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Strategies to reduce sodium levels in European seabass sausages

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ABSTRACT

Considering the increasing demand towards "ready-to-cook" processed seafood products, recognised as being potential contributors to high sodium (Na) intake by consumers, this study aimed to assess the effect of sodium chloride (NaCl) reduction on physicochemical, microbiological and sensory properties of European seabass (*Dicentrarchus labrax*) sausages stored in chilling conditions during 5 weeks. Three formulations were tested in comparison with a control (100% NaCl, CTR): (i) 50% NaCl+50% ME (oleoresins microcapsules) (F1); (ii) 50% NaCl+50% KCl (F2); and (iii) only 50% NaCl (F3). The NaCl reduction mainly affected the texture and the salty taste, resulting in softer and perceived as less salty sausages after processing. However, hardness differences disappeared after 5 weeks. It seems that an antioxidant protection was obtained in sausages formulated with oleoresins microcapsules. No or low growth of psychrotrophic and mesophilic bacteria was observed (≤2.40 log CFU/g). Decreasing NaCl content and/or partially replacing it (50%) by KCl or oleoresins microcapsules seem to be suitable solutions to reduce Na (30.9–36.3%) levels, while maintaining the chilled sausages quality for 5 weeks. The partial replacement of NaCl by KCl also allows obtaining a product richer in K (997.2 mg/100 g), which ingestion may contribute for a cardiovascular protective effect.

1. Introduction

Sodium chloride (NaCl) is widely used in the food industry due to its low cost and diverse functionalities, including: a) acts as preserving agent by inhibiting microbial growth (Connell, 1980; Okoronkwo et al., 2014); b) enhances or modifies the taste perception of other ingredients (Gillette, 1985; Aaslyng et al., 2014); and c) has an important role as texture and colour enhancer, as well as binding and emulsifier agent (Pedro and Nunes, 2019).

However, high sodium (Na) intake has been linked to raised blood pressure (hypertension) and also with other health disabilities, such as kidney disease, renal stones, osteoporosis, stomach cancer and obesity (He and MacGregor, 2010; WHO (World Health Organization), 2017).

To avoid Na consumption above the recommended levels in Europe (<2 g/day, equivalent to 5 g of NaCl in adults and children), the World Health Organization (WHO) has established a global target of 30% average Na intake reduction in the population by 2025 (WHO , 2012, 2018; EFSAPanel et al., 2019). Thus, there is a strong need for the food industry to offer processed products with reduced Na levels. However, the reduction of NaCl content in processed products is a huge challenge since it can be hampered by consumer taste preferences and compromised by other sensory properties, such as texture and colour, as well as

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microbial safety and product shelf life (Pedro and Nunes, 2019). Hence, different strategies to reduce Na in processed foods have been studied. Among these, the reduction of NaCl and its partial substitution by different salts, such as food grade KCl and/or taste enhancers (e.g. plant aqueous extracts, algae extracts) have been proposed by different authors (Yamaguchi et al., 1984; Katsiari et al., 1997; Inguglia et al., 2017; Giese et al., 2019). The partial replacement of NaCl by other salts (i.e. KCl, $CaCl_2$, $MgCl_2$, K-lactate, etc.) is the most suggested approach. Within these salts, KCl has been considered as a good substitute of NaCl because it allows to obtain similar functional and microbiological properties (Vidal et al., 2019). However, the use of higher levels of KCl is mainly limited by its acrid, metallic and bitter taste. Therefore, the best option is to use well balanced mixtures of KCl and NaCl, maintaining a Na reduction range from 25 to 50% (relatively to NaCl) (Belohlawek et al., 1984; Cepanec et al., 2017).

Within Na reduction strategies, enhancers have been recently tested to improve taste or reduce bitterness. These include yeast extract, lactates, monosodium glutamate (MSG) and nucleotides, amongst others. Within these, MSG is the most common and widely used. Algae and plant aqueous extracts have also been studied in order to develop a salt substitute with low Na content (Lee et al., 2011; Mitchell, 2019). Nonetheless, previous studies focusing on the substitution of NaCl in processed seafood products by using extracts of aromatic plants and spices (such as oleoresins microcapsules considered good alternatives to NaCl (Serrano et al., 2020)) were not found in literature, and the use of KCl mainly focused on salted cod and smoked products (Martínez-Alvarez et al., 2005; Fuentes et al., 2012; Giese et al., 2019; Muñoz et al., 2020).

Fish sausages are processed products increasingly found in the European market that are rich in Na (Cardoso et al., 2019), being key players in gastronomy and revealing good sensory properties. In addition, these products have high potential due to its convenience (ready-to-eat) and absence of bones, allow an upgrading of processed seafood (e.g. of the less valuable portions of fish), as well as are often used as model to test new ingredients and/or additives. For this reason, the aim of this study was to assess the effect of NaCl reduction on the quality (i.e. the physicochemical, microbiological and sensory properties) of European seabass (*Dicentrarchus labrax*) sausages (used as model of seafood products) up to 5 weeks of chilled storage.

2. Materials and methods

2.1. Raw material and ingredients

Approximately 30 kg of European seabass (each: 600–800 g) farmed in Spain was purchased in a Portuguese supermarket (Lisbon) and immediately transported to the laboratory. Fish was weighted, manually gutted, washed, drained and filleted. In addition, the ventral part was removed. The suppliers of ingredients used as well as the different formulations of seabass sausages are shown in Table 1. The CTR treatment was formulated according to Cardoso et al. (2008) with 2.31% NaCl, while three other batches were formulated with 50% NaCl reduction. This percentage of NaCl reduction was selected after previous trials testing different concentrations of NaCl, KCl (food grade) and oleoresins microcapsules were carried out (data not shown).

