	91.02 ± 2.19^{a}	1.91 ± 0.39^{a}	3.67 ± 0.59^{bc}	2.05 ± 1.29^{a}	2.99 ± 0.02^{a}
10% NE	93.20 ± 0.44^{a}	1.32 ± 0.07^{b}	3.26 ± 0.25^{c}	2.80 ± 0.51^{a}	2.87 ± 0.03^{a}
15% NE	90.53 ± 0.46^{a}	1.8 ± 0.03^{ab}	4.62 ± 0.07^{ade}	2.83 ± 0.44^{a}	2.72 ± 0.02^{a}
20% NE	89.59 ± 0.38^{a}	2.10 ± 0.08^{a}	5.07 ± 0.04^{ae}	3.53 ± 0.36^{a}	2.73 ± 0.06^{a}

Results are expressed as mean ± standard deviation of 3 determinations.
Means with different letters were significantly different at $p < 0.05$.

Table Antioxidant activity of gelatin based-films loaded with rutin oil-in-water nanoemulsions (NE) at different levels.

	Antioxidant activity		
Film type	DPPH (μg/mL)	ABTS (mM TE/g dry film)	FRAP (mM TE/g dry film)
Control	0.07 ± 0.01^{a}	0.49 ± 0.1^{a}	0.1 ± 0.01^{a}
5% NE	0.26 ± 0.01^{b}	3.47 ± 0.1^{b}	81.1 ± 2.3^{b}
10% NE	0.66 ± 0.01^{c}	6.97 ± 0.1^{c}	112.3 ± 3.1^{c}
15% NE	1.30 ± 0.01^{d}	8.96 ± 0.1^{d}	168.3 ± 2.5^{d}
20% NE	1.97 ± 0.01^{e}	12.33 ± 0.2^{e}	198.3 ± 3.4^{e}
β-carotene[1000 ppm]$_{aq.}$	0.21 ± 0.01^{g}	0.83 ± 0.1^{g}	9.32 ± 1.6^{g}

Results are expressed as mean ± standard deviation of 3 determinations.
Means with different letters were significantly different at $p < 0.05$.

Active films with emulsified active compound

Films of gelatin with rutin emulsified in water (Dammak et al., 2017).

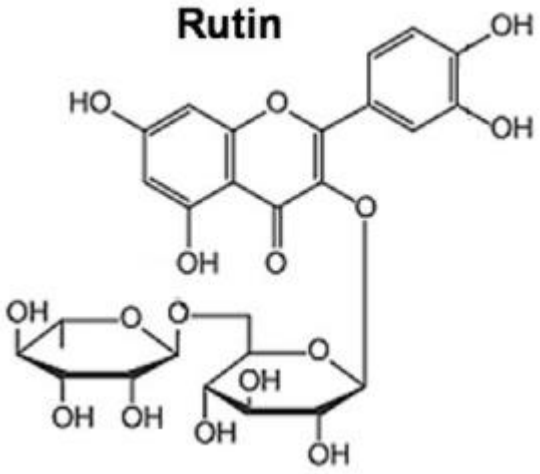

Table Color parameters … nanoemulsion (NE) at va… h rutin oil-in-water

Film type	Film color L^*	Transparency values
Control	90.41 ± 1.…	3.08 ± 0.00[a]
5% NE	91.02 ± 2.…	2.99 ± 0.02[a]
10% NE	93.20 ± 0.…	2.87 ± 0.03[a]
15% NE	90.53 ± 0.…	2.72 ± 0.02[a]
20% NE	89.59 ± 0.…	2.73 ± 0.06[a]

Results are expressed as …
Means with different let…

Table Antioxidant activity of gelatin based-films loaded with rutin oil-in-water nanoemulsions (NE) at different levels.

Film type	Antioxidant activity		
	DPPH (μg/mL)	ABTS (mM TE/g dry film)	FRAP (mM TE/g dry film)
Control	0.07 ± 0.01[a]	0.49 ± 0.1[a]	0.1 ± 0.01[a]
5% NE	0.26 ± 0.01[b]	3.47 ± 0.1[b]	81.1 ± 2.3[b]
10% NE	0.66 ± 0.01[c]	6.97 ± 0.1[c]	112.3 ± 3.1[c]
15% NE	1.30 ± 0.01[d]	8.96 ± 0.1[d]	168.3 ± 2.5[d]
20% NE	1.97 ± 0.01[e]	12.33 ± 0.2[e]	198.3 ± 3.4[e]
β-carotene[1000 ppm]$_{aq.}$	0.21 ± 0.01[g]	0.83 ± 0.1[g]	9.32 ± 1.6[g]

Results are expressed as mean ± standard deviation of 3 determinations.
Means with different letters were significantly different at $p < 0.05$.

