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3 **Production of low-fat frankfurter sausage using a new fat replacer:**
4 **reconstituted agar hydrogel**

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6
7 **Running title:** Production of low-fat frankfurter sausage
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11
12 **Abstract**

13 There is a growing trend towards healthy meat products containing lower fat content. The use of
14 fat replacers can solve the quality problems of low-fat products. This study aimed to produce low-
15 fat frankfurter sausage using a new fat replacer. Thus, in the first stage, reconstituted agar hydrogel
16 (RAH) was produced by adding water to agar cryogel after homogenization. In the second stage,
17 RAH was used as a fat substitute in frankfurter sausage at 0, 25, 50, 75, and 100%, and the
18 characteristics of the resulting low-fat sausage, including chemical composition, water holding
19 capacity, cooking loss, texture characteristics, porosity, color, percentage of fat after frying, oil
20 absorption, pH over time and sensory evaluation were examined. Results showed that RAH could
21 form two kinds of gel by temperature changes: a low-set gel at 55°C and a high-set gel at 90°C.
22 Substitution of oil in the sausages caused an increase in moisture content, porosity, oil absorption,
23 and cooking loss. On the other hand, this replacement reduced fat content, cutting force, water
24 holding capacity, fat percentage after frying, and texture properties such as hardness, cohesiveness,
25 springiness, gumminess, and chewiness. The results of pH measurements over time showed that
26 sulfated antimicrobial groups in the agar structure delayed the spoilage of sausages containing
27 RAH compared to the control sample. Sensory evaluation showed that RAH-containing sausages
28 were not significantly different from the control sample in terms of color, juiciness, and texture.
29 However, flavor and overall acceptance increased significantly under the influence of this
30 substitution ($P < 0.05$). So, consumers selected a sample with 25% replacement as the optimal
31 sample. As a result, RAH can be successfully used as a fat replacer in low-fat products with
32 desirable quality characteristics.

33 **Keywords:** Fat substitute, Cryogel, Reconstituted hydrogel, Low-fat sausage.

34

35 **Introduction**

36 Frankfurters are emulsion-type sausages that are widely consumed. In restructured meat
37 products, fat plays an important role in stabilizing meat emulsions, reducing cooking loss,
38 improving water holding capacity, and providing organoleptic quality (Rather et al. 2015). It also
39 provides essential nutrients such as fat-soluble vitamins and essential fatty acids and plays an
40 important role in the flavor and texture of meat products. On the other hand, a high intake of
41 saturated fats increases the risk of obesity and cancer. It is also closely linked to high blood
42 cholesterol and coronary heart disease.(Rather et al. 2015; Safa et al. 2015; Shin et al. 2022; Souza
43 et al. 2019). With an awareness of their nutrition and health, consumers today prefer to eat healthier
44 meat products with fewer calories. From a nutritional point of view, it is desirable to reduce the
45 high amount of fat in meat products. But, the elimination or reduction of fats in these products
46 reduces the sensory quality of the final product and mainly affects its texture (Chen et al. 2021; Y.
47 Kumar 2021; Pietrasik and Soladoye 2021). In this regard, manufacturers have introduced several
48 changes in an effort to compensate for the negative effects of reducing fat levels in reconstructed
49 meat products. These modifications include the use of fat substitutes, which can be used to produce
50 low-fat meat products with the same characteristics of high-fat meat products in terms of
51 appearance and taste. FDA-approved fat replacers include carbohydrate-based, protein-based, and
52 fat-based fat replacers (Y. Kumar 2021).

53 **Hydrogels are three-dimensional networks of homopolymers or copolymers whose hydrophilic**
54 **structure enables them to hold large amounts of water in their three-dimensional network. Softness,**
55 **elasticity, swelling, non-dissolution in water, absorbent nature, flexibility (due to the presence of**
56 **a lot of water in the structure), and the ability to store water are important characteristics of**
57 **hydrogels. Natural hydrocolloids such as agar, alginate, gellan, guar, tragacanth, and pectin are**
58 **natural polymers used to prepare hydrogels (Klein and Poverenov 2020; Nayak and Das 2018;**
59 **Ahmed 2015). Agar, among these, is widely used in the food industry. Agar forms a gel with a**
60 **high hydration capacity and can lose a lot of water without changing the gel structure. It can**
61 **maintain its gel at high temperatures. It is considered the strongest gel-forming substance. Agar**
62 **has sufficient resistance to hydrolysis to such an extent that in meat preservatives that require**

63 sterilization at a temperature higher than 121 C, treatment can be done successfully without
64 hydrolysis of agar (Phillips and Williams 2009).