The oleoresins microcapsules used as ingredient were formulated according to Serrano et al. (2020). Briefly, a mixture of dry aromatic plants and spices composed of *Allium schoenoprasum* L. (2 g), *Anethum graveolens* L. (2 g), *Capsicum frutescens* (4 g) and *Mentha pulegium* L. (2 g), selected from herbal seasoning used in culinary preparations of fish, was obtained. Oleoresins were extracted from these dry aromatic plants and spices by using a Soxhlet equipment. The oleoresin extracts (1:20 (v/v)) were encapsulated in inulin and maltodextrin (inulin/maltodextrin: oleoresin 8:2 (v/v) ratio).

Table 1
Ingredients used in the preparation of seabass sausages.

Raw material and ingredients	Suppliers	Formulation (%)			
		CTR[a]	F1	F2	F3
European seabass	Local supermarket	57.13	57.13	57.13	57.79
Fibre (Fibruline XL)	Cosucra, S.A.	5.24	5.24	5.24	5.30
Ice	–	25.16	25.16	25.16	25.45
Inner pea fibre (Swelite®)	Cosucra, S.A.	3.88	3.88	3.88	3.92
Potato starch	KMC	3.14	3.14	3.14	3.18
NaCl	Local supermarket	2.31	1.15	1.15	1.17
Milk protein concentrate (MPC-85)	Formulab Aditivos Alimentares	1.26	1.26	1.26	1.27
Soy protein concentrate	SOJAPROTEIN	1.05	1.05	1.05	1.06
Frankfurter taste	Givaudan	0.21	0.21	0.21	0.21
Dextrose	ROQUETTE Laisa España, S.A.	0.42	0.42	0.42	0.42
Ascorbic acid	Shandong Luwei Pharmaceutical Co., LTD	0.10	0.10	0.10	0.11
Smoke aroma	Ruitenberg Ingredients	0.10	0.10	0.10	0.11
Oleoresins microcapsules[b]	INIAV, I.P.[c]	–	1.15	–	–
Food grade KCl	Quimics Dalmau	–	–	1.15	–

[a] Control (CTR: 100% NaCl) formulated according to Cardoso et al. (2008); F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.
[b] Formulated according to Serrano et al. (2020).
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2.2. Sausage processing

The raw material and ingredients were mixed step by step in a refrigerated vacuum homogenizer (model UM12, Stephan and Söhne, Hameln, Germany). The mixture was transferred to a hydraulic filler (model EB-12, Mainca Equipamientos Cárnicos, S.L., Granollers, Spain) and encased under pressure into cellulose sausage casings. Immediately after, cellulose casings were twisted and tied manually. Then, sausages were cooked in an oven (Combi-Master CM6, Rational Grosskuchen Technik, GmbH, Landsberg am Lech, Germany) at 75 °C for 15 min. Subsequently, they were taken from the oven and cooled in a water/ice bath. The cellulose casings were removed and sausages were vacuum-packed (model A300/52, Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). Finally, sausages were pasteurized in the same oven for 15 min at 90 °C, cooled in a water/ice bath and kept under refrigeration (2 ± 1 °C) for five weeks until further analyses.

2.3. Analyses

Quality of seabass sausages was evaluated by physicochemical, microbiological and sensory analyses. Samples were taken for the different analyses, being performed at least in duplicate, on day 0 and after 3 and 5 weeks of storage. For each set of conditions, quality was assessed in two packages containing 3 sausages each. The proximate chemical composition and minerals (K and Na) were only assessed on day 0.

2.3.1. Proximate chemical composition and energy value

Moisture, ash and free fat contents were determined according to the Association of Official Analytical Chemists methods (AOAC, 1998). Briefly, moisture was determined by oven (ULE 500, Memmert, Schwabach, Germany) drying of sample overnight at 105 ± 1 °C, whereas ash was obtained by incineration of dry sample in a muffle furnace (TYP.MR170, Heraeus, Hanau, Germany) for 16 h at 500 ± 25 °C. Free fat was determined through the Soxhlet extraction method (in a Soxhlet apparatus, Behr Labor-Technik, Dusseldorf, Germany)

using diethyl ether solvent (at approximately 40 °C; 7h), and by weighing the fat residue after drying in a 105 ± 1 °C air oven. Crude protein was calculated from total nitrogen using the conversion factor of 6.25 (FAO, 2003). Total nitrogen was analysed according to the Dumas method (Saint-Denis and Goupy, 2004) in an automatic nitrogen analyser (LECO FP-528, LECO Corp., St. Joseph, USA) calibrated with EDTA. Nitrogen was released by combustion at 850 °C and detected by thermal conductivity. Total carbohydrates were determined by difference and the energy value was estimated using Food and Agriculture Organization factors (FAO, 1989).

2.3.2. Macroelements

Potassium (K) and sodium (Na) contents were determined by flame atomic absorption spectrophotometry (Spectr AA 55B spectrophotometer, Varian, Palo Alto, CA, USA) with a background deuterium correction, based on the method described by Jorhem (2000). The concentrations were calculated using linear calibration obtained from absorbance measurements of, at least, five different concentrations of standard solutions (KNO_3 and $NaNO_3$, dissolved in 0.5 M HNO_3).

