



UNIVERSITÀ
DEGLI STUDI
DI UDINE

Università degli studi di Udine

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

Original

Availability:

This version is available <http://hdl.handle.net/11390/1144758> since 2020-07-16T16:19:02Z

Publisher:

Published

DOI:10.1016/j.lwt.2018.10.068

Terms of use:

The institutional repository of the University of Udine (<http://air.uniud.it>) is provided by ARIC services. The aim is to enable open access to all the world.

Publisher copyright

(Article begins on next page)

Manuscript Number: LWT-D-18-02608

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

Article Type: Research paper

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

Corresponding Author: Professor Monica Anese,

Corresponding Author's Institution: University of Udine

First Author: Marilisa Alongi

Order of Authors: Marilisa Alongi; Sofia Melchior; Monica Anese

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.

Suggested Reviewers: Ana Maria Estevez
Dept Agroind & Enol, University of Chile, Santiago, Chile
aestevez@uchile.cl
Expert of dietary fibre

ML Sudha
Dept of Flour Milling Baking & Confectionery, CSIR, Cent Food Technol Res Inst, Mysore 570020, Karnataka, India
sudhaml@hotmail.com
Expert of vegetable by product reuse

Lara Manzocco
Dipartimento di Scienze Agroalimentari, Ambientali e Animali, University of Udine, Italy
lara.manzocco@uniud.it
Expert of food processing and formulation

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

3 Marilisa Alongi, Sofia Melchior, Monica Anese*

4

5 Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via

6 Sondrio 2/A, 33100 Udine, Italy

7

8

9 *Corresponding author. E-mail address: monica.anese@uniud.it

10

11 **Abstract**

12 The present research aimed at enriching short dough biscuits with apple pomace to evaluate the
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

1. Introduction

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

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

It is noteworthy that each year 10 million tons apple pomace are obtained from apple juice 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). α -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, $\text{CaCl}_2(\text{H}_2\text{O})_2$, Na_2CO_3 , NaHCO_3 , NaCl, KCl, KH_2PO_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_{od} and AP_{fd}, respectively, while apple pomace

78 recovered at industrial scale was oven-dried and named AP_{ind}. 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 CaCl₂(H₂O)₂ (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 CaCl₂(H₂O)₂ (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

phenol content was determined according to Singleton, Orthofer, and Lamuela-Raventos (1999). Briefly, 100 μ L apple pomace extract was added to 900 μ L water, 5 mL Folin-Ciocalteu's reagent and 3.5 mL of Na_2CO_3 (150 g/L). The mixture was incubated for 2 h at 25 °C and absorbance was measured at 765 nm. Total phenolic content was calculated as $\text{mg}_{\text{gallic acid equivalent (GAE)}}/\text{g}_{\text{dry weight}}$.

2.7. Sugars

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

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

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

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_{od} and AP_{fd} are reported in Table 2. Both AP_{od} and
182 AP_{fd} 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_{od} 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_{GAE}/g_{dry weight} in AP_{od}, while a higher content was detected in
194 AP_{fd} (2.0 ± 0.1 mg_{GAE}/g_{dry weight}), 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_{od} and AP_{fd} 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_{od}, as compared to AP_{fd}, 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_{od} 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_{od} at 10 and 20% levels are presented in Table
216 3. As the concentration of AP_{od} 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 ± 0.1 , 1.6 ± 0.1 and 1.7 ± 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_{od} sugar (Table 2), increased as as apple pomace content increased. Even if
232 glucose was found in AP_{od}, 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_{od}-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_{od} 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_{od}. 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_{od}-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_{od} (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_{od}-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_{dry weight} after 90 min, was recorded in white bread. Glucose
262 concentration in control biscuits increased up to 120 mg/g_{dry weight} after 120 min. On the contrary,
263 the maximum glucose concentration recorded for AP_{od}-containing biscuits corresponded to 98 and
264 97 mg/g_{dry weight} 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 ± 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 ± 1.8 and 60.8 ± 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_{od}
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_{od} 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_{od} effectively reduced the glycemic index of biscuits, further trials were carried out to
295 understand if apple pomace deriving from industrial processing (AP_{ind}) presented performances
296 similar to those of AP_{od} obtained at laboratory scale. Table 4 reports the major functional properties
297 of AP_{ind}, 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_{ind} presented functional properties similar to what observed for AP_{od} (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_{od}. 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

320 **Acknowledgements**

321 Authors are grateful to Ms. Francesca Zuccolo and Ms. Surubhi Mazzon for contributing to
322 analyses.

323

324 **References**

- 325 American Diabetes Association. (2004). Nutrition principles and recommendations in diabetes.
326 *Diabetes Care*, 27, S36–S46.
- 327 Brennan, C. S. (2005). Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition and*
328 *Food Research*, 49, 560–570.
- 329 Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. S. (2005).
330 Glycaemic index methodology. *Nutrition Research Reviews*, 18, 145–171.

