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Adding Value to Stalked Barnacles from Berlengas Nature Reserve (Portugal) by the Development of a New Food

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Abstract: In this work, a new and natural food product, stalked barnacle (*Pollicipes Pollicipes*) pâté enriched with blackberry, was developed to valorise the rejected stalked barnacle. To evaluate the addition of blackberry fruits (*Rubus ulmifolius* Schott.) as a natural preservative on pâté quality, four pâté sample groups were considered: a negative control without a synthetic additive (CTR), a positive control with butylated hydroxytoluene (BHT), a group with blackberry extract (blackberry), and a group with a mixture of BHT and blackberry (blackberry + BHT). In addition, the effect of pasteurization (80 °C for 30 min) versus sterilization (121 °C for 30 min) on the pâté quality were evaluated. The bioactive evaluation expressed by the total phenolic content (TPC) and antiradical activity by the DPPH radical scavenging associated with oxidative stability determined by thiobarbituric acid reacting substances (TBARS) were performed. The impact of packaging opening followed by refrigerated storage (4 ± 1 °C) simulating the consumer behavior at home, was assessed. All the heat-treated stalked barnacle pâté samples were found microbially safe with an interesting content of total phenolic, the highest ones being the CTR and Blackberry + BHT, ranging from 58.79 to 55.38 mg GAE/100 g. After the package opening of the barnacle pâté sample of Blackberry + BHT, it revealed a superior inhibition of TBARS (0.46 mg MDA/100 g) after 7 days at refrigerated storage compared to the other samples. These results state the efficacy of blackberry in minimizing the lipid oxidation of stalked barnacle pâte. This study showed the potential for rejected stalked barnacle to be valorised and improve the sustainability of resources.

Keywords: seafood; waste reduction; sustainability; preservation; heat treatment

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1. Introduction

The stalked barnacle (*Pollicipes pollicipes*) is an important economic resource of the rocky shores of Portugal and Spain [1] and is heavily exploited by both professional and recreational fishers. In Portugal, *P. pollicipes* can be found in three protected areas with specific harvesting legislation, namely, the Berlengas Nature Reserve (also classified as the Biosphere Reserve of UNESCO), Luiz Saldanha Marine Park, and Sudoeste Alentejano and Costa Vicentina Nature Park. In the Berlengas Nature Reserve the management plan for stalked barnacle harvesting, which considers geographical and seasonal limitations, the limited number of professional licensed fishermen, as well as the minimum size for stalked barnacle, harvesting bag size limitations and a maximum daily quantity per fisher, lead to a strict harvesting control of this valuable food source [2]. Additionally, in both countries, the stalked barnacle can attain high selling prices, ranging from 20 to 200 euros per kg [3]. Regarding the Portuguese legislation, if the half of the total volume of harvested specimens does not present a 'nail' length equal to or greater than 23 mm, defined as the maximum distance between the outer edge of the rostrum and carina plates of the 'nail', these barnacles should not be commercialized or integrated into the fresh supply chain. These rejected stalked barnacles result in waste not only for the professional fisherman but, above all, for

the environment. In this sense, the strategy of valorisation, promoting a circular economy by the development of a new food product using as the main raw material the rejected barnacles without the minimal size for commercial purposes, is very promising.

In the process of developing a new food product, the search for natural preservative ingredients that can act as antioxidant compounds, and food with less or no synthetic additives while maintaining quality and food safety during storage, is one of the most important challenges in the food industry [4]. Usually, food with antioxidants achieves an extended shelf-life by preventing and/or retarding the food quality deterioration, such as the observation of discoloration by oxidation reactions. The blackberry fruits (*Rubus ulmifolius* Schott.) are a known rich source of antioxidants essentially due to the presence of phenolic compounds such as flavonoids and anthocyanins. These compounds augment the retardation or prevent oxidative deterioration and prolong the shelf-life period of food products [5,6].

Therefore, the purpose of the present study was to develop a new seafood product by using rejected barnacles (with low economic value) and the addition of a natural source of bioactive compounds, blackberry fruits, contributing to the achievement of a natural food product—pâté without synthetic additives and with a health benefit to consumers, thus lowering the environmental impact of this fishing activity. Additionally, the impact of pasteurization versus sterilization, usually applied in these kinds of products, are herein compared. Analytical data such as the physical–chemical properties, total phenolic content, and the antiradical activity expressed by the DPPH scavenging activity, as well the microbial development and oxidative stability by thiobarbituric acid reactive substances (TBARS) values of all barnacle pâté samples, were collected. Another main problem that occurred in stored pâté products, affecting the physical–chemical properties and sensorial quality, was the development of undesirable compounds such as peroxy radicals and fatty acid peroxides [7]. A preliminary study using the pasteurized barnacle pâté with blackberry after package opening and stored for 7 days was also made, and the consumer behavior was simulated.

2. Materials and Methods

2.1. Raw Materials Used in the Pâté Formulation

The stalked barnacles were harvested in the Berlengas Nature Reserve (Portugal) by professional fishermen. Upon arrival at the fishing port of Peniche, the barnacles were screened according to the legal requirements for harvesting [2]. Afterwards, the barnacles that did not meet the pre-requisites were transported in a refrigerator container (5 ± 1 °C) to the Marine Sciences R&D, Education, and Knowledge Dissemination Centre and were used for pâté preparation. The remaining ingredients used in the pâté formulation were locally available in the supermarket (Peniche, Portugal) and included: oil, potato puree, milk, butter, salt, black pepper, and blackberries.

Process for Obtaining Barnacle Pâté Enriched with Blackberry

The process for obtaining stalked barnacle pâté enriched with blackberry included three main operations, as shown in Figure 1: firstly, the cooking of the stalked barnacle, then the pâté preparation, and finally the treatment applied.

Firstly, the stalked barnacle was cooked in boiling water with salt for 1 min, then cooled to room temperature by immersion on a water/ice bath for 5 min. The non-edible part of barnacle, nail, and stalk outer layer were removed and the edible part was used for pâté preparation.

Secondly, the pâté was prepared by mixing the potato puree, the edible barnacle crushed, and oil in the proportions of 30%, 62%, and 8%, respectively (Table 1).

