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Research Article

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Fucus vesiculosus extracts as natural antioxidants for improvement of physicochemical properties and shelf life of pork patties formulated with oleogels

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Abstract

BACKGROUND: There is limited information in the literature concerning the feasibility of using algal extracts as natural additives for improvement of the quality and shelf-life of meat products. Hence, a *Fucus vesiculosus* extract (FVE) at the concentrations of 250 mg kg^{-1} (FVE-250), 500 mg kg^{-1} (FVE-500) and 1000 mg kg^{-1} (FVE-1000) were added to pork patties with linseed oil oleogel as a fat replacer.

RESULTS: Total polyphenol content of FVE was determined to be 20 g phloroglucinol equivalents 100 g^{-1} extract. Antioxidant values ranged from 37.5 μmol of Trolox equivalents (TE) g^{-1} (FRAP assay) to 2111 μmol TE g^{-1} extract (ABTS assay). Regarding oxidation stability, FVE-1000 showed the lowest values of thiobarbituric acid-reactive substance and carbonyl content. On the other hand, FVE did not improve color, surface discoloration or odor attributes of patties during storage. Sensory evaluation revealed that there was no significant difference among all studied samples.

CONCLUSION: Although FVEs have a high polyphenol content and antioxidant activities, they are not effective oxidation inhibitors for long-term storage of meat products. Therefore, additional measures or compounds should be considered when FVE is the only antioxidant in meat products.
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Keywords: seaweed extract; lipid and protein oxidation; physicochemical parameters; sensory analysis; antioxidant activity

INTRODUCTION

Changes in muscle foods due to oxidative reactions, both in lipids and proteins, have a strong impact on meat quality, causing a reduction in shelf life as well as a considerable loss of nutrients.[1] Oxidation reactions may occur in a variety of food products, including meat and meat products, as phenomena responsible for the deterioration of nutritional and sensory quality.[1] The changes in organoleptic attributes may result in consumer dissatisfaction and product rejection. For instance, according to Carpenter *et al.*[2] variations in color parameters could affect consumers' purchase decisions, as the product color is often regarded as an indicator of product freshness and quality.[3] Proteins can also be deteriorated by oxidation, leading to loss of amino acids and alterations in their functionality, with negative consequences for product texture.[4]

The scientific community, as well as the meat industry, considers the spoiling of muscle foods as the result of oxidative reactions to be a challenge, leading them to develop strategies to slow down these processes.[1] To keep oxidation reactions in lipids and/or proteins under control, synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), are

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added to meat, fats and oils.[5] However, because of possible toxic effects on human health, serious concerns are raised regarding the addition of these substances to food products.[6]

To overcome this inconvenience, as well as, following consumer trends, to consume more natural products, certain substitutes for synthetic antioxidants have been introduced.[7] Plant extracts or plant-derived compounds increasingly represent alternatives to synthetic additives, which could contribute to delaing oxidation and stabilizing color and texture in foods.[1] The marine environment has been shown to be rich in natural compounds that are rare in terrestrial ecosystems. For example, notably macroalgae and their extracts have many functional properties[8] and constitute a valuable source of antioxidant agents, such as carotenoids, polyphenols, alkaloids, tocopherol and terpenes.[9] *Fucus vesiculosus* is rich in phlorotannins which are particular polyphenolic compounds of brown algae.[10] Some phlorotannins have shown higher antioxidant capacity than commercial antioxidants and are of interest as natural antioxidant alternatives .

Beef burgers are popular products throughout the world.[11] However, consumers tend to perceive animal fats and meat as unhealthy.[11] The replacement of these fats with vegetable oils turns out to be a successful strategy to improve the nutritional profile of muscle foods. Linseed oil might be a good alternative to animal fat for production of these food products. It is the major seed oil, with an α-linolenic acid content of ca 50–55%. Moreover, it has an α-linoleic acid content of ca 15–18%, demonstrating a positive balance between polyunsaturated, monounsaturated and saturated fatty acids (FAs).[12] Unfortunately, the replacement of hard fats with oils usually leads to a decrease in product quality, resulting in altered properties, concerning mainly textural attributes and sensory characteristics.[13] Consequently, the structuring of edible oils, while respecting their fat composition, is actively researched. The use of gelling agents has recently proved to be an efficient approach for this purpose. These agents are organic liquids, known as oleogels, that are encased in thermoreversible three-dimensional gel networks.[14] These oleogels maintain the FA profile of the oils, as well as the functionality and texture of the final product.

In this context, the aim of this investigation was to evaluate the effectiveness of *F. vesiculosus* extracts to extend the shelf life of pork patties manufactured with linseed oil oleogels and packaged under conditions of modified atmosphere during refrigerated storage. The physicochemical properties and sensory attributes of seaweed-based pork patty formulations were compared with control samples that were formulated without any antioxidant or with BHT.

MATERIAL AND METHODS

Algal material

The brown seaweed *F. vesiculosus* (Kingdom: Chromista, Phylum: Ochrophyta, Class: Phaeophyceae and Order: Fucales) was supplied by Portomuiños company (A Coruña, Spain). The algal material was collected at the Galician Atlantic coast, in the area of Camariñas, a village close to the town of A Coruña (NW Spain). It was subjected to milling with a conventional homemade mincer until particles ≤0.8 mm were obtained using a porous mesh. After this treatment, the ground seaweed was packaged in plastic bags under vacuum at 75% and stored at −20 °C until further use.

Obtaining *F. vesiculosus* extracts

The algal material was extracted with a mixture of water/ethanol (50:50, v/v) in the proportion of 1:10 (w/v) for 30 min, using an ultrasound bath at room temperature. The solid residue was separated from the solvent by centrifugation for 10 min at 3000 x*g* and 4 °C. The supernatant was collected and filtered under vacuum using a simple laboratory filter. The filtrate was subjected to vacuum at 40 °C using a rotary evaporator to remove the ethanol. Finally, the aqueous extract was freeze-dried and kept at −20 °C until further use. The seaweed extract was resuspended in water:ethanol (50:50, v/v) before its use.

Preparation of linseed oil oleogel

The oleogel used in the present study consisted of an oil phase and a mixture of two structuring agents (Ɣ-oryzanol and β-sitosterol). The oil phase was commercial linseed oil (Vitaquell®) containing 72% polyunsaturated (ca 55% α-linoleic), 19% monounsaturated and 9% saturated FAs. The Ɣ-oryzanol and β-sitosterol were mixed in a ratio of 60:40 (w/w). This ratio corresponds to a mole ratio of 1:1 and was reported to be the ratio that allows formation of the firmest transparent gel. Ɣ-Oryzanol and β-sitosterol were purchased from Oryza Co. (Japan) and Sigma-Aldrich (France), respectively. Both structuring agents were dispersed by stirring until solubilization, together with the linseed oil, at 80 °C for 30 min. Then, the mixture was left to cool at room temperature until formation of the gel.

