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Title: Reducing the glycemic index of short dough biscuits by using apple pomace as a functional ingredient

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Abstract: The present research aimed at enriching short dough biscuits with apple pomace to evaluate the effect on glycemic index. Apple pomace was dehydrated and milled to a powder, which was characterized for soluble and insoluble dietary fiber, and for phenolic content. Apple pomace was used to partially replace wheat flour (10 and 20% w/w) in biscuits, which were characterized for their sensory properties and submitted to in vitro digestion to predict the glycemic index. Results indicated that the apple pomace contained impressive amounts of dietary fiber (nearly 40%), mainly represented by insoluble fiber (more than 25%). Apple pomace led to a significant reduction in the expected glycemic index of reformulated biscuits. The conventional biscuit presented a glycemic index of 70 and was thus classified as high glycemic index food. Substituting wheat flour by 10 and 20% with apple pomace reduced biscuit glycemic index to 65 and 60 respectively, thus ranking the product within the intermediate glycemic index foods.

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Dear Editor,

I would like to submit the manuscript entitled “Reducing the glycemic index of short dough biscuits by using apple pomace as a functional ingredient” by Marilisa Alongi, Sofia Melchior, Monica Anese for consideration for publication in LWT - Food Science and Technology.

The manuscript reports on the formulation of short dough biscuits with reduced glycemic index by partially replacing wheat flour with apple pomace. Reusing apple pomace, which is a by-product of apple juice processing, within food formulation would concomitantly satisfy the need for dietary strategies to manage type 2 diabetes and for the valorization of food discards.

Best regards

Monica Anese

## **Highlights**

Partially replacing wheat flour with apple pomace reduced biscuits glycemic index

Glycemic index decreased from 70 to 65 and 61 upon 10 and 20% wheat flour replacement

Industrially obtained apple pomace behaved analogously to the lab prepared one

1 **Reducing the glycemic index of short dough biscuits by using apple pomace as a functional ingredient**

2

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10

11 **Abstract**

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13 effect on glycemic index. Apple pomace was dehydrated and milled to a powder, which was  
14 characterized for soluble and insoluble dietary fiber, and for phenolic content. Apple pomace was  
15 used to partially replace wheat flour (10 and 20% w/w) in biscuits, which were characterized for  
16 their sensory properties and submitted to *in vitro* digestion to predict the glycemic index. Results  
17 indicated that the apple pomace contained impressive amounts of dietary fiber (nearly 40%), mainly  
18 represented by insoluble fiber (more than 25%). Apple pomace led to a significant reduction in the  
19 expected glycemic index of reformulated biscuits. The conventional biscuit presented a glycemic  
20 index of 70 and was thus classified as high glycemic index food. Substituting wheat flour by 10 and  
21 20% with apple pomace reduced biscuit glycemic index to 65 and 60 respectively, thus ranking the  
22 product within the intermediate glycemic index foods.

23

24 **Keywords:** type 2 diabetes; formulation; apple pomace; by-product, short dough biscuits

25

26

## 27        **1. Introduction**

28        During the last century dietary habits have considerably changed, leading to an excessive  
29        consumption of highly refined sugars and high-calorie-density foods. Such dietary change, together  
30        with sedentary lifestyle, has been correlated with the increasing incidence of chronic metabolic  
31        diseases. Among these, one of the most alarming is type 2 diabetes, which is expected to become  
32        the 7<sup>th</sup> death cause by 2030 worldwide (WHO, 2003). As type 2 diabetes is characterized by chronic  
33        hyperglycemia, dietary changes have been suggested to limit its occurrence (American Diabetes  
34        Association, 2004; WHO, 2003). Several foods have demonstrated antidiabetic properties, due to  
35        the presence of bioactive compounds, which mainly act by modulating digestive enzyme activity  
36        and intestinal transit rate, resulting in a low glycemic index. Phenolic compounds have been  
37        reported to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase, thus slowing down carbohydrates digestion and  
38        glucose absorption (Kwon, Apostolidis, & Shetty, 2008; Ríos, Francini, & Schinella, 2015). The  
39        latter is also affected by dietary fiber that increases matrix viscosity and modifies gastrointestinal  
40        transit, resulting in a reduced accessibility of starch to enzymatic hydrolysis (Brennan, 2005;  
41        Juvonen *et al.*, 2009).

42        A promising formulation approach for type 2 diabetes management is thus using functional  
43        ingredients rich in phenolics and dietary fiber, which are typically contained in fruit and vegetables  
44        (Stamataki *et al.*, 2016; Laguna, Salvador, Sanz, & Fiszman, 2011; Plazzotta, Calligaris, &  
45        Manzocco, 2018). These are often processed, generating tons of discards that are generally disposed  
46        of or used to produce energy, but that are still rich in bioactives (Rodríguez, Jiménez, Fernández-  
47        Bolaños, Guillén, & Heredia, 2006). Biochemical processing or chemical extraction have also been  
48        proposed to recover such value-added compounds, but they are costly and require an efficient  
49        management framework. Reusing processing discards, upon only negligible changes, might be a  
50        promising approach to obtain foods designed to address specific functions.

