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Use of seaweed powder (*Undaria* sp.) as a functional ingredient in low-fat pork burgers

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ABSTRACT

Seaweeds are a valuable resource for food development due to their nutritional composition and technological properties. This work aimed to evaluate *Undaria* sp. powder (UP) properties and its performance as a functional ingredient in low-fat pork burgers. UP proximal composition, physicochemical, and functional properties were evaluated. UP showed a monomodal particle size distribution, low moisture, and low water activity. It showed both high mineral (26.5 g/100 g db) and total dietary fiber (40.685 g/100 g db) contents. UP lipid level was low (5.2 g/100 g db). The fatty acid profile presented high proportions of SFA and MUFA (48.8 % and 38.2 %, respectively), followed by PUFA (13 %), with palmitic (C16:0; 36.9 %) and oleic acids (C18:1n-9; 34.4 %) in the highest abundance. Concerning UP properties, it retained more water than lipids; its total phenolic content reached 15.50 mg GAE/g, and the antioxidant capacity was 29.42 and 34.50 μmol TE/g for DPPH and ABTS methods, respectively. Burgers were manufactured with UP or milk protein concentrate (MPC) as emulsifiers, packaged, and stored frozen. Formulation and storage time effects on the product's physicochemical and techno-functional properties were evaluated. The UP addition to burgers increased yields, hardness, and chewiness, while reduced shrinkages in comparison with control burgers with MPC. Although the color was adversely affected by the formulation, it was balanced with carmine addition. Even though the UP burgers showed higher antioxidant capacity, lipid oxidation increased during storage and with UP incorporation. UP addition did not affect the product's fatty acid profiles or fat contents. Therefore, UP could be an adequate strategy for developing meat products with improved techno-functional and antioxidant properties.

1. Introduction

Consumers' unwillingness to change their dietary habits and the constantly increased demand for fast foods has stimulated the development of frequently-consumed products, such as meat products, that have been re-designed to make them healthier by fat reduction or

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substitution [1,2]. These modifications could also transform them into an excellent vehicle for bioactive compound delivery [3,4] and therefore become more closely aligned with dietary recommendations while preserving their technological characteristics. In addition, and following current consumer trends, the reformulation of these products could be a market opportunity since it is attractive and meets their demands.

In replacement of animal fat, vegetable or marine oils were widely added to low-fat meat products through pre-emulsification using different agents [5–8]. Generally, the meat industry uses a wide range of ingredients with specific technological properties, to improve the appearance, taste, and texture of products, as well as their nutritional value. For example, milk proteins are often added to meat products to improve their juiciness, texture, and flavor. Functional properties of milk protein concentrate (MPC) are crucial for their wide use in food and other industries, particularly for their water-binding, thickening, viscosity, and emulsification properties [9].

Currently, continuous demand from modern consumers is pushing the food industry to decrease or even eliminate the use of synthetic additives in manufacturing [10–12]; thus, numerous research and technological applications are now focused on natural counterparts [13–16]. In this regard, seaweeds, with low-calorie content and high content of key nutrients, chiefly dietary fiber, and health-promoting compounds [17,18], can be used to overcome some technological problems associated with these reformulated meat products, through their fat and water binding properties.

Also, the design of meat-based functional foods including the use of whole seaweed dehydrated or powdered would have various advantages over the inclusion of isolated compounds from seaweeds in meat production. The entire seaweed promotes the simultaneous presence of different components with both health-beneficial effects and technological advantages, some of them widely used in the food industry [17,19]. Besides, it would help promote the consumption and the economic potential of seaweeds in Western countries, particularly of *Undaria* sp., which is found in abundance on the Argentinean coast, and where the population is not accustomed to using them as a culinary ingredient [20].

Specifically, the addition of 2.5–5.5 g/100 g of whole brown seaweed *Undaria pinnatifida* (Wakame) (Phaeophyceae) in healthier meat products such as patties [21–23] and gel/emulsion meat systems [24,25] was previously studied. The resulting products showed firmer and chewier structures with better water and fat-binding properties, with a different effect on product texture if they were patties or gel/emulsion systems, presenting reasonable overall acceptability. Particularly in patties, López-López et al. [22] found that the addition of 3 g/100 g wakame resulted in an adequate product characteristics with no negative effect on their sensory properties.

Therefore, this work aimed to characterize the physicochemical and functional properties of *Undaria* sp. powder (UP) obtained from the Argentine Patagonian coast to show their potential use as an ingredient. Moreover, its inclusion as a potential functional and healthy ingredient in low-fat pork burgers in replacement of milk protein concentrate was evaluated, studying the techno-functional properties of the final product and its behavior during storage.

2. Materials and methods

2.1. Materials

All chemicals and solvents used were analytical grades. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were acquired from Sigma-Aldrich (CABA, Argentina). Sodium hydroxide (NaOH), sodium carbonate ($NaCO_3$), potassium hydroxide (KOH), and potassium persulfate ($K_2S_2O_8$) were obtained from Cicarelli (Santa Fe, Argentina). Acetone was obtained from Dorwill S.A. (CABA, Argentina). Folin–Ciocalteu's reagent and gallic acid were obtained from BioPack (CABA, Argentina).

Undaria sp. powder (UP) obtained from the Argentine Patagonian coast was provided by Soriano S.A. (Gaiman, Chubut, Argentina). The seaweed powder was fractioned, vacuum packed in Cryovac BB4L bags (Sealed Air Co., Buenos Aires, Argentina; PO_2: 35 cm^3 m^{-2} $día^{-1}$ bar^{-1} at 23 °C), and frozen at −20 °C until used (up to three months).

Commercial lean pork cuts (top inside round, integrated by *adductor femoris* and *semimembranosus* muscles; pH: 5.61 ± 0.01) were used. The meat without visible fat and connective tissue was passed through a mincer with a 0.95 cm plate (Meifa 32, Buenos Aires, Argentina). Minced meat lots of 341 g were packed in polyethylene bags with a hermetic seal, stored frozen at −20 °C until used, no more than three weeks. As a lipid source, refined high-oleic acid (HO) sunflower oil (Granix S.A., Vicente López, Argentina) was used. Milk protein concentrate (MPC, 80 % protein, Milkaut, Santa Fe, Argentine), analytical-grade sodium chloride (NaCl) and sodium tripolyphosphate (TPP) (Anedra, Research GA, Argentina), carmine red natural coloring solution (Nat-Car-01, Naturis S.A, Buenos Aires, Argentine), were used.