Manufacture of pork patties

A total of 125 patties divided into 25 units per treatment (5 treatments) were manufactured in the pilot plant at the Centro Tecnolóxico da Carne, Ourense, Spain. Different treatments were carried out as follows: patties with seaweed *F. vesiculosus* extract (FVE) as antioxidant incorporated at 250 (FVE-250), 500 (FVE-500) and 1000 (FVE-1000) mg kg^{-1}; patties with BHT added at 200 mg kg^{-1} as conventional synthetic antioxidant; and patties without antioxidant (CO) as control. Patties were manufactured with prime cuts of pig loin purchased in a local market. The meat samples were chopped under refrigeration in a mincer machine (La Minerva, A/E 22R, Bologna, Italy) and then mixed with linseed oil oleogel, salt and water in the proportions indicated in Table 1. Patty samples of 60 g were produced using a burger maker (A-2000, Gaser, Girona, Spain). After production, the patties were wrapped in polystyrene trays of 300 mm thickness under 80% O_2 and 20% CO_2, using a LARI3/Pn T-VG-R-SKIN heat sealer (Ca. Ve.Co., Palazzolo, Italy). Polyethylene film [thickness 74 mm, with permeability less than 2 mL (m^2 bar day^{-1}) $^{-1}$] appropriate for gas mixtures was used (Viduca, Alicante, Spain) and stored at 2 ± 1 °C under light in order to simulate the storage condition of supermarkets. Lux values varied in the range of 15–20 as a function of the tray position (Digital luxometer HT 306, Faenza, Italy). A conventional light source was used in this study, i.e. no wavelength or range of lengths, such as UV, was filtered. Pork patties were analyzed at the initial day for chemical composition and FA profile, and at 0, 7, 11, 15 and 18 days of storage for physicochemical parameters (pH and color) and stability to oxidation [values of thiobarbituric acid-reactive substance (TBARs) and protein oxidation]. In addition, odor and visual acceptance were evaluated at all sampling time points, whereas preference tests were performed at the beginning of storage [5 patties (units) per treatment]. At each sampling time point, 4 units of each type were taken for analysis.

Total phenolic content (TPC) and evaluation of the antioxidant activity of the seaweed extracts

TPC and total antioxidant capacity (evaluated with radical cation decolorization assay (ABTS), oxygen radical absorbance capacity

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Table 1. Ingredient proportions used in the manufacture of pork patties with several added antioxidants

	Treatment				
Ingredient (g 100 g^{-1})	CO	BHT	FVE-250	FVE-500	FVE-1000
Pork loin	87	87	87	87	87
Linseed oil oleogel	5	5	5	5	5
Salt	1.04	1.04	1.04	1.04	1.04
Water	6.96	6.96	6.96	6.96	6.96
BHT	0	0.02	0	0	0
Seaweed extract	0	0	0.025	0.05	0.1

Abbreviations: BHT, addition of 200 mg kg^{-1} tert-butyl-4-hydroxytoluene; CO, control without any antioxidant; FVE-250, FV-500 and FV-1000, addition of *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1}, respectively.

assay (ORAC), radical scavening assay (DPPH) and ferric reducing antioxidant power (FRAP) assays) were determined according to methods previously described in detail by Agregán *et al.*[9] who specialize in research on algal samples.

Chemical composition and fatty acid (FA) profile of pork patties

Protein, moisture and ash content were determined following ISO recommended standards, i.e. ISO 937:1978,[15] ISO 1442:1997,[16] ISO 936:1998,[17] respectively. Fat was extracted using an extractor (Ankom XT10, ANKOM Technology Corp., Macedon, NY, USA) and was quantified following the official AOCS procedure Am 5–04.[18] The FA profile was determined according to methods described by Fernandes *et al.*[7] using a gas chromatograph equipped with an FID detector (GC Agilent 7890 N; Agilent Technologies Spain, S.L., Madrid, Spain).

Determination of physical parameters of pork patties

A portable digital pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration probe was employed to measure the pH of pork patties. The color parameters of the CIELAB color space system, including lightness (L*), redness (a*) and yellowness (b*), were determined using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) equipped with a pulsed xenon arc lamp filtered to D65 illuminant lighting conditions, with a zero-degree viewing angle geometry and aperture size of 8 mm. The color was measured at six different points on the patty surface after 30 min of exposure to the environmental atmosphere.

Determination of protein and lipid oxidation indices

Protein oxidation was evaluated based on the procedure described by Mercier *et al.*[19] and results were expressed as nmol carbonyl mg^{-1} protein. The stability of pork patty lipid was assessed using the TBARs test, following the method proposed by Vyncke[20] and expressing the results as mg malonaldehyde (MDA) kg^{-1} pork patty.

Sensory evaluation of raw and cooked pork patties

Sensory analysis of raw and cooked patties was carried out by 16 trained panellists who were selected from the *Centro Tecnolóxico da Carne*. A test of acceptance of raw patties was conducted at 0, 7, 11, 15 and 18 days of storage, as well as a test of acceptance of and preference for cooked patties. In the acceptance test, panelists accepted or rejected the samples, and in the preference test, panelists classified the samples from best to worst according to taste. In the acceptance test, panelists scored raw samples based on color, surface discoloration and odor, and they scored cooked samples with respect to odor and taste using a 5-point hedonic scale (from 1, excellent to 5, not acceptable). In the test of preference for cooked samples, panelists with a score of 1, favorite. Patties were cooked in an oven (Rational Combimasterplus CMP61, Germany) until a core temperature of 70 °C was reached. Sensory tests were carried out in an individual cabin illuminated with white light. Samples were served on disposable plastic dishes marked with 3-digit random numbers.[21] Water and unsalted toasted bread were provided at the beginning and between tests of samples to remove residual flavors from the mouth.