2.3.2.1. Nutritional contribution (NC). The NC of seabass sausages in terms of Na and K was determined considering a portion of 150 g and the dietary reference values recommended by the European Food Safety Authority (EFSA, 2016; EFSAPanel et al., 2019), according to the following formula: $NC\ (\%) = 100\ \text{x}\ \frac{C\ \text{x}\ M}{AI}$, where C = mean concentration of the macroelement in mg/kg; M = typical meal portion (150 g); and AI = adequate intake (mg/day).

2.3.3. Lipid oxidation

Thiobarbituric acid reactive substances (TBARs) were determined according to the Vyncke method modified by Ke et al. (1984), from a trichloroacetic acid (7.5%) extract. Results were calculated using a standard curve prepared with five different concentrations of 1,1,3, 3-tetraethoxypropane.

2.3.4. Colour

Colour determination was performed in sausages slices (20 mm thickness and 26 mm diameter) in the model MACBETH COLOUR-EYE 3000 colorimeter (Macbeth, New Windsor, NY, USA), previously calibrated with a white standard plate. The L^*, a^* and b^* coordinates from CIELAB system were recorded. In this system, L^* denotes lightness on a scale of 0 (black) to 100 (white); the a^* values describe the intensity from green (-) to red (+); and the b^* values from blue (-) to yellow (+). Whiteness was calculated according to Schubring (2009) by the following equation: $Whiteness = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$.

2.3.5. Texture

The texture analysis was carried out on a TA.XTplus analyser (Stable Micro Systems, Surrey, UK) using the TA.XTplus software. The Texture Profile Analysis (TPA) was applied using a 30 kg load cell; the sausages were cut into slices (20 mm thickness and 26 mm diameter) and the samples were compressed twice up to 50% of the original height with a cylindrical probe of 50 cm diameter and applying a constant speed of 2 mm/s. Hardness, cohesiveness and chewiness were obtained. The puncture test was performed using a 5 kg load cell and a 5 mm diameter spherical probe, which penetrated the sample in the centre at constant speed of 1.1 mm/s. The rupture force (g) and the deformation at rupture (cm) were determined and the gel strength was obtained by multiplying both parameters.

2.3.6. Water holding capacity (WHC)

WHC was determined following the method described by Sánchez-González et al. (2008). Each sample analysed (approximately 2 g) comprised 3 slices of independent sausages. The slices were chopped into small cubes (3 × 3 mm), wrapped in two overlaid Whatman No.1 filter papers (previously weighted) and centrifuged at 3000 g (43.7 rpm) for 10 min at 18 °C (Kubota 6800, Kubota Corp., Tokyo, Japan). After centrifugation, the sample was removed, and the filter papers were weighed again.

The WHC of samples was calculated by the weight of the liquid released and expressed as the amount of water retained by the sample using the following equation

$$WHC\ (\%) = 100 \times \left[W_s \times \left(\frac{H}{100}\right) - (W_f - W_i)\right] \Big/ \left[W_s \times \left(\frac{H}{100}\right)\right]$$, where

W_s = weight of sample analysed (approximately 2 g); W_f and W_i = weight of filter papers after and before centrifugation, respectively; and H = Sample moisture (%).

2.3.7. Water activity (a_w)

a_w was determined in small slices of sausages at 20 °C using a water activity meter (Rotronic-Hydrolab, Rotronic Measurement Solutions, Bassersdorf, Schweiz).

2.3.8. pH

The pH values of fish sausages were measured instrumentally by inserting a combined glass electrode for solids (Hanna FC200, Hanna Instruments, Inc., Woonsocket, USA) directly into the sausage.

2.3.9. Total viable counts (TVC)

TVC (mesophilic and psychrotrophic) were performed according to ISO 4833-1:2013 (total mesophilic flora) and ISO 17410-1:2019 (psychrotrophic microorganisms) by plating in Plate Count Agar (BIOKAR Allonne, France) followed by incubation for 3 days at 30 °C and 10 days at 6.5 °C, respectively.

Slices of three sausages from the same package were aseptically taken and pooled until a 25 g portion was obtained. Then, to prepare the initial suspension, 10 g of this pooled test portions were aseptically weighted in a sterile bag and homogenised with 90 g of sterile Tryptone-Salt Broth (BIOKAR Allonne, France) for 60 s in a stomacher blender (Stomacher Star Blender LB 400, VWR, Leuven, Belgium). Decimal dilutions (up to 10^{-3}) were prepared in Tryptone-Salt Broth (BIOKAR Allonne, France).

2.3.10. Sensory evaluation

Sensory assessment was done in the test room of IPMA's sensory laboratory equipped according to ISO 8589 (2007). The quantitative descriptive method was applied using seven trained panellists (selected from the trained panel of 10 assessors) and a 9-point scale (0 – absent; 1 – very slight; 2 – slight; 3 – slight-moderate; 4 – moderate; 5 – moderate-strong; 6 – strong; 7 – strong-extreme; 8 – extreme) to rate the intensity of several attributes/descriptors (Meilgaard et al., 2016). Considering the potential effects of the salt reducing strategies applied, sensory test was focused on the taste, in particular salty and bitter taste, and texture properties (firmness, succulence, elasticity, cohesiveness and adhesiveness). Furthermore, for a complete characterisation of the sensory quality of the products after processing (t0 – initial) and during the refrigerated storage, other relevant attributes/descriptors were assessed: odour (typical sausage, fish, aromatic plants (due to the use of oleoresins microcapsules) and uncharacteristic), colour and taste (sausage, fish and uncharacteristic). Sausages were taken out from their packages 30 min before testing, cut into slices (15 mm thickness and 26 mm diameter) and presented to the panellists in white coded dishes sequentially in a random order.