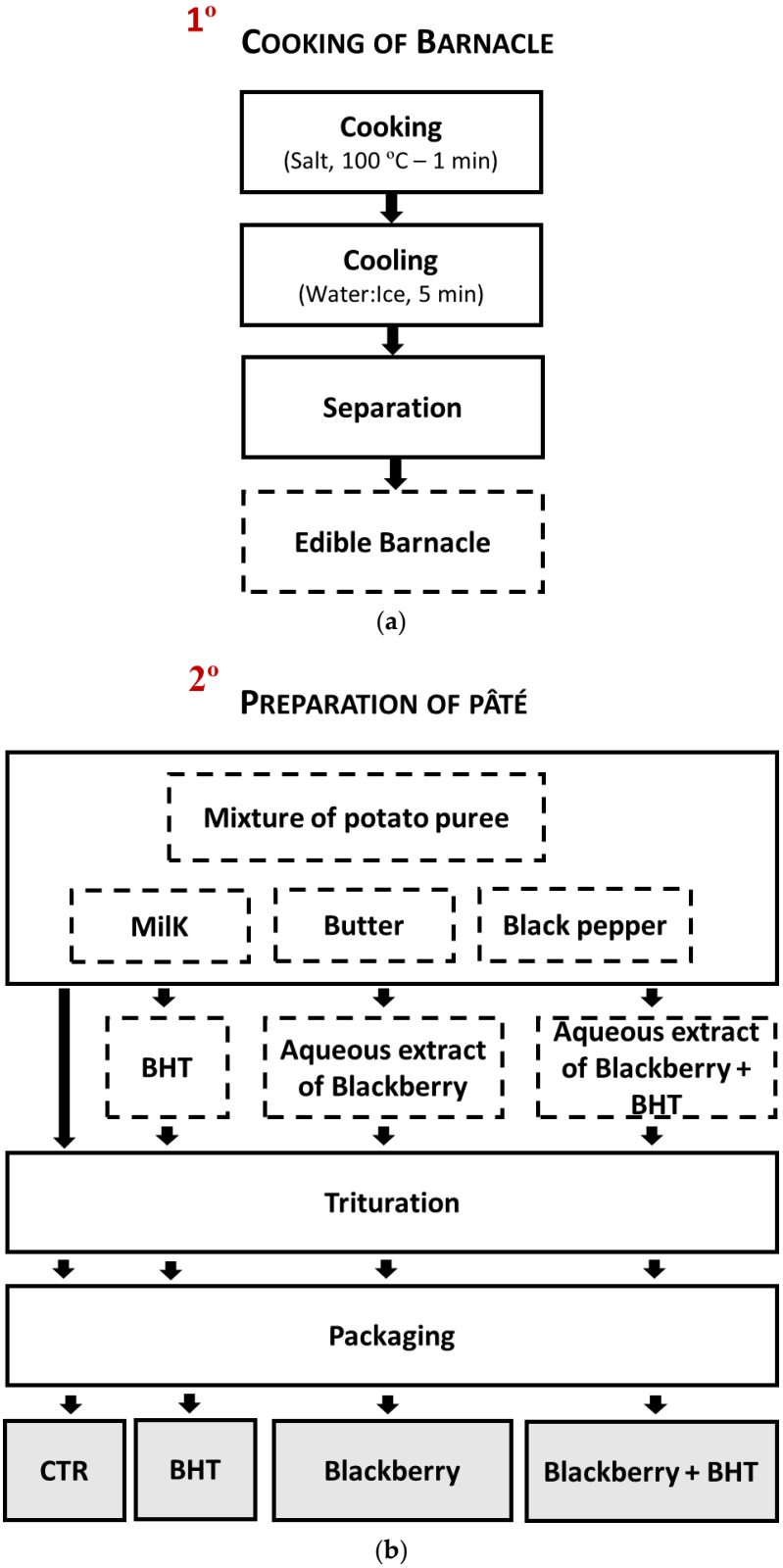
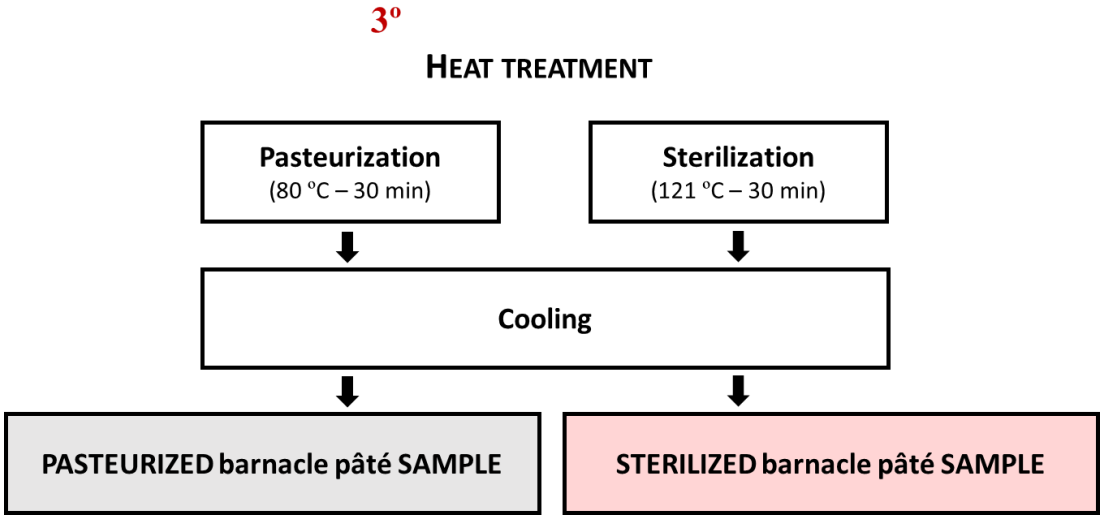


Figure 1. *Cont.*



(**c**)

Figure 1. The flow diagram of the main steps of processing ((**a**) cooking of barnacle; (**b**) preparation of pâte; (**c**) heat treatment) of stalked barnacle pâté samples (negative control: CTR, positive control: BHT, natural enriched: blackberry, mixture of natural and synthetic additive (Blackberry + BHT)).

Table 1. The formulation of stalked barnacle (*Pollicipes pollicipes*) pâté samples (CTR—without synthetic additive; BHT—with synthetic additive; blackberry—with blackberry extract and blackberry + BHT—with synthetic additive and blackberry).

Ingredients	CTR (g/100 g)	BHT (g/100 g)	Blackberry (g/100 g)	Blackberry + BHT (g/100 g)
Edible barnacle	62	62	62	62
Potato starch	7	7	7	7
Milk	10	10	10	10
Margarine	1	1	1	1
Black pepper	0.002	0.002	0.002	0.002
Water	12	12 *	12 ^{A}	12 ^{A}*
Oil	8	8	8	8

The superscripts (^{A}) indicate the presence of 2.5% of blackberry and (*) indicate the synthetic additive BHT at 0.01%, taking in to account the whole mass of the product.

After homogenization of 2.5 g of fruits in 12 mL of water, the aqueous blackberry solution was added into the mixture. The synthetic additive BHT was also added into the formulation, according to European legislation [8].

Afterwards, two preservation treatments, pasteurization and sterilization, were performed on the pâté packaged in glass containers (±100 g). Pasteurization was realized in a water bath (Thermo Scientific, TSSWB15, Porto, Portugal) at 80 °C for 30 min, according to the method previously described in Pinheiro et al. (2020) [9], and sterilization in an autoclave (Raypa, AES-18, Barcelona, Spain) at 121 °C for 30 min. After the heat treatment step all container samples were placed on an ice/water bath for cooling.

To evaluate the effect of the blackberry addition to the barnacle pâté, four pâté formulations in three different batches were considered: the negative control—without a synthetic additive (ID: CTR), positive control—with a synthetic additive (ID: BHT), a batch with blackberry extract (ID: Blackberry), and a batch with a mixture of synthetic and natural extract (ID: Blackberry + BHT).

Since the shelf-life period is one mandatory and required piece of information in food labels together with the safe consumption period after package opening while stored at appropriate conditions, both these properties should be considered to ensure consumer safety. Thus, we also presented a preliminary study using the pasteurized barnacle pâté en-

riched with blackberry after package opening that was stored at a refrigerated temperature (4 ± 1 °C) until the analysis days 2, 5, and 7.

2.2. Methods

2.2.1. Evaluation of Quality of Stalked Barnacle Pâté Enriched with Blackberry

Quality parameters such as the pH value, water activity (aw), color, and texture of stalked barnacle samples were collected at the moment of package opening and at analysis days 2, 5, and 7 at the refrigerated temperature.

The pH of stalked barnacle pâté samples was measured at room temperature (21 ± 1 °C) using a pH meter (SP70P, SympHony, Radnor, PA, USA). The results were expressed as an average of three determinations per pâté sample.

The aw of stalked barnacle pâté samples was measured with a portable water activity analyzer (Rotronic, HP23-AW-A, Bassersdorf, Swiss) and the results were expressed as an average of three determinations per pâté sample.

The color of stalked barnacle pâté samples was assessed by a tristimulus colorimeter (Minolta chroma Meter, CR-400, Osaka, Japan) as described in Pinheiro et al. (2020) [9]. Briefly, the instrument was calibrated using a white standard tile (L* = 97.10, a* = 0.19, b* = 1.95), D65 illuminant, and 2° standard observer. The results of the color parameters (L*a*b*) were expressed as an average of six determinations per pâté sample.

Texture was determined according to the modified method described by Estévez et al. (2006) [10]. The penetration test was performed using a Texture Analyzer (TA.HDi, Stable Microsystem Ltd., Godalming, UK), a 5 kg load cell, and a stainless steel cylinder probe with a 10 mm diameter. The penetration test was performed at a speed of 1.5 mm/s and penetration distance of 8 mm. Hardness (maximum peak force in gram) and adhesiveness (g/s) were used as indicators of texture. The texture was measured at room temperature to avoid storage temperature effects on the analysis. The texture results were expressed as an average of six determinations per pâté sample.

2.2.2. Evaluation of Total Phenolic Content, Antiradical Activity, and Oxidative Stability of Stalked Barnacle Pâté Enriched with Blackberry

The total phenolic content (TPC), antiradical activity, and oxidative stability of stalked barnacle pâté samples were determined at the moment of package opening and at analysis days 2, 5, and 7 at a refrigerated temperature.

The total phenolic content (TPC) and antiradical activity by the DPPH method (DPPH) were determined after the preparation/extraction of pâtés samples. Briefly, the extraction was realized by a mixture of the pâtés samples and ethanol in a ratio of 1:10 (*w*:*v*) after incubation overnight (12–24 h) in refrigerated storage. Then, the extracts were centrifuged (Eppendorf, 5810R, Hamburg, Germany) at 8000 rpm for 10 min (4 °C) and the supernatants were protected from light until the analysis.

The total phenolic content (TPC) of stalked barnacle pâté samples was determined by the Folin–Ciocalteu method [11] adapted to a 96-well plate assay: 20 µL of sample/standard was mixed with 100 µL of diluted Folin–Ciocalteau reagent (1/10, *v*/*v*) and after 4 min, 80 µL of a Na_2CO_3 solution (7.5%, (*m*/*v*)) was added. After 2 h at room temperature, the reaction product was measured at 750 nm using a microplate reader (Synergy H1 Multi-Mode Micro-plate Reader, BioTek® Instruments, Winooski, VT, USA). Gallic acid was used to prepare a standard calibration within the concentration range of 0.05 to 2.5 mg/mL. The TPC was expressed as milligrams of gallic acid equivalents per 100 g of pâté (mg GAE/100 g) and the average of three measurements per pâté sample are herein reported.

