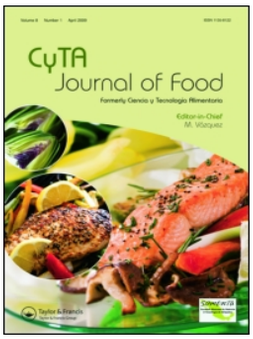

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Stability of Lutein Obtained from *Muriellopsis sp* biomass and used as a natural colorant and antioxidant in a mayonnaise-like dressing sauce

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ABSTRACT

Lutein is present in higher plants and algae. It may confer protection against the progression of chronic and eye diseases. Currently, lutein supplements are supplied to the world market in the form of capsules. We obtained a lutein oleoresin from the biomass of the microalgae *Muriellopsis sp*. This was added to a home-made mayonnaise. The samples were stored for three months at 5 ± 2°C. The lutein content was quantified by high-performance liquid chromatography (HPLC), and the antioxidant concentration was determined by the total polyphenol content (Folin-Ciocalteau) and the oxygen radical absorbance capacity (ORAC). Pigment degradation followed a first-order kinetics, with k = 0.0068 days-1, and t½ = 102 days. The chromatic coordinates L*, a*, b* indicated high pigment stability in the matrix. These results indicate that, due to its high antioxidant capacity, lutein derived from the Muriellopsis sp could represent a potential substitute for pigments, such as β-carotene, in oily matrices.

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Sauces; lutein; antioxidant properties; HPLC; *Muriellopsis*sp

PALABRAS CLAVES
Aderezos; luteína; propiedades antioxidantes; HPLC; *Muriellopsis sp.*

Estabilidad de la Luteína obtenida desde la biomasa de *Muriellopsis sp*. y usada como colorante natural y antioxidante en un aderezo tipo mayonesa

RESUMEN

La luteína está presente en algas y plantas superiores. Confiere protección contra la progresión de enfermedades crónicas y oculares. Actualmente, suplementos de luteína se suministran al mercado mundial en forma de cápsulas. Obtuvimos una oleorresina de luteína desde la biomasa de la microalga *Muriellopsis sp*. la que se añadió a una mayonesa casera. Las muestras se almacenaron por tres meses a 5 ± 2°C. El contenido de luteína se cuantificó a través de cromatografía líquida de alta resolución (HPLC), y la concentración antioxidante se determinó por el contenido de Polifenoles Totales (Folin-Ciocalteau) y capacidad de absorción de radicales de oxígeno (ORAC). La degradación del pigmento siguió una cinética de primer orden, con k=0.0068 días^{-1} y t½=102 días. Las coordenadas cromáticas L*, a*, b* indican alta estabilidad del pimento en la matriz. Estos resultados indican que, debido a su alta capacidad antioxidante, la luteína proveniente de *Muriellopsis sp*. podría representar un potencial sustituto para pigmentos, tales como β-caroteno en matrices oleosas.

1. Introduction

Food coloring agents can be used to restore or intensify the original color of a food, correct natural variation, confer a particular identity to food or provide it with an attractive appearance (Aberoumand, 2011; Carocho & Ferreira, 2013; Parra, 2011). As such, they play a fundamental role in the preparation of food, either for technical reasons or to satisfy organoleptic requirements. This has stimulated research on food pigments, with a focus on toxicity, side effects and the replacement of artificial colors with natural dyes (Restrepo, 2007). Nowadays, consumers are more health conscious about what they eat and what goes into their foods. Consumers are increasingly demanding "clean labels" on food and beverage products, and the food industry has attempted to formulate new products from natural ingredients (Chareonthaikij, Uan-On, & Prinyawiwatkul, 2016), including flavouring and coloring agents (Nachay, 2016; Sukkwai, Chonpracha, Kijroongrojana, & Prinyawiwatkul, 2017).

Most of the natural compounds in commercial use are extracted from marine sources, such as algae and micro-algae (Blunt, Copp, Keyzers, Munro, & Prinsep, 2013; Borowitzka, 2013). In the microalga *Muriellopsis sp*. (class *Porphyridiophyceae*), lutein accounts for at least 65% of total carotenoids, which also include β-carotene and violaxanthin, making this species particularly rich in lutein and thus converting it into an important potential source of this pigment for commercial use (Meléndez-Martínez, Vicario, & Heredia, 2004).

Lutein ($C_{40}H_{56}O$) is a pigment of a yellow-orange color that cannot be produced by the human body and must therefore be obtained from food or supplements. With a recommended daily intake of 4 to 20 mg (Beatty, Nolan, Kavanagh, & O´Donovan, 2004) the consumption of lutein-fortified food and drinks are on the rise (Abdel-Aal, Akhtar, Zaheer, & Ali, 2013; Rocha et al., 2017). On the market, it comes in different presentations, including easily dissolvable powders or emulsions, which can be added to a range of foods, including beverages (Rocha et al., 2017), pasta, baked goods such as bread and

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pastries, and fruit jellies (Abdel-Aal, 2013). In addition, it is added to cakes and juices, and to dairy products such as yogurt and milk with varying fat contents (Parra, 2011). The advantage of using lutein as a dietary supplement is that it is a powerful antioxidant, as confirmed by a number of studies on the benefits of lutein for human health (Kijlstra, Tian, Kelly, & Berendschot, 2012; Monego, Barcellosda Rosa, & Cícero Do Nascimento, 2017) and a growing body of research supports the notion that its antioxidant capacity plays a protective role in delaying the development of chronic diseases (Fiedor & Burda, 2014; SanGiovanni & Neuringer, 2012). A novel study carried out by Asker and Awad (2019) isolated and identified a new marine microalgae class Trebouxiophyceae, using HTS and 18S rRNA techniques, which has the potential to produce a large amount of carotenoids, included lutein as the major component (~82.8%). In contrast to other lutein producers, this strain is potentially promising for the production of lutein and its future applications in Food Industry. Currently, this pigment is used in the manufacturing of drugs, cosmetics, natural dye (Jin, Polle, Lee, Hyun, & Chang, 2003) and as a food additive (e.g. for the pigmentation of egg yolks) (Harp & Barrows, 2015).