2.4. Statistical analysis

Statistical analysis was performed using the STATISTICA software version 12 (StatSoft. Inc., Tulsa, OK, USA). The effect of NaCl reduction after processing (t = 0) on proximate composition and macroelements was evaluated by one-way analysis of variance (ANOVA). The influence

of such reduction and storage time on the other quality parameters analysed (lipid oxidation, physical, sensory and microbiological) was tested by factorial ANOVA. Tukey's HSD test was applied in groups multiple comparison. Statistical significance was considered at $P < 0.05$ for all analyses (Zar, 2010).

3. Results and discussion

3.1. Proximate chemical composition and energy value

The proximate chemical composition of seabass sausages obtained with different formulations (t = 0) is shown in Table 2. The moisture content ranged from 70.1 to 68.4%, being the significantly lower value found for F1 likely due to the fact that oleoresins microcapsules are less hygroscopic compared to the tested salts (Zieger et al., 2017; Serrano et al., 2020). Regarding ash, values ranged from 2.1 to 1.6%, and the significantly lower value can be ascribed to the reduction of NaCl content (to half) without substitutes addition in F3. No appreciable differences were found between formulations for protein, which values were close to 12–13%. The highest fat content observed in F1 (5.8%) compared to the other formulations (~3%) may be due to the presence of fat in oleoresins used in the preparation of microcapsules and explain the increase in energy value in this formulation. The carbohydrates content was approximately 12% in all formulations. Similar results of proximate chemical composition were found by other authors who studied salt reduction (to half) in cod sausages (Cardoso et al., 2009).

3.2. Macroelements

As expected, Na content found in the CTR formulation was significantly higher than that observed in the other three formulations (Table 3). The Na reductions found below 50% (i.e. between 30.9% and 36.3%) in F1, F2 and F3 can be ascribed to the fact that fish (72.31 ± 4.91 mg Na/100 g) and other ingredients used (e.g. smoke aroma, frankfurter taste) also contain Na. However, reductions higher than 50% can have a major impact not only on sensory characteristics and technological properties but also on safety (Horita et al., 2014). Similar results were obtained by other researchers, who also studied reductions of added NaCl by 50% (e.g. using blends of KCl) in seafood processed products (i.e. Na reduction of approximately 31%; Fuentes et al., 2012; Horita et al., 2014). Furthermore, the Na reductions observed in the present work were above 25%, which allows to claim that these seabass sausages can be easily commercialized by the seafood industry as products reduced in Na content, according to the Regulation (EC) No. 1924/2006 (European Parliament, 2006). Meeting this requirement is also a prerequisite for the use of the health claim that "reducing the consumption of Na contributes to the maintenance of normal blood pressure" (EC, 2012).

On the other hand, K content reached the highest value in the formulation with 50% of KCl (F2), as expected, attaining an uptake of 59.1% compared to the CTR (Table 3). This slightly higher uptake than expected can be explained by the fact that K naturally occurs in fish (329.88 ± 19.83 mg K/100 g).

It is also important to show that the Na/K ratio was 0.42 in F2 sausages, i.e. it is in the range recommended by WHO (<1) for maintaining a healthy cardiovascular condition (Whelton, 2014).

$$^{*}K\ uptake = \frac{(K\ content\ (F2) - K\ content\ (CTR)) \times 50}{K\ uptake\ expected^{**}}$$

$$^{**}K\ uptake\ expected = \frac{(KCl\ amount\ used\ in\ F2/Kg\ of\ fish \times K\ molecular\ weight)}{KCl\ molecular\ weight}$$

3.2.1. Nutritional contribution

The consumption of 150 g (usual portion) of seabass sausages produced with 100% of NaCl (CTR) contributes with 12.1% (K) and 49.5% (Na) of the daily adequate intake for adults, and 38.6% (K) and 76.2% (Na) of this intake for children (Table 4). In contrast, the formulation of seabass sausages with KCl (F2) allows to increase greatly the NC of K (NC = 42.7% for adults and NC = 136.0% for children). Moreover, the formulations F1, F2 and F3 also allow to decrease significantly the NC of Na compared to the CTR. The NC of Na is approximately 33% and 51% for adults and children, respectively in these new formulations, corresponding to 1.1–1.2 g of NaCl, which is well below the limit value recommended by EFSA (2019) (5 g of salt).

It has been demonstrated that a K intake of 3.500 mg (90 mmol)/day has beneficial effects on blood pressure in adults (EFSA, 2016). Hence, the consumption of the new seabass sausages can have human health benefits (e.g. for individuals with cardiovascular ailments) associated to the decrease of Na content and at the same time (in the case of F2) the increase of K content.

3.3. Lipid oxidation

The NaCl reduction (after processing, t = 0) did not influence seabass sausages lipid oxidation (Table 5). However, it is important to highlight that the presence of oleoresins microcapsules (F1) seemed to confer an antioxidant protection, significantly after 3 and 5 weeks of storage,

Table 2
Proximate composition of different seabass sausage formulations (t = 0).

