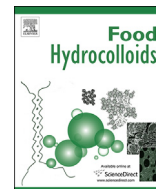




Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Green tea polyphenols- β -lactoglobulin nanocomplexes: Interfacial behavior, emulsification and oxidation stability of fish oil

Mariana von Staszewski, Victor M. Pizones Ruiz-Henestrosa, Ana M.R. Pilosof*

CONICET, Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 24 May 2013

Accepted 9 July 2013

Keywords:

Green tea polyphenols

 β -lactoglobulin

Emulsions

Antioxidant

Interfacial properties

ABSTRACT

Green tea polyphenols have a good antioxidant capacity but poor interfacial activity. β -lactoglobulin (β -lg) was used as an emulsifier agent and also as a carrier molecule by spontaneous nanocomplexes formation with green tea polyphenols. Oil-in-water emulsions containing liver fish oil rich in ω -3 fatty acids were formulated using these nanocomplexes at pH 6. The interfacial behavior of these complexes showed that both surface pressure and dilatational properties decreased as compared with pure β -lg. However, the initial droplet size and stability of emulsions were improved in the presence of the nanocomplexes. Moreover, the oxidative stability of liver fish oil was improved by the presence of polyphenols.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

There is a strong demand of natural bioactive ingredients with health benefits. The beneficial effects of long chain ω -3 polyunsaturated fatty acids (LC ω -3 PUFA), in particular of eicosapentaenoic acid (EPA, 20:5 ω -3) and of docosahexaenoic acid (DHA, 22:6 ω -3) which are abundant in fish oil, are well documented for a wide range of benefits including cardiovascular health (Abeywardena & Head, 2001; McLennan & Abeywardena, 2005; Weitz, Weintraub, Fisher, & Schwartzbard, 2010). However, the incorporation of these types of bioactives can also bring about a challenge since PUFA degradation through auto-oxidation during processing and storage, easily leads to rancidity volatiles formation (Flick & Martin, 1992; Hsieh & Kinsella, 1989; Sanguansri & Augustin, 2006; Sun, Wang, Chen, & Li, 2011). Rancidity flavors still constitute one of the main objections in the production and commercialization of fish oil containing food products (Jacobsen et al., 1999).

The use of natural antioxidant agents instead of synthetic ones is an effective and healthier methodology to control rancidity in oils and food. Green tea polyphenols are known as antioxidant agents (Dreosti, 2000) and their activity depends on the type of system into which they are incorporated (aqueous, oil or emulsified

systems). For example, polyphenols localization and concentration in different regions of a multiphase system will depend on their polarity, solubility and affinity for structural constituents (micelles, liposomes, etc.) (Gramza & Korczak, 2005). Regarding the antioxidant activity of green tea polyphenols in food matrices, they have been studied in meat, fish and vegetable oils (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2001). These food systems are very sensitive to lipid oxidation and polyphenols have shown higher antioxidant capacity than other synthetic and natural antioxidants used in the food industry like BHA, BHT, α -tocopherol, vitamin E and other vegetable extracts (Yilmaz, 2006). Additionally, polyphenols are receiving more and more attention because of their beneficial functions to human bodies. Thus, it is becoming popular to design food products containing polyphenols as functional foods.

Polyphenols, besides their antioxidant protective properties, are also expected to affect both the dispersion degree and the physical stability of the dispersed phase because they can interact with proteins. The interactions between proteins and polyphenols have been extensively studied in solution (Charlton et al., 2002; Jöbstl, O'Connell, Fairclough, & Williamson, 2004; Kanakis et al., 2011; Lin, Chen, Cheng, & Chen, 2004; Poncet-Legrand et al., 2006; Richard, Lefeuvre, Descendit, Quideau, & Monti, 2006; Siebert, Troukhanova, & Lynn, 1996), but very few studies were carried out to understand the effect of polyphenols on the adsorption properties of proteins at the interfaces.

The aim of this study was to evaluate the effect of green tea polyphenols- β -lactoglobulin nanocomplexes on the interfacial and colloidal properties of liver fish oil-in-water emulsions. Also the

* Corresponding author. Tel.: +54 11 4576 3377; fax: +54 11 4576 3366.

E-mail addresses: apilosof@di.fcen.uba.ar, anirenata@yahoo.com.ar (A.M.R. Pilosof).

physical and oxidative stability of these emulsions were analyzed. β -lactoglobulin (β -lg) was chosen as emulsifier agent since its behavior in ultrasound prepared emulsions was characterized in a previous work (Camino, Sanchez, Rodríguez Patino, & Pilosof, 2012). A pH of 6.0 was selected to obtain the smallest and most stable nanocomplexes between β -lg and polyphenols (von Staszewski et al., 2012).

2. Materials and methods

2.1. Materials

BioPURE β -lactoglobulin was supplied by DAVISCO Foods International, Inc. (Le Sueur, Minnesota). Its composition was: protein (dry basis) 97.8% being β -lactoglobulin 93.6% of total proteins, fat 0.3%, ash 1.8% and moisture 5.0%.

Green tea extract powder (Sunphenon® 90MD) from Taiyo International, Inc. (Minneapolis, Minnesota) contained >95% total polyphenols, >75% total catechins, >45% Epigallocatechin gallate (EGCG) and <6.0% caffeine.

Liver oil from Argentinean fish was kindly donated by South Oils (Mar del Plata, Argentina) and according to the supplier it has 75.53% (w/w) of fatty acids, being 44.8% polyunsaturated, 35.1% monounsaturated and 20.2% saturated. This oil has a high proportion of LC ω -3 PUFA (38.4%), with 23.4% of DHA and 7.7% of EPA (Modified AOCS Official Method Ce 1b-89). The oil was vacuum filtered through Whatman filter paper No. 41 (particle retention 20–25 μ m) prior to use. No further purification was used in order to study the performance of polyphenols- β -lg nanocomplexes in a real interface. To check differences in the interfacial behavior of nanocomplexes that could be attributed to the heterogeneous composition of fish oil, we proceeded as explained before (Camino et al., 2012). β -lg and impurities may compete for the interface, but β -lg dominates the surface pressure even at short adsorption times.

