

1 **Physicochemical composition and nutritional**  
2 **properties of foal burgers enhanced with**  
3 **healthy oils emulsion hydrogels**

4 **Running title: Foal burgers with healthy emulsion hydrogels**

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17 **Summary**

18        This study investigates the effect of animal fat replacement by oil mixture emulsion  
19 hydrogels on quality characteristics of foal burgers. Three batches were manufactured:  
20 control (CON) - 100% of pork fat; treatment 1 and 2 (T1 and T2) - pork fat was totally  
21 replaced using oil mixture emulsions, avocado (T1) or pumpkin seed (T2) mixed with  
22 algal oil. These fat replacements were accompanied by a significant decrease in fat  
23 content ( $P<0.001$ ) and colour parameters ( $P<0.05$ ). Any significant differences in texture  
24 were observed in reformulated patties, except for gumminess ( $P<0.05$ ) and chewiness  
25 ( $P<0.001$ ). Moreover, a healthier fatty acid profile was reached ( $P<0.001$ ), saturated fat  
26 decreased, mono- (T1) and polyunsaturated fatty acids (T2) increased and an  
27 improvement of all health indices was observed. However, the sensory acceptability of  
28 burgers was unaffected ( $P>0.05$ ). Thus, these fat reformulations represent a promising  
29 strategy to obtain healthier foal burgers with improved nutritional characteristics without  
30 affecting sensory properties.

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32 **Keywords:** foal **meat** product; healthy burger; animal fat replacers; physicochemical  
33 properties; fatty acid profile; sensory acceptance

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## 37 **Introduction**

38           Nowadays, there is a major awareness of the relationship between diet and health.  
39   Thus, meat and meat products have gradually acquired a negative connotation, since it is  
40   well known that they have a high amount of fat, mainly saturated fatty acids (SFA),  
41   cholesterol and other compounds that can damage human health (Barros *et al.*, 2020a).  
42   Therefore, in the last decade, both the meat industry and the scientific community have  
43   made great efforts to limit the use of animal fat in the production of meat products. There  
44   are mainly 3 strategies to replace animal fat with healthier lipids (Domínguez *et al.*,  
45   2021b; Heck *et al.*, 2021; López-Pedrouso *et al.*, 2021). Multiple studies have used  
46   microencapsulated oils (Lorenzo *et al.*, 2016; Heck *et al.*, 2018; Vargas-Ramella *et al.*,  
47   2020c), oleogels (Moghtadaei *et al.*, 2018; Martins *et al.*, 2019, 2020) and hydrogels  
48   (Heck *et al.*, 2019; Barros *et al.*, 2020b,a; Vargas-Ramella *et al.*, 2020b) with the aim of  
49   reformulating meat products. However, recent reviews point out that the use of emulsion  
50   hydrogels have several advantages for incorporating oils as substitutes for animal fat in  
51   comparison with the use of encapsulation techniques or oleogels (Domínguez *et al.*,  
52   2021a,b). In addition, it is also highlighted that the incorporation of an oil mixture instead  
53   of pure oil, allows the optimization of the nutritional value of the final product, limiting  
54   its impact on technological or sensory properties (Domínguez *et al.*, 2021a).  
55   Consequently, the reformulation of the foal burger is proposed by incorporating an oil  
56   mixture immobilized in an emulsion hydrogel. In particular, for this study, algal oil was  
57   employed and it was mixed with avocado or pumpkin seed oils to obtain alginate-based  
58   emulsion hydrogels.

59           Algal oil is generally used as good source of long-chain omega-3 fatty acids, like  
60   eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids  
61   (Lorenzo *et al.*, 2017). On the other hand, avocado and pumpkin seed oils are untapped

62 sources of health food: avocado oil is a relevant source of monounsaturated fatty acids  
63 (MUFA) such as oleic acid (Flores *et al.*, 2019), while in pumpkin seeds oil,  
64 polyunsaturated fatty acids (PUFA) (mainly linoleic acid) and MUFA (mostly oleic acid)  
65 fractions are the most abundant (Aksoylu Özbek and Günç Ergönül, 2020).

