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Research article

The effect of seasoning with herbs on the nutritional, safety and sensory properties of reduced-sodium fermented Cobrançosa cv. table olives

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Abstract: This study aimed at evaluating the effectiveness of seasoning Cobrançosa table olives in a brine with aromatic ingredients, in order to mask the bitter taste given by KCl when added to reduced-sodium fermentation brines. Olives were fermented in two different salt combinations: Brine A, containing 8% NaCl and, Brine B, a reduced-sodium brine, containing 4% NaCl + 4% KCl. After the fermentation the olives were immersed in seasoning brines with NaCl (2%) and the aromatic herbs (thyme, oregano and calamintha), garlic and lemon. At the end of the fermentation and two weeks after seasoning, the physicochemical, nutritional, organoleptic, and microbiological parameters, were determined. The olives fermented in the reduced-sodium brines had half the sodium concentration, higher potassium and calcium content, a lower caloric level, but were considered, by a sensorial panel, more bitter than olives fermented in NaCl brine. Seasoned table olives, previously fermented in Brine A and Brine B, had no significant differences in the amounts of protein (1.23% or 1.11%), carbohydrates (1.0% or 0.66%), fat (20.0% or 20.5%) and dietary fiber (3.4% or 3.6%). Regarding mineral contents, the sodium-reduced fermented olives, presented one third of sodium, seven times more potassium and three times more calcium than the traditional olives fermented in 8% NaCl. Additionally, according to the panelists' evaluation, seasoning the olives fermented in 4% NaCl + 4% KCl, resulted in a decrease in bitterness and an improvement in the overall evaluation and flavor. *Escherichia coli* and *Salmonella* were not found in the olives produced.

Keywords: *Olea europaea*; Cobrançosa cultivar; reduced-sodium table olives; KCl; aromatic herbs

Abbreviations: *a*: Color parameter indicating redness (+)/greenness (−); a_W: Water activity; A_{DPPH}: Absorbance of the DPPH● solution; ANOVA: Analysis of variance; AOAC: Association of Official Analytical Chemists; AS: Absorbance of the sample solution; A1: Olives brined and fermented in 8% NaCl; A2: Olives immersed in 2% NaCl and seasoned after fermentation in 8% NaCl; *b*: Color parameter indicating yellowness (+)/blueness (−); B1: Olives brined and fermented in 4% NaCl + 4% KCl; B2: Olives immersed in 2% NaCl and seasoned after fermentation in 4% NaCl + 4% KCl; CFU: Colony Forming Unit; *Chroma*: Purity or saturation of the color; DM: Dry matter; $DPPH^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl free radical; EC_{50}: Extract solution concentration which provides 50% inhibition; *Hue angle*: Color nuance; *L*: Color parameter indicating lightness, 0—black to 100—white; *p*: Probability value; TE: Trolox Equivalent solution; TPC: Total phenolic content

1. Introduction

Several epidemiological and clinical studies have been shown that high salt intake increases not only blood pressure but also the risk of stroke, left ventricular hypertrophy and proteinuria. Daily salt intake reduction can delay or prevent the incidence of antihypertensive therapy, facilitate blood pressure reduction in hypertensive patients receiving medical therapy, and may represent a simple cost-saving mediator to reduce cardiovascular morbidity and mortality [1].

Due to the correlation between diet and these chronic diseases, according to health agencies, reducing sodium intake is among the most urgent challenges to implement in dietary habits. This program implies removing or partially replacing NaCl with other mineral salts in the new food formulations. However, its reduction or replacement reduces the salty taste of foods, noticeably decreasing their flavor, changing their typical textural properties and may compromise the microbiological and physicochemical stability of food products [2]. Potassium chloride is the most investigated mineral salt as a substitute of sodium chloride [2–7] but its use results in a bitter, astringent and metallic taste of the reformulated foods [8].

Aromatic herbs are natural sources of flavors and colors and they have been used globally for centuries in cuisine preparations without adding significant energy, sugar, saturated fat, or sodium to food. They have also been used for medicinal purposes for a very long time. Indeed, several culinary herbs are now known to have beneficial effects on human health, such as being digestive stimulants, anti-inflammatory, antimicrobial, antioxidant and anticarcinogenic. Some of these properties are attributed to the polyphenol compounds [9–11]. Most of the antioxidant activity results from their reaction with free radicals created during the initiation stage of autoxidation, others form complexes with metal ions [12].