Various tests and methods have been developed and adapted to specifically assess the presence and activity of antioxidants in foodstuffs, nutraceuticals, dietary supplements, and biological fluids (Huang, Ou, & Prior, 2005). The ORAC assay measures the radical chain breaking ability of antioxidants by monitoring the inhibition of peroxyl radical-induced oxidation. Peroxyl radicals are the predominant free radicals found in lipid oxidation in foods and biological systems under physiological conditions. Hence, ORAC values are considered by some to be of biological relevance as a reference for antioxidant effectiveness.

In last years, some research have been developed based on research of food products elaborated with microalgae biomass, rich in polyunsaturated fatty acids and antioxidants effects (Batista et al., 2012). Diverse works expose studies about functional foods like pasta, biscuits and vegetarian mayonnaises (Batista et al., 2017).

Diverse factors influence the mayonnaise market: health, obesity issues, government regulations and retail sector growth (International Markets Bureau, 2013). Healthy reductions of fats, oils, sugar, carbohydrates, and additions of healthy ingredients will have a positive impact on the growth of the mayonnaise market. For this reason, the mayonnaise manufacturers seek to develop more healthy products for the consumers (Bakhtavoryan, R. (2017). Brand-Level Demand Analysis of Mayonnaise in Northeast Texas. In 2017 Annual Meeting, February 4- 7, 2017).

Therefore, it was selected as a food model for this study whose objective was directed to the determination of the dose and stability of the lutein oleoresin extracted from the lyophilized biomass of the microalga Muriellopsis sp .; added to this food matrix, as a substitute for synthetic dyes and/or β-carotene, currently used by leading companies in the food market.

2. Material and methods

2.1. Materials

Three different commercially available traditional mayonnaises (A, B and C) with a ~90% similarity in their ingredients (vegetable oil, water, pasteurized whole egg, lemon juice concentrate, calcium disodium EDTA, antioxidants such as E-385, BHA, Propyl gallate, and β-carotene as a colorant) were purchased from the local market. A home-made style mayonnaise (test mayonnaise) was developed as part of this study and colored through the addition of lutein oleoresin (LO), in order to simulate the color of the commercially available mayonnaise, to which it was compared.

Chicken eggs, 100% vegetable oil, and lemon juice were purchased from the local market in Antofagasta, Chile. Acetone (HPLC grade), dimethyl sulfoxide, methanol (HPLC grade), n-hexane (HPLC grade), and light petroleum were purchased from Winkler Ltda. (Santiago, Chile).

2.2. Preparation of an uncolored home-made style test mayonnaise

We produced less than 1 kg of an uncolored home-made style mayonnaise (test mayonnaise), which provided the basis for our coloring experiments, as follows. 13.8% of egg white and yolks were mixed at 210 rpm for 2 min in a blender (Oster, model 4172, Mexico). 84.4% of 100% vegetable oil was added to this mixture in various servings at regular intervals. Finally, 0.95% of lemon juice and 0.79% of fine table salt were added. The obtained product was homogenized at 210 rpm for 3 min and stored in a covered glass recipient in a domestic refrigerator at 5 ± 2°C, until the addition of the coloring agent.

2.3. Lutein oleoresin production

A lutein oleoresin was prepared from lyophilized biomass of *Muriellopsis sp*, at a mass fraction of 20% (w/w) in 100% vegetable oil. The resulting solution was manually stirred with a glass rod for 3 min, until forming an oily solution of a reddish color, followed by ultrasound homogenization (COLE PARMER, CP130) at an amplitude of 40 oscillations and a pulse repetition frequency of 6 pulses per second, over a period of 10 min with the application of ice to avoid temperature increases and possible degradations.

2.4. Turmeric oleoresin production

A turmeric oleoresin was prepared at a mass fraction of 30% (w/w) of commercial ground turmeric dissolved in 100% vegetable oil. The mixture and homogenized. After filtering, a translucent yellow fluorescent solution was obtained. In previous studies, the preparation and addition of turmeric oleoresin had good results in the simulation CIELAB color coordinates of other commercial mayonnaises. The turmeric oleoresin was added in varying amounts to batches of uncolored mayonnaise (see below), with the sole purpose of adjusting all batches to a similar final color, in synergy with the lutein oleoresin.

2.5. Colorimetric analysis

The color parameters of all mayonnaise samples were measured using a Spectrocolorimeter HunterLab (Model ColorFlex, Hunterlab Associates Laboratory, Inc., Virginia, USA), using the CIEL*a*b* color system, where asterisks are part of the letter coding according to CIELAB (Mayer, Ginesin, & Machtei, 2017). The differences in color between our uncolored mayonnaise and the three commercially available mayonnaise samples were evaluated based on the averages for each chromatic coordinate, L*, a*, and b*, as well as on ΔE* (Equation 1). The commercial mayonnaise

with the lowest values for b* and ΔE* was considered the best model to be matched by our lutein-enriched test mayonnaise.

$$\Delta E^* = \sqrt{\left(L_i^* + L_o^*\right) + \left(a_i^* - a_o^*\right)^2 + \left(b_i^* - b_o^*\right)^2} \quad (1)$$

To match the color of the commercial model mayonnaise, five 630 g-batches (B1-B5 formulations) of the test mayonnaise were prepared and dyed with different amounts of lutein and turmeric oleoresins (Table 1). Following this, the CIEL*a*b* color parameters of each batch were analyzed and ΔE* with respect to the commercial model iscalculated using Equation 1. The batch with the smallest color difference ΔE* with respect to the model was selected as the best color match. Difference in color over the storage period of three months was calculated for each sample of dyed test mayonnaise. Sample colors were measured on days 0, 4, 5 and 6 of the first week, then once a week during weeks 2 to 13.