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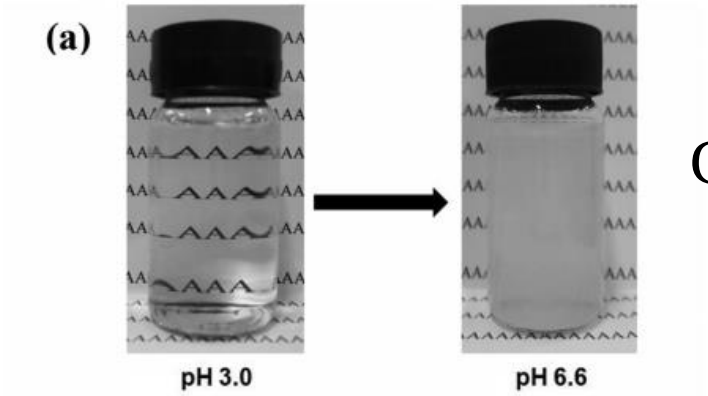
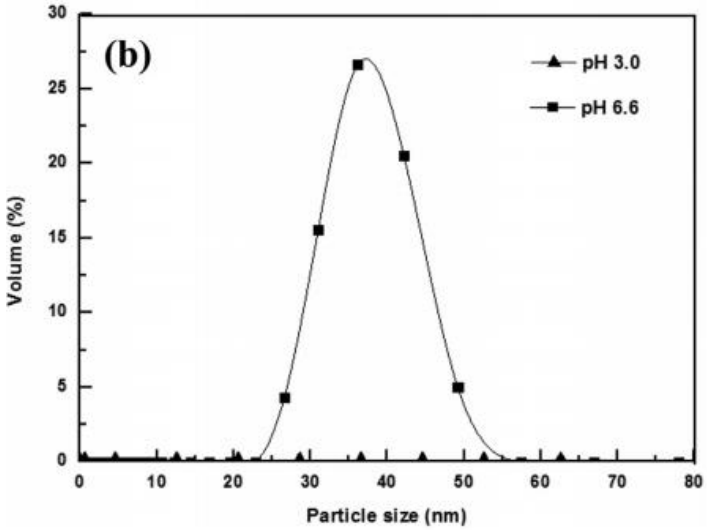
Another approach: Pickering emulsion

Pickering emulsions are formed when solid colloidal particles are adsorbed to the interface of emulsion's droplets, stabilizing them (Manoharan, 2006).



ChiNP characteristics



Particle-size distribution graph of chitosan nanoparticles.

Source: Dammak & Sobral. (2018)

d= 38.4±11 nm

Span= 0.25

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The experimental and predicted mean droplet size ($d_{3,2}$) and emulsion stability index (*ESI*) of prepared emulsions.

Run No.	Chitosan NP cc. (%)	Lecithin cc. (%)	O/W ratio (−)	Experimental *ESI* (−)	Predict *ESI* (−)	Experimental $d_{3,2}$ (μm)	Predicted $d_{3,2}$ (μm)
1	−1 (0.2)	−1 (1)	0 (0.275)	0.45	0.36	6.91	8.19
2	1 (2)	−1 (1)	0 (0.275)	0.521	0.45	6.8	5.58
3	−1 (0.2)	1 (7)	0 (0.275)	0.49	0.56	7.5	8.72
4	1 (2)	1 (7)	0 (0.275)	0.3	0.39	8.64	7.36
5	−1 (0.2)	0 (4)	−1 (0.05)	0.4	0.43	6	6.11
6	1 (2)	0 (4)	−1 (0.05)	0.42	0.43	3.2	5.81
7	−1 (0.2)	0 (4)	1 (0.5)	0.39	0.43	16.48	6.11
8	1 (2)	0 (4)	1 (0.5)	0.31	0.28	10.3	10.19
9	0 (1.1)	−1 (1)	−1 (0.05)	0.238	0.30	6.82	5.43
10	0 (1.1)	1 (7)	−1 (0.05)	0.65	0.55	5.3	3.97
11	0 (1.1)	−1 (1)	1 (0.5)	0.28	0.38	7.56	8.89
12	0 (1.1)	1 (7)	1 (0.5)	0.32	0.26	11.26	12.65
13	0 (1.1)	0 (4)	0 (0.275)	0.8	0.78	3.1	2.81
14	0 (1.1)	0 (4)	0 (0.275)	0.76	0.78	2.9	2.81
15	0 (1.1)	0 (4)	0 (0.275)	0.82	0.78	2.52	2.81
16	0 (1.1)	0 (4)	0 (0.275)	0.78	0.78	2.68	2.81
17	0 (1.1)	0 (4)	0 (0.275)	0.72	0.78	2.87	2.81

* The values (−1), (0) and (1) are coded levels.

