Innovative strategies to reduce sodium levels in processed seafood products

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ABSTRACT

Considering the increasing demand towards “ready-to-cook” processed seafood products, recognised as being potential contributors to high 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 texture (hardness, chewiness, cohesiveness, gel strength and rupture force) and the salty flavour, resulting in softer and less salty sausages after processing. However, hardness differences faded after 5 weeks. It seems that some 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 are effective 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 (Na/K ratio=0.42), which ingestion will contribute for a cardiovascular protective effect.

Keywords: Sodium (Na), Sodium chloride (NaCl), Potassium chloride (KCl), Oleoresins microcapsules, European seabass sausages, Quality

1. Introduction

Salt (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 (Okoronkwo et al., 2014); b) enhances or modifies the flavour perception of other ingredients (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 (in the form of NaCl) has been linked to raised blood pressure (hypertension), which is considered the most relevant risk factor for cardiovascular diseases that are the main cause of worldwide death, taking out an estimated 17.9 million lives each year (WHO, 2017). Moreover, high Na intake has also been associated with other health disabilities, such as kidney disease, renal stones, osteoporosis, stomach cancer and obesity (He and MacGregor, 2010). Due to the harmful effects of high Na intake in health, recently the World Health Organization (WHO) and the European Food Safety Authority (EFSA) Panel on Nutrition, Novel Food and Allergens (NDA) have recommended a Na intake lower than 2 g/day (equivalent to 5 g of NaCl) in adults and children (WHO, 2012; EFSA, 2019).

To avoid Na consumption above the recommended levels in Europe, the WHO has established a global target of 30% average Na intake reduction in the population by 2025 (WHO, 2018). Thus, there is a strong need for the food industry to offer processed products with low 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 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 flavour enhancers (e.g. plant aqueous extracts, algae extracts) have been proposed by different authors (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 the best substitute of NaCl because it allows to obtain similar functional and microbiological properties (Vidal et al., 2019). However, KCl use is mainly limited by its acrid, metallic and bitter side taste at higher levels. Therefore, the best option is to use well balanced mixtures of KCl and NaCl, maintaining a Na reduction range from 25-50% (relatively to NaCl) (Cepanec et al., 2017).

Within Na reduction strategies, flavour enhancers have been recently tested to improve flavour 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 (Mitchell, 2019). Nonetheless, previous studies focusing on the substitution of NaCl in processed seafood products by using extracts of aromatic plants and spices 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).

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 allow an upgrading of processed seafood, and 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) up to 5 weeks of chilled storage. Such reduction was performed by lowering its levels and/or partially replacing it (50%) by other ingredients, i.e. KCl (food grade) and an oleoresin extracted from aromatic plants and spices, encapsulated in inulin and maltodextrin microcapsules (Serrano et al., 2020).

2. Materials and Methods

2.1. Raw material and ingredients

European seabass (600-800 g), about 30 kg, 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 sausages were formulated according to Cardoso et al. (2008) with 2.31% NaCl, while the three other sausages were formulated with 50% NaCl reduction.

Table 1. Ingredients used in the preparation of seabass sausages.

Raw material and ingredients	**Suppliers**	**Formulation (%)**			
		CTR*	**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 flavour	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	INIAV	---	1.15	---	---
Food grade KCl	Quimics Dalmau	---	---	1.15	---

*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

2.2. Sausage preparation

The raw material and ingredients were mixed step by step in a refrigerated vacuum homogenizer, model UM12 (Stephan and Söhne, Hameln, Germany). Afterwards, the mixture was transferred to a model EB-12 hydraulic filler (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 a Combi-Master CM6 oven (Rational Grossküchen Technik, GmbH, Landsberg am Lech, Germany) at 75 ^{o}C for 15 minutes. Subsequently, they were taken from the oven, cooled in a water/ice bath, their cellulose casings removed and vacuum-packed using a Multivac model A300/52 (Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). Finally, sausages were subjected to

125 pasteurization in the same oven for 15 minutes at 90 ^{o}C, cooled in a water/ice bath and
126 kept under refrigeration (2±1 ^{o}C) for five weeks.
127