2.2. Methods

2.2.1. Particle size and Z-potential measurements

Particle size analysis experiments were carried out using a Dynamic Laser Light Scattering (DLS) instrument (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, UK) provided with a He-Ne laser (633 nm) and a digital correlator, Model ZEN3600. Measurements were carried out at a fixed scattering angle of 173°. Samples were contained in a disposable polystyrene cuvette.

The zeta potential of solutions and emulsions was measured using the laser Doppler velocimetry (LDV) technique (measurement range from 5 nm to 10 μ m). In this technique, a voltage was applied across a pair of electrodes placed at both ends of a cell containing the particle dispersion. Samples were diluted in their corresponding buffer before loading them in the cell and temperature was set at 25 °C. The assays were carried out in triplicate.

2.2.2. Dynamic interfacial properties

All the experiments were carried out in an automatic drop tensiometer at 25 °C by circulating water from a thermostat). A droplet was formed with the aqueous solution (constant volume at 12 mm³) at the tip of a capillary that was into a cell filled with the oil. Measurements were done until the adsorption equilibrium was reached (around 180 min). The glass materials in contact with the oil phase and the aqueous solutions were properly cleaned in order to avoid any contamination by surface-active substances.

2.2.2.1. Dynamic interfacial tension. Time-dependent surface pressure (π) of adsorbed β lg/polyphenols films were determined at the O/W interface with an automatic drop tensiometer PAT-1

(Sinterface Technologies, Berlin, Germany). The surface tension (γ) was calculated through the analysis of the droplet profile (Labourdenne et al., 1994). The surface pressure is $\pi = \gamma^\circ - \gamma$, where γ° is the sub-phase interfacial tension (24 mN/m) and γ the interfacial tension of solution at each time (θ). The average standard accuracy of the interfacial tension is roughly 0.1 mN/m and the reproducibility of the results, for at least two measurements, was better than 1%.

During the first step of the adsorption of the components, it can be obtained the diffusion rate constant by using a modified form of the Ward and Tordai equation (Equation (1)) (Pizones Ruiz-Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2008).

$$\pi = 2C_0kT \left(\frac{k_{\text{diff}}\theta}{3.14} \right)^{1/2} \quad (1)$$

where C_0 is the concentration in the aqueous phase, k is the Boltzmann constant, T is the absolute temperature, k_{diff} is the diffusion coefficient and θ is the time. A plot of π vs. $\theta^{1/2}$ should be linear if diffusion controls the adsorption process, and the slope would represent k_{diff} .

2.2.2.2. Surface dilatational properties. The surface viscoelastic parameters (surface dilatational modulus, E , and its elastic, E_d , and viscous, E_v , components), were measured as a function of time, at 3% of deformation amplitude of the drop volume ($\Delta A/A$) and 0.05 Hz of angular frequency (ω). Previously, the percentage area change has been determined to be in the linear region (data not shown). A sinusoidal perturbation was induced at the interface by injecting and extracting liquid into the drop. A Fourier transformation was performed so as to obtain the dilatational parameters of the interfacial film.

The surface dilatational modulus derived from the change in surface tension (dilatational stress), σ (Equation (2)), resulting from a small change in surface area (dilatational strain), A (Equation (3)), may be described by Equation (4) (Lucassen & Van Den Tempel, 1972):

$$\sigma = \sigma_0 \sin(\omega\theta + \delta) \quad (2)$$

$$A = A_0 \sin(\omega\theta) \quad (3)$$

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d \ln A} = E_d + iE_v \quad (4)$$

where σ_0 and A_0 are the stress and strain amplitudes, respectively, and δ is the phase angle between stress and strain.

The dilatational modulus is a complex quantity, which is composed of real and imaginary parts. The real part of the dilatational modulus or storage component is the dilatational elasticity, $E_d = |E|\cos \delta$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity $E_v = |E|\sin \delta$. The dilatational modulus, E , is a measure of the total unit material dilatational resistance to deformation (elastic + viscous).

2.2.3. Emulsion preparation

Green tea polyphenols (0.25–1% w/w)- β -lg (3% w/w) nanocomplexes were prepared by dissolving the proper amount of each powder in phosphate buffer (pH 6.0, 0.01 M) at room temperature and stirring for 30 min. If necessary, pH was adjusted with HCl or NaOH. The solutions were allowed to stand overnight at 4 °C to assure nanocomplexes formation.

The O/W emulsions were prepared at 10:90 ratio by applying ultrasounds for 20 min. An ultrasonic processor Vibra Cell VCX 750

model (Sonics & Materials Inc., Newtown, Connecticut, USA) at a frequency of 20 kHz and amplitude of 20% was used. A 13 mm (1/2 inch) high grade titanium alloy probe threaded to a 3 mm tapered microtip was used to sonicate 5 mL of sample in a 15 mL glass tube reactor that was glycerine-jacketed at 0.5 °C with a circulating constant temperature cooling bath (Polystat, Cole–Parmer). At this temperature, the heat produced during sonication was dissipated by keeping the temperature of the sample below 25 °C.

2.2.4. Droplet size distribution

The droplet size distribution of emulsions was measured by light scattering using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit (Malvern Instruments, Worcestershire, United Kingdom). The pump speed was set in 1000 rpm. The refractive index (RI) of the disperse phase (1.47) and its absorption parameter (0.001) was used. The RI of the interfacial layer was not taken into account because when considered, the change produced was less than 1%, since the thickness of an adsorbed layer is about 2–10 nm (McClements, 1999). Droplet size is reported as the volume-surface mean diameter or Sauter diameter ($D_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$) and the equivalent volume-mean diameter or De Broucker diameter ($D_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets of diameter d_i (Galazka, Dickinson, & Ledward, 1996; Gu, Decker, & McClements, 2005; Guzey, Kim, & McClements, 2004; Huang, Kakuda, & Cui, 2001; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). D_{32} provides a measure of the mean diameter where most of the particles fall in between (Gu et al., 2005). D_{43} is related with changes in particle size involving destabilization processes so it is more sensitive to fat droplet aggregation (Palazolo, 2006; Relkin & Sourdet, 2005) than D_{32} .