Statistical analyses

Statistical analysis of the results was conducted using the IBM SPSS Statistics® 23.0 program (IBM Corporation, Somers, NY, USA). One-way analysis of variance (ANOVA) was applied to all variables assessed in this study. Time and different treatments were taken to be fixed variables and chemical composition (moisture, fat, protein and ash content), FA profile, pH, color and oxidation parameters (TBARs values and protein oxidation) were taken to be independent variables. The least square means (LSM) were separated using Duncan's *post hoc* test (significance level $P < 0.05$). Friedman's test (significance level $\alpha < 0.05$) was applied to cooked patties in the sensorial analysis to determine the panelists' preferences.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant capacity of *F. vesiculosus* extracts

The TPC of FVEs was ca 20 g of phloroglucinol equivalents (PGE) 100 g^{-1} extract. These results contrast with those obtained by Agregán *et al.*[8] who reported very low contents of phenolic compounds in aqueous extracts of several brown seaweeds (*Bifurcaria bifurcate, F. vesiculosus* and *Ascophyllum nodosum*), with values ranging from 0.96 to 1.99 g PGE 100 g^{-1} FVE. Wang *et al.*[22] found 17.6 g PGE 100 g^{-1} FVE in a aqueous extracts of *F. vesiculosus*, which is similar to that found in the present study. According to these authors,[22] phenolic compounds are more easily extractable with polar organic solvents than with water. Aqueous mixtures of methanol, ethanol or acetone were recommended for this purpose,[23] the use of which resulted in a yield of 24.2 g PGE 100 g^{-1} FVE in a 70% (v/v) acetone extract from *F. vesiculosus*, not higher than that found in the current investigation. Moreover, almost all brown algae tested in the study carried out by

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Waterman and Mole[23] had higher amounts of polyphenols than the red and green algae.

Similarly, according to Jiménez-Escrig *et al.*,[24] the brown seaweeds tested had higher TPCs than the red seaweeds, whereas the organic polar solvents were found to be more efficient in extracting polyphenolic compounds than water. However, no TPC value obtained for the seaweed extracts analyzed exceeded 3.5 g PGE 100 g^{-1} FVE, which is considerably lower than the values obtained in the present study for the FVEs, even when a combination of organic polar solvents, such as methanol and water, was used at the same ratios as applied in the current investigation. Jiménez-Escrig *et al.*[24] and Farvin and Jacobsen[25] reported high TPCs in all the *Fucus* species investigated. Generally, brown algae are richer in phenolic compounds than other types of algae due to their high content of phlorotannins, a family of polyphenol compounds exclusively found in brown algae in special vesicles (physodes) within the cells.

The antioxidant value obtained using the ABTS test was 2111 ± 22.63 μmol TE g^{-1} FVE (IC_{50}, 0.65 ± 0.01). In a previous study, Agregán *et al.*[8] reported lower values, ranging from 100 to 1100 μmol TE/g Trolox equivalents FVE in aqueous extracts of three brown seaweeds (*A. nodosum, F. vesiculosus* and *Bifurcaria bifurcata*) using the same assay (which showed the FVE value to be the highest). In this case, the lower values could be related to the lower polyphenol contents, since all these extracts had much lower concentrations of polyphenols than the extract used in the present study. Moreover, the positive correlation between polyphenol content and antioxidant capacity has been well documented.[22,25]

As for the ORAC assay, a value of 1598.5 ± 34.65 μmol TE g^{-1} FVE was found, which is comparable to that reported by Wang *et al.*[22] (i.e. 1417 μmol TE g^{-1} FVE obtained with a 70% acetone extract) for *A. nodosum*. However, higher values were found by these authors when they used 70% acetone to obtain the extracts from *F. vesiculosus* and *Fucus serratus* (2567 and 2567 μmol TE/g^{-1} FVE, respectively), and also in water extracts of the *Ascophylum nodosum* (2000 μmol TE/g^{-1} FVE). However, Agregán *et al.*[8] reported a considerably lower value for the water extract from *F. vesiculosus* (756.5 μmol TE/g^{-1} FVE).

The DPPH assay of FVE revealed values of 278 ± 1.41 μmol TE g^{-1} extract (IC_{50}, 3.47 ± 0.01 g L^{-1}). In this context, Agregán *et al.*[8] also used the DPPH assay to measure antioxidant capacity, reporting a lower value in the water extract from *F. vesiculosus* (135.31 μmol TE/g FVE) with a consequently higher value for its IC_{50} (4.19 g L^{-1}), as the lower IC_{50} value of the extract reveals higher radical-scavenging activity. Farvin and Jacobsen[25] found that the water extracts of 2 *Fucus* species, namely *F. vesiculosus* and *F. serratus*, had the highest antioxidant activities (IC_{50} of 0.0083 g L^{-1} in both cases) among other studied species. The ethanol extracts of these algal species also exhibited the highest radical-scavenging activities, with an IC_{50} of 0.0099 g L^{-1} and 0.0092 g L^{-1} for *F. vesiculosus* and *F. serratus*, respectively. The IC_{50} values reported in the study by Farvin and Jacobsen[25] were lower than those obtained for the FVEs analyzed in the current study, indicating a higher antioxidant capacity of these extracts in the previously conducted research. In another study, Rajauria *et al.*[26] found lower antioxidant capacities also in water and methanol extracts from *Himanthalia elongata* alga, in comparison with those of the previous extracts.[25] According to Connan *et al.*,[27] environmental factors and intrinsic features of algae, such as light or salinity and age or length, could lead to notable variations in the phenolic content of algae that may be responsible for the differences observed in their antioxidant capacities.

A value of 37.5 ± 2.12 μmol TE g^{-1} extract was recorded when a FRAP assay of the FVE was used. This result was very close to the results reported by Rajauria *et al.*[26], i.e. 41.15 μmol TE g^{-1} FVE and 46.75 μmol TE g^{-1} FVE obtained with 40% and 60% methanol extracts, respectively, from *H. elongata* brown seaweed. The FRAP assay was also used by Agregán *et al.*[8] to measure the antioxidant capacity of seaweeds, and a slightly higher value was found in the aqueous extract from *F. vesiculosus* (51.66 μmol TE/g FVE) compared to the values in extracts from *A. nodosum* and *B. bifurcata* (7.52 and 26.93 μmol TE g^{-1} FVE respectively).

Effect of FVE incorporation on the proximate composition and FA profile of pork patties

As shown in Table 2, which gives the chemical composition (protein, fat, moisture and ash content) and FA profile of pork patties, no significant differences in chemical composition were observed among the different formulations ($P > 0.05$). Moreover, the different concentrations of FVE in patties did not influence the percent values for moisture, fat, protein and ash. These results are in agreement with those reported by Rodríguez Carpena *et al.*,[28] who found no changes regarding the proximate composition of raw pork meat patties after incorporation of Mediterranean berries or avocado by-products.