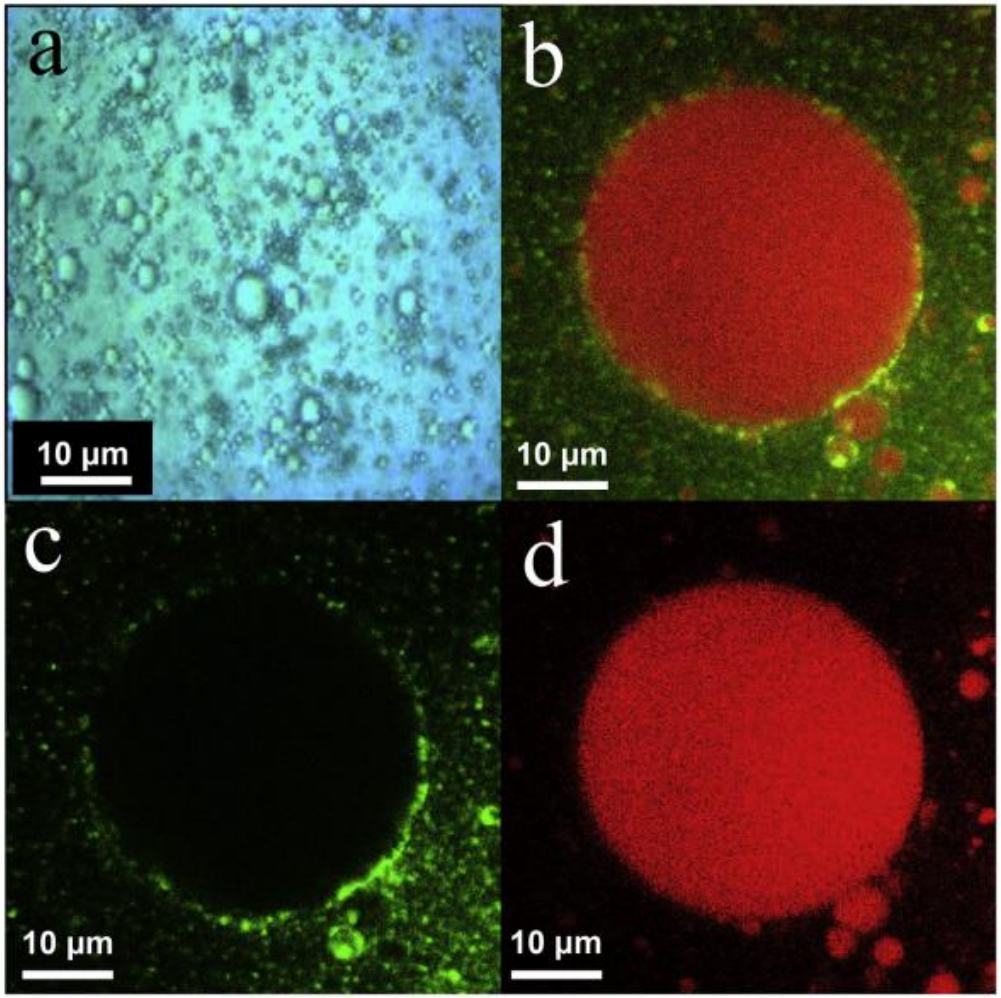
Optimal conditions regarding ESI:

ChiNP = 1.1% (w/w)

Lecithin= 4.4% (w/w)

O/W ratio= 0.24 (v/v). Source: Dammak & Sobral., (2018)

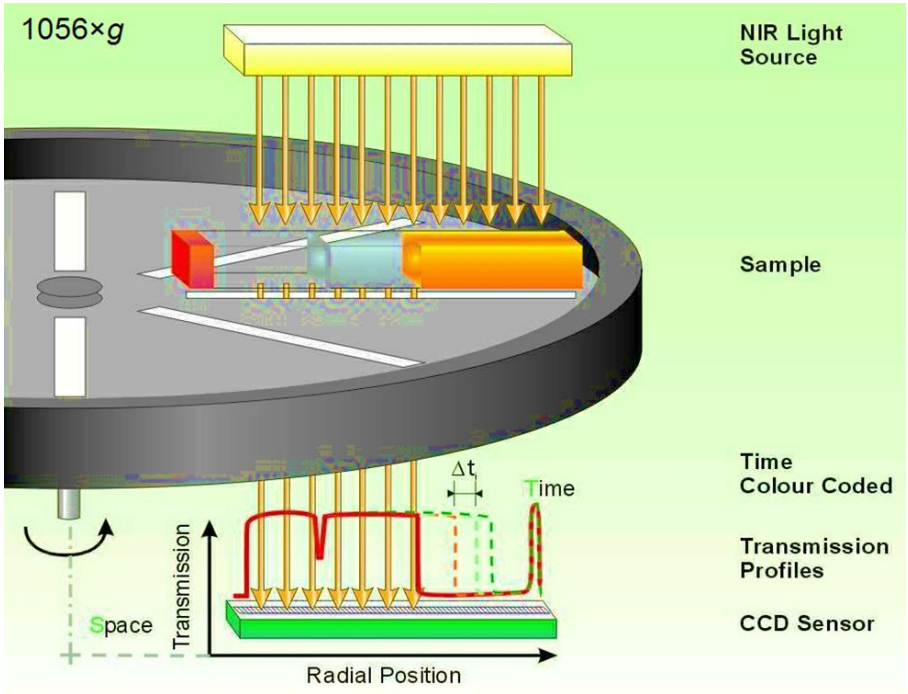
Pickering emulsions carrying hesperidin characteristics