(g/100 g)	Formulation			
	CTR	F1	F2	F3
Moisture	69.80 ± 0.32^{b}	68.36 ± 0.17^{a}	70.08 ± 0.26^{b}	70.09 ± 0.09^{b}
Ash	2.08 ± 0.00^{b}	2.13 ± 0.04^{b}	2.10 ± 0.02^{b}	1.64 ± 0.12^{a}
Protein	12.81 ± 0.02^{b}	12.19 ± 0.16^{a}	12.25 ± 0.18^{a}	12.75 ± 0.05^{b}
Fat	2.87 ± 0.08^{a}	5.84 ± 0.02^{b}	3.43 ± 0.60^{a}	3.10 ± 0.09^{a}
Carbohydrates*	12.44 ± 0.38^{b}	11.49 ± 0.00^{a}	12.15 ± 0.53^{a,b}	12.42 ± 0.11^{b}
Energy value (kcal/100 g)	131.74 ± 0.96^{a}	151.89 ± 0.52^{b}	133.14 ± 3.94^{a}	133.46 ± 0.60^{a}

Results are given as means values ± standard deviations. For each parameter, different superscript letters indicate significant differences ($P < 0.05$) between formulations. CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl; *Calculated by difference.

Table 3
Na and K concentration of different seabass sausage formulations (t = 0).

Parameters	Formulation			
	CTR	F1	F2	F3
Na (mg/100 g)[1]	660.32 ± 8.86^{b}	452.12 ± 10.67^{a}	420.85 ± 25.71^{a}	456.47 ± 7.28^{a}
Na reduction (%)	–	31.53	36.27	30.87
K (mg/100 g)[2]	283.29 ± 17.29^{a}	245.69 ± 5.52^{a}	997.16 ± 17.82^{b}	268.62 ± 14.98^{a}
K uptake (%)*	–	–	59.14	–
Na:K	2.33	1.84	0.42	1.70

Results are given as mean values ± standard deviations. For each parameter, different superscript letters indicate significant differences between formulations ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl; [1]Detection limit = 0.09; Proficiency Test = FAPAS Test 01120, Nutritional Components in Canned Meat, January–March 2018 (Fera Science Ltd., York, UK); Certified (average ± uncertainty) = 0.60 ± 0.03 mg/kg; Present work (average ± standard deviation) = 0.55 ± 0.02 mg/kg [2]Detection limit = 0.01; Certified reference material = Dorm-4, fish protein certified reference material for trace metals (National Research Council of Canada, Canada); Certified (average ± uncertainty) = 15500 ± 1000 mg/kg; Present work (average ± standard deviation) = 14500 ± 495 mg/kg.

Table 4
Nutritional contribution (NC) of seabass sausages in terms of Na and K, taking into account a meal portion of 150 g.

Macroelements	Age	Adequate Intake (mg/day)	NC (%)			
			CTR	F1	F2	F3
Na	Men/Women (≥18 years)	2000	49.52 ± 0.67^{b}	33.91 ± 0.80^{a}	31.56 ± 1.93^{a}	34.24 ± 0.55^{a}
	Children (4–6 years)	1300	76.19 ± 1.02^{b}	52.17 ± 1.23^{a}	48.56 ± 2.97^{a}	52.67 ± 0.84^{a}
K	Men/Women (≥18 years)	3500	12.14 ± 0.74^{a}	10.53 ± 0.24^{a}	42.74 ± 0.77^{b}	11.51 ± 0.64^{a}
	Children (4–6 years)	1100	38.63 ± 2.36^{a}	33.50 ± 0.76^{a}	135.98 ± 2.43^{b}	36.63 ± 2.04^{a}

Results are given as mean values ± standard deviations. Different superscripts letters within a row represent significant differences between formulations ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.

Table 5
Thiobarbituric acid reactive substances (TBARs) values in seabass sausages refrigerated for 5 weeks.

Storage time (weeks)	TBARs (mg MDA/kg)			
	CTR	F1	F2	F3
0 (Initial)	2.76 ± 0.25^{c,d}	2.23 ± 0.14^{a,b,c}	2.44 ± 0.07^{b,c,d}	2.59 ± 0.63^{b,c,d}
3	2.99 ± 0.12^{d}	2.00 ± 0.08^{a,b}	2.61 ± 0.05^{b,c,d}	2.68 ± 0.18^{c,d}
5	3.00 ± 0.05^{d}	1.77 ± 0.22^{a}	3.04 ± 0.06^{d}	2.66 ± 0.09^{c,d}

Results are given as mean values ± standard deviations. Different superscript letters indicate significant differences between formulations and storage time ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.

which can be linked to the amount of polyphenol compounds present in oleoresins (Serrano et al., 2020). Furthermore, all TBARs values were close to 3 mg MDA/kg, indicating a very good quality of seabass sausages according to Schormüller (1969).

3.4. Colour and texture

The results of colour and texture of seabass sausages over the storage time can be found in Table 6. The addition of oleoresins microcapsules (F1) and the NaCl reduction (50%) without substitutes addition (F3) lead to higher whiteness values compared to CTR after processing. In the case of F1, such values were also significantly higher than those observed in the CTR over the entire storage time. On the other hand, whiteness values were similar between the CTR and F2 (KCl addition) after processing and during storage. Other researchers also have shown that the partial NaCl replacement by KCl has no significant effect on the colour of processed products such as sausages (Cardoso et al., 2009; Fuentes et al., 2011). Furthermore, there was a significant increase in whiteness after 3 weeks of storage in all formulations, remaining the values high (84–86) after 5 weeks.