The monounsaturated FAs (MUFAs) were the most abundant type of FA among all formulations, with an average percentage of 41.10% of the total FAs analyzed, followed by saturated (SFA) and polyunsaturated FA (PUFA) compounds, with an average percentage of 31.95 and 25.74%, respectively. As expected, the percentage of PUFAs was considerably higher than those found by other authors in previous studies regarding pork meat without added vegetable oils.[29] This difference in FA profile may be attributed to the replacement of pork fat by linseed oil in the patties as seed oils are well known to possess high amounts of PUFAs. Similar findings were reported by Delgado-Pando *et al.*[30] after replacement of pork fat with olive, linseed or fish oils, and also by Rodríguez-Carpena *et al.*[31] after replacement of pork fat with sunflower oil.

Oleic acid (C18:1*n*-9c), the most abundant MUFA and one of the main FAs determined after all treatments, was found in highest amounts in the CO patties. All patties contained substantial amounts of palmitic (C16:0) and stearic (C18:0) acids as the most abundant SFAs, at an average of 19.64 and 10.93%, respectively. Similar results were obtained in studies on porcine meat. According to Pateiro *et al.*,[29] who investigated fat oxidation in enriched pâtés, percentages of ca 20–22% and 10–12% for palmitic and stearic acids respectively, were observed. On the other hand, linoleic (C16:2*n*-6) and linolenic (C18:3*n*-3) acids, the most abundant PUFAs, were also present at a high percentage, with average values of 13.16% and 11.79% respectively. These results were predictable, taking into account that linseed oil is very rich in linoleic (15–18%) and linolenic (50–55%) acids. Concerning a low-fat pork liver pâté, Delgado-Pando *et al.*[30] reported a significant ($P < 0.05$) increase in linoleic and linolenic acids when the pork fat was totally or partially replaced by a mixture of olive, linseed or fish oils.

Omega-6 and omega-3 FAs are essential fatty acids for humans because of the inability of the human body to synthesize them. Therefore, these FAs must be ingested via the intake of foods or supplements. Nevertheless, the consumption of diets with high amounts of omega-6 PUFAs and very high *n*-6/*n*-3 ratios promotes several diseases, including cardiovascular diseases and certain types of cancer.[32] On the other hand, high PUFA levels

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Table 2. Effect of addition of *Fucus vesiculosus* extract and BHT on proximate composition and fatty acid (FA) profile (% of total FAs) of pork patties on one given day [mean ± standard error ($n = 4$)]

	Treatment						
	CO	BHT	FVE-250	FVE-500	FVE-1000	SEM	Sig.
Moisture (%)	64.55 ± 0.90	64.92 ± 0.47	65.17 ± 0.98	65.24 ± 0.20	64.63 ± 1.42	0.19	ns
Fat (%)	13.98 ± 1.23	13.34 ± 0.69	13.52 ± 1.05	13.75 ± 0.46	13.51 ± 0.66	0.18	ns
Protein (%)	17.22 ± 1.13	16.81 ± 0.44	16.70 ± 0.27	16.54 ± 0.28	16.60 ± 0.14	0.13	ns
Ash (%)	2.05 ± 0.04	2.10 ± 0.03	2.03 ± 0.04	2.04 ± 0.04	2.09 ± 0.05	0.01	ns
			FA profile				
C14:0	1.03 ± 0.01^{b}	0.98 ± 0.02^{a-c}	1.00 ± 0.02^{a}	1.01 ± 0.01^{a-c}	1.00 ± 0.02^{a-c}	0.01	*
C16:0	19.99 ± 0.24^{b}	19.24 ± 0.14^{a-c}	19.65 ± 0.27^{b}	19.70 ± 0.11^{b}	19.64 ± 0.33^{b}	0.07	**
C16:1*n*-7	1.86 ± 0.01^{b}	1.79 ± 0.02^{a-c}	1.79 ± 0.03^{a-c}	1.85 ± 0.03^{b}	1.82 ± 0.03^{a,b}	0.01	**
C17:0	0.18 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.00	ns
C17:1*n*-7	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.15 ± 0.00	0.15 ± 0.01	0.00	ns
C18:0	11.05 ± 0.19	10.79 ± 0.18	10.97 ± 0.18	10.93 ± 0.12	10.93 ± 0.20	0.04	ns
C18:1*n*-9 t	0.18 ± 0.01	0.17 ± 0.00	0.18 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.00	ns
C18:1*n*-9c	36.18 ± 0.08^{c}	35.38 ± 0.26^{a-c}	35.76 ± 0.38^{a,b}	36.06 ± 0.18^{b,c}	35.68 ± 0.31^{a,b}	0.08	**
C18:1*n*-7c	2.45 ± 0.07	2.46 ± 0.02	2.46 ± 0.13	2.47 ± 0.15	2.45 ± 0.14	0.02	ns
C18:2*n*-6	12.92 ± 0.06	13.25 ± 0.21	13.08 ± 0.23	13.18 ± 0.48	13.38 ± 0.16	0.06	ns
C20:0	0.20 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.19 ± 0.01	0.20 ± 0.00	0.00	ns
C20:1*n*-9	0.69 ± 0.03	0.70 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.69 ± 0.03	0.01	ns
C18:3*n*-3	11.28 ± 0.46	12.68 ± 0.63	11.94 ± 1.01	11.40 ± 0.31	11.63 ± 1.00	0.19	ns
C20:2*n*-6	0.39 ± 0.00	0.39 ± 0.01	0.40 ± 0.02	0.40 ± 0.01	0.40 ± 0.02	0.00	ns
C20:4*n*-6	0.36 ± 0.03^{a-c}	0.41 ± 0.01^{b}	0.39 ± 0.01^{a,b}	0.40 ± 0.03^{b}	0.40 ± 0.01^{b}	0.01	*
SFA	32.44 ± 0.44^{b}	31.38 ± 0.27^{a-c}	31.99 ± 0.46^{a,b}	32.01 ± 0.21^{a,b}	31.95 ± 0.55^{a,b}	0.11	*
MUFA	41.50 ± 0.14^{c}	40.65 ± 0.28^{a-c}	41.01 ± 0.36^{a,b}	41.38 ± 0.27^{b,c}	40.96 ± 0.35^{a,b}	0.09	**
PUFA	24.96 ± 0.39^{a}	26.73 ± 0.51^{b}	25.81 ± 0.77^{a,b}	25.38 ± 0.39^{a-c}	25.81 ± 0.84^{a,b}	0.18	*
∑*n*-3	11.28 ± 0.46	12.68 ± 0.63	11.94 ± 1.01	11.40 ± 0.31	11.63 ± 1.00	0.19	ns
∑*n*-6	13.68 ± 0.07	14.05 ± 0.22	13.87 ± 0.26	13.98 ± 0.51	14.17 ± 0.17	0.07	ns
n-6/*n*-3	1.21 ± 0.06	1.11 ± 0.07	1.17 ± 0.11	1.23 ± 0.07	1.23 ± 0.12	0.02	ns