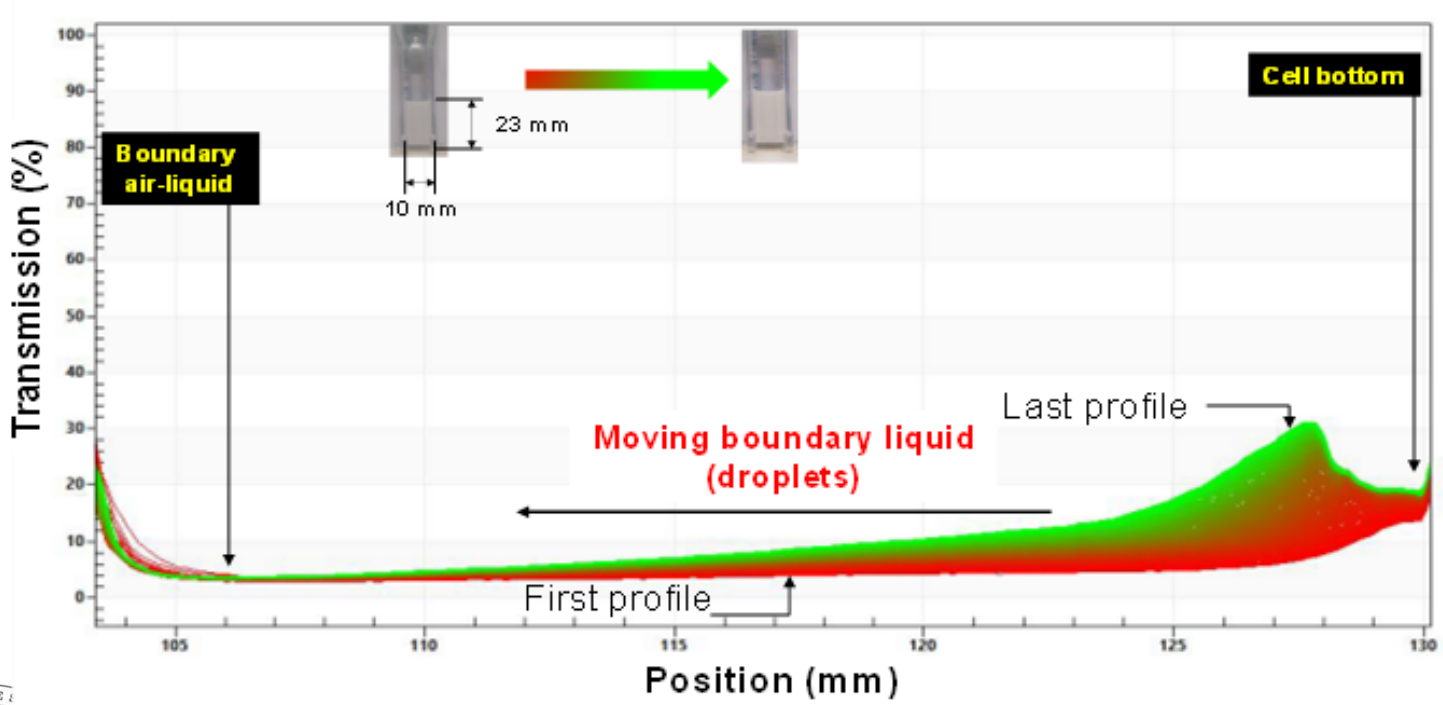
(a) Optical micrograph of emulsion droplets prepared under optimal conditions. (b) Confocal laser-scanning microscopy (CLSM) micrograph of emulsion droplets after labeling of oil and chitosan nanoparticles. (c) CLSM micrograph of labeled chitosan nanoparticles. (d) CLSM micrograph of labeled oil phase.