Concerning textural properties, hardness and chewiness values were significantly higher in the CTR (100% NaCl) than in the other three formulations after processing (t = 0) (Table 6). Previous studies also reported similar results and explained that this behaviour seems to be linked with the capacity of salt to solubilise proteins and, thus, to form a stronger and more cohesive network. Hence, a lower NaCl concentration in F1, F2 and F3 may have implied less solubilized protein and consequently, insufficient aggregation to form the strong protein network that cause the highest hardness and chewiness values in the CTR (Schmidt et al., 2017). Such significant pattern remained at 3 weeks of storage. Nonetheless, a significant increase of hardness and chewiness was found between 0 and 5 weeks of storage in all formulations, except in the CTR. Consequently, similar hardness and chewiness values were achieved in all formulations at 5 weeks of storage. Zamudio-Flores et al. (2015) also observed that the hardness and chewiness of Frankfurt turkey sausages (formulated with a NaCl content similar to F1, F2 and F3 sausages) increased with storage time (20 days at 4 °C).

Table 6
Whiteness and texture of seabass sausages refrigerated for 5 weeks.

	Formulation	Storage time (weeks)		
		0 (Initial)	3	5
Whiteness	CTR	81.74 ± 0.04^{a}	84.18 ± 0.00^{b,c}	84.26 ± 0.63^{b,c}
	F1	83.64 ± 0.10^{b}	85.74 ± 0.19^{d}	85.64 ± 0.15^{d}
	F2	82.01 ± 0.02^{a}	84.69 ± 0.50^{b,c,d}	83.88 ± 0.62^{b}
	F3	83.66 ± 0.49^{b}	85.18 ± 0.08^{c,d}	84.22 ± 0.54^{b,c}
Hardness (N)	CTR	94.03 ± 7.76^{d,e}	95.94 ± 6.65^{e}	83.25 ± 2.94^{c,d}
	F1	53.34 ± 2.92^{a}	63.58 ± 2.26^{a,b}	77.60 ± 4.30^{c}
	F2	58.62 ± 3.78^{a}	71.65 ± 2.43^{b,c}	78.35 ± 2.76^{c}
	F3	63.49 ± 1.62^{a,b}	64.69 ± 5.96^{a,b}	82.43 ± 2.57^{c,d}
Chewiness (N)*	CTR	37.08 ± 4.13^{d,e}	35.23 ± 1.67^{d}	43.24 ± 1.38^{e}
	F1	16.72 ± 1.43^{a,b}	17.94 ± 0.94^{a,b,c}	37.17 ± 3.27^{d,e}
	F2	22.73 ± 2.15^{b,c}	23.70 ± 1.31^{c}	39.80 ± 1.54^{d,e}
	F3	19.97 ± 1.94^{a,b,c}	15.23 ± 0.80^{a}	40.30 ± 2.57^{d,e}
Rupture Force (g)	CTR	421.68 ± 4.06^{f}	478.97 ± 6.66^{h}	449.56 ± 5.13^{g}
	F1	313.51 ± 2.16^{a}	353.49 ± 8.77^{c}	369.03 ± 5.39^{d}
	F2	349.42 ± 5.44^{c}	411.54 ± 2.18^{f}	421.59 ± 3.14^{f}
	F3	333.51 ± 1.10^{b}	393.33 ± 2.47^{e}	393.26 ± 4.42^{e}
Gel strength (g·cm)	CTR	206.44 ± 4.81^{f}	235.74 ± 5.82^{g}	224.63 ± 3.53^{g}
	F1	156.42 ± 5.90^{a,b}	166.05 ± 6.36^{b,c}	167.27 ± 5.08^{b,c}
	F2	167.22 ± 0.15^{b,c}	195.85 ± 5.77^{e,f}	194.39 ± 1.74^{e,f}
	F3	150.69 ± 2.40^{a}	186.83 ± 0.68^{d,e}	173.94 ± 4.95^{c,d}

Results are given as mean values ± standard deviations. For each parameter, different superscript letters indicate significant differences between formulations and storage time ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl; *Hardness x cohesiveness x springiness.

The reasons given above probably also explain the higher and significant cohesiveness values (data not shown) found in the CTR than in F1 and F3 after processing (from 0.49 to 0.55) and after 3 weeks of storage (from 0.39 to 0.51). Similar cohesiveness values (around 0.6) were also observed after 5 weeks of storage in all formulations.

The puncture test showed that rupture force and gel strength (Table 6) were significantly higher in the CTR than in the other formulations after processing. Horita et al. (2014) also mentioned that a higher concentration of NaCl increases the ionic strength and, as a consequence, a more uniform and denser protein matrix is formed,

increasing the gel strength. Such significant pattern remained after 3 and 5 weeks of storage. Furthermore, a significant increase of rupture force and gel strength was observed at a certain point during storage in all formulations (not significant for F1 in gel strength) probably due to the aggregation of proteins that may have become stronger.

3.5. Water holding capacity and water activity

Regarding WHC, F1, F2 and F3 showed significantly lower values (73.26%, 69.72% and 70.07%, respectively) than the CTR (75.98%) after processing (Fig. 1). It is well known that in emulsified products, the WHC of the matrix is strongly influenced by the ionic strength and functional properties of proteins. So, when NaCl is reduced by 50% and not replaced by other salts (the case of F3) or replaced by other ingredients with lower ionic strength (the case of F1 and F2), such strength decreases and, as a consequence, a less uniform and denser protein matrix is formed, decreasing the WHC (Horita et al., 2014). In the case of F1 and F3, such significant pattern remained during all storage time. Additionally, a significant WHC decrease was observed during storage in CTR and F1 sausages. However, all values obtained after 3 and 5 weeks of storage are close to 70%.