51        It is noteworthy that each year 10 million tons apple pomace are obtained from apple juice  
52        production worldwide. Apple pomace consists of flesh, peels, seeds, and stalks and contains a great

53 amount of high value-added compounds, mainly represented by dietary fiber (Wolfe & Liu, 2003).  
54 The aim of the present study was to elucidate whether a partial replacement of wheat flour by apple  
55 pomace in a short dough biscuit decreased its glycemic index.

56

## 57 **2. Materials and methods**

58

### 59 *2.1.Apparatus and chemicals*

60 The following apparatuses were used to carry out the experimental trials: vacuum oven (Bicasa,  
61 Milano, Italy), oven (Electrolux Professional, Pordenone, Italy), mill (Retsch, Hann, Germania),  
62 kneading machine (Kenwood, Milano, Italy), food laminating machine (Imperia & Monferrina,  
63 Roma, Italy), centrifuge (Brea, California, USA), spectrophotometer (Shimadzu Corporation,  
64 Kyoto, Japan), micro-centrifuge (Hittich, Tuttlingen, Germania), tristimulus colorimeter (Minolta,  
65 Osaka, Japan), texture analyzer (Instron LTD, High Wycombe, UK), freeze-drier (Edwards Alto  
66 Vuoto, Milano, Italia).  $\alpha$ -Amylase from *Bacillus* sp., porcine pepsin, porcine pancreatin, porcine  
67 bile extract, amyloglucosidase from *A. niger*, L-(+)-arabinose, D-(-)-fructose, D-(+)-glucose, sucrose,  
68 HCl, NaOH,  $\text{CaCl}_2(\text{H}_2\text{O})_2$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ , NaCl, KCl,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgCl}_2(\text{H}_2\text{O})_6$ ,  $(\text{NH}_4)_2\text{CO}_3$  and  
69 total dietary fiber assay kit were purchased from Sigma Aldrich (Milano, Italy). Folin-Ciocalteu's  
70 reagent, acetonitrile and ethanol 98% (v/v) were purchased from Carlo Erba Reagents (Milano, Italy).  
71 Deionized water (System advantage A10®, Millipore S.A.S, Molsheim, France) was used.

72

### 73 *2.2.Apple pomace preparation*

74 Apple pomace, consisting of peel, pulp, seeds, and stem, was recovered during the extraction of  
75 *Golden delicious* apple juice at laboratory or industrial scale. It was immediately dehydrated by  
76 using a vacuum oven (75 °C and 0.1 MPa) or a freeze-drier. Oven-dried and freeze-dried apple  
77 pomaces obtained at laboratory scale were named AP<sub>od</sub> and AP<sub>fd</sub>, respectively, while apple pomace



78 recovered at industrial scale was oven-dried and named AP<sub>ind</sub>. Samples were milled (particle size <  
79 200 µm) and stored at 20 °C and 5% RH.

80

### 81 *2.3.Short dough biscuits preparation*

82 Biscuit without (Control) or with 10% (R10) or 20% (R20) apple pomace were prepared (Table 1).  
83 The ingredients were mixed by a kneading machine and the dough was left to stand for 30 min at 4  
84 °C. The dough was rolled out into a 2-mm layer and 50 mm diameter discs were obtained. Samples  
85 were baked at 140 °C for 15 min, removed from the oven, cooled to room temperature and sealed  
86 under vacuum in flexible poly laminate pouches for storage in dark conditions until analysis.

87

### 88 *2.4.In vitro digestion*

89 *In vitro* digestion was carried out according to the protocol proposed by Minekus *et al.* (2014), with  
90 minor changes. The simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids were  
91 prepared and stored at 4 °C. The fluids were preheated to 37 °C just before *in vitro* digestion.  
92 Biscuits were milled for 30 s and 1 g sample was placed into a 50-mL falcon tube with 0.1 g L-(+)-  
93 arabinose as internal standard. The oral phase was started by adding 6 µL of CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> (0.3 M),  
94 194 µL of water and 800 µL of a 6.4 mg/mL α-amylase solution, prepared in SSF and providing 75  
95 U/mL activity in the final mixture. The sample was maintained at 37 °C under stirring for 2 min. At  
96 the end of the oral phase, the pH was adjusted to 3.0 with 40 µL HCl (1 M). Subsequently, 140 µL  
97 water and 1.82 mL of a 0.31 mg/mL pepsin solution, prepared in SGF and providing 2,000 U/mL  
98 activity in the final mixture, were added to start the gastric phase. The mix was stirred at 37 °C for 2  
99 h. At the end of the gastric phase, the pH was adjusted to 7.0 with 30 µL NaOH (1 M). The  
100 intestinal phase was initiated by adding 8 µL CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> (0.3 M), 262 µL of water, 3.2 mL of  
101 22.15 mg/mL pancreatin solution, prepared in SIF and providing 100 U/mL activity in the final  
102 mixture, and 0.5 mL of 160 mM bile extract prepared in SIF. The mix was stirred at 37 °C for 2 h.  
103 A secondary intestinal phase was conducted by adding 0.1 mL amyloglucosidase to the digestion mix,

