

19 **Abstract**

20 In the Mediterranean area, being pedoclimatic conditions more favorable to durum than common
21 wheat cultivation, a bread-making tradition from durum wheat has been established. Durum
22 wheat bread has a compact texture, with lower specific volume than common wheat bread. Due
23 to health implications, several studies were carried out to reduce the content of NaCl in common
24 wheat bread, however without considering durum wheat bread. The aim of this work was to
25 assess the effect of salt reduction on quality and acceptability of durum wheat bread, with regard
26 to specific volume, sensory features and aroma profile. Breads prepared with 5, 10, 15, 20 g/kg
27 NaCl were submitted to consumer test. Control bread (20 g/kg salt) was the most appreciated,
28 followed (>80% consumers) by bread with 10 g/kg salt, which showed a significantly ($p<0.05$)
29 higher specific volume, but lighter crust and weaker aroma (lower amounts of Maillard reaction
30 products and fusel alcohols).

31
32 **Key words:** bread; re-milled semolina; sodium chloride; rheofermentometer; volatile
33 compounds

34

35 **1. Introduction**

36 Epidemiological studies suggest that, among other determinants, high dietary intake of salt
37 (sodium chloride) contributes to hypertension which, in turn, is a major risk factor in the
38 development of cardiovascular diseases (Bibbins-Domingo et al., 2010). The Committee on
39 Medical Aspects of Food and Nutrition Policy (COMA) recommended a reduction of the dietary
40 intake of salt from 9 g/day (3.6 g/day of sodium), recorded in the UK, to 6 g/day (Wyness,
41 Butriss, & Stanner, 2012). This reduction was considered to be an achievable goal, rather than an
42 optimal or ideal level of consumption. The World Health Organization and the Food and
43 Agriculture Organisation recommended a daily salt intake of 5 g/day as a worldwide guideline
44 (WHO & FAO, 2003).

45 In the 18th century, salt was needed primarily for food preservation and its usage in bread
46 making was limited, never exceeding 10 g kg⁻¹ (Quilez & Salas-Salvado, 2012). In the 20th
47 century, the advent of systems to preserve foods other than salting, coupled with the
48 industrialization and standardization of bread making, induced to raise the salt content of bread
49 to 20 g kg⁻¹, as an easy way to improve flavor and reduce mould spoilage (Belz et al., 2012;
50 Quilez & Salas-Salvado, 2012). Currently, the level of salt used in bread making is between 18
51 and 22 g kg⁻¹, therefore bread is considered one of the key contributors of dietary intake of salt,
52 accounting for approximately 25% of total (Joossens, Sasaki, & Kesteloot, 1994).

53 Reporting sodium or salt content in the label of foods is now mandatory (European Parliament &
54 Council of the European Union, 2011). According to the traffic-light labeling system, baked
55 goods containing more than 15 g kg⁻¹ of salt have a “red light” on the label, and those with 3-15
56 g kg⁻¹ of salt a yellow one (Food Standard Agency, 2007). Therefore, lowering the salt intake by
57 reformulating processed foods, including bread, has become a worldwide trend (Wyness et al.,
58 2012). Nutrition claims such as “low sodium/salt”, “very low sodium/salt” and “sodium/salt-
59 free” have also been ruled, which can be applied to foods containing 1.2, 0.4 and 0.05 g kg⁻¹ of

60 sodium, or the equivalent value for salt, respectively (European Parliament & Council of the
61 European Union, 2006).

62 Numerous studies – see the reviews of Belz et al. (2012) and Silow, Alex, Zannini, & Arendt
63 (2016) – have been carried out at the purpose of decreasing the salt content of bread prepared
64 from common wheat flour (*Triticum aestivum* L.), assessing the lowest possible level in the
65 range 13-17 g kg⁻¹ (Conner, Booth, Clifton, & Griffiths, 1988; Girgis et al., 2003). Another
66 strategy consisted in using salt replacers, i.e. magnesium and potassium salts, also complemented
67 by taste enhancers. The replacers allowed maintaining better technological quality, but sensory
68 limitations due to bitterness of the potassium salts were reported (Salovaara, 1982; Raffo et al.,
69 2018). In brown bread, instead, the partial replacement of sodium chloride with potassium,
70 magnesium and calcium salts was more acceptable (Charlton, Macgregor, Vorster, Levitt, &
71 Steyn, 2007).

72 Salt concentration, however, is not only influential on the sensory acceptability of food in terms
73 of taste. In case of bread, salt is an essential ingredient, being crucial for a proper development of
74 dough structure. The interaction of salt with flour components such as gluten is very important to
75 form a high quality bread crumb. Both too low and too high salt content in dough can be
76 undesirable. Beck, Jekle, & Becker (2012a) observed that a dough prepared without salt showed
77 significantly lower farinograph stability than a dough containing 10-20 g NaCl kg⁻¹ wheat flour.
78 No significant differences were observed within the range 10-20 g NaCl kg⁻¹ wheat flour,
79 whereas a further increase of farinograph stability was observed at levels of NaCl as high as 30-
80 40 g kg⁻¹ wheat flour. Preston (1989) reported that dough prepared with 0.05-0.10 M NaCl had
81 higher dough strength than dough with no salt. Higher concentrations (0.5-1.0 M) of NaCl
82 further increased dough strength, whereas in case of more chaotropic salts, such as NaI and
83 NaSCN, it was observed a decrease. He, Roach, & Hosney (1992) observed lower mixograph
84 mixing time and peak height in the dough obtained with pure water than in a dough containing
85 15 g kg⁻¹ NaCl, and recorded further significant increases at 40 g kg⁻¹. Similar results were