On the other hand, the a_w values were similar after processing and during storage in all formulations (ranging only from 0.948 ± 0.000 to 0.961 ± 0.001). The levels of a_w and WHC obtained were lower than those found in previous studies for similar products, which may be due, for example, to the different sausages formulation (e.g. different fish species used), fish biological condition and/or different temperature and duration of thermic treatment (Schmidt and Fontana, 2007; Dincer and Cakli, 2010; Filho et al., 2010).

3.6. pH and total viable counts (TVC)

Generally, the different formulations as well as the storage time did not significantly affect pH, which ranged from 6.1 to 6.3 in all sausages (e.g. from 6.17 ± 0.01 (CTR) to 6.31 ± 0.01 (F3) after processing (t = 0); and 6.09 ± 0.01 (CTR, t = 5) to 6.22 ± 0.01 (F2, t = 3) between 3 and 5 weeks of storage). These values are within the pH range reported in previous studies for fat and salt reduced sausages (Jin et al., 2018).

Despite the high a_w found in all sausages, psychrotrophic colony counts were always under the detection limit (<1 log CFU/g). On the other hand, mesophilic microorganisms were occasionally detected in some formulations (Fig. 2). However, colony counts were always lower than 2.40 log CFU/g, i.e., far below the acceptability limit (6 log CFU/g) (Huss et al., 2003). Such results demonstrated that, regardless the NaCl reduction, the application of vacuum packaging, heat treatment (15 min at 90 °C) and refrigerated storage allowed to obtain safe products from a microbiological point of view and consequently, with quality not compromised.

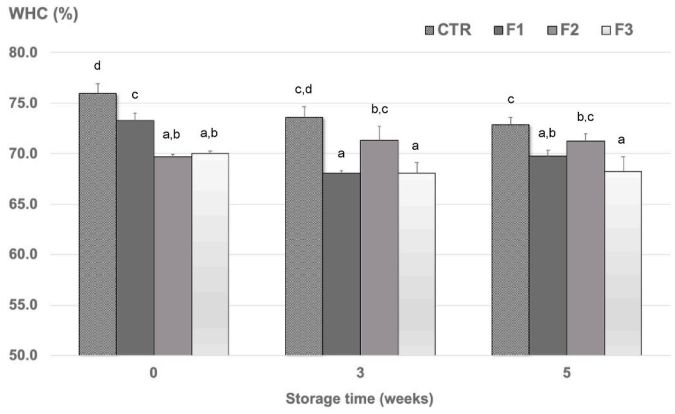

Fig. 1. Water Holding Capacity (WHC) of seabass sausages refrigerated for 5 weeks (mean values; error bars indicate the standard deviations). Different lower-case letters indicate significant differences between formulations and storage time ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.

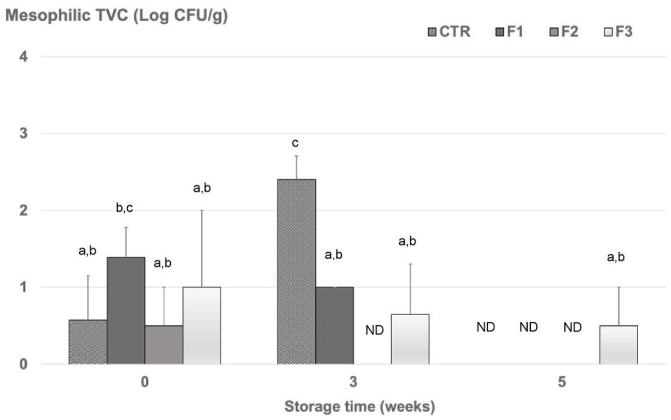

Fig. 2. Mesophilic total viable counts (TVC) in seabass sausages refrigerated for 5 weeks (mean values; error bars indicate the standard deviations). Different lower-case letters indicate significant differences between formulations and storage time ($P < 0.05$). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl. ND – Not detected.

3.7. Sensory analysis

Sensory results obtained after processing (t = 0) of sausages are shown in Fig. 3A. The odour and colour of sausages were not influenced by NaCl reduction. In all formulations, panellists detected a moderate or moderate-strong sausage odour and taste and considered the fish odour and taste as well as the aromatic plants odour as imperceptible. A slight-moderate cream-white colour was also identified in all sausages. On the other hand, the salty taste was the only taste descriptor significantly affected by the amount of NaCl used in the formulations. The sausages formulated with less 50% NaCl were scored with slight salty taste (1.5–1.8) while the CTR (100% NaCl) was rated as moderate (4.0). No odour or taste considered uncharacteristic or unpleasant (e.g. bitter) was detected in seabass sausages. Previous studies also reported that the colour and taste, with the exception of the salty taste (as in this study), were not affected by the partial replacement of NaCl by blends of salts that included 50% of KCl (Jin et al., 2018).

The firmness results tended to follow the pattern observed in instrumental measurement (see section 3.4). CTR sausages were scored with moderate-strong firmness (5.2), while the others with slight-moderate and moderate (3.3–4.2). However, these differences were not significant. All sausages were rated with similar values of adhesiveness (very slight), elasticity (slight-moderate to moderate), cohesiveness and succulence (slight to slight-moderate) (Fig. 3A).