The DPPH scavenging activity was determined according to methodology reported by Brand-Williams et al. (1995) [12]. Briefly, 50 µL of sample/standard was reacted with 150 µL of DPPH solution (150 µmol/L) for 30 min in the dark, at room temperature. Subsequently, the absorbance was measured at 517 nm in a microplate reader (Synergy H1 Multi-Mode Microplate Reader, BioTek® Instruments, Winooski, VT, USA). The percentage of inhibition was expressed as the DPPH radical scavenging activity (%RSA).

Lipid oxidation of the barnacle pâté samples was evaluated according to the methodology of thiobarbituric acid reacting substances (TBARS) described in Rosmini et al. (1996) [13] at the moment of package opening and at analysis days 2, 5, and 7 of the refrigerated temperature storage. Briefly, 15 g of each pâté sample was homogenized with a solution of trichloroacetic acid at 7.5%, propyl gallate and ethylenediamine tetra acetic acid (EDTA) and then filtrated by Whatman no. 4 filter paper. Then 5 mL of TBA was added to the filtrate, vortexed, and incubated in a boiling water bath at 100 °C for 40 min. After cooling, the absorbance was measured at 530 nm (BioTek Instruments, Winooski, VT, USA). The standard curve was prepared using a 1,1,3,3-tetraethoxypropane (TEP) solution and the results were expressed as mg of malondialdehyde (MDA) per kg of barnacle pâté, resulting in an average of three determinations per each pâté sample.

2.2.3. Evaluation of the Microbial Load of Stalked Barnacle Pâté Enriched with Blackberry

The microbiological assessment of all stalked barnacle pâté samples was determined by total viable mesophilic, psychotropic, and Enterobacteriaceae determination. Briefly, twenty-five grams of sample and 225 mL of peptone water were transferred into sterile stomacher filter bags and homogenized in a stomacher (Interscience, St Nom, France) for 10 min. Afterwards, a ten-fold dilution series was prepared and plates containing the agar medium were inoculated. The total viable mesophilic counts [14] were determined using plate count agar (PCA) and incubated at 30 °C for 72 h. The methodology for the psychotropic count [15] was similar to the total viable mesophilic counts, varying the temperature and the incubation time, 5 °C and 96 h, respectively. In Enterobacteriacea determination [16] about 2 mL of each successive dilution was transferred to Petri dishes with a VRBG medium at 44–47 °C, in duplicates. Then the plates were incubated at 37 °C for 24 ± 2 h and the colonies were counted from two successive dilutions, with between 15 and a maximum of 150 colonies and the results were expressed as the number of colonies forming units (CFU.g^{-1}). The obtained microbiological results were analyzed according to the European regulation [17] and the microbiological criteria for food products were considered.

2.2.4. Evaluation of the Nutritional Composition of Stalked Barnacle Pâté Enriched with Blackberry

The nutritional composition (moisture, protein, fat, carbohydrates, ash, and fiber) of barnacle pâté samples enriched with blackberry was determined following the AOAC methods [18].

The moisture content of pâté samples was determined by drying the homogenized sample in an oven (Binder, Bohemia, NY, USA) at 105 °C to a constant weight. The moisture was calculated as follows:

$$\text{Moisture } (\%) = \left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \right) \times 100 \quad (1)$$

The crude protein was performed by the Kjeldahl method (N × 6.25). Briefly, 1 g of sample was digested in Kjeldahl digestion flasks with 20 mL of concentrated sulphuric acid and 15 g of Kjeldahl catalyst. The digestion flask was heated at 380 °C for 45 min in a Kjeldahl digester (FOSS, Hillerod, Denmark). The flask was allowed to cool at room temperature and the distillation was carried out in a Büchi Distillation Unit (mod. B-324) (Büchi, New Castle, DE, USA) and 0.1 N HCl standard solution was used as the titration acid. Fat was determined by extracting 5 g of the sample with petroleum ether using a Soxhlet apparatus (Behr, Labor Technik, Düsseldorf, Germany), ash content was determined by incineration at 600 ± 15 °C, and carbohydrates were calculated by the difference as is next shown:

$$\text{Carbohydrates } (\%) = 100 - (\text{g moisture} + \text{g protein} + \text{g fat} + \text{g ash}) \quad (2)$$

The total energy was calculated using the following equation:

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}) \quad (3)$$

Moreover, the crude fiber was determined by acid and basic digestion of 1 g of defatted sample where samples were first added to 150 mL of sulfuric acid (1.25%), then were stirred, boiled for 30 min, and then filtered with Whatman n.° 4. Sequentially the basic digestion was realized, with the same solvent proportions, using the sodium hydroxide (1.25%). The residue thus obtained was dried at 105 °C for 8 h until a constant weight and ashed in a muffle at 550 °C for 5 h, then cooled and weighted. The difference between the ash weight subtracted from the weight of the insoluble matter was expressed as a crude fiber percent using the original weight content.

2.3. Statistical Analysis

Analysis of variance (two-way ANOVA) was performed to assess the significance ($p < 0.05$, Tukey test) of the heat treatment (sterilization or pasteurization) on quality, bioactive composition, and microbial load of barnacle pâté samples (negative—CTR and positive—BHT control, blackberry, and a mixture of BHT and blackberry). Pearson's correlation test was conducted to determine the linear relationships between the variables. All the obtained data were presented as an average ± standard deviation. The statistical analysis was performed using the Statistica version 8.0 software (Statsoft Inc., Tulsa, OK, USA) [19].

3. Results and Discussion

3.1. Quality of Stalked Barnacle Pâté Enriched with Blackberry

Preservation treatments, such as pasteurization and sterilization, are useful for extending the shelf-life of food, however, the quality attributes of food, such as texture and color, can be affected negatively by these heat treatments leading to an unpleasant sensorial perception in consumers [20].

The impacts of heat treatment on the pH value, water activity (aw), and texture of the stalked barnacle pâté samples are reported in Table 2.

Table 2. The pH, a_w, and texture [Hardness (g), adhesiveness (g/s)] of stalked barnacle pâté samples (CTR, BHT, Blackberry, Blackberry + BHT) after heat treatment.

Heat Treatment	Pâté Sample	pH	a_w	Hardness (g)	Adhesiveness (g/s)
Pasteurization	CTR	6.58 ± 0.00^{d}	0.98 ± 0.00^{a}	35.92 ± 2.10^{a}	-36.40 ± 11.91^{a}
	BHT	6.62 ± 0.01^{e}	0.99 ± 0.00^{a}	$33.04 \pm 1.42^{a,d,e}$	-39.06 ± 5.20^{a}
	Blackberry	6.49 ± 0.00^{a}	0.98 ± 0.00^{a}	34.80 ± 1.53^{a}	-34.92 ± 5.23^{a}
	Blackberry + BHT	6.45 ± 0.01^{c}	0.99 ± 0.00^{a}	$34.09 \pm 1.44^{a,e}$	-49.25 ± 22.00^{a}
Sterilization	CTR	6.48 ± 0.00^{a}	0.99 ± 0.00^{a}	$27.48 \pm 1.62^{b,c}$	-40.21 ± 9.92^{a}
	BHT	6.30 ± 0.01^{b}	0.98 ± 0.00^{a}	$29.25 \pm 2.36^{b,c,d}$	-35.84 ± 12.91^{a}
	Blackberry	6.47 ± 0.00^{a}	0.98 ± 0.00^{a}	$29.96 \pm 3.75^{c,d,e}$	-38.47 ± 18.19^{a}
	Blackberry + BHT	6.28 ± 0.00^{b}	0.99 ± 0.00^{a}	25.43 ± 2.49^{b}	-41.12 ± 15.71^{a}

Values are presented as mean ± standard deviation. In the same column, different letters indicate significant differences between pâté samples (p-value < 0.05, Tukey test).