2.2. Undaria sp. powder characterization

2.2.1. Physicochemical characteristics

Particle size distribution (Mastersizer 2000, Malvern Instruments Ltd., U.K.) of UP was measured and calculated from a light scattering pattern. A refractive index of 1.33 was used for the dispersant medium and 1.52 for the UP particles. Average particle size was calculated using volume-weighted mean diameter ($D_{4,3}$) according to Eq. (1):

$$D_{4,3} = \frac{\sum_{i=1}^{n} d_i^4 n_i}{\sum_{i=1}^{n} d_i^3 n_i} \quad (1)$$

where d_i is the effective diameter of the ith particle (μm) and n_i is the number of particles with d_i diameter (μm). Determinations were performed in duplicate and average results were reported.

Also, the polydispersity index (Span) was calculated using the Eq. (2):

$$\text{Span} = \frac{d(0.9) - d(0.1)}{d(0.5)} \quad (2)$$

where d(0.1), d(0.5), and d(0.9) represent 10 %, 50 %, and 90 % of the total measured volume of particles, respectively.

Water activity was determined in duplicate at 25 °C using AquaLab Dew Point Series 4 4TEV equipment (Decagon Devices INC. MA, USA).

Total moisture, ash, lipid, and protein (Kjeldahl factor: 5.00; [26]) contents were determined in duplicate using AOAC [27] procedures. Kit Megazyme (Megazyme International Ltd., Wicklow, Ireland) was used to determine total dietary fiber content.

The fatty acid (FA) profile was determined in the UP-lipid fraction extracted by the Folch method [28] and saponified with 2 N KOH to obtain the fatty acid methyl esters. The extracts were analyzed by GC-FID (Agilent Technologies, 7890 A, USA) using a DB-23 capillary column (30 m long, 0.25 mm diameter × 250 μm thickness), flow 0.65582 mL/min. Helium was used as carrier gas. The injector conditions were: 250 °C, 8.54 psi, total flow 35.345 mL/min, septum purge flow 2 mL/min, and split ratio: 50:1. The detector conditions were: 280 °C, hydrogen flow 40 mL/min, airflow 350 mL/min, make-up flow (He) 25 mL/min. A temperature ramp was made, with an initial temperature of 50 °C. In the first stage, a rate of 25 °C/min was used up to 175 °C and then 4 °C/min from 175 °C to 230 °C, maintaining the latter for 15 min. The total running time was 34.75 min. Fatty acids were identified by comparison of the retention times of each peak with an external standard Supelco 37 Component FAME Mix, Cat. No.18919–1 AMP, (Sigma Aldrich, Argentina) was previously analyzed in the same conditions. The results were expressed as a percentage of total fatty acids. With the FA

profiles, Atherogenic and Thrombogenic Indexes (AI and TI, respectively) were calculated [29].

2.2.2. Functional properties and pigments

Water (WRC) and oil (ORC) retention capacities were measured by a centrifugation method described by Gómez-Ordoñez et al. [30]. Both parameters were expressed as g water or oil bound/g UP, respectively.

Swelling Grade (SG) was performed by using 0.125 g UP mixed with 2.5 mL of water in graduated tubes for 24 h. Therefore, the volume occupied by the hydrated UP was read and the SG was expressed as mL occupied/g UP. All the measurements were made in duplicate.

Chlorophylls and carotenoid contents were quantified according to Lichtenthaler [31] on extracts prepared from 0.4 g of UP with 5 mL of acetone:water (80:20). The results were expressed as μg/g UP.

Total phenolic content (TPC) was determined on ethanolic extracts from UP by the Folin-Ciocalteu colorimetric method [32] using gallic acid (GA) as standard. Briefly, UP-extract (0.2 mL) and 2 % p/v Na_2CO_3 in NaOH 0.1 N (2 mL) were mixed and kept 2 min in the dark. 0.2 mL of Folin-Ciocalteu's reagent solution (1:1 in water) was added and incubated for 30 min at room temperature in darkness. The absorbance was measured at 725 nm with a spectrophotometer (T60 Vis spectrophotometer, PG Instruments, UK) against distilled water as blank. The results were expressed as gallic acid equivalents (mg GAE/g UP).

Assessment of antioxidant capacity (AC) was performed using two different procedures: $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging methods. The UP-ethanolic extract was assayed within an appropriate range of dilutions and Trolox was used as standard. The reaction time for each procedure was previously determined by following the absorbance change of the sample in time until obtaining a stable absorbance value.

DPPH• radical scavenging capacity: A concentration of extracts or standard (0.1 mL) was added to 3.9 mL of DPPH• ethanolic solution (25 mg/L). Mixtures were shaken vigorously and left to stand for 24 h in darkness at room temperature. The reduction of DPPH• radical was measured at 515 nm with a spectrophotometer (T60 Vis spectrophotometer, PG Instruments, UK). Results were expressed as Trolox equivalents (μmol TE/g UP).

$ABTS^{\bullet+}$ radical scavenging activity: The $ABTS^{\bullet+}$ radical was generated by the reaction of diammonium salt (ABTS, 7 mmol) in water, with $K_2S_2O_8$ (2.45 mmol) overnight in the dark at room temperature. The absorbance of the mixture was adjusted to 0.70 ± 0.03 at 734 nm. Different concentrations of extracts or standard (0.1 mL) were mixed with 3.9 mL $ABTS^{\bullet+}$ solution, shaken, and left to react for 3 h in the dark. The reduction of $ABTS^{\bullet+}$ radical was determined by reading the absorbance at 734 nm with a spectrophotometer (T60 Vis spectrophotometer, PG Instruments, UK), using the extraction solvents as blank. Results were expressed as Trolox equivalents (μmol TE/g UP).

2.3. Burgers reformulated with Undaria sp. powder

2.3.1. Burger elaboration

Low-fat pork burgers were prepared according to Pennisi Forell et al. [33] with 10 g/100 g of pre-emulsified HO-sunflower oil using 3 g/100 g of milk protein concentrate (MPC) in the Control burger (CO-B) or UP in the UP-burger (UP-B) as emulsifiers. The selected amount of UP (3 g/100 g) used as an emulsifier has been based on the positive results obtained in previous studies, which indicate that this amount does not exert negative effects on the reformulated products [22]. Water (10 g/100 g) and salts (1 g NaCl/100 g and 0.2 g TPP/100 g) were kept constant in both products. TPP was added to improve binding and water-holding properties. Water was incorporated to replace part of the fat. In UP-burger, carmine red (0.0032 g/100 g) addition was needed to compensate for the seaweed color, so it was diluted and incorporated into the added water. The main HO-sunflower oil fatty acids were C18:1n-9 (82 ± 4 %), C18:2n-6 (10 ± 4 %), C16:1n-7 (5.01 ± 0.09 %) and C18:0 (2.8 ± 0.20 %).