The specific surface area (SSA) and the polydispersity obtained by the software are also reported. The polydispersity is calculated as: $(D_{0.9} - D_{0.1})/D_{0.5}$ where 10, 50 and 90% of the oil volume in the emulsion is contained in droplets with diameters below or equal to $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$, respectively. The SSA is calculated using the diameter D_{32} (Carrera Sánchez & Rodríguez Patino, 2005; Cornec et al., 1998). The droplet sizes are reported as the average and standard deviation of ten readings made per sample.

The flocculation degree (FD) was calculated from the size parameters as Palazolo, Sorgentini, and Wagner (2005):

$$FD(\%) = [(D_{43} \text{ without SDS} - D_{43} \text{ with SDS}) / D_{43} \text{ with SDS}] \times 100 \quad (5)$$

2.2.5. Emulsion stability

The global stability of emulsions was determined through the use of a vertical scan analyzer Turbiscan MA 2000 (Formulation, Toulouse, France). This equipment allows the optical characterization of any type of dispersion (Mengual, Meunier, Cayré, Puech, & Snabre, 1999). The reading head is composed of a pulsed near-IR light source ($\lambda = 850$ nm) and two synchronous detectors. The transmission detector receives the light, which goes through the sample (0°), while the back-scattering detector receives the light back scattered by the sample (135°).

We proceeded as described in Camino and Pilosof (2011) for determining creaming and flocculation processes during 21 days of storage at ambient temperature (25 °C). The results were reported as the average and standard deviation of at least two samples.

2.2.6. Lipid hydroperoxides

Emulsions Lipid hydroperoxides were determined according to Shanta and Decker (1994) by mixing the emulsion (0.3 mL) with 1.5 mL of isooctane:2-propanol (2:1, v/v), vortexing three times for 30 s and centrifuging for 2 min at 1000 g. 200 μ L of the supernatant

were taken and 2.8 mL of a methanol:1-butanol (3:1, v/v) solution were added, followed by 15 μ L of ferrous iron solution (prepared by adding equal amounts of 0.132 M BaCl₂ and 0.144 M FeSO₄). After 20 min, the absorbance of the solutions was measured at 510 nm using a T70 UV/Vis Spectrometer (PG Instruments Ltd, Leicestershire, England). Hydroperoxides concentration was determined using a standard calibration curve prepared with hydrogen peroxide.

2.3. Statistical analysis

The data were statistically analyzed with the program Statgraphic 5.1 plus. All the measurements were conducted and reported as means \pm 95% confidence limits. Statistical analysis were performed using *t*-test and one-way analysis of variance (ANOVA) to identify which groups were significantly different from other groups ($P < 0.05$).

3. Results and discussion

3.1. β -lg-polyphenol interactions in solution

Dynamic light scattering, based on the Brownian motion of particles, is a good technique to reveal the existence of bio-molecules association (Lin et al., 2004) and in the present work was used to characterize the formation of nanocomplexes between β -lg and polyphenols. The volume size distribution of β -lg (0.3% w/w) at pH 6.0 is shown in Fig. 1. Pure polyphenols solutions can not be characterized by DLS because they are very small molecules (MW < 500) and form colored solutions. β -lg showed a monomodal distribution broadening from 3 to 15 nm and the maximum peak at 25 °C (6.5 nm) is consistent with β -lg dimer which is the predominant form at neutral pH (McKenzie & Sawyer, 1967). This is in accordance with values determined by Griffin and Griffin (1993), who found a hydrodynamic diameter, $d(H)$, of 6.19 nm for the β -lg dimer. However the population also included β -lg monomers, minor proteins of higher molecular weight/size present in the β -lg sample and β -lg structures larger than dimers.

The higher size particle distribution of the mixture of β -lg and polyphenols (0.1% w/w) indicates the binding of β -lg with polyphenols and the formation of nanocomplexes. The binding between polyphenols and proteins occurs at exposed hydrophobic and approximately planar side chains, suggesting that it is dominated

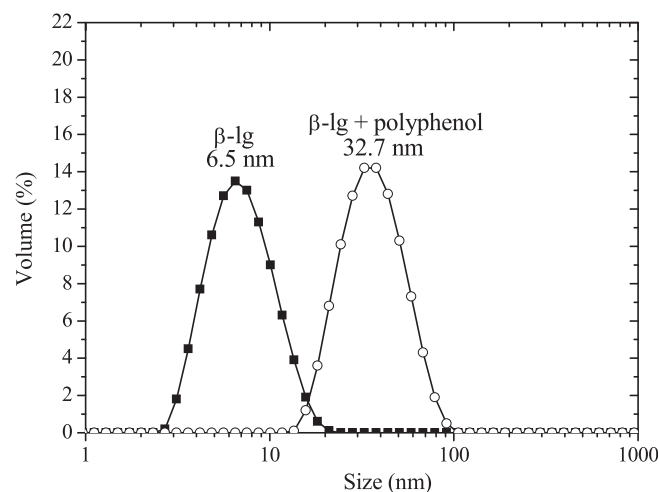


Fig. 1. Volume size distributions for β -lg (0.3% w/w) (■) and β -lg (0.3% w/w)-polyphenols (0.1% w/w) nanocomplexes (○) at 25 °C and pH 6.

by stacking of the polyphenolic rings against hydrophobic surfaces (Charlton et al., 2002).

3.2. β -lg-polyphenol complexes behavior at the oil-water interface: dynamic adsorption and viscoelasticity of films

Polyphenols- β -lg nanocomplexes would behave different from pure protein during their diffusion, penetration and rearrangement at the oil-water interface. Thus, the interfacial study of β -lg and polyphenols is of great importance since its direct impact on the emulsification properties.