128 ## 2.3. Analyses

129 Quality of seabass sausages was evaluated by physicochemical, microbiological and
130 sensory analyses. Samples were taken for the different analyses, being performed at
131 least in duplicate, on day 0 and after 3 and 5 weeks of storage. For each set of conditions,
132 quality was assessed at least in 3 sausages. The proximate chemical composition and
133 minerals (K and Na) were only assessed on day 0.
134

135 ### 2.3.1. Proximate chemical composition and energy value

136 Moisture, ash and free fat were determined according to the Association of Official
137 Analytical Chemists methods (AOAC, 1998). Briefly, moisture was determined by oven
138 (ULE 500, Memmert, Schwabach, Germany) drying of sample overnight at 105±1 ^{o}C,
139 whereas ash was obtained by incineration of dry sample in a muffle furnace (TYP.MR170,
140 Heraeus, Hanau, Germany) for 16 h at 500±25 ^{o}C. Free fat was determined through the
141 Soxhlet extraction method (in a Soxhlet apparatus, Behr Labor-Technik, Dusseldorf,
142 Germany) using diethyl ether solvent (at approximately 40 ^{o}C; 7h), and by weighing the
143 fat residue after drying in a 105±1 ^{o}C air oven. Crude protein was calculated from total
144 nitrogen using the conversion factor of 6.25 (FAO, 2003). Total nitrogen was analysed
145 according to the Dumas method (Saint-Denis and Goupy, 2004) in an automatic nitrogen
146 analyser (LECO FP-528, LECO Corp., St. Joseph, USA) calibrated with EDTA. Nitrogen was
147 released by combustion at 850 ^{o}C and detected by thermal conductivity. Total

148 carbohydrates were determined by difference and the energy value was estimated using
149 Food and Agriculture Organization factors (FAO, 1989).
150
151 **2.3.2. Macroelements**
152 Potassium (K) and sodium (Na) contents were determined by flame atomic absorption
153 spectrophotometry (Spectr AA 55B spectrophotometer, Varian, Palo Alto, CA, USA) with
154 a background deuterium correction, based on the method described by Jorhem (2000).
155 The concentrations were calculated using linear calibration obtained from absorbance
156 measurements of, at least, five different concentrations of standard solutions (KNO_3 and
157 $NaNO_3$, dissolved in 0.5 M HNO_3).
158
159 **2.3.2.1 Nutritional contribution (NC)**
160 The NC of seabass sausages in terms of Na and K was determined considering a portion
161 of 150 g and the dietary reference values recommended by the European Food Safety
162 Authority (EFSA, 2016, 2019), according to the following formula: $NC(\%) = 100 \text{ x } \frac{C \ x \ M}{AI}$,
163 where C = mean concentration of the macroelement in mg/kg; M = typical meal portion
164 (150 g); and AI = adequate intake (mg/day).
165
166 **2.3.3. Lipid oxidation**
167 Lipid oxidation was determined by the 2-thiobarbituric acid index (TBA), one of the most
168 used methods. The procedure was performed according to the Vyncke method modified
169 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 (3x3 mm), wrapped in two overlaid Whatman No.1 filter papers (previously weighted) and centrifuged at 3000 g (43.7 rpm) for 10 minutes at 18 ^{o}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, $WHC\ (\%) = 100 \times \left[W_s \times \left(\frac{H}{100}\right) - (W_f - W_i)\right] / \left[W_s \times \left(\frac{H}{100}\right)\right]$, 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 ^{o}C using a water activity meter (Rotronic-Hydrolab, Rotronic Measurement Solutions, Bassersdorf, Schweiz).

212 **2.3.8. pH**

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

216

217 **2.3.9. Total viable counts (TVC)**

218 TVC (mesophilic and psychrotrophic) were performed according to ISO 4833-1:2013
219 (total mesophilic flora) and ISO 17410-1:2019 (psychrotrophic microorganisms) by
220 plating in Plate Count Agar (BIOKAR Allonne, France) followed by incubation for 3 days
221 at 30 ^{o}C and 10 days at 6.5 ^{o}C, respectively.
222 Slices of three sausages from the same package were aseptically taken and pooled until
223 a 25 g portion was obtained. Then, to prepare the initial suspension, 10 g of this pooled
224 test portions were aseptically weighted in a sterile bag and homogenised with 90 g of
225 sterile Tryptone-Salt Broth (BIOKAR Allonne, France) for 60 seconds in a stomacher
226 blender (Stomacher Star Blender LB 400, VWR, Leuven, Belgium). Decimal dilutions (up
227 to 10^{-3}) were prepared in Tryptone-Salt Broth (BIOKAR Allonne, France).