66 Thus, the objective of this study was to assess the **effect of animal fat replacement**  
67 by healthy oils emulsions hydrogels on proximate composition, physicochemical  
68 parameters, fatty acids profile and sensory acceptability of foal burgers.

## 69 **Materials and Methods**

### 70 *Elaboration of alginate-based emulsion hydrogels*

71 The study was performed in the Centro Tecnológico da Carne (CTC) (Ourense,  
72 Spain). Algal, avocado and pumpkin seeds oils were employed in the processing of  
73 emulsions. Algal oil, containing a high omega-3 concentration (418.3 mg/g  
74 docosahexaenoic acid; DHA; C22:6n-3), was kindly provided by Solutex Corporation  
75 (Madrid, Spain). Avocado oil (Ethnos, Sevilla, Spain) and pumpkin seed oil (Ecosana,  
76 León, Spain) were purchased from a local market. In the present study, two types of  
77 alginate-based hydrogels were processed with Prosella powder as gelling agent (Prosella  
78 VG NF4, Coli Ingredients, Mittelhausen, France) and elaborated one day before the  
79 processing of burgers (de Carvalho *et al.*, 2019; Barros *et al.*, 2020b): Treatment 1 (T1)  
80 and Treatment 2 (T2) hydrogels. These emulsions contained algal oil (2.25 g/100 g  
81 emulsion) mixed with avocado (T1) or pumpkin seed oil (T2) (35.05 g/100 g emulsion).  
82 The fatty acids composition of pork back fat and oils are shown in Table 1.

83 **The Prosella powder can be employ as animal fat replacer and consisted of jellifying**  
84 **agents (calcium sulphate and sodium alginate), wheat glucose syrup (7.4%), a stabilizer**  
85 **(disodium diphosphate, added P<sub>2</sub>O<sub>5</sub>: 9.58%) and an antioxidant (sodium ascorbate),**  
86 **which retain oils in its structure. Thus, for hydrogel preparations, water (56 g/100g) (pH**

87 7.7 and 10 °f) and algal and avocado or pumpkin seed oils (37.3 g/100 g) were mixed for  
88 1 min in a bowl cutter (Sirman, mod C15VV, Marsango, Italy). Successively, the Prosella  
89 powder (6.7 g/100 g) was added and homogenized during 3 min and put in a bowl to rest  
90 for 2 h. Once the mix was jellified, it was refrigerated at 4 °C until the manufacture of the  
91 burgers. The final proportion of the emulsions were: water (56 g/100 g), algal and  
92 avocado or pumpkin seed oil (37.3 g/100 g) and the prosella powder (6.7 g/100 g).

### 93 ***Burger manufacture***

94 For the present research, three different batches of patties were manufactured in the  
95 pilot plant (Figure 1): Control (CON) - containing 100% pork back fat as fat source (10  
96 g/100 g) and other two experimental batches in which animal fat was totally replaced by  
97 the alginate-based hydrogels (10 g/100 g) containing algal oil mixed with avocado oil  
98 (T1) or pumpkin seed oil (T2), depending on the batch. The other ingredients used in all  
99 formulations were lean foal meat (82 g/100 g) (provided by Cárnicas Mutiloa, Rocaforte,  
100 Navarre, Spain), salt (1.05 g/100 g) and water (7 g/100 g). In the case of control samples,  
101 pork back fat was selected since it is commonly used as fat source in meat products  
102 (Vargas-Ramella *et al.*, 2020b). The foal burger processing were carried out according to  
103 the procedure reported by Barros *et al.* (2020). It was produced 9 replicates for each  
104 formulation and the same elaboration was replicate three times, on different days (9  
105 samples per treatment x 3 experimental treatments x 3 manufacture process runs). After  
106 processing, the samples were collected and evaluated for their proximate composition,  
107 physicochemical parameters, fatty acids profile and sensory analysis.

### 108 ***Physicochemical, lipid oxidation and composition analysis***

109 The proximate composition, pH, colour, cooking loss and texture profile analysis  
110 (TPA) were determined following the procedures described by Vargas-Ramella *et al.*  
111 (2020b). The energy content was calculated according to European Commission

112 Regulation (Regulation (EU) No 1169/2011, 2011). Lipid oxidation was evaluated  
113 through thiobarbituric acid reactive substances (TBARS) index using the method  
114 described by Vyncke (1975) and values were expressed as mg MDA/kg sample.

