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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1522199> since 2017-05-22T08:37:05Z

Published version:

DOI:10.1016/j.lwt.2015.03.113

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This is the accepted version of the following article:

Bertolino M., Belviso S., Dal Bello B., Ghirardello D., Giordano M., Rolle L., Gerbi V., Zeppa G. (2015). Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. *LWT-Food Science and Technology*, 63, 1145–1154 DOI: 10.1002/jsfa.4593.

which has been published in final form at

[<http://onlinelibrary.wiley.com/doi/10.1016/j.lwt.2015.03.113/pdf>]

**INFLUENCE OF THE ADDITION OF DIFFERENT HAZELNUT SKINS ON THE
PHYSICOCHEMICAL, ANTIOXIDANT, POLYPHENOL AND SENSORY PROPERTIES
OF YOGURT**

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Keywords: Hazelnut skin, Yogurt, Antioxidant, Phenolic acid, Shelf-life

Chemical compound studied in this article: 2-2-diphenyl-1-picrylhydrazyl, Free Radical (Pub Chem
CID: 2735032); gallic acid (PubChem CID: 370); protocatechuic acid (PubChem CID: 72);
procyanidin B1 (PubChem CID: 11250133); gallocatechin gallate (PubChem CID: 199472); 3-
coumaric acid (PubChem CID: 637541); 2-coumaric acid (PubChem CID: 637540); rutin (PubChem
CID: 5280805).

Abstract

Skins obtained from three different varieties (Georgia, San Giovanni and Tonda Gentile Trilobata) of
roasted hazelnuts (*Corylus avellana* L.) were used at two different percentages (3% and 6%) in yogurt

production to increase the dietary fibre and polyphenol content. The effects on the physico-chemical characteristics, antioxidant capacity, phenolic compounds, and sugar and organic acid content during 3 weeks of storage at 4 °C were evaluated, and a preference test was performed with consumers at the end of storage.

The amount of skin and the variety used significantly influenced all of the physico-chemical parameters and were associated with consumer preference. Concerning the dietary fibre content, total polyphenol content and antioxidant capacity, all of which affect the functional ability of food products, the highest values obtained were for all of the products contained a hazelnut skin content of 6%. Among the cultivars, the highest values obtained were for yogurt with the Georgia hazelnut skin. Although 6% hazelnut skin yogurts displayed the highest functional ability, a decreased consumer preference was observed; yogurt with 3% San Giovanni and Tonda Gentile Trilobata hazelnut skins had the maximum consumer rating.

41

42 **1. Introduction**

The production of hazelnuts in 2012 was 914.447 *10⁹ kg. Turkey was the world's largest producer and contributed 72% of the total production, followed by Italy (9.3%), the United States (3.3%) and Georgia (2.7%) (FAOSTAT, 2012). Two different by-products are obtained during the transformation of hazelnuts through the post-harvesting processes - shells and hazelnut skin - among these, only the shell has a direct commercial value as a heating source. Hazelnut skin, representing approximately 2.5% of the total kernel weight (Alasalvar et al., 2009), is a rich source of dietary fibre as well as phenolic compounds with antioxidant properties (Del Rio, Calani, Dall'Asta, & Brighenti, 2011). The definition of dietary fibre and its beneficial effects on human health has been considerably debated and related to physiological considerations (EFSA, 2010). Dietary fibre is categorized into two groups according to water solubility: water-soluble dietary fibre (SDF) and water-insoluble dietary fibre (IDF). SDF forms a viscous solution that results in increased viscosity in the intestine, leading to slowed intestinal transit, delayed gastric emptying and slowed glucose and sterol absorption, whereas

55 IDF has a high water-holding capacity that contributes to increased faecal bulk. Currently, an average
56 daily fibre intake of 25 g for adults and 10 g (1-3 years old) to 21 g (17 years old) for children is
57 recommended.

58 Antioxidants are notably important compounds in food science due to their ability to prevent lipid
59 oxidation in foods and to decrease the negative effects of reactive oxygen species on physiological
60 functions in humans. Polyphenols, which are widely distributed in plants, are among the most studied
61 natural antioxidants due to consumer preference for natural products. Currently, a daily polyphenol
62 intake of 1 gram is reported (Scalbert, Manach, Morand, Rémésy & Jiménez, 2005). Recently,
63 hazelnut skin itself or its phenolic extracts have been added to vanilla ice cream, bread or coffee to
64 investigate the effects on the final products in terms of fat replacement, as a source of dietary fibre
65 and as a potential source of antioxidants, respectively.. The application of hazelnut skin to ice cream
66 demonstrated that it could improve product overrunning, but it resulted in greater susceptibility to
67 melting and was not preferred by consumers (Dervisoglu, 2006). The use of hazelnut skin in bread
68 revealed that a concentration of 5% did not considerably affect the rheological properties of the dough
69 or the final product and produced acceptable results from the sensory panel (Anil, 2007). Contini,
70 Baccelloni, Frangipane, Merendino, and Massantini (2012) emphasized that phenolic extracts from
71 hazelnut skins increased the antiradical activity of coffee due to an increase in the total polyphenol
72 content.

73 Therefore, the aim of this work was to evaluate the possibility of using hazelnut skin as a source of
74 dietary fibre and antioxidants in yogurt. The use of hazelnut skin in yogurt could have a dual benefit
75 by employing a food industrial by-product for human nutrition, thereby reducing industrial waste. In
76 addition, it could augment the consumption of fibre and antioxidant compounds in all sectors of the
77 population owing to the popularity of yogurt around the world (61.248×10^9 kg yogurt production -
78 FAOSTAT, 2012).