228

229 **2.3.10. Sensory evaluation**

230 Sensory evaluation was done in a test room by seven trained panellists. Sausages were
231 taken out from their packages 30 minutes before, cut into slices (15 mm thickness and
232 26 mm diameter) and presented to the panellists in white coded dishes sequentially in
233 a random order. Each panellist scored the intensity of the following
234 attributes/descriptors, on 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) (Meilgaard et al., 2016): sausage odour, fish odour, aromatic plants odour, uncharacteristic odour, white colour, cream colour, sausage flavour, fish flavour, salty flavour, bitter flavour, uncharacteristic flavour, firmness, succulence, elasticity, cohesiveness and adhesiveness.

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 period on quality parameters (chemical, 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). 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 value 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. The differences in the proximate chemical composition can also be attributed to the heterogeneity of the batter, which may not be a true emulsion (Horita et al., 2014). Similar results were found by other authors who studied salt reduction (to half) in cod sausages (Cardoso et al., 2009).

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.

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 highest reduction was observed in F2 (36.3%), followed by F1 (31.5%) and F3 (30.9%). Thus, a simple 50% reduction in

277 the added NaCl did not correspond to a 50% reduction in Na, which can be ascribed to
278 the fact that fish (72.31±4.91 mg Na/100 g) and other ingredients used (e.g. smoke
279 aroma, frankfurter flavour) also contain Na. However, reductions higher than 50% can
280 have a major impact not only on sensory characteristics and technological properties
281 but also on safety (Horita et al., 2014). Similar results (Na reductions of 27.6% and
282 approximately 35%) were obtained by other researchers, who also studied reductions
283 of added NaCl by 50% (e.g. using blends of KCl) in seafood processed products (Fuentes
284 et al., 2012; Horita et al., 2014, respectively). But, Na reductions observed in the present
285 work were above 25%, which allows to claim that these seabass sausages can be easily
286 commercialized by the seafood industry as products with reduced Na content, according
287 to the Regulation (EC) No. 1924/2006 (European Parliament, 2006). Meeting this
288 requirement is also a prerequisite for the use of the health claim that "reducing the
289 consumption of Na contributes to the maintenance of normal blood pressure" (EC,
290 2012).
291 On the other hand, K content reached the highest value in the formulation with 50% of
292 KCl (F2), as expected, attaining an uptake of 59.1% compared to the CTR (Table 3). This
293 slightly higher uptake than expected can be explained by the fact that K naturally occurs
294 in fish (329.88±19.83 mg K/100 g) and the heterogeneity of the batter (Horita et al.,
295 2014).
296 It is also important to show that the Na/K ratio was 0.42 in F2 sausages, i.e. it is in the
297 range recommended by WHO (<1) for maintaining a healthy cardiovascular condition
298 (Whelton, 2014).
299

300 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

301 Results are given as mean values ± standard deviations. For each parameter, different superscript letters
302 indicate significant differences between formulations (P<0.05). CTR: 100% NaCl; F1: 50% NaCl + 50% ME
303 (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl; [1]Detection limit = 0.09; Proficiency Test
304 = FAPAS Test 01120, Nutritional Components in Canned Meat, January–March 2018 (Fera Science Ltd.,
305 York, UK); Certified (average ± uncertainty) = 0.60±0.03 mg/kg; Present work (average ± standard
306 deviation) = 0.55±0.02 mg/kg. [2]Detection limit = 0.01; Certified reference material = Dorm-4, fish protein
307 certified reference material for trace metals (National Research Council of Canada, Canada); Certified
308 (average ± uncertainty) = 15500±1000 mg/kg; Present work (average ± standard deviation) = 14500±495
309 mg/kg.
310