### 115 ***Fatty acids analysis***

116 The fatty acids were quantified following the procedure described by Barros *et al.*  
117 (2020b). Separation and quantification of the FAMES was carried out using a gas  
118 chromatograph (GC-System 7890B; Agilent Technologies Spain, S.L., Madrid, Spain)  
119 with flame ionization detector (FID), following the chromatographic conditions reported  
120 by Barros *et al.* (2020b) and data were expressed as g/100 g of fat. The health indices of  
121 foal burgers were calculated: n-6/n-3 and PUFA/SFA ratios, atherogenic (AI)  $[C12:0 +$   
122  $(4 * C14:0) + C16:0] / [(\Sigma MUFA) + (\Sigma PUFA)]$  and thrombogenic (TI)  $[C14:0 + C16:0 +$   
123  $C18:0] / [(0.5 * \Sigma MUFA) + (0.5 * n-6) + (3 * n-3) + (n-3/n-6)]$  indices (Ulbricht and  
124 Southgate, 1991) and hypocholesterolemic/hypercholesterolemic ratio (h/H)  $[\Sigma(C18:1n-$   
125  $9, C18:1n-7, C18:2n-6, C18:3n-3, C20:3n-6, C20:4n-6) / \Sigma(C14:0 + C16:0)]$  (Fernández  
126 *et al.*, 2007). It is well known that some fatty acids can help to prevent or promote  
127 coronary thrombosis and atherosclerosis based on their effect on low-density lipoprotein  
128 (LDL) concentration and serum cholesterol. In particular, AI and TI indices reflect the  
129 effects of fatty acids on cardiovascular risk, while the h/H ratio indicates the functional  
130 effects of fatty acids on cholesterol metabolism (Ulbricht and Southgate, 1991; Fernández  
131 *et al.*, 2007).

### 132 ***Consumer evaluation***

133 For sensorial analysis, a total of 39 consumers (with age between 29 and 40 and  
134 from both genders) from Ourense (Spain) participated in the test. Restrictions caused by  
135 the world state of emergency (September 2020) limited the participation of a major  
136 number of tasters, although it was obtained an appropriate number, according to

137 Mammasse and Schlich (2014). This work consists in a preliminary consumer analysis  
138 realized using a home use test (HUT). This methodology was another election caused by  
139 the limitations of the pandemic state. The aim of this study was to evaluate consumers'  
140 acceptance and preference of the distinct foal burgers elaborated. The treatments were  
141 evaluated in raw and cooked samples. Each consumer tasted three samples, one for each  
142 formulation, in a single session. Consumers evaluated the foal burgers by the acceptance  
143 test using a 7-point hedonic scale, which ranged from "1-disliked much" to "7-liked  
144 much", for the following attributes: in raw burgers – visual aspect and odor and in cooked  
145 burgers – cooked odor, texture (firmness), juiciness, greasy character, flavor and overall  
146 acceptability. In addition, it was asked to order the sample according to their preference  
147 (UNE-EN ISO 8589:2010/Amd 1:2017, 2017) using a 3-point scale (1=less favourite ad  
148 3= most favourite). Moreover, specific instructions were provided to consumers.  
149 Furthermore, the samples were coded with 3-digit random numbers and it was indicated  
150 randomly in which order to taste samples in order to avoid the possible effects of the order  
151 of presentation.

### 152 ***Statistical analysis***

153 Statistical analyses were performed using the SPSS statistical software (SPSS 25.0,  
154 Chicago, IL, USA). Normal distribution and variance homogeneity were previously  
155 tested (Shapiro-Wilk). Data were submitted for analysis of variance (ANOVA), where  
156 the parameters were set as dependent variables, treatments (fat source) were considered  
157 as fixed effects and replications (the experiment was repeated three times) as a random  
158 effect, while for sensory acceptance consumers were additionally included in the model  
159 as a random effect (each panellist tasted three samples, one for each treatment, in a single  
160 session). The pairwise differences between least-square means were evaluated by  
161 Duncan's method. Differences were considered significant if  $P < 0.05$ . The statistical

162 evaluation for the preference test was performed using the Friedman test, with Newell  
163 and McFarlene tables ( $\alpha=0.05$ ). When a significant effect ( $P<0.05$ ) was found, least  
164 significant difference (LSD) test was used as a multiple comparison test.