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80 **2. Materials and Methods**

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2.1. Hazelnut skin (HS) samples

The skins of three different hazelnut (*Corylus avellana* L.) varieties (“Tonda Gentile Trilobata - TGT”, “San Giovanni” cultivars from Italy, and “Georgia” from Georgia) were obtained from the Nocciolo Marchisio S.p.A. (Cortemilia, CN, Italy). The roasting process was conducted under three different conditions (temperature: 155, 150 and 155 °C; time 37, 35, 39 min, respectively). Conventional procedures were applied by the processor in an industrial continuous-working oven, where the skins were separated from the roasted kernels by vigorously rubbing them against themselves, followed by skin removal via vacuum.

2.2. Chemicals

All reagents and solvents were purchased from Sigma-Aldrich (Milano, Italy). All chemicals were reagent-grade, and ultrapure water was produced with a Milli-Q System (Millipore, Milan, Italy).

2.3. HS preparation

HS were collected just after industrial processing and transported to the laboratory in vacuum bags. HS were milled and sieved to obtain a particle fraction of 0.5 mm using an ultra-centrifugal mill Retsch ZM 200 (Retsch GmbH, Haan, Germany). The resulting products were stored at 4 °C.

2.4. Chemical composition of HS and fortified yogurt

The moisture content was determined using a Radwag MAC 210/NH thermo-balance (Radwag, Radom, Poland) at 105 °C. The total protein content (conversion factor 6.25) was obtained according to the Kjeldahl method using a UDK 130A system (Velp Scientifica, Usmate, Italy). The lipid fraction was extracted using a Soxhlet Velp Extraction System SER 148 (Velp Scientifica, Usmate, Italy) for 6 h using *n*-hexane as solvent. The ash content was determined in a muffle furnace according to the AOAC (1990) method. The carbohydrate value was estimated by the difference. Dietary fibre (TDT,

107 SDF and IDF) was measured using the Megazyme Total Dietary analysis kit according to the
108 enzymatic gravimetric method proposed by Lee, Prosky, and Devries, (1992). Compositional
109 analyses of fortified yogurt were run 24 hours after yogurt production. All analyses were performed
110 in triplicate.

111

112 2.5. *Yogurt preparation*

113 A single lot of stirred yogurt was prepared from UHT whole milk (fat 3.6%; protein 3.1% and
114 carbohydrates 4.8%) purchased at the local market. Milk was placed into a vat and allowed to cool at
115 42 °C and was subsequently inoculated with the starter culture YO-MIX 401 (Santamaria, Burago di
116 Molgora, Italy), which is a combination of *Streptococcus thermophilus* and *Lactobacillus delbrückii*
117 subsp. *Bulgaricus*. Incubation was carried out at 42 °C until the pH was 4.8 (approximately 6.5 h).
118 After the desired pH was reached, the fermentation was interrupted by cooling the vat to 20 °C. The
119 coagulum was then broken with a stainless steel skimmer. The HS content of the yogurt was directly
120 adjusted (0, 3 and 6 g were added to obtain 100 g of yogurt designated as the control 0%, 3% and 6%,
121 respectively) in single pots. Yogurt was kept at 4 °C and analysed on days 1, 7, 14 and 21 of storage.

122

123 2.6. *Analysis of the physico-chemical characteristics of yogurt*

124 The pH of the samples was measured with a Crison microph 2002 pH-meter (Crison Strumenti SpA,
125 Carpi, Italy). The titratable acidity was determined by the potentiometric method according to the
126 IDF standard (IDF, 1991) and expressed as the lactic acid %. Yogurt syneresis was determined by the
127 centrifugation method of Celik, Bakırcı, and Şat (2006), with several modifications. Twenty grams
128 of yogurt were centrifuged at $16800 \times g$ for 20 min at 4 °C using a Megafuge 11 R centrifuge (Thermo
129 Fischer Scientific, Waltham, MA, USA). Syneresis was expressed as the volume of separated whey
130 per 100 mL of yogurt. All of the analyses were performed in triplicate.

131

132 2.7. *Microbiological analysis*

133 Microbiological analyses of yogurt were performed to determine the influence of the HS addition on
134 the starter. Streptococci were counted on M-17 agar (Oxoid, Basingstoke, Hampshire, England) and
135 were incubated aerobically at 37 °C for 24 h. Lactobacilli were counted on MRS agar (Lab M Limited,
136 Heywood, Lancashire, United Kingdom) under anaerobic incubation at 37 °C for 48 h. The samples
137 were analysed in duplicate.

138

139 2.8. *Antioxidant capacity of yogurt*

140 2.8.1. *Bioactive compounds extraction*

141 Yogurt extracts were prepared according to McCue and Shetty (2005), with slight modifications.
142 Briefly, each yogurt sample (10 g) was diluted with distilled water (2.5 ml) and centrifuged (16800 ×
143 g, 40 min, 4 °C). The supernatant was harvested and filtered through a 0.45-µm polypropylene
144 membrane filter (VWR, Milan, Italy). Extraction was conducted in triplicate, and extracts were stored
145 at 4 °C in amber glass vials until further analyses.

146

147 2.8.2. *Total phenolic content assay*

148 The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay as reported by
149 Apostolidis, Kwon, and Shetty (2007) after the reaction samples were centrifuged (16800 × g, 10
150 min, 20 °C), and the absorbance of the supernatant was measured at 725 nm with a UV-Visible
151 spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Milan, Italy). The results were expressed as
152 µg gallic acid equivalents (GAE) per gram of sample (calibration curve linearity range: $r = 0.997$).