Fig. 2 shows the interfacial pressure evolution with adsorption time for β -lg and its mixtures with polyphenols (polyphenols alone did not present surface activity). The evolution of interfacial pressure showed a rapid diffusion of the protein to the oil-water interface, due to the high protein concentration (3% w/w), high enough to saturate the interface. In all cases surface activity was lowered by increasing polyphenols concentration. One possible explanation has to be with the lower diffusion coefficients of nanoparticles at increasing polyphenols concentration (19.4, 18.0, 16.2 and 10.0 $\text{mN m}^{-1} \text{s}^{-0.5}$ for 0, 0.25, 0.5 and 1% w/w polyphenols, respectively). These k_{diff} values were obtained by applying the Equation (1) that describes the change in interfacial pressure with time during the first step of the adsorption. As the diffusion step was very fast ($\pi > 10 \text{ mN/m}$ at the beginning of the experiment), k_{diff} were estimated from the first value in the π vs. $t^{1/2}$ plot as indicated by Martinez, Carrera Sanchez, Rodríguez Patino, and Pilosof (2009). Thus, it can be assumed that the change in the adsorption kinetics has its origin in the diffusion of components to oil-water interface. Additionally, at longer times (once the equilibrium was reached and the adsorbed components are organized at the interface), the values of the interfacial pressure of β -lg-polyphenols nano-complexes were lower than those of pure protein. As stated previously, polyphenols get stacked to hydrophobic side chains of the aminoacids in such a way that these hydrophobic domains could not be fully available to penetrate the interface. Sausse, Aguié-Béghin, and Douillard (2003) also observed that polyphenols prevent the adsorption of a fraction of total casein and suggested that polyphenols generate an energy barrier to the adsorption process. Generally, the protein conformational change during adsorption leads to a decrease in the protein superficial energy. The interactions between protein and polyphenols may reduce the protein flexibility and prevent the necessary protein conformational change to insert in the interface.

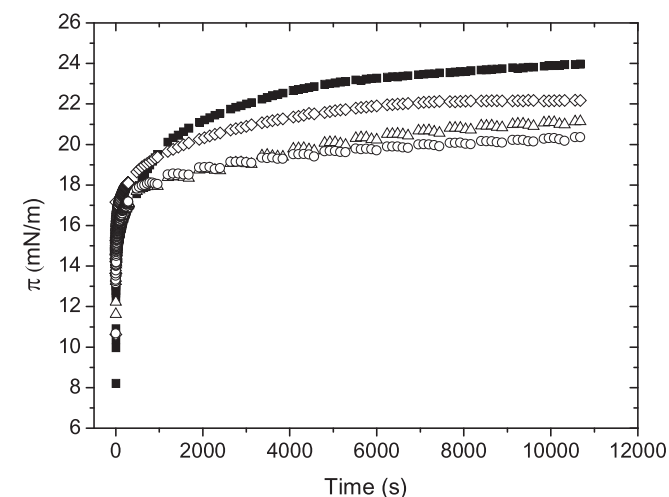


Fig. 2. Interfacial pressure as a function of adsorption time for β -lg 3% (■), β -lg 3%-polyphenol 0.25% (◇), β -lg 3%-polyphenol 0.5% (Δ) and β -lg 3%-polyphenol 1% (○).

The surface rheology of β -lg film was also sensitive to the presence of polyphenols. Fig. 3 shows the evolution of the dilatational elasticity E_d (mN/m) of interfacial films with time. It was observed that this parameter increased over time, which is consistent with an increment in the viscoelastic character of these films as the number of interactions between the adsorbed molecules increase (Dickinson, 1999; Pizones Ruiz-Henestrosa et al., 2008). Also the amount of adsorbed molecules increases with time and the rearrangement of these components at the interface lead to a higher interfacial density and thus to an E_d increment. The presence of polyphenols induced a decrease of E_d values as compared to single β -lg films, indicating an antagonistic effect regarding the elasticity of the films. The formation of β -lg-polyphenols nano-complexes would not contribute to a good developing of a viscoelastic film with good rheological properties because the polyphenols stacked to the hydrophobic side chains of the aminoacids, as indicated previously. Also, as previously suggested by Wüstneck, Moser, and Muschliok (1999) and Benjamins, Cagna, and Lucassen-Reynders (1996), the best conditions to form an interfacial structure with high mechanical stability are those in which protein conformational changes are not restricted. The intra and intermolecular hydrophobic interactions between proteins, needed to form an elastic film, would be restricted by the binding of polyphenols.

3.3. Emulsification of liver fish oil by β -lg-polyphenol nanocomplexes

3.3.1. Emulsion formation

Fig. 4 shows the monomodal droplet size distribution for O/W emulsions, formed by high intensity ultrasound, with increasing polyphenols concentration. The presence of polyphenols decreased the droplet size of the emulsions, which is beneficial for emulsions stability. At all polyphenol concentrations tested smaller droplet sizes were obtained as compared with β -lg alone, being the 0.5% (w/w) the optimum concentration.

Table 2 shows the average diameters, the polydispersity and the specific surface area (SSA) for recently prepared β -lg (3% w/w)-polyphenols (0.25–1% w/w) emulsions. Smaller average diameters are obtained in the presence of polyphenols. The SSA was higher when the values of D_{32} were smaller due to the smaller the oil droplets are for the same total oil volume, the total interfacial area will rise directly (Carrera Sánchez, & Rodríguez Patino, 2008).

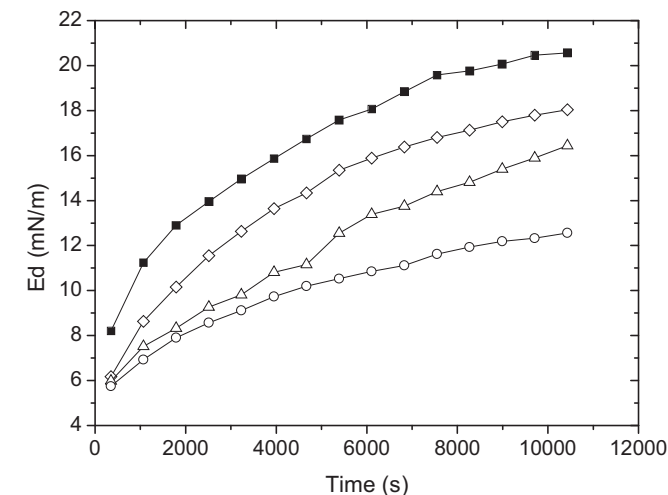


Fig. 3. Dilatational elastic modulus evolution, E_d , at pH 6 for β -lg 3% (■), β -lg 3%-polyphenol 0.25% (◇), β -lg 3%-polyphenol 0.5% (Δ) and β -lg 3%-polyphenol 1% (○).