86 reported by Danno & Hosney (1982) in trials where NaCl was increased from 20 to 50 g kg⁻¹,
87 although He, Roach, & Hosney (1992) observed that flour of different baking quality (i.e.
88 containing weak, average and strong gluten) responded differently to salt increase, with stronger
89 flour showing greater dough strength increases. Salt has an influence also on fermentation rate,
90 due to the effect of osmotic pressure on yeast growth. Absence of salt leads to excessive
91 fermentation, causing gassy and acidic dough which, in turn, results in loaves with poor texture
92 and an open grain. Amounts of salt higher than 2% result in a decrease of leavening ability, so
93 that less sugars are metabolized, leading to darker crust during baking (Belz, Ryan, & Arendt,
94 2012).

95 In the Mediterranean area, pedoclimatic conditions are more favorable to durum wheat (*Triticum*
96 *turgidum* subsp. *durum* (Desf.) Husnot) than common wheat cultivation. According to an ancient
97 tradition, particularly consolidated in Southern Italy, bread is prepared from durum wheat re-
98 milled semolina (Pasqualone, 2012), which has finer particle size (65-70% of particles below
99 180 µm), greater content of damaged starch and higher hydration rate than semolina for pasta-
100 making, as well as higher tenacity to extensibility (P/L) alveograph ratio than common wheat
101 flour (Giannone et al., 2018). Durum wheat bread has peculiar sensory features, compared to
102 white bread prepared from common wheat. Bread crumb has a yellowish color, due to the
103 presence of carotenoid pigments (Giannone et al., 2018). In addition, due to the typically high
104 P/L ratio of the raw material used (Giannone et al., 2018), durum wheat bread is characterized by
105 a compact texture – sometimes excessively close – with lower specific volume and harder crumb
106 than white bread (Pasqualone et al., 2011). Some durum wheat breads, such as Altamura bread
107 and Dittaino bread, have been awarded of the Protected Designation of Origin (PDO) mark for
108 their high quality level and typicality (Pasqualone, Summo, Bilancia, & Caponio, 2007;
109 Pasqualone, Alba, Mangini, Blanco, & Montemurro, 2010; Giannone et al., 2018).

110 Though the reduction of dietary intake of salt is highly recommended, and despite the large
111 amount of studies in common wheat bread, no studies have been carried out so far to assess the

112 effect of reducing salt content in bread prepared from durum wheat, which has gluten
113 characteristics quite different from those of common wheat. Besides the positive effect on health,
114 decreasing salt content could also moderate the compactness of durum wheat bread. The aim of
115 this work was, therefore, to assess the effect of the reduction of salt content on quality and
116 acceptability of durum wheat bread.

117

118 **2. Materials and methods**

119

120 *2.1 Bread production*

121 Bread-making trials were carried out using commercial re-milled semolina of durum wheat
122 (*Triticum turgidum* L. subsp. *durum* (Desf.) Husnot) provided by Molino Mininni, Altamura,
123 Italy, which was obtained by blending different batches of wheat in the milling industry. The
124 quality characteristics of re-milled semolina were the following: 12.8 g 100 g⁻¹ protein; 11.7 g
125 100 g⁻¹ dry gluten; 0.8 g 100 g⁻¹ ash; 12.7 g 100 g⁻¹ moisture; alveograph P/L ratio = 2.1;
126 alveograph W = 203 × 10⁻⁴ J. All the values were within the usual range of variability for this
127 commercial category (Pasqualone, Caponio, & Simeone, 2004; Giannone et al., 2018).
128 Compressed fresh yeast (*Saccharomyces cerevisiae*, 'Pinnacle' yeast, AB Mauri, Casteggio,
129 Italy) was provided by the local bakery where the bread-making trials were carried out (Panificio
130 Bartolo Digesù, Bari, Italy). Food grade refined sea salt (99.5% NaCl, Piazzolla Sali, Margherita
131 di Savoia, Italy) was purchased at a local retailer. Tap water used in the trials had hardness =
132 21 °f; conductivity at 20 °C = 441 µS/cm and dry residue at 180 °C = 308 mg L⁻¹. Bread rolls at
133 four different salt (NaCl) levels were prepared according to Licciardello et al. (2017), with minor
134 modifications. In detail, re-milled semolina was kneaded for 17 min at 25 °C by a diving arms
135 kneader (Dell'Oro, Valmadrera, Lecco, Italy) with 10 g kg⁻¹ compressed yeast, salt (5, 10, 15, or
136 20 g kg⁻¹), and water (595 g kg⁻¹). All amounts were on semolina weight basis. The amount of
137 water was preliminary assessed through the farinograph test according to the AACC 54-21

138 method (AACC, 2000), to reach the farinograph consistency of 500 Brabender Units (Brabender
139 instruments, Duisburg, Germany). Specifically, the amount of water needed in case of 5, 10, 15,
140 and 20 g kg⁻¹ salt accounted for 596, 596, 595 and 594 g kg⁻¹ water, respectively. However, since
141 the differences were very slight and not statistically significant, the average value (595 g kg⁻¹ of
142 water) was considered in all the trials for a simpler procedure, being the whole bread-making
143 process done in a bakery and not in a laboratory. After kneading all the ingredients, the dough
144 was rested in bulk for 1.5 h, then was manually scaled into 100 g pieces, rolled out, proofed for
145 90 min at 32 °C and 66% RH (Pavailler Engineering proofer, Galliate, Italy), and finally baked
146 in an electric oven (RP, Rinaldi, Massa, Italy) at 240 °C for 20 min. Bread-making trials were
147 repeated twice.