Source: Dammak & Sobral. (2018)

Physical stability of emulsions: Determined using a analytical centrifuge (Lumisizer®)



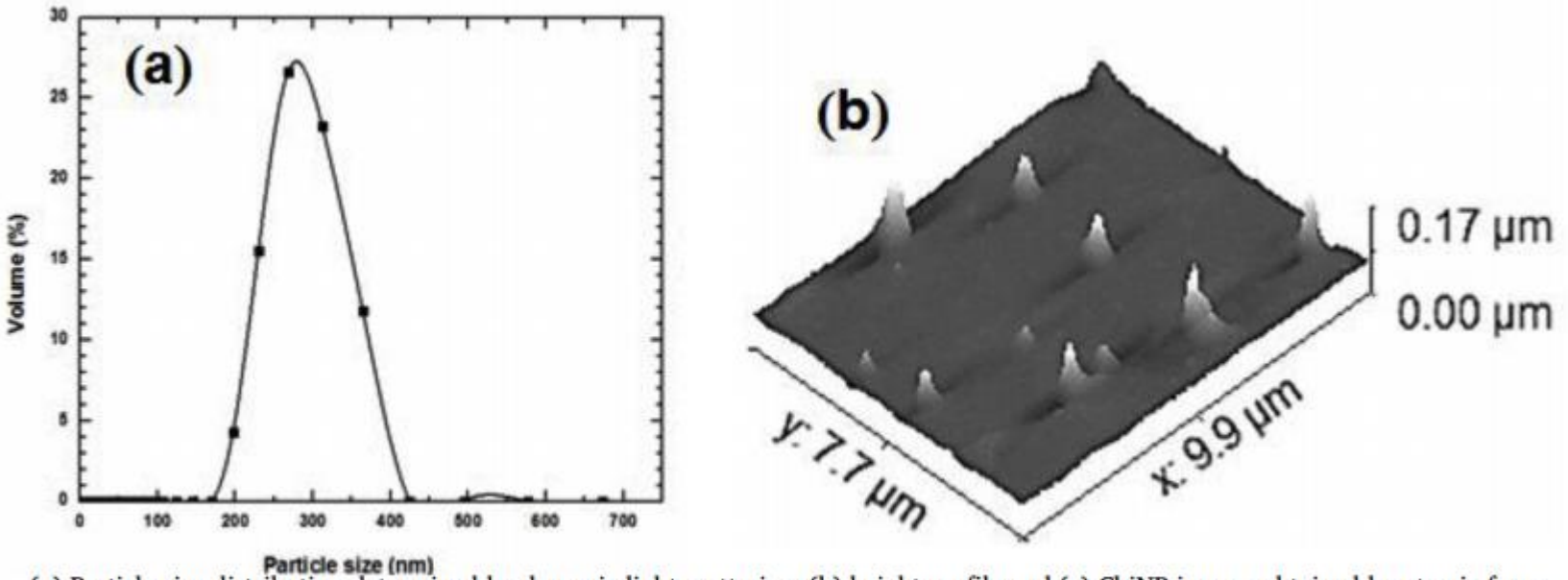
Physical stability of Pickering emulsions carrying hesperidin characteristics



Source: Dammak & Sobral. (2018)