## 165 **Results and Discussion**

### 166 *Physicochemical analysis of foal burgers*

167 Proximate composition and physicochemical results of the foal burgers are shown  
168 in Table 2. The [pork backfat](#) replacement by oil emulsion hydrogels resulted in a  
169 significant ( $P<0.001$ ) increase in moisture and ash values and a decrease in fat and protein  
170 contents. A similar trend was reported by previous studies for moisture and ash in beef  
171 burgers reformulated with algal oil (Alejandre *et al.*, 2017) and/or wheat germ oil (Barros  
172 *et al.*, 2020a), with tiger nut oil (Barros *et al.*, 2020b) and with chia and linseed oil (Heck  
173 *et al.*, 2019) compared to conventional formulations (high animal fat percentage).  
174 Conversely, Martins *et al.* (2019) found a decrease in moisture and ash in pork patties  
175 with partial (75%) animal fat replacement. In the present case, according to recent studies  
176 (Barros *et al.*, 2020a,b), the increase in moisture and ash was due to the amount of water  
177 (56 g/100 g) and prosella powder (6.7 g/100 g) used to prepare the different emulsions.  
178 On the other hand, lipid values significantly decreased ( $P<0.001$ ) with animal fat  
179 replacement (from 9.86 g/100 g in CON to 7.38 g/100 g T1 burgers) and treatments  
180 achieved a level of reduction in fat of about 25% (T1) and 23% (T2) compared to control  
181 group. These results can be expected considering that pork back fat was substitute for an  
182 oil-in-water emulsions which only contained 37.2% of oil. This outcome is in agreement  
183 with those previously reported by other authors (Alejandre *et al.*, 2017; Heck *et al.*, 2019;  
184 Barros *et al.*, 2020b,a; Vargas-Ramella *et al.*, 2020b), who reformulated burgers with  
185 hydrogelled emulsions. Similarly, [T1 and T2 burgers reported a significant \( \$P<0.001\$ \)](#)  
186 [reduction in protein content](#). This result is consistent with data [published](#) in literature by



187 other authors, who replaced (partially or totally) pork back fat by emulsion hydrogels in  
188 burgers (Heck *et al.*, 2019; Barros *et al.*, 2020b; Vargas-Ramella *et al.*, 2020b), or by fish  
189 oil in liver pâté (Domínguez *et al.*, 2017b). Nevertheless, some studies observed an  
190 opposite trend (de Oliveira Fagundes *et al.*, 2017; Barros *et al.*, 2020a), reporting a  
191 significant increase in protein content of reformulated beef burgers with vegetable oils.  
192 In our study, the protein content diminution in T1 and T2 burgers could be related to the  
193 fact that animal fat contains about 10% of proteins (Heck *et al.*, 2019), whereas no  
194 proteins were added to the emulsion hydrogel (containing only water, oil and gelling  
195 agents). All formulations studied (Table 2) can be claimed as “high protein content”  
196 according to European Regulation (EC, 2006), since at least 20% of the energy value of  
197 the product is provided by protein.

198         This variation in proximate composition among the three batches was reflected in  
199 energy parameters, as could be expected. T1 and T2 samples reported the lowest energy  
200 content values compared to CON samples ( $P<0.001$ ), recording a decrease of 14-15%. It  
201 is widely known that fat represents the most important component of calorie content.  
202 Thus, a diminution in fat content (as occurs in the reformulated burgers) provides a  
203 decrease in calorie content. These outcomes agree with data reported by previous  
204 researchers (Alejandre *et al.*, 2017; Barros *et al.*, 2020b,a).