148

149 *2.2 Rheofermentometer analysis of dough*

150 The proofing properties of dough at different salt levels were assessed by the F3
151 rheofermentometer (Tripette et Renaud, Chopin Technologies, Villeneuve-la-Garenne, France).
152 The analysis determines the total gas production of yeast and dough volume at standard
153 barometric pressure over time. The method conforms to the AACC 89-01 (2000) standard for the
154 measurement of yeast activity and gas production. An amount of 315 g of dough, prepared as
155 described in paragraph 2.1, was submitted to the analysis according to the conditions of the
156 Chopin protocol reported in the rheofermentometer instruction manual (Chopin Technologies,
157 2004), i.e. at 28.5 °C for 3 h, with a 2000 g weight. The instrument records two curves during
158 dough fermentation and rising, one describing the development of the dough and another
159 depicting the production and retention of gas. The following indices were measured: i) maximum
160 dough height (H_m); ii) time needed to reach the maximum dough height (T_I); iii) dough height
161 after 3 h (h); iv, v, vi) volume of gas produced (V_T), lost (V_L), and retained (V_R) at the end of the
162 test (3 h); vii) gas retention coefficient (V_R/V_T). Analyses were carried out in triplicate.

163

164 *2.3 Consumer test*

165 A consumer test involving 65 habitual bread consumers (28 male and 32 female, enrolled among
166 students and employees of University of Bari, Italy, aged from 21 to 60 y) was effected
167 according to ISO 8587 (2006) to rank preference and assess willingness to purchase bread
168 samples. Each consumer received: a white dish containing a square portion (4 cm side, 1 cm
169 height) of each bread type (identified by an alphanumeric code); a glass of water; the
170 evaluation sheet and a pencil. Each consumer had to taste the four bread types, with a break
171 between each sample and cleansing the palate with water, then had to rank the samples according
172 to preference from the most appreciated (score = 1), to the least appreciated (score = 4). The
173 consumers were also asked to express their willingness to purchase each bread sample.

174

175 *2.4 Determination of specific volume*

176 The loaf volume of bread samples was determined by rapeseed displacement as in the AACC
177 method 10-05.01 (2000). Bread specific volume was calculated as volume to weight ratio.
178 Analyses were carried out in triplicate.

179

180 *2.5 Color determinations*

181 Crust color was analyzed by using the CR-300 Chromameter (Minolta, Osaka, Japan) under the
182 illuminant D65 as in Giannone et al. (2018). Color parameters L^* (brightness), a^* (redness), and
183 b^* (yellowness) were determined. Brown index was calculated as $100 - L^*$ (Giannone et al.,
184 2018). Five replications were carried out for each determination.

185

186 *2.6 Quantitative descriptive analysis (QDA) of sensory properties*

187 Quantitative Descriptive Sensory Analysis of bread samples containing 10 and 20 g kg⁻¹ salt was
188 performed by a panel consisting of 8 trained members in the conditions described in a previous
189 work (Pasqualone et al., 2007). Descriptors of taste (salty, sweet, and bitter), appearance (crust

190 color), texture (crumb consistency and crumb grain), and odor (toasted, as perceived on crust,
191 and yeasty, on crumb) were considered. The descriptors were rated on an anchored line scale that
192 provided a 0-9 score range (0 = minimum; 9 = maximum intensity). The definitions of each
193 descriptor and the scale anchors are reported in Pasqualone et al. (2007). Sensory sessions were
194 carried out in triplicate.

195

196 *2.7 Determination of volatile compounds*

197 An amount of 0.50 ± 0.05 g of bread crust, cut in pieces of 2-3 mm, was submitted to the
198 determination of volatile compounds by solid phase micro-extraction (SPME) coupled to gas-
199 chromatography/mass spectrometry (GC/MS) as reported by Giannone et al. (2018) with minor
200 modifications. In detail, the SPME analysis was made by using an Agilent 6850 gas-
201 chromatograph equipped with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc.,
202 Santa Clara, CA, USA) and with a HP-Innowax (Agilent Technologies Inc., Santa Clara, CA,
203 USA) polar capillary column (60 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness), in the
204 following conditions: SPME fiber size and material = 75 μ m carboxen/polydimethylsiloxane
205 (CAR/PDMS) (Supelco, Bellefonte, PA, USA); time and temperature of fiber exposure to
206 sample headspace = 50 min at 35 °C; desorption time = 6 min; GC injector temperature =
207 280 °C; flow = 2.0 mL min⁻¹. The GC temperature program was set as follows: 35 °C for 8 min,
208 increased by 5 °C/min to 50 °C (held for 5 min), increased by 5.5 °C/min to 230 °C (held for 5
209 min). The interface temperature was 230 °C. Mass spectra were recorded by electronic impact at
210 70 eV, in the mass range m/z 33–200. Peak identification was performed by computer matching
211 with the reference mass spectra of National Institute of Standards and Technology (NIST) and
212 Wiley libraries. The semi-quantitative data (peak areas expressed as total ion counts - TIC) were
213 used to compare the samples, as in Giannone et al. (2018). The analysis was carried out in
214 triplicate.

215

216 2.8 Statistical analyses

217 The data obtained were submitted to statistical analysis using XLStat software (Addinsoft, NY).
218 Analysis of variance (ANOVA) followed by Tukey's HSD test was used to compare bread
219 samples. The results of preference ranking test were analysed by the non-parametric Friedman
220 test. Consumers' willingness to purchase was modelled by binary logistic regression.