And finally: Active film-Pickering emulsion

ChiNP characteristics

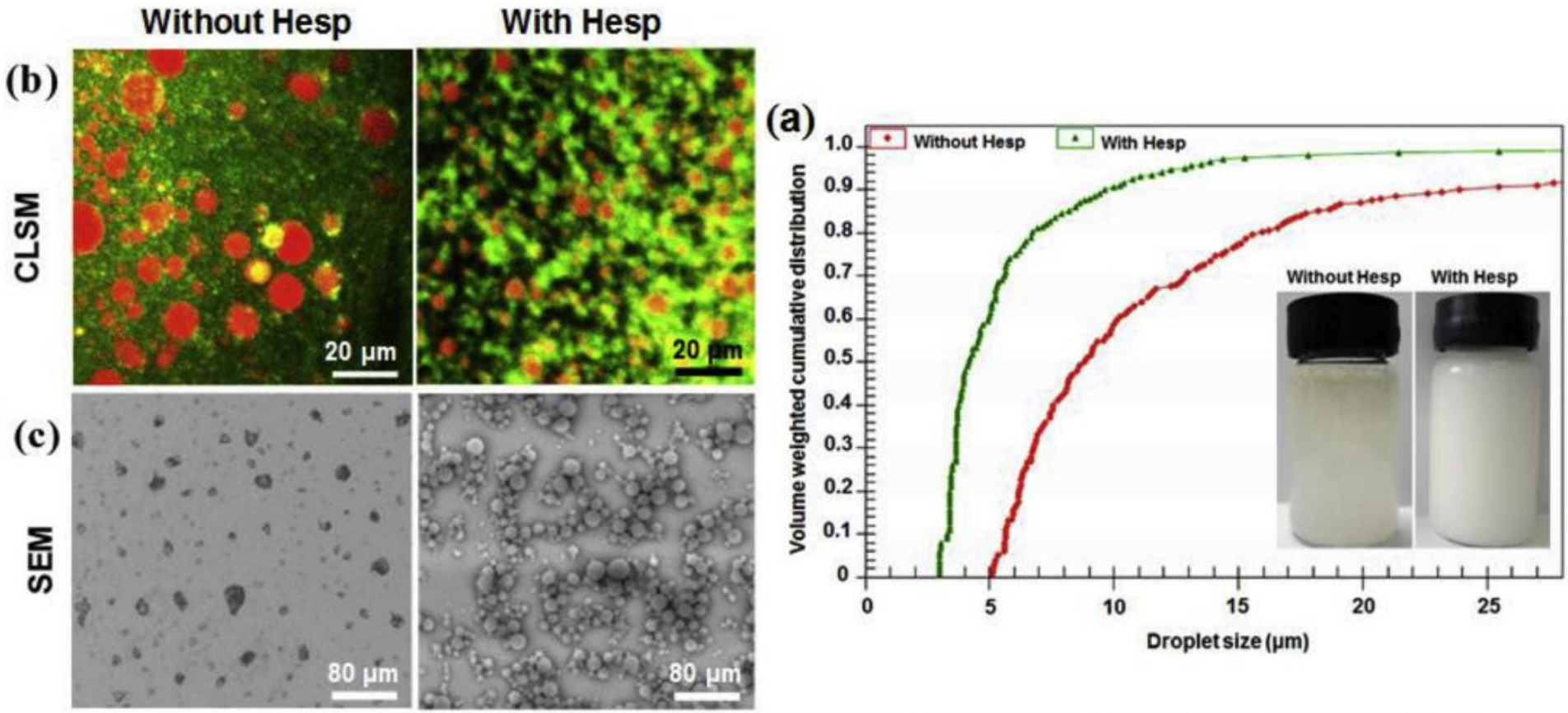


(a) Particle size distribution determined by dynamic light scattering; (b) height profile and (c) ChiNP images obtained by atomic force microscopy. Sample was diluted at 1:150 in distilled water.

d= 280±11 nm and Span= 0.4±0.1

Source: Dammak et al. (2019)

Pickering emulsions carrying hesperidin characteristics



(a) Appearance of the ChiNP-stabilized emulsions without and with Hesp after 2 h of storage and droplet size distribution, (b) CLSM micrographs, and (c) SEM images of ChiNP-stabilized Pickering emulsions.

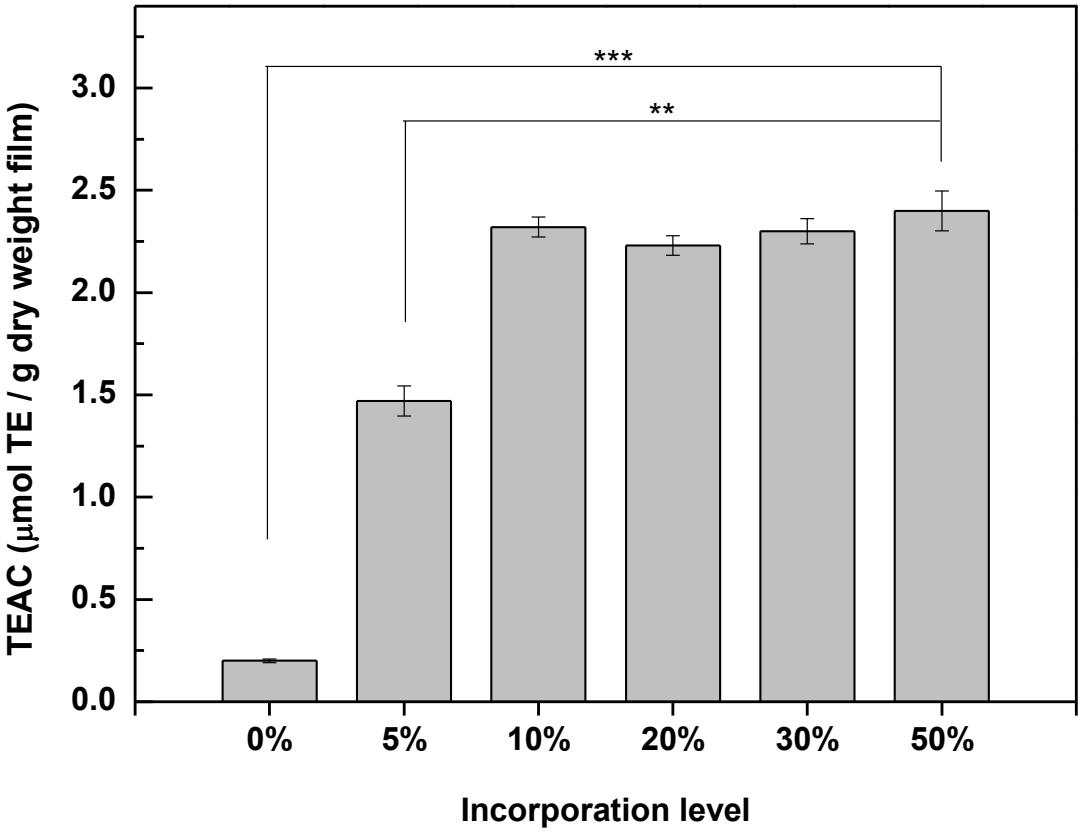
Source: Dammak et al. (2019)

The mean droplet size, polydispersity index and hesperidin retention of O/W Emulsions prepared with and without hesperidin at two homogenization speeds.