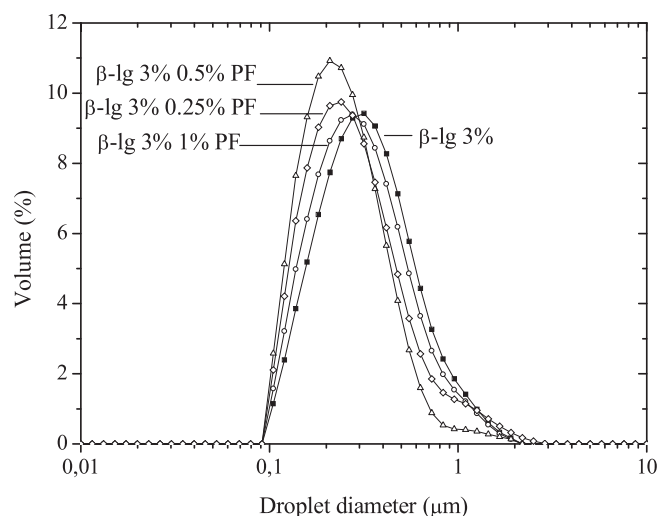


Fig. 4. Volume droplet size distribution for O/W emulsions formulated with β -lg 3% (■), β -lg 3%-polyphenol 0.25% (◇), β -lg 3%-polyphenol 0.5% (Δ) and β -lg 3%-polyphenol 1% (○). PF: polyphenols.

D_{43} diameters, related with possible flocculation, and the polydispersity were lower in the presence of polyphenols.

3.3.2. Creaming, flocculation and coalescence of emulsions

The creaming, flocculation and coalescence stabilities were analyzed from the back-scattering (BS%) profiles during the stationary storage at ambient temperature. Fig. 5 shows as an example the BS% profiles for a β -lg (3% w/w)-polyphenols (0.5% w/w) emulsion over time. It can be observed the clarification phenomenon in the bottom of the tube and the creaming at the top. The creaming/flocculation kinetics constants (K_{creaming}) obtained from the backscattering profiles for the emulsions are included in Table 3. It can be observed that the creaming rate decreased with the increment of polyphenols concentration up to an optimal level of 0.5% (w/w). Exceeded this value, creaming rate becomes equal to those emulsions with single β -lg. This behavior takes correlation with the initial droplet size of emulsion (Table 1).

Also, it is evident that the particle size does not change over time as can be observed in the middle part of the tube where lines are superimposed. If there was a change in droplet size, which would indicate a coalescence phenomenon, then a gradual decrease would be observed in the backscattering towards the increase in the droplet size. Also, the coalescence could not be evidenced through the increase in the D_{32} diameter during storage at ambient temperature (Table 2), related with the average size of all the particles in the emulsion (Gu et al., 2005). The low variation of D_{32} at all polyphenol concentrations during 20 days of storage, confirms the absence of coalescence and aware creaming to a flocculation process. When oil-in-water emulsions are prepared with proteins as unique emulsifying agent, coalescence becomes a slow destabilization mechanism compared with flocculation and

Table 1

Size distribution parameters (average diameter of Sauter (D_{32}), of De Broucker (D_{43}), polydispersity and specific surface area (SSA)) for β -lg (3% w/w)-polyphenols (0.25–1% w/w) emulsions. Maximum standard deviation: 1%.

	0%	0.25%	0.5%	1%
D_{32} (μm)	0.303	0.255	0.226	0.233
D_{43} (μm)	0.418	0.338	0.282	0.301
Polydispersity	1.710	1.556	1.293	1.356
SSA (m ² /g)	19.8	23.5	26.5	25.8

Table 2

Average diameters (D_{32}) for β -lg (3% w/w)-polyphenols (0.25–1% w/w) emulsions during storage at ambient temperature. Maximum standard deviation: 1%.

Polyphenol (% w/w)	Recently prepared	Day 7	Day 14	Day 21
0	0.309	0.308	0.307	0.308
0.25	0.281	0.281	0.281	0.280
0.5	0.237	0.239	0.239	0.238
1	0.260	0.260	0.259	0.262

creaming (Britten & Giroux, 1991). This is favored by the repulsion between oil droplets making slower the general destabilization process. The flocculation degree (Equation (5)) decreased with polyphenols possibly due to an increase in the net charge of the emulsified oil droplets, which repel each other (Table 3).

No relationship can be observed between the interfacial behavior and the emulsion properties since polyphenols decreased the surface pressure and viscoelasticity of the films but, on the other hand contributed to better emulsions. Besides interfacial properties, the viscosity of emulsions also plays an important role in the stability (Asano & Sotoyama, 1999). Nevertheless, the determined viscosity could neither explain the emulsion performance of polyphenols- β -lg nanocomplexes. It may be concluded that the increased emulsion stability may be attributed to charge effects of the nano-complexes as shown in Table 3.