311 **3.2.1. Nutritional contribution**

312 The consumption of 150 g (usual portion) of seabass sausages produced with 100% of
313 NaCl (CTR) contributes with 12.1% (K) and 49.5% (Na) of the daily AI for adults, and
314 38.6% (K) and 76.2% (Na) of this intake for children (Table 4). In contrast, the new
315 formulation of seabass sausages with KCl (F2) allows to increase greatly the NC of K
316 (NC=42.7% for adults and NC=136.0% for children). Moreover, the three novel
317 formulations (F1, F2 and F3) also allow to decrease significantly the NC of Na compared
318 to the CTR. The NC of Na is approximately 33% and 51% for adults and children,
319 respectively in these new formulations, corresponding to 1.1-1.2 g of salt, which is well
320 below the limit value recommended by EFSA (2019) (5 g of salt).
321 It has been demonstrated that a K intake of 3.500 mg (90 mmol)/day has beneficial
322 effects on blood pressure in adults (EFSA, 2016). Hence, the consumption of the new
323 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.

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

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 some antioxidant protection, significantly after 3 and 5 weeks of storage. Furthermore, all thiobarbituric acid reactive substances (TBARs) values were close to 3 mg MDA/kg, indicating a very good quality of seabass sausages according to Schormüller (1969).

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)	$2.76\pm0.25^{c,d}$	$2.23\pm0.14^{a,b,c}$	$2.44\pm0.07^{b,c,d}$	$2.59\pm0.63^{b,c,d}$
3	2.99 ± 0.12^{d}	$2.00\pm0.08^{a,b}$	$2.61\pm0.05^{b,c,d}$	$2.68\pm0.18^{c,d}$
5	3.00 ± 0.05^{d}	1.77 ± 0.22^{a}	3.04 ± 0.06^{d}	$2.66\pm0.09^{c,d}$

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

3.4. Colour and texture

The results of colour and texture of seabass sausages over the storage period 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 case of F1, such significant pattern remained 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

362 stronger and more cohesive network. Hence, a lower NaCl concentration in F1, F2 and
363 F3 may have implied less solubilized protein and consequently, insufficient aggregation
364 to form the strong protein network that cause the highest hardness and chewiness
365 values in the CTR (Schmidt et al., 2017). Such significant pattern remained at 3 weeks of
366 storage. Nonetheless, a significant increase of hardness and chewiness was found
367 between 0 and 5 weeks of storage in all formulations, except in the CTR. Consequently,
368 similar hardness and chewiness values were achieved in all formulations at 5 weeks of
369 storage. Likely, more proteins aggregated during storage in F1, F2 and F3, forming a
370 similar network to that observed in the CTR at 5 weeks. Zamudio-Flores et al. (2015) also
371 observed that the hardness and chewiness of Frankfurt turkey sausages (formulated
372 with a NaCl content similar to F1, F2 and F3 sausages) increased with storage time (20
373 days at 4 ºC).
374 The reasons given above probably also explain the higher and significant cohesiveness
375 values (data not shown) found in the CTR than in F1 and F3 after processing (from 0.49-
376 0.55) and after 3 weeks of storage (from 0.39-0.51), as well as the similar cohesiveness
377 values (around 0.6) observed after 5 weeks of storage in all formulations.
378 The puncture test showed that rupture force and gel strength (Table 6) were significantly
379 higher in the CTR than in the other formulations after processing. Horita et al. (2014)
380 also mentioned that a higher concentration of NaCl increases the ionic strength and, as
381 a consequence, a more uniform and denser protein matrix is formed, increasing the gel
382 strength. Such significant pattern remained after 3 and 5 weeks of storage. Furthermore,
383 a significant increase of rupture force and gel strength was observed at a certain point
384 during storage in all formulations (not significant for F1 in gel strength) probably due to
385 the aggregation of proteins that may have become stronger (as already mentioned).