2.6. Lutein hydrolysis

According to the method described by Cerezal, Barragán, Palma, and Ortiz (2015), 3 g of the lutein-dyed test mayonnaise was mixed with 2 mL of acetone, 1 mL of dimethyl sulfoxide, 2 mL of n-hexane and 1 mL of saturated saline in 14 mL glass centrifuge tubes. Samples were vortexed for 1 min at 3000 rpm (Wizard, VELP Scientifica, Italy) before centrifuging for 3 min at 3500 rpm (Centrifuge 5702, Eppendorf AG, Germany).

The hexane-phase supernatant was separated into a clean tube with 1.0 g anhydrous sodium sulfate and acetone before drying at 50°C in a rotary evaporator (RE 52A. Xian Heb Biotechnology, China) for 5 min. Following this, the sample's absorbance was measured in a spectrocolorimeter (SP 2000 UV, Spectrophotometer Spectrum. China) at 446 nm. Three milliliters of the sample were transferred to a round-bottomed flask, in order to remove the acetone in a rotary evaporator at 50°C. An alkaline hydrolysis was carried out according to Cerezal et al. (2015), 0.1 mL KOH (1%) and 2 mL of methanol were added to the sample before vortexing for 1 min at 1600 rpm. Lutein was hydrolyzed at room temperature in darkness and under N_2 for 15 h. KOH and methanol were eliminated by washing with 2 mL of petroleum ether and 2 mL of saturated saline solution. The petroleum ether extract was then dried through the addition of 1 g anhydrous sodium sulfate, and the solvent was removed by injection of N_2 gas. Each sample was subsequently dissolved in acetone and filtered through a nylon membrane (pore size 0.22 µm) before its absorbance was measured at 446 nm in a spectrophotometer. Following this, the sample was injected into the HPLC chromatograph.

Table 1. Mass concentrations of lutein and turmeric oleoresins (%) in batches B1-B5 of home-made style test mayonnaise.

Tabla 1. Concentraciones másicas de oleorresinas de luteína y cúrcuma (%) en las series B1-B5 de la mayonesa casera de prueba.

	Mass concentration (%) (w/v)	
Batch	Lutein	Turmeric
B1	0.2	0.2
B2	0.2	0.25
B3	0.37	0.2
B4	0.37	0.1
B5	0.25	0.1

B1-B5: Batch = Different formulations.
B1-B5: Series = Diferentes formulaciones.

2.7. Lutein quantification by HPLC

Lutein quantification was performed on a Hitachi 7100 liquid chromatograph (Japan) equipped with three pumps and a UV-VIS detector. Samples corresponded to 20 µL aliquots of extracted and hydrolyzed pigment, which were analyzed on a Hibar®RT 150–4.6 LiChrospher®RP-18 5 µm column at 23°C, using a gradient of methanol (A), acetone (B) and water (C), as follows: 60:17:23 A: B: C at time 0, for 5 min, and then 60:10: 30 A: B: C by 5 min, and finally, 60:17:23 for 5 min. The mobile phase was pumped at a flow rate of 1 mL min^{-1} and the response was detected at a wavelength of 446 nm. Lutein was identified by a comparison of retention times with a standard calibration curve.

2.8. Kinetics of lutein degradation

In order to determine the kinetics of lutein degradation over time, lutein quantification results over the three-month storage period were used to chart $\ln(C/C_o)*100$ against time, where C_o is the initial concentration and C is the concentration remaining at the time of each measurement. The reaction rate coefficient (k) and half-life ($t_{½}$) were obtained for the process of degradation (Huang & Von Elbe, 1987).

2.9. Proximal analysis of colored mayonnaise

Mayonnaise samples were analyzed for % moisture, proteins, and lipids. All the determination was conducted according to the method described in ISP Manual for Food Analysis (Instituto de Salud Pública, 1995). This assay was performed in order to elaborate a home-made mayonnaise under preparation standards related to the proximal profile of commercial mayonnaises.

2.10. Oxygen radical absorbance capacity assay

The ORAC assay is considered more biologically relevant than diphenylpicrylhydrazyl (DPPH) and other similar protocols and is especially useful for extracts when multiple constituents coexist and complex reaction mechanisms are involved (Huang et al., 2005). Although it was originally developed for measurement of hydrophilic antioxidants (Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005), ORAC assay has also been adapted to detect both lipophilic and hydrophilic antioxidants by altering the radical source and solvent. Huang, Ou, Hampsch-Woodill, Flanagan, and Deemer (2002) developed and validated a modified ORAC assay for lipophilic antioxidants. This modified ORAC assay has been successfully employed for measuring ORAC values of tocopherols, tocotrienols, γ-oryzanol, resveratrol, lipophilized tea catechins, and various food and biological samples (Huang et al., 2002).

Finally, the value of ORAC was considered to be present because these sources of carotenoids, although they express limited antioxidant activity towards peroxyl radicals, are strong antioxidants against singlet oxygen and, therefore, are necessary in the daily diet. Zulueta, Esteve, and Frígola (2009) have indicated that with the ORAC method the xanthophylls (lutein and zeaxanthin) had values greater

than β-carotene, which might be because of the presence of OH groups in the terminal rings.

2.11. Total polyphenol content

The total polyphenol content was determined following the Folin-Ciocalteau method, with modifications by Singleton & Rossi (Singleton & Rossi, 1965).

2.12. Statistical analyses

All measured values were represented as means (X) with their respective standard deviations (S). The means of two samples were compared using the student t statistic. Where there were more than two samples to compare, an analysis of variance was carried out. Where significant differences were found, Duncan's multiple range test was performed (Gutiérrez & de la Vara, 2003). All statistical analyses were performed at a p-value <0.05.