3.4. Lipid oxidation

Oxidation of oils is a major cause of their deterioration, and hydroperoxides formed by the reaction between oxygen and the unsaturated fatty acids are the primary products of this reaction. The hydroperoxide concentrations of the fish oil-in-water emulsions stabilized with polyphenols- β -lg nanocomplexes were measured during storage at 37 °C (Fig. 6). The concentration of hydroperoxides of fish oil emulsions increased from 20 to 214 meq/kg oil in the emulsion stabilized by single β -lg. On the other hand, emulsions formed with polyphenols- β -lg nanocomplexes never exceeded 40 meq/kg oil of hydroperoxides during the whole storage, proving their outstanding performance as antioxidants even in combination with a protein. The relative low hydroperoxide level in the emulsions containing polyphenols would therefore indicate that fish oil was relatively stable to oxidation during storage at

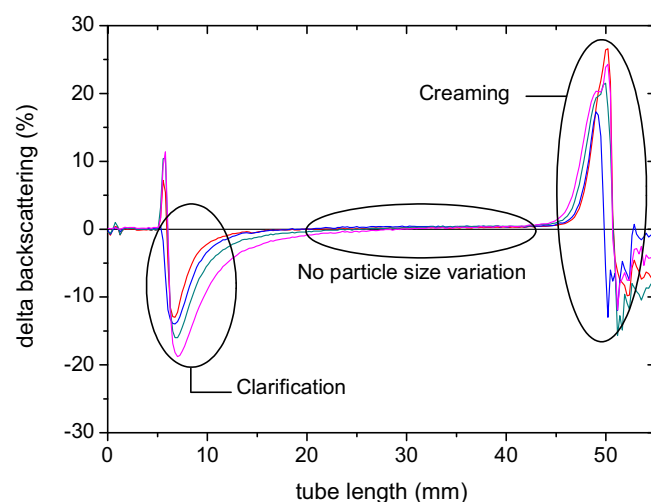


Fig. 5. Changes in back-scattering profiles (BS%) as a function of sample height with storage time (samples were stored for 20 days) of β -lg 3%-polyphenol 0.5% emulsions at pH 6. The creaming kinetics was analyzed in the 7–20 mm zone.

Table 3

Stability parameters of O/W for β -lg (3% w/w)-polyphenols (0.25–1% w/w) O/W emulsions. Maximum standard deviation: 5%.

	0%	0.25%	0.5%	1%
Z-potential (mV)	–59.6	–62.9	–67.9	–68.4
Viscosity (mPa/s)	1.326	1.355	1.356	1.335
K_{creaming} (mm/h)	0.0105	0.0087	0.0068	0.0105

37 °C. Similar results were obtained by [Almajano, Delgado, and Gordon \(2007\)](#), when assessing mixtures of green tea catechins and albumin as antioxidant systems in sunflower oil-in-water emulsions.

It has been reported that predominantly nonpolar antioxidants, such as α -tocopherol, ascorbyl palmitate, and carnosol, were found to be more effective in oil-in-water emulsions than in bulk oil, while the opposite has been observed for predominantly polar antioxidants, such as trolox, ascorbic acid, carnosic acid, and rosmarinic acid ([Porter, 1993](#)). According to this polar paradox, polyphenols may not be effective antioxidants in an oil-in-water emulsion. However, [Pazos, Gallardo, Torres, and Medina \(2005\)](#) also observed that grape polyphenols behaved as effective antioxidants in fish oil-in-water emulsions and related their effectiveness to the capacity of polyphenols to establish hydrophobic and hydrophilic interactions. They have hydrophobic cores with hydrophilic hydroxyl groups and may expose both or any of the two regions, resulting together with the protein in surfactant-like accumulation active oil-water interfaces.

The interfacial region, which is the contact region between the dispersed lipids and the aqueous phase, represents a critical area for oxidation development. Thus, the combination of β -lg and polyphenols would be beneficial since the antioxidants are trapped and located in the interface. The distribution in the emulsified system of surface-active compounds (emulsifiers, polar lipid oxidation products, amphiphilic antioxidants) that adsorb at the oil/water interface influence noticeably lipid oxidation ([Genot, Meynier, & Riaublanc, 2003](#); [Waraho, McClements, & Decker, 2011](#)). On the other hand, some authors ([Berton, Genot, Guibert, & Ropers, 2012](#); [Berton, Genot, & Ropers, 2011](#); [Berton, Ropers, Bertrand, Viau, & Genot, 2012](#)) reported that the interfaces covered by proteins offered worse protection of lipid droplets against oxidation than interfaces covered by a single layer of surfactants because the protein-covered interfaces exhibit a limited effective coverage and are more heterogenous and thus give access

for the aqueous soluble initiators or free radicals, to the lipid phase. Polyphenols may also help to avoid this problem by acting as cross-linkers between β -lg molecules and forming an interfacial film that assures the best oxidative stability since nanocomplexes can act as a physical barrier to prevent prooxidant penetrating and diffusing.

4. Conclusions

Green tea polyphenols- β -lg nanocomplexes could be used as efficient emulsifiers in liver fish oil emulsions with the additional benefit of acting as antioxidants and bioactive compounds. Future studies will be focused in understanding the behavior of these systems under physiological conditions as it has been reported in the literature that green tea may modify emulsion behavior of dietary fat in the gastrointestinal tract (changes in fat digestion and absorption) ([Shishikura, Khokhar, & Murray, 2006](#)).

Acknowledgments

This research was supported by Universidad de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica and Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