221

222 3. Results and discussion

223

224 3.1 Effect on rheofermentometer indices of dough

225 The rheofermentometer was used to measure the development of durum wheat dough at different
226 salt levels, during a 3-h fermentation. This method allows evaluating the fermentative capacity of
227 flours, as well as yeast activity, by providing information on both gas production and ability of
228 dough to retain the produced gas. As expected, the lowest dough development was observed at
229 the highest salt level, due to inhibiting activity of salt on yeasts (Belz, Ryan, & Arendt, 2012). A
230 progressively more pronounced development was observed as the salt content decreased (Table
231 1).

232 In detail, higher salt levels caused a decrease of the volume of CO₂ produced (V_T), which
233 resulted in lowering both the gas production curve (H'_m) and the maximum height of dough
234 (H_m). Besides, the increase of salt content prolonged the time needed for reaching the maximum
235 dough height (T'_i), which was significantly longer in dough containing 20 g kg⁻¹ salt than in the
236 other, less salty, types of dough. Beck, Jekle & Becker (2012b) observed a similar increase of H_m
237 in dough made of common wheat flour, when salt amounts were progressively lowered from 40
238 to 0 g kg⁻¹. However, in our salt content range, i.e. 5-20 g kg⁻¹, Beck et al. (2012b) registered H_m
239 values accounting for 73-82 mm, which were higher than our results (accounting for 45-55 mm).
240 Although the effect of different yeast activity and fermentative attitude of flour cannot be
241 excluded, the observed difference in H_m was probably due to the high alveograph P/L ratio of re-

242 milled semolina, which inhibited the dough to expand more freely. The presence of tenacious
243 and little extensible gluten is quite common in re-milled semolina (Giannone et al., 2018;
244 Pasqualone et al., 2011), also due to breeding programs for improving the pasta-making
245 performances of durum wheat (De Vita et al., 2007).

246 While inhibiting fermentation, however, salt had a strengthening effect on gluten (Preston,
247 1989), therefore it helped to retain the gas produced. Due to its ionic nature, salt positively
248 influences the interactions between gluten strands in the dough (Beck et al., 2012a). Therefore, at
249 higher salt levels, longer time (T_x) was needed before that some gas was lost through the dough
250 network (Table 1). As a consequence, at 20 g kg⁻¹ salt level, the height of dough observed at the
251 end of the rheofermentometer test (h) was very similar (only 6% decrease) to the maximum
252 dough height recorded (H_m) in the whole trial. Lower amounts of salt, instead, caused a decrease
253 of h by 24-35%. Finally, the gas retention coefficient (V_R/V_T) was slightly higher in presence of
254 higher salt contents, but without significant differences with the other samples.

255 To sum up, due to an improvement of gluten network, the saltiest dough showed a more effective
256 ability in retaining the gas released by fermentation, resulting in a low volume of lost gas (V_L).
257 However, at the same time the saltiest dough produced the lowest volume of gas (V_T) and, as a
258 consequence, it showed the lowest volume of retained gas (V_R) at the end of fermentation.

259

260 *3.2 Effect on consumer appreciation*

261 The mean appreciation scores of breads at decreasing salt levels are reported in Figure 1. The
262 consumers were asked to rank bread samples according to their preference, from the most
263 appreciated (score = 1) to the least appreciated (score = 4). Control bread (20 g kg⁻¹) was the
264 most appreciated by consumers, but without significant differences with bread containing 15 g
265 kg⁻¹ salt. It would be therefore possible to decrease the salt amount of durum wheat bread
266 formulation to 15 g kg⁻¹ without significantly affecting consumers appreciation. Similarly,

267 studies carried out in common wheat bread pointed out an optimal level of salt in the range 13-17
268 g kg⁻¹ (Conner et al., 1988; Girgis et al., 2003).

269 Bread with 10 g kg⁻¹ salt had an intermediate appreciation score, whereas bread with 5 g kg⁻¹ salt
270 was rejected by the majority of consumers. A non-linear relationship was observed (Fig. 1)
271 between the mean ranking assigned to breads by consumers and their salt contents: these
272 findings point out that a substantial reduction of salt results in a limited decrease of acceptability
273 compared to bread prepared with 20 g kg⁻¹ salt.

274 The consumers were also asked to express their willingness (P) to purchase each bread type.
275 Binary logistic regression was used to analyze the responses. The obtained regression equation
276 was the following:

$$277 \quad P(\text{purchase}) = \frac{\exp(Y')}{1 - \exp(Y')}$$

278 where

$$279 \quad Y' = -1.147 + 0.2624 \times \text{NaCl} (g \text{ kg}^{-1})$$

280
281 All goodness-of-fit tests (Deviance, Pearson and Hosmer-Lemeshow) allowed to reject the *null*
282 hypothesis for the obtained model. The curve of the probability to purchase bread as a function
283 of salt content (Fig. 2) allows to estimate that more than 80% of consumers would purchase
284 bread prepared with 10 g kg⁻¹ salt. This kind of bread would contain half the salt content usually
285 employed in bread-making, and even less than half the salt content used in Southern Italy (where
286 the trials have been carried out), where the majority of bakers include 25 g kg⁻¹ salt in bread
287 formulation. Such a decrease would effectively contribute to lowering the sodium dietary intake.
288 On the basis of the results of the consumer test, bread with 10 g kg⁻¹ salt was submitted to further
289 investigations, in comparison with control bread, to evaluate the effect of salt lowering on bread
290 quality features other than taste.