153

154 2.8.3. *DPPH radical scavenging capacity of yogurt*

155 The free radical scavenging activity (RSA) of the extracts was determined according to the procedure
156 reported by von Gadow, Joubert, and Hansmann (1997) using the stable 2,2-diphenyl-1-
157 picrylhydrazyl radical (DPPH[•]). Briefly, 75 µL of sample extract was added to 3 mL of a 6.1×10^{-5}

158 M DPPH[•] methanol solution and incubated for 1 h at room temperature in the dark. After this time
159 and after a centrifugation step (16800 × g, 10 min, 20 °C), the decrease in absorbance at 515 nm was
160 recorded against methanol as a control; a methanol solution of DPPH[•] was used as a blank. The
161 inhibition percentage (IP) of the DPPH[•] by the antioxidant extracts was calculated according the
162 formula

$$163 \quad IP = [(A_{0min} - A_{60min})/A_{0min}] \times 100$$

164 where A_{0min} is the absorbance of the blank at t = 0 min and A_{60 min} is the absorbance of the samples at
165 60 min. The results were expressed as μM Trolox equivalents (TE) per gram of sample by means of
166 a dose-response curve for Trolox (0-350 μM).

167

168 *2.9.HPLC-DAD Phenolic compound analysis*

169 HPLC-DAD analysis was performed by using a Thermo-Finnigan Spectra-System HPLC system
170 (Thermo-Finnigan, Waltham, USA) equipped with a P2000 binary gradient pump system, a SCM
171 1000 degasser, an AS 100 automatic injector, an UV6000LP DAD and ChromQuest software for data
172 processing. Separation was achieved on a C₁₈ RP Lichrosphere 250 × 4.6 mm, 5-μm (Merck, Milan,
173 Italy) column equipped with a C₁₈ RP Lichrosphere 5-μm guard column (Merck, Milan, Italy). The
174 mobile phase was composed of trifluoroacetic acid/ultrapure water (0.1:99.9, v/v) (A) and methanol
175 (B). The flow rate was 1 mL/min, and the injection volume was 20 μL. The elution program was as
176 follows: 95% A as the initial condition, maintained for 2 minutes; 80% A for 8 min; 25% A for 57
177 min; 0% A for 13 min; and 95% A for 5 min. DAD spectra were recorded in full scan mode over a
178 wavelength range of 200 to 400 nm. Identification was achieved by comparing the retention times
179 and spectra with authentic standards (Fig. 1). Each compound was quantified as mg/Kg sample by
180 means of calibration with external standards: gallic acid, protocatechuic acid, procyanidin B1,
181 gallocatechin gallate, 3-coumaric acid and rutin purchased from Sigma-Aldrich (Milan, Italy) and 2-
182 coumaric acid purchased from Extrasynthese (Genay Cedex, France).

183

184 2.10. *HPLC-UV-RI Organic acids and sugars analysis*

185 The content of organic acids and sugars was determined according to the method of Adhikari, Grün,
186 Mustapha, and Fernando (2002). The HPLC system (Thermo Quest, San Jose, CA) was equipped
187 with a P4000 isocratic pump, a multiple autosampler AS3000 fitted with a 20-μL loop, a UV detector
188 (UV100) set at 210 nm, and a refractive index detector (Spectra System RI-150, Thermo Electro
189 Corporation). The detectors were connected in series. Data were collected using ChromQuest ver. 3.0
190 (Thermo Finningan). The mobile phase was 0.01 N H₂SO₄, and the analyses were performed
191 isocratically at 0.8 mL/min and 65 °C with a 300 × 7.8 mm i.d. cation exchange column (Aminex
192 HPX-87H) equipped with a cation H⁺ microguard cartridge (Bio-Rad Laboratories, Hercules, CA).
193 Identification was achieved by comparison with the retention times of authentic standards: lactose,
194 glucose, galactose, pyruvic acid, lactic acid, malic acid and citric acid purchased from Sigma-Aldrich
195 (Milan, Italy).

196

197 2.11. *Preference test*

198 To assess the sensory acceptability of the yogurts, twenty consumers (40% male and 60% female,
199 aged between 24 and 65 years) were recruited at the Dipartimento di Scienze Agrarie, Forestali e
200 Alimentari of Turin University. Written informed consent was obtained from each subject after the
201 experiments were described.

202 The test was performed inside an air conditioned room with white light at approximately 21 °C.
203 Yogurt samples (10 g) were served blinded in a transparent plastic cup coded with a random three-
204 digit number. Samples were served in a completely randomized order. Consumers were asked to rate
205 their preference for odour, taste, flavour, texture and acceptability. Preference was expressed on a 5-
206 point hedonic scale ranging from “dislike extremely” (1) to “like extremely” (5) (Peryam & Pilgrim,
207 1957). Paper score-sheets were used for data collection.

208

209 2.12. *Data analysis*

210 A one-way analysis of variance (ANOVA) with Duncan's test for mean comparison was used to
211 highlight significant differences among the yogurt samples. All calculations were performed with the
212 STATISTICA software for Windows (Release 7.0; StatSoft Inc., Tulsa, OK, USA).

213

214 **3. Results**

215

216 *3.1. Chemical composition of HS*

217 Table 1 shows the chemical composition of HS. According to the results, total dietary fibre was the
218 major component, amounting to a mean of 55%. A mean of 86% of the fibre was composed of
219 insoluble fibre, with significant differences among the varieties. The lipid content ranged from 109.96
220 $\pm$ 1.68 g/Kg for Georgia samples to 187.55 g/kg for San Giovanni samples. The values were similar
221 to those reported by Anil (2007) as well as Turhan, Sagir and Ustun (2005) for other varieties.

222 The TPC values assessed in hazelnut skin extracts significantly characterized the varieties. The
223 highest values were measured in the Georgia skin extracts, and the lowest values were found in the
224 San Giovanni skin extracts; nevertheless, there were no significant difference for TGT.

225 The results of the RSA assays revealed a different trend - the RSA had the highest values reported
226 for the Georgia sample, followed by San Giovanni and TGT.

227 The use of different extraction methods and/or different data expression methods prevented the
228 comparison of our TPC and RSA results with those published by other authors.