65 So far, carbohydrate-based fat replacers have been widely used in meat products. For example,
66 Rather et al.,(2015) used xanthan as a fat replacer in goshtaba. It was observed that oxidative
67 stability and product quality of goshtaba increased with this hydrocolloid due to the antioxidant
68 activity of xanthan. Also, hardness and taste intensity decreased with increasing xanthan
69 concentration. Rather et al., (2016) investigated the effect of guar gum as a fat replacer on some
70 quality parameters of the mutton product. The results of this experiment were reduced lipid
71 oxidation, increased water holding capacity, reduced hardness and flavor intensity, and increased
72 chewiness due to reduced fat levels. Abbasi et al., (2019) used tragacanth gum as a fat replacer in
73 sausages. Delays in the formation of reactive oxidation materials (thiobarbituric acid), low
74 carbonyl content at the end of storage, and thus increased oxidative stability and improved texture
75 quality were some of the results of this replacement. Öztürk-Kerimoğlu (2021) used a gel system
76 comprising pea protein and agar (PAG) as fat substitutes in meat emulsions. Fat replacement
77 reduced total fat content while increased moisture, protein, and dietary fiber content. Emulsion
78 stability and cook yield increased with increasing replacement rate. The lightness and yellowness
79 of the modified samples were higher than the control samples, but their redness was lower than
80 the control samples. Complete fat replacement with PAG gave a softer but more adhesive texture.
81 In general, this replacement had favorable effects on the functional properties of meat emulsions.
82 Jayarathna et al., (2022) investigated the effect of replacing vegetable oil with garlic inulin on the
83 quality characteristics of chicken sausages. Sausages containing inulin had a higher score of taste
84 and overall acceptance. The ash content, moisture, and protein content of sausages increased with
85 increasing inulin levels. In contrast, the fat content decreased from 13.67% to 4.47% in products
86 containing 3% inulin. Also, products containing 2% inulin had lower brightness than the control
87 sample. As a result, garlic inulin can be successfully used as a fat substitute in sausages without
88 changing the quality parameters of the meat.

89 Since the consumption of low-fat meat products has received a lot of attention from consumers,
90 this study considers using a new form of agar hydrocolloid as a fat replacer. For this purpose, the
91 production of reconstituted agar hydrogel from its cryogel, which is not previously used in any
92 other studies, and replacing sausage fat with produced RAH will be evaluated.

93

94 **Materials and methods**

95 **Production of reconstituted agar hydrogel**

96 Agar solution (1%. w/v) was prepared by adding agar powder to distilled water at 45 °C
97 gradually. Then, the solution was stirred for one hour at 95 °C until the agar was completely
98 hydrated. The agar solution was immediately aerated with a homogenizer (T-25, IKA, Germany)
99 at 11000 rpm for 15 min and then was cooled to 45 °C to form the agar gel (Ellis et al. 2017). After
100 the gel formation, it was stored at -18 °C for 24 h and then dried by freeze-drying method to form
101 agar cryogel. Water was added to agar cryogel (90%, w/w), and the mixture was homogenized at
102 11000 rpm for 3 min to obtain a solid fat-like texture called reconstituted agar hydrogel (RAH).
103 The RAH was kept in a refrigerator (4°C) after production until sausage production (less than
104 24H).

105

106 **Characterization of reconstituted agar hydrogel**

107 The microstructure of agar cryogel was investigated by obtaining electron images from the
108 surface and cross-section of agar cryogel using a field emission-scanning electron microscope (FE-
109 SEM) (Quanta FEG-450, FEI, USA). Agar cryogel was broken into liquid nitrogen to prepare a
110 cross-section of the sample. Then, the surface and cross-section of the agar cryogel were covered
111 with gold and imaged at a voltage of 20 kV (Fayaz et al. 2017; Plazzotta et al. 2019).

112 To determine water holding capacity (WHC), Eppendorf weight without (a) and with RAH (b)
113 was recorded. Then, the Eppendorf was centrifuged at 10000 rpm for 15 min. Afterward, the
114 Eppendorf was turned upside down on filter paper for 3 min to remove separated water from RAH,
115 and the Eppendorf was weighed again (c). WHC of the RAH was calculated using the following
116 equations (Fayaz et al. 2017)

117
$$\text{Water migration (\%)} = (b-a) - (c-a) / (b-a) \quad (1)$$

118
$$\text{Water holding capacity (\%)} = 100 - \text{water migration} \quad (2)$$

119 To determine WHC at different temperatures, RAH samples were stored at 30, 60, and 90 °C for
120 45 min before WHC determination.

121 The back extrusion method was applied to determine the hardness of the RAH tissue. A texture
122 analyzer device (SDM1, Santam, Iran) equipped with a 100 kg cell at a speed of 100 mm/min was
123 used. The maximum force required to penetrate the RAH tissue at a depth of 6 mm was determined
124 (Giacintucci et al. 2018). The texture hardness of solid fat was also measured.

125 RAH color was evaluated using a HunterLab colorimeter (ZE6000, Nippon Denshoko, Japan)
126 based on the L*, a*, and b* color system. The color of solid fat was also determined.

127 128 **Preparation of low-fat sausages using reconstituted agar hydrogel**

129 Chicken meat (25% chicken breast and 75% chicken thigh) and other ingredients were obtained
130 from a local market. According to a commercial sample formulation, the required ingredients were
131 mixed in a food processor (FP3010, Brown, Germany) according to Table 1 to produce control
132 frankfurter sausage. First, chicken meat, grated bell pepper and garlic were mixed for 3 min. Then,
133 salt, phosphate, and crushed ice were mixed for 1 min. Sodium nitrite dissolved in cold water and
134 crushed ice were added to the cutter. Soy protein isolate, powdered milk, and crushed ice were
135 then mixed for 1 min. Finally, gluten, oil, wheat flour, starch, crushed ice, spices, and ascorbic
136 acid were added in order. Then, the mixture was filled in polyamide coating with a diameter of 5
137 cm and a thickness of 0.1 mm and placed in boiling water for one hour until the center temperature
138 of the sausage reached 72-75 °C. After cooking, the samples were cooled to room temperature
139 (25°C) and kept in the refrigerator (4°C) for seven days. Low-fat sausages were produced by
140 replacing the oil in the sausage formulation with RAH at 25, 50, 75, and 100% replacement.