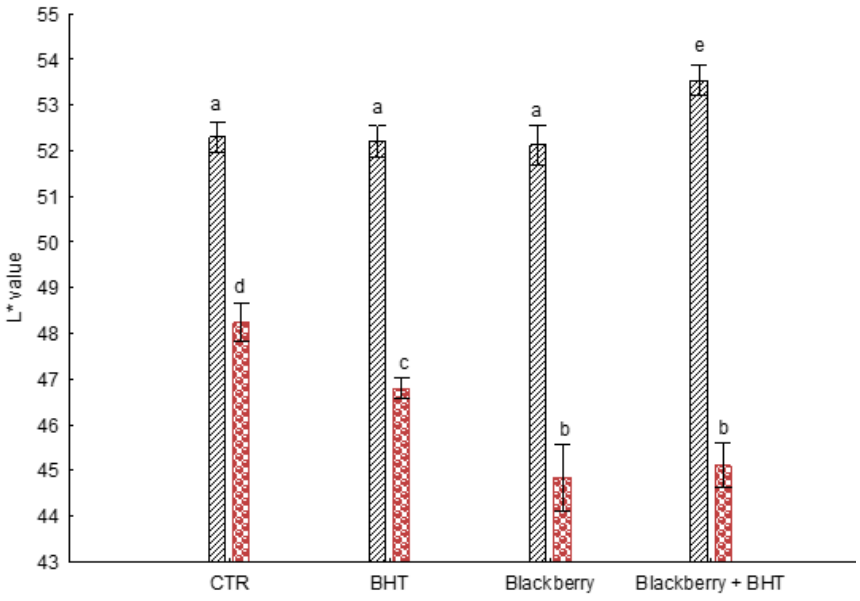
In pasteurized samples, the negative (CTR) and positive (BHT) controls of the barnacle pâté revealed the highest and the most significant ($p < 0.05$, Tukey test) pH value compared to the sample enriched with blackberry and combined with the synthetic additive (Blackberry + BHT). This difference can be an efficiency indicator of adding blackberry extract to barnacle pâté to achieve a shelf-life extension, since products with a lower pH value present the highest probability of an extended shelf-life [21]. Concordantly, after sterilization, a significant ($p < 0.05$, Tukey test) reduction in the pH value was found both

in the control pâté samples (CTR and BHT) and the enriched pâté samples (blackberry and blackberry + BHT). In the pasteurized and sterilized enriched pâté sample (blackberry), a similar pH value ($p > 0.05$, Tukey test) was obtained that guarantees the product quality. In a study reported by Munekata et al. (2017) [22], the pH value of pork liver pâté was not significantly different ($p > 0.05$, Tukey test) after enrichment with a natural antioxidant such as beer residue, chestnut leaves, and peanut skin.

Considering the food quality, the water activity (aw) is another important attribute that indicates the behavior and susceptibility of the pâté to decay [23]. Despite no significant difference ($p > 0.05$, Tukey test) among the aw in all heat-treated pâté samples, the lowest value of 0.98 was found in the blackberry heat-treated pâté.

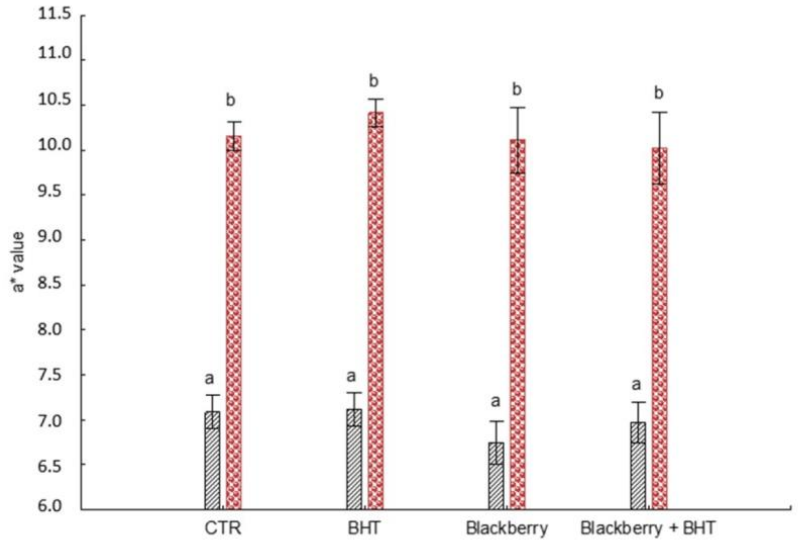
Texture is one of the physical properties used by consumers to assess the food quality especially in pâté products, which are an example of how this attribute can influence the quality of perception and acceptability [24]. By observation of the texture parameters obtained in heat-treated samples, a non-significant difference ($p > 0.05$, Tukey test) was achieved in the adhesiveness of barnacle pâté. A hardness reduction in all four barnacle pâté samples subject to sterilization, due the action of the highest temperature compared to pasteurization, was observed. However, the hardness of the sterilized sample of enriched barnacle was significantly ($p < 0.05$, Tukey test) highest when compared to the negative control, 29.96 $\pm$ 3.75 and 27.48 $\pm$ 1.62, respectively. The persistence of the stalked barnacle pâté texture could be the result of the internal structure of starch and protein and the heat treatment applied in the processing technology as referenced by Rezler et al. (2021) [25] in pork liver by the substitution of fat with modified starch.

The color of the heat-treated barnacle pâté samples was significantly affected ($p < 0.05$, Tukey test) as can been observed by the luminosity and a* color values, seen in Figure 2A,B, respectively.



(**A**)

Figure 2. *Cont.*



(**B**)

Figure 2. Luminosity (**A**) and a* color parameters (**B**) of pasteurized ( ) and sterilized ( ) stalked barnacle pâté samples (CTR—negative control, BHT—positive control, blackberry, and blackberry + BHT). The error lines indicate standard deviation and different letters express the significant change between barnacle pâté samples (p-value < 0.05; Tukey test).

Both heat treatments applied, contributed to color changes in the control (CTR and BHT), and enriched (blackberry and blackberry + BHT) barnacle pâté samples. As observed in Figure 2, the pasteurized pâté samples revealed the highest value in the luminosity but with no significant difference ($p > 0.05$, Tukey test) between them. The higher luminosity value in pasteurized pâté samples shows that the product became lighter/less dark when compared to the sterilized pâté samples. This behavior agrees with the performance of the redness factor represented by a* color values. In a study reported by Dutra et al. (2013) [26], the cooked ham-type pâté produced with sheep meat, where the luminosity of the samples decreased and a* value increased, exhibited a dark and red color. The study developed by Ganhão et al. (2010) [27] in pork burger enriched with several fruits' extract such as *Rubus ulmifolius*, stated that the augment in the redness was due to the richness of the red fruit color. This augment can contribute to a pleasant pâté color. Barnacle pâté enriched with blackberry (blackberry) and BHT (blackberry + BHT) displayed the lowest value of luminosity when compared to the negative control (CTR). This difference can be associated with the blackberry addition and its rich content of compounds such as anthocyanin [28,29]. In this study, the synthetic additive was introduced to obtain whiteness, expressed by the highest value of luminosity, especially in pasteurized samples. However, in sterilized pâté samples a significant difference was observed between BHT samples and both enriched pâté samples ($p < 0.05$, Tukey test).

3.2. Phenolic Content, Antiradical Activity, and Oxidative Stability of Stalked Barnacle Pâté

One of the main goals of adding blackberry in barnacle pâté is the enrichment of the pâte in essential and beneficial compounds, such as the phenolic compounds, in a processed food product [22,30]. The phenolic content in stalked barnacle pâté samples after pasteurization and sterilization can be observed in Figure 3.

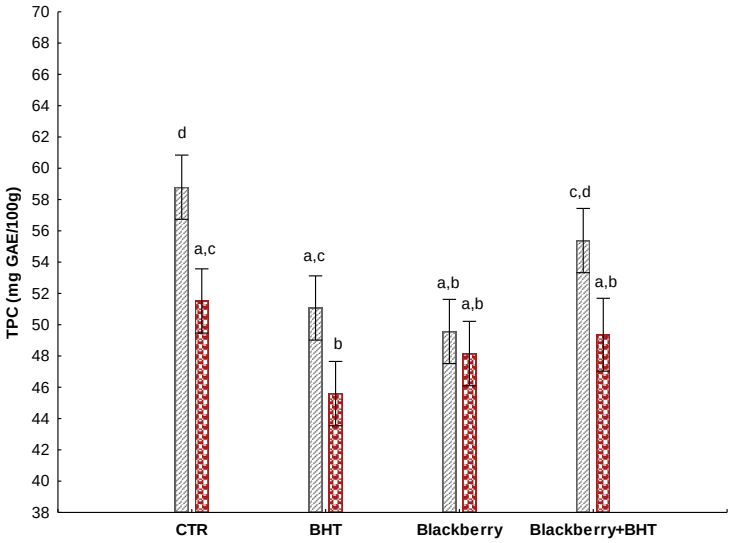


Figure 3. The phenolic content (mg GAE/100 g) of pasteurized ( ) and sterilized ( ) stalked barnacle pâté samples (CTR—negative control, BHT—positive control, blackberry, and blackberry + BHT). Error lines indicate the standard deviation and different letters express the significant change between barnacle pâté samples (p-value < 0.05; Tukey test).

The obtained phenolic content in pasteurized pâté samples was in the range of 58.78 to 49.56 mg GAE/100 g on CTR and blackberry, respectively, and in sterilized pâté samples from 51.52 and 45.60 mg GAE/100 g^1 on CTR and BHT, respectively. In both heat-treated samples, the negative control revealed the highest phenolic content, decreasing after the addition of the synthetic and natural additives. The decrease in phenolic content did not represent a significant difference between the sterilized samples and when both were combined (blackberry + BHT). However, and despite the slight difference in phenolic content between both heat-treated pâté samples, a reduction in these valuable compounds was observed when the products were subjected to heat treatment at a higher temperature, such as in the sterilization process [31]. Comparing both heat-treated stalked barnacle pâtés enriched with blackberry, a non-significant (p > 0.05, Tukey test) change in the phenolic content was observed. This fact can be associated with the protective effect associated with the rich phenolic content of blackberry fruits. In studies reported by Acosta-Montoya et al. (2010) [32] and Schulz et al. (2019) [33] the rich phenolic content of blackberries was reported, meaning that these extracts can be used as a natural and antiradical potential ingredient to new food formulations as a functional food ingredient.