The fruits of *Olea europaea* L. are processed to obtain table olives and olive oil, which are essential in the Mediterranean Diet food pattern. Table olives are a fermented product consumed and enjoyed worldwide consumed in large quantities as snacks, with vegetables in the form of salads and equally in cooked foods especially before the popularity of western food [13,14]. Sodium chloride is a key component on the olive fermentation processing and is generally used at concentrations of 8–10%, constituting a significant source of Na in the final product. However, reduced-sodium table olives have been prepared successfully [3–6,8].

According to Saúde et al. [6] olives from Maçanilha Algarvia cultivar fermented in a brine blend of sodium and potassium chlorides, 4% each, led to final products with lower fat, carbohydrates, dietary fiber and energy value, with similar amounts of phenolic compounds as those brined in 8% NaCl. Besides, these table olives received the same scores regarding all the

organoleptic attributes except for bitterness. Thus, to compensate the increase of bitterness and to satisfy the consumer preference, the inclusion of herbs in this type of ready to eat product, should be studied, as was shown in prior works where herbs were used to improve acceptability of low-sodium foods [15]. Seasoning table olives after fermentation is a common practice around the Mediterranean area and can be done according to various recipes depending on the tradition of each region. Seasoned olives are in general, highly appreciated due to their distinctive characteristics [16,17].

In southern Portugal, one of the cultivars mainly used, nowadays, to produce table olives is Cobrançosa, which drupes weights 4.4 ± 0.7 g and has a pulp/stone ratio of 3.2 ± 0.6, with an edible portion of 76 ± 3%, where five major fatty acids were identified, palmitic (22.0 ± 1.0 mol%), palmitoleic (0.09 ± 0.02 mol%), stearic (0.25 ± 0.96 mol%), oleic (69.0 ± 2.0 mol%) and linoleic (9.0 ± 1.0 mol%) [18]. Not all olive cultivars are suitable for table olive processing since the characteristics of the fruits depend on genetic, agronomic and environmental factors [19–21]. However, Cobrançosa cv produces fruits with the biometric and texture characteristics suitable to obtain split table olives [18]. These olives are split through a mechanical splitting process and then fermented by a natural fermentation in 8% NaCl brines. At the end of fermentation, when they partly lose their natural bitterness, olives are washed and immersed in new brines with lower salt concentration (~2% NaCl), seasoned with aromatic herbs and packed for commercialization [18].

The objective of the present study was to evaluate the effectiveness of aromatic herbs (thyme, oregano and calamintha), garlic and lemon to mask the bitter taste of table olives of Cobrançosa cultivar, fermented in a reduced-sodium brine containing 4% NaCl + 4% KCl. It is intended that the seasoning may increase consumers' satisfaction and preference for the reduced-sodium table olives.

2. Materials and methods

2.1. Sampling, preparation of split olives and fermentation

Olives were handpicked in the crop year 2016, when the drupes were semi-ripe with a purple color. Only healthy fruits, without any kind of infection or physical damage, were selected. After arriving at the factory, the drupes were washed with water and split (mechanical splitting) in a local medium-size factory (Hélder Madeira—Indústria e Comércio de Azeitonas, Unipessoal Lda., Tavira, Portugal) and transported to the Instituto Superior de Engenharia, Universidade do Algarve, Faro, Portugal, where they were separated in two fermentation trials, with brines of approximately similar osmotic pressures: (A) 8% NaCl and (B) 4% NaCl + 4% KCl. The fermentations were left to progress naturally, during 225 days, at room temperature, in a pilot scale of 8.0 kg of olives per 7.4 l of brine. The experiments were done in duplicate in food grade polyethylene fermenters. At the end of fermentation, olives were separated into batches A1 and A2, B1 and B2. Those from A1 and B1 fermenters were immediately analyzed for physicochemical, nutritional and sensorial characteristics. The table olives from A2 and B2 batches were prepared differently. They were washed and immersed in new brines (2% NaCl), seasoned with fresh garlic (0.6% w/w), fresh lemon juice (1.2% w/w), and dry aromatic herbs (*Thymus* sp. (0.06% w/w), *Origanum* sp. (0.04% w/w) and *Calamintha nepeta* (0.02% w/w). They were also analyzed for nutritional and sensorial characteristics. All batches were analyzed for microbiological characteristics in order to evaluate their safety and spoilage potential.