291

292 *3.3 Effect on bread quality characteristics*

293 Specific volume is one of the most important quality characteristics of bread, related to crumb
294 softness. Soft and well developed bread loaves show high specific volumes. The value of
295 specific volume observed in bread prepared with 20 g kg⁻¹ salt was low, indicating a very
296 compact inner structure, typical of bread obtained exclusively from durum wheat re-milled
297 semolina, without blending with common wheat flour (Giannone et al., 2018; Pasqualone et al.,
298 2011) (Table 2). Lowering the salt content from 20 to 10 g kg⁻¹, a significant increase of specific
299 volume was observed, essentially due to a positive effect on dough fermentation, as shown by
300 rheofermentometer data. Similar results were observed by other authors who studied the effect of
301 salt decrease in common wheat bread (Beck et al., 2012b). Specific volume of bread paralleled
302 the trend observed in the rheofermentometer indices of dough development (H_m and H'_m), as
303 well as in the volume of gas produced (V_T), and retained (V_R), during rheofermentometer test.
304 Significant correlations between bread specific volume and rheofermentometer indices were
305 reported by Beck et al. (2012b).

306 Brown, appealing crust is another appreciated characteristic of durum wheat bread. Bread with
307 lower salt content showed a less colored crust. All colorimetric indices (a^* , b^* and $100-L^*$)
308 significantly increased as salt level increased, due to a more intense Maillard browning (Moreau,
309 Bindzus, & Hill, 2009; Silow et al., 2016). Several mechanisms have been proposed to explain
310 the positive effect of salt on bread color, including the inhibition of fermentation, with
311 consequent higher amounts of sugars left, prone to be involved in Maillard and caramelization
312 reactions during baking (Belz et al., 2012).

313 Among the sensory features, the highest scores were attributed to crumb consistency and crust
314 color. Bread prepared with 20 g kg⁻¹ salt showed the typical sensory characteristics of durum
315 wheat bread, with highly consistent crumb and brown crust (Giannone et al., 2018; Licciardello
316 et al., 2017). The variation in salt content had a significant impact on the sensory profile: in
317 addition to the expected effect on taste, with higher scores for “salty” and lower for “sweet”
318 descriptor as the level of salt increased, significant differences were observed in texture and

319 odor. In particular, paralleling the values of specific volume, bread with 10 g kg⁻¹ salt showed a
320 less consistent crumb than bread with 20 g kg⁻¹ salt. Crumb grain of the least salty bread showed
321 also a more open structure with larger pores, but the difference with the other bread type was not
322 statistically significant.

323 The sensory evaluation of crust color, which was darker in bread containing 20 g kg⁻¹ salt,
324 agreed with the colorimeter data.

325 The odor descriptors “yeasty” and “toasted”, ascertained in crumb and crust, respectively, were
326 significantly affected by salt variation, with higher score for yeasty and lower for toasted in
327 bread with 10 g kg⁻¹ salt than in the saltiest formulation. The increase of yeasty odor intensity as
328 salt content decreased has been reported also in common wheat bread (Raffo et al., 2018).

329 Besides the effect of fermentation, which was more intense in reduced-salt bread as shown by
330 rheofermentometer data, a flavor-enhancing effect of salt has been reported, probably due to the
331 ability of salt in influencing water activity. The water restriction by salt results in the
332 concentration of flavor molecules in solution and affects their volatility (Costa-Corredor, Serra,
333 Arnau, & Gou, 2009). In addition, the increase of ionic strength, caused by the presence of salt,
334 influences the chemical bonds within the food system and, therefore, the flavor sensation
335 (Hutton, 2002).

336

337 *3.4 Effect on bread volatile compounds*

338 One of the most appreciated sensory characteristics of bread is its aroma, which depends on the
339 combination of various factors: type of ingredients and yeast, extent of mechanical-enzymatic
340 degradation due to kneading and leavening, and intensity of the thermal reactions occurring
341 during baking. The SPME/GC-MS analysis of volatile compounds of crust allowed identifying
342 several aroma compounds, including alcohols, aldehydes, ketones, carboxylic acids, lactones,
343 furan compounds, and pyrazines (Table 3).

344 Strecker aldehydes (mainly 2-methylpropanal, 3-methylbutanal and 2-methylbutanal, deriving
345 respectively from valine, leucine and isoleucine), 3-methyl-butanol, 2-furan-methanol (furfuryl
346 alcohol), furfural, methylpyrazine, acetic acid and ethanol were the most abundant compounds in
347 all samples.

348 Furans, together with pyrazines, pyrroles, and Strecker aldehydes, typically arise from thermal
349 degradation of sugars and Maillard reaction, therefore are quite common in the aroma profile of
350 bread crust or cookies (Giannone et al., 2018; Giarnetti et al., 2015). These compounds were
351 detected in durum wheat bread in previous researches (Giannone et al., 2018; Licciardello et al.,
352 2017). Other compounds, such as 3-methyl-butanol (isoamyl alcohol), 2-methyl-1-propanol
353 (isobutyl alcohol), and 2-methylpropanoic acid derive from yeast metabolism (Hansen &
354 Schieberle, 2005). Small amounts of sulfur compounds were also observed, such as carbon
355 disulfide and dimethyl sulfide. The latter derived from the decomposition of methional, the
356 Strecker aldehyde of methionine (Giannone et al., 2018).