References

- Abeyswardena, M. Y., & Head, R. J. (2001). Long chain n-3 polyunsaturated fatty acids and blood vessel function. *Cardiovascular Research*, 52(3), 361–371.
- Almajano, M. P., Delgado, M. E., & Gordon, M. H. (2007). Albumin causes a synergistic increase in the antioxidant activity of green tea catechins in oil-in-water emulsions. *Food Chemistry*, 102, 1375–1382.
- Asano, Y., & Sotoyama, K. (1999). Viscosity change in oil/water food emulsions prepared using a membrane emulsification system. *Food Chemistry*, 66, 327–331.
- Benjamins, J., Cagna, A., & Lucassen-Reynders, E. H. (1996). Viscoelastic properties of triacylglycerol/water interfaces covered by proteins. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 114, 245–254.
- Berton, C., Genot, C., Guibert, D., & Ropers, M. H. (2012). Effect of lateral heterogeneity in mixed surfactant-stabilized interfaces on the oxidation of unsaturated lipids in oil-in-water emulsions. *Journal of Colloid and Interface Science*, 377, 244–250.
- Berton, C., Genot, C., & Ropers, M. H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. *Journal of Colloid and Interface Science*, 354, 739–748.
- Berton, C., Ropers, M. H., Bertrand, D., Viau, M., & Genot, C. (2012). Oxidative stability of oil-in-water emulsions stabilised with protein or surfactant emulsifiers in various oxidation conditions. *Food Chemistry*, 131, 1360–1369.
- Britten, M., & Giroux, H. J. (1991). Coalescence index of protein-stabilised emulsions. *Journal of Food Science*, 56, 792–795.
- Camino, N. A., & Pilosof, A. M. R. (2011). Hydroxypropylmethylcellulose at the oil-water interface. Part II. Submicron emulsions as affected by pH. *Food Hydrocolloids*, 25, 1051–1062.
- Camino, N. A., Sanchez, C. C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2012). Hydroxypropylmethylcellulose- β -lactoglobulin mixtures at the oil-water interface. Bulk, interfacial and emulsification behavior as affected by pH. *Food Hydrocolloids*, 27(2), 464–474.
- Carrera Sánchez, C., & Rodríguez Patino, J. M. (2008). Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution. *Food Hydrocolloids*, 19(3), 407–416.
- Charlton, A. J., Baxter, N. J., Lokman Khan, M., Moir, A. J. G., Haslam, E., Davies, A. P., et al. (2002). Polyphenol/peptide binding and precipitation. *Journal of Agricultural and Food Chemistry*, 50, 1593–1601.
- Cornec, M., Wilde, P., Gunning, P., Mackie, A., Husband, F., Parker, M., et al. (1998). Emulsions stability as affected by competitive adsorption between an oil-soluble emulsifier and milk proteins at the interface. *Journal of Food Science*, 63, 39–43.
- Dickinson, E. (1999). Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces*, 15(2), 161–176.
- Dreosti, I. E. (2000). Antioxidant polyphenols in tea, cocoa, and wine. *Nutrition*, 16, 692–694.
- Flick, G. F., & Martin, J. (1992). *Lipid oxidation in food advances in seafood biochemistry*. Technomic Publishing Company.
- Galazka, V. B., Dickinson, E., & Ledward, D. A. (1996). Effect of high pressure on the emulsifying behaviour of β -lactoglobulin. *Food Hydrocolloids*, 10, 213–219.
- Genot, C., Meynier, A., & Riaublanc, A. (2003). Lipid oxidation in emulsions. In A. Kamal-Eldin (Ed.), *Lipid oxidation pathways* (pp. 190–244). Champaign: AOCS Press.

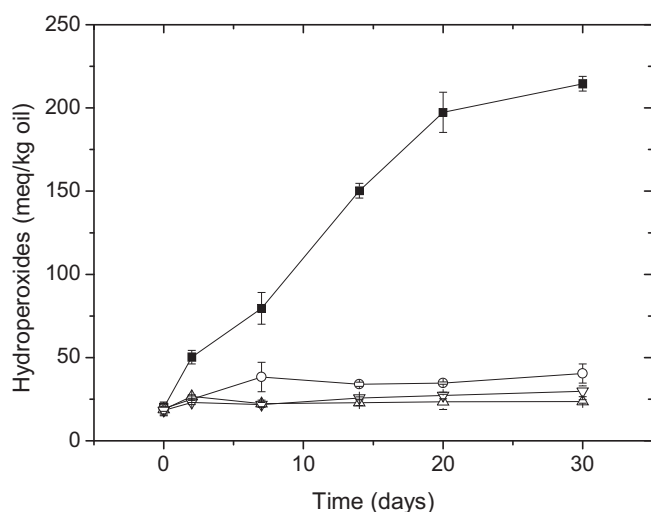


Fig. 6. Lipid hydroperoxides production in O/W emulsions formulated with β -lg 3% (■), β -lg 3%-polyphenol 0.25% (◇), β -lg 3%-polyphenol 0.5% (△) and β -lg 3%-polyphenol 1% (○).