All solvents and reagents for analysis were of a chromatographic or analytical grade.

2.2. Physicochemical analyses

Samples of 20 randomly chosen olives were manually de-pitted, chopped and homogenized in an Ultra-Turrax T25, IKA-Laborthechnik (Staufen, Germany). The olive paste obtained was immediately analyzed.

The physicochemical analyses were carried out in three replicates.

The water activity (a_W) of olive paste was measured at 25 ºC using a lithium chloride humidity sensor Rotronic DT Hygroskop (DMS-100H, Bassersdorf, Switzerland).

The pH measurements of olive paste were done using a digital Crison instrument, GLP 21 pH meter (Barcelona, Spain), at 21 ºC.

The total acidity of the olive paste, expressed as g of lactic acid/100 g olive (%, w/w), was obtained as the sum of free and combined acidities determined by the titration method of Fernández-Díez et al. [22] with some modifications [6]. A 10 g aliquot of the olive paste was macerated in 50 mL of distilled water, at 20 ºC, for 30 min and then filtered using a Macherey-Nagel MN 615 (Ø 70 mm) paper filter (Düren, Germany). A 5 mL aliquot of olive extract added to 25 mL of distilled water was titrated with 0.1 N NaOH, up to pH 8.2, or with 0.1 HCl, down to pH 2.6, to obtain the free or combined acidities, respectively.

The total salt content of olive paste was calculated from the total mineral content obtained by nutritional analyses and expressed as %-equivalent to g NaCl/100 g olive.

The concentration of reducing sugars in the olive paste, expressed as g glucose equivalent/kg olive, was determined according to an adaptation of the Miller's method [23], described by Maldonado et al. [24], with some modifications [6]. In summary, an aliquot of 1.0 mL olive extract was mixed with 1.0 mL dinitrosalicylic acid in test tubes, which were immersed in successive boiling water a bath and an ice bath for 10 min each. Next, 10 mL of distilled water was added to each tube and the absorbance was measured at 546 nm, using a Genesys^{TM} 10 series spectrophotometer (Waltham, MA, USA). A standard curve with glucose in the range 0.2–0.4 g/L was also run along with the test samples.

The total phenolic content (TPC) of the olive paste was determined according to the procedure described by Saúde et al. [6]. Firstly, total phenolic extraction was done by stirring (Edmund Buhler KL2, 150 rpm, Hechingen, Germany) 10 g of olive paste with 15 mL of pure methanol, in the dark, at room temperature for 24 h, followed by centrifugation (Sigma 3K20, Osterode am Harz, Germany) at 1600 g, 20 ºC for 15 min, and filtration, and brought to a final volume of 25 mL with pure methanol. The TPC was then determined with the Folin-Ciocalteu assay [25] with some modifications. Briefly, 100 µL of the phenolic extract was added to 500 µl of Folin-Ciocalteu (0.2 N) (Scharlau Chemie. SA, Sentmenat, Spain). The mixture was shaken and, after 2 min, 400 µl of 7.5% Na_2CO_3 was added, and a final volume of 5 mL was completed with distilled water and thoroughly mixed. After incubation in the dark for 60 min, the absorbance at 765 nm was measured in the spectrophotometer using a blank that was prepared as the sample, replacing the extract with equal volume of methanol. TPC was expressed as g gallic acid equivalent per kg, by means of a calibration plot using pure gallic acid monohydrate 98% (Fluka, Buchs, Switzerland) as a standard at different concentrations (0.015, 0.0325, 0.075, 0.125, 0.25, 0.50 mg/mL).

Antioxidant activity: The capacity to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) was monitored according to the Boskou et al. [26] method, with some modifications [18]. A 0.1 mL aliquot of olive extract solutions with different concentrations were mixed vigorously

with 3.9 mL of methanolic solution (6×10^{-5} M) containing DPPH$^{\bullet}$ radicals and left to stand for 60 min in the dark at room temperature. The absorbance was taken at 517 nm in the spectrophotometer. DPPH$^{\bullet}$ scavenging effect was calculated as the percentage of DPPH discoloration using Eq 1:

$$\% \text{ scavenging effect} = \frac{A_{DPPH}-A_S}{A_{DPPH}} \times 100 \quad (1)$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH$^{\bullet}$ solution. The extract solution concentration providing 50% inhibition, EC_{50}, was calculated from the plot of % scavenging effects against extract solution concentrations. A standard curve was done by using Trolox standard solutions at concentrations ranging from 25 to 1000 μM. The DPPH$^{\bullet}$ scavenging effect percentage of the test samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox Equivalent (TE) as μmol TE/100 g olive.