386

387 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}

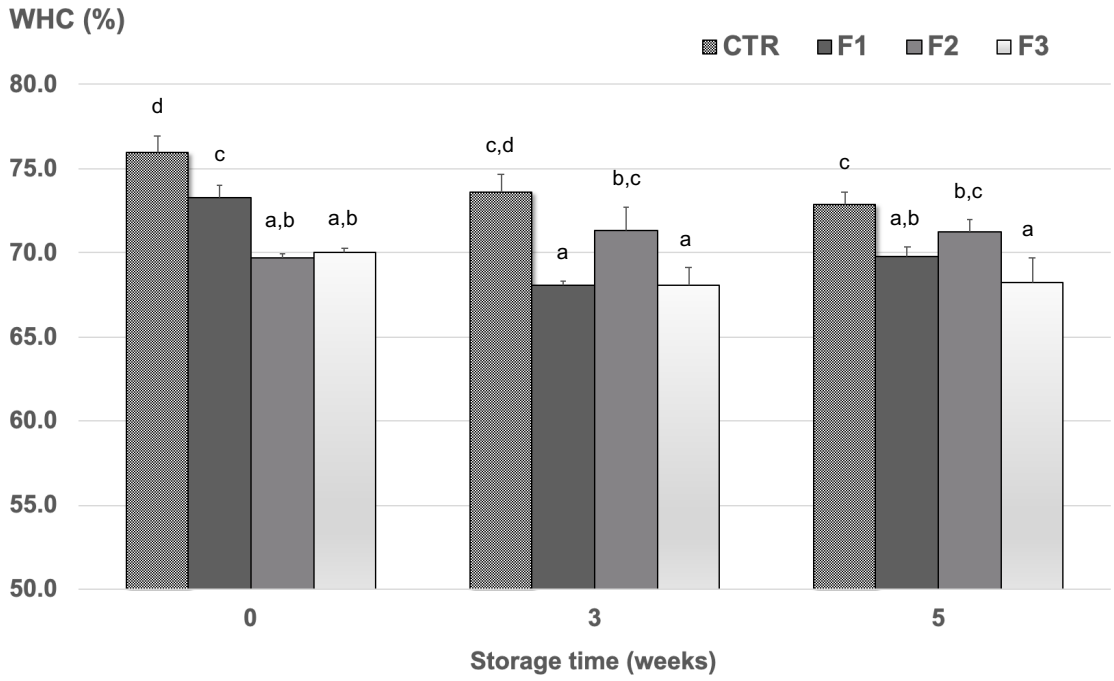
388 Results are given as mean values ± standard deviations. For each parameter, different superscript letters
389 indicate significant differences between formulations and storage period (P<0.05). CTR: 100% NaCl; F1:
390 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl; *Hardness x
391 cohesiveness x springiness

392

393 ## 3.5. Water holding capacity and water activity

394 Regarding WHC, F1, F2 and F3 showed significantly lower values (73.3%, 69.7% and
395 70.1%, respectively) than the CTR (76.0%) after processing (Figure 1). It is well known
396 that in emulsified products, the WHC of the matrix is strongly influenced by the ionic
397 strength and functional properties of proteins. So, when NaCl is reduced by 50% and not
398 replaced by other salts (case of F3) or replaced by other ingredients with lower ionic

399 strength (case of F1 and F2), such strength decreases and, as a consequence, a less
400 uniform and denser protein matrix is formed, decreasing the WHC (Horita et al., 2014).
401 In case of F1 and F3, such significant pattern remained during all storage period.
402 Additionally, a significant WHC decrease was observed during storage in CTR and F1
403 sausages. However, all values obtained after 3 and 5 weeks of storage are close to 70%.



404

405 Figure 1. Water Holding Capacity (WHC) of seabass sausages refrigerated for 5 weeks
406 (mean values; error bars indicate the standard deviations). Different lower-case letters
407 indicate significant differences between samples (P<0.05). CTR: 100% NaCl; F1: 50% NaCl
408 + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.
409

410 On the other hand, the a_w values were similar after processing and during storage in all
411 formulations (ranging only from 0.95±0.00 to 0.96±0.00). The levels of a_w and WHC
412 obtained were lower than those found in previous studies for similar products, which
413 may be due to a higher heat treatment duration applied by these authors (35 and 80
414 minutes, respectively) (Dincer and Cakli, 2010; Filho et al., 2010).

415

3.6. pH and total viable counts (TVC)

Generally, the different formulations as well as the storage period 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 (Figure 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 ^{o}C) and refrigerated storage allowed to obtain safe products from a microbiological point of view and consequently, with quality not compromised.