3. Results and discussion

Due to current market preferences for products of natural origin (Méndez, Pérez, Montañéz, Martínez, & Aguilar, 2011), the lutein used here was obtained from the biomass of the microalga *Muriellopsis sp*. The hydrophobic character of this pigment confers it a high affinity for the lipid-based matrix used here, resulting in a high stability of the color of the mayonnaise over a storage period of three months. Lutein thus proves to be a good coloring agent for lipid-based foods and a promising substitute for β-carotene and FD&C Yellow 5 (tartrazine), which are currently used for the same purpose.

3.1. Coloring of a mayonnaise-like dressing with lutein oleoresin

In order to develop a mayonnaise with a color similar to that of currently available commercial alternatives, we performed a colorimetric study of the products available on the market. We found that commercial mayonnaises A and B had 38% higher values on the blue-yellow scale (chromatic coordinate b*) than mayonnaise C (Table 2), a quantitative and qualitative reflection of the visible color difference between these products.

All three chromatic coordinates differed significantly ($p < 0.05$) between the commercially available mayonnaise and our uncolored test mayonnaise (Table 2). As expected, the uncolored test mayonnaise had higher lightness values and lower values for both a* and b*, clearly separating it from the commercial mayonnaise. The color difference with respect to the model mayonnaise C, ΔE*, was 6.50, lower than the ΔE* values for the comparison to the other commercial mayonnaises, A and B. These differences could be due to the color of mayonnaise may be graded from light yellow or to ochre yellow which depends on amount of egg yolk, edible vegetable oil and possible adjustments with mustard according to each preparation of commercial mayonnaise (Huang, Wang, Han, Meng, & Lu, 2016; Sikimic, Popov-Raljic, Zlatkovic, & Lakic, 2010). Fernandes and Mellado (2018), who replaced egg yolk by freeze-dried chia mucilage in mayonnaises, point out that the luminosity (L*), it was directly proportional to the increase in moisture, which proved to be lower when replacing or decreasing the amount of egg yolk in the sample. In order to achieve a coloration similar to that of the commercially available products, different amounts of lutein and turmeric were therefore added to the uncolored test mayonnaise (see Table 1). The values of the chromatic coordinates L*, a*, b* for each of the different batches of lutein-dyed test mayonnaise, B1 to B5, as well as the resulting color difference ΔE* with respect to the model mayonnaise C are shown in Table 3.

While it is true that the values of L*, a*, b* in mayonnaise samples depend on the amount of ingredients (egg yolk, vegetable oil), Fernandes and Mellado (2018) compare the values of their mayonnaise samples with a commercial mayonnaise which has values of L* = 91.44 ± 0.46; a* = 1.05 ± 0.04; b* = 22.09 ± 0.08, similar to those exposed in B-5 (L* = 85.61 ± 0.13; a* = 1.62 ± 0.07; b* = 21.81 ± 0.09).

A value of ΔE* <5.0 is thought to be imperceptible to the human eye (Hong, Han, & Krochta, 2004; Obón, Castellar, Alacid, & Fernández-López, 2009), and the colored batches were therefore tested against this criterion. Although 60% of the colored mayonnaise batches had ΔE* values <3.0 with

Table 2. Chromaticity coordinates and color difference of commercial mayonnaise and mayonnaise-type home unpigmented control.

Tabla 2. Coordenadas cromáticas y diferencia de color de Mayonesa comercial y mayonesa casera control sin pigmentar.

	Type of mayonnaise			
	Commercial mayonnaises			
Chromaticity coordinates	A	B	C	UDTM
L*	85.34^{a} ± 0.09	86.05^{b} ± 0.08	88.43^{c} ± 0.09	89.04^{d} ± 0.14
a*	5.45^{a} ± 0.14	4.80^{b} ± 0.08	1.16^{c} ± 0.13	0.33^{d} ± 0.12
b*	36.81^{a} ± 0.18	36.33^{b} ± 0.14	22.25^{c} ± 0.18	15.73^{d} ± 0.20
Color difference (ΔE*)	21.7*	21.3**	**6.5***	

UDTM, uncolored test mayonnaise.
UDTM, mayonesa control no pigmentada.
X ± S: Mean±standard deviation. Superscript letters indicate significant differences ($p < 0.05$).
*. – ΔE for the comparison between UDTM and commercial mayonnaise A.
**. – ΔE for the comparison between UDTM and commercial mayonnaise B.
***. – ΔE for the comparison between UDTM and commercial mayonnaise C.
Asterisks are part of the letter coding according to CIELAB (Mayer et al., 2017).
X ± S: Promedio±desviación estándar. Letras en super-índice indicant diferencias significativas ($p < 0.05$).
*. – ΔE para la comparación entre UDTM y mayonesa comercial A.
**. – ΔE para la comparación entre UDTM y mayonesa comercial B.
***. – ΔE para la comparación entre UDTM y mayonesa comercial C.
Las asteriscos son parte del código de letras de acuerdo a CIELAB (Mayer et al., 2017).

Table 3. Chromaticity coordinates of test mayonnaise batches B1-B5 after the addition of different amounts of the two oleoresins, and of the commercial model mayonnaise C.

Tabla 3. Coordenadas de cromaticidad de las series de mayonesa control B1-B5 después de la adición de diferentes cantidades de las oleorresinas y la del modelo comercial de mayonesa C.