Additionally, the sensory properties of sausages were not significantly affected by the storage time (Fig. 3A and B) and thus their sensory quality was maintained up to 5 weeks.

Overall, the different strategies applied (i.e. reducing the NaCl content and/or partially replacing it (50%) by KCl or oleoresins microcapsules) only influenced the salty taste of sausages. Such result suggests that the amount of oleoresins microcapsules tested did not confer any taste that masked the NaCl absence. However, since there is an increase in the marketing of products with reduced Na content, expectations regarding the consumers' acceptance (sensory criterion) of the new seabass sausages are high, which is crucial for the market success of new products. Thus, the formulations reduced in NaCl can be optimized and discriminative followed by affective tests should be carried out in future studies to support the proposed strategies.

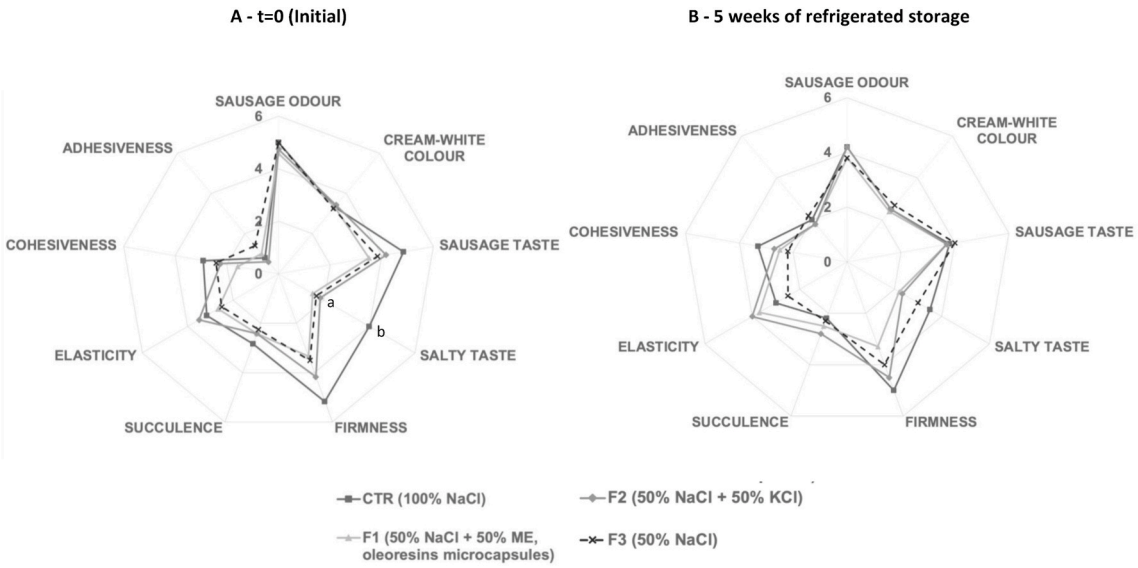

Fig. 3. Sensory profile of seabass sausages after A) processing, t = 0 (initial) and B) 5 weeks of refrigerated storage. Results corresponds to mean values ($0.8 \leq SD \leq 2.0$). 9-point intensity scale: 0 (absent), 2 (slight), 4 (moderate), 6 (strong) and 8 (extreme). For salty taste (t = 0), different lower-case letters indicate significant differences between the CTR (b) and the other three formulations (a).

4. Conclusions

The NaCl 50% reductions mainly affected the texture (although not perceived by the sensory panel) and the salty taste, resulting in softer and perceived as less salty sausages. However, such hardness differences disappeared after 5 weeks of chilling storage. The WHC was lower in the formulations with 50% less NaCl compared to the CTR. On the other hand, the NaCl reduction strategies had no microbiological effects over 5 weeks, showing that the application of vacuum packaging, heat treatment (15 min at 90 °C) and refrigerated storage allowed to obtain safe products. Additionally, the use of oleoresins microcapsules seemed to confer an antioxidant protection. It is also important to highlight that the consumption of 150 g (usual portion) of seabass sausages produced with 50% of NaCl + 50% KCl contributed to important daily intakes of potassium - a nutrient with beneficial effects on blood pressure - in adults and children (NC = 42.7% and NC>100%, respectively).

Finally, the proposed strategies are effective solutions to produce high quality products (up to 5 weeks) claimed as reduced in Na content (reduction > 25%), which can be easily implemented by the seafood industry. Nevertheless, semi-industrial scale trials and the use of new taste enhancers should be considered in the coming studies related to the Na reduction in seafood processed products.

CRediT authorship contribution statement

Anabel Estévez: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Carolina Camacho:** Investigation, Writing – review & editing. **Tatiana Correia:** Investigation. **Vera Barbosa:** Investigation, Writing – review & editing. **António Marques:** Funding acquisition, Writing – review & editing. **Helena Lourenço:** Investigation. **Carmo Serrano:** Resources, Writing – review & editing. **Margarida Sapata:** Resources. **Maria Paula Duarte:** Investigation, Resources, Writing – review & editing. **Carla Pires:** Investigation, Writing – review & editing. **Amparo Gonçalves:** Conceptualization, Investigation, Writing – review & editing. **Maria Leonor Nunes:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Helena Oliveira:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Supervision, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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