205         Considering lipid oxidation, any significant differences ( $P>0.05$ ) among batches  
206 were detected on TBARs values. However, according to previous studies (Alejandre *et al.*  
207 *et al.*, 2017; Barros *et al.*, 2020a,b), the control formulation showed the highest TBARs  
208 concentrations (0.30 mg MDA/kg) in comparison to reformulated burgers, T1 (0.25  
209 mg/MDA/kg) and T2 (0.26 mg MDA/kg). Either way, results were all below sensory  
210 threshold limits at which consumers perceived rancidity (de Carvalho *et al.*, 2019). It is  
211 well known that highly unsaturated fatty acids (UFA) are more susceptible to the

212 oxidative degradation than SFA (Domínguez *et al.*, 2019). However, despite the high  
213 concentration of UFA in avocado, pumpkin seed and algal oils (Table 1), T1 and T2  
214 burgers were not affected by oxidation. Barros *et al.* (2020) confirmed that oleic acid (the  
215 major fatty acid in avocado oil) is less sensitive to oxidative processes than PUFAs, which  
216 could explain our results. Moreover, according to recent studies (Alejandre *et al.*, 2017;  
217 Serdaroğlu *et al.*, 2017; Barros *et al.*, 2020a,b), the presence of natural antioxidants in the  
218 oils and the protective action of the emulsion hydrogel (immobilized oil) against  
219 oxidizing agents could justify our outcomes.

220 Instrumental colour ( $L^*$ ,  $a^*$  and  $b^*$ ) of foal burgers was affected by the  
221 incorporation of oil emulsion hydrogels ( $P < 0.05$ ). This trend is consistent with data  
222 reported by other authors (de Souza Paglarini *et al.*, 2019; de Carvalho *et al.*, 2020;  
223 Vargas-Ramella *et al.*, 2020c), who observed significant differences on colour values in  
224 meat products reformulated with different vegetables oils as animal fat replacers. On the  
225 contrary, analogous studies (Pires *et al.*, 2019; Barros *et al.*, 2020a) did not detect  
226 significant difference on colour parameters among the different treatments. However, our  
227 results and the presence of distinct outcomes in literature could be related to the different  
228 oil characteristics and composition, the emulsion properties and the other ingredients used  
229 in the meat product formulation (Barros *et al.*, 2020a). In particular, Table 2 showed that  
230 pork fat replacement decreased significantly  $L^*$  and  $a^*$  values, while T1 burgers reported  
231 the highest  $b^*$  values. In all cases, the lowest values were detected in T2 burgers, whose  
232 values are in line with the characteristic greenish colour of pumpkin seed oil (visual  
233 assessment) (Figure 1).

234 As regards pH, values were not affected by the animal fat replacement for oils  
235 emulsion hydrogels ( $P > 0.05$ ). These results are in accordance with previous authors  
236 (Martins *et al.*, 2019; Pires *et al.*, 2019; Barros *et al.*, 2020a), who observed that fat source

237 did not significantly affect the meat products reformulated with vegetable oil emulsions.  
238 Moreover, reformulated burgers reported significantly ( $P<0.01$ ) lower cooking loss  
239 values compared to CON treatment (25.01% in T1 and 22.85% in T2 vs. 27.14% in CON  
240 treatments). Similarly, recent findings observed that cooking loss decreased in beef  
241 burgers elaborated with vegetable oils as back fat replacers (Gómez *et al.*, 2018;  
242 Moghtadaei *et al.*, 2018; Barros *et al.*, 2020b). In our case, this diminution could be  
243 justified by the use of alginate in the emulsion hydrogel, which acted as barrier against  
244 liquid loss during cooking in (Moghtadaei *et al.*, 2018).