Surface color analysis was done on 20 randomly chosen olives using a Dr. Lange Spectro-colour (Berlin, Germany) colorimeter, according to Saúde et al. [6]. The color parameters *L*, *a* and *b* were measured. *Hue angle* ($\tan^{-1} b/a$) and *chroma* $(a^2 + b^2)^{0.5}$ parameters were calculated.

A compression test was done, according to [18], on 10 randomly chosen olives to evaluate their hardness, using a texture profile analyzer Brookfield, LFRA 1500 (Middleboro, USA) with a 2 mm compression probe (TA39), at a constant rate of 0.5 mm/s and a total deformation of 2 mm. The maximum force (hardness, N) required to compress the sample was obtained by the software Texture ProLite V1.1.

2.3. Nutritional analyses

The dry matter (DM) of olive paste samples (15 g each) were obtained by oven-drying at 105 ± 2 ℃ until constant weights were reached, according to AOAC 934.01 method [27]. The moisture content was calculated by difference: 100%-%DM.

Total nitrogen was determined by the Kjeldahl method according to AOAC 920.152 [28], using approximately 5 g of olive paste samples. A conversion factor of 6.25 was used to convert the measured nitrogen content to protein content, expressed as percentage in the olive paste.

Total fat content of the previously dehydrated samples (5 g each) were determined according to AOAC 948.22 method [29] in a Soxhlet apparatus, using *n*-hexane and a minimum extraction time of 13 h. The results were expressed as the percentage of fat in the olive paste.

The percentage of ash in the olive paste was determined by incineration of 5 g of samples in a muffled furnace at 550 ± 15 ℃ until consistent weight was obtained, according to AOAC 940.26 method [30].

Dietary fiber content, expressed as a percentage in the olive paste, was done according to AOAC 978.10 method [31], using 3 g of fat free samples, which were submitted to successive digestion with acid (H_2SO_4, 1.25%) and alkali (NaOH, 1.25%), drying and incineration.

Carbohydrate content, expressed as a percentage in the olive paste, was determined by difference: 100% - % sum of moisture, protein, fat, fiber and ash contents.

The energy value, expressed as kilocalories per 100 g of olive was calculated using the conversion factors for protein (4), fat (9), carbohydrates (4) and dietary fiber (2) contents [32].

The mineral contents of Na, K and Ca were determined by flame photometry (Jenway, PFP 7, Essex, England) according to the method described by [6], using a standard calibration curve at 0.8–20 mg/L of Na, K and Ca. Each measurement was carried out in three replicates and the results were expressed as percentage of cation in the olive paste.

2.4. Microbiological analyses

The table olives obtained at the end of each fermentation trial and after seasoning were studied in relation to the microbiological safety parameters, *Salmonella* sp. and *Listeria monocytogenes*, as well as the hygienic parameter *Escherichia coli.* The mesophilic microorganisms and the fungi (yeasts and filamentous fungi) were also enumerated.

Salmonella spp. and *Listeria monocytogenes* were evaluated according to the ISO 6579:2002 (Microbiology of food and animal feeding stuffs—Horizontal methods for the detection of *Salmonella* spp.) and ISO 11290 1:1996 FDAM1:2004 (Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of *Listeria monocytogenes*—Part 1: Detection method.) The mesophilic microbial counts were enumerated according to the ISO 4833 (2003) (Microbiology of food and animal feeding stuffs—Horizontal methods for enumeration of microorganisms—Colony-count at 30 °C). The fungi were enumerated following the ISO 21527-1 (2008) (Microbiology—General guidance for counted of yeasts and molds. Colony count technique at 25 °C).

Escherichia coli and the total coliform were counted, according to González et al. [33], using Chromocult Agar (Merck, Darmstadt, Germany), following incubation at 37 °C during 24 h.