For burger elaboration, overnight-thawed minced meat (approximately 18 h at 4 °C) was grounded in a commercial food processor (Universo, Rowenta, Germany) equipped with a 14 cm blade for 5 min at the highest speed. NaCl and TPP were added to the ground meat and processing continued for 2 min. Meanwhile, emulsifiers, either MPC or UP (according to formulation) were solubilized in cold distilled water, homogenized for 30 s with a hand-held food processor (Braun, Buenos Aires, Argentina), and rested for 10 min for better hydration. Then, oil was added and emulsified for 1.5 min. The emulsion obtained was added to the meat-salts mixture, mixing all the ingredients for 4 min at the highest speed. Batters were stored for 1 h at 4 °C.

Burgers (40 ± 1 g) were formed using a mold (5 cm diameter and 1.2 cm high), wrapped separately in polyethylene cling film, and placed over aluminum trays in polyethylene bags with hermetic seal. Burgers were frozen and stored at −20 °C for up to 6 months, the typical expected shelf life of this type of product without losing quality. The burgers manufacture was replicated twice using the same ingredients and processing conditions.

2.3.2. Burger characterization

At each storage time, five burgers from each formulation were removed from the freezer and immediately cooked (without previously defrosting) in a commercial electric double-side grill (Model 3882, Oster, China) at 210 °C. The burgers were cooked for 3 min, the time needed to reach 71 °C of internal temperature and ensure the product's microbiological safety [34]. Cooked burgers were cooled at room temperature before their analysis.

The cooking yield was determined according to Argel et al. [26] as the percentage of weight kept after the cooking treatment (five replicates by formulation and manufacture replicate). Burger shrinkage (%) [35] was determined by measuring the diameter (D) and thickness (T) of raw and cooked burgers (five replicates by formulation and manufacture replicate).

Instrumental juiciness was defined as the percentage of liquid drained from cooked burgers submitted to a pressure of 100 N for 2 min in a TA-xt2i texturometer (Texture analyzer, Stable Micro Systems, UK) [36]. The measurement was made in triplicate for each formulation.

Texture Profile Analysis (TPA) was performed on cooked burgers in a controlled temperature room (20 °C). Samples (1.5 cm thick and 1.7 cm diameter) from the center of the burgers were compressed twice to 30 % of their original height between flat plates using a TA-xT2i Texture Analyzer (Texture analyzer, Stable Micro Systems, UK) with a 75 mm diameter probe (SMSP/75) operating at 0.5 mm/s, interfaced with a computer, using the software supplied by Texture Technologies Corp. Eight measurements were taken for each formulation and mean values reported. Hardness (peak force of first compression cycle, N), adhesiveness (negative force area of the first byte represented the work necessary to pull the compressing plunger away from the sample, J), cohesiveness (ratio of positive areas of the second cycle to area of the first cycle, J/J, dimensionless), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm/mm, dimensionless), chewiness (hardness x cohesiveness x springiness, N) and resilience (area during the withdrawal of the first compression divided by the area of the first compression, J/J, dimensionless) were determined.

The color was measured at room temperature on the internal surface of cooked burgers, using a Chroma Meter CR-400 colorimeter (Minolta Co., Ramsey, New Jersey, USA), and CIE-LAB parameters (L*, a*, and b*) were determined. Sixteen measures were taken for each formulation. The colorimeter was calibrated using a white tile (L* = 98.45, a* = −0.10, b* = −0.13; Minolta calibration plate), using an 8 mm aperture, illuminant D65 at a standard observation of 2°.

Thiobarbituric acid-reactive substances (TBARS) values were determined in duplicate according to Pennisi Forell et al. [33] to evaluate the lipid oxidation. Results were expressed as mg malonaldehyde (MDA)/kg product.

At 6 months of frozen storage, the burger's antioxidant capacity

(AC), lipid content, and FA profile were evaluated. Antioxidant capacities were determined on extracts following the Mancini et al. [37] procedure. Briefly, 5 g of the minced cooked burger were mixed for 2 min with 10 mL of 96 % ethanol in tubes covered with aluminum foil. The liquid phase was centrifuged for 10 min at 10,500*g* and the precipitate was discarded. The extracts were made in duplicate for each formulation. The AC was determined on the supernatant using the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods already described. The results were expressed as μmol TE/g burger. The cooked burger's lipid content and FA profile were evaluated by the methodology described before.

2.4. Statistical analysis

Experimental data were reported as mean values and between parenthesis the standard error of the mean (SEM). Analysis of variance (ANOVA) followed by Tukey's HSD test for burger characterization was performed using InfoStat software. Differences in means and F-tests and Pearson correlation coefficients (R) for relationships between various parameters were calculated and considered significant when $P < 0.05$.

3. Results and discussion

3.1. Undaria sp. powder characterization

The particle size of food materials affects their physicochemical and functional properties, being a critical parameter in the texture, appearance, and functional properties in product manufacture. The smaller the particle size, the better the water and oil binding capacities of the food material [38]. UP showed a monomodal behavior characterized by an average volume-weighted mean diameter ($D_{4,3}$) of 334 μm and a polydispersity index (Span) of 2.34.

The proximal composition and fatty acids profile of UP are shown in Table 1. Seaweed's proximal composition depends on the species, place of cultivation, atmospheric conditions, and harvesting period [39].

Table 1
Proximal composition and fatty acid profile (% of total fatty acids) of *Undaria* sp. powder.