104 which was maintained under stirring at 37 °C for 2 h. After 20, 60, 90 and 120 min from the beginning  
105 of the secondary intestinal phase, a sample was collected and the *in vitro* digestion was stopped by  
106 adding ethanol 98% (1:4 v/v). Samples were centrifuged at 10,000 g for 5 min at 4 °C. The  
107 supernatant was collected and analyzed for sugar content, as described in paragraph 2.5.3. White  
108 bread (62.3% flour, 34.6% water, 1.2% salt, 1.0% bakery yeast, 0.9% sugar, w/w) was also *in vitro*  
109 digested and analyzed for sugar content.

110

#### 111 2.4.1. *AUC and glycemic index computation*

112 Glucose release during the secondary digestion phase was plotted against time and the area under  
113 curve (AUC) was obtained (Matthews *et al.*, 1990). The glycemic index was computed (Wolever,  
114 Jenkins, Jenkins, & Josse, 1991) as reported in Equation 1.

115

$$116 \quad GI = \frac{AUC_s}{AUC_r} \times 100 \quad (1)$$

117

118 where  $AUC_s$  and  $AUC_r$  represent respectively the area under curve of glucose release relevant to the  
119 sample or to the reference food, i.e. white bread (Brouns *et al.*, 2005).

120

#### 121 2.5. *Solids and dietary fiber*

122 Total solids were determined by gravimetric method (AOAC, 1995). Soluble and insoluble dietary  
123 fiber was analyzed by using the total dietary fiber assay kit (AOAC International, Method 985.29,  
124 1997), and expressed on dry weight basis.

125

#### 126 2.6. *Total phenolic content*

127 Five g apple pomace were extracted in 75 mL water, for 60 min at 100 °C. The mixture was cooled  
128 down and centrifuged at 7,000 g for 10 min at 4 °C to collect the apple pomace extract. Total

129 phenol content was determined according to Singleton, Orthofer, and Lamuela-Raventos (1999).  
130 Briefly, 100  $\mu$ L apple pomace extract was added to 900  $\mu$ L water, 5 mL Folin-Ciocalteu's reagent  
131 and 3.5 mL of  $\text{Na}_2\text{CO}_3$  (150 g/L). The mixture was incubated for 2 h at 25 °C and absorbance was  
132 measured at 765 nm. Total phenolic content was calculated as  $\text{mg}_{\text{gallic acid equivalent (GAE)}/\text{g}_{\text{dry weight}}}$ .

133

#### 134 2.7. *Sugars*

135 The apple pomace extract and the supernatant collected after *in vitro* digestion of biscuits and bread  
136 were analyzed according to Englyst *et al.* (1999). Sugar were separated by an HPLC (Agilent 1260  
137 Infinity Quaternary LC, Agilent Technologies, Germany) equipped with an auto-injector (1260  
138 ALS), a chromatographic column (Amino 100 A, 5  $\mu$ m, 250 mm, 4.6 mm, SephaChrom, Rho,  
139 Italy), a temperature control system (1260 TCC) and a quaternary pump (1260 Quat Pump),  
140 generating a flow rate of 1 mL/min. The mobile phase was represented by water and acetonitrile  
141 (30:70, v/v) and the injection volume was 20  $\mu$ L. Monosaccharides were detected by a refractive  
142 index detector (1260 RID). A solution of D-(+)-glucose (0.05 g/mL), D-(-)-fructose (0.025 g/mL)  
143 and sucrose (0.05 g/mL) was diluted to 1:5, 1:10, 1:20 (v/v), added with L-(+)-arabinose (0.01  
144 g/mL) as internal standard and used for calibration.

145

#### 146 2.8. *Water-holding capacity (WHC) and oil-adsorption capacity (OAC)*

147 Apple pomace WHC was determined according to Sudha, Baskaran, and Leelavathi (2007), with  
148 slight modifications. Aliquots of 0.05 g of apple pomace were mixed with 1 mL water in a  
149 microcentrifuge tube, centrifuged at 13,000 *g* for 30 min, and decanted the excess water. The  
150 sample was weighed, and WHC was expressed as  $\text{g}_{\text{water}}/\text{g}_{\text{dry weight}}$ . OAC was similarly determined,  
151 by using sunflower oil instead of water. OAC was expressed as  $\text{g}_{\text{oil}}/\text{g}_{\text{dry weight}}$ .