229

230 *3.2. Chemical composition of yogurt*

231 Table 2 shows the chemical composition of the yogurts. The overall composition of the yogurts was
232 significantly different ($p < 0.001$). In particular, yogurt with HS was associated with a mean decreased
233 humidity of 2.9% and 6.0% for the 3% and 6% HS treatments, respectively, but the differences
234 observed among the different varieties were not statistically significant. These results are in

235 accordance with those obtained by García-Pérez, Lario, Fernández-López, Sayas, Pérez-Alvarez and
236 Sendra, (2005) who added citrus fibre to yogurt.

237 The addition of hazelnut skin was also associated with a decrease in protein, lipids, carbohydrates
238 and ash.

239 As expected, the addition of HS was associated with the dietary fibre level in the final product.
240 Furthermore, the dietary fibre content increased with the mean values of 94.65 ± 28.19 g/Kg and
241 165.19 ± 4.91 g/Kg in yogurt with 3% and 6% HS, respectively. Among the varieties, the highest
242 concentration was observed in yogurt fortified with Georgia, but no differences were observed
243 between San Giovanni and TGT cultivar HS. Similar data for total dietary fibre showing an increase
244 in yogurt due to added fibre were obtained by do Espírito Santo et al. (2012) and Tseng and Zhao
245 (2013). The results showed an increase in total dietary fibre for all of the matrices used, and as
246 expected, the fibre content in the final product increased with an increasing percentage of the
247 ingredients studied.

248 For the soluble and insoluble dietary fibre content, the highest concentrations were observed for both
249 yogurt samples with different percentages of Georgia HS.

250

251 *3.3. Physico-chemical characteristics of yogurt*

252 The pH, titratable acidity and syneresis of yogurts are reported in Table 3. The pH of all products
253 dropped slightly ($p < 0.001$) during storage independent of the HS addition. Among the products, the
254 6% Georgia fortified yogurt showed the lowest pH reduction during storage (0.19 unit), while the 6%
255 TGT fortified yogurt had the highest pH reduction (0.28 unit). The mean reduction was 0.24 units
256 and was lower than that reported in other studies in which different types of by-products were added
257 to yogurt (García-Pérez et al., 2005; Tseng & Zhao, 2013), but was slightly higher than that found by
258 others when different pure dietary fibres were added (Dello Staffolo, Bertola, Martino, & Bevilacqua,
259 2004). Moreover, a significant difference ($p < 0.001$) between the types and percentages of HS used

260 was present between the first and the second week of storage, but at the end (3 weeks), only the yogurt
261 with 3% TGT HS was different from the others.

262 For syneresis, the addition of HS was associated with increased whey separation compared to the
263 control at all storage times ($p < 0.001$) due to the rearrangement of the gel matrix being associated with
264 the high content of insoluble dietary fibre in the HS, as previously observed by García-Pérez et al.
265 (2005) and Tseng and Zhao (2013). Among the two percentages of HS, regardless of the varietal used,
266 a difference with a mean value of 9% was observed. Only the Georgia 6% and the TGT 3% fortified
267 yogurts showed significantly different values during storage.

268 For titratable acidity, the incorporation of HS in the yogurts was associated with statistically
269 significant differences between the products for all storage periods. The 6% TGT fortified yogurt
270 showed the highest increase in acidity during storage (0.81 unit), and the 3% TGT fortified yogurt
271 had the lowest (0.06 unit).

272

273 3.4. Microbiological analysis

274 As shown in Fig. 2, the addition of HS to yogurt did not affect the survival of the starter strains; after
275 21 days of storage, both strains had a concentration higher than that required by the Codex
276 Alimentarius (10^7 CFU/g). In particular, in the fortified yogurts, *S. thermophilus* reached a mean
277 concentration of $8.67 \log_{10}$ CFU/mL, which was higher than the control ($8.38 \log_{10}$ CFU/mL). *L.*
278 *bulgaricus* was present at a mean concentration of $7.73 \log_{10}$ CFU/mL in fortified yogurt compared
279 to $7.64 \log_{10}$ CFU/mL in the control.

280 The viability of *S. thermophilus* decreased during refrigerated storage (Fig. 2 A & B), but by less than
281 1 CFU/mL. TGT HS was associated with the highest reduction, while the lowest reduction was
282 observed for Georgia 3% and San Giovanni 6%.

283 The viability of *L. bulgaricus* decreased during refrigerated storage (Fig. 2 C & D), but was less than
284 1 CFU/mL and less than that observed for the *S. thermophilus*, except for TGT 3% and 6%.

285 As observed for *S. thermophilus*, TGT HS was associated with the highest reduction in *L. bulgaricus*;
286 the lowest was observed for Georgia 3% and San Giovanni 6%.

287

288 3.5. Total phenolic content and antioxidant capacity of yogurt

289 Table 4 shows the total phenolic content and the free radical scavenging activity of the yogurts.
290 During the storage period, the TPC observed for the control yogurt dropped significantly ($p < 0.001$)
291 due to bacterial metabolic activity associated with a reduction/modification of the non-phenolic
292 compound that reacted with the Folin-Ciocalteu reagent (Everette, Bryant, Green, Abbey, Wangila,
293 & Walker, 2010).

294 Fortified yogurts showed statistically significant differences at each storage time ($p < 0.001$), and
295 among the samples, a statistically significant increase was observed during storage. This increase is
296 in accordance with the results obtained by Zainoldin and Baba (2009) for yogurt fortified with dragon
297 fruit, but contrasts with results obtained by other researchers for yogurt fortified with grape pomace
298 (Tseng & Zhao, 2013), different grape berries and callus extract (Karaaslan, Ozden, Vardin, &
299 Turkoglu, 2011) and *Berberis boliviana* anthocyanins (Wallace & Giusti, 2008). Addition of 3% HS
300 increased the total phenolic compound concentrations by 36.5, 29.4, and 27.4% for TGT, Georgia
301 and San Giovanni, respectively. Addition of 6% HS increased the concentration by 30.9, 26.7 and
302 26.3% for TGT, San Giovanni and Georgia, respectively.