Parameter[a]	*Undaria* sp. powder
Moisture (g/100 g)	7.9 (0.2)
Proteins (g/100 g) db[b]	6.5 (0.2)
Lipids (g/100 g) db	5.2 (0.1)
Ash (g/100 g) db	26.5 (0.2)
Total dietary fiber (g/100 g) db	40.68 (0.002)
Fatty acids (%)	
C14:0	6.0 (0.1)
C15:0	0.27 (0.02)
C16:0	36.9 (0.2)
C16:1n-7	2.01 (0.04)
C17:0	0.21 (0.02)
C17:1n-7	1.17 (0.04)
C18:0	4.72 (0.08)
C18:1n-9	34.4 (0.2)
C18:2n-6	4.46 (0.03)
C18:3 n-6	1.06 (0.00)
C18:3n-3	0.59 (0.01)
C20:0	0.64 (0.08)
C20:1n-9	0.6 (0.1)
C20:4n-6	4.5 (0.1)
C20:5n-3	2.33 (0.03)
SFA	48.8 (0.4)
MUFA	38.2 (0.2)
PUFA	13.0 (0.2)
n-3	0.59 (0.01)
n-6	10.1 (0.2)

[a] Standard error of the mean is given between parentheses.
[b] db: data expressed as dry matter basis; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

UP presented a low moisture content caused by the seaweed drying and low water activity, adequate to its conservation by retarding the microbial growth, lipid oxidation, and antioxidant compounds degradation, and also preserving its desirable qualities [40]. The UP protein content was within the range reported for brown seaweeds (3–15 % of dry weight) and lower than green and red species [41]. UP mineral content was high, in agreement with Peñalver et al. [39], who demonstrated that seaweeds could have between 8 and 40 % of mineral of their dry weight, with a great abundance of essential minerals such as Na, Ca, Mg, K, P, Fe, Zn, Mn, and Cu.

Seaweeds, known for being rich in carbohydrates, mainly structural polysaccharides in the cell walls and storage polysaccharides in plastids, have interesting nutritional properties since their total dietary fiber, may reach up to 38 % (dry weight) depending on the species [42]. They could be a valuable fiber source for Western-countries´ diets that generally are rich in refined products and poor in fiber [43]. In concordance with the above, the UP's total dietary fiber content was 40.68 g/100 g db, a considerable proportion compared to terrestrial vegetables [39].

UP had low-lipid content (5.2 %). However, its FA profile (Table 1) showed that the saturated fatty acids (SFA) were the most abundant fatty acids of this seaweed (48.8 %), followed also by high proportions of monounsaturated fatty acids (MUFA) and relatively low amounts of polyunsaturated fatty acids (PUFA) (38.2 % and 13.0 %, respectively). Regarding the individual fatty acids, palmitic acid (C16:0) presented the highest amount (about 37 % of total FA), followed by oleic (C18:1n-9; 34.4 %), miristic (C14:0; 6 %), and stearic (C18:0), linoleic (C18:2n-6) and arachidonic (C20:4n-6) fatty acids with similar values (about 4–5 % each). Moreover, eicosapentaenoic fatty acid (EPA; C20:5n-3) was detected in UP and represented 2.33 % of total fatty acids. Our results agree with those reported by Boulom et al. [44], who found in the SFA fraction of *Undaria pinnatifida* that C16:0 was the major fatty acid, followed by C14:0 and C18:0. Similar to our findings, the same authors reported high amounts of C18:1n-9 and C20:5n-3. In contrast, these authors found that the content of PUFA was higher than MUFA in *Undaria pinnatifida* samples. It is also important to note that in that study, the authors observed also very high differences in the fatty acid composition of different seaweed plant parts, which could explain the differences between our data and that reported by them. Other recent research also studied the fatty acid composition of *Undaria pinnatifida* [45], but in this case, the authors reported that PUFA were the predominant fatty acids, which high amounts of γ-linoleic acid (C18:3n-6), C20:5n-3, docosahexaenoic acid (DHA; C22:6n-3), α-linoleic acid (C18:3n-3), and C18:2n-6. The SFA fraction was the second most abundant group, mainly composed of C16:0 and MUFA was characterized by high amounts of C18:1n-9 [45], in agreement with our results.

On the other hand, the seaweed's functional properties mainly principally depend on the amount and chemical nature of their dietary fiber content [46], and their ability to absorb and retain water. WRC was higher than ORC (Table 2), showing that the UP retained water better than lipids, as also the high SG indicated. These properties could be related to the hydrophilic nature of the charged polysaccharides of soluble dietary fiber, mainly fucans, in brown seaweeds such as *Undaria* sp. These results were in agreement with those obtained by Gomez-Ordoñez et al. [30]. However, these results are difficult to compare because they are also dependent on the experimental conditions (temperature, pH, time, and centrifugation) as well as sample preparation and particle size [47].

The results for pigments, total phenolic content, and antioxidant activity of UP are also shown in Table 2. Chlorophyll a and carotenoids were the main pigments quantified, reaching values of 357 μg/g and 123.1 μg/g respectively. Thus, due to the combination of both types of pigments, part of the green of the chlorophyll is masked resulting in a brownish color. One of the main carotenoids available in different species of brown seaweeds such as *Undaria* sp. is fucoxanthin, which was associated with an antioxidant, photo-protective, anticancer, anti-

Table 2
Physicochemical and functional characteristics (water activity (aw), water (WRC) and oil (ORC) retention capacities, swelling grade (SG), pigments, total phenolic content (TPC), and antioxidant capacity (AC)) of *Undaria* sp. powder.

Parameter[a]	*Undaria* sp. powder
A_w (25 °C)	0.5495 (0.0005)
WRC (g water/g)	6.6 (0.1)
ORC (g oil/g)	0.99 (0.07)
SG (ml/g)	13.4 (0.3)
Chlorophyll a (μg/g)	357 (4)
Chlorophyll b (μg/g)	41 (1)
Total chlorophylls (μg/g)	399 (4)
Carotenoids (μg/g)	123.1 (0.9)
TPC (mg GAE/g)	15.5 (0.5)
AC-ABTS method (μmol TE/g)	34.5 (0.8)
AC-DPPH method (μmol TE/g)	29.4 (0.3)

[a] Standard error of the mean is given between parentheses.

inflammatory, and other preventive effects [39].

Seaweeds are considered a good source of phenolic compounds [17,18,48,49], one of the most important classes of natural antioxidants which also possess many biological activities [50]. The UP total phenolic content reached 15.5 mg GAE/g with the methodology used, that implies ethanolic extracts that were higher than those informed for different extracts from *Undaria pinnatifida* [50,51]. The variation between the different studies could be related to the type and conditions during extraction, since these parameters also have a very decisive influence on the total phenolic content, in addition to other factors like species, geographical origin or the area of cultivation, seasonal, physiological, and environmental variations.

The antioxidant capacity evaluation is strongly dependent on the evaluation method so it is generally analyzed with several in order to obtain a global evaluation of the real and total antioxidant activity [52]. The UP antioxidant capacity reached values of 29.4 and 34.5 μmol TE/g for DPPH and ABTS methods, respectively. According to Jiménez-Escrig et al. [53], brown seaweeds showed better scavenging capacity than the red ones, but commercial seaweeds showed lower antioxidant capacity than fresh seaweeds probably due to some deleterious effect of the drying process and storage on the content of bioactive compounds.