357 A significant effect of salt level on the volatile profile was observed. Higher amounts of Strecker
358 aldehydes, 2-methylfuran, methylpyrazine and diacetyl (2,3-butanedione) were detected in bread
359 with 20 g kg⁻¹ salt, due to more relevant Maillard browning associated to higher salt levels
360 (Moreau et al., 2009), as confirmed by color determinations. Acetaldehyde and acetic acid,
361 mainly deriving from fermentation, showed higher amounts in reduced-salt breads, in accordance
362 with the increased fermentation rates. Fusel alcohols (2-methyl-1-propanol and 3-methyl-1-
363 butanol), produced by yeasts either from amino acids through the Ehrlich pathway, or from
364 carbohydrates during branched-chain amino acids synthesis (Watanabe, Fukuda, Asano, & Ohta,
365 1990) were unexpectedly detected in higher amounts in breads with 20 g kg⁻¹ salt, probably due
366 to environmental effects on the synthetic pathways of these compounds, already pointed out by
367 transcriptomic studies. In particular, Schoondermark-Stolk et al. (2006) reported that at slightly
368 acidic pH, as in the dough of the present study (data not shown), the production of 3-methyl-1-

369 butanol by *Saccharomyces cerevisiae* increased at higher salt contents (though at pH 3.0 the
370 highest levels were observed in absence of salt).

371 Control bread also showed higher levels of hexanal, imputable to catalytic action of salt on lipid
372 oxidation (Snirivasan & Xiong, 1996). Hexanal, in fact, takes origin from the oxidation of
373 linoleic acid, which is present in the fatty fraction of semolina.

374 Overall, the differences observed in the volatile profile of bread crusts agreed with the sensory
375 evaluation of crust odor descriptor “toasted”.

376

377 **4. Conclusions**

378 With the exception of bread prepared with 5 g salt per kg⁻¹ of re-milled semolina, the quality
379 changes observed in reduced-salt breads did not compromise either their acceptability or the
380 willingness to purchase expressed by consumers. The modifications induced by salt reduction in
381 durum wheat bread were similar to those reported in literature for common wheat bread, and
382 were essentially attributable to an increased fermentative capacity. Specifically, a reduction of
383 salt by 50% (from 20 to 10 g kg⁻¹), which would help fulfilling healthier dietary levels,
384 negatively influenced aroma profile and color of bread, leading to a less intensely colored crust
385 and a weaker toasted aroma, but had a positive effect on bread specific volume and crumb
386 consistency.

387 The latter findings are of particular interest in the production of durum wheat bread, which is
388 known for being very compact due to the high alveograph P/L ratio of re-milled semolina. In
389 fact, the raise of specific volume and the decrease of crumb consistency might meet the
390 expectation of those people used to softer products such as white bread, therefore increasing the
391 number of potential consumers of durum wheat bread.

392 Further studies are underway for assessing the effects of salt reduction in bread obtained from
393 tetraploid wheats other than durum, such as emmer wheat (*Triticum dicoccon* Schrank).

394

395 **Acknowledgements**

396 The authors acknowledge the financial support of the Italian Ministry of Education, University
397 and Research (MIUR), program PRIN 2015 (grant number 2015SSEKFL) “Processing for
398 healthy cereal foods”, and of University of Bari, Aldo Moro, within the “IDEA Giovani” Project
399 titled “Technological and biotechnological strategies to obtain low-sodium bakery leavened
400 products enriched in beta-glucans”. Mr. Bartolo Digesù (Digesù bakery, Bari, Italy) is
401 acknowledged for accurate preparation of bread samples.

402

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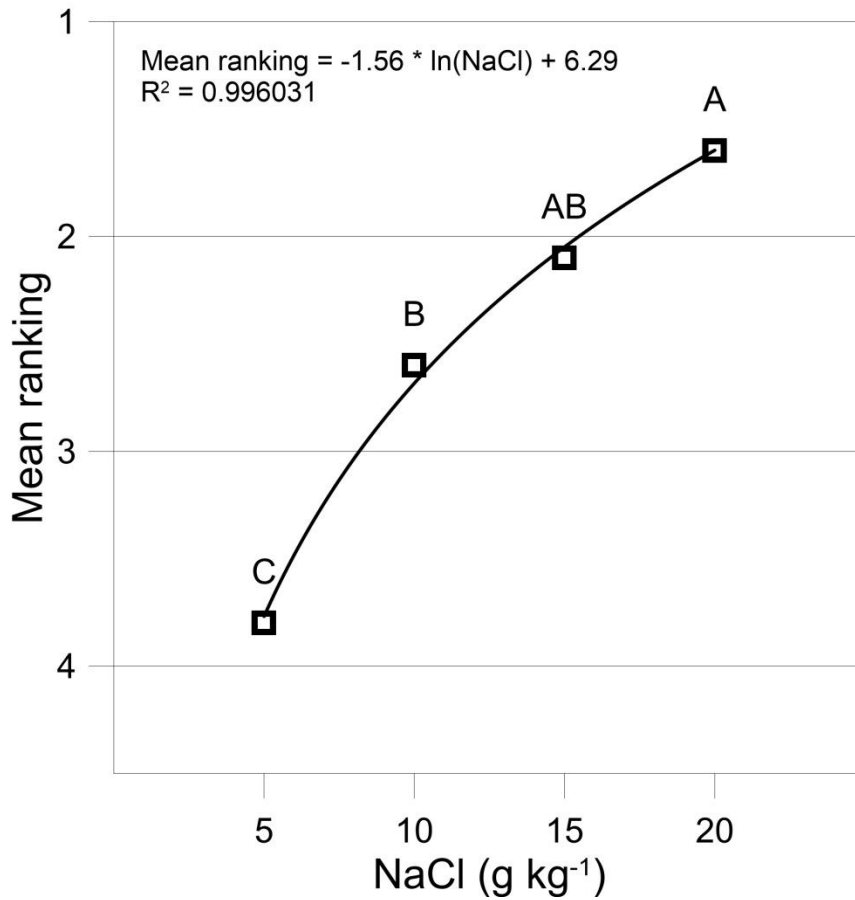
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- 516

517 **Figure captions**

518

519 **Figure 1.** Mean ranking of four durum wheat bread types, containing different salt (NaCl)
520 amounts, according to the preference expressed by consumers. Rank = 1 corresponded to the
521 maximum appreciation; rank = 4 corresponded to the minimum appreciation.