303 During storage, the RSA of control samples significantly increased ($p < 0.005$), possibly because
304 bacterial metabolic activity caused a breakdown of macromolecules that could react with the DPPH[•]
305 reagent.

306 Fortified yogurts showed storage trends similar to those observed for TPC. In storage, the addition of
307 3% HS showed an increased RSA of 41.6, 52.4, and 69.4% for San Giovanni, Georgia, and TGT,
308 respectively, and the addition of 6% HS showed an increased RSA of 30.6, 39.5 and 73.6% for
309 Georgia, San Giovanni and TGT, respectively.

310

311 3.6. *Phenolic compounds profile*

312 The most abundant phenolic compound was procyanidin B1, followed by protocatechuic acid, gallic
313 acid, gallocatechin gallate, rutin and 3-coumaric acid (Table 5). 2-coumaric was detected only in the
314 Georgia HS samples, but was not quantified. None of the phenolic compounds found in the fortified
315 yogurts were detected in the control samples.

316 Yogurts with 6% HS showed a higher concentration of phenolic compounds (except for coumaric
317 acid and gallocatechin gallate) than those with 3% HS. The compounds detected were unchanged
318 during storage in almost all samples. An increase in gallic acid (in the San Giovanni and TGT cultivars
319 at both percentages), protocatechuic acid (in the TGT cultivar at 6% HS) and rutin (in San Giovanni
320 cultivar at 3% HS and TGT cultivar at 6% HS) during storage could be attributed to an increase in
321 compound solubilization into the yogurt, probably due to the decrease of pH during storage (Stalikas,
322 2007), followed by major extraction in water. Statistically significant variations in procyanidin B1
323 and protocatechuic acid were found among the HS varieties at each sampling time. The lowest
324 concentrations were detected in San Giovanni HS, whereas the highest were observed in Georgia HS.
325 Statistically significant differences for gallic acid were found among the HS varieties at each storage
326 time. The lowest concentration was detected in Georgia HS, while the highest was observed in TGT
327 HS.

328 The highest rutin concentrations were detected at days 7 and 21 in yogurts with 6% San Giovanni
329 HS, while the lowest were found in yogurts made with 3% San Giovanni and TGT HS (< LOQ).

330

331 3.7. *Organic acid and sugar profiles*

332 Table 6 shows the sugar and organic acids concentration of the yogurts. No statistically significant
333 differences in the lactose concentration were observed among the samples at any sampling time. The
334 3% HS was associated with higher lactose degradation, as indicated by a higher bacterial count at
335 each storage time (Fig. 2). Statistically significant differences for the control, Georgia 3% and 6%

336 and San Giovanni 3% samples were observed, in which lactose degradation was 6.7, 9.0, 7.2 and
337 6.9%, respectively.

338 Statistically significant differences for glucose and galactose were observed for both the varieties at
339 each storage time and for each sample during storage, except in the San Giovanni 6% sample. In
340 particular, the control samples evidenced an increase in the galactose concentration of 11.4% during
341 the storage period, while in the other samples, the galactose concentration decreased with a mean
342 percentage of 22.2% and 20.0% for 3% and 6% HS, respectively. The highest degradation was
343 observed in TGT yogurt samples and the lowest in the San Giovanni samples.

344 An increase in the glucose concentration was observed in the control and the 3% and 6% San
345 Giovanni HS samples during the storage period, amounting to 159.5, 6.4 and 23.3%, respectively. In
346 the other samples, a decrease occurred that amounted to a mean percentage of 43.5 and 120.0% for
347 the 3% and 6% HS samples, respectively. The highest degradation was observed in the Georgia
348 samples and the lowest in the TGT samples.

349 For citric acids, no significant differences were observed, indicating that starter bacteria do not utilize
350 citrate, possibly because they are a Cit⁻ strain as previously mentioned by Adhikari, Grün, Mustapha,
351 and Fernando, (2002).

352 During the storage time, the concentration of pyruvic acid increased. However, this increase was not
353 constant during storage, possibly because it is an intermediary product of bacterial metabolism and
354 its concentration normally fluctuates during storage as a function of bacterial activity. Lactic acid
355 showed a statistically significant increase during storage. Regardless of variety, the mean increase
356 observed was 10.0% and 14.4% for 3% and 6% HS, respectively. Among the varieties, the highest
357 increase was observed in 3% San Giovanni and 6% TGT.

358 Malic acid was not detected in the control samples because it is an acid derived from HS. Statistical
359 differences were observed between the varieties and the HS levels. As expected, an increased
360 concentration of HS in yogurt was associated with a higher concentration of malic acid. Among the

361 varieties, the highest concentration was detected in the San Giovanni samples and the lowest in the
362 Georgia samples.

363

364 *3.8.Sensory analysis*

365 Fig. 3 shows the consumer acceptance of yogurts. The fortification of yogurt with the HS was
366 associated with a statistically significant effect ($p < 0.001$) on all of the parameters analysed except for
367 odour. The control sample was acceptable. For all of the parameters analysed, the control scored the
368 central value of the scale (3 = neither like nor dislike). Consumers preferred 3% HS to 6% HS. This
369 preference can possibly be explained because HS was associated with increased liquidity of the
370 samples (see syneresis value Table 3).

371 For the 3% HS samples, the San Giovanni and TGT cultivar scores always achieved the central scale
372 value for the 6% HS samples. The San Giovanni cultivar had the highest score for all of the
373 parameters, but only the odour achieved the central scale value.

374 In general, the observed low acceptance of the fortified yogurts was not surprising because similar
375 results have been previously observed in other studies in which different types of fibre were used.
376 Tseng and Zhao (2013) observed that the use of fibre was associated with a lower value for flavour,
377 texture and consistency. Hashim, Khalil, and Afifi (2009) reported that the addition of fibre was
378 associated with lower ratings for firmness, smoothness and flavour. Sendra, Fayos, Lario, Fernández-
379 Lopez, Saras-Barberá, and Pérez-Alvarez, (2008) observed that the addition of fibre was associated
380 with reduced creaminess and decreased overall acceptability.