3.2. Burger characterization

Thus, taking into account the aforementioned properties of UP, it was used to reformulate burgers. Fig. 1(a-c) shows the evolution of burger yield, shrinkage, and instrumental juiciness during frozen storage. Burger yields were higher than 80 % at the initial of the storage, being the highest for burgers with UP (UP-B). During the 1st month of storage yields slightly diminished for both formulations. Beyond that moment, they showed no variations with storage time, and UP-B yields were higher than the control samples (CO-B) ($P < 0.05$).

In agreement with this, UP-B size reductions or shrinkages were lower ($P < 0.05$) than CO-B ones at all storage times (Fig. 1b), possibly due to the meat-protein denaturation which causes water and fat losses during cooking [54]. For both formulations, it moderately increased beyond the 3rd month of frozen storage. Regarding instrumental juiciness (Fig. 1c), formulation, storage time, and their interaction were significant ($P < 0.05$), being lower for UP-B compared with CO-B ones for all storage time points. When correlations among parameters were evaluated, a positive one was found between instrumental juiciness and shrinkage ($R = 0.70$), while negative correlations were observed between yield and each (−0.69 and −0.77, with instrumental juiciness and shrinkage, respectively). These findings revealed that liquid within the matrix was easily lost when the instrumental juiciness values were high, resulting in lower yields. Therefore, UP incorporation in low-fat burgers could be used to increase yields and reduce shrinkages in comparison to the milk protein concentrate commonly used. According to Cofrades

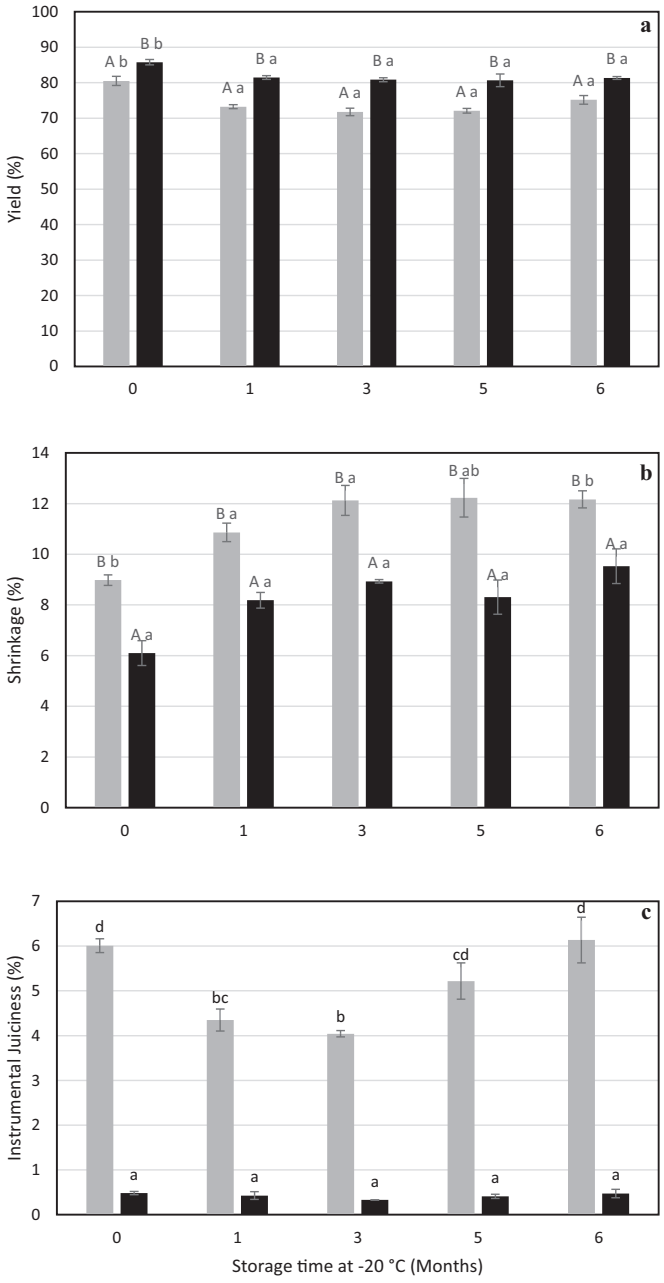

Fig. 1. Yield (a), shrinkage (b), and instrumental juiciness (c) of low-fat pork-meat burgers formulated with milk protein concentrate (■ CO-B) or *Undaria* sp. powder (■ UP-B) as a function of the frozen storage time. For (a) and (b): $^{A-B}$ Means with the same superscript do not differ significantly ($P > 0.05$) between formulations; $^{a-b}$ Means with the same superscript do not differ significantly ($P > 0.05$) between storage time. For (c): $^{a-b}$ Means with the same superscript do not differ significantly ($P > 0.05$) as the formulation × time interaction was significant.

et al. [55], UP improved the water- and fat-binding properties of the meat matrix due to major components like fiber (40.68 g/100 g db, Table 1). Similar results were previously obtained by López-López et al. [22] in low-salt low-fat beef patties including 3 % of Wakame (*Undaria pinnatifida*), and by Jeon and Choi [21] for pork patties with up to 4 % of Sea mustard (*Undaria pinnatifida*), who also found no effect on the flavor preference or overall acceptance despite panelists describing an algae flavor in the reformulated meat product.

Texture profile analysis (Fig. 2a-f) indicated that when UP was incorporated into the burgers, the hardness and chewiness significantly