2.5. Sensory evaluation

A sensory panel, consisting of 14 different judges, 8 women and 6 men, with ages between 19 and 52 years old, were trained to familiarize themselves with sensory table olives attributes, following the "Guidelines for taster and panel leader training in the sensory assessment of table olives and panel management" [34] and the procedure described in [6]. They were asked to score the attributes, appearance, firmness, flavor, aroma, bitterness, acidity, saltiness and overall sensorial evaluation, using an acceptability test based on a 7-point hedonic scale, where 1—unacceptable and 7—excellent in appearance, flavor, aroma and overall sensorial evaluation, or 1—very low (or without) and 7—excessive in bitterness, acidity, firmness and saltiness. Samples were coded with random 3 digit numbers. Six olives were given to the assessors in individual booths, in an air-conditioned sensory evaluation room at 20 °C, under incandescent white lighting. Rinsing with water was used to clean out tastes between samples. The sensorial analysis was organized according to the methodology described in [35].

2.6. Statistical analyses

The experimental values were expressed as the means of several measurements (depending on the method of analysis) and the standard deviation. Regarding physicochemical characteristics, the comparison between the mean variables was done using the Student's *t*-test. Analysis of variance (ANOVA) was carried out for nutritional composition, microbial parameters and sensorial

evaluation and the average values were compared using a Scheffe's multiple-range test. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were done using SPSS statistical software version 24.0 (IBM SPSS Statistics 24.0, Faro, Portugal).

3. Results and discussion

3.1. Physicochemical characteristics

At the end of fermentation, split Cobrançosa table olives brined in both salt combinations (A—8% NaCl and B—4% NaCl + 4% KCl), presented no significant differences for almost all physicochemical characteristics, except for water activity, total acidity and salt content (Table 1).

Table 1. Physicochemical characteristics of split Cobrançosa table olives brined in 8% NaCl (A) and 4% NaCl + 4% KCl (B).

Physicochemical characteristics	A—Table Olives (8% NaCl)	B—Table Olives (4% NaCl + 4% KCl)
a_W	0.938 ± 0.001^a	0.951 ± 0.001^b
pH	4.460 ± 0.000^a	4.515 ± 0.005^a
Total acidity (%, w/w)	1.80 ± 0.04^a	1.97 ± 0.01^b
Total salt content (% equivalent NaCl, w/w)	3.91 ± 0.09^b	3.65 ± 0.12^a
Reducing sugars content (g/kg)	1.76 ± 0.02^a	1.76 ± 0.01^a
Total phenolic content (g/kg)	1.55 ± 0.05^a	1.68 ± 0.04^a
EC_{50} (mg/mL)	0.68 ± 0.03^a	0.72 ± 0.07^a
Texture and Color parameters		
Hardness (N)	2.8 ± 0.7^a	2.9 ± 0.6^a
L	38 ± 2^a	38 ± 2^a
a	3.1 ± 0.7^a	3.9 ± 0.9^a
b	7 ± 1^a	7 ± 1^a
Hue angle	65 ± 6^a	60 ± 8^a
Chroma	7.5 ± 0.9^a	8 ± 1^a

Different letters in the same row indicate significant differences according to a Student's *t* test ($p < 0.05$)

Although brines with approximately the same osmotic pressures were used, the water activity of olives processed in A brine (0.938 ± 0.001) is lower than the value observed in B brine (0.951 ± 0.001), probably due to their lower moisture (67.6 ± 0.1 or 69.3 ± 0.6, respectively) (Table 2) and, therefore, higher salt contents (3.91 ± 0.09 or 3.65 ± 0.12 %-equivalent to g NaCl/100 g olive, respectively) in the pulp. This may be explained by the lower content of sodium and the presence of potassium cations in B brine, which affect the diffusion of salt into the olive flesh during the fermentation. Similar values of water activity were obtained for Giarraffa olives brined with 8% NaCl [36].

Table 2. Nutritional composition of split Cobrançosa table olives (/100 g): A1—olives brined in 8% NaCl; A2—olives immersed in 2% NaCl and seasoned after fermentation in 8% NaCl; B1—olives brined in 4% NaCl + 4% KCl; B2—olives immersed in 2% NaCl and seasoned after fermentation in 4% NaCl + 4% KCl.