- Gramza, A., & Korczak, J. (2005). Tea constituents (*Camellia sinensis* L.) as antioxidants in lipid systems. *Trends in Food Science & Technology*, 16(8), 351–358.
- Griffin, W. G., & Griffin, M. C. A. (1993). Time-dependent polydispersity of growing colloidal aggregates: predictions from dynamic light scattering. *Journal of the Chemical Society, Faraday Transactions*, 89, 2879–2889.
- Gu, Y., Decker, E., & McClements, D. J. (2005). Production and characterization of oil-water emulsions containing droplets stabilized by multilayer membranes consisting of β -lactoglobulin, κ -carrageenan and gelatin. *Langmuir*, 21, 5752–5760.
- Guzey, D., Kim, H. J., & McClements, D. J. (2004). Factors influencing the production of O/W emulsions stabilized by β -lactoglobulin-pectin membranes. *Food Hydrocolloids*, 18, 967–975.
- Hsieh, R., & Kinsella, J. (1989). Oxidation of polyunsaturated fatty acids: mechanisms, products and inhibition with emphasis on fish. *Advances in Food Research and Nutrition Research*, 33, 233–341.
- Huang, X., Kakuda, Y., & Cui, W. (2001). Hydrocolloids in emulsions: particle size distribution and the interfacial activity. *Food Hydrocolloids*, 15, 533–542.
- Jacobsen, C., Hartvigsen, K., Lund, P., Meyer, A. S., Adler-Nissen, J., & Holsborg, J. (1999). Oxidation in fish-oil-enriched mayonnaise. 1. Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis. *European Food Research and Technology*, 210, 13–30.
- Jöbstl, E., O'Connell, J., Fairclough, P. A., & Williamson, M. P. (2004). Astrigency – a molecular model for polyphenol/protein binding. *Fibre Diffraction Review*, 12, 66–69.
- Kanakis, C. D., Hasni, I., Bourassa, P., Tarantilis, P. A., Polissiou, M. G., & Tajmir-Riahi, H. A. (2011). Milk β -lactoglobulin complexes with tea polyphenols. *Food Chemistry*, 127, 1046–1055.
- Labourenne, S., Gaudry-Rolland, N., Letellier, S., Lin, M., Cagna, A., Esposito, G., et al. (1994). The oil-drop tensiometer: potential applications for studying the kinetics of (phospho)lipase action. *Chemistry and Physics of Lipids*, 71(2), 163–173.
- Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., & Mazoyer, J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids*, 17, 455–462.
- Lin, H.-C., Chen, P.-C., Cheng, T.-J., & Chen, R. L. C. (2004). Formation of tannin-albumin nano-particles at neutral pH as measured by light scattering techniques. *Analytical Biochemistry*, 325, 117–120.
- Lucassen, J., & Van Den Tempel, M. (1972). Dynamic measurements of dilational properties of a liquid interface. *Chemical Engineering Science*, 27(6), 1283–1291.
- Martinez, M. J., Carrera Sanchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2009). Bulk and interfacial behaviour of caseinoglycomacropeptide (GMP). *Colloids and Surfaces B: Biointerfaces*, 71, 230–237.
- McClements, D. J. (1999). Emulsion ingredients. In D. J. McClements (Ed.), *Food emulsions: Principles, practice and techniques* (pp. 83–124). Florida, USA: CRC Press.
- McKenzie, H. A., & Sawyer, W. H. (1967). Effect of pH on β -lactoglobulin. *Nature*, 214, 1101–1104.
- McLennan, P. L., & Abeywardena, M. Y. (2005). Membrane basis for fish oil effects on the heart: linking natural hibernators to prevention of human sudden cardiac death. *Journal of Membrane Biology*, 206(2), 85–102.
- Mengual, O., Meunier, G., Cayré, I., Puech, K., & Snabre, P. (1999). Turbiscan MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta*, 50, 445–456.
- Palazolo, G. (2006). *Formación y estabilidad de emulsiones O/W preparadas con proteínas nativas y desnaturizadas de soja*. vol. PhD thesis. Argentina: Universidad Nacional de La Plata.
- Palazolo, G. G., Sorgentini, D. A., & Wagner, J. R. (2005). Coalescence and flocculation in o/w emulsions of native and denatured whey soy proteins in comparison with soy protein isolates. *Food Hydrocolloids*, 19(3), 595–604.
- Pazos, M., Gallardo, J. M., Torres, J. L., & Medina, I. (2005). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chemistry*, 92, 547–557.
- Pizones Ruiz-Henestrosa, V., Carrera Sánchez, C., & Rodríguez Patino, J. M. (2008). Effect of sucrose on functional properties of soy globulins: adsorption and foam characteristics. *Journal of Agricultural and Food Chemistry*, 56, 2512–2521.
- Poncet-Legrand, C., Edelmann, A., Putaux, J. L., Cartalade, D., Sarni-Manchado, P., & Vernhet, A. (2006). Poly (L-proline) interactions with flavan-3-ols units: Influence of the molecular structure and the polyphenol/protein ratio. *Food Hydrocolloids*, 20, 687–697.
- Porter, W. L. (1993). Paradoxical behaviour of antioxidants in food and biological systems. In G. M. Williams (Ed.), *Antioxidants: Chemical, physiological, nutritional, and toxicological aspects* (pp. 93–122). Princeton, NJ, USA: Princeton Scientific.
- Relkin, P., & Sourdet, S. (2005). Factors affecting fat droplet aggregation in whipped frozen protein-stabilized emulsions. *Food Hydrocolloids*, 19, 503–511.
- Richard, T., Lefevre, D., Descendit, A., Quideau, S., & Monti, J. P. (2006). Recognition characters in peptide-polyphenol complex formation. *Biochimica et biophysica acta*, 1760, 951–958.
- Sanguansri, L., & Augustin, M. A. (2006). Microencapsulation and delivery of omega-3 fatty acids. In J. Shi (Ed.), *Functional food ingredients and nutraceuticals: Processing technologies* (pp. 297–327). CRC Press/Taylor and Francis Group.
- Sausse, P., Aguié-Béghin, V., & Douillard, R. (2003). Effects of epigallocatechin gallate on β -Casein adsorption at the air/water interface. *Langmuir*, 19, 737–743.
- Shanta, N. C., & Decker, E. (1994). Rapid, sensitive, iron based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International*, 77(2), 421–425.
- Shishikura, Y., Khokhar, S., & Murray, B. S. (2006). Effects of tea polyphenols on emulsification of olive oil in a small intestine model system. *Journal of Agricultural and Food Chemistry*, 54, 1906–1913.
- Siebert, K. J., Troukhanova, N. V., & Lynn, P. Y. (1996). Nature of polyphenol-protein interactions. *Journal of Agricultural and Food Chemistry*, 44, 80–85.
- von Staszewski, M., Jara, F. L., Ruiz, A. L. T. G., Jagus, R. J., Carvalho, J. E., & Pilosof, A. M. R. (2012). Nanocomplex formation between β -lactoglobulin or caseinomacropeptide and green tea polyphenols: Impact on protein gelation and polyphenols antiproliferative activity. *Journal of Functional Foods*, 4(4), 800–809.
- Sun, Y., Wang, W., Chen, H., & Li, C. (2011). Autoxidation of unsaturated lipids in food emulsion. *Critical Reviews in Food Science and Nutrition*, 51, 453–466.
- Tang, S., Kerry, J. P., Sheehan, D., Buckley, D. J., & Morrissey, P. A. (2001). Antioxidant effect of added tea catechins on susceptibility of cooked red meat, poultry, and fish patties to lipid oxidation. *Food Research International*, 34, 651–657.
- Waraho, T., McClements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science & Technology*, 22(1), 3–13.
- Weitz, D., Weintraub, H., Fisher, E., & Schwartzbard, A. Z. (2010). Fish oil for the treatment of cardiovascular disease. *Cardiological Reviews*, 18, 258–263.
- Wüstneck, R., Moser, B., & Muschiolik, G. (1999). Interfacial dilational behaviour of adsorbed β -lactoglobulin layers at the different fluid interfaces. *Colloids and Surfaces B: Biointerfaces*, 15, 263–273.
- Yilmaz, Y. (2006). Novel uses of catechins in foods. *Trends in Food Science & Technology*, 17, 64–71.