245         Considering texture parameters, only gumminess and chewiness were affected  
246 ( $P<0.05$ ) by healthier fat reformulation. Hardness, springiness and cohesiveness were  
247 similar in all treatments ( $P>0.05$ ). In particular, T2 burgers reported the lowest values in  
248 gumminess ( $P<0.05$ ) and chewiness ( $P<0.001$ ) compared to the other two formulations.  
249 Whereas, T1 burgers presented an opposite behaviour, showing a firmer texture and the  
250 highest values, especially in gumminess and chewiness. This trend is according to the  
251 results of other authors, who reported an increment in chewiness (de Oliveira Fagundes  
252 *et al.*, 2017; Heck *et al.*, 2019) and in gumminess (de Oliveira Fagundes *et al.*, 2017) in  
253 reformulated batches. On the other hand, our results disagree with data reported by recent  
254 studies (Alejandre *et al.*, 2019; Paglarini *et al.*, 2019; Barros *et al.*, 2020b; dos Santos *et*  
255 *al.*, 2020; Vargas-Ramella *et al.*, 2020b), where any significant differences were observed  
256 in reformulated meat products containing oil emulsions as animal fat replacers. On the  
257 whole, in our study, it is complicated to relate the different behaviour of batches to a  
258 single factor, since relevant differences in proximate composition were detected (protein,  
259 lipid, and ash content) (Barros *et al.*, 2020a). Moreover, the distinct features of selected  
260 oils, the different physicochemical characteristics between animal fat and oil-in-water

261 emulsions employed and their interaction with meat could explain part of the textural  
262 differences among batches.

### 263 ***Fatty acids and health indices of foal burgers***

264 The fatty acids contents (g/100 g fat) and health indices of the different foal burgers  
265 are shown in Table 3 (only those represented >0.1%). Unsurprisingly, the replacement of  
266 pork back fat by T1 and T2 alginate-based emulsion hydrogels affected the lipid profile  
267 of burgers. As regards SFA, palmitic (C16:0) and stearic (C18:0) acids were the most  
268 abundant, especially in CON batches. However, coinciding with the literature (Heck *et*  
269 *al.*, 2019; Barros *et al.*, 2020a,b; Vargas-Ramella *et al.*, 2020a,b), the substitution of  
270 animal fat by healthy oil formulations produced a statistically significant reduction of  
271 SFA fraction ( $P<0.001$ ) compared to the conventional meat products. These outcomes  
272 are related with the significant lower values of C16:0 and C18:0 obtained in the  
273 reformulated samples. In this manner, it is obtained an important reduction of SFA with  
274 atherogenic, hypercholesterolemic (C16:0) and thrombogenic (C16:0 and C18:0) effects  
275 (Fernández *et al.*, 2007; Montesano *et al.*, 2018).

276 MUFA content also resulted be affected by the type of fat source employed  
277 ( $P<0.001$ ). Among them, oleic acid (C18:1n-9) represented the prevalent fatty acids in all  
278 of the cases, with concentrations ranging from 29.29 to 35.69 g/100g of fat, where the  
279 highest values belonging to T1 batch. Similarly, other individual MUFA such as  
280 palmitoleic (C16:1n-7) and cis-vaccenic acids (C18:1n-7) showed the highest values in  
281 T1 burgers. These outcomes agree with what previously reported by Rodríguez-Carpena  
282 *et al.* (2012), studying the partial substitution (50%) of animal fat by avocado oil in burger  
283 patties. Additionally, significant MUFA increment was observed in other studies, where  
284 tiger nut (Barros *et al.*, 2020b; Vargas-Ramella *et al.*, 2020b), canola (Alejandre *et al.*,

285 2019; Vargas-Ramella *et al.*, 2020a), olive and soybean oils (Vargas-Ramella *et al.*,  
286 2020a) emulsion hydrogels were investigated as animal fat replacers.