Nutritional composition	A1	A2	B1	B2
Energy (kcal)	211 ± 5^{b}	199 ± 2^{a}	198 ± 4^{a}	196 ± 4^{a}
Water (g)	67.6 ± 0.1^{a}	71.5 ± 0.4^{c}	69.3 ± 0.6^{b}	71.7 ± 0.4^{c}
Protein (g)	1.24 ± 0.06^{b}	1.11 ± 0.04^{a}	$1.17 \pm 0.04^{a,b}$	$1.23 \pm 0.03^{a,b}$
Carbohydrate (g)	3.46 ± 0.09^{b}	0.66 ± 0.06^{a}	3.37 ± 0.3^{b}	1.0 ± 0.3^{a}
Fat (g)	20.7 ± 0.8^{a}	20.5 ± 0.4^{a}	19.3 ± 0.3^{a}	20.0 ± 0.7^{a}
Dietary Fiber (g)	2.7 ± 0.9^{a}	3.6 ± 0.2^{a}	2.8 ± 0.2^{a}	3.4 ± 0.3^{a}
Ash (g)	4.28 ± 0.02^{c}	2.69 ± 0.05^{a}	4.03 ± 0.01^{b}	2.60 ± 0.04^{a}
Na (mg)	1.390 ± 0.04^{d}	0.820 ± 0.02^{c}	0.660 ± 0.04^{b}	0.300 ± 0.01^{a}
K (mg)	0.142 ± 0.004^{b}	0.046 ± 0.000^{a}	0.830 ± 0.03^{d}	0.320 ± 0.006^{c}
Ca (mg)	0.059 ± 0.001^{b}	0.041 ± 0.003^{a}	0.252 ± 0.009^{d}	0.124 ± 0.005^{c}

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test ($p < 0.05$).

Total acidity is also different whether olives were fermented in A or B brines (1.80 ± 0.04 or 1.97 ± 0.01% (w/w), respectively). During fermentation, citric and malic acids diffuse from olive pulp and are solubilized in the brine. Simultaneously, organic acids are produced by the activity of fermentative microorganisms in reducing sugar metabolization. Probably, these biochemical processes were also influenced by the partial replacement of sodium by potassium in the brine. Although the different acidities observed, both table olives and their brines showed the same pH values of 4.5 and 4.3, respectively. The brines' pH results are in accordance to the Trade Standard Applying to Table Olives [37], which states that olive fermentations should lead to brines' pH values of 4.3 or less.

After 225 days of fermentation, reducing sugars were still detected in both table olives, at a content of 1.76 g/kg (Table 1), which may result in concerns regarding the occurrence of extemporaneous fermentation during the storage period. The presence of aerobic mesophilic microorganisms and yeasts, when reducing sugars are available, may result in an impaired shelf-life [38].

Table olives represent an important supply of antioxidant phenolic compounds and their consumption is recommended every day in a reasonable portion, such as a handful [13,14]. In fact, since a portion of split Cobrançosa table olives is approximately 50 g, it provides about 80 mg of total phenolic compounds (1.6 g/kg, Table 1), which is almost 10% of the estimated total phenolic intake in Spain (1171 mg/person/day) [39]. The antioxidant activity, measured by the concentration providing 50% inhibition, EC_{50} parameter, was the same (0.7 mg/mL) whether olives were fermented in A or B brines, which suggests that both brines interfere in the same way on the degree of diffusion of phenolic compounds from the drupes and their antioxidant activity. Similar values of total phenolic content were obtained with cracked Maçanilha Algarvia [6] and Giarraffa table olives [36]. Additionally, analogous values of EC_{50} were measured in Maçanilha Algarvia [6]. Ambra et al. [8] also observe that the brines containing potassium did not significantly affect the presence of phenolic compounds in the table olives of Nocellara del Belice cultivar.

The texture and color parameters of table olives fermented in A and B brines were not significantly different. The presence of potassium cation in the brine did not change de hardness of

the olive pulp, nor the olive surface luminescence, as they present the same brightness *L* values. The similar values of *a*, *b*, *hue angle* and *chroma* parameters observed for A and B brined olives, indicate the same soft yellow-red color and an equal intensity of color.

3.2. Nutritional composition

After 225 days of fermentation, the nutritional composition of split Cobrançosa table olives brined in 8% NaCl and 4% NaCl + 4% KCl indicated that water was the major component, followed by fat. Protein, dietary fiber, ash and carbohydrates were found in lower amounts (Table 2).