141 142 **Characterization of low-fat sausages**

143 **Chemical composition**

144 The chemical composition of control and low-fat sausages, including moisture, fat, protein, and
145 ash, were measured one day after production by freeze-drying, Soxhlet, Kjeldahl, and dry ashing
146 methods, respectively, according to AOAC standard (2005) (Horwitz and Latimer 2005).

147 148 **Water holding capacity (WHC)**

149 Four pieces of filter paper with dimensions of 2.5 × 2.5 cm² were folded and placed in an
150 Eppendorf tube and weighed (w₁). The sausage sample (w₃) was placed in it and centrifuged at
151 14000 rpm for 10 min. After the sample was separated from the Eppendorf, the filter paper and
152 Eppendorf tube were weighed again (w₂). WHC was calculated according to the following
153 equation (Santana et al. 2015):

$$154 \text{WHC (\%)} = 100 - ((w_2 - w_1) * 100 / w_3) \quad (3)$$

155 156 **Cooking loss**

157 Circular pieces of sausage with a thickness of 1 cm were cut and weighed (w_1). Then, they were
158 deep-fat fried for 30 s. The surface oil of the sausages was removed using filter paper, and the
159 sausage samples were weighed again after cooling (w_2). Cooking loss was calculated as follows
160 (Lee and Chin 2016):

$$161 \text{Cooking loss (\%)} = (w_1 - w_2) / w_1 \quad (4)$$

162 163 **Texture analysis**

164 Sausage samples were cut with a diameter of 1.37 cm and a height of 1.6 cm with a v-shaped
165 blade to perform the cutting test. Then, they were tested with a Warner-Bratzler blade at a constant
166 speed of 100 mm/min using a texture analysis device (SDM1, Centam, Iran) (Abbasi et al. 2019).

167 Texture profile analysis (TPA) of different sausages was also evaluated using a texture analysis
168 device (Instron, America) at 25 °C. First, the sausages were cut into cylinders with diameters and
169 heights of 1.37 and 1.6 cm, respectively. Then, a stainless-steel cylindrical probe with a diameter
170 of 3 cm was used for TPA testing. Compression of 50%, a number of cycles of 2 pcs, load cell of
171 100 N, speed of 50 mm/min, and trigger force of 0.07 N were applied to perform this test. The
172 parameters of hardness, cohesiveness, springiness, gumminess, and chewiness were extracted from
173 the obtained profiles (Fernández-López et al. 2006; Kouzounis et al. 2017; Surasani et al. 2022).

174 175 **Oil absorption**

176 Sausage samples were fried according to the previous section method. The fat content of raw (F_1)
177 and fried sausage samples (F_2) was measured by the AOAC method (2005) using the following
178 equation:

$$179 \text{Oil absorption (\%)} = (F_2 - F_1) / F_1 \quad (5)$$

180 181 **pH over time**

182 Sausage (2g) was mixed with distilled water (8 ml) and homogenized at 11000 rpm for one min.
183 The pH of the samples was determined at intervals of 0.5, 3, 5, 9, 12, and 16 days after production
184 using a manual pH meter (Acton, Malaysia) (Lee and Chin 2016).

185 186 **Sensory evaluation**

187 Control sausage and low-fat sausages with 25 and 50 % RAH as fat replacers were identified
188 with three-digit codes and were simultaneously presented to 25 evaluators from different age

189 groups. The evaluators evaluated parameters of juiciness, flavor, texture, color, and overall
190 evaluation based on seven-point hedonic scales, where the 7 number indicated a very good sample
191 and the 1 number indicated a weak sample.(de Souza Paglarini et al. 2021; Gómez-Estaca et al.
192 2019; M. Kumar and Sharma 2004).

193

194 **Results and discussion**

195 **Characterization of agar cryogel**

196 Surface and cross-section images of agar cryogel are shown in Figure 1a and b, respectively, to
197 evaluate its microstructure using scanning electron microscopy. Figure 1b well shows the porous
198 network structure of agar cryogel. Hydrogen bonds solely cause the formation of the agar gel
199 network. This gel network loses its water rapidly due to the sublimation during the drying process
200 but retains its network structure, which is well seen in Figure 1b and was confirmed by the report
201 of Patel *et al.*(2013) in the agar cryogel structure that shows the intensity of the hydrogen bonds
202 present in the network. The gel structure of agar is intimately connected with the intensity of
203 hydrogen bonds. The ability of agar to form a stable gel relies on the establishment of a network
204 of hydrogen bonds between its molecular components. This is the same network that can trap water
205 and cause the creation of hydrogel, confirming the high capacity of water storage.