331 Carson, K. J., Collins, J. L., Penfield, M. P. (1994). Unrefined, dried apple pomace as a potential
 332 food ingredient. *Journal of Food Science*, 59, 1213-1215.

333 Chen, H., Rubenthaler, G. L., Leung, H. K., & Baranowski, J. D. (1988). Chemical, physical, and
 334 baking properties of apple fiber compared with wheat and oat bran. *Cereal Chemistry*, 65,
 335 244–247.

336 De Toledo, N. M. V., Nunes, L. P., Da Silva, P. P. M., Spoto, M. H. F., & Canniatti-Brazaca, S. G.
 337 (2017). Influence of pineapple, apple and melon by-products on biscuits: physicochemical and
 338 sensory aspects. *International Journal of Food Science and Technology*, 52, 1185–1192.

339 Devisetti, R., Ravi, R., & Bhattacharya, S. (2015). Effect of hydrocolloids on quality of proso millet
 340 biscuit. *Food and Bioprocess Technology*, 8, 2298–2308.

341 Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1999). Rapidly
 342 available glucose in foods: an in vitro measurement that reflects the glycemic response.
 343 *American Journal of Clinical Nutrition*, 69, 448–454.

344 Figuerola, F., Hurtado, M. L., Estevez, A. M., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates
 345 from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food*
 346 *Chemistry*, 91, 395–401.

347 Gullón, B., Falqué, E., Alonso, J. L., & Parajó, J. C. (2007). Evaluation of apple pomace as a raw
 348 material for alternative applications in food industries. *Food Technology and Biotechnology*,
 349 45, 426–433.

350 Joshi, A. U., Liu, C., Sathe, S. K. (2015). Functional properties of select seed flours. *Food Science*
 351 *and Technology*, 60, 325-331.

352 Juvonen, K. R., Purhonen, A.-K., Salmenkallio-Marttila, M., Lahteenmaki, L., Laaksonen, D. E.,
 353 Herzig, K.-H., ... Karhunen, L. J. (2009). Viscosity of oat bran-enriched beverages influences
 354 gastrointestinal hormonal responses in healthy humans. *Journal of Nutrition*, 139, 461–466.

355 Kwon, Y., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α -
 356 amylase and α -glucosidase for management of hyperglycemia linked to type 2 diabetes.

- 357 *Journal of Food Biochemistry*, 32, 15–31.
- 358 Laguna, L., Salvador, A., Sanz, T., & Fiszman, S. M. (2011). Performance of a resistant starch rich
 359 ingredient in the baking and eating quality of short-dough biscuits. *LWT - Food Science and*
 360 *Technology*, 44, 737–746.
- 361 Lu, Y., & Foo, L. Y. (1997). Identification and quantification of major polyphenols in apple
 362 pomace. *Food Chemistry*, 59, 187–194.
- 363 Martins, S. I. F. S., Jongen, W. M. F., & Van Boekel, M. A. J. S. (2001). A review of Maillard
 364 reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*,
 365 11, 364–373.
- 366 Matejová, S., Fikselová, M., Čurlej, J., & Czako, P. (2016). Application of by-products in the
 367 development of foodstuffs for particular nutritional uses. *Journal of Central European*
 368 *Agriculture*, 17, 1306–1319.
- 369 Matthews, J. N. S., Altman, G. D., Campbell, M. J., Royston, P. (1990). Analysis of serial
 370 measurements in medical research. *British Medical Journal*, 300, 230-235.
- 371 Miao, M., Jiang, B., Cui, S. W., Zhang, T., & Jin, Z. (2015). Slowly digestible starch – A review.
 372 *Critical Reviews in Food Science and Nutrition*, 55, 1642–1657.
- 373 Minekus, M., Alming, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... Brodkorb, A.
 374 (2014). A standardised static in vitro digestion method suitable for food-an international
 375 consensus. *Food & Function*, 5, 1113-1124.
- 376 Plazzotta, S., Calligaris, S., & Manzocco, L. (2018). Application of different drying techniques to
 377 fresh-cut salad waste to obtain food ingredients rich in antioxidants and with high solvent
 378 loading capacity. *LWT - Food Science and Technology*, 89, 276-283.
- 379 Popov-Raljić, J. V, Mastilović, J. S., Laličić-Petronijević, J. G., Kevrešan, Ž. S., & Demin, M. A.
 380 (2013). Sensory and color properties of dietary biscuits with different fiber sources during 180
 381 days of storage. *Hem Ind*, 67, 123–134.
- 382 Rana, S., Gupta, S., Rana, A., Bhushan, S. (2015). Functional properties, phenolic constituents and