Due to consumers' demand for natural food without or with a significant low content of synthetic additives while maintaining an extended shelf-life, the need for strategies to address and evaluate these demands are mandatory. The evaluation of the antioxidant capacity of foods has been essential to assess and predict, not only a food product shelf-life, but also the health benefits associated with the consumption of these new food products, such as cardiovascular disease prevention and others [34]. Antiradical activity expressed by the DPPH scavenging radical capacity can be observed in Figure 4.

Despite the similar values in the inhibition of radical activity in both groups of heat-treated barnacle pâtés, the blackberry samples presented the highest value in the pasteurized (44.35%) and sterilized (44.85%) groups, followed the blackberry + BHT, BHT, and CTR samples. The barnacle pâté samples subjected to pasteurization, with the exception of the CTR sample, showed an increase in the antiradical activity expressed by the DPPH scavenging activity observed when compared to the sterilized samples. Munekata et al. (2017) [22] studied the impact of beer, chestnut leaves, and peanut skin extracts in liver pork pâté and no significant difference between lipid stability was found between all samples, despite the phenolic content and antioxidant capacity expressed in natural extracts reported in previous studies [7].

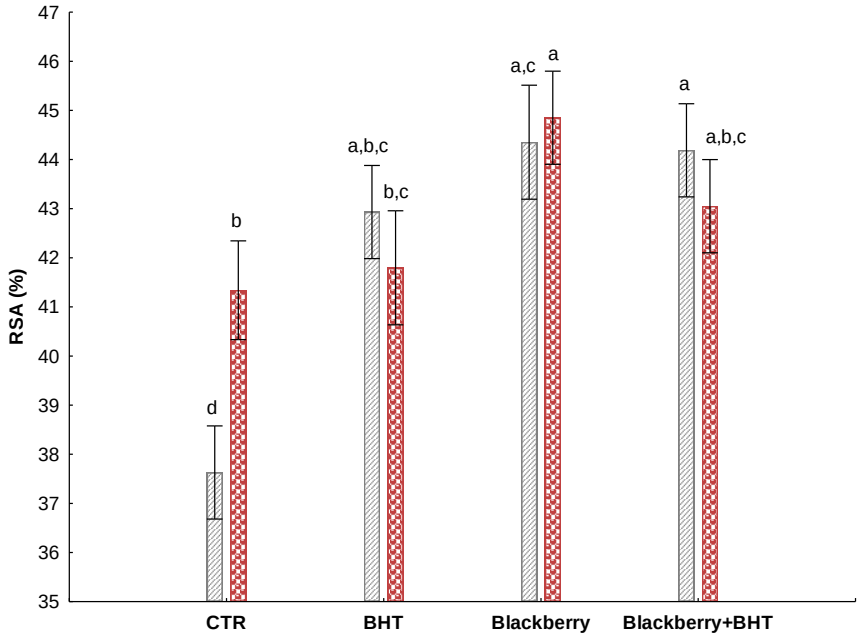


Figure 4. The antiradical activity expressed by DPPH radical scavenging activity (RSA, %) of pasteurized ( ) and sterilized ( ) stalked barnacle pâté. Error lines indicate the standard deviation and different letters express the significant change between barnacle pâté samples (p-value < 0.05; Tukey test).

The influence of the heat treatment on lipid oxidation of barnacle pâté samples can be observed in Figure 5.

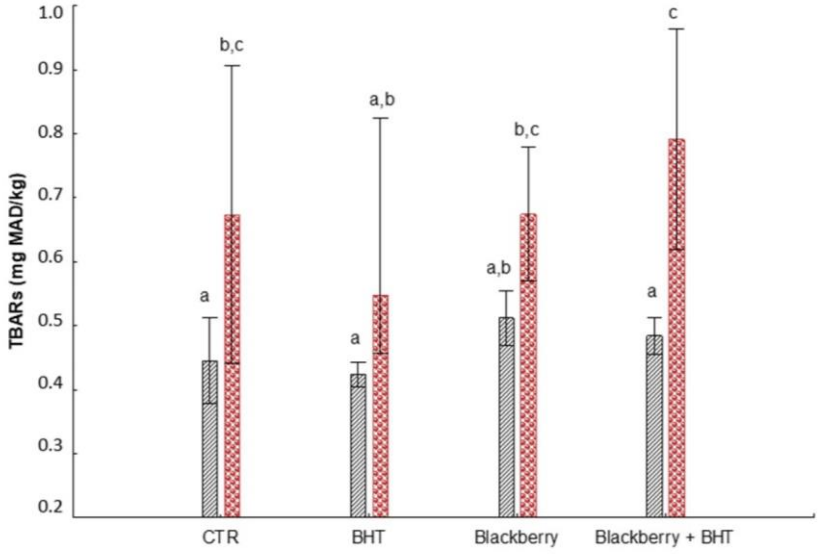


Figure 5. Thiobarbituric acid reacting substances (TBARS, mg MAD/kg) of pasteurized ( ) and sterilized ( ) stalked barnacle pâté samples (CTR—negative control, BHT—positive control, blackberry, and blackberry + BHT). Error lines indicate the standard deviation and different letters express the significant change between barnacle pâté samples (p-value < 0.05; Tukey test).

Regarding the obtained values of the thiobarbituric acid reactive substances (TBARS) between the heat-treated sample groups, an increase was observed in the sterilized barnacle pâtés, ranging from 0.64 to 0.79 mg MAD/kg. In the pasteurized barnacle pâté samples, the range value of TBARS was lower, between 0.42 and 0.51 mg MAD/kg. Factors such as more processed products, elevated fat contents, and a low concentration of natural antioxidants

have been defined as promoters of lipid oxidation in processed foods such as pâté [35,36]. The highest value of TBARS found in sterilized barnacle pâté samples has been previously reported by Ganhão et al. (2013) [37]. In this study, the addition of natural extracts from *Arbutus unedo* L., *Crataegus monogyna* L., *Rosa canina* L., and *Rubus ulmifolius* Schott were evaluated regarding the inhibition of lipid oxidation in raw, cooked, and cooked and chilled (2 °C/12 days) porcine burger patties and after cooking, an augment of 41% of TBARS was observed in control samples.

3.3. Microbiological Quality of Stalked Barnacle Pâté Enriched with Blackberry

Following the guidelines of the European legislation [38] and guide values established by the Portuguese National entity (Doctor Ricardo Jorge National Health Institute—INSA) [39] on the microbiological quality criteria for pâté products, the results of the total viable mesophilic, psychrotrophic, and Enterobacteriacea were satisfactory, since in all the heat-treated pâté sample groups the obtained results were less than 10^6 CFU/g in both and not detected in 10 g, respectively (Table 3). The microbial behavior in the heat-treated pâté samples was expected since the temperature raise both in pasteurized and sterilized pâté products usually leads to an inhibition in the microorganism's growth [9].

Table 3. The microbiological quality (Log cfu/g) of stalked barnacle pâté samples (CTR, BHT, blackberry, and blackberry + BHT) after the heat treatment.

Heat Treatment	Pâté Samples	Total Viable Mesophilic Counts	Psychrotrophic	Enterobacteriacea
Pasteurization	CTR	0.0 ± 0.0	0.0 ± 0.0	N.D.
	BHT	0.0 ± 0.0	0.0 ± 0.0	N.D.
	Blackberry	0.0 ± 0.0	0.0 ± 0.0	N.D.
	Blackberry + BHT	2.1 ± 0.0	0.0 ± 0.0	N.D.
Sterilization	CTR	0.0 ± 0.0	0.0 ± 0.0	N.D.
	BHT	0.0 ± 0.0	0.0 ± 0.0	N.D.
	Blackberry	0.0 ± 0.0	0.0 ± 0.0	N.D.
	Blackberry + BHT	0.0 ± 0.0	0.0 ± 0.0	N.D.

N.D.—Not detected.

Regarding the microbiological hazards of this processed food, both the hygiene standards of the operator and tools of the processing are essential, especially when combined with adequate maintenance to prevent cross-contamination. However, pathogenic microorganisms such as *Listeria* and *Salmonella* should be evaluated in future studies to obtain a safe product with a significantly improved shelf-life.

3.4. Evaluation of Stalked Barnacle Pâté after Package Opening and Stored at a Refrigerated Temperature

The combination of stalked barnacle and blackberry herein proposed resulted in a natural enrichment in the pâté presenting an interesting nutritional value without compromising the final quality of the processed product (Table 4).

Table 4. The nutritional composition of pasteurized stalked barnacle pâté enriched with blackberry.