152

153 *2.9. Color*

154 Color was analyzed using a tristimulus colorimeter and expressed in CIE units as L\*  
155 (lightness/darkness), a\* (redness/greenness) and b\* (yellowness/blueness) (Clydesdale, 1978).

156

157 *2.10. Firmness and thickness*

158 Dough firmness was determined by penetrating 3 mm a 20-mm-thick dough layer at 100 mm/min  
159 with a 6.2 mm cylindrical probe attached to a 0.1 kN unit. Biscuit firmness was determined by  
160 compressing 10 g sample at 150 mm/min speed with a ten-blade Kramer shear cell attached to a 5.0  
161 kN unit. Force-distance curves were recorded (Automated Materials Testing System, Version 5,  
162 Series IX, Instron Ltd.), and firmness was taken as the maximum force required to compress  
163 samples. The thickness of biscuits before and after baking was also measured.

164

165 *2.11. Sensory analysis*

166 Thirty panelists were involved in the sensory evaluation under laboratory conditions. A 7-point  
167 hedonic scale (1 low intensity, 7 high intensity) was used and panelists were instructed to compare  
168 the sample (R10 and R20) to the reference (control), which was attributed 4 points. Seven  
169 parameters representative of quality attributes (Popov-Raljić, Mastilović, Laličić-Petronijević,  
170 Kevrešan, & Demin, 2013), i.e. texture, crispiness, sweetness, sourness, shortbread flavor, baked  
171 flavor and fruit flavor, were considered.

172

173 *2.12. Statistical analysis*

174 Data were reported as mean  $\pm$  standard deviation of at least three measurements on two replicated  
175 samples. Analysis of variance (ANOVA) was performed with significance level set to  $p < 0.05$ ; the  
176 Bartlett procedure was used to test the homogeneity of variances, using R software, version 3.4.3  
177 (The R Foundation for Statistical Computing, 2018).

178

179 **3. Results and discussion**

180 *3.1. Physical and chemical properties of apple pomace*

181 Results of chemical and physical analyses of AP<sub>od</sub> and AP<sub>fd</sub> are reported in Table 2. Both AP<sub>od</sub> and  
182 AP<sub>fd</sub> presented a low moisture content, accounting for nearly 5%. Dry matter was mainly  
183 represented by TDF (37%), consisting of SDF and IDF with a ratio 1:3, in agreement with the  
184 literature (Carson, Collins, & Penfield, 1994; Rana, Gupta, Rana, & Bhushan, 2015). TDF was  
185 determined only in the AP<sub>od</sub> sample as drying is not expected to affect its content. TDF was in the  
186 same range as reported by other authors, i.e. 30 to 50% of total solids (Yan & Kerr, 2013; Shea *et*  
187 *al.*, 2015; Sudha *et al.*, 2007). The wide span of fiber concentration described in the literature can be  
188 attributed to the high matrix variability. Additionally, since apple pomace residues from apple juice  
189 processing, its composition is affected not only by the extrinsic (e.g. climatic conditions) and  
190 intrinsic (e.g. variety, ripeness degree) factors influencing apple growth, but also by storage  
191 conditions and dehydration techniques (Gullón, Falqué, Alonso, & Parajó, 2007). The latter, and in  
192 particular thermal processing, are expected to induce phenolics degradation (Lu and Foo, 1997).  
193 TPC actually accounted for 1.06 mg<sub>GAE</sub>/g<sub>dry weight</sub> in AP<sub>od</sub>, while a higher content was detected in  
194 AP<sub>fd</sub> (2.0 ± 0.1 mg<sub>GAE</sub>/g<sub>dry weight</sub>), in agreement with literature findings (Wolfe, Wu, & Liu, 2003).  
195 Despite low-temperature dehydration technologies could prevent phenolics degradation, they  
196 require higher costs when compared to high-temperature methods, which represent thus a more  
197 feasible option for food industries aiming at valorizing processing by-products.  
198 Consistently with literature data, fructose was the major component of AP<sub>od</sub> and AP<sub>fd</sub> sugars,  
199 accounting for 66 and 64% of total sugar content, respectively, while glucose and sucrose accounted  
200 for 25 and 24, and 9 and 12%, respectively (Gullón *et al.*, 2007).  
201 Color parameters indicated a golden yellow hue of both samples. A lower lightness was observed in  
202 AP<sub>od</sub>, as compared to AP<sub>fd</sub>, which resulted also less reddish. As observed by other authors (Martins,  
203 Jongen, & Van Boekel, 2001; Yan & Kerr, 2013), exposure to high temperature during drying may  
204 induce color changes of apple pomace due to the Maillard reaction.