522 Different letters mean a significant difference at $p < 0.05$ according to Friedman non-parametric
523 test.



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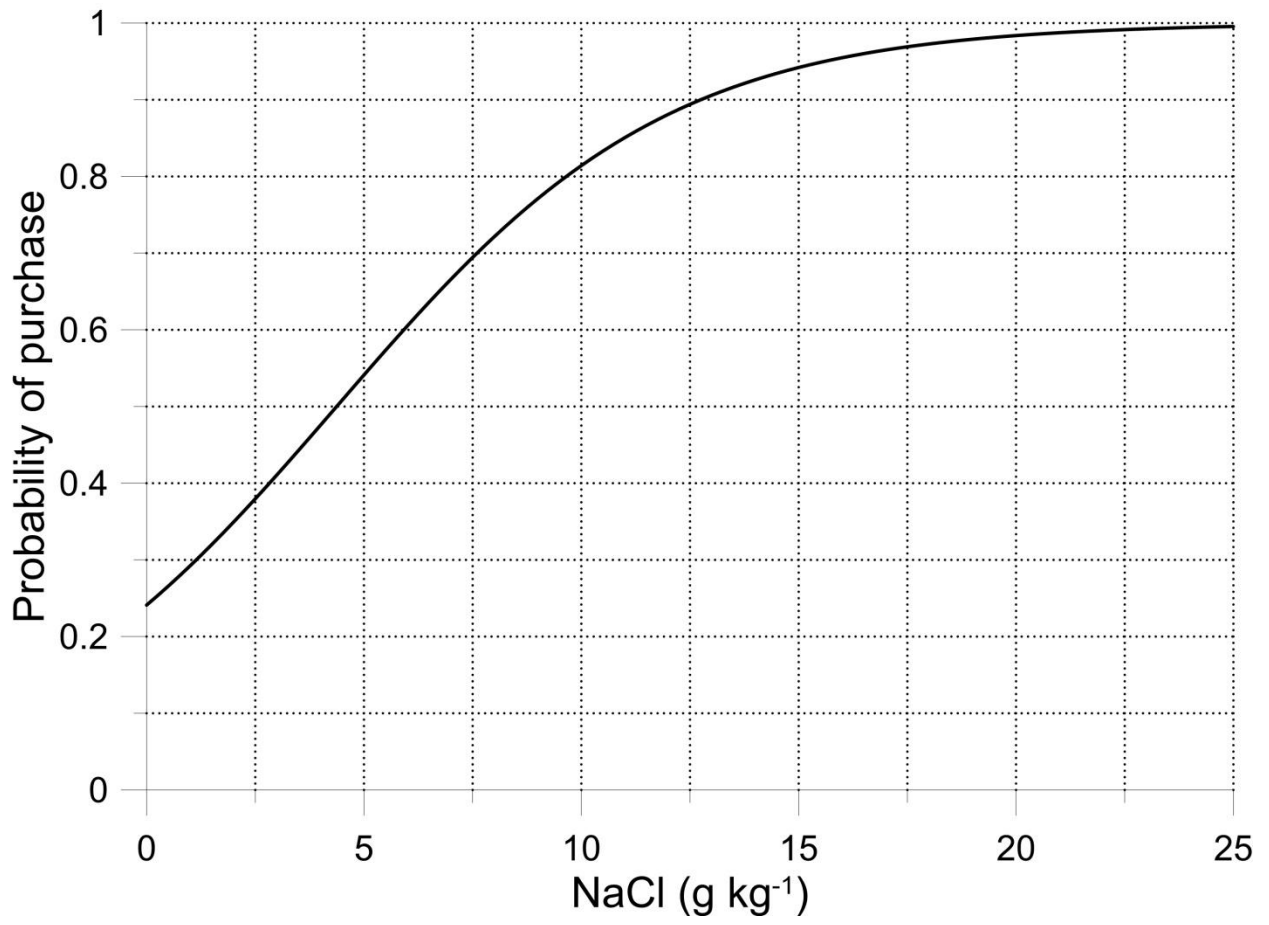
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532 **Figure 2.** Curve describing the willingness to purchase bread as a function of salt content.



533

534

535 **Table 1.**
 536 Mean and standard deviation of dough height and volume of gas produced and retained during
 537 fermentation, determined by rheofermentometer. Dough was prepared with re-milled durum
 538 wheat semolina at different salt (sodium chloride) levels.

