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The nutritional quality of wholegrain and multigrain breads is not necessarily better 1

- 2 than white breads: the case of gluten-free and gluten-containing breads
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13 Abstract

14 Despite the importance of breads through the history, the wide range of options might lead to a choice dilemma from health-conscious consumers when purchasing bread. In this study, 15 commercial white, wholegrain and multigrain regular breads, sold in Europe, were collected, and 16 classified into gluten-free and gluten-containing categories. For gluten-free-breads, no significant 17 18 differences were found in energy, saturated fatty acids, sugar, fiber and salt between white and 19 wholegrain breads regardless of the mention "multigrain". For gluten-containing, carbohydrates 20 and fibers differed between white and wholegrain breads, while when considering multigrain 21 presence all the nutritional composition varied significantly. Nevertheless, the mentions 22 wholegrain and multigrain on gluten-free and gluten-containing breads do not guarantee a better 23 nutritional quality compared to white bread. Gluten-free breads showed increased fiber, and 24 decreased carbohydrates, sugar and energy which are comparable to gluten-containing 25 wholegrain breads. This underlines the improvement of gluten-free breads and suggests further 26 investigations to increase protein content.

27 Keywords: bread, multigrain, wholegrain, fiber, gluten-free, gluten-containing

28 1. Introduction

Bread is a staple food consumed worldwide and it is traditionally made using basic ingredients 29 30 (i.e., wheat flour, water and yeast) providing essential nutrients (e.g., energy, protein and 31 carbohydrates) in human diet. Obesity and other metabolic and cardiovascular diseases have been 32 related to bread consumption. Nevertheless, bread is still a main asset in worldwide diet despite 33 the lifestyles' changes. Bread's recipes and processes have been subjected to constant 34 transformation to respond people demands. This rapid adaptation of the breads to cultures, 35 countries and lifestyles, make breads an interesting case of food study and considering their 36 impact on the diet and health, it is important a deeper analysis of what is in the market and if those 37 breads are filling the social demands regarding aspects like nutrients, healthiness, sustainability. 38 The use of additional ingredients beside those basic and the advent of new technologies have 39 allowed a wide diversification of bread products over the centuries (Paciulli et al. 2021). As nutrients vehicles, breads have been the election carrier to solve nutrients deficiencies in the 40 population through enrichment or fortification. Sensory appealing is driven by the use of different 41 42 ingredients to create new flavors (and sensory experiences) such as microalgae, pulses, 43 sourdoughs, and wholegrain flours (Naqash et al. 2017; Graça et al. 2018; Stantiall and Serventi 44 2018).

Wholegrains are considered as significant components of a healthy diet since they are valuable 45 sources of phytochemicals such as phenolic compounds, antioxidants, phytic acid and sterols, 46 47 water-soluble fiber (such as β -glucan and arabinoxylan), minerals, and vitamins (Mridula et al. 48 2015). There is increasing evidence that the consumption of wholegrain can be related to 49 reductions in markers of overweight, obesity and type 2 diabetes (Della Pepa et al. 2018; Wang et al. 2020). Nevertheless, European Food Safety Authority (EFSA) noted that a cause and effect 50 relationship could not be established between wholegrain and specific health effects due to 51 52 variability of results of the randomized controlled trials submitted to substantiate this association 53 (Agostoni et al. 2010). Precise association is difficult to determine due to different 54 epidemiological methodologies, different methods of determining wholegrain intake, and 55 different definitions of a wholegrain food (Kissock et al. 2021). In Europe, there is no legally 56 recognized definition for wholegrain foods. For instance, wholegrain foods must contain \geq 50% 57 of dry matter from wholegrain ingredients in Scandinavian countries, at least \geq 51% in UK, \geq 90% 58 in Germany, and 100% in some countries such as Netherlands and Spain (Ross et al. 2017). In 59 2010, The Healthgrain European Union Integrated Research Project recommended that a food may be labeled as "wholegrain" if it contains \geq 30% wholegrain ingredients in the overall product 60 61 and contains more wholegrain than refined grain ingredients on a dry-weight basis (Van Der Kamp et al. 2014). This definition was intended to be useful in the context of nutritionalguidelines and for labelling purposes.

64 In Europe, there is no legislation regarding labelling wholegrain food products (Mathews and Chu 2020). The more recent European commission notice (2017/C 393/05) provided guidelines on the 65 application of the principle of quantitative ingredients declaration (European Commission 66 Directorate-General for Health and Food Safety 2017). Thus, if "wholegrain" is mentioned on the 67 68 label, the levels of whole-grain ingredients must be listed on the packaging as part of mandatory 69 information. However, this information is mentioned within the list of ingredients list, and thus it 70 might limit its visibility to consumers interested in products containing more wholegrain (Ross et 71 al. 2017). In 2021, Whole Grain Initiative recommended that foods containing 25-50% whole-72 grain ingredients based on dry weight, may make a front-of-pack claim on the presence of 73 wholegrain but cannot be designated 'wholegrain' in the product name (Van Der Kamp et al. 74 2021) (Van Der Kamp et al. 2021). They recommend to apply the rule of nutrient claim in a food 75 (such as "source of" and "high" dietary fiber), where the amount required for the "high" 76 qualification is twice the amount required for "source of" (Van Der Kamp et al. 2021)).