205 The hydration properties of apple pomace fell in the same range reported by other authors, namely  
206  $1.6\text{-}8.4 \text{ g}_{\text{water}}/\text{g}_{\text{dry weight}}$  for WHC and  $1.2\text{-}2.0 \text{ g}_{\text{oil}}/\text{g}_{\text{dry weight}}$  for OAC (Figuerola *et al.*, 2005; Sudha *et*  
207 *al.*, 2007; Rana *et al.*, 2015). Apple pomace presented a 2.5-fold higher WHC than wheat flour  
208 (Joshi, Liu, & Sathe, 2015). The good hydration properties of apple pomace could be attributed to  
209 its higher content in soluble dietary fiber (more than 10%), when compared to wheat flour, in which  
210 SDF generally accounts for less than 5% (Taneyo, Di Silvestro, Dinelli, & Gianotti, 2017).

211

### 212 *3.2. Effect of apple pomace use on biscuit physical, chemical and sensory properties*

213 Apple pomace was used to partially substitute wheat flour in biscuits. Only AP<sub>od</sub> was used, due to  
214 the higher feasibility of conventional dehydration techniques. The physical and chemical properties  
215 of biscuits prepared by replacing wheat flour with AP<sub>od</sub> at 10 and 20% levels are presented in Table  
216 3. As the concentration of AP<sub>od</sub> increased, the thickness of biscuits decreased, when compared to  
217 the conventionally formulated biscuit (i.e. control), in agreement with the literature (Sudha *et al.*,  
218 2007). The limited volumetric increase can be attributed to the strong WHC of apple fiber (Table 2)  
219 which in turn might be related to changes in dough firmness (Chen, Rubenthaler, Leung, &  
220 Baranowski, 1988). The latter significantly increased when wheat flour was partially replaced by  
221 apple pomace, corresponding to  $1.1 \pm 0.1$ ,  $1.6 \pm 0.1$  and  $1.7 \pm 0.1$  N for control, R10 and R20  
222 respectively, potentially impinging volume increase during baking. In fact, firmness of R10 and  
223 R20 biscuits (Table 3) resulted lower ( $p < 0.05$ ) than that of the control sample. Similarly, Matejová,  
224 Fikselová, Čurlej, and Czako (2016) observed a reduction in biscuit firmness when wheat flour was  
225 replaced with apple, buckwheat and grape pomaces. Such a difference is not expected to rely on  
226 moisture, which was comparable for all biscuits (i.e.  $3.5 \pm 0.6\%$ ), but could depend on other  
227 interactions occurring within the matrix. The partial removal of wheat flour reduced gluten content,  
228 thus limiting gluten development during mixing and resulting in softer biscuits (Devisetti, Ravi, &  
229 Bhattacharya, 2015).

230 As expected, the control sample only contained sucrose, while the concentration of fructose, which  
231 was the major AP<sub>od</sub> sugar (Table 2), increased as as apple pomace content increased. Even if  
232 glucose was found in AP<sub>od</sub>, it was not detected in reformulated biscuits, due to its depletion upon  
233 Maillard reaction occurring during baking (Martins *et al.*, 2001).

234 The lightness of AP<sub>od</sub>-containing biscuits significantly decreased when compared to the control,  
235 while a\* and b\* concomitantly increased, indicating a more pronounced browning of biscuits  
236 containing apple pomace, due to the presence of glucose and fructose, which represent reactants of  
237 the Maillard reaction. The latter is well known to induce browning in baked foods, caused by the  
238 development of melanoidins and other Maillard reaction products. These compounds also affect the  
239 sensory properties of baked foods, leading to characteristic flavors (Martins *et al.*, 2001). To  
240 understand if and to what extent reformulation intervention affected the perception of biscuit  
241 sensory properties, some representative descriptors (Popov-Raljić *et al.*, 2013) were evaluated based  
242 on a hedonic scale and results are reported in Fig. 1. Wheat flour replacement by AP<sub>od</sub> did not affect  
243 the perception of most descriptors, such as firmness, crispiness, sweetness, sourness, and shortbread  
244 flavor (Fig. 1). On the contrary, the baked and fruit flavors were differently perceived in biscuits  
245 containing 10 and 20% APP<sub>od</sub>. Both reformulated biscuits presented significantly higher scores  
246 ( $p < 0.05$ ) for the baked flavor when compared to the control, probably due to the faster evolution of  
247 the Maillard reaction in AP<sub>od</sub>-containing biscuits (Martins *et al.*, 2001). The fruit flavor resulted  
248 significantly more intense ( $p < 0.05$ ) in biscuits containing the highest amount of AP<sub>od</sub> (20%), while  
249 the lower concentration (10%) did not affect its perception when compared to control. An  
250 improvement in the sensory profile of bakery goods obtained with a partial substitution of wheat  
251 flour with apple pomace were observed by other authors (De Toledo, Nunes, Da Silva, Spoto, &  
252 Canniatti-Brazaca, 2017), who described also an improvement of nutritional properties in bread and  
253 biscuits.