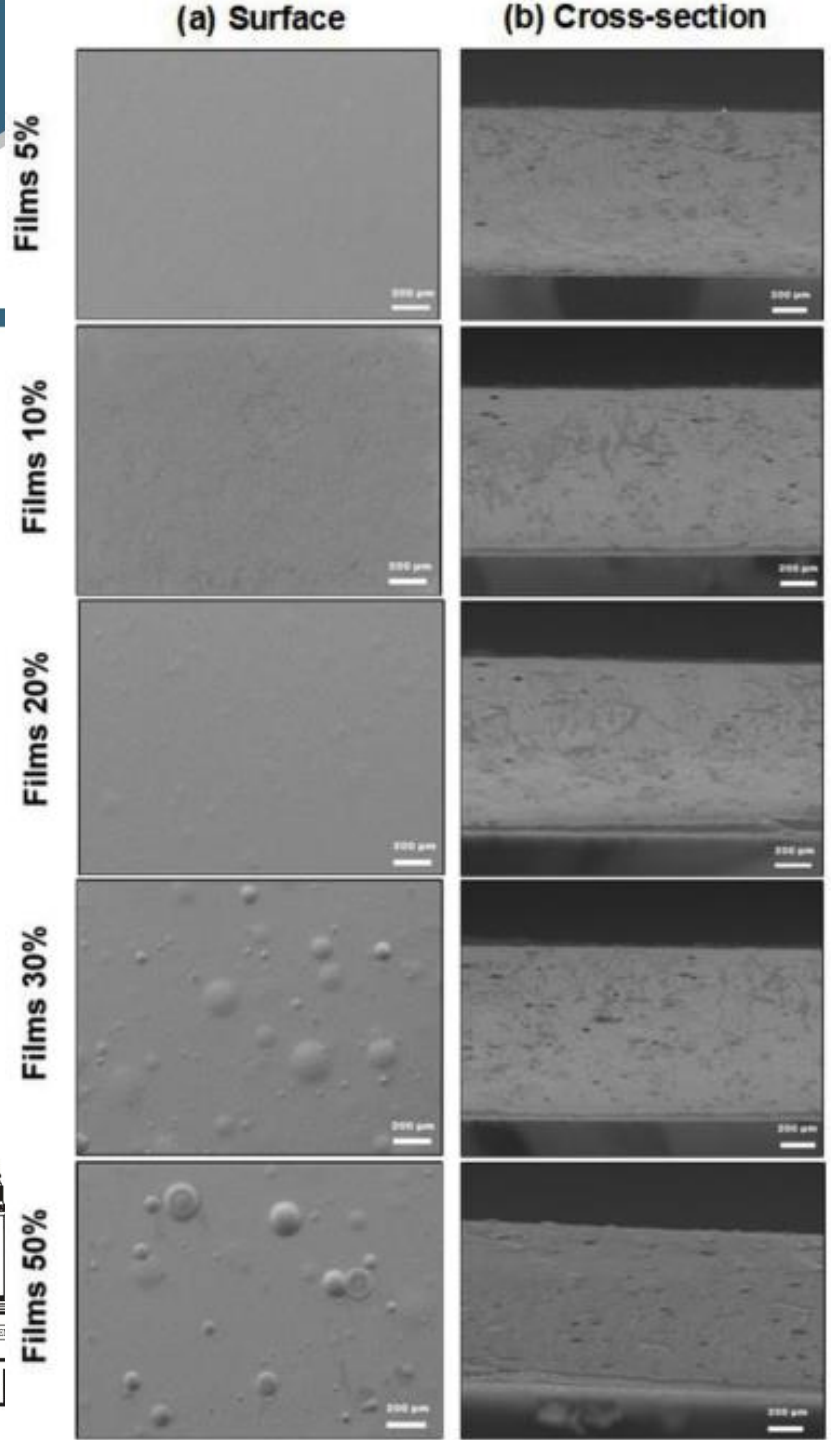
	Homogenization speed 5,000 rpm		Homogenization speed 10,000 rpm	
	Without hesperidin	With hesperidin	Without hesperidin	With hesperidin
Mean droplet size (μm)	**15.3 ± 1.2^{a}**	**8.3 ± 1.7^{c}**	**9.5 ± 2.0^{b}**	**5.4 ± 2.0^{c}**
Polydispersity index (-)	**0.7 ± 0.1^{a}**	**0.4 ± 0.3^{c}**	**0.5 ± 0.1^{b}**	**0.3 ± 0.1^{d}**
Hesperidin retention (mg/mL)	**-**	**0.81 ± 0.02^{a}**	**-**	**0.72 ± 0.02^{b}**

Mean ± standard deviations from triplicate determinations.
$^{a-d}$ Different letters in the same row indicate significant differences at $p < 0.05$.
Source: Dammak et al. (2019)

Active films characteristics



Source: Dammak et al. (2019)



(a) Top-surface and (b) cross-section SEM images of films incorporated with different levels of Pickering emulsions. Levels of Pickering emulsions are indicated in figures.