77 The market for wholegrain breads is growing (Market Data Forecast 2021) particularly amongst 78 obese, diabetic and elderly populations (Călinoiu and Vodnar 2018; Capurso and Capurso 2019). 79 Wholegrains breads are generally considered healthier choices compared to white breads made 80 with refined flours (Avberšek Lužnik et al. 2019). This topic is still debatable, and results differ among studies conducted comparing the impact of these breads intake on human health. 81 82 Considering glycemic index, whole wheat bread is recommended over the white bread (Nirmala Prasadi and Joye 2020; Romão et al. 2021). It is assumed that the mechanism of action of 83 wholegrains is associated with their dietary fibers promoting satiety and contributing into 84 lipogenesis and fat storage (McRae 2017). Several studies reported contradictory results, where 85 86 some findings sustained that no significant difference between refined white bread and whole wheat bread (Jenkins et al. 1988; Zafar et al. 2020), while other reported a lower glycemic index 87 88 in wholegrain breads (Avberšek Lužnik et al. 2019). Furthermore, it was reported that replacing 89 refined flours with other grains or seeds such as pulses might result to reduced glycemic of gluten-90 free and gluten-containing breads (Zafar et al. 2020; Boukid et al. 2019; Udani et al. 2009). 91 Commonly, breads made with blends of flours are commercially designed "multigrain" as they 92 contain more than one type of grains such as wheat, maize, millet, sorghum, oat, barley, maize, 93 and rice (Sagar and Pareek 2021). Some of the multigrain flours may include wholegrain 94 ingredients but the term multi-grain does not necessarily ensure that the food certainly contained wholegrain ingredients (Mridula et al. 2015). Consumers might be confused between "multigrain" 95 96 and "wholegrain", which are not interchangeable terms.

97 Therefore, the present study has a dual objective. The first aim is to evaluate the nutritional 98 composition of commercial regular breads sold in the European market by retrieving information from their packaging and to investigate differences in terms of energy, macronutrients 99 (carbohydrates, sugars, fat, saturates "SFA", proteins and fibers) and salt contents among gluten-100 101 free and gluten containing bread products. This study considered both gluten-free and glutencontaining products separately to identify similarities/ dissimilarities among white, multigrain and 102 103 wholegrain breads. Then, a section was allocated to compare gluten-free to gluten-containing 104 products to evaluate if the efforts in formulations and processing of gluten-products enabled parity 105 in nutritional value with gluten-containing products. This analysis will further provide a better 106 understanding of the nutrient's intake of the European population through the consumption of a 107 staple food, in this case bread.

108 **2.** Material and methods

109 2.1. Data collection & extraction

The search was carried out in January 2022, by consulting the Mintel Global New Product 110 111 Database (Mintel GNPD-Mintel Group Ltd., London, UK, https://portal.mintel.com/portal/). The Mintel GNPD search was conducted using the parameters specified in Table 1 available during 112 2021 in Europe (44 countries including UK). Out of the super-category of "foods", the search was 113 114 concerted on the category "Bread & Bread Products". The term "slices" was added as a filter to narrow the research to regular French ("pain de mie") leavened white breads. Similar setting was 115 116 used for multigrain breads (made with more than two types of grains) with the use of "multigrain 117 bread" as product name. Non multigrain products refer to product made using one type of grain. 118 Wholegrain breads were retrieved using the same setting with specifying that product designation 119 include the mention "wholegrain". Non wholegrain products refer to products made only with refined flours and no wholegrain flour was used. Nutritional facts, i.e., energy (kcal/100 g), total 120 121 fat (g/100 g), saturated fatty acids-SFA (g/100 g), carbohydrates (g/100 g), sugars (g/100 g), fiber (g/100 g); protein (g/100 g), and salt (g/100 g), were set as a filter for all the products. The results 122 123 of all searches were exported to Microsoft Excel (Microsoft Office, Washington, WA, USA). 124 Furthermore, brands and countries of products are reported in Table S1. The search was conducted for gluten-containing and gluten-free products. For gluten-containing (GC) and gluten-free (GF) 125 products, breads were grouped as white bread (WB), wholegrain (WGB) and containing 126 127 multigrain (MG) or non-containing multigrain (NMG).

128 2.2. Data extraction

- 129 For all the selected products, the nutritional labelling of all products, energy (kcal/100 g), total fat
- 130 (g/100 g), saturated fatty acids-SFA (g/100 g), carbohydrates (g/100 g), sugars (g/100 g), fiber
- 131 (g/100 g); protein (g/100 g), and salt (g/100 g) were collected. All products country, brand and
- 132 list of ingredients were also collected.