Rheofermentometer parameter	Salt level (g kg ⁻¹ re-milled semolina)			
	5	10	15	20
<i>Curve of dough development</i>				
Maximum dough height (H_m) (mm)	55±1a	53±1a	49±1b	45±1c
Time to reach maximum dough height (T_l) (min)	88±2b	90±2b	90±2b	121±3a
Dough height after 3 h (h) (mm)	36±1b	37±1b	38±1b	42±1a
Decrease of dough height after 3 h compared to T_l [$(H_m-h)/H_m$] (%)	35±2a	30±1a	24±1b	6±0c
<i>Curve of gas production and retention</i>				
Volume of gas produced (V_T) (mL)	1595±10a	1570±11b	1558±12b	1529±10c
Volume of gas lost (V_L) (mL)	359±4a	347±2b	339±4c	317±3d
Volume of gas retained (V_R) (mL)	1236±6a	1223±7ab	1219±8b	1212±7b
Gas retention coefficient [V_R/V_T] (%)	77±2a	78±2a	78±1a	79±2a
Maximum height of gas production curve (H'_m) (mm)	83±3a	79±2a	74±2b	69±1c
Time to reach maximum height of gas production curve (T'_l) (min)	45±1c	46±1c	51±2b	57±2a
Time needed to start losing gas (T_x) (min)	55±2c	58±2b	63±2a	64±2a

539 Different letters in row indicate a significant difference at $p < 0.05$.

540

541 **Table 2.**
 542 Mean and standard deviation of specific volume, color indices and main sensory features of
 543 durum wheat bread at different salt (sodium chloride) levels.

Parameter	Salt level (g kg ⁻¹ re-milled semolina)	
	10	20
Specific volume (mL g ⁻¹)	3.61±0.18a	3.02±0.11b
<i>Crust color indices</i>		
Yellow index (<i>b</i> *)	24.55±0.36b	28.74±0.55a
Red index (<i>a</i> *)	4.01±0.17b	8.07±0.48a
Brown index (100- <i>L</i> *)	26.09±0.62b	33.27±0.81a
<i>Sensory features</i>		
Crumb grain	5.3±0.6a	4.2±0.5a
Crumb consistency	4.7±0.5b	6.1±0.6a
Crust color	4.5±0.4b	5.9±0.5a
Salty taste (crumb)	1.2±0.4b	2.8±0.4a
Sweet taste (crumb)	2.3±0.3a	1.5±0.2b
Bitter taste (crust)	0.5±0.2a	1.1±0.3a
Yeasty odor (crumb)	6.1±0.5a	4.9±0.4b
Toasted odor (crust)	2.5±0.3b	3.8±0.4a

544 Different letters in row indicate a significant difference at $p < 0.05$.
 545

546 **Table 3.**
 547 Volatile compounds (peak areas expressed as total ion chromatogram $\times 10^6$) detected in the crust
 548 of durum wheat bread at different salt (sodium chloride) levels.

Compound	Salt level (g kg ⁻¹ re-milled semolina)				ANOVA (<i>p</i> -Value)
	10		20		
	Mean	SD	Mean	SD	
<i>Alcohols</i>					
Ethanol	1227.7	215.5	1150.2	159.1	n.s.
2-Methyl-1-propanol	59.6	15.5	132.2	34.0	0.01
3-Methyl-1-butanol	229.5	41.6	619.8	148.9	0.01
<i>Aldehydes</i>					
Acetaldehyde	39.5	8.5	19.7	2.5	0.01
2-Butenal	17.6	2.9	3.0	1.3	0.001
2-Methylpropanal	593.1	178.9	752.8	109.3	n.s.
2-Methylbutanal	104.2	21.9	331.5	89.4	0.01
3-Methylbutanal	353.4	187.1	1025.6	383.0	0.05
Hexanal	0.0	0.0	7.3	1.2	0.001
<i>Ketones</i>					
2-Propanone-1-hydroxy	208.6	17.2	142.1	10.6	0.001
Butanone	27.8	9.8	43.1	11.7	n.s.
2-Butanone-3-hydroxy	123.0	65.1	201.4	44.9	n.s.
2,3-Butanedione	169.7	47.0	253.9	47.9	0.05
2,3-Pentanedione	21.2	7.3	59.9	31.3	n.s.
<i>Carboxylic acids</i>					
Acetic acid	208.8	31.3	164.0	17.8	0.05
Propanoic acid	29.3	3.9	32.1	5.7	n.s.
2-Methylpropanoic acid	32.5	10.1	117.5	19.5	0.001
2,2-Dimethylpropanoic acid	44.0	5.3	34.3	22.4	n.s.
<i>Esters</i>					
Ethyl-acetate	13.9	13.0	19.4	5.5	n.s.
<i>Lactones</i>					
γ -Butyrolactone	18.3	4.7	13.8	6.2	n.s.
<i>Furan compounds</i>					
Furan	15.7	4.9	12.3	2.3	n.s.
2-Methylfuran	8.2	10.2	24.0	7.6	0.05
2-Furanmethanol	449.6	109.9	337.0	46.5	n.s.
Furfural	291.6	70.1	224.8	12.0	n.s.
<i>Pyrazines</i>					
Pyrazine	78.4	26.9	61.6	11.9	n.s.
Methylpyrazine	156.9	72.0	279.2	58.8	0.05
Ethylpyrazine	47.2	18.9	60.2	26.8	n.s.
2,3-Dimethylpyrazine	10.0	1.5	10.7	4.1	n.s.
<i>Pyrroles</i>					
Pyrrole	53.3	14.8	49.5	4.6	n.s.
1-Methyl-1-H-pyrrole	12.7	6.8	12.8	4.5	n.s.
<i>Sulfur compounds</i>					
Carbon disulfide	0.9	0.3	1.3	0.2	n.s.
Dimethyl sulfide	2.0	0.6	2.3	0.8	n.s.