Abbreviations: BHT, addition of 200 mg kg^{-1} tert-butyl-4-hydroxytoluene; CO, control without any antioxidant; FVE-250, FV-500 and FV-1000, addition of *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1} respectively;
SEM, standard error of mean;
Sig., significance: *, $P < 0.05$; **, $P < 0.01$; ns, not significant;
SFA, Σ (C14:0 + C16:0 + C17:0 + C18:0 + C20:0) ;
MUFA, Σ (C16:1*n*-7 + C17:1*n*-7 + C18:1*n*-9 t + C18:1*n*-9c + C18:1*n*-7c + C20:1*n*-9) ;
PUFA, Σ (C18:2*n*-6 + C18:3*n*-3 + C20:2*n*-6 + C20:4*n*-6);
Σn-6, Σ (C18:2*n*-6 + C20:2*n*-6 + C20:4*n*-6);
Σn-3, Σ (C18:3*n*-3);
$^{a-c}$Means in the same row not followed by a common superscript letter are significantly different ($P < 0.05$; Duncan's test).

and low *n*-6/*n*-3 ratios induce suppressive effects.[32] Pork patties in the present study showed *n6*/*n*-3 ratios between 1 and 2 for all treatments, without significant differences ($P > 0.05$). These results are in agreement with the recommendation given by Simopoulos[32] that the target *n*-6/*n*-3 ratio in a healthy diet should be balanced between 1:1 and 2:1.

Effect of FVE on physical properties of pork patties during storage

The results of the evaluation of pH and color parameters in pork patties during refrigerated storage are shown in Table 3. The different patty formulations led to significant pH differences between values at Day 0 ($P < 0.001$) and Day 18 ($P < 0.01$). In this regard, Lorenzo *et al.*[3] reported higher pH values during storage of pork patties manufactured with chestnut and seaweed extracts as compared to patties manufactured with tea and grape seed extracts. On the other hand, in the current study, refrigerated storage modified the pH values of patties in all formulations, with the exception of the FVE-500 treatment. The pH values were significantly different in the CO- and BHT- ($P < 0.01$) treated patties, and in the FVE-250- ($P < 0.05$) and FVE-1000- ($P < 0.001$) treated patties. A marked decrease was observed at day 11, with the exception of the BHT treatment, after which the pH increased sharply until Day 15. Following FVE-250 treatment, however, the pH began to increase at Day 15. Similar results were observed in pork patties with added natural extracts during refrigerated storage.[3]

Different formulations of patties, as well as storage time, also affected the color parameters L*, a* and b*. However, the lightness (L*) did not seem to follow any specific trend over storage time, despite a significant ($P < 0.05$) change after the BHT, FVE-250 and FVE-1000 treatments. The L* values were not affected by different formulations, as previously reported by Lorenzo *et al.*[3] Regarding redness (a*), a progressive loss of red surface color of the patties was noted over storage. Similar trends were observed in other investigations with meats from different species.[7] The reduction of redness on the patty surface during storage confirmed the appearance of a phenomenon of fading as this process is marked mainly by the loss of a* values,[3] which is attributed to the metmyoglobin

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Table 3. Effect of addition of *Fucus vesiculosus* extract and BHT on the evolution of pH and color parameters (L*, a* and b*) during refrigerated storage of pork patties [mean ± standard error (n = 4)]

	Treatment						
Day	CO	BHT	FVE-250	FVE-500	FVE-1000	SEM	Sig.
0	5.65 ± 0.02 ^{a,1}	5.68 ± 0.02 ^{a,b,1}	5.70 ± 0.02 ^{b,1,2}	5.71 ± 0.02 $^{1-3}$	5.75 ± 0.02 ^{c,2}	0.01	***
7	5.73 ± 0.04 ^{2,3}	5.66 ± 0.10 ^{1}	5.74 ± 0.05 ^{2}	5.75 ± 0.08	5.74 ± 0.04 ^{2}	0.02	ns
11	5.68 ± 0.03 ^{1,2}	5.66 ± 0.01 ^{1}	5.67 ± 0.04 ^{1}	5.65 ± 0.01	5.65 ± 0.02 ^{1}	0.01	ns
15	5.74 ± 0.02 ^{3}	5.77 ± 0.05 ^{2}	5.66 ± 0.06 ^{1}	5.76 ± 0.09	5.72 ± 0.03 ^{2}	0.01	ns
18	5.74 ± 0.04 ^{a,b,3}	5.78 ± 0.02 ^{c,2}	5.76 ± 0.04 ^{b,c,2}	5.71 ± 0.01 $^{a-c}$	5.72 ± 0.01 ^{a,2}	0.01	**
SEM	0.01	0.02	0.01	0.01	0.01		
Sig.	**	**	*	ns	***		
0	62.09 ± 1.28	63.11 ± 1.84 ^{1,2}	62.01 ± 0.19 ^{1}	61.15 ± 0.51	60.06 ± 3.02 ^{1}	0.41	ns
7	61.61 ± 1.99	65.54 ± 0.20 ^{2}	62.31 ± 2.32 ^{1}	63.48 ± 3.81	61.51 ± 1.39 ^{1,2}	0.57	ns
11	59.80 ± 2.47	63.84 ± 3.43 ^{1,2}	63.51 ± 1.53 ^{1,2}	62.47 ± 1.72	64.94 ± 1.76 ^{2}	0.61	ns
15	64.02 ± 1.53	61.37 ± 1.58 ^{1}	65.72 ± 2.27 ^{2}	62.02 ± 2.35	63.43 ± 2.20 ^{1,2}	0.53	ns
18	61.52 ± 1.78	60.09 ± 3.16 ^{1}	61.59 ± 1.29 ^{1}	62.79 ± 2.41	61.99 ± 1.92 ^{1,2}	0.48	ns
SEM	0.48	0.64	0.48	0.51	0.57		
Sig.	ns	*	*	ns	*		
0	12.03 ± 0.41 ^{b,3}	11.10 ± 0.96 ^{a,b,3}	9.88 ± 0.64 ^{a,3}	10.25 ± 0.77 ^{a,4}	10.33 ± 1.36 ^{a,4}	0.25	*
7	9.35 ± 1.40 ^{2}	8.91 ± 0.62 ^{2}	9.17 ± 2.27 ^{2,3}	8.35 ± 1.53 ^{3}	8.70 ± 1.07 ^{3}	0.31	ns
11	8.86 ± 1.40 ^{2}	8.19 ± 0.65 ^{1,2}	7.88 ± 0.68 ^{2}	8.08 ± 0.79 ^{2,3}	7.18 ± 0.95 ^{2}	0.22	ns
15	6.07 ± 0.87 ^{1}	7.19 ± 0.29 ^{1}	5.62 ± 0.86 ^{1}	6.63 ± 1.05 ^{2}	7.02 ± 0.59 ^{2}	0.21	ns
18	4.72 ± 0.58 ^{a,1}	7.36 ± 1.30 ^{b,1}	4.94 ± 0.52 ^{a,1}	5.07 ± 0.78 ^{a,1}	5.54 ± 0.65 ^{a,1}	0.27	**
SEM	0.62	0.36	0.50	0.45	0.42		
Sig.	***	***	***	***	***		
0	22.51 ± 0.64 ^{b,3}	21.58 ± 0.51 ^{a,b,3}	20.75 ± 0.77 ^{a,2}	20.92 ± 1.07 $^{a-c}$	21.09 ± 0.81 $^{a-c}$	0.21	*
7	19.86 ± 1.07 ^{2}	19.62 ± 1.69 ^{1,2}	20.55 ± 1.62 ^{2}	19.81 ± 2.60	20.85 ± 1.99	0.39	ns
11	19.36 ± 0.44 ^{2}	20.71 ± 0.64 ^{2,3}	19.67 ± 0.70 ^{1,2}	20.33 ± 1.12	20.06 ± 1.30	0.21	ns
15	17.91 ± 1.65 ^{a,1}	19.10 ± 0.76 ^{a,b,1}	19.18 ± 1.17 ^{a,b,1,2}	19.95 ± 1.17 $^{1-3}$	20.78 ± 0.91 $^{1-3}$	0.32	*
18	17.65 ± 0.30 ^{1}	18.91 ± 0.71 ^{1}	18.04 ± 1.47 ^{1}	18.65 ± 1.06	19.22 ± 0.71	0.23	ns
SEM	0.44	0.30	0.33	0.35	0.29		
Sig.	***	**	*	ns	ns		