133 2.3. Statistical data analysis

134 The statistical analysis was carried out using the Statistical Package for Social Sciences software (IBM SPSS Statistics, Version 25.0, IBM corp., Chicago, IL, USA). All data were checked for 135 136 normality using the Shapiro-Wilk test. Energy and nutrient contents per 100 g of products were analyzed using Kruskal-Wallis non-parametric one-way ANOVA for independent samples with 137 138 multiple pairwise comparisons when considering 4 bread categories (NMG-WB, NMG-WGB, MG-WP and MG-WGB) for gluten-free and gluten-containing products. Mann-Whitney non-139 parametric test were used for two independent samples, white "WB" (NMG-WB+MG-WB) vs 140 141 wholegrain "WGB" (NMG-WGB+MG-WGB). A principal component analysis (PCA) was 142 performed based on the correlation matrix to compare gluten-free breads to those gluten-143 containing.

Criteria	Gluten- free white bread	Gluten-free wholegrain bread	Gluten-free multigrain white bread	Gluten-free multigrain wholegrain bread	Gluten- containing white bread	Gluten-containing wholegrain bread	Gluten-containing multigrain white bread	Gluten-containing multigrain wholegrain bread
Code	GF-NMG- WB	GF-NMG- WGB	GF-MG-WB	GF-MG-WGB	GC-NMG-WB	GC-NMG-WGB	GC-MG-WB	GC-MG-WGB
Sub- Category	Bread & Bread Products							
Product name	White bread	Wholegrain bread	White bread	Wholegrain bread	White bread	Wholegrain bread	White bread	Wholegrain bread
Filter	Text: Slices	Text: Slices	Text: Multigrain Slices	Text: Multigrain Slices	Ingredient: wheat flourText: slices	 Ingredient: wheat Text: slices 	 Ingredient: wheat flour Text: slices, multigrain 	 Ingredient: wheat flour Text: slices, multigrain
Claim	Gluten- free	Gluten-free	Gluten-free	Gluten-free	-	-	-	-
Region				Euro	pe			
Nutrition (from the label)	Energy (kc	al/100 g); Fat (g/1	00 g); Saturated F	Fatty acids-SFA (g/1	00 g); Carbohydrates((g/100 g).	(g/100) g; Sugars (g/10	0 g); Fiber (g/100 g); P	rotein (g/100 g); Salt

Table 1. Search strategy used on Mintel Global New Product Database.

<u>GC-NMG-WB: gluten-containing non multigrain white bread; GC-NMG-WG: gluten-containing non multigrain wholegrain bread; GC-MG-WB; gluten- containing multigrain white bread; GC-MG-WB: gluten-free non multigrain white bread; GF-NMG-WG: gluten-free
</u>

148 <u>non multigrain wholegrain bread; GF-MG-WB; gluten-free multigrain white bread; GF-MG-WGB: gluten-free multigrain wholegrain bread.</u>

3. Results and discussion

152 **3.1** Nutritional profile of gluten-free breads

153 Nutritional profile of gluten-free GF-WB (n = 71) and GF-WGB (n = 53) sold in the EU market is

outlined in Figure 1. To understand if products designed "multigrain" differ from breads without

this designation, GF-WB (n=71) were divided into GF-NMG-WB (n=44) and GF-MG-WB

156 (n=27). Similarly, GF-WGB (n=53) were divided into GF-NMG-WGB (n=49) and GF-MG-

157 WGB (n=4). Figure 2 illustrates the results of the four categories examined.

158 Results of energy showed no significant difference between GF-WB and GF-WGB (Figure 1), 159 and neither when going deeper within the four groups (GF-NMG-WB, GF-MG-WB, GF-NMG-160 WGB and GF-MG-WGB) (Figure 2). Fat content was found higher in WGB compared to WB (Figure 1). This variability can be attributed in part to higher amount of fat in wholegrains due to 161 the preservation of germ rich in fat and in second part to differences in the amounts of fat deriving 162 163 from flours/ starches/ seeds in bread formulations. More specifically, GF-NMG-WGB had the 164 highest amount, and no significant differences were found among GF-NMG-WB, GF-MG-WB, 165 and GF-MG-WGB (Figure 2). Nevertheless, no significant differences were found in SFA 166 between WGB and WB (Figure 1). Likewise, Figure 2 showed that even dividing these two 167 categories in those designed multigrain and those without, similar results were observed. This 168 underline that similar amounts of SFA were added to gluten-free formulations. Indeed, vegetable 169 oils mostly sunflower oil and rapeseed oil were commonly used in these formulations. As 170 illustrated in Figure 1, carbohydrates were found higher in GF-WB compared to GF-WGB. This 171 difference is due to higher amounts of starchy ingredients used in GF-WB. GF-NMG-WGB 172 showed the lowest amount, while the remaining categories showed similar amounts, due to the 173 inclusion of higher amounts of wholegrain flours. Indeed, NWG-WGB are made from wholegrain 174 rice flour (n=26), whole buckwheat flour (n=10), wholegrain amaranth flour (n=7), wholegrain 175 millet flour (n=4), and whole quinoa flour (n=2) (Table 2). Compared to GF-NMG-WB, similar 176 ingredients were used in GF-MG-WB and the use of wholegrain flours was at low levels. 177 Probably, this can contribute to the absence of difference between both categories besides limited 178 number of samples designed "multigrain" white breads. On the other hand, GF-NMG-WB were 179 made chiefly from refined flours [rice flour (n=40), millet flour (n=11), quinoa flour (n=13), 180 sorghum flour (n=1), buckwheat flour (n=11), oat flours (n=1), and teff flour (n=1)] and starches [corn starch (n=40), rice starch (n=17), potato starch (n=22), and tapioca starch (n=12)]. 181 Furthermore, few products contained wheat starch (1 product) and oat ingredients (4 products), 182 183 presumably due to their unsafe reputation for celiac patients. Noteworthy, wheat starch and oat 184 ingredients can be considered suitable for a gluten-free diet (if the maximum gluten contamination