Source: Dammak et al., (2019)

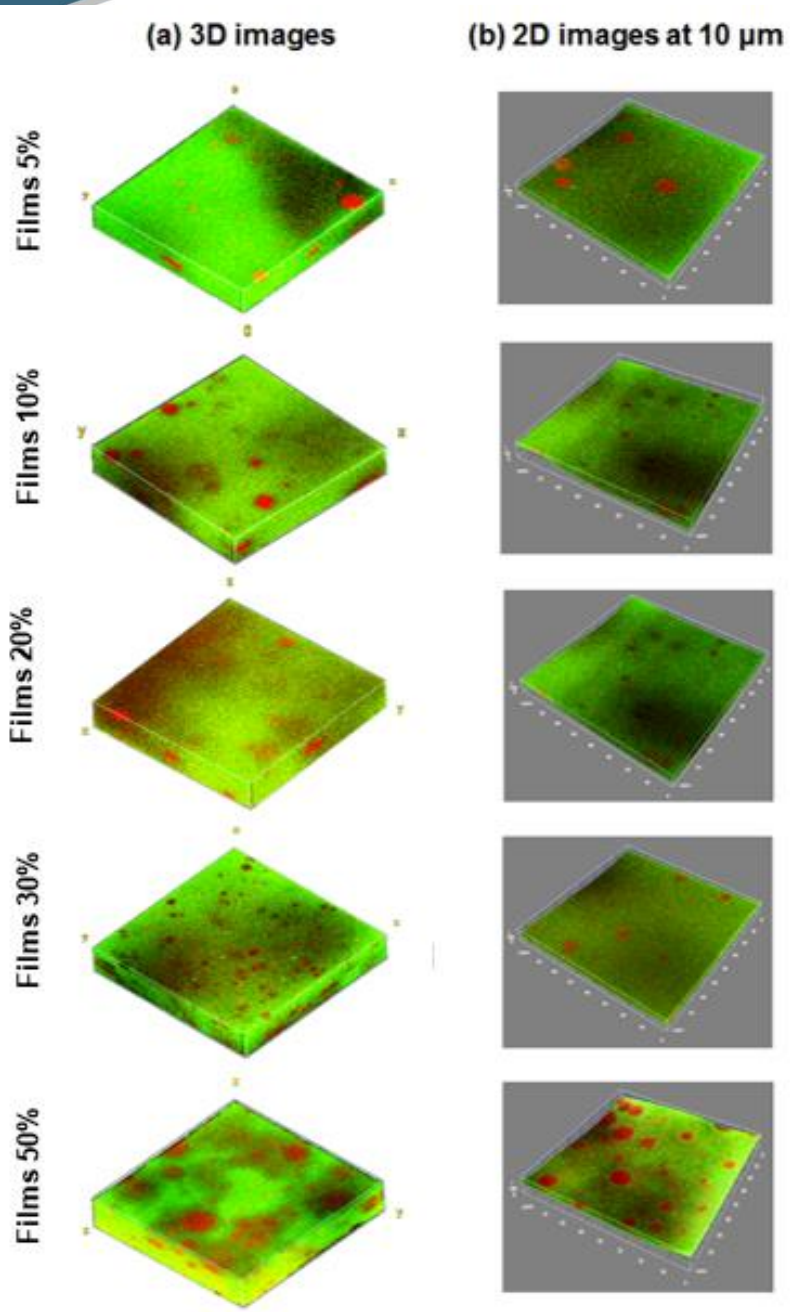


Figure. (a) 3D CLSM images of films with different levels of Pickering emulsions; (b) 2D CLSM images at a penetration depth of 10 µm of films with Pickering emulsions. Scale bar is of 20 µm.

Source: Dammak et al. (2019)

Calculated mean droplet size and polydispersity index of oil droplets incorporated into the active gelatin films.

Incorporation level (g oil/ 100 g gelatin)	Mean droplet size (μm)	Polydispersity index
5%	6.4 ± 2.2[a]	0.4 ± 0.02[a]
10%	7.2 ± 2.3[a]	0.3 ± 0.03[a]
20%	6.2 ± 1.8[a]	0.3 ± 0.04[a]
30%	7.2 ± 2.1[a]	0.4 ± 0.03[a]
50%	8.4 ± 2.5[a]	0.5 ± 0.05[a]

Mean ± standard deviations from triplicate determinations.
Different letters in the same column indicate significant differences at $p < 0.05$.

Source: Dammak et al. (2019)

Final Remarks

We worked a lot, but a lot of research still needs to be done to have a material with high potential for application in the food industry.

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References

Ours references can be viewed in our CV:

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Thank you very much

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