206

207 **Characterization of reconstituted agar hydrogel**

208 The WHC of the reconstituted hydrogel prepared from agar cryogel was $99.39 \pm 0.11\%$. The high
209 WHC of RAH is due to the agar structure, which can retain a high amount of water in its structure.
210 The porous network structure of agar cryogel, shown in Figure 1, confirms the high-water holding
211 capacity of RAH. The results of WHC of RAH samples at 30, 60, and 90 °C were 99.65 ± 0.17 ,
212 92.1 ± 1.33 , and 99.38 ± 0.11 , respectively. The water holding capacity of the RAH decreased
213 significantly with the temperature increasing to 60 °C. However, it increased significantly again
214 as the temperature rose to 90 °C ($p < 0.05$). The agar gel network is created by only weak hydrogen
215 bonds retaining water in its network. Therefore, the RAH network is weakened by temperature
216 increasing up to 60 °C due to the weakening of hydrogen bonds. So, the WHC of the reconstituted
217 hydrogel at 60 °C was significantly reduced. A very interesting and different phenomenon
218 happened to the RAH compared to the agar gel observed during this test. The RAH turned into a
219 gel at 90 °C, and the water was entrapped entirely into the structure of the reconstituted hydrogel.

220 Thus, the water holding capacity of RAH increased. It seems that the process of converting agar
221 gel to cryogel has caused changes in the physical structure of the agar, providing hydrophobic
222 interaction between RAH chains, which results in the formation of gel at high temperatures.
223 Therefore, it can be said that RAH has a different property compared to agar powder, which is the
224 ability of RAH to form two kinds of gel by temperature changes: 1) a low-set gel, which is
225 thermoreversible, forms by cooling the RAH solution heated up to about 55 °C, and 2) a high-set
226 gel, which is irreversible, forms by heating RAH solution up to 90 °C. This phenomenon is similar
227 to the unique property of curdlan, which is not seen in other hydrocolloids. Curdlan can also form
228 two types of gels: low-set gel (forms at about 55 °C) and high-set gel (forms at about 80 °C).
229 Hydrophobic reactions between curdlan molecules at high temperatures cause a network structure
230 to hold water inside (Phillips and Williams 2009).

231 Texture analysis of RAH was obtained by the back extrusion method at a depth of 6 mm. Results
232 showed that the maximum force applied on the RAH was 4.00 ± 0.28 N. The hardness of the solid
233 fat, measured by the same method, was 2.2 N. Comparison of the hardness of RAH with solid fat
234 helps estimate the hardness of RAH. The similarity of these two numbers also indicates the
235 possibility of using RAH as a substitution for solid fats in low-fat products.

236 The color parameters L^* , a^* , and b^* of the RAH were 54.83, -1.08, and 9.11, respectively. Results
237 shows that the RAH sample has low lightness. Since homogenization is used to prepare RAH, the
238 entry of a large volume of air into the reconstituted hydrogel network causes its color to darken.
239 The negative value of a^* parameter in the RAH sample indicates a slight green tint of the RAH
240 sample. The b^* parameter of RAH shows the yellowness of reconstituted hydrogel. The color of
241 RAH was very similar to solid fat ($L^*=58.24$, $a^*=-2.42$, and $b^*=13.22$), which is an advantage of
242 using RAH as a fat substitution in low-fat foods.

243

244 **Characterization of low- fat sausages**

245 **Chemical composition and WHC**

246 The chemical composition of prepared sausages containing different levels of RAH as fat replacers
247 is shown in Table 2. The moisture content increased significantly with the replacement of sausage
248 oil with RAH due to high water in the RAH structure. It is attributed to the ability of agar to retain
249 much water in its structure during the preparation and curing process (Phillips and Williams 2009).

250 Rather et al., (2015) reported that reconstituted meat products containing xanthan as a fat replacer

251 had more moisture content than the control sample due to reduced fat content and increased
252 hydrocolloid content in the formulation. The fat content of sausage samples decreased with
253 increasing the replacement ratio of oil with RAH. Rather et al., (2015) also reported that the fat
254 content decreased in meat formulations containing xanthan as a fat replacer. In general, the use of
255 hydrocolloids in different types of sausages effectively has reduced the fat content of products
256 (Berizi et al. 2017; Glisic et al. 2019; Jayarathna et al. 2022; Kim et al. 2019; Souza et al. 2019).
257 The fat content of samples with 100% replacing oil with RAH was not zero due to the fat content
258 of chicken (2.9%) used in the formulation. The results obtained from the ash and protein contents
259 showed that the sausages containing RAH and the control sample were not significantly different
260 in terms of ash and protein contents ($p < 0.05$). Because agar does not contain protein. Also, agar
261 ash content did not affect the ash content of the final sausage because only one percent of agar was
262 used in the RAH making. Similarly, Salajegheh et al. (2018) showed that fat reduction did not
263 significantly affect the level of ash and protein in sausage.

264 The results of Table 2 show no significant difference in WHC between the control sample and
265 sausages containing 25 and 50% RAH, but the WHC of samples significantly reduced when the
266 replacement increased to 75 and 100%. Agar is a hydrocolloid and has a high-water absorption
267 and storage capacity. Therefore, it was expected that the WHC of the samples did not change in
268 the low amount of replacing the fat with RAH (25 and 50%). However, the amount of water in the
269 samples containing 75 and 100 % oil replacement with RAH was very high (Table 2). Therefore,
270 the amount of protein available was insufficient to create a suitable emulsion holding a high
271 volume of water. Zhu et al. (2023) used oleogel based on flaxseed gum/arabic gum as a fat
272 substitute in sausage. They observed that the WHC of the control group was lower than the sausage
273 samples containing 25 and 50% oleogel replacement. They stated that a higher WHC is beneficial
274 for retaining water molecules in the gel structure and reducing the cooking loss of sausages.