Comparing washed and seasoned table olives with unwashed table olives, i.e., A2 *vs* A1 and B2 *vs* B1, almost all nutrients were significantly ($p < 0.05$) different, except fat and dietary fiber. In A2 and B2 olives, water mean values were higher due to the washing procedure carried out before immersion in the seasoned brine of low salt content (2% NaCl). Moreover, the mean values of the other nutrients were lower because they were partially leached during washing. Fat and dietary fiber are not water-soluble and, therefore, were not affected by washing treatment. The energy value of A2 olives was significantly ($p < 0.05$) lower than A1, due to the loss of protein and carbohydrates resulting from lixiviation. Although the same trend was observed with B2 and B1 olives, the difference between protein contents is not statistically significant. So, in terms of nutritional composition, table olives are richer at the end of fermentation and before washing. However, for commercialization, they are always washed and immersed in a low salt content brine.

Regarding table olives fermented in different brine compositions, A1 *vs* B1, there are only significant differences ($p < 0.05$) in terms of micronutrients and water content. Olives brined in 4% NaCl + 4% KCl (B1) had half the sodium concentration, six times more potassium and four times more calcium, thus having a lower ash content than those fermented in 8% NaCl brine. In addition, although the differences between macronutrients mean values were not statistically significant, the lower contents of protein, carbohydrates and fiber led to a significantly lower caloric level in B1 olives than in those fermented in 8% NaCl (A1). Thus, fermentation carried out in a low sodium content brine as B1 (4% NaCl + 4% KCl) results in reduced-sodium table olives being nutritionally more desirable.

Finally, and according to the previous discussion, comparing both seasoned table olives brined in 4% NaCl + 4% KCl (B2) and 8% NaCl (A2), there are no significant differences regarding macronutrients, except for mineral contents, with B2 presenting one third of sodium, seven times more potassium and three times more calcium than A2.

3.3. Microbial characteristics

The decrease in the amount of NaCl, by its replacement with other salts or by the utilization of seasonings, such as aromatic herbs, may result in problems of microbiological safety. For this reason, at the end of the fermentation processes and after the seasoning, the table olives obtained were analyzed to detect *Salmonella* sp., *L. monocytogenes* and *E. coli*. Additionally, the presence of coliforms and fungi (yeasts and filamentous fungi) were also counted at the end of fermentation and after seasoning (Table 3). Yeasts were predominant and no filamentous fungi, coliforms including *E. coli*, *Salmonella* sp., and *L. monocytogenes* were found in the table olives produced in both brines and after seasoning. The levels of the aerobic mesophilic microorganisms and the yeasts were in the

range of those described by Pires-Cabral et al. [18] and Romeo et al. [36] and in the review of Arroyo-López et al. [40] for yeasts. These values were within the limits expected for this type of fermented food [37].

Table 3. Microbial characteristics and safety parameters of split Cobrançosa table olives: A1—olives brined in 8% NaCl; A2—olives immersed in 2% NaCl and seasoned after fermentation in 8% NaCl; B1—olives brined in 4% NaCl + 4% KCl; B2—olives immersed in 2% NaCl and seasoned after fermentation in 4% NaCl + 4% KCl (ND-Not detected).

Microbial groups (Log CFU/g olives)	A1	A2	B1	B2
Aerobic Mesophilic	5.80 ± 0.06	4.96 ± 0.02	5.09 ± 0.01	5.34 ± 0.00
Yeasts	5.98 ± 0.07	5.04 ± 0.03	5.54 ± 0.00	5.54 ± 0.02
Filamentous fungi	<1	<1	<1	<1
E. coli	<1	<1	<1	<1
Salmonella sp.	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND

3.4. Sensory evaluation

The results of the organoleptic assessment of split Cobrançosa table olives, after 225 days of fermentation, are shown in Table 4.

Table 4. Sensorial evaluation of split Cobrançosa table olives: A1—olives brined in 8% NaCl; A2—olives immersed in 2% NaCl and seasoned after fermentation in 8% NaCl; B1—olives brined in 4% NaCl + 4% KCl; B2—olives immersed in 2% NaCl and seasoned after fermentation in 4% NaCl + 4% KCl.