254

255 3.3. Effect of apple pomace use on biscuit glycemic index

256 Conventional and AP<sub>od</sub>-containing biscuits were *in vitro* digested to assess the effect of  
257 reformulation on the predicted glycemic index. Fig. 2 shows glucose concentration during the  
258 second intestinal phase of *in vitro* digestion.

259 Glucose concentration increased during *in vitro* digestion for all samples, presenting a sharper  
260 growth during the first 20 min of the second intestinal phase. As expected, the maximum glucose  
261 concentration, accounting for 155 mg/g<sub>dry weight</sub> after 90 min, was recorded in white bread. Glucose  
262 concentration in control biscuits increased up to 120 mg/g<sub>dry weight</sub> after 120 min. On the contrary,  
263 the maximum glucose concentration recorded for AP<sub>od</sub>-containing biscuits corresponded to 98 and  
264 97 mg/g<sub>dry weight</sub> for R10 and R20, respectively, after 20 min.

265 Glucose concentration data collected during the second intestinal phase of *in vitro* digestion were  
266 used to estimate the glycemic index of control, R10, and R20. The conventional biscuit (control)  
267 presented a glycemic index of  $70.4 \pm 0.2$  and was thus classified as high glycemic index food.  
268 Substituting flour by 10 (R10) and 20 (R20) percent significantly ( $p < 0.05$ ) reduced biscuit glycemic  
269 index to  $65.7 \pm 1.8$  and  $60.8 \pm 1.9$  respectively, thus ranking the product within the intermediate  
270 glycemic index foods (American Diabetes Association, 2004).

271 The reduction in the glycemic index, which resulted significant in biscuits with the highest AP<sub>od</sub>  
272 content, can be attributed to the considerable TDF content of this by-product (Table 1). Total  
273 dietary fiber is well known to contribute to glycemic index reduction by several mechanisms.  
274 Soluble dietary fiber can increase matrix viscosity at gastrointestinal level, contributing to the  
275 formation of a gel. The latter can envelop starch grains, protecting them from the amylolytic  
276 activity of digestive enzymes and thus impinging the release of free glucose, resulting in a reduced  
277 glycemic response (Brennan, 2005; Juvonen *et al.*, 2009). Despite insoluble dietary fiber does not  
278 directly influence postprandial glucose excursions, it plays a part in affecting the glycemic  
279 response, as it was demonstrated to affect gut transit time and was associated with a significant  
280 reduction of type 2 diabetes risk (Weickert & Pfeiffer, 2018; Wilfart, Montagne, Simmins, Noblet,



281 & Van Milgen, 2007). Since AP<sub>od</sub> presented a higher WHC than that reported in the literature for  
282 wheat flour, this by-product could entrap the water contained in the dough. Consequently, during  
283 biscuit baking, starch gelatinization would be partially prevented, inducing the retention of a high  
284 concentration of native starch. The latter can also be defined as resistant starch, since it is  
285 inaccessible to digestive enzymes due to the persistence of crystalline form (Miao, Jiang, Cui,  
286 Zhang, & Jin, 2015). In other words, resistant starch cannot be hydrolyzed in the gastrointestinal  
287 tract to release free glucose, thus not contributing to the glycemic response (Englyst *et al.*, 1999).  
288 Despite sugar content increased from 46 (control) to 60 (R10) and 65% (R20), a significant  
289 reduction in the glycemic index estimate was found when apple pomace was used to partially  
290 replace wheat flour. A further reduction in the glycemic index could thus be pursued by balancing  
291 the amount of sucrose used in biscuit formulation with sugars deriving from apple pomace.

292

### 293 *3.4.Characterization and use of apple pomace deriving from industrial processing*

294 Since AP<sub>od</sub> effectively reduced the glycemic index of biscuits, further trials were carried out to  
295 understand if apple pomace deriving from industrial processing (AP<sub>ind</sub>) presented performances  
296 similar to those of AP<sub>od</sub> obtained at laboratory scale. Table 4 reports the major functional properties  
297 of AP<sub>ind</sub>, potentially affecting biscuit formulation.

298 Despite TDF content of apple pomace obtained from the industrial process resulted higher than that  
299 found in apple pomace produced at laboratory scale (Table 2), it was in the same magnitude span  
300 and presented the same SDF:IDF ratio (i.e. 1:3) as reported by other authors (Carson *et al.*, 1994;  
301 Rana *et al.*, 2015). Analogously, WHC and OAC values corresponded to those relevant to apple  
302 pomace. Since AP<sub>ind</sub> presented functional properties similar to what observed for AP<sub>od</sub> (Tables 2  
303 and 4), this by-product was used to replace by 20% the flour in biscuit (Table 1). The glycemic  
304 index was then assessed and resulted analogous (62.0) to that observed for biscuits containing the  
305 same amount of AP<sub>od</sub>. Based on these results, it can be stated that the by-product of apple juice

306 production obtained at the industrial level could be exploited as an ingredient to reduce the  
307 glycemic index of biscuits.