	BATCHES					
Chromaticity coordinates	B1	B2	B3	B4	B5	Type C
L*						$88.43^{e} \pm 0.09$
a*	$-0.58^{a} \pm 0.05$	$0.66^{b} \pm 0.07$	$2.58^{e} \pm 0.09$	$1.71^{d} \pm 0.06$	$1.62^{d} \pm 0.07$	$1.16^{c} \pm 0.13$
b*	$23.72^{c} \pm 0.08$	$23.84^{c} \pm 0.08$	$25.01^{e} \pm 0.05$	$24.25^{d} \pm 0.09$	$21.81^{a} \pm 0.09$	$22.25^{b} \pm 0.18$
ΔE*	2.8	3.3	3.8	2.9	2.7	

X ± S: Mean ± standard deviation. Superscript letters indicate significant differences ($p < 0.05$).
B1-B5: Batch = Different Formulations.
ΔE* = Referred to the comparation between Batches and Mayonnaise type C.
Asterisks are part of the letter coding according to CIELAB (Mayer et al., 2017).
X ± S: Promedio ± desviación estándar. Letras en super-índice indican diferencias significativas ($p < 0.05$).
B1-B5: Series = Diferentes Formulaciones.
E = Referido a la comparación entre las formulaciones con la mayonesa tipo C.
Asteriscos son parte del código de letras de acuerdo a CIELAB (Mayer et al., 2017).

respect to the commercial model, the ΔE* was lowest for B5, which was therefore chosen to simulate the color of the commercial mayonnaise C.

Based on the amounts used to produce batch B5, 0,36% LO, was used to color test mayonnaise. The color parameters L*, a* and b* for commercial mayonnaise C, uncolored test mayonnaise, and the test mayonnaise dyed according to the B5 recipe are shown in Figure 1(a).

3.2. Color stability in the lutein-colored test mayonnaise

Due to the effect of a lipid-based environment on carotenoid stability in the presence of oxidative processes, the presence of other compounds in foods, as well as that of the lipids in the matrix could influence lutein stability (Meléndez-Martínez, Vicario, & Heredia, 2007). We therefore evaluated the chromatic stability of the lutein-colored mayonnaise over time by measuring the decrease of the values for the chromatic coordinates L*, a* and b* from t = 0 to t = 13 weeks. As shown in Figure 1(a), the parameter L*, which measures the lightness of the sample, remained stable without significant variation over the three-month storage period, demonstrating that the color of the pigmented test mayonnaise remained unchanged during this period. The chromatic coordinates a* and b* also remained practically constant over the entire 13 weeks (see Figure 1(a)).

Figure 1(b) shows the ΔE* of the lutein-pigmented test mayonnaise over time for the three-month storage period at 5 ± 2°C. While the ΔE* of the mayonnaise increased slightly over the storage period, it only exceeded 3.0 on days 47 and 84. The variation of ΔE* in the pigmented test mayonnaise follows the minimal fluctuations of the chromatic coordinates; however, these variations do not call the stability of the pigment into question. Due to the effect of a lipid-based environment on carotenoid stability in the presence of oxidative processes, the presence of other compounds in foods, as well that of the lipids of the matrix, could influence the pigment's stability (Meléndez-Martínez et al., 2004).

The changes in ΔE* found in the lutein-pigmented test mayonnaise over time are well below the threshold considered to indicate product instability (ΔE* ≥ 5.0) (Obón et al., 2009), and most of the values are ≤3.0, representing color differences that are not easily detected by the human eye (Hong et al., 2004). We found that the change in ΔE* was completely dependent on the reduction of the "yellowness" (Δb*). This is in line with a study by Lennersten and Lingnert (2000), who showed that β-carotene in mayonnaise is degraded by exposure to light of different wavelengths. Therefore, the higher the concentration of the coloring agent in the mayonnaise, and the more light is absorbed,

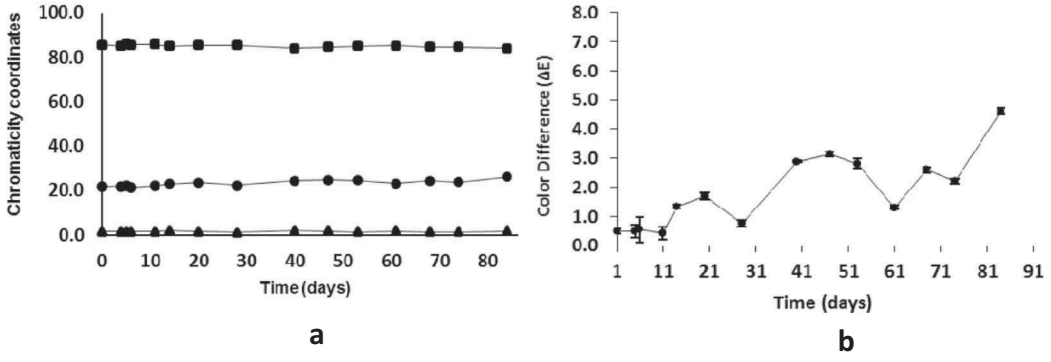

Figure 1. (a) Variation of the chromatic coordinates L* (■; lightness), a* (▲; position along the red-green axis) and b* (●; position along the yellow-blue axis) in the lutein-dyed mayonnaise over the storage period of three months at 5 ± 2°C. (b) ΔE* of the lutein-dyed mayonnaise, measured against the commercial model, over the three-month storage period. Values shown represent the means of three repeats ± standard deviation.

Figura 1. (a) Variación de las coordenadas cromáticas (■; luminosidad), a* (▲; posición a lo largo del eje rojo-verde) and b* (●; posición a lo largo del eje amarillo-azul) en la mayonesa pigmentada con luteína, durante el período de almacenamiento de tres meses a 5 ± 2°C. (b) ΔE* de la mayonesa pigmentada con luteína, medida en comparación al modelo comercial, durante el tiempo de almacenamiento de tres meses. Los valores mostrados representan el promedio de tres repeticiones ± desviación estándar.

the greater the degradation of the coloring agent, and the greater the changes to ΔE*. Our results indicate that the pigment remained stable over the time of storage, without there being any significant changes to the chromatic coordinates (L*, a* and b*). Based on this very minor change observed in ΔE* over time, the use of lutein as a coloring agent in commercial mayonnaise is reasonable.