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382 **4. Conclusions**

383 This study demonstrated that HS can be utilized as an alternative source of antioxidants and dietary
384 fibre to fortify yogurt. The addition of HS and the percentage added contributes to the dietary fibre
385 content and antioxidant capacity of the final product, as well as to all of the other physico-chemical
386 parameters considered. During storage, the antioxidant capacity of fortified products was increased

387 with respect to the control, and no modification of the phenolic compounds was observed. Thus, it is
388 possible to conclude that the functional ability of these products is stable or increased during storage.
389 The yogurt with the 3% San Giovanni and TGT HS achieved the highest score from the consumers.
390 By consuming 100 g of products fortified with 3% of these two varieties, consumers obtain the 37%
391 dietary fibre intake recommended by the European Union and the respective 0.4 and 0.6 %,
392 polyphenol intake reported by the scientific literature.

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394 **Acknowledgments**

395 This study was funded by the project “Ricerca & innovazione per il Miglioramento della Sostenibilità
396 della Filiera Agro-alimentare ECOFOOD – Finanziamento PSR-FEASR – cofinanziamento dall’UE,
397 dal Ministero dell’Economia e delle Finanze e dalla Regione Piemonte”. Paper n° 19.

398 The authors also thank Nocciolificio Marchisio for generously providing the hazelnut skin samples.

399

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Fig. 1: HPLC-DAD chromatograms of yogurts added with 6% of hazelnut skin at 7th days of storage. a) Georgia; b) Tonda Gentile Trilobata; c) San Giovanni hazelnut varieties. 1 = gallic acid; 2 = protocatechuic acid; 3 = procyanidin B1; 4 = gallocatechingallate; 5 = 3-coumaric acid; 6 = 2-coumaric acid; 7 = rutin identified compounds.

Fig. 2: *Streptococcus thermophilus* (A) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (B) counts in fortified yogurts with 0% (control) and 3% of hazelnut skins during 3 weeks of storage at 4 °C. *Streptococcus thermophilus* (C) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (D) counts in fortified yogurts with 0% (control) and 6% of hazelnut skins during 3 weeks of storage at 4 °C.

□ 0% (Control) and ■ 3% Georgia, ■ 6% Georgia, ■ 3% San Giovanni, ■ 6% San Giovanni, ■ 3% Tonda Gentile Trilobata, □ 6% Tonda Gentile Trilobata hazelnut varieties fortification.

Fig. 3: Linking of odour, texture, taste, flavour and acceptance expressed by 20 consumers for the control and fortified yogurts.

□ 0% (Control) and ■ 3% Georgia, ■ 6% Georgia, ■ 3% San Giovanni, ■ 6% San Giovanni, ■ 3% Tonda Gentile Trilobata, □ 6% Tonda Gentile Trilobata hazelnut varieties fortification. Histograms with different letters were significantly different at $p < 0.05$.

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Table 1: Chemical composition, total phenolic content (TPC) and DPPH radical scavenging activity (RSA) of hazelnut skin (HS)^W.

Composition	Hazelnut varieties			Significance
	Georgia	San Giovanni	TGT	
Humidity (g/Kg)	43.13 ± 0.15 ^a	60.20 ± 0.16 ^b	47.14 ± 0.15 ^a	**
Protein (g/kg dw)	93.90 ± 1.36	91.67 ± 0.83	88.46 ± 1.14	ns
Total lipid (g/kg dw)	109.86 ± 1.68 ^a	187.55 ± 1.45 ^c	171.95 ± 1.58 ^b	***
Carbohydrates (g/kg dw)	174.57 ± 34.28 ^a	183.33 ± 1.00 ^b	190.98 ± 2.10 ^b	***
Ash (g/kg dw)	21.56 ± 0.52 ^a	25.96 ± 0.53 ^b	24.66 ± 0.64 ^b	***
Total dietary fibre (g/kg dw)	568.44 ± 5.53 ^b	543.26 ± 14.57 ^a	542.85 ± 29.70 ^a	**
Soluble dietary fibre (g/kg dw)	87.57 ± 1.79 ^c	54.26 ± 4.60 ^b	45.12 ± 2.10 ^a	***
Insoluble dietary fibre (g/kg dw)	499.30 ± 3.48 ^b	464.54 ± 4.10 ^a	466.60 ± 4.96 ^{a,b}	***
TPC (GAE µg/g dw)	195.76 ± 4.93 ^b	153.29 ± 5.95 ^a	160.05 ± 2.84 ^a	***
RSA (TE µM/g dw)	1004.98 ± 21.23 ^b	984.66 ± 16.78 ^b	854.47 ± 21.59 ^a	***

^W Data are expressed as mean ± SD (n = 3). Means followed by different letters were significantly different at $p < 0.05$.

Abbreviations: TGT = Tonda Gentile Trilobata, dw = dry weight; GAE = gallic acid equivalent and TE = trolox equivalent.

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant.