	Overall evaluation	*Appearance*	*Flavor*	*Saltiness*	*Acidity*	*Bitterness*	*Aroma*	*Firmness*
A1	$4.6 \pm 1.1^{a,b}$	4.7 ± 1.4^{a}	$4.4 \pm 1.2^{a,b}$	4.6 ± 0.9^{a}	4.2 ± 0.9^{a}	$4.5 \pm 0.9^{a,b}$	4.9 ± 1.2^{a}	4.1 ± 1.2^{a}
A2	$4.5 \pm 1.2^{a,b}$	5.1 ± 1.2^{a}	4.8 ± 1.4^{b}	4.4 ± 0.7^{a}	4.0 ± 0.9^{a}	4.3 ± 1.0^{a}	5.3 ± 1.0^{a}	4.0 ± 1.3^{a}
B1	3.7 ± 1.1^{a}	4.8 ± 1.3^{a}	3.8 ± 1.0^{a}	4.1 ± 1.1^{a}	4.7 ± 1.1^{a}	5.3 ± 1.4^{b}	4.5 ± 1.2^{a}	4.6 ± 1.2^{a}
B2	4.7 ± 0.8^{b}	5.5 ± 1.0^{a}	$4.6 \pm 1.1^{a,b}$	3.9 ± 0.6^{a}	4.5 ± 1.4^{a}	$4.5 \pm 0.7^{a,b}$	5.3 ± 1.1^{a}	4.5 ± 1.2^{a}

Each value of sensory attributes is the mean ± standard deviation of samples tasted by 14 different judges, using an acceptability test based on a 1 to 7 point hedonic scale.

Different letters in the same column indicate significant differences according to a Scheffe's multiple-range test ($p < 0.05$).

Olives processed in brines with 8% NaCl or 4% NaCl + 4% KCl, with or without seasoning, received the same scores regarding all the attributes except for overall evaluation, flavor and bitterness, which was significantly different in the four sets of olives ($p < 0.05$).

The taste panel considered that olives fermented in the 4% NaCl + 4% KCl brine without washing and seasoning (B1) were below the reasonable limit (4 on the 7-point hedonic scale) regarding overall evaluation and flavor attributes. In fact, overall evaluation of B1 olives was scored with the lowest value, significantly different ($p < 0.05$) from washed and seasoned olives (B2), which were the most appreciated. The flavor attribute of B1 olives had also the lowest score, significantly different ($p < 0.05$) from seasoned olives fermented in 8% NaCl (A2).

Regarding bitterness, panellists attributed the worst evaluation to olives brined in 4% NaCl + 4% KCl (B1). It has been described that KCl in brines increases the bitter perception as reported by Marsílio et al. [41] and Ambra et al. [8]. However, in the present study, after washing and seasoning, these olives (B2) were not statistically different ($p < 0.05$) from those brined in 8% NaCl (A1 and A2). The partial replacement of sodium by potassium had a negative effect on the perception of bitterness, probably associated with the leaching and hydrolysis of oleuropein during fermentation and with the natural bitterness of KCl. Nevertheless, the addition of herbs to these (B1) table olives favorably masked this attribute.

Fermentation in a NaCl and KCl brine and subsequent seasoning of the obtained table olives (B2) results in a good quality product, corresponding sensorial and emotionally to the consumer expectations and preferences, as they are similar to those traditionally produced in brine fermentation of 8% NaCl and seasoned afterwards. In addition, these table olives present lower sodium and higher potassium contents, which is also important for consumer acceptability. The inclusion of aromatic herbs will also increase their antioxidant effect. In fact, antioxidant compounds, such as caffeic acid, p-coumaric acid, rosmarinic acid, caffeoyl derivatives, cavacrol and flavonoids were found in oregano and gallic acid, caffeic acid, rosmarinic acid, thymol, phenolic diterpenes and flavonoids in thyme herbs [12].

The characterization of seasoned split Cobrançosa table olives, produced in traditional brine fermentation (8% NaCl) and in a lower sodium content (4% NaCl + 4% KCl), which was the aim of this study, can be useful to improve the visibility of a food product of the Mediterranean diet as well as its future applications.

4. Conclusion

People from Mediterranean countries are habitual consumers and fond of table olives, which is part of their traditional daily diet. The partial replacement of sodium with potassium chloride in the fermentation brines of Cobrançosa olives resulted in final products with lower sodium, higher potassium and calcium contents, but with a bitter flavor. The subsequent immersion in new seasoned brines, led to a final product with improved sensorial characteristics, less bitter, low sodium content, as well as fortified in potassium and calcium, with similar amounts of macronutrients, but lower caloric value. These olives presented a microbial quality that accomplished the microbial criteria of food hygiene and safety and their nutritional characteristics make them interesting ingredients in traditional and innovative applications.

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Conflict of interest

The authors declare no conflicts of interest.

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