Abbreviations: BHT, addition of 200 mg kg^{-1} tert-butyl-4-hydroxytoluene; CO, control without any antioxidant; FVE-250, FV-500 and FV-1000, addition of *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1}, respectively; SEM, standard error of mean; Sig., significance;
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant
$^{a-c}$Means in the same row (different treatments on the same storage day) not followed by a common superscript letter are significantly different ($P < 0.05$; Duncan's test);
$^{1-3}$Means in the same column (same treatment in different storage days) not followed by a common superscript number are significantly different ($P < 0.05$; Duncan's test).

produced through oxidation of myoglobin. From the beginning of storage, patties showed a progressive brownish appearance on the surface until the final storage state. Brown color in pork meat is assigned a* values ranging between 4.6 and 10.8.[33]

Overall, the addition of antioxidants to pork patties affected redness with progressing time, although differences were only significant at the beginning ($P < 0.05$) and at the end ($P < 0.01$) of the storage period. At Day 0, patties manufactured with eaweed extract showed lower a* values than CO patties. On the contrary, at Day 18 the result was reversed, i.e. the seaweed antioxidants supported the protection of red color on the patty surface. This stabilizing effect on a* values was also found by other researchers using different natural antioxidants, such as grape seed,[3] oregano,[7,34] borage, rosemary[34] and avocado extracts.[28] Finally, yellowness (b*) also decreased over storage time, although less markedly than redness. These results were in good agreement with those obtained by Fernandes *et al.*[7] in sheep patties with added BHT and oregano as antioxidants. The different formulations affected the b* values of patties during refrigerated storage. Greater retention of the color yellow was achieved in treatments with seaweed extract as compared to CO.

Barbut *et al.*[13] found that replacement of beef fat with canola oil oleogels made with different amounts of ethylcellulose and sorbitan monostearate induced differences in the lightness and redness of frankfurters. In this context, L* values were increased with fat replacement by oleogel in most of the treatments, although the differences were not significant ($P > 0.05$). Conversely, a* values decreased significantly ($P < 0.05$) in formulations with oleogel. Similar results were obtained for dry fermented sausages with partial pork back-fat replacement by konjac gel.[35] On the other hand, according to Barbut *et al.*,[13] the use of canola oil that was not in gel form had a significant ($P < 0.05$) effect on color, in that the L* value increased and the a* value decreased with respect to CO. Considering that the addition of a seed (canola) oil generated changes in the surface color of the final patty product, it can be assumed that the addition of linseed oil to our samples induced some modification in the color of patties. This hypothesis is supported by the results reported by Utrilla *et al.*[36] who found that higher olive oil

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Table 4. Change of TBARS values during refrigerated storage of pork patties manufactured with different antioxidants [mean ± standard error (n = 4)]

	Treatment						
Day	CO	BHT	FVE-250	FVE-500	FVE-1000	SEM	Sig.
	$0.13 \pm 0.00^{e,1}$	$0.10 \pm 0.00^{c,1}$	$0.09 \pm 0.00^{b,1}$	$0.12 \pm 0.00^{d,1}$	$0.08 \pm 0.00^{a,1}$	0.00	***
0	0.87 ± 0.17^{2}	0.58 ± 0.11^{2}	0.74 ± 0.14^{2}	$0.66 \pm 0.13^{1,2}$	0.76 ± 0.15^{2}	0.04	ns
7	$1.44 \pm 0.01^{d,2}$	$0.78 \pm 0.09^{a,3}$	$1.07 \pm 0.01^{b,2}$	$1.19 \pm 0.01^{c,2}$	$1.09 \pm 0.01^{b,2}$	0.05	***
11	$3.92 \pm 0.63^{b,3}$	$0.96 \pm 0.15^{a,4}$	$3.70 \pm 0.59^{b,3}$	$3.66 \pm 0.52^{b,3}$	$3.54 \pm 0.57^{b,3}$	0.30	***
15	$4.09 \pm 0.66^{b,3}$	$1.00 \pm 0.16^{a,4}$	$3.87 \pm 0.62^{b,3}$	$3.76 \pm 0.75^{b,3}$	$3.69 \pm 0.59^{b,3}$	0.31	***
18	0.38	0.08	0.37	0.46	0.35		
SEM	***	***	***	***	***		
Sig.	$0.13 \pm 0.00^{e,1}$	$0.10 \pm 0.00^{c,1}$	$0.09 \pm 0.00^{b,1}$	$0.12 \pm 0.00^{d,1}$	$0.08 \pm 0.00^{a,1}$	0.00	***