275 **Cooking loss**

276
277 The results of Figure 2 show that the cooking loss of the samples increased with increasing the
278 replacement ratio. WHC is one of the most important factors affecting the cooking loss of the
279 sausages. The lower the WHC, the higher the cooking loss due to the higher water evaporation
280 from the product during frying. On the other hand, reducing the amount of oil in sausages increases
281 the moisture content as the emulsion cannot be fully formed, and as a result, the cooking loss

282 increases (Salajegheh et al. 2018). The water content of sausage samples increased by increasing
283 the oil replacement with RAH (Table 2). So, the water of samples containing higher amounts of
284 water evaporated more during frying, which increased the porosity of the fried samples and the
285 cooking loss. Jiménez-Colmenero et al. (2010) added a combination of marine spaghetti
286 gel/kongac to frankfurters to reduce fat and salt. It was observed that this combination increased
287 cooking loss in gel/emulsion systems. These researchers attributed this effect to the early melting
288 of the hydrocolloids and higher water release from the konjac gel during baking to 68°C. Dong
289 Kim et al. (2019) also reported that adding konjac gel to low-fat sausage decreased the cooking
290 loss of low-fat sausage but still had more cooking loss than normal-fat sausage.

291 **Texture analysis**

292 The results of TPA of sausages containing different amounts of RAH are shown in Table 2. The
293 hardness of the samples decreased with increasing the percentage of RAH in the samples, which
294 may be due to the increase of moisture content of sausages after replacing oil with RAH. The
295 springiness and cohesiveness also decreased with increasing the replacement amount of RAH. The
296 fat content is one of the many factors affecting the springiness and cohesiveness of the sausages.
297 The lower the fat content of the sample, the lower these parameters. Therefore, the reduction of
298 these texture parameters can be due to the decrease in fat content (Mendoza et al. 2001). The
299 gumminess was another texture parameter that decreased with increasing the content of RAH in
300 the sausages due to the reduction of hardness and cohesiveness. Chewiness reduction after
301 replacing oil with RAH was due to the decrease in gumminess (Fernández-López et al. 2006).

302 The result of the cutting test is also shown in Table 2, which indicates that the higher the amount
303 of RAH in the sausages, the lower the force required to cut the samples and, thus, the higher the
304 cutting ability. The cohesiveness and hardness of the sausages decreased after replacing oil with
305 RAH, which can reduce the force required to cut the samples. On the other hand, reducing the
306 amount of fat causes the protein structure not to open completely during the preparation of
307 sausages, which will create weaker gels and thus increase the cutting ability of the samples. Zhu
308 et al.(2023) examined the textural characteristics of sausages formulated with the replacement of
309 pork back fat by flaxseed gum/arabic gum oleogel. It was observed that the hardness of sausages
310 formulated with oleogels was higher compared to the control group. But the hardness of emulsified
311 sausages decreased when the replacement ratio of fat with oleogels increased from 25% to 100%.
312 The cohesiveness and chewiness of sausages showed a similar trend to hardness. However, there

313 was no significant difference in springiness between the control group and the sausage emulsified
314 with oleogel.

315 **Oil absorption and fat content after frying**

316 The oil absorption of sausages containing 0, 25, and 50 % RAH was 16.35 ± 1.12 , 45.87 ± 5.36 ,
317 and 46.48 ± 4.33 , respectively. Results show a significant increase in oil absorption of sausages
318 after replacing oil with RAH. However, there is no significant difference between the oil
319 absorption of samples containing 25 and 50% RAH. The results of WHC showed that replacing
320 oil with RAH reduced the WHC of the samples, which could be a good reason for the oil absorption
321 increase. As higher amounts of water evaporate during the frying in samples with lower WHC and
322 cause higher porosity, higher amounts of oil will absorb in the sausages.

323 After frying, the fat content of sausages was 22.51 ± 0.5 , 21.88 ± 1.01 , and 16.41 ± 0.7 in sausages
324 containing 0, 25, and 50% RAH, respectively. The results show that oil replacement with RAH
325 significantly affected the fat content of the samples after frying ($p < 0.05$). The fat content of the
326 samples after frying was significantly lower in the samples containing RAH as a fat replacer. In
327 the control sample, which is high in fat, the fat content can be expected to increase after frying;
328 however, the amount of fat after frying did not decrease significantly in the samples containing
329 25% RAH, which is due to the higher oil absorption of this sample compared to the control sample.
330 The results of this test show that although the oil absorption of samples containing RAH increased;
331 however, due to the less oil used in the formulation of low-fat sausages, the fat content of the final
332 sample in low-fat sausages is less than the control sample, which shows the positive effect of using
333 RAH on fat reduction in low-fat products. These results were consistent with the findings of Zhu
334 et al. (2023), who used flaxseed gum-based oleogel as a fat substitute in emulsion sausages. They
335 observed that increasing the concentration of flaxseed gum increased the rate of oil loss
336 significantly due to the tighter network structure of the oleogels.