3.3. Lutein quantification by HPLC

The pigment stability in our pigmented test mayonnaise was determined by the degradation of lutein over time. To assess this, we performed high-performance liquid chromatography (HPLC) of stability of lutein, between 1 and 13 weeks, where the chromatograms showed that although the areas of the peaks decreased over the time, retention times were maintained in the weeks of stability study (Figure 2). We found a first-order degradation kinetics with a reaction rate coefficient k = - 0.0068 days^{-1}, a coefficient of determination R^2 = 0.8241, and a half-life $t_{½}$ of 102 days (Figure 3). This behavior of lutein molecules inside the mayonnaise's oil-protein matrix is consistent with the stability as measured by the chromatic coordinates L*, a*, and b* (Figure 1(a)) and results in good color stability while stored at 5 ± 2°C.

The (minor) degradation of this pigment seen here could be due to its polyunsaturated nature, which makes it susceptible to thermal and oxidative processes during manufacturing and storage and may result in a loss of its biological and nutritional properties (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Argüelles-Monal, 2004; Meléndez-Martínez et al., 2007). In line with this, both the structural complexity of esterified xanthophylls and the composition of the matrix they are embedded in have been shown to influencing their stability (Rodriguez-Amaya, 1993). Since mayonnaise is a structurally complex matrix on a protein/lipid base, our findings support this hypothesis, which is of direct significance to the stability of carotenoids in oxidative processes (Meléndez-Martínez et al., 2004).

3.4. Quantification of moisture, lipid and protein contents of the lutein-dyed test mayonnaise

We compared the moisture levels and the lipid and protein contents of our lutein-pigmented test mayonnaise (M1) to different mayonnaise, for which studies were available, including palm oil mayonnaise (M2), soybean oil mayonnaise (M3), mustard oil mayonnaise (M4) (Palma, Aziz, Chawdhury, Uddin, & Alam, 2004), corn oil mayonnaise (M5) (Suhail, Rasool, Hussain, & Alam, 2013), peanut oil mayonnaise (M6) (Johnston et al., 2003) and a commercial mayonnaise from the local market (M7) (Figure 4).

The values obtained for our lutein-pigmented test mayonnaise lay well within the range of the values obtained for the

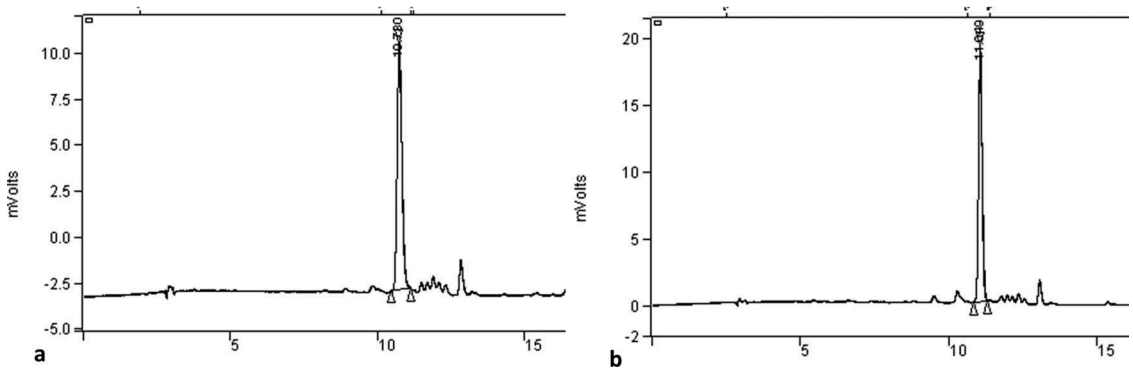

Figure 2. Chromatograms of the reading of lutein in mayonnaise during the stability analysis using HPLC method. (a) First week of stability study with a retention time (10.78 min). (b) Last week of stability study with a retention time (11.05 min).

Figura 2. Cromatogramas de las lecturas de luteína en mayonesa durante el análisis de estabilidad utilizando el método de HPLC. (a) Primera semana del estudio de estabilidad con un tiempo de retención (10.78 minutos). (b) última semana del estudio de estabilidad con un tiempo de retención (11.05 minutos).

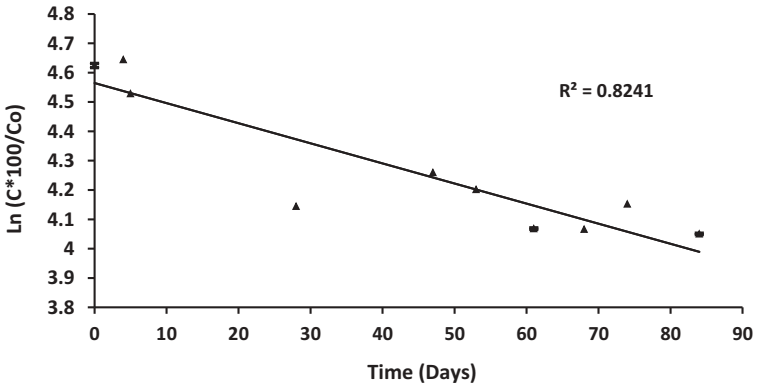

Figure 3. Degradation kinetics (First-order) of lutein over 90 days of storage of the mayonnaise. Values shown represent the means of three repeats ± standard deviation.

Figura 3. Cinética de degradación (primer orden) de luteína durante los 90 de almacenamiento de la mayonesa. Los valores mostrados representan los promedios de tres repeticiones ± de desviación estándar.