383 antioxidant potential of industrial apple pomace for utilization as active food ingredient. *Food*
384 *Science and Human Wellness*, 4, 180-187.

385 Ríos, J., Francini, F., & Schinella, G. (2015). Natural products for the treatment of type 2 diabetes
386 mellitus. *Planta Medica*, 81, 975–994.

387 Rodríguez, R., Jiménez, A., Fernández-Bolaños, J., Guillén, R., & Heredia, A. (2006). Dietary fibre
388 from vegetable products as source of functional ingredients. *Trends in Food Science and*
389 *Technology*, 17, 3–15.

390 Shea, N. O., Ktenioudaki, A., Smyth, T. P., Mcloughlin, P., Doran, L., Auty, M. A. E., ...
391 Gallagher, E. (2015). Physicochemical assessment of two fruit by-products as functional
392 ingredients: apple and orange pomace. *Journal of Food Engineering*, 153, 89–95.

393 Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and
394 other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in*
395 *Enzymology*, 299, 152–178.

396 Stamataki, N. S., Nikolidaki, E. K., Yanni, A. E., Stoupaki, M., Konstantopoulos, P., Tsigkas, A.-
397 P., ... Karathanos, V. T. (2016). Evaluation of a high nutritional quality snack based on oat fl
398 akes and inulin: effects on postprandial glucose, insulin and ghrelin responses of healthy
399 subjects. *Food & Function*, 7, 3295–3303.

400 Sudha, M. L., Baskaran, V., & Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and
401 polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*,
402 104, 686–692.

403 Taneyo, D., Di Silvestro, R., Dinelli, G., & Gianotti, A. (2017). Effect of sourdough fermentation
404 and baking process severity on dietary fibre and phenolic compounds of immature wheat flour
405 bread. *LWT - Food Science and Technology*, 83, 26–32.

406 Weickert, M. O., & Pfeiffer, A. F. (2018). Impact of dietary fiber consumption on insulin resistance
407 and the prevention of type 2 diabetes. *The Journal of Nutrition*, 148, 7–12.

408 WHO. (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organization*

409 *Technical Report Series*, 916.

410 Wilfart, A., Montagne, L., Simmins, H., Noblet, J., & Van Milgen, J. (2007). Digesta transit in
411 different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by
412 wheat bran. *British Journal of Nutrition*, 98, 54–62.

413 Wolever, T. M. S., Jenkins, D. J. A., Jenkins, A. L., Josse, R. J. (1991). The glycemic index:
414 methodology and clinical implications. *The American Journal of Clinical Nutrition*, 54, 846-
415 854.

416 Wolfe, K. L., & Liu, R. H. (2003). Apple peels as a value-added food ingredient. *Journal of*
417 *Agricultural and Food Chemistry*, 51, 1676–1683.

418 Yan, H., & Kerr, W. L. (2013). Total phenolics content, anthocyanins, and dietary fiber content of
419 apple pomace powders produced by vacuum-belt drying. *Journal of the Science of Food and*
420 *Agriculture*, 93, 1499–1504.

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_{od}) 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_{od}) 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_{od}) or freeze-dried (AP_{fd}) apple pomace powders.