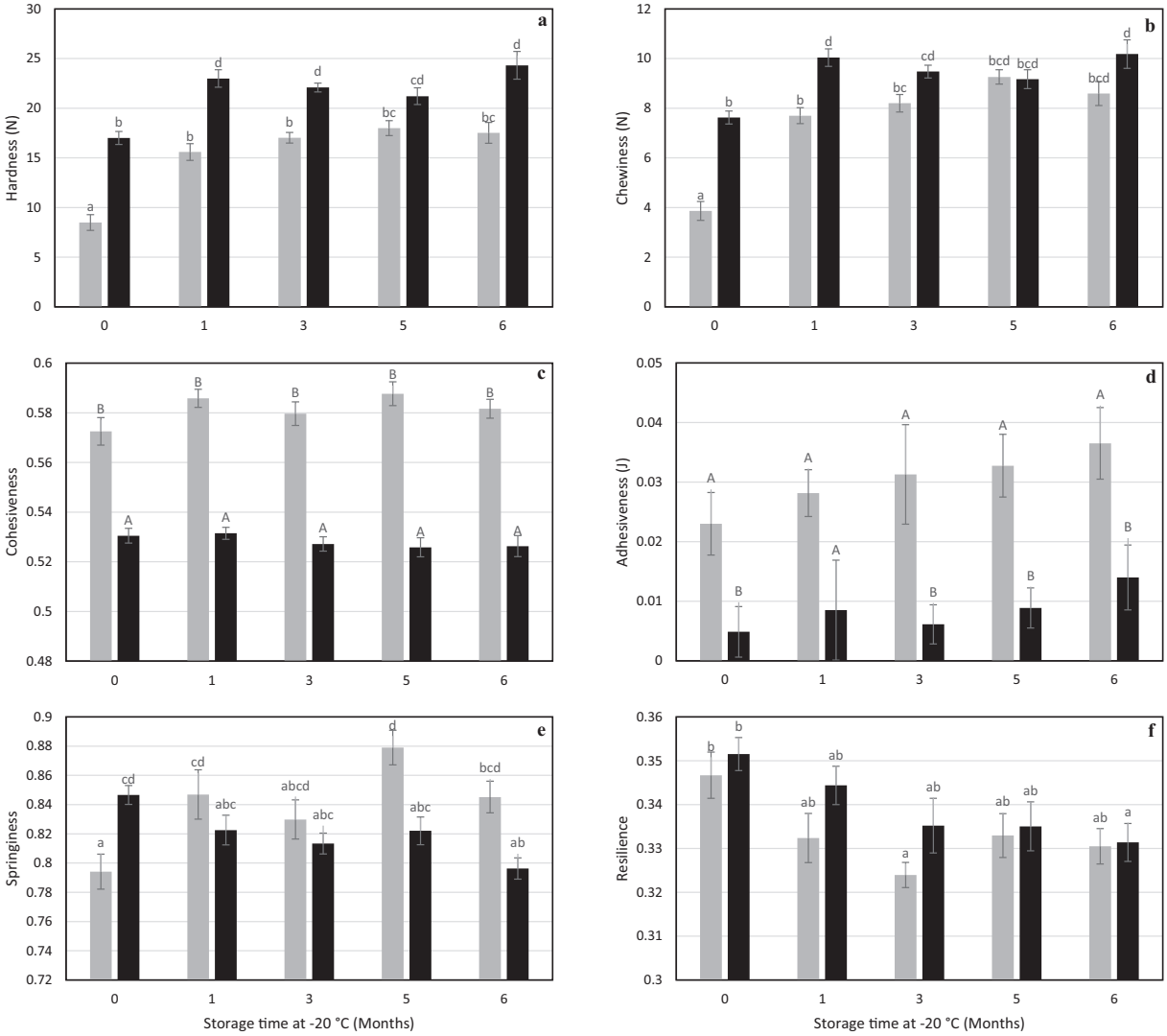

Fig. 2. Texture Profile Analysis (TPA) parameters a) hardness, b) chewiness, c) cohesiveness, d) adhesiveness, e) springiness, and f) resilience, of cooked low-fat pork-meat burgers formulated with milk protein concentrate (■ CO-B) or *Undaria* sp. powder (■ UP-B) as a function of the frozen storage time. For (a), (b), (e), and (f): $^{a-b}$ Means with the same superscript do not differ significantly ($P > 0.05$) as the formulation x time interaction was significant. For (c) and (d): $^{A-B}$ Means with the same superscript do not differ significantly ($P > 0.05$) between formulations; $^{a-b}$ Means with the same superscript do not differ significantly ($P > 0.05$) between storage time.

increased ($P < 0.05$), and springiness ($P < 0.1$), cohesiveness, and adhesiveness were reduced ($P < 0.05$) compared to CO-B. As both formulations included similar ingredients and only differ in the ingredient used as an emulsifier (MPC and UP, in CO-B and UP-B, respectively), the change in their texture parameters were principally caused by the role played by the ingredient, in the case of UP, and by its principal components, chiefly dietary fiber content. The effects of this macrocomponent from terrestrial and marine sources have been studied alone or combined with other ingredients in meat products, and the results depend on the type and concentration of fiber added. In agreement with our findings, several authors found that the incorporation of seaweed in meat products generally increases hardness [56–59] and in consequence, chewiness.

Frozen storage had a significant effect on some TPA parameters ($P < 0.05$). In both burger formulations, hardness and chewiness increased up to 1 month at −20 °C and remained constant thereafter. This effect was related to yield variations that were significant at 1 month but not afterwards, demonstrating that changes in the main meat-matrix occurred during this storage period.

Changes in hardness and loss of juiciness could be related to myofibrillar protein denaturation that reduces their water holding capacity, thus the products became less juicy and harder due to the aggregated myofibrillar proteins [36]. According to this, a negative correlation was found between hardness and juiciness ($R = -0.71$).

Similar results were reported by López-López et al. [60] on frankfurters added with Sea Spaghetti after 14 days of chilled storage. However, according to Cofrades et al. [61], the effect of the seaweed on the meat product texture depends on the type of final product (burgers or gel/emulsion systems).

Only the formulation significantly affected the cohesiveness and the adhesiveness, both being higher in CO-B than in UP-B. Youssef et al. [62] found that high cohesiveness values, related to the “strength” of the internal bonds required to maintain the structure of the sample, and could be related to a greater cooking loss, which would lead to the development of a more cohesive protein matrix. This may explain the higher cohesiveness of CO-B that correlated with lower yields compared to UP-B, in which the high amount of fiber present likely interfered with the meat-matrix internal bonds.

Among burger color parameters (Fig. 3a-c), L* and a* were affected by formulation (decrease with UP addition; $P < 0.05$), storage time (L*