- level do not exceed 20 ppm) in Europe based on European Commission Regulation (EC) 41/2009
- 186 (European Commission Regulation (EC) 41/2009 2009).
- 187 Regarding sugar content, no significant difference was found among the bread groups in Figure
 1 and Figure 2. Probably, this can be attributed to similar amounts of added sugars to gluten-free
- 100 1 and 1 igure 2. 1100abiy, this can be attributed to similar amounts of added sugars to gru
- regular breads as substrate for yeasts during fermentation (Roman et al. 2019).
- 190 Noteworthy, GF-WGB and GF-WB showed high values of fiber content (up to 16 g/ 100g), which 191 is due to the different types of purified fibers that are added in the recipes, namely sugar beet 192 fiber, psyllium, plantain fiber, citrus fruits fiber, apple fiber, chickpea fiber, corn fiber, and 193 bamboo fiber, and inulin (Table 2). No significant differences in fiber content were found between 194 GF-WB and GF-WGB probably due the frequent use of plant fibers in both categories as 195 previously reported (Allen and Orfila 2018a). These fibers are incorporated to improve the 196 nutritional value of gluten-free breads made chiefly from starchy ingredients and thus to reduce 197 rapidly digestive starch and potentially glycemic index (Djordjević et al. 2021). Particularly, 198 psyllium was frequently incorporated due to its effectiveness in reducing risks of health issues 199 such as hypercholesterolemia, type 2 diabetes, obesity, constipation, diarrhea, hemorrhoids, and 200 irritable bowel syndrome (McRorie et al. 2021). Additionally, this ingredient is used in breads as 201 a natural and clean label alternative to gums and hydrocolloids that must be labelled as additives 202 (Belorio and Gómez 2020). Furthermore, they play a functional role in increasing water holding 203 capacity of the dough and increasing breadcrumb moistness (Sciarini et al. 2017). Furthermore, 204 seeds rich in fibers were added in their intact forms (for topping) and as flours (as functional 205 ingredient). Among oilseeds, sunflower seed, linseed, sesame seed, flaxseed, poppy seed, chia, 206 nigella seeds, and pumpkin seeds are the most used. These seeds were reported to improve the 207 nutritional characteristics of gluten-free breads by contributing into fiber contents (Huerta et al. 208 2019) and functional properties as fat substitutes, due to their water absorption capacity and 209 rheological properties (Korus et al. 2015; Huerta et al. 2019). Depending on their level of addition, 210 they contribute into the modulation of the rheology of the doughs and the end-quality of breads 211 including volume, texture, and organoleptic characteristics (De Lamo and Gómez 2018). Figure 212 2 showed that GF-NMG-WGB and GF-MG-WGB had slightly higher amounts of fiber compared 213 to NMG-WB and MG-WB. The differences can be potentially attributed to the use of wholegrain 214 flours as a main ingredient.

Protein was found higher in GF-WGB compared to GF-WB. Specially, GF-NMG-WB had the lowest value, while the remaining categories were statistically similar. These differences might be attributed to the different content of proteins of the flours used. Furthermore, protein fortification is a common practice in gluten-free bread formulations. Proteins are added in different forms isolates, such as soy proteins, whey protein and egg white. Furthermore, legumes

- and seeds can contribute into the increase of protein (Huerta et al. 2019). No significant difference
- in salt contents were observed. In general, in gluten free breads, salt content is added as a flavor
- enhancer and do play a minor functional role, in opposition to what occurs in gluten-containing
- breads.