338 **pH over time**

339 The pH during storage of sausages prepared with different percentages of oil replacement with
340 RAH is presented in Table 3. The pH of all three samples decreased over time. However, this
341 decrease was not significant until the ninth day ($p < 0.05$). While it significantly decreased until
342 the 16th day due to the microbial spoilage over time with lactic acid bacteria, which are the
343 dominant microorganisms in meat products. The pH difference between the three samples was not
344

345 significant until the ninth day, which shows that replacing sausage oil with RAH did not cause the
346 samples to become more perishable. Interestingly, from the ninth to the twelfth day, when the pH
347 decreased in all three samples, the intensity of this decrease was higher in the control sample than
348 in the samples containing RAH. Therefore, the results show that replacing sausage oil with RAH
349 slowed down the spoilage of the samples. It seems that the presence of low amounts of sulfate in
350 the agarose structure of agar acts as an antimicrobial agent and slows down the spoilage of sausages
351 containing agar compared to the control sample (Liu et al. 2017).

352

353 **Sensory evaluation**

354 The sensory evaluation of sausages prepared with different amounts of RAH is presented in Table
355 4. The color, juiciness, and texture of sausages containing RAH were not significantly different
356 from the control samples. However, there was a significant difference between the samples in
357 terms of aroma and taste and overall acceptance ($p < 0.05$). Interestingly, the aroma and taste of
358 the sample containing 25% RAH were significantly more desirable than those of the control
359 sample. Fat-soluble flavoring agents seem well sensed in the control sample due to the high
360 amounts of oil in the formulation. However, water-soluble flavoring agents are not released well,
361 so many flavors do not feel good in the control. However, the total fat-soluble and water-soluble
362 flavors are well released and sensed in the sample containing 25% RAH due to the desirable
363 balance between hydrophobicity and hydrophilicity of the sample, so the flavor improves at this
364 percentage of replacement. Finally, the overall evaluation shows that the samples containing 25%
365 RAH and control samples are similar. The sample containing 50% RAH, still from the consumer's
366 point of view, had no significant difference from the control sample in terms of overall acceptance.

367 Kim et al. (2019) used konjac gel and three herbal powders (aloe vera, cactus pear, or wheat germ)
368 as pork fat substitutes to prepare low-fat frankfurter sausages. It was observed that cactus pear
369 konjac gel can be used as a frankfurter fat substitute with a positive effect on color, taste, smell,
370 and overall acceptability.

371

372 **Conclusions**

373 Considering the increasing awareness and concern of consumers regarding the harm of high-fat
374 and high-calorie meat products that cause the spread of some diseases, using a way to reduce the
375 amount of fat helps to overcome these concerns. On the other hand, eliminating or reducing the

376 fats in the product reduces the quality of sensory properties in the final product and especially
377 affects its texture. The use of fat substitutes can be a way to solve the quality problems of these
378 products. Regenerated agar hydrogel (RAH) was used instead of oil to prepare frankfurter sausage
379 to achieve this goal. Using RAH instead of oil positively affected the chemical compounds,
380 including moisture and fat, texture, color, and sensory evaluation of sausage samples. Also, due to
381 sulfated antimicrobial groups in the agar structure, the spoilage occurred later in the sausage
382 samples containing RAH than in the control sample. Therefore, RAH can be used in meat products
383 to reduce the amount of fat and, at the same time, maintain the quality characteristics of the
384 product. Sensory evaluators gave better scores to the sample with 25% RAH. Finally, sausages
385 with two percent replacement rates of 25 and 50 % can be introduced as the best low-fat sausages.

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499

Table 1. Formulation of different sausages prepared with RAH replacer.

Ingredient	Percentage of oil replacement with RAH				
	0	25	50	75	100
Chicken meat	45	45	45	45	45
Sunflower oil	18	13.5	9	4.5	0
RAH	0	4.5	9	13.5	18
Salt (NaCl)	1.7	1.7	1.7	1.7	1.7
Sodium polyphosphate	0.57	0.57	0.57	0.57	0.57
Sodium nitrite	0.01	0.01	0.01	0.01	0.01
Ascorbic acid	0.02	0.02	0.02	0.02	0.02
Soy protein isolate	0.95	0.95	0.95	0.95	0.95
Gluten	1.12	1.12	1.12	1.12	1.12
Wheat starch	1.86	1.86	1.86	1.86	1.86
Milk powder	1.07	1.07	1.07	1.07	1.07
Wheat flour	4.95	4.95	4.95	4.95	4.95
Garlic	1.15	1.15	1.15	1.15	1.15
Sugar	0.28	0.28	0.28	0.28	0.28
Ice	20	20	20	20	20
Bell pepper	2	2	2	2	2
Spices*	1.32	1.32	1.32	1.32	1.32

500 *Spices contained pepper, nutmeg, ginger, coriander seeds, thyme, chicken flavor, liquid smoke, and pepper flavor.

501

502 **Table 2.** Chemical composition, WHC, and texture analysis of sausages containing different
503 amounts of RAH.