287 Moreover, data showed statistically significant differences ( $P<0.001$ ) in PUFA  
288 concentrations among treatments and T2 burgers reported the highest values compared to  
289 CON and T1 samples. Linoleic acid (C18:2n-6) was the most abundant in the three  
290 formulations ( $P<0.001$ ), although it was predominant in T2 batch. This outcome could be  
291 expected since pumpkin seed oil has a high linoleic content as showed in Table 1 (39.60  
292 g/100g of oil), which explained also the highest omega-6 (n-6) concentration in T2  
293 samples. Whereas, among omega-3 fatty acids (n-3),  $\alpha$ -linolenic acid (C18:3n-3) was the  
294 most abundant in T1 samples ( $P<0.001$ ). In addition, the presence of algal oil in the  
295 emulsion hydrogels increased omega-3 fatty acids (n-3) of reformulated burgers  
296 ( $P<0.001$ ). In fact, as **previously mentioned**, it is recognized that marine oils contains high  
297 amounts of long-chain n-3 (LC n-3), as eicosapentaenoic (EPA, C20:5n-3) and  
298 docosahexaenoic (DHA, C22:6n-3) acids (Munekata *et al.*, 2020). This is confirmed and  
299 reflected in our data, where reformulated burgers contained 94.80 mg EPA+DHA/100 g  
300 of burger (T1 samples) and 98.73 mg EPA+DHA/100 g of burger (T2 samples) (data not  
301 shown). Thus, they could be claimed as “source of omega-3 fatty acids” and “high omega-  
302 3 content”, according to the European Parliament (Regulation (EU) No 116/2010, 2010),  
303 which establishes a minimum of 40 mg and of 80 mg of the sum of EPA and DHA per  
304 100 g of product, respectively. Our results are consistent with those obtained by other  
305 authors (Alejandre *et al.*, 2019; de Souza Paglarini *et al.*, 2019; Heck *et al.*, 2019; Barros  
306 *et al.*, 2020a,b; Vargas-Ramella *et al.*, 2020b), who observed a relevant increase of PUFA  
307 proportions in meat products reformulated with vegetable and/or marine oils as pork back  
308 fat substitutes. In particular, in line with the oils employed, **the** authors observed an  
309 increase of omega-6 and/or omega-3 fractions, **similar to ours**.

310 Therefore, as general conclusion, it seems that the lipid profile of the burgers  
311 reflects the fatty acid composition of the fat source employed in their formulation. As  
312 well as, several studies about reformulated burgers (de Oliveira Fagundes *et al.*, 2017;  
313 Heck *et al.*, 2017, 2019; de Carvalho *et al.*, 2019; Barros *et al.*, 2020b,a; Vargas-Ramella  
314 *et al.*, 2020b) reported the same conclusions.

315 Regarding the nutritional values of foal burgers (Table 3), T1 burgers recorded the  
316 lowest n-6/n-3 ratio (3.01) among all formulations ( $P<0.001$ ). As described above, T1  
317 emulsion hydrogel modified both n-6 and n-3 contents in foal burgers, reducing the n-  
318 6/n-3 ratio and obtaining a value minor than 4, according to the recommendation of the  
319 n-6/n-3 ratio (Simopoulos, 2004). Whereas, T2 and CON samples exceeded the  
320 recommended ratio by 2.91 and 2.64, respectively. However, it is worth mentioning that  
321 results derived by n-6/n-3 ratio should not to be considered alone. Moreover, it is relevant  
322 to observe that pork back fat replacement by T1 and T2 emulsion hydrogels increased the  
323 PUFA/SFA ratio ( $P<0.001$ ), recording values above 0.4 (0.46 in T1 and 0.85 in T2), as  
324 recommended (Wood *et al.*, 2008). These outcomes represented an important  
325 improvement in the nutritional characteristics of the fatty acids composition of foal  
326 burgers. Similar results were obtained by other authors, replacing animal fat with  
327 vegetable fat sources in meat products (Heck *et al.*, 2019; Pires *et al.*, 2019).

328 In addition, following the recommendations, healthy products should have AI and TI as  
329 low as possible (Ulbricht and Southgate, 1991), while h/H should be high. In our study,  
330 in both reformulated burgers were observed a decrease of TI and AI indices and an  
331 increase of h/H index compared to CON treatment ( $P<0.001$ ), evidencing the  
332 improvement of the lipid profile obtained with the substitution of animal fat by healthy  
333 vegetable oil emulsion hydrogels. Similar to the present study, other authors found a  
334 reduction in TI and AI and an increment of h/H index in meat products applying vegetable

335 oil emulsions as fat replacers (Domínguez *et al.*, 2016, 2017a,b; Heck *et al.*, 2019; Barros  
336 *et al.*, 2020b). Thus, the reformulations employed confers healthier characteristics to the  
337 final products. As regards algal oil, although it presents high SFA and low MUFA  
338 contents, it has an elevated PUFA content, being a precious source of omega-3 fatty acids  
339 as commented above (Table 1). Thus, the presence of algal oil in the emulsion hydrogels  
340 surely favored and took part to the improvement of the lipid profile of our reformulated  
341 burgers, also by a nutritional standpoint.