308

#### 309 **4. Conclusion**

310 Results acquired in the present study demonstrated the efficacy of a formulation approach aimed to  
311 reduce food glycemic index by reusing vegetables discards. This would concomitantly satisfy the  
312 need for dietary strategies to manage type 2 diabetes and for the valorization of food by-products.  
313 Reusing them within food formulation would not only reduce food discards, thus limiting the food  
314 production environmental impact, but could also deliver nutritional advantages. To this regard,  
315 further research would be required to characterize bioactives contained in processing by-products,  
316 as well as to minimize the effect of further processing, required to convert by-products into  
317 ingredients, on bioactives content. Nonetheless, the functionality of new formulations should be  
318 validated *in vivo*.

319

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323

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421

422 **Captions for figures**

423

424 **Fig. 1.** Sensory scores attributed to biscuits containing 0 (control), 10 (R10) and 20 (R20) percent  
425 apple pomace (AP<sub>od</sub>) on flour basis.

426

427 **Fig. 2.** Glucose concentration as a function of time during the second intestinal phase, relevant to  
428 biscuits containing 0 (control), 10 (R10) and 20 (R20) percent of apple pomace (AP<sub>od</sub>) on flour  
429 basis.

430

**Table 1**

Composition of biscuit dough samples. Ingredients are listed according to the adding sequence. Wheat flour was substituted by 0 (control), 10 (R10) and 20 (R20) percent (w/w) of apple pomace on flour basis.

Ingredients (% w/w)	Control	R10	R20
Egg	20.7	20.7	20.7
Sucrose	17.2	17.2	17.2
Sunflower oil	8.6	8.6	8.6
Wheat flour	51.6	46.4	41.3
Apple pomace	-	5.2	10.3
NaCl	0.2	0.2	0.2
Baking powder	1.7	1.7	1.7



**Table 2**

Dry matter, total (TDF), soluble (SDF) and insoluble (IDF) dietary fiber, fructose, glucose and sucrose, total phenolic (TPC), color, water holding (WHC) and oil absorbing (OAC) capacity of oven-dried (AP<sub>od</sub>) or freeze-dried (AP<sub>fd</sub>) apple pomace powders.

		AP <sub>od</sub>	AP <sub>fd</sub>
Dry matter (%)		94.6 ± 0.1 <sup>b</sup>	95.1 ± 0.1 <sup>a</sup>
TDF (%)		36.6 ± 0.2	n.d.
	SDF	9.2 ± 0.2	n.d.
	IDF	27.4 ± 0.1	n.d.
Sugar (mg/g <sub>dry weight</sub> )	Fructose	115.6 ± 0.5 <sup>a</sup>	116.6 ± 0.5 <sup>a</sup>
	Glucose	44.4 ± 0.2 <sup>a</sup>	42.2 ± 0.1 <sup>b</sup>
	Sucrose	14.9 ± 0.2 <sup>b</sup>	21.1 ± 0.2 <sup>a</sup>
TPC (mg <sub>GAE</sub> /g <sub>dry weight</sub> )		1.1 ± 0.1 <sup>b</sup>	2.0 ± 0.1 <sup>a</sup>
Color	L*	77.8 ± 0.5 <sup>b</sup>	84.9 ± 0.4 <sup>a</sup>
	a*	2.6 ± 0.2 <sup>a</sup>	-0.7 ± 0.3 <sup>b</sup>
	b*	22.5 ± 0.4 <sup>b</sup>	25.1 ± 0.4 <sup>a</sup>
Hydration properties	WHC (g <sub>water</sub> /g <sub>dry weight</sub> )	4.7 ± 0.2 <sup>a</sup>	3.3 ± 0.1 <sup>b</sup>
	OAC (g <sub>oil</sub> /g <sub>dry weight</sub> )	1.0 ± 0.6 <sup>a</sup>	1.0 ± 0.1 <sup>a</sup>

n.d.: not determined

**Table 3**

Sugar content, thickness, firmness, and color of biscuits containing 0 (Control), 10 (R10) and 20 (R20) percent apple pomace powder ( $AP_{od}$ ) on flour basis.

Sample	Thickness (mm)	Firmness (kN)	Sugar (mg/g <sub>ss</sub> )			Color		
			Fructose	Glucose	Sucrose	L*	a*	b*
Control	4.4±0.5 <sup>a</sup>	1.2±0.1 <sup>a</sup>	n.d.	n.d.	46.3±1.3 <sup>b</sup>	86.1±0.5 <sup>a</sup>	5.0±0.4 <sup>b</sup>	16.2±0.8 <sup>b</sup>
R10	2.8±0.2 <sup>b</sup>	0.8±0.0 <sup>c</sup>	8.9±0.1 <sup>b</sup>	n.d.	51.5±1.4 <sup>ab</sup>	73.3±0.4 <sup>b</sup>	10.8±0.3 <sup>a</sup>	22.1±0.6 <sup>a</sup>
20	2.1±0.1 <sup>c</sup>	1.0±0.1 <sup>b</sup>	11.5±0.0 <sup>a</sup>	n.d.	52.3±0.1 <sup>a</sup>	72.4±0.3 <sup>c</sup>	11.3±0.1 <sup>a</sup>	21.6±0.4 <sup>a</sup>

n.d.: not detected.