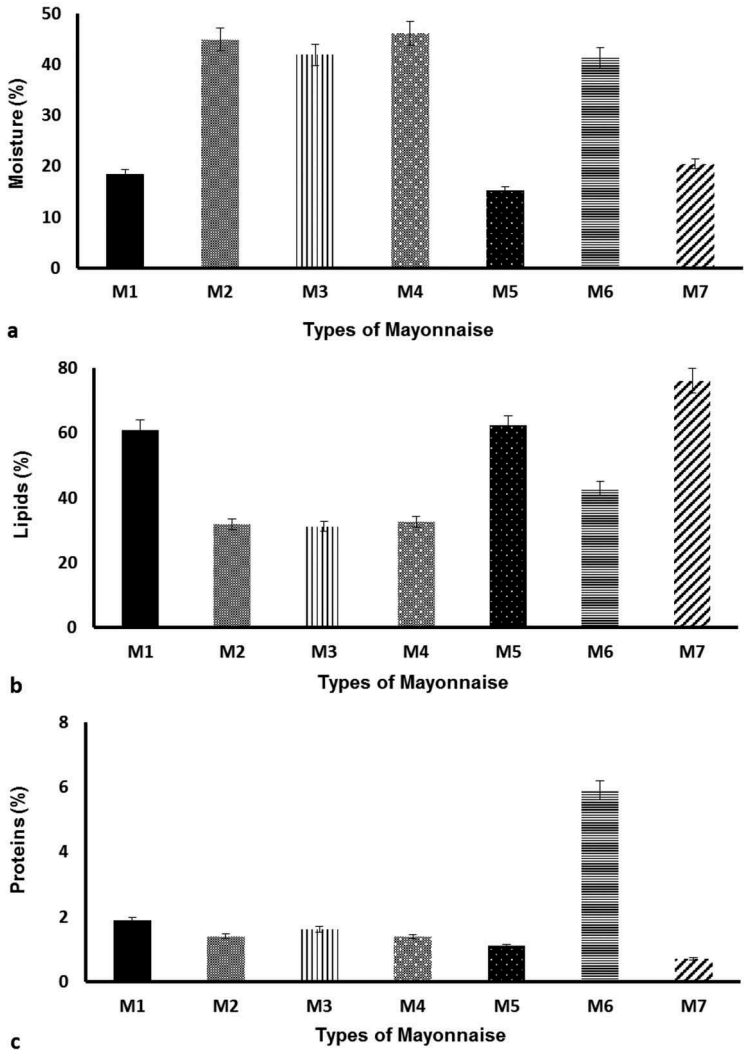

Figure 4. Comparison of % moisture (a), % lipids (b), and % proteins (c) in the lutein-dyed test mayonnaise (M1), palm oil mayonnaise (M2), soybean oil mayonnaise (M3), mustard oil mayonnaise (M4), corn oil mayonnaise (M5), peanut oil mayonnaise (M6) and a commercially available mayonnaise from the local market (M7).

Figura 4. Comparación de % de humedad (a), % de lípidos (b), y % de proteínas (c) en la mayonesa pigmentada con luteína (M1), mayonesa de aceite de palma (M2), mayonesa de aceite de soya (M3), mayonesa de aceite de mostaza (M4), mayonesa de aceite de maíz (M5), mayonesa de aceite de maní (M6) y una mayonesa comercialmente disponible en el mercado local (M7).

comparison mayonnaises, demonstrating that the characteristics of the mayonnaise developed here are typical of this type of food matrix, as currently available on the market. The moisture content of the lutein-pigmented mayonnaise developed here (M1) was 18.5%, below that of its commercially available counterpart (M7; 20.5%) and above that of maize oil mayonnaise (M5; 12.5%). Furthermore, the moisture content of our lutein-pigmented mayonnaise was approximately half that of palm oil mayonnaise (M2), soybean oil mayonnaise (M3) and mustard oil mayonnaise (M4; Figure 4(a)).

It should be considered that a higher relative content of polyunsaturated fatty acids is perhaps causing less stability of the dressing due to higher incidence of peroxidation (Kamal-Eldin, 2006).

The lipid contents showed an opposite trend to that of the moisture contents (Figure 4(b). Supplementary Material). The lipid content of our test mayonnaise was 61.0%, well above that of M6 (42.9%) and only little below that of the maize oil mayonnaise M5 (62.3%). The highest lipid content (76.1%) was found in the commercial mayonnaise (M7).

Protein contents of all mayonnaises fell within a range of 0.5% to 2% (M1, 1.9%; M2, 1.4%; M3, 1.6%; M4, 1.4%; M5, 1.1%; M7, 0.7%) (Figure 4(c)), with the exception of M6, whose protein content of 5.9% exceeded that of the other types of mayonnaise more than three times.

These characteristics allow placing our lutein-pigmented test mayonnaise within the range of the characteristics of similar products, both those available on the market and those that have been developed in research with oils obtained from different sources.

3.5. Antioxidant capacity

The ORAC assay is based on the principle of free radical damage to a fluorescent probe, such as fluorescein (Huang et al., 2002). This assay is particularly useful for samples containing multiple ingredients, which display complex reaction kinetics (Karadag, Dzcelik, & Saner, 2009).