Nutritional Composition	Barnacle Pâté Enriched with Blackberry
Moisture (%)	71.9 ± 0.2
Fat (%)	11.5 ± 0.3
Protein (%)	9.0 ± 0.2
Carbohydrate (%)	3.8 ± 0.1
Ash	1.99 ± 0.01
Energy value (kcal/100 g)	155.0 ± 0.3

Values are presented as mean ± standard deviation.

The presented findings are in concordance with another study of an innovative product where the potential of Arbutus unedo as a natural additive in limpet's pâté was studied, resulting in a product with an important nutritional composition and bioactive composition [9] when compared to other pâté types. In a study developed by Andronikov et al. (2017) [40], the relative composition of three different pâté products, made of pork, beef, and a combination of vegetables, was investigated. In this study and due to the main raw materials used in the pâté formulation, a significant change in the protein, fat, and carbohydrates was observed, reaching a higher energy value when compared to the known barnacle pâtés.

Table 5 summarizes the impact of the opening of the packaging and consequent refrigerated storage (at 4–5 °C) on the physical–chemical properties, antiradical activity, and oxidative stability of pasteurized pâté samples.

Table 5. The impact of opening the packaging followed by refrigerated storage for 7 days of pasteurized stalked barnacle pâté (CTR, BHT, blackberry, blackberry + BHT).

Quality Attribute	Pâté Sample	Storage Period (Days)			
		0 *	2	5	7
pH	CTR	6.58 ± 0.00 ^{de}	6.73 ± 0.00 ^{g}	6.75 ± 0.00 ^{gh}	6.76 ± 0.00 ^{hi}
	BHT	6.62 ± 0.01 ^{e}	6.60 ± 0.00 ^{f}	6.56 ± 0.00 ^{dc}	6.58 ± 0.01 ^{de}
	Blackberry	6.49 ± 0.00 ^{b}	6.74 ± 0.00 ^{g}	6.73 ± 0.00 ^{g}	6.78 ± 0.01 ^{i}
	Blackberry + BHT	6.45 ± 0.01 ^{a}	6.55 ± 0.00 ^{c}	6.58 ± 0.00 ^{de}	6.56 ± 0.01 ^{cd}
a_w	CTR	0.98 ± 0.00 ^{defg}	0.98 ± 0.00 ^{a}	0.98 ± 0.00 ^{a}	0.98 ± 0.00 ^{bcd}
	BHT	0.99 ± 0.00 ^{gh}	0.99 ± 0.00 ^{fgh}	0.98 ± 0.00 ^{ab}	0.98 ± 0.00 ^{abc}
	Blackberry	0.98 ± 0.00 ^{def}	0.99 ± 0.00 ^{h}	0.98 ± 0.00 ^{ab}	0.98 ± 0.00 ^{cde}
	Blackberry + BHT	0.99 ± 0.00 ^{efgh}	0.98 ± 0.00 ^{bcd}	0.97 ± 0.00 ^{a}	0.98 ± 0.00 ^{bcd}
L* color	CTR	52.30 ± 0.34 ^{bcd}	53.29 ± 0.34 ^{cde}	53.26 ± 0.87 ^{cde}	53.79 ± 0.27 ^{e}
	BHT	52.21 ± 0.34 ^{abcd}	53.39 ± 0.62 ^{cde}	52.31 ± 0.61 ^{bcd}	53.57 ± 0.57 ^{de}
	Blackberry	52.13 ± 0.43 ^{abc}	51.58 ± 0.35 ^{ab}	50.83 ± 0.70 ^{a}	52.55 ± 0.36 ^{bcde}
	Blackberry + BHT	53.54 ± 0.34 ^{de}	51.57 ± 1.28 ^{ab}	51.73 ± 0.97 ^{ab}	52.81 ± 0.53 ^{bcde}
a* color	CTR	7.09 ± 0.18 ^{cd}	7.30 ± 0.16 ^{d}	7.17 ± 0.08 ^{cd}	6.93 ± 0.18 ^{abcd}
	BHT	7.12 ± 0.19 ^{cd}	7.02 ± 0.19 ^{bcd}	7.08 ± 0.14 ^{cd}	7.01 ± 0.16 ^{bcd}
	Blackberry	6.74 ± 0.24 ^{abc}	6.70 ± 0.31 ^{abc}	6.88 ± 0.21 ^{abcd}	6.82 ± 0.18 ^{abcd}
	Blackberry + BHT	6.97 ± 0.23 ^{bcd}	6.47 ± 0.41 ^{a}	6.70 ± 0.25 ^{abc}	6.58 ± 0.14 ^{ab}
b* color	CTR	6.51 ± 0.22 ^{abcdef}	7.14 ± 0.25 ^{f}	6.54 ± 0.30 ^{bcdef}	6.60 ± 0.23 ^{cdef}
	BHT	6.69 ± 0.19 ^{cdef}	7.08 ± 0.41 ^{ef}	6.57 ± 0.30 ^{bcdef}	6.55 ± 0.21 ^{bcdef}
	Blackberry	6.01 ± 0.23 ^{abc}	6.39 ± 0.47 ^{abcde}	5.81 ± 0.34 ^{a}	6.10 ± 0.23 ^{abc}
	Blackberry + BHT	6.86 ± 0.29 ^{def}	6.25 ± 0.59 ^{abcd}	5.85 ± 0.42 ^{ab}	5.99 ± 0.27 ^{abc}
°*hue*	CTR	42.58 ± 0.44 ^{bcdef}	44.35 ± 0.85 ^{efg}	42.35 ± 1.31 ^{bcde}	43.61 ± 0.50 ^{cdefg}
	BHT	43.22 ± 0.36 ^{cdefg}	45.20 ± 1.07 ^{g}	42.83 ± 0.87 ^{bcdef}	43.03 ± 1.05 ^{bcdef}
	Blackberry	41.72 ± 0.50 ^{abc}	43.61 ± 0.97 ^{cdefg}	40.14 ± 1.21 ^{a}	41.78 ± 0.70 ^{abc}
	Blackberry + BHT	44.53 ± 0.63 ^{fg}	43.91 ± 1.14 ^{defg}	41.09 ± 1.19 ^{ab}	42.31 ± 0.85 ^{bcd}

Table 5. *Cont.*

Quality Attribute	Pâté Sample	Storage Period (Days)			
		0 *	2	5	7
Hardness (g)	CTR	35.92 ± 1.44 ^{abcd}	35.77 ± 1.39 ^{def}	34.24 ± 1.25 ^{bcdef}	39.70 ± 1.17 ^{f}
	BHT	34.80 ± 1.53 ^{a}	36.81 ± 1.84 ^{abcd}	37.16 ± 3.23 ^{abcd}	41.86 ± 3.44 ^{f}
	Blackberry	33.04 ± 1.42 ^{abc}	35.47 ± 0.53 ^{abcd}	35.70 ± 1.99 ^{abcde}	42.86 ± 2.15 ^{ef}
	Blackberry + BHT	35.92 ± 2.10 ^{ab}	40.32 ± 3.16 ^{abc}	38.35 ± 2.18 ^{abc}	42.11 ± 2.42 ^{cdef}
TPC (mg/100 g)	CTR	58.79 ± 3.80 ^{cd}	55.30 ± 2.54 ^{abc}	62.60 ± 4.21 ^{b}	60.79 ± 4.56 ^{cd}
	BHT	51.07 ± 1.89 ^{ab}	54.22 ± 2.60 ^{abc}	56.21 ± 1.61 ^{bc}	54.66 ± 6.48 ^{abc}
	Blackberry	49.56 ± 3.18 ^{a}	58.14 ± 4.00 ^{cd}	56.99 ± 2.52 ^{bcd}	56.05 ± 3.93 ^{bc}
	Blackberry + BHT	55.38 ± 1.39 ^{abc}	58.91± 4.42 ^{cd}	59.04 ± 1.09 ^{cd}	55.21 ± 6.23 ^{abc}
DPPH (%)	CTR	37.69 ± 2.67 ^{a}	42.26 ± 0.69 ^{bd}	41.58 ± 1.42 ^{cde}	44.23 ± 0.89 ^{cefg}
	BHT	42.93 ± 0.58 ^{bcdef}	44.93 ± 0.89 ^{gh}	42.52 ± 0.85 ^{bcd}	44.79 ± 0.97 ^{gh}
	Blackberry	44.35 ± 0.70 ^{cdefg}	46.09 ± 0.73 ^{h}	43.54 ± 0.46 ^{bcdefg}	44.67 ± 0.91 ^{fgh}
	Blackberry + BHT	44.19 ± 0.49 ^{cefg}	45.07 ± 0.90 ^{gh}	42.79 ± 0.67 ^{bcde}	44.41 ± 0.83 ^{efgh}
TBARS (mg MDA/kg)	CTR	0.45 ± 0.06 ^{abc}	0.49 ± 0.06 ^{bcd}	0.42 ± 0.03 ^{a}	0.43 ± 0.04 ^{ab}
	BHT	0.42 ± 0.02 ^{ab}	0.45 ± 0.07 ^{abcd}	0.46 ± 0.03 ^{abcd}	0.48 ± 0.05 ^{abcd}
	Blackberry	0.51 ± 0.04 ^{d}	0.50 ± 0.02 ^{cd}	0.48 ± 0.03 ^{abcd}	0.48 ± 0.03 ^{abcd}
	Blackberry + BHT	0.48 ± 0.03 ^{bcd}	0.47 ± 0.04 ^{abcd}	0.43 ± 0.02 ^{ab}	0.46 ± 0.06 ^{abcd}

Values represent the mean ± standard deviation. (*) The storage day of 0 corresponds to the moment of package opening and then it was stored in refrigerated storage until the analysis days 2, 5, and 7. Different letters in the same column, for each quality attributes, express the significant change between the barnacle pâté samples (p-value < 0.05; Tukey test).