Abbreviation: BHT, addition of 200 mg kg^{-1} tert-butyl-4-hydroxytoluene; CO, control without any antioxidant; FVE-250, FV-500 and FV-1000, addition of *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1} respectively;
SEM, standard error of mean;
Sig., significance; ***, $P < 0.001$; ns, not significant;
[a-e]Means in the same row (different treatments on the same storage day) not followed by a common superscript letter are significantly different ($P < 0.05$; Duncan's test);
[1-4]Means in the same column (same treatment on different storage days) not followed by a common superscript number are significantly different ($P < 0.05$; Duncan's test).

content in dry-ripened sausages produced higher and lower values of b* and a*, respectively, at the beginning of ripening.

Oxidative stability during refrigerated storage of pork patties

Lipid oxidation was quantified in patty samples using the TBARs assay (Table 4). All treatments resulted in a significant ($P < 0.001$) increase in TBARs values during refrigerated storage. According to the results of the present study, patties containing BHT and FVE-1000 had higher oxidative stability than the CO and FVE-250- and FVE-500-containing patties (Table 4).

TBARs values increased slightly until day 11, after which an abrupt increase was noted for up to 15 days. Lorenzo *et al.*[3] and Sánchez-Escalante *et al.*[34] reported similar changes in TBARs values in patties with added natural antioxidants during refrigerated storage. The latter authors found a sharp increase in oxidation from Day 4 (ca 0.8 mg MDA kg^{-1} sample) to Day 12 (ca 3.8 MDA kg^{-1} sample) in the CO. In the present study, oxidation showed a sharp increase with comparable TBARs values (0.87 ± 0.17 and 3.92 ± 0.63 mg MDA kg^{-1} sample respectively). After Day 12, Sánchez-Escalante *et al.*[34] observed a stabilization of, and even a slight decrease in, the TBARs values. On the other hand, they noted that these values for some of the patties with added natural antioxidants, such as oregano, rosemary and rosemary with ascorbic acid, remained very stable until Day 16, with values below 1 MDA kg^{-1} sample, and that the values increased sharply until the end of storage, reaching values between 1 and 3 mg MDA kg^{-1} sample, lower than those found by us at Day 18 for patties with added seaweed extract.

The evolution of protein oxidation in pork patties during storage is shown in Table 5. A significant ($P < 0.01$) increase in protein oxidation over time was found with all treatments. Similar findings were reported by other authors.[7,28] Protein oxidation began on Day 7 and increased until the end of storage. The CO patties were the most sensitive to protein oxidation, reaching values of 6.81 ± 0.61 nmol carbonyl mg^{-1} protein at Day 18. The BHT treatment was the most effective, resulting in patties with the lowest carbonyl values. The treatments with FVE reduced the protein oxidation significantly with respect to the CO without antioxidant at the last day of storage, reaching carbonyl values between 0.77 and 1.15 nmol mg^{-1} protein. The FVE-1000 treatment also resulted in carbonyl values significantly ($P < 0.05$) lower than those of the CO at day 15. This result suggests that addition of FVE decreased the final carbonyl concentrations, especially at 1000 mg extract kg^{-1} sample. Other natural extracts, such as peel and seed extracts from avocado[28] and oregano extracts[7] entailed a delay in carbonyl formation when they were added to pork, beef or sheep meats. Nutritional quality and tenderness are appreciated attributes of meat which are affected by protein oxidation. For this reason its inhibition is important to protect raw meat against these undesirable effects.[7] The phenolic content found in the FVE probably protected patties against oxidative stress, which delayed the appearance of degradation products.

Effect of FVE on sensory properties of pork patties

Figures 1 and 2 show the findings of the sensory evaluation of cooked and raw pork patties at Day 0 and at Days 0, 7, 11, 15 and 18, respectively. Samples cooked at Day 0 did not show any significant differences in odor and taste, independent of treatment. The most significant results can be correlated to the attribute odor, with the highest acceptation received for the FVE-500-treated sample, while no differences in taste preference were found among the CO and the FVE-500- and FVE-1000-treated samples. Bañón *et al.*[37] reported similar findings with no appreciable differences in odor and taste preferences at Day 0 in low-sulfite beef patties formulated with green tea and grape seed extracts. Regarding sample preference, no consistent differences were observed among the samples chosen by the panelists as the small differences found in the acceptance test did not seem to influence preference.

Regarding evolution of the sensory properties during refrigerated storage, the surface color and odor attributes of raw samples from all groups studied were given acceptable values until Day 11. From that day on, the acceptability of patties decreased in all treatment groups until it reached the score of 'hardly acceptable' at the end of storage. From Day 11 on, the patties containig BHT received appreciation of color and surface discoloration scores close to acceptability. On the other hand, the FVE-1000 treatment received more favorable color appreciation scores at Day 18 than the rest of

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Table 5. Evolution of protein oxidation during refrigerated storage of pork patties manufactured with different antioxidants [mean ± standard error (n = 4)]

	Treatment						
Day	CO	BHT	FVE-250	FVE-500	FVE-1000	SEM	Sig.
	3.57 ± 0.10 ^{1}	3.51 ± 0.19 ^{1}	3.49 ± 0.50 ^{1}	3.53 ± 0.19 ^{1}	3.47 ± 0.14 ^{1}	0.06	ns
0	3.79 ± 0.31 ^{1}	3.52 ± 0.06 ^{1}	3.68 ± 0.05 ^{1,2}	3.55 ± 0.10 ^{1}	3.53 ± 0.15 ^{1}	0.04	ns
7	4.58 ± 0.32 ^{b,2}	3.58 ± 0.04 ^{a,1}	4.34 ± 0.18 ^{b,2}	4.51 ± 0.44 ^{b,2}	4.45 ± 0.51 ^{b,2}	0.11	**
11	5.94 ± 0.17 ^{c,3}	3.79 ± 0.09 ^{a,1,2}	5.40 ± 0.77 ^{b,c,3}	5.31 ± 0.18 ^{b,c,3}	5.14 ± 0.29 ^{b,3}	0.19	***
15	6.81 ± 0.61 ^{c,4}	4.04 ± 0.39 ^{a,2}	6.04 ± 0.39 ^{b,3}	5.72 ± 0.27 ^{b,3}	5.66 ± 0.54 ^{b,3}	0.23	***
18	0.31	0.06	0.27	0.22	0.22		
SEM	***	**	***	***	***		
Sig.	3.57 ± 0.10 ^{1}	3.51 ± 0.19 ^{1}	3.49 ± 0.50 ^{1}	3.53 ± 0.19 ^{1}	3.47 ± 0.14 ^{1}	0.06	ns