Parameter	RAH amounts (%)				
	0	25	50	75	100
Moisture (%)	59.00 ± 1.47 ^e	62.50 ± 0.70 ^d	65.30 ± 0.23 ^c	66.70 ± 0.19 ^b	67.90 ± 0.14 ^a
Fat (%)	19.30 ± 0.35 ^a	15.00 ± 0.14 ^b	11.20 ± 0.14 ^c	6.40 ± 0.28 ^d	1.30 ± 0.14 ^e
Protein (%)	12.90 ± 0.33 ^a	12.20 ± 0.50 ^a	12.10 ± 0.87 ^a	12.40 ± 0.38 ^a	12.09 ± 0.57 ^a
Ash (%)	2.99 ± 0.15 ^a	3.19 ± 0.16 ^a	3.46 ± 0.48 ^a	3.38 ± 0.53 ^a	3.39 ± 0.09 ^a
WHC (%)	77.65 ± 2.16 ^a	76.31 ± 1.21 ^a	76.36 ± 0.81 ^a	70.83 ± 2.54 ^b	67.38 ± 1.75 ^c
Hardness (kg)	17.23 ± 1.46 ^a	11.86 ± 0.92 ^b	12.06 ± 0.24 ^b	9.42 ± 0.80 ^c	9.75 ± 0.32 ^c
Springiness (cm)	6.83 ± 0.14 ^a	6.51 ± 0.27 ^b	5.83 ± 0.30 ^c	5.60 ± 0.22 ^{cd}	5.34 ± 0.14 ^d
Cohesiveness	0.58 ± 0.07 ^a	0.55 ± 0.03 ^a	0.50 ± 0.04 ^b	0.41 ± 0.01 ^c	0.34 ± 0.01 ^d
Gumminess (kg)	10.13 ± 1.37 ^a	6.36 ± 0.43 ^b	6.15 ± 0.62 ^b	3.94 ± 0.31 ^c	3.38 ± 0.19 ^c
Chewiness	69.28 ± 9.47 ^a	43.17 ± 2.57 ^b	35.86 ± 3.28 ^c	22.12 ± 1.37 ^d	18.10 ± 1.19 ^d
Cutting test	2.83 ± 0.46 ^a	1.95 ± 0.36 ^b	1.80 ± 0.25 ^b	1.26 ± 0.18 ^c	1.21 ± 0.15 ^c

504 Lowercase letters in each row indicate a significant difference between the samples (p < 0.05)

505

506

507 **Table 3.** pH of sausages containing different amounts of RAH during storage.

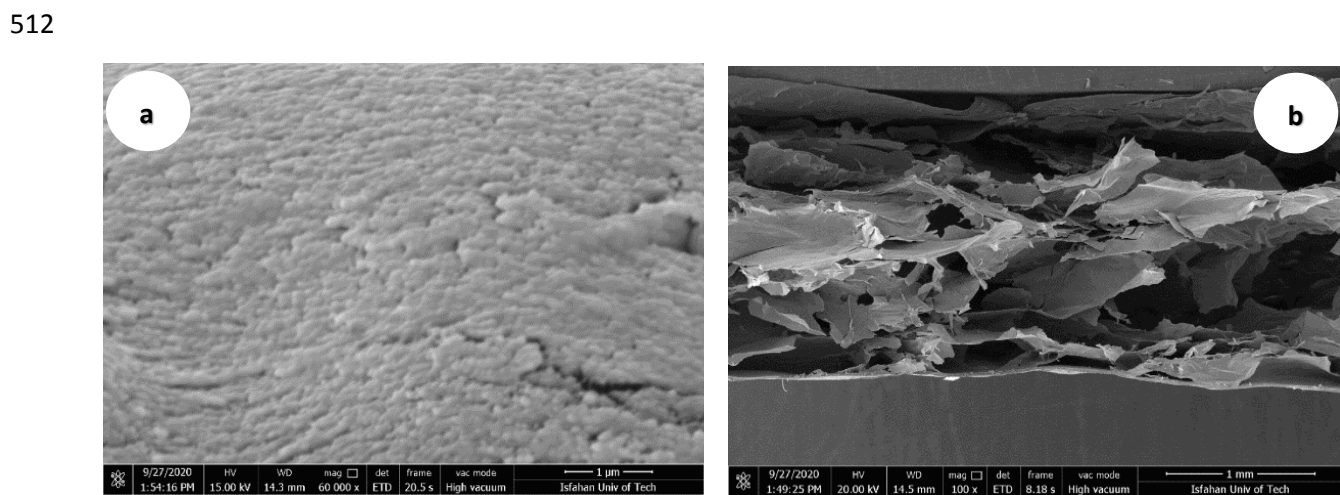
Time (day)	RAH amounts (%)		
	0	25	50
0.5	6.12 ± 0.015 ^{a-c}	6.11 ± 0.004 ^{a-c}	6.10 ± 0.014 ^b
3	6.12 ± 0.003 ^{a-c}	6.12 ± 0.003 ^{a-c}	6.13 ± 0.004 ^a
5	6.12 ± 0.007 ^{a-c}	6.13 ± 0.006 ^{a-c}	6.14 ± 0.006 ^a
9	6.07 ± 0.023 ^c	6.08 ± 0.009 ^b	6.12 ± 0.009 ^{a-c}
12	5.22 ± 0.049 ^f	5.81 ± 0.072 ^d	6.17 ± 0.042 ^a
16	5.18 ± 0.03 ^f	5.64 ± 0.11 ^e	5.6 ± 0.022 ^e

508 Lowercase letters indicate a significant difference between samples ($p < 0.05$).

509
510 **Table 4.** Sensory evaluation of sausages containing different amounts of RAH.