483 Table 2: Chemical composition of yogurts with 0% (control), 3% and 6% content in hazelnut skin (HS) ^w.

Composition				Hazelnut varieties											
	Geogia					San Giovanni					TGT				
	0% (Control)		3% HS		6% HS		3% HS		6% HS		3% HS		6% HS	Significance	
Humidity (g/Kg)	858.17 ± 0.76	c	833.72 ± 0.74	b	809.26 ± 0.71	a	834.23 ± 0.74	b	810.29 ± 0.71	a	833.84 ± 0.74	b	809.51 ± 0.72	a	***
Protein (g/kg dw)	261.00 ± 0.57	d	232.24 ± 0.51	c	210.78 ± 0.52	b	232.29 ± 0.40	c	210.75 ± 0.44	b	231.41 ± 0.53	c	209.30 ± 0.53	a	***
Total lipid (g/kg dw)	303.09 ± 23.84	c	269.75 ± 19.58	a,b,c	244.95 ± 16.38	a	283.46 ± 19.57	b,c	268.78 ± 16.35	a,b,c	280.55 ± 19.57	a,b,c	263.76 ± 16.37	a,b	***
Carbohydrates (g/kg dw)	382.90 ± 18.99	b	346.94 ± 17.75	a	320.21 ± 18.19	a	348.95 ± 15.79	a	323.58 ± 13.45	a	349.88 ± 15.84	a	325.30 ± 13.53	a	***
Ash (g/kg dw)	59.70 ± 1.93	c	53.11 ± 1.49	b	48.22 ± 1.17	a	53.96 ± 1.48	b	49.67 ± 1.15	a	53.67 ± 1.47	b	49.18 ± 1.14	a	***
Total dietary fibre (g/kg dw)	- ± -	a	98.14 ± 0.77	b	171.13 ± 1.37	d	92.41 ± 2.75	b	161.50 ± 4.89	c	93.39 ± 4.81	b	162.93 ± 8.47	c	***
Soluble dietary fibre (g/kg dw)	- ± -	a	15.12 ± 0.30	e	26.36 ± 0.52	f	9.23 ± 0.93	c	16.13 ± 1.63	e	7.76 ± 0.35	b	13.54 ± 0.61	d	***
Insoluble dietary fibre (g/kg dw)	- ± -	a	86.21 ± 0.52	c	150.31 ± 0.90	f	79.02 ± 0.70	b	138.10 ± 1.26	d	80.28 ± 0.70	b	140.06 ± 1.25	e	***

^wData are expressed as mean ± SD (n = 3). Means followed by different letters were significantly different at *p* < 0.05.

Abbreviations: TGT = Tonda Gentile Trilobata, dw = dry weight.

Significance: * *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001.

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Table 3: pH, acidity (express as lactic acid %) and syneresis (express as whey %) of yogurt during 3 week of storage at 4 °C^W.

				Storage period (days)																			
Parameter	Hazelnut varieties	HS %		1					7					14					21				Significance
pH	Control	0	A	4.46 ± 0.02	a		B	4.38 ± 0.01	b		A	4.29 ± 0.00	c		B	4.24 ± 0.01	d	***					
	Geogia	3	A	4.47 ± 0.01	d		A	4.37 ± 0.00	c		A	4.29 ± 0.01	b		B	4.24 ± 0.00	a	***					
		6	A	4.46 ± 0.02	c		C	4.43 ± 0.01	c		C	4.32 ± 0.01	b		B	4.27 ± 0.01	a	***					
	San Giovanni	3	A,B	4.48 ± 0.01	d		A	4.37 ± 0.01	c		A,B	4.30 ± 0.01	b		B	4.25 ± 0.01	a	***					
		6	B	4.52 ± 0.03	c		C	4.43 ± 0.01	b		B	4.29 ± 0.01	a		B	4.26 ± 0.03	a	***					
	TGT	3	A,B	4.48 ± 0.02	c		A	4.36 ± 0.00	b		C	4.33 ± 0.00	b		A	4.21 ± 0.01	a	***					
		6	B	4.52 ± 0.03	c		D	4.45 ± 0.00	b		A	4.28 ± 0.01	a		B	4.24 ± 0.02	a	***					
Significance			*				***				***				*								
Acidity	Control	0	A	0.98 ± 0.03	a		A	1.18 ± 0.03	a,b		B,C	1.40 ± 0.15	b		A,B	1.46 ± 0.20	b	***					
	Geogia	3	B	1.07 ± 0.05			A	1.29 ± 0.08			A,B	1.24 ± 0.17			A,B	1.49 ± 0.09		ns					
		6	C	1.14 ± 0.02			A	1.31 ± 0.10			B,C	1.41 ± 0.19			A,B	1.54 ± 0.06		ns					
	San Giovanni	3	C	1.17 ± 0.00	a		A	1.17 ± 0.00	a		B,C	1.54 ± 0.15	b		A,B,C	1.68 ± 0.22	b	*					
		6	C	1.14 ± 0.02			B	1.68 ± 0.43			C	1.69 ± 0.28			B,C	1.76 ± 0.01		ns					
	TGT	3	C,D	1.20 ± 0.05			A	1.17 ± 0.21			A	0.99 ± 0.25			A	1.26 ± 0.51		ns					
		6	D	1.25 ± 0.05	a		A	1.35 ± 0.17	a,b		B,C	1.42 ± 0.21	a,b		C	2.06 ± 0.02	b	*					
Significance			***				*				*				*								
Syneresis	Control	0	A	35.34 ± 0.10	b		A	32.98 ± 0.58	a		A	31.76 ± 0.95	a		A	32.32 ± 0.10	a	*					
	Geogia	3	B	40.52 ± 0.26			B	40.77 ± 1.30			B	40.41 ± 0.25			B	41.58 ± 0.60		ns					
		6	D	46.73 ± 0.11	a		D	51.75 ± 0.18	c		C,D	49.93 ± 0.03	b		D	52.94 ± 0.08	d	***					
	San Giovanni	3	B	40.76 ± 0.04			B	41.66 ± 0.43			B	40.72 ± 0.95			B	41.37 ± 0.31		ns					
		6	E	48.24 ± 0.76			D	51.83 ± 0.49			C	48.55 ± 0.81			C	50.18 ± 1.37		ns					
	TGT	3	C	43.79 ± 0.83	b		B	40.87 ± 0.07	a		B	39.87 ± 0.23	a		B	40.94 ± 0.94	a	*					
		6	F	51.75 ± 0.18			C	50.30 ± 0.15			D	50.21 ± 0.90			C	51.09 ± 0.41		ns					
Significance			***				***				***				***								

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^W Data are expressed as mean ± SD (n = 3).