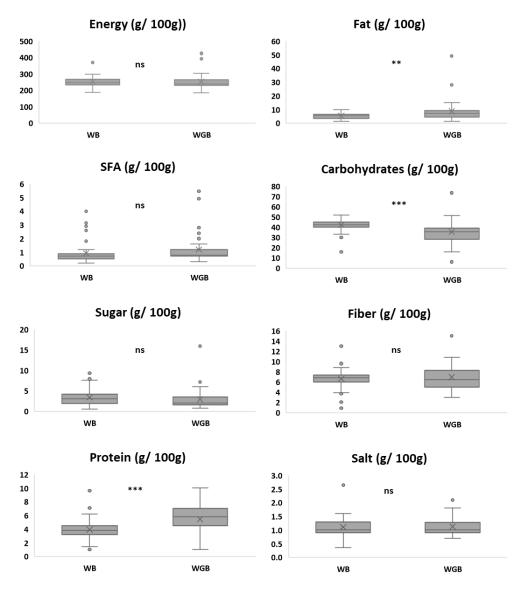
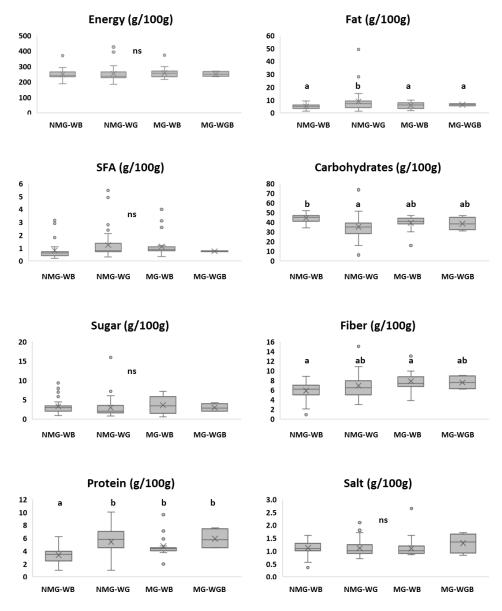


Figure 1: Nutritional profile of white (WB) (N = 71) and wholegrain (WGB) (N = 53) gluten-free breads sold in the EU market. The box-plot legend: the box is limited by the lower (Q1 = 25th) and upper (Q3 = 75th) quartile; the median is the horizontal line dividing the box; Whiskers above and below the box indicate the 10th and 90th percentiles; outliers: the points outside the quartile 10–90th percentiles. p < 0.05, **p < 0.01, ***p < 0.001, ns non-significant (p > 0.05). WB: white brad; WGB: wholegrain bread.



233 Figure 2: Nutritional profile of gluten-free white (N=44) and wholegrain (N=49) breads without the 234 designation "multigrain" vs multigrain white (N = 27) and multigrain wholegrain (N = 4) breads sold in the 235 EU market. The box-plot legend: the box is limited by the lower (Q1 = 25th) and upper (Q3 = 75th) quartile; 236 the median is the horizontal line dividing the box; Whiskers above and below the box indicate the 10th and 237 90th percentiles; outliers: the points outside the quartile 10-90th percentiles. Different letters indicate 238 significant differences among bread types, p < 0.05. ns non-significant (p>0.05). NMG-WB: non 239 multigrain white bread; NMG-WG: non multigrain wholegrain bread; MG-WB; multigrain white 240 bread; MG-WGB: multigrain wholegrain bread.

Table 2: Grains and seeds used in making gluten-free breads.

	White bread	Wholegrain bread	Multigrain white bread	Multigrain wholegrain bread
Wholegrain flours	-	Wholegrain rice flour (n=26), whole buckwheat flour (n=10), wholegrain amaranth flour (n=7), wholegrain millet flour (n=4), whole quinoa flour (n=2),	Wholegrain rice flour (n=2) (0.9 and 1.4%),	Whole rice flour (n=2) (1.4-34%), wholegrain, buckwheat flour (7%) (n=2)
Refined grain flours	Rice flour (n=40), millet flour (n=11), quinoa flour (n=13), sorghum flour (n=1), buckwheat flour (n=11), oat flours (n=1), teff flour (n=1),	Rice flour (n=46), millet flour (n=11), amaranth flour (n=5), quinoa flour (n=15), buckwheat flour (n=12), carob flour (n=2), oat flour (n=1), chestnut flour (n=3),	Rice flour (n=22), millet flour (n=9), amaranth flour (n=1), quinoa flakes/flour (n=12), sorghum flour (n=6), buckwheat flour (n=7) carob flour (n=2), oat flakes/flours (n=2),	Millet flour (n=2), buckwheat flour (n=2), rice flour (n=3),
Pulses flours	Lentil flour (n=1)	Lentil flour (n=1), bean flour (n=2)	Bean flour (n=1), lentil flour (n=1)	Lupine flour (n=1)
Starches	Corn starch (n=40), rice starch (n=17), potato starch (n=22), tapioca starch (n=12), wheat starch $(n=1)$	Corn starch (n=22), rice starch (n=7), potato starch (n=8), tapioca starch (n=4), cassava starch (n=6)	Corn starch (n=24), rice starch (n=17), potato starch (n=5), tapioca starch (n=3)	Rice starch (n=2), corn starch (n=2),
Fiber	Psyllium (n=28), citrus fruits fiber (n=2), apple fiber (n=6), bamboo fiber (n=4)	Sugar beet fiber (n=3), psyllium (n=18), plantain fiber (n=2), citrus fruits fiber (n=2), apple fiber (n=6), chickpea fiber (n=1), corn fiber (n=1), bamboo fiber (n=3), inulin (n=4)	Sugar beet fiber (n=1), psyllium (n=1), plantain fiber (n=1), citrus fruits fiber (n=3), apple fiber (n=5)	Psyllium (n=2)
Seeds	Soybean flour (n=22), flaxseed flours (n=4), linseed (n=7), pumpkin seeds (n=1), carob seed flour (n=1), chestnut flour (n=1)	Sunflower seed flour (n=20), linseed flour (n=22), flaxseeds flour(n=5), chia seeds (n=20), sesame seeds (n=12), pumpkin seeds (n=11)	Soybean flour (n=15), sunflower seed (n=26), linseed (n=22), sesame seed (n=1), flaxseed (n=6), poppy seed (n=5), chia (n=2), nigella seeds (n=1), pumpkin seeds (n=3), chestnut flour (n=2)	Sunflower seeds (n=4), soy flour (n=1), linseed (n=3), chia seeds (n=2),