**Table 4**

Total (TDF), soluble (SDF) and insoluble dietary fiber (IDF), water holding (WHC) and oil absorbing capacity (OAC) of apple pomace powder deriving from industrial processing (AP<sub>ind</sub>).

TDF (%)		47.2 ± 0.1
	SDF (%)	12.7 ± 0.1
	IDF (%)	34.5 ± 0.1
Hydration properties	WHC (g <sub>water</sub> /g <sub>dry weight</sub> )	5.0 ± 0.4
	OAC (g <sub>oil</sub> /g <sub>dry weight</sub> )	1.2 ± 0.1

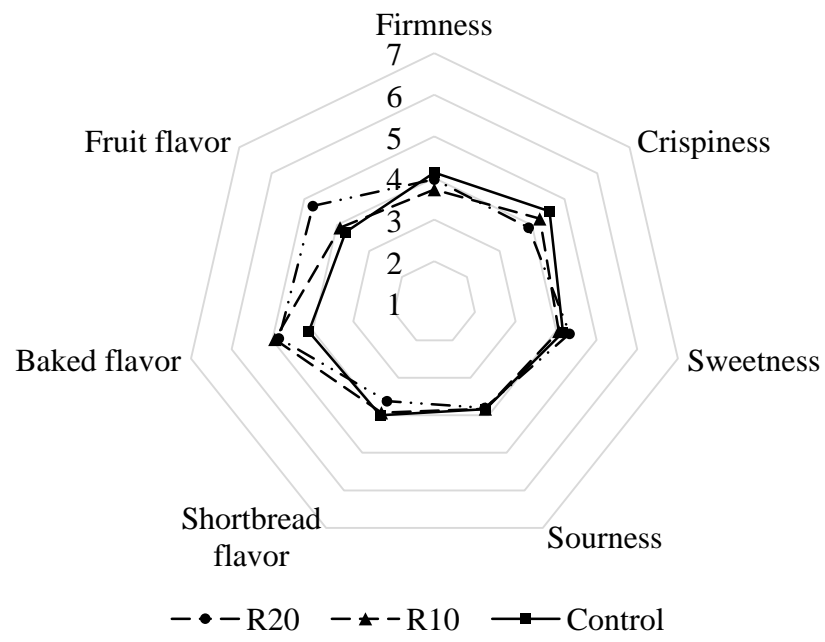


Fig. 1.

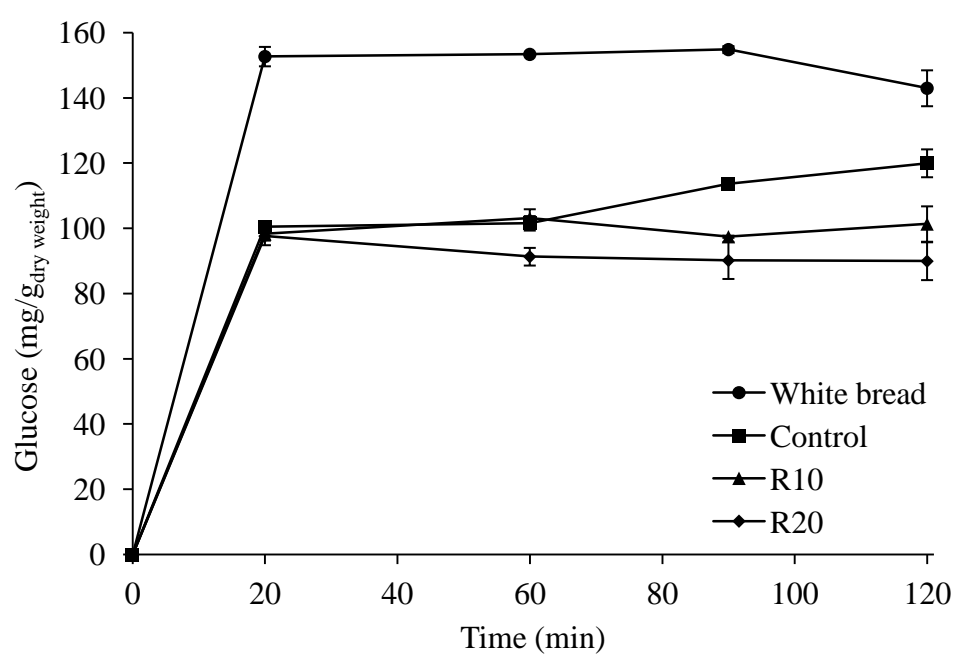


Fig. 2.