In order to evaluate the antioxidant capacity of lutein, added to our mayonnaise in the form of an oleoresin, we studied the antioxidant capacity of the lyophilized biomass of *Muriellopsis sp.*, the original source of the lutein used to prepare the oleoresin used here. Its antioxidant capacity, expressed as the total polyphenol content and ORAC of the

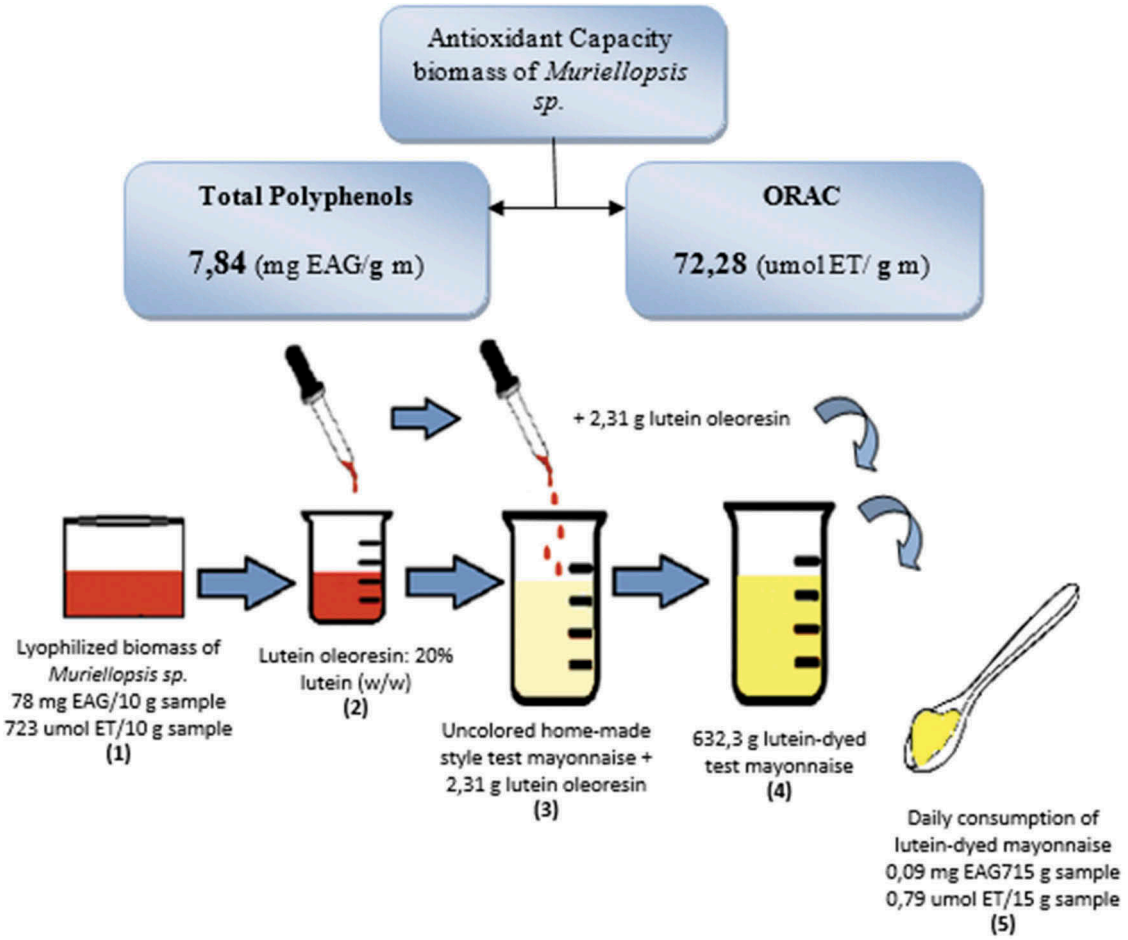

Figure 5. Antioxidant capacity of the lyophilized biomass of *Muriellopsis sp*., expressed as total polyphenol content and ORAC (1), and of the lutein oleoresin contained in 15 g of lutein-dyed test mayonnaise, the average daily intake of mayonnaise according to the Chilean Ministry of Health (MINSAL) (5).

Figura 5. Capacidad antioxidante de la biomasa liofilizada de Muriellopsis sp., expresada como contenido de Polifenoles totales y ORAC (1), y de la oleorresina de luteína contenida en 15 g de mayonesa control pigmentada con luteína, el promedio diario de consumo de mayonesa de acuerdo al Ministerio de Salud Chileno (MINSAL) (5).

lutein-enriched mayonnaise sample, was of 7.84 mg (GAE)/100 g sample and 72.28 µmol TE/100 g sample, respectively. The recommended daily dietary total antioxidant capacity is 3500 TE (Trolox equivalents); the average intake of antioxidants is of 100 mg/day. According to the Chilean Ministry of Health (MINSAL), the average daily consumption of mayonnaise in Chile is 15 g/person (Zacarías et al., 2011). Based on these data, each daily serving of our mayonnaise supplemented with *Muriellopsis sp*. would provide 0.09 mg GAE total polyphenols and 0.79 µmol TE (Figure 5), a suitable average daily antioxidant intake. As a dietary supplement with a demonstrated antioxidant capacity, which is consumed on a regular basis, this functional dressing would make a positive contribution to human nutrition.

The ORAC values reported in the current study (72.28 µmol TE/100 g sample) are comparable with those obtained from 45 a 577 µmol TE/g DW, ORAC values of extracts of 11 microalgal strains, which are producers carotenoids (Ahmed et al., 2014).

4. Conclusions

Our home-made style test mayonnaise achieved the color of traditional commercial mayonnaise through the addition of the pigment lutein. As shown by the low dispersion of the data along the three chromatic coordinates L*, a* and b*, its color remained near constant over the three-month storage at 5 ± 2°C, with only very minor color variations that cannot be detected by the human eye. Overall, our results therefore demonstrate that lutein remains very stable at the interior of a mayonnaise-type protein/lipid matrix.

Six hundred and thirty two grams of our lutein-enriched test mayonnaise had a polyphenol content of 3.63 mg GAE and an ORAC of 33.40 µmol/TE, allowing a polyphenol intake of 0.09 mg EAG/day and an antioxidant capacity of 0.79 µmol ET/day based on consumption data from the Chilean ministry of health (Zacarías et al., 2011).

Disclosure statement

No potential conflict of interest was reported by the authors.

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