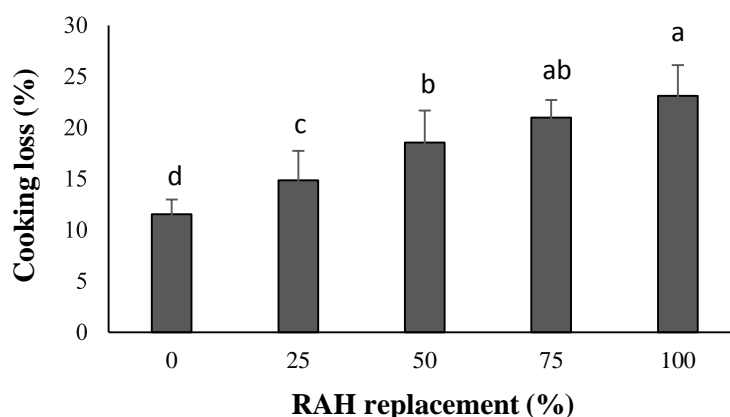
Test	RAH amounts (%)		
	0	25	50
Color	6.6 ± 0.57 ^a	6.36 ± 0.9 ^a	6.24 ± 0.92 ^a
Aroma and taste	5.88 ± 1.01 ^b	6.56 ± 0.82 ^a	6.04 ± 0.97 ^{ab}
Juiciness	6.36 ± 0.57 ^{ab}	6.56 ± 0.58 ^a	6 ± 1.11 ^b
Texture	6.56 ± 0.58 ^a	6.44 ± 0.76 ^a	6.28 ± 0.84 ^a
Overall evaluation	6.28 ± 0.61 ^{ab}	6.6 ± 0.5 ^a	6.12 ± 0.78 ^b

511 Lowercase letters indicate a significant difference between samples ($p < 0.05$)



513 **Figure 1.** FE-SEM images of agar cryogel from a) surface and b) cross-section.

514



516

517 **Figure 2.** Cooking loss of sausages prepared with different amounts of RAH. Small letters
518 indicate a significant difference between the samples ($p < 0.05$).
519

519

520 تولید سوسیس فرانکفورتی کم چرب با استفاده از یک جایگزین چربی جدید: هیدروژل آگار بازسازی شده

521 راضیه رنجبران، و هاجر شکرچی زاده

522 روند رو به رشدی به سمت محصولات گوشتی سالم با محتوای چربی کمتر وجود دارد. استفاده از جایگزین های چربی
523 می تواند مشکلات کیفی محصولات کم چرب را برطرف کند. این مطالعه با هدف تولید سوسیس فرانکفورتی کم چرب با
524 استفاده از یک جایگزین چربی جدید انجام شد. بنابراین، در مرحله اول، هیدروژل آگار بازسازی شده (RAH) با افزودن آب
525 به کرایوژل آگار پس از همگن سازی تولید شد. در مرحله دوم، RAH به عنوان جایگزین چربی در سوسیس فرانکفورتی
526 در غلظت های 0، 25، 50، 75 و 100 درصد استفاده شد و ویژگی های سوسیس کم چرب حاصل از جمله ترکیب شیمیایی،
527 ظرفیت نگهداری آب، کاهش پخت، ویژگی های بافت، تخلخل، رنگ، درصد چربی پس از سرخ کردن، جذب روغن، pH
528 در طول زمان و ارزیابی حسی مورد بررسی قرار گرفت. نتایج نشان داد که RAH می تواند دو نوع ژل را با تغییرات دما
529 تشکیل دهد: یک ژل کم گیر در دمای 55 درجه سانتی گراد و یک ژل با ست شدن بالا در دمای 90 درجه سانتی گراد.
530 جایگزینی روغن در سوسیس و کالباس باعث افزایش رطوبت، تخلخل، جذب روغن و از دست دادن پخت می شود. از سوی
531 دیگر، این جایگزینی باعث کاهش محتوای چربی، نیروی برش، ظرفیت نگهداری آب، درصد چربی پس از سرخ کردن و
532 خواص بافتی مانند سختی، چسبندگی، فنری، صمغی و جویدنی شد. نتایج اندازه گیری pH در طول زمان نشان داد که گروه
533 های ضد میکروبی سولفات در ساختار آگار، فساد سوسیس های حاوی RAH را نسبت به نمونه شاهد به تاخیر انداخت.
534 ارزیابی حسی نشان داد که سوسیس های حاوی RAH از نظر رنگ، آبدار بودن و بافت تفاوت معنی داری با نمونه شاهد
535 ندارند. با این حال، طعم و پذیرش کلی به طور قابل توجهی تحت تأثیر این جایگزینی افزایش یافت ($P < 0/05$). بنابراین
536 مصرف کنندگان نمونه ی با 25 درصد جایگزینی را به عنوان نمونه بهینه انتخاب کردند. در نتیجه، RAH می تواند با
537 موفقیت به عنوان جایگزین چربی در محصولات کم چرب با ویژگی های کیفی مطلوب استفاده شود.