#### 342 ***Consumer evaluation of foal burgers***

343 Table 4 presents the acceptance test results for the different burger treatments. Any  
344 significant differences ( $P>0.05$ ) among formulations were detected by consumers in the  
345 acceptance test, reporting similar values for all sensory parameters. Data showed a  
346 tendency among batches only for flavour attribute ( $P<0.1$ ), where T1 samples recorded  
347 the lowest values compared with the other two batches (3.7 for T1 vs. 4.6 for CON and  
348 T2 samples). The low acceptability of T1 samples could be justified by its characteristic  
349 flavour, which is not described as a typical burger flavor. However, when overall  
350 acceptance was studied, although T2 and CON obtained the same scores (4.6) and higher  
351 than T1 burgers (3.8), no differences were detected among the three formulations  
352 ( $P>0.05$ ). It is possible to affirm that all formulations were considered “accepted”,  
353 recording values higher than 3.5 (acceptability limit). Thus, these outcomes indicated that  
354 the use of T1 and T2 emulsion hydrogels as animal fat replacers did not alter the sensory  
355 acceptability of foal burgers. Our results are consistent with previous studies realized by  
356 other researchers, who reported that 100% animal fat substitution by algae oil (Alejandre  
357 *et al.*, 2017), tiger nut oil (Barros *et al.*, 2020b; Vargas-Ramella *et al.*, 2020b), chia or  
358 linseed oils (Vargas-Ramella *et al.*, 2020b) emulsion hydrogels in burgers did not affect  
359 the consumer acceptability.

360 As regard preference test, it allows to understand how differently the consumer's  
361 perception of the three burger formulations was. Total scores of preferences (number in  
362 brackets in Table 5) showed that CON and T2 samples were the most chosen (most  
363 favourite) by the consumers, while the T1 burgers were the least favourite. However,  
364 Friedman's test indicated that total preference was unaffected ( $P>0.05$ ) by the type of fat  
365 source included in the formulations ( $F_{\text{test}}<F=0.05$ ). Thus, taking into account the results  
366 obtained from the sensorial analysis, T1 and T2 emulsions could represent successful  
367 pork back fat replacers since their incorporations did not modify the global acceptance of  
368 the final products.

### 369 **Conclusions**

370 The use of T1 and T2 emulsions as pork back fat substitutes was able to reduce the  
371 fat amount, increase the product yield and elaborate healthier burgers, according to their  
372 characteristics. Reformulated burgers showed a significant reduction of the energy  
373 content according to the proximate composition changes. Both alginate-based hydrogel  
374 emulsions reduced SFA content, T1 formulation increased MUFA content (especially  
375 oleic acid), while T2 raised PUFA fraction (particularly linolenic acid) in foal burgers.  
376 The presence of algal oil in both emulsions provided an added value to the products, since  
377 increased the omega-3 content (in particular DHA), allowing to claim the reformulated  
378 burgers as "high omega-3 content" and "source of omega-3". Furthermore, both  
379 formulations improved health indices, obtaining PUFA/SFA, TI, AI and h/H values in  
380 line with the health recommendations. In addition, T1 burgers reduced n-6/n-3 ratio,  
381 obtaining a value  $<4$ , as recommended. Moreover, the inclusion of these hydrogels  
382 showed to not affect sensory acceptability. Further studies are necessary to improve  
383 technological features of the reformulated burgers (as colour and texture). Thus, as a



384 general conclusion, the use of emulsion hydrogels is a promising strategy to develop  
385 healthy burger without affecting sensory properties.

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### 396 **Ethical Guidelines**

397 **Ethics approval was not required for this research.**

### 398 **Conflict of interest**

399 The authors declare that there are no conflicts of interest.

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486 **ionic gelation. The lipid reformulation did not affect hardness and improved**  
487 **important technological properties, such as cooking loss and fat retention. In**  
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