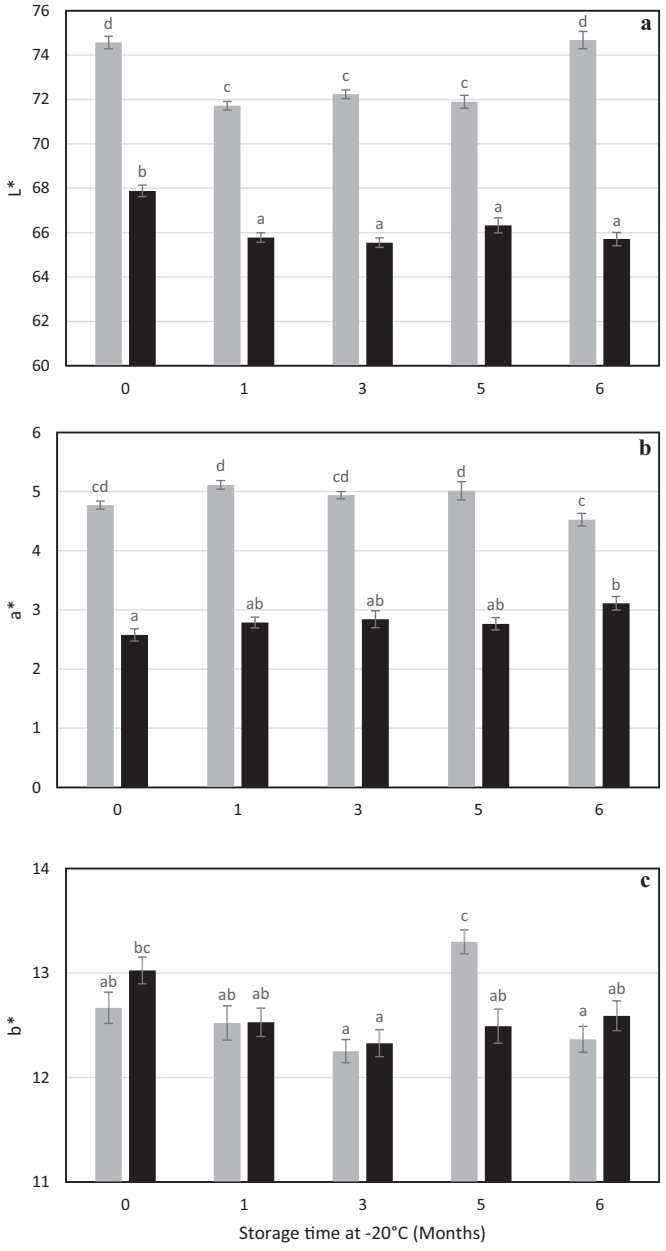

Fig. 3. Color parameters changes a) L*, b) a*, and c) b* of cooked low-fat pork-meat burgers formulated with milk protein concentrate (■ CO-B) or *Undaria* sp. powder (■ UP-B) as a function of the frozen storage time. [a-b] Means with the same superscript do not differ significantly (P > 0.05).

decrease and a* increase with the storage; P < 0.05), and interaction, while b* was only affected by storage time and interaction.

The color of reformulated meat products is determined by fat content, added water, and pigmentation of the meat used [63]. As these ingredients were at the same levels in both formulations, the changes in their color parameters were caused principally by the MPC replacement by UP, plus the addition of the carmine solution. The combination of the seaweed pigments, mainly chlorophyll *a* and carotenoids (Table 2), with the carmine solution almost balanced the resulting burger color. Nevertheless, UP-B presented significantly lower lightness (L*) and redness (a*) for all storage times, while b* was less affected (Fig. 3). Kim et al. [64] found similar color changes in reduced-fat low NaCl meat emulsion systems added with *Undaria pinnatifida*. Cofrades et al. [25] also reported that the addition of seaweeds (*Undaria pinnatifida*, *Himantalia elongata*, - *Phaeophyceae*, *and Porphyra umbilicalis* -*Rhodophyta*) caused a decrease of L* in comparison with the control and that the color variations were consistent with the color characteristics of the brown or red seaweeds used in the formulation. Therefore, the color differences found by us are related to the typical color of the UP used in the burger formulation.

The antioxidant capacity of UP-B evaluated by the DPPH method was significantly higher than the CO-B (1.04 and 0.78 μmoles TE/g, respectively), nevertheless, it was no different ($P > 0.05$) by the ABTS method. Therefore, UP appears to provide the burger with bioactive molecules, probably the phenolic compounds and the pigments (Table 2) that improved its antioxidant properties. Hentati et al. [65] found a similar effect on fish burgers' antioxidant capacity by the addition of different seaweeds. However, although the antioxidant activity (measured with the DPPH method) was higher in the UP-B, these samples showed lower lipid oxidation stability. Lipid oxidation of cooked burgers at different storage times is shown in Table 3. For both formulations, TBARS values tended to increase with frozen storage. This trend is expected since it is well known that the radical activity and lipid degradation in the different lipid oxidation phases (initiation, propagation and termination) increase with the storage time [66]. The same behavior was observed by multiple authors, who reported a significant increase in lipid oxidation during the storage phase of different meat products [67–69]. In the present study, after manufacture (month 0), non-significant differences were observed among batches. However, the variations between the beginning and the end of the storage were up to 0.251 mg MDA/kg for UP-B (from 0.161 to 0.412 mg MDA/kg). In contrast, the increase of lipid oxidation in CO-B samples was very low (from 0.155 to 0.202 mg MDA/kg). This fact demonstrated that the antioxidant properties of the UP were not sufficient to protect the burgers from oxidative reactions. This could be due to some extracts, although presented high amounts of polyphenols, they could have pro-oxidant effects in meat products [15]. Similar trends were observed in a previous study in which the addition of 3 % *Undaria pinnatifida* to healthy beef burgers did not decrease the lipid oxidation in comparison with control samples during frozen storage [22].

In any case, it is important to highlight that in both types of samples, the oxidation level was low, and it remained below the limit considered as detectable by the consumer. In this sense, various authors affirm that the limit for the consumer to detect rancid flavors or off-odors in fresh meat is 2–2.5 mg MDA/kg [66,70,71]. A more restrictive level of deterioration of the rancid flavor in meat products was established between 0.5 and 0.6 mg MDA/kg [69,72,73]. TBARS values in all samples of the present research were between 0.140 and 0.412 mg MDA/kg, below the aforementioned limits, which ensures that consumers will not observe rancid tastes or odors in any of the samples. Thus, it was concluded that the lipid oxidation in low-fat cooked pork burgers formulated with both, MPC and UP is not a limiting factor for frozen stability. López-López et al. [22] found similar results in low-fat low-sodium beef patties with wakame (3 %), although their values were higher (0.43–0.87 mg MDA/kg) than those observed in this study. The low oxidation in our samples could be related to the high MUFA (>63 %) and relatively low PUFA (<8.3 %) content in both types of burgers (Table 4). It is well known that

Table 3
Lipid oxidation of low-fat cooked pork burgers expressed as TBARS (mg MDA/kg) for samples formulated with milk protein concentrate (CO-B) or *Undaria* sp. powder (UP-B) as a function of the frozen storage time.