BHT, addition of 200 mg kg^{-1} tert-butyl-4-hydroxytoluene; CO, control without any antioxidant; FVE-250, FV-500 and FV-1000, addition of *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1} respectively;
SEM, standard error of mean;
Sig., significance; ***, $P < 0.001$); ns, not significant;
$^{a-e}$Means in the same row (different treatments on the same storage day) not followed by a common superscript letter are significantly different ($P < 0.05$; Duncan's test);
$^{1-4}$Means in the same column (same treatment on different storage days) not followed by a common superscript number are significantly different ($P < 0.05$; Duncan's test).

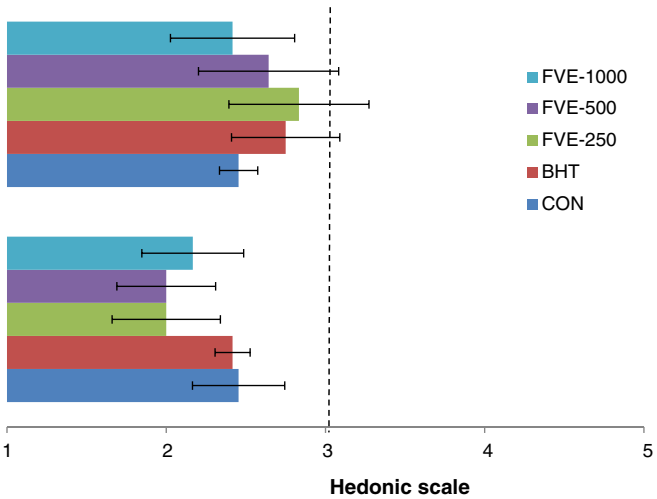

Figure 1. Average sensory scores given to pork patties containing different added extracts.

treatments, but always behind acceptability. Based on the results obtained it may be concluded that FVE, at the concentrations used during the shelf-life of the pork patties, did not improve any of the attributes studied, i.e. color, surface discoloration and odor.

CONCLUSIONS

In this study, *F. vesiculosus* extracts were used for the first time as natural preservatives in pork patties. The incorporation of FVE at the concentration of 1000 mg kg^{-1} into pork patties successfully protected the samples against oxidation, although this treatment was not as effective as the synthetic BHT at the concentration of 200 mg kg^{-1}. One of the key benefits of the preservation technique proposed here, i.e. the use of seaweed extract, was the absence of apparent differences in the sensory attributes of the raw patties studied. Furthermore, the FVE-500 treatment generated the best sensory scores for odor in the cooked product. Despite these results, the limited protection afforded by FVE against oxidation seen under the conditions reported in the present study makes this extract unsuitable for use in meat products. Therefore, increasing the antioxidant power of the extract seems to be the best way to improve fat and protein protection in meat products against oxidation during storage. Further research should explore other potential benefits derived from the incorporation of FVE into meat products, such as nutritional benefits for the consumer.

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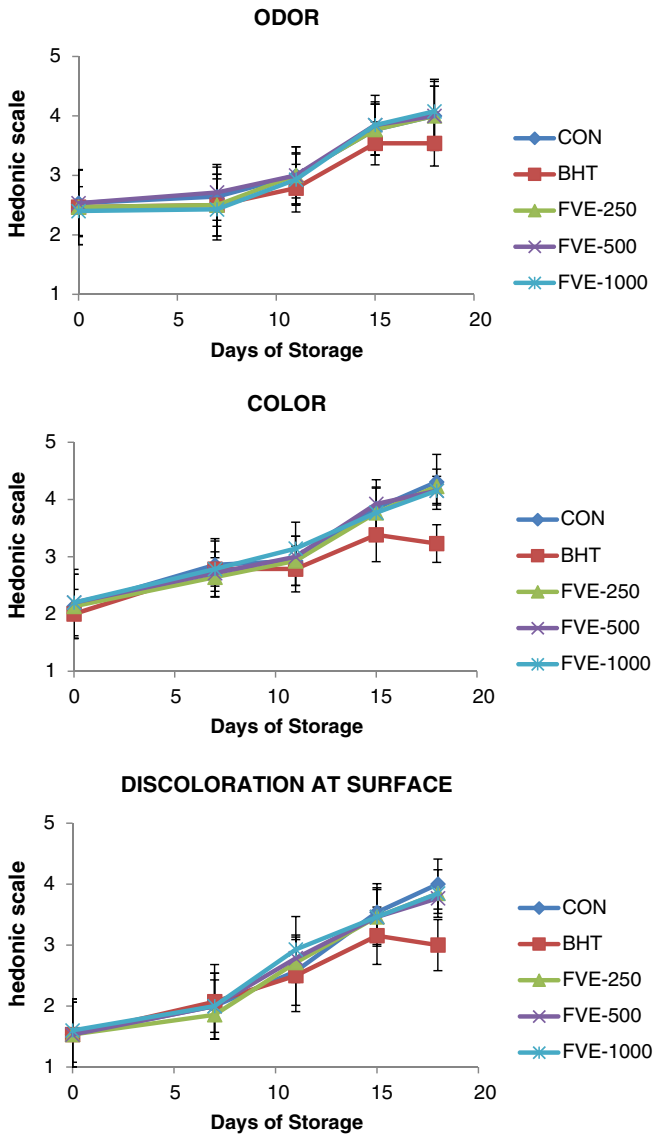

Figure 2. Evolution of color, surface discoloration and odor attributes of raw pork patties with different added extracts during refrigerated storage. Hedonic scale used: 1, excellent; 2, good; 3, acceptable; 4,hardly acceptable; 5, not acceptable. Abbreviations: BHT, tert-butyl-4-hydroxytoluene; CO, control; FVE-250, -500 and -1000, *Fucus vesiculosus* extract at 250, 500 and 1000 mg kg^{-1}, respectively.

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