3.2 Nutritional profile of gluten-containing breads

245 Figure 3 illustrates the nutritional profile of gluten-containing GC-WB (n = 57) and GC-WGB

246 (n = 59) sold in the EU market. GC-WB were further subdivided into GC-NMG-WB (n=29) and

247 GC-MG-WB (n=28), to discriminate between those non-containing or containing multigrains.

- 248 GC-WGB (n=59) were classified into GC-NMG-WGB (n=28) and GC-MG-WGB (n=31).
- 249 No significant differences were observed in energy, fat, SFA, sugars, protein and salt, except 250 carbohydrates (Figure 3). Even though not all products have reported the amounts of wholegrains 251 in their list of ingredients, it can be speculated that the added amounts were quite low and did not 252 induce relevant changes into the nutritional profile. It can be also hypothesized that the high intra-253 variability within white breads vs wholegrains breads due to different formulations (Table 3) and 254 different manufactures (Table S1) masked the inter-variability. This aligns with previous results 255 focused on products sold in the Italian market showing WG inclusion cannot be always considered 256 a marker of the overall nutritional quality of foods (Dall'asta et al. 2022). Figure 4 particularly 257 shows that GC-MG-WB provided the highest energy which can be related to its highest fat and SFA contents compared to the rest types. Carbohydrates were found significantly higher in GC-258 WB compared to GC-WGB (Figure 3), and more particularly GC-MG-WGB showed significantly 259 260 the lowest value, while other breads were found similar. This can be attributed to the high amounts 261 of wholegrain included in the formulations (up to 62%, Table 2) resulting in lower carbohydrates 262 and sugars. Fiber content was found significantly lower in GC-NMG-WB compared to the rest of 263 breads. Protein content was found similar, independently on the presence/absence of the mention 264 "multigrain". No differences were found in salt content, except GC-MG-WB that had the highest 265 amount.

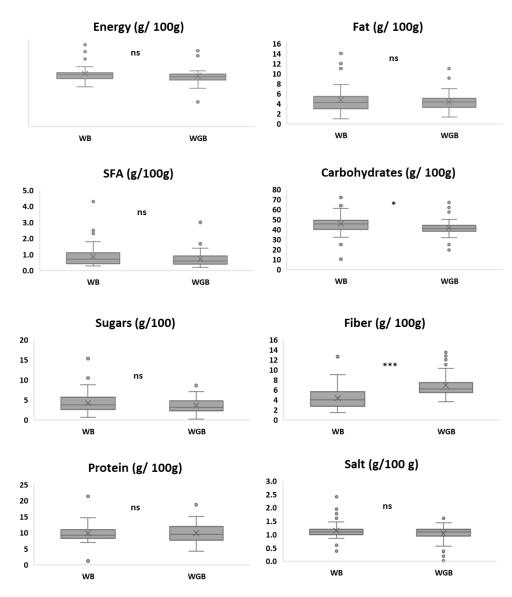
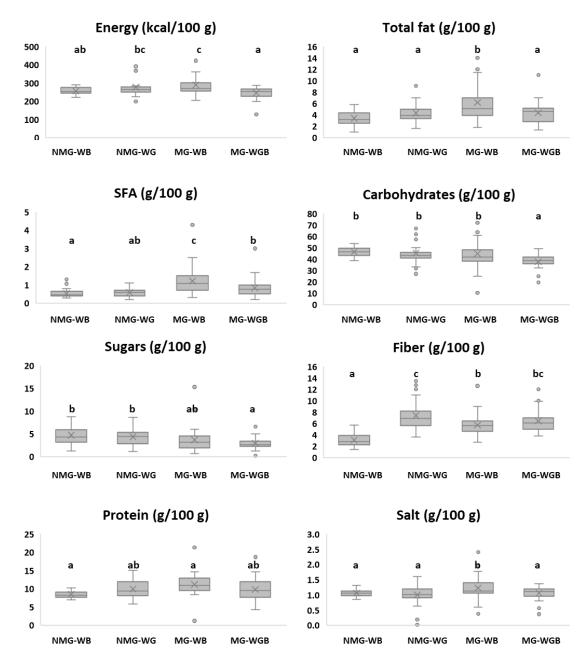