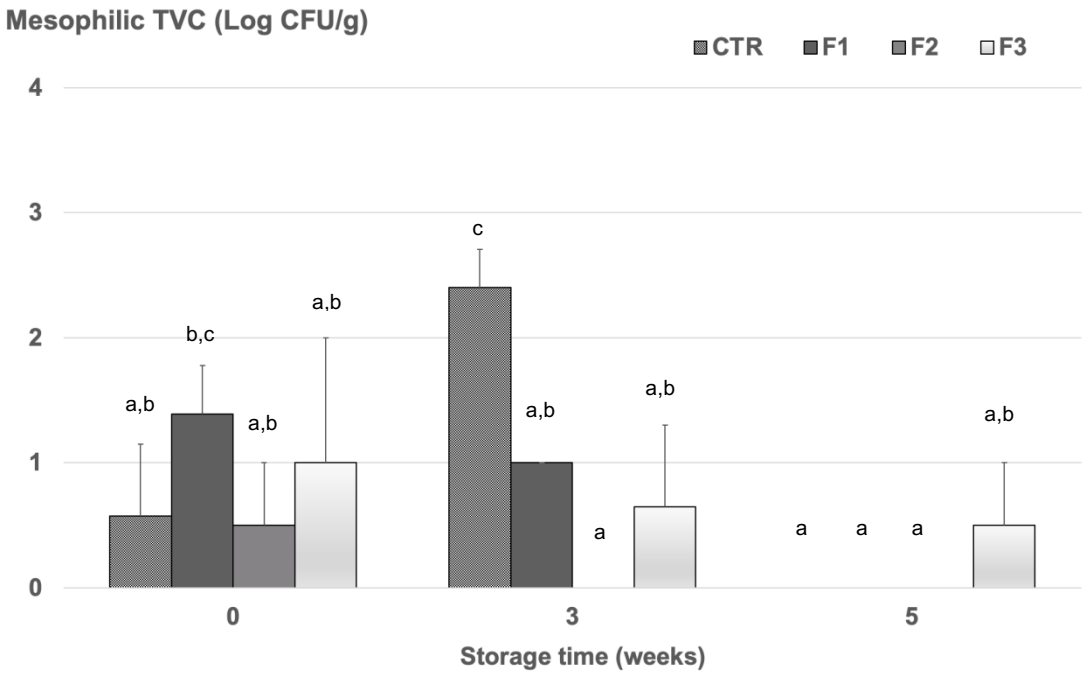
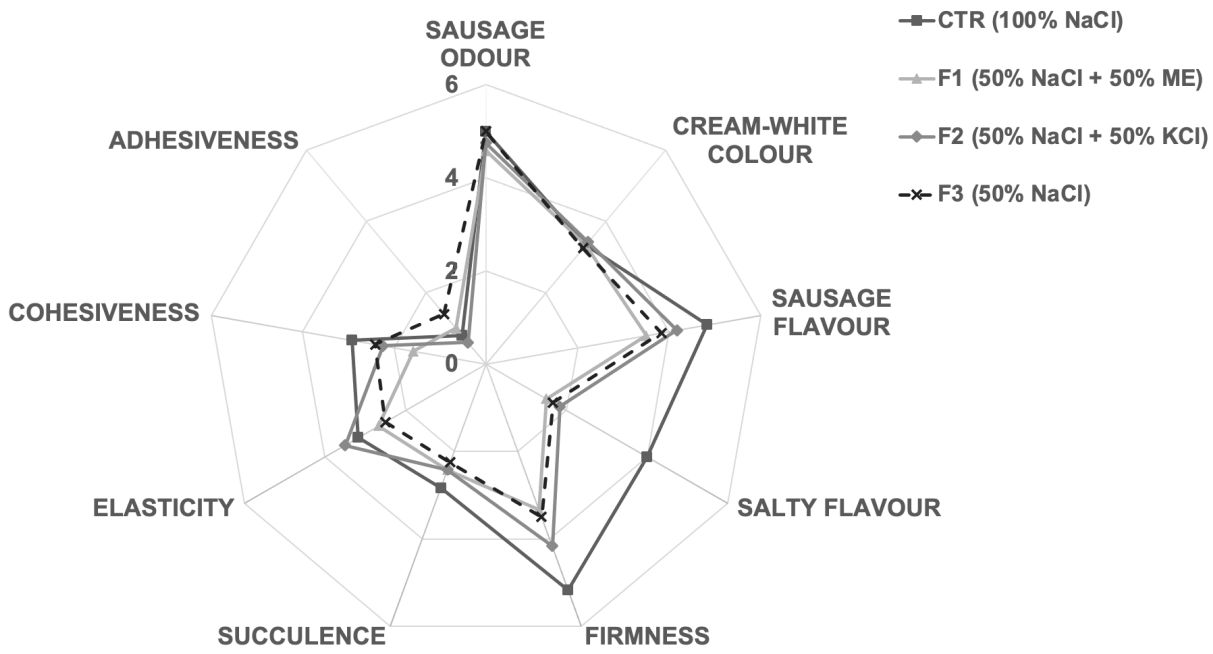