In all pâté samples a significant difference ($p < 0.05$, Tukey test) in the pH value was encountered immediately after the opening of the package. Comparing the pH of all studied samples, the highest and lowest value was observed in the BHT and blackberry + BHT of pâté with 6.62 and 6.45, respectively. Based on the pH range mentioned by Jay et al. (2005) [41], as the optimum pH range is 6.6 to 7.5 for microbial development, our pâté samples had the adequate value to ensure quality and preservation safety. At the end of the storage, the pâté sample with the addition of synthetic additive maintained the pH value and the sample with the mixture of natural and synthetic additive varied from 6.62 to 6.58 and 6.45 to 6.56, respectively. The pH value of barnacle pâté samples changed during storage after package opening, as previously described in other pâté studies, such as those done on liver pork pâté formulation enriched with natural antioxidants [22] and on pig liver pâtés with the addition of tea, chestnut, and grape seed extracts [42]. On the other hand, the water activity (aw) of the pâté samples did not significantly change ($p > 0.05$) between the initial pâté formulation and the storage period observed. Considering the color and texture, two quality attributes that consumers usually appreciate when tasting the pâté product, only the second changed during storage, reaching the end of storage as a less soft product. Despite the observed increase in the pâté texture, the spreadability properties remained. Similar textural behavior has been observed in other pâté products such as those studied by Kambarova et al. (2021) [43] in turkey pâté supplemented with a plant-based protein (rice, oat, or corn).

Comparing the antiradical activity expressed by the DPPH radical scavenging activity of negative CTR, a non-significant ($p > 0.05$, Tukey test) increase was obtained in the positive CTR sample, in blackberry alone, and in blackberry combined with BHT. In addition, a similar behavior of antiradical activity was observed between the blackberry alone and the combined blackberry and BHT pâté samples, indicating an advantage for the

product preservation by using this ingredient as a natural source of antiradical/antioxidant compounds. Regarding the difference between the initial and final antiradical activities of the barnacle pâté samples, the significantly highest differences ($p < 0.05$, Tukey test) were observed in CTR and BHT and the lowest ($p > 0.05$, Tukey test) differences were found in blackberry and blackberry + BHT.

These differences between the pâté samples demonstrate the beneficial effects of oxidation inhibition of one product enriched with natural antiradical/antioxidant compounds as reported by the same research group in a study of limpet pâté enriched with Arbutus unedo [9] and in liver pâté fortified with added rosemary extract [23], where in natural enriched pâté samples, a higher DPPH scavenging activity was observed after 15 days compared to CTR samples. After heat processing, a lower value of TBARS (0.42 ± 0.02) in the pâté sample with the synthetic additive (BHT) was observed. Regarding the pâté sample enriched with blackberry and despite the higher TBARS value after processing, after opening and during storage at the refrigerated temperature, this prevented the unwanted secondary metabolite development in the processed product, thus preserving the product quality attributes such as aroma. This interesting behavior can be explained by the blackberry richness in antiradical compounds leading to a higher inhibition of the referred undesirable compounds. Identical action was found in wild fruits from the Mediterranean area, especially with blackberries and strawberries, in raw pork burger patties [27]. The obtained results highlight the importance of not only investigating the potential of new natural sources of antiradical/antioxidant compounds for food applications, but above all, to use, evaluate, and validate their beneficial effects in food products, as presented in this study. These findings contributed to the increase in the potential of the antiradical/antioxidant richness of blackberries for quality maintenance and oxidative stability in barnacle pâté without synthetic additives. The possible replacement of synthetic antioxidant additives, such as BHT, is a means to guarantee e food safety combined with the reduction in health hazards.

4. Conclusions

The present work identified both a valuable way of giving economic value to rejected barnacles and an answer to the consumer demand for natural food products with extended shelf-life using new and natural antiradical/antioxidant resources for synthetic additive replacement. So, a healthy and functional food was produced with added value in the food supply chain, thus developing new markets and attractive consumers. Simultaneously it also studied the common techniques of pasteurization and sterilization for extending the shelf-life and maintenance of the food quality. In this sense, the current study compared the impact of two heat treatments, pasteurization versus sterilization, regarding the quality, oxidative stability, bioactive content, and microbial load of one innovative food product, the barnacle pâté enriched with natural antiradicals/antioxidants from the blackberries. The color of stalked barnacle pâté is affected since a darkness change and a decrease in the phenolic content in all samples after sterilization was denoted. Both the stalked barnacle pâtés enriched with blackberry and blackberry + BHT were more resistant in both heat treatments, revealing the potential of combining these two ingredients from the sea and earth, the stalked barnacle and blackberry, respectively. The addition of this natural source of these antiradicals/antioxidants from blackberries, preserve the pâté quality even after air exposure and subsequent storage at refrigerated temperatures. Future studies will use sensorial evaluations to validate the consumer acceptability of pasteurized barnacle pâtés enriched with blackberry.

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References

1. Sousa, A.; Jacinto, D.; Penteado, N.; Pereira, D.; Silva, T.; Castro, J.J.; Leandro, S.M.; Cruz, T. Temporal variation of the fishers' perception about the stalked barnacle (Pollicipes pollicipes) fishery at the Berlengas Nature Reserve (Portugal). *Reg. Stud. Mar. Sci.* **2020**, *38*, 101378. [CrossRef]
2. Portaria n.º 232. Primeira alteração à Portaria n.º 378/2000 de 27 de junho, que aprova o regulamento da apanha do perceve (Pollicipes pollicipes) na Reserva Natural das Berlengas, Diário da República n.º 113/2011. Série I de 2011-06-14. 2011, pp. 3105–3109. Available online: https://dre.pt/dre/detalhe/portaria/232-2011-670052?_ts=1672272000034 (accessed on 12 October 2020).
3. Cruz, T.; Jacinto, D.; Sousa, A.; Penteado, N.; Pereira, D.; Fernandes, J.N.; Silva, T.; Castro, J.J. The state of the fishery, conservation and management of the stalked barnacle Pollicipes pollicipes in Portugal. *Mar. Environ. Res.* **2015**, *112*, 73–80. [CrossRef] [PubMed]
4. Sellimi, S.; Ksouda, G.; Benslima, A.; Nasri, R.; Rinaudo, M.; Nasri, M.; Hajji, M. Enhancing colour and oxidative stabilities of reduced-nitrite turkey meat sausages during refrigerated storage using fucoxanthin purified from the Tunisian seaweed Cystoseira barbata. *Food Chem. Toxicol.* **2017**, *107*, 620–629. [CrossRef]
5. Toshima, S.; Hirano, T.; Kunitake, H. Comparison of anthocyanins, polyphenols, and antioxidant capacities among raspberry, blackberry, and Japanese wild Rubus species. *Sci. Hortic.* **2021**, *285*, 110204. [CrossRef]
6. Martillanes, S.; Rocha-Pimienta, J.; Cabrera-Bañegil, M.; Martín-Vertedor, D.; Delgado-Adámez., J. Application of phenolic compounds for food preservation: Food additive and active packaging. *Phenolic Compd.-Biol. Act.* **2017**, *3*, 39–58. [CrossRef]
7. Lorenzo, J.M.; Sineiro, J.; Amado, I.R.; Franco, D. Influence of natural extracts on the shelf life of modified atmosphere-packaged pork patties. *Meat Sci.* **2014**, *96*, 526–534. [CrossRef] [PubMed]
8. Commission Regulation (EU) Nº 1129/2011 of 11 November 2011-Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by Establishing a Union List of Food Additives. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1129&from=EN (accessed on 10 January 2019).
9. Pinheiro, J.; Rodrigues, S.; Mendes, S.; Maranhão, P.; Ganhão, R. Impact of aqueous extract of Arbutus unedo fruits on limpets (Patella spp.) pâté during storage: Proximate composition, physicochemical quality, oxidative stability, and microbial development. *Foods* **2020**, *9*, 807. [CrossRef]
10. Estévez, M.; Ventanas, S.; Cava, R. Effect of natural and synthetic antioxidants on protein oxidation and colour and texture changes in refrigerated stored porcine liver pâté. *Meat Sci.* **2006**, *74*, 396–403. [CrossRef]
11. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
12. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity—A Review. *LWT Food Sci. Technol.* **1995**, *28*, 25–30.
13. Rosmini, M.R.; Perlo, F.; Pérez-Alvarez, J.A.; Pagán-Moreno, M.J.; Gago-Gago, A.; López Santoveña, F. TBA test by an extractive method applied to "paté". *Meat Sci.* **1996**, *42*, 103–110. [CrossRef]
14. *NP 4405*; Microbiologia alimentar: Regras Gerais para a Contagem de Microrganismos. Contagem de Colónias a 30 °C. Instituto Português da Qualidade, Ministério da Economia, da Inovação e do Desenvolvimento: Caparica, Portugal, 2002.
15. *NP-2307*; Regras gerais para contagem de microrganismos psicrotróficos. Instituto Português da Qualidade, Ministério da Economia, da Inovação e do Desenvolvimento: Caparica, Portugal, 1987.