Time (months)	TBARS (mg MDA/kg)	
	CO-B	UP-B
0	0.155^{ab} (0.006)	0.161^{ab} (0.007)
1	0.140^{a} (0.01)	0.285^{c} (0.004)
3	0.150^{ab} (0.002)	0.350^{d} (0.03)
5	0.180^{ab} (0.01)	0.410^{e} (0.004)
6	0.202^{b} (0.005)	0.412^{e} (0.004)

[a-b] means with the same superscript do not differ significantly ($P > 0.05$).

Table 4
Fatty acid profile (% of total fatty acids) and ratios of nutritional relevance of the cooked Control (CO-B) and *Undaria* sp. powder added (UP-B) burgers.

Fatty acid (%)*	CO-B	UP-B
C14:0	0.44[a] (0.05)	0.50[b] (0.10)
C15:0	nd	nd
C16:0	19.0[a] (1.0)	18.3[a] (0.70)
C16:1n-7	0.36[a] (0.02)	0.40[a] (0.02)
C17:0	nd	nd
C17:1n-7	nd	nd
C18:0	7.20[a] (0.90)	6.60[a] (0.50)
C18:1n-9	62.9[a] (0.60)	64.0[a] (0.90)
C18:2n-6	8.00[a] (2.0)	7.70[a] (0.50)
C18:3n-6	nd	nd
C18:3n-3	0.31[a] (0.04)	0.34[a] (0.05)
C20:0	0.51[a] (0.07)	0.50[a] (0.10)
C20:1n-9	nd	nd
C20:4n-6	nd	nd
C20:5n-3	nd	nd
SFA	27.2[a] (0.60)	25.9[a] (1.0)
MUFA	63.3[a] (0.61)	64.4[a] (0.92)
PUFA	8.31[a] (2.0)	8.04[a] (0.6)
n-3	0.31[a] (0.05)	0.34[a] (0.06)
n-6	8.00[a] (2.1)	7.70[a] (0.51)
n-6/n-3	26.8[a] (5.0)	22.7[a] (3.0)
AI	0.29[a] (0.02)	0.28[a] (0.02)
TI	0.73[a] (0.03)	0.69[a] (0.03)

[a-b] means with the same superscript within the same row do not differ significantly ($P > 0.05$). nd: no detected.
* Standard error of the mean is given between parentheses.

the extent of unsaturation in the fatty acids plays a vital role in their oxidation susceptibility [66]. Therefore, the fatty acid composition of the burgers of our research is low susceptible to lipid oxidation.

Finally, the fat contents of cooked C- and UP-burgers, evaluated at 6 months of frozen storage, did not differ ($P > 0.05$) and showed a mean level of 13.45 g/100 g. These values are in agreement with the estimated values considering the fat content of the ingredients, their proportion in each formulation, and their cooking yield, showing that there were no lipid losses after cooking. As there were no significant differences between the burgers' lipids content, UP was equivalent to the MPC as an emulsifier in this meat system. In addition, burgers' lipid levels were mainly determined by the two principal lipid sources in the products, the lean-pork meat, and the HO-sunflower oil, both at the same level in both formulations.

Similarly to the total fat content, the fatty acids (FA) profile did not show differences between CO-B and UP-B samples (Table 4). In fact, any of the eight individual FA nor the total saturated, monounsaturated or polyunsaturated fatty acids presented differences ($P > 0.05$) between burger samples.

According to the FA profiles obtained at 6 months of storage, oleic acid (C18:1n-9) was the fatty acid found in the highest proportion in both formulations. This fact directly reflects the composition of the oil used to make the burgers, since, as indicated in the material and methods section, high-oleic sunflower oil has 82 % of C18:1n-9. In addition, it is well known that in pork fat, the content of this fatty acid also tends to be the majority. Additionally, although the content of this fatty acid did not show significant differences, the UP-B presented slightly higher amounts of C18:1n-9 than CO-B, which are related to relatively more amount of this acid in the UP.

The second most abundant fatty acid was palmitic (C16:0; ~19 %), followed by linoleic (C18:2n-6; ~8 %) and stearic (C18:0; ~7 %) with similar values. In the present study, as aforementioned, the burgers of both batches presented the characteristic fatty acid composition of the lipid sources used in their formulation (HO sunflower oil and meat fat). Additionally, the same formulation in both cases was used, except for the MPC and UP. However, the low amount of these ingredients added to the burgers (3 %) and also the low lipid content of UP (5.2 %; Table 1) determine that these variations had minimal or no effects on the fatty acid composition.

Finally, due to the lack of significant differences in fatty acids profile, the nutritional indices presented also the same values for both, control and UP burgers. Although the values were not significant, UP-B had a lower n-6/n-3 ratio than CO-B. Similarly, the AI and TI values of UP-B were slightly lower than CO-B, but not significant. Our TI and AI values were lower than those reported by other authors in meat emulsion systems reformulated with 5.6 % of wakame [24], but the n-6/n-3 ratio was very higher than those found by these authors.

4. Conclusions

Concerning the use of milk protein concentrate as an emulsifier and meat-product additive, UP resulted in higher yields and lower shrinkages due to better water retention. The changes in texture parameters, mainly the increase in hardness, were principally associated with its principal components, the dietary fiber, while an acceptable color of the burger was maintained using a carmine solution to counteract the possible negative effects of the seaweed pigments (mainly chlorophylls and carotenoids). With the UP incorporation, the cooked burger resulted in higher antioxidant capacity, but this enhanced antioxidant activity was not reflected in lipid oxidation stability. However, despite the higher lipid oxidation values obtained in the reformulated samples, the fatty acid profiles were not impacted by the inclusion of seaweed and therefore their effect on the nutritional quality was insignificant. In addition, the use of UP did not influence the total fat and fatty acids contents. As a general conclusion, the addition of UP improved the techno-functional properties and the antioxidant capacity of low-fat pork burgers with pre-emulsified oil without affecting their nutritional quality and could be a satisfactory strategy for developing healthier meat products. Despite this, it is important to highlight that more studies are necessary, mainly focused on sensory characteristics, to observe how reformulation influences the consumer acceptability of the final product.

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CRediT authorship contribution statement

Nadia Florencia Nagai: Formal analysis, Writing – original draft. **José M. Lorenzo:** Writing – review & editing. **Natalia Ranalli:** Conceptualization, Formal analysis, Writing – original draft. **José Ángel Pérez-Álvarez:** Writing – review & editing. **Nestor Sepulveda:** Writing – review & editing. **Rubén Domínguez:** Writing – original draft, Writing – review & editing. **Eva M. Santos:** Writing – review & editing. **Alfredo Teixeira:** Writing – review & editing. **Silvina Cecilia Andrés:** Conceptualization, Formal analysis, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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