Figure 2. Mesophilic total viable counts (TVC) in seabass sausages refrigerated for 5 weeks (mean values; error bars indicate the standard deviations). Different lower-case letters indicate significant differences between samples (P<0.05). CTR: 100% NaCl; F1: 50% NaCl + 50% ME (oleoresins microcapsules); F2: 50% NaCl + 50% KCl; F3: 50% NaCl.

3.7. Sensory analysis

Sensory results obtained after processing (t=0) of sausages are shown in Figure 3. 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 flavour and considered the fish odour and flavour 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 flavour 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 flavour while the CTR (100% NaCl) was rated as moderate. No odour or flavour considered uncharacteristic or unpleasant (e.g. bitter) was detected in seabass sausages. Previous studies also reported that the colour and

448 flavour, with the exception of the salty flavour (as in this study), were not affected by
449 the partial replacement of NaCl by blends of salts that included 50% of KCl (Jin et al.,
450 2018).
451 The firmness results tended to follow the pattern observed in instrumental
452 measurement (see section 3.4). CTR sausages were scored with moderate-strong
453 firmness, while the others with slight-moderate and moderate. However, these
454 differences were not significant. All sausages were rated with similar values of
455 adhesiveness (very slight), elasticity (slight-moderate to moderate), cohesiveness and
456 succulence (slight to slight-moderate).
457 Additionally, the sensory properties (odour, colour, flavour and texture) of sausages
458 were not affected by the storage period (data not shown).
459 Overall, the different strategies applied (i.e. reducing the NaCl content and/or partially
460 replacing it (50%) by KCl or oleoresins microcapsules) only influenced the salty flavour
461 of sausages. Such result suggests that the oleoresins microcapsules did not confer any
462 flavour that masked the NaCl absence. However, since there is an increase in the
463 marketing of products with low Na content, expectations regarding the consumers´
464 acceptance (sensory criterion) of the new seabass sausages are high, which is crucial for
465 the market success of new products.
466



467

468 Figure 3. Sensory profile of seabass sausages (after processing, t=0). Results corresponds
469 to mean values (0.8≤SD≤2.0). 9-point intensity scale: 0 (absent), 2 (slight), 4 (moderate),
470 6 (strong) and 8 (extreme).

471

472 ## 4. Conclusions

473 The NaCl 50% reductions mainly affected texture and the salty flavour, resulting in softer
474 and less salty sausages. However, such hardness differences faded after 5 weeks of
475 chilling storage. The WHC was lower in the formulations with 50% less NaCl compared
476 to the CTR. On the other hand, the NaCl reduction strategies had no microbiological
477 effects over 5 weeks, showing that the application of vacuum packaging, heat treatment
478 (15 min at 90 ^{o}C) and refrigerated storage allowed to obtain safe products. Additionally,
479 the use of oleoresins microcapsules seemed to confer some antioxidant protection. It is
480 also important to highlight that the consumption of 150 g (usual portion) of seabass
481 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 flavour enhancers should be considered in the coming studies related to the Na reduction in seafood processed products.

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.

CRediT authorship contribution statement

Anabel Estévez: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Carolina Camacho, Vera Barbosa, Carla Pires:** Investigation, Writing - review & editing. **Tatiana Correia, Helena Lourenço:** Investigation. **António Marques:** Funding acquisition, Writing - review & editing. **Carmo Serrano:** Resources, Writing - review & editing. **Margarida Sapata:** Resources. **Maria Paula Duarte:** Investigation, Resources, 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.

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