16. *ISO 21528-1*; Microbiology of the food chain—Horizontal method for the detection and enumeration of Enterobacteriaceae—Part 1, Detection of Enterobacteriaceae. ISO: Geneva, Switzerland, 2017.
17. Regulamento (CE). 2073 da Comissão de 15 de Novembro de 2005 relativo a critérios microbiológicos aplicáveis aos géneros alimentícios. 2005. Available online: https://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:02005R2073-20190228&from=SV (accessed on 10 July 2020).
18. AOAC—Association of Official Analytical Chemists. *Official Methods of Analysis of the Association of the Analytical Chemists*; AOAC: Rockville, VA, USA, 2016.
19. StatSoft Inc. *Statistica (Data Analysis Software System)*; Version 8; StatSoft Inc.: Tulsa, OK, USA, 2007.
20. Lorenzo, J.M.; Vargas, F.C.; Strozzi, I.; Pateiro, M.; Furtado, M.M.; Sant´Ana, A.S.; Rocchetti, G.; Barba, F.J.; Dominguez, R.; Lucini, L.; et al. Influence of pitanga leaf extracts on lipid and protein oxidation of pork burger during shelf-life. *Food Res. Int.* **2018**, *114*, 47–54. [CrossRef] [PubMed]
21. Andrés-Bello, A.; Barreto-Palacios, V.; GarcíaeGovia, P.; Mir-Bel, J.E.; Martínez-Monzó, J. Effect of pH on color and texture of food products. *Food Eng. Rev.* **2013**, *5*, 158–170. [CrossRef]
22. Munekata, P.E.S.; Domínguez, R.; Campagnol, P.C.B.; Franco, D.; Trindade, M.A.; Lorenzo, J.M. Effect of natural antioxidants on physicochemical properties and lipid stability of pork liver pâté manufactured with healthy oils during refrigerated storage. *J. Food Sci. Technol.* **2017**, *54*, 4324–4334. [CrossRef]
23. Bilska, A.; Kobus-Cisowska, J.; Kmiecik, D.; Danyluk, B.; Kowalski, R.; Szymanowska, D.; Gramza-Michalowska, A.; Szcepaniak, O. Cholinesterase inhibitory activity, antioxidative potential and microbial stability of innovative liver pâté fortified with rosemary extract (*Rosmarinus officinalis*). *Electron. J. Biotechnol.* **2019**, *40*, 22–29. [CrossRef]
24. Xiong, G.; Wang, P.; Zheng, H.; Xu, X.; Zhu, Y.; Zhou, G. Effects of plant oil combination substituting pork back-fat combined with pre-emulsification on physicochemical, textural, microstructural and sensory properties of spreadable chicken liver pâté. *J. Food Qual.* **2016**, *39*, 331–341. [CrossRef]
25. Rezler, R.; Krzywdzinska-Bartkowiak, M.; Platek, M. The influence of the substitution of fat with modified starch on the quality of pork liver pâtés. *LWT Food Sci. Technol.* **2021**, *135*, 110264. [CrossRef]
26. Dutra, M.P.; Palhares, P.C.; Silva, J.R.O.; Ezequiel, I.P.; Ramos, A.L.S.; Perez, J.R.O.; Ramos, E.M. Short communication—Technological and quality characteristics of cooked ham-type pâté elaborated with sheep meat. *Small Rumin. Res.* **2013**, *115*, 56–61. [CrossRef]
27. Ganhão, R.; Estévez, M.; Kylli, P.; Heinonnen, M.; Morcuende, D. Characterization of selected wild mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. *J. Agric. Food Chem.* **2010**, *58*, 8854–8861. [CrossRef]
28. Da Silva, L.P.; Pereira, E.; Prieto, M.A.; Simal-Gandara, J.; Pires, T.C.S.P.; Alves, M.J.; Calhelha, R.; Barros, L.; Ferreira, I.C.F.R. *Rubus ulmifolius* Schott as a novel source of food colorant: Extraction optimization of coloring pigments and incorporation in a bakery product. *Molecules* **2019**, *24*, 2181. [CrossRef] [PubMed]
29. Schulz, M.; Chim, J.F. Nutritional and bioactive value of Rubus berries. *Food Biosci.* **2019**, *31*, 100438. [CrossRef]
30. Hangun-Balkir, Y.; Mckenney, M.L. Determination of antioxidant activities of berries and resveratrol. *Green Chem. Lett. Rev.* **2012**, *5*, 147–153. [CrossRef]
31. Olas, B. Berry Phenolic Antioxidants—Implications for Human Health? *Front. Pharmacol.* **2018**, *9*, 78. [CrossRef]
32. Acosta-Montoya, O.; Vaillant, F.; Cozzano, S.; Mertz, C.; Pérez, A.M.; Castri, M.V. Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages. *Food Chem.* **2010**, *119*, 1497–1501. [CrossRef]
33. Schulz, M.; Seraglio, K.T.; Betta, F.D.; Nehring, P.; Valese, A.C.; Daguer, H.; Gonzaga, L.V.; Costa, A.C.O.; Fett, R. Blackberry (Rubus ulmifolius Schott): Chemical composition, phenolic compounds and antioxidant capacity in two edible stages. *Food Res. Int.* **2019**, *122*, 627–634. [CrossRef]
34. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive compounds and antioxidant activity in different types of berries. *Int. J. Mol. Sci.* **2015**, *16*, 24673–24706. [CrossRef]
35. Russell, E.A.; Lynch, A.; Lynch, P.B.; Kerry, J.P. Quality and shelf life of duck liver pate as influenced by dietary supplementation with alpha-tocopheryl acetate and various fat sources. *J. Food Sci.* **2003**, *68*, 799–802. [CrossRef]
36. Delgado-Pando, G.; Cofrades, S.; Ruiz-Capillas, C.; Triki, M.; Jiménez-Colmenero, F. Enriched n-3 PUFA/konjac gel low-fat pork liver pâté: Lipid oxidation, microbiological properties and biogenic amine formation during chilling storage. *Meat Sci.* **2012**, *92*, 762–767. [CrossRef]
37. Ganhão, R.; Estévez, M.; Armenteros, M.; Morcuende, D. Mediterranean berries as inhibitors of lipid oxidation in porcine burguer patties subjected to cooking and chilled storage. *J. Integr. Agric.* **2013**, *12*, 1982–1992. [CrossRef]
38. Regulamento (CE) n.º 1441 da Comissão de 5 de Dezembro de 2007 que altera o Regulamento (CE) n.º 2073/2005 relativo a critérios microbiológicos aplicáveis aos géneros alimentícios. 2007. Available online: https://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32007R1441&from=PT (accessed on 10 July 2020).
39. Instituto Nacional de Saúde Doutor Ricardo Jorge. *Interpretação de Resultados de Ensaios Microbiológicos em Alimentos Prontos Para Consumo e em Superfícies do Ambiente de Preparação e Distribuição Alimentar: Valores-guia*; INSA IP: Lisboa, Portugal, 2019.
40. Andronikov, D.; Mojsov, K.; Janevski, A.; Kuzelov, A.; Sofijanova, E.; Indzhelieva, D. Proximate composition, microbiological quality and sensory characteristics in different types of pâtés. *Sci. Work. Univ. Food Technol.* **2017**, *64*, 80–83.

41. Jay, J.M.; Loesonen, M.J.; Golden, D.A. *Modern Food Microbiology*, 7th ed.; Springer: New York, NY, USA, 2005.
42. Pateiro, M.; Lorenzo, J.M.; Amado, I.R.; Franco, D. Effect of addition of green tea, chestnut and grape extract on the shelf-life of pig liver pâté. *Food Chem.* **2014**, *147*, 386–394. [CrossRef] [PubMed]
43. Kambarova, A.; Nurgazezova, A.; Nurymkhan, G.; Atambayeva, Z.; Smolnikova, F.; Rebezov, M.; Issayeva, K.; Kazhibaeva, G.; Asirzhanova, Z.; Moldabaeva, Z. Improvement of quality characteristics of turkey pâté through optimization of a protein rich ingredient: Physicochemical analysis and sensory evaluation. *Food Sci. Technol.* **2021**, *41*, 203–209. [CrossRef]