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

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 _{ss})			Color		
			Fructose	Glucose	Sucrose	L*	a*	b*
Control	4.4±0.5 ^a	1.2±0.1 ^a	n.d.	n.d.	46.3±1.3 ^b	86.1±0.5 ^a	5.0±0.4 ^b	16.2±0.8 ^b
R10	2.8±0.2 ^b	0.8±0.0 ^c	8.9±0.1 ^b	n.d.	51.5±1.4 ^{ab}	73.3±0.4 ^b	10.8±0.3 ^a	22.1±0.6 ^a
20	2.1±0.1 ^c	1.0±0.1 ^b	11.5±0.0 ^a	n.d.	52.3±0.1 ^a	72.4±0.3 ^c	11.3±0.1 ^a	21.6±0.4 ^a

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_{ind}).

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

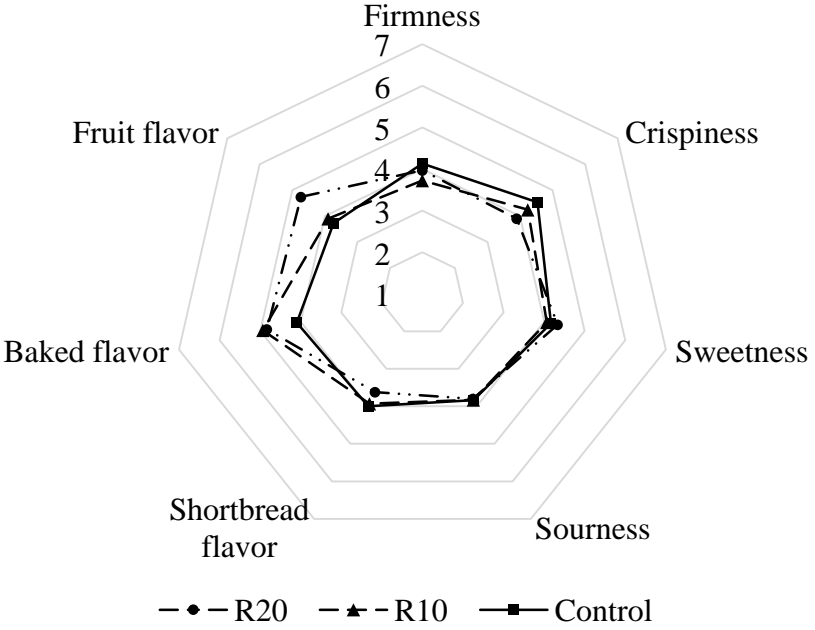


Fig. 1.

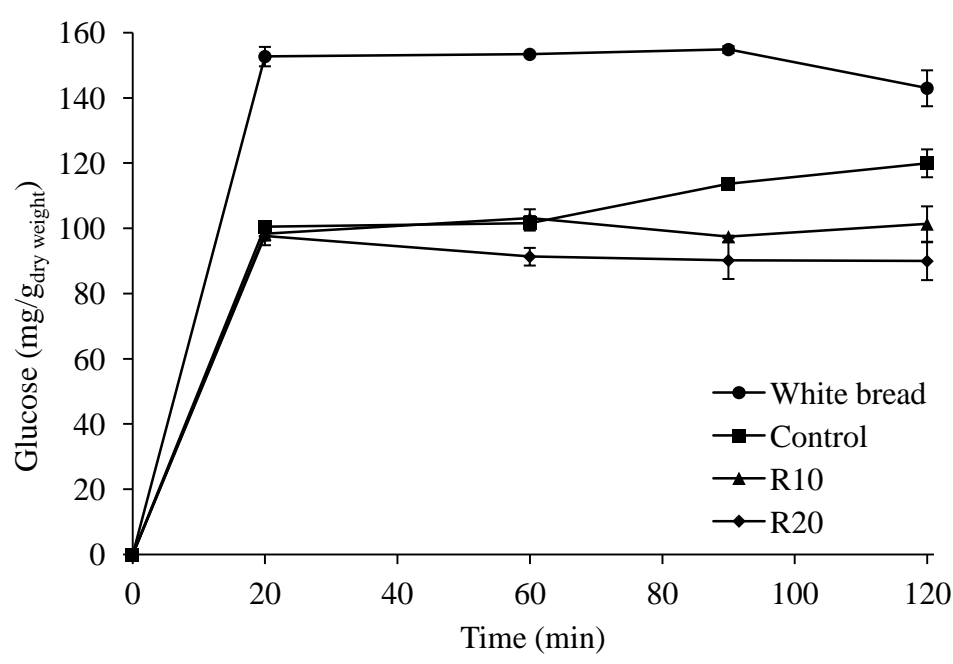


Fig. 2.