Figure 3: Nutritional profile of white (N = 57) and wholegrain (N = 59) gluten-containing breads sold in the EU market. The box-plot legend: the box is limited by the lower (Q1 = 25th) and upper (Q3 = 75th) quartile; the median is the horizontal line dividing the box; Whiskers above and below the box indicate the 10th and 90th percentiles; outliers: the points outside the quartile 10–90th percentiles. *p < 0.05, **p < 0.01, ***p < 0.001, ns non-significant (p > 0.05). WB: white bread; WGB: wholegrain bread.



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Figure 4: Nutritional profile of white (N = 29), wholegrain (N = 28), white multigrain (N = 28), and multigrain wholegrain (N = 31) gluten-containing breads sold in the EU market. The box-plot legend: the box is limited by the lower (Q1 = 25th) and upper (Q3 = 75th) quartile; the median is the horizontal line dividing the box; Whiskers above and below the box indicate the 10th and 90th percentiles; outliers: the points outside the quartile 10–90th percentiles. Different letters indicate significant differences among bread types, p < 0.05. <u>NMG-WB: non multigrain white bread; NMG-WG: non multigrain wholegrain bread; MG-WB; multigrain white bread; MG-WGB: multigrain bread.</u>

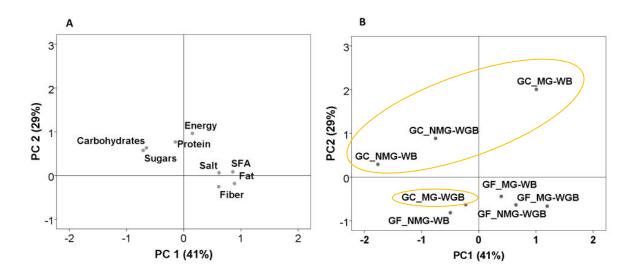
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	White bread	Wholegrain bread	Multigrain white bread	Multigrain wholegrain bread
Whole cereal grain flours	Whole wheat flour (n=2)	Whole wheat flour (n=28) (26- 100%), wholegrain wheat flour sourdough (n=2) (20%), sprouted wholegrain wheat flours (n=1) (12%), semi-whole flour (n=1), wheat bran (n=2) Wholegrain rye flour (15-57%) (n=2), wholegrain sprouted rye grains (19%) (n=1), wholegrain rye malt (n=2),	Wholegrain wheat flour (n=4) (2.4-20%), wholegrain rye flour (n=2) (1.2%),	Wholewheat flour (n=15) (9-62%), wholegrain rye flour (n=8), wheat bran (n=3), wheat germ (n=7), wheat fiber (n=2), oat fiber (n=2),
Refined cereal grains flours	Wheat flour (60-73%) (n=29), fermented wheat flour (n=1), rye flour (n=1), barley flour (n=1)	Wheat Flour (n=28), malted rye flour (n=2), rye flour (n=7), black rice flour (n=1), oat flour (n=1), malted and roasted barley flour (n=2), corn flour (n=1), barley flour (n=4), spelt wheat flour (n=1), buckwheat flour (n=1), kamut flour (n=2), quinoa flour (n=2), millet flour (n=2)	Wheat flour (n=28) (25-58%), barley flour (n=24), oat flour (n=19), rye flour (n=21), spelt flour (n=1), buckwheat flour (n=3), quinoa flour (n=1), millet (n=5), corn (n=7)	Wheat flour (n=31), rye flour (n=14), barley flour (n=27), oat flour (n=31), spelt (n=3), buckwheat (n=7), corn (n=10), rice (n=), quinoa (n=2), millet (n=12), amaranth (n=1), corn (n=1)
Oil seeds	Soybean flour (n=12), seed mix (n=1) (sunflower seed, sesame seed, linseed)	Sunflower seed flour (n=2), linseed flour (n=2), flaxseeds flour (n=2), chia seed (n=1)	Soybean flour (n=2), sunflower seed (n=24), linseed (n=24), sesame seed (n=24), flaxseed (n=6), poppy seed (n=5), chia (n=2)	sunflower seeds (n=27), soy flour (n=5), linseed (n=24), pumpkin seed (n=4), sesame seeds (n=9), poppy seeds (n=2), chia seeds (n=2),
Pulses	Mix of pulses flour $(n=1)$ (black bean red bean, navy bean, chickpea, yellow lentil), fava flour $(n=1)$, bean flour (n=1)	-	Lupin flour (n=1)	-

284	Table 3: Grains and	l seeds used in mak	king gluten-conta	ining breads.
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286 **3.3.** Gluten-containing vs gluten free

287 Considering gluten-free and gluten-containing categories, PCA was performed to visualize the 288 variability/ similarity in nutritional profiles of the different bread types (Figure 5). The total accumulative variance from the first two principal components accounted for 70% of the total 289 290 variance; the first component accounted for 41%, and the second component accounted for 29%. 291 The first component was expressed as function of fats, SFA, sugars, carbohydrates, salt and fibers, 292 while the second component was expressed as a function of energy, proteins, carbohydrates, 293 sugars, fat and fibers (Figure 5A). The projection of the bread types on the factorial space enabled 294 the separation of gluten-free and gluten-containing breads based on PC2 (Figure 5B). Indeed, it 295 was readily evident the aggregation of gluten-free products in the negative side of PC2, while 296 gluten-containing products were located in the upper side except GC-MG-WGB. This shows the significant variability in the nutritional composition between both categories. Indeed, protein 297 298 content was found lower in the gluten-free breads in alignment with previous studies focused on 299 products sold in Australian supermarkets (Wu et al. 2015; Hughes et al. 2020). Further studies are still required to increase protein content through the use of pulses or their protein isolates to avoid 300 301 beany flavors at high level of addition that might hamper their organoleptic properties (Boukid et 302 al. 2021). Notably, GC-MG-WGB were located near the gluten-free breads due to similarities in 303 nutritional profiles, i.e., low carbohydrates and high fibers compared to those gluten-containing 304 breads. Unexpectedly, gluten-free breads were less dense energy in consistence with previous 305 findings focused on breads sold in the Italian market (Angelino et al. 2020). This can be due to 306 the efforts made for improving the nutritional quality of gluten-free bakery products such as 307 reducing starchy ingredients (Morreale et al. 2018). Previously reported studies indicated that 308 gluten free breads were higher in fat and sugar contents (Matos and Rosell 2015; Allen and Orfila 309 2018b), Present analysis indicates that recipes of commercial products have been improved making and resulted more balanced and closer to the gluten-containing breads. Similar 310 conclusions were drawn for Norwegian products showing that fat and sugars contents were not 311 312 different between the groups (Myhrstad et al. 2021).



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314 Figure 5: Principal component analysis (PCA) describing the intra-category variability of bread products 315 based on their nutritional profile (energy (kcal/100 g), total fat (g/100 g), saturates (g/100 g), total 316 carbohydrates (g/100 g), sugars (g/100 g), protein (g/100 g), fiber (g/100 g) and salt (g/100 g)). Loading plots of Principal Component (PC) 1 and 2 (A) and rotated principal scores of bread types projected into 317 the first two principal components PC1 and PC2 (B). GC NMG-WB: gluten-containing non multigrain 318 white bread; GC NMG-WG: gluten-containing non multigrain wholegrain bread; GC MG-WB; 319 gluten- containing multigrain white bread; GC_MG-WGB: gluten-containing multigrain wholegrain bread; 320 321 GF NMG-WB: gluten-free non multigrain white bread; GF NMG-WG: gluten-free non multigrain 322 wholegrain bread: GF MG-WB: gluten-free multigrain white bread: GF MG-WGB: gluten-free 323 multigrain wholegrain bread.

325

326 4. Conclusion

Gluten-free and gluten-containing breads labeled "wholegrain" did not show significant 327 differences in nutritional profiles (except for fat, carbohydrates and proteins in the case of gluten-328 329 free breads and carbohydrates and fibers in case of gluten-containing breads) compared to white 330 breads. This aligns with the results of the survey of ingredients, showing high similarity and small 331 differences in terms of ingredients. In most cases, when comparing "multigrain" vs "non multigrain", no relevant changes were observed in terms of nutritional value and ingredients. This 332 333 indicates that this designation "wholegrain" and "multigrain" did not imply an added nutritional 334 value and thus it can be deduced that the terminology is mostly used for marketing motives. 335 Despite the nutritional improvement in gluten-free breads showing low carbohydrates, low energy 336 and high fiber contents, similar to gluten-containing wholegrain breads, protein content is still a 337 limitation that needs to be further addressed to enable consumers a nutritionally equivalent products, especially for patients genetically predisposed to adverse reaction to gluten. The use of 338 339 fruits, vegetables, algae, insects and to upgrade the quality of gluten-free products is a public 340 health priority. There is also opportunity for the use of wholegrain for an improved nutritional

- quality, but it needs to be ensured the added value of wholegrains and not only on-pack promotion.
- 342 Thus, clear regulation of health or nutrition claims of wholegrain mention in association with
- 343 fiber content might improve on-pack information and thereby attract more consumers. This in

344 turn will boost food manufacturers to use wholegrain and thus increase fiber intake.

345

346 Conflicts of Interest

347 The authors declare no conflict of interest.

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