Abbreviations: HS % = hazelnut skin content (%), TGT = Tonda Gentile Trilobata.

Means followed by different lowercase letters in same row within each concentration were significantly different at $p < 0.05$; means followed by different capital letters in same column within each storage time were significantly different at $p < 0.05$.

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant.

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Table 4: Total phenolic content (TPC) and DPPH radical scavenging activity (RSA) of yogurt during 3 week of storage at 4 °C^W.

				Storage period (days)															
Parameter	Hazenul varietals	HS %		1				7				14				21			Significance
TPC (GAE µg/g dry matter)	Control	0	A	8.06 ± 0.28	b,c		A	7.82 ± 0.02	b		A	8.33 ± 0.07	c		A	7.23 ± 0.15	a	***	
	Georgia	3	B	10.64 ± 0.61	a		B	11.51 ± 0.35	a		B	13.65 ± 0.10	b		B	13.77 ± 0.21	b	***	
		6	C	15.38 ± 1.36	a		C	17.27 ± 1.38	a,b		E	20.89 ± 0.44	c		C	19.43 ± 1.84	b,c	**	
	San Giovanni	3	B	10.30 ± 0.12	a		B	10.72 ± 0.59	a		B	12.71 ± 0.15	b		B	13.12 ± 0.37	b	***	
		6	C	14.10 ± 0.96	a		C	16.48 ± 1.10	b		C	17.07 ± 0.55	b		C	17.86 ± 0.80	b	**	
	TGT	3	B	10.67 ± 0.03	a		B	11.49 ± 0.52	a,b		B	13.56 ± 1.90	b,c		B	14.56 ± 0.16	c	**	
		6	C	14.12 ± 0.47	a		C	16.42 ± 0.51	b		D	18.97 ± 0.28	c		C	18.48 ± 0.25	c	***	
	Significance			***				***				***			***				
RSA (TE µM/g dry matter)	Control	0	A	9.73 ± 0.41	a		A	8.89 ± 0.32	a		A	10,00 ± 0.18	a		A	12.02 ± 1.09	b	**	
	Georgia	3	B	19.50 ± 0.78	a		B	20.15 ± 0.33	a		B	24.67 ± 0.51	a,b		B,C	29.71 ± 3.97	b	***	
		6	C,D	29.40 ± 2.75	a		C	31.80 ± 2.22	a,b		F	39.16 ± 1.17	c		D	38.41 ± 3.76	b,c	**	
	San Giovanni	3	B	17.84 ± 1.20	a		B	18.95 ± 0.97	a		B	23.22 ± 0.10	b		B	25.27 ± 1.66	b	***	
		6	D	25.44 ± 2.28	a		C	29.49 ± 2.33	a,b		D	31.71 ± 1.28	b,c		C,D	35.49 ± 1.08	c	***	
	TGT	3	B	20.01 ± 0.14	a		B	21.71 ± 0.91	a		C	28.35 ± 0.61	b		C,D	33.89 ± 2.30	c	***	
		6	C	27.24 ± 1.85	a		C	31.26 ± 0.92	a,b		E	35.48 ± 0.45	b		E	47.29 ± 3.00	c	***	
	Significance			***				***				***			***				

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^W Data are expressed as mean ± SD (n = 3).

Abbreviations: HS % = hazelnut skin content (%), TGT = Tonda Gentile Trilobata, GAE = Gallic acid equivalent, TE = Trolox equivalent.

Means followed by different lowercase letters in same row within each concentration were significantly different at $p < 0.05$; means followed by different capital letters in same column within each storage time were significantly different at $p < 0.05$.

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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Table 5: Phenolic compound concentration (mg/kg) of yogurt during 3 week of storage at 4 °C^W.

				Storage period (days)												
Parameter	Hazelnut varieties	HS %	1			7			14			21			Significance	
Gallic acid	Georgia	3	A	4.21 ± 0.91		A	5.89 ± 0.31		A	5.89 ± 0.10		A	7.22 ± 1.71		ns	
		6	B,C	10.62 ± 2.01		B	14.02 ± 0.81		B	14.61 ± 0.11		C	15.19 ± 0.91		ns	
	San Giovanni	3	A,B	6.11 ± 0.32	a	A	7.41 ± 0.50	a,b	A	7.10 ± 0.42	a,b	A,B	8.32 ± 0.21	b	*	
		6	B	9.51 ± 0.41	a	C	17.42 ± 1.40	a,b	B	12.61 ± 2.91	a,b	C	16.71 ± 1.61	b	*	
	TGT	3	A,B	8.33 ± 1.01	a	B	10.71 ± 0.92	a,b	B	12.02 ± 0.12	a,b	B,C	13.14 ± 1.60	b	*	
		6	C	15.53 ± 1.71	a	D	22.53 ± 0.60	a	C	20.81 ± 0.22	a	D	26.71 ± 1.60	b	**	
Significance		**			***			***			***					
Protocatechuic acid	Georgia	3	B	15.21 ± 1.20		B	18.71 ± 0.70		C	20.11 ± 0.40		B	23.31 ± 4.41		ns	
		6	C	30.71 ± 5.81		C	38.82 ± 2.92		D	42.89 ± 0.60		C	43.12 ± 0.70		ns	
	San Giovanni	3	A	4.61 ± 0.22		A	5.61 ± 0.22		A	5.73 ± 0.60		A	6.60 ± 1.10		ns	
		6	A,B	8.51 ± 0.00		A	10.91 ± 0.60		B	11.04 ± 2.01		A	12.52 ± 0.91		ns	
	TGT	3	A,B	9.50 ± 1.80		A	11.42 ± 1.91		B	14.51 ± 0.10		A	14.44 ± 1.71		ns	
		6	B	15.41 ± 0.10	a	B	22.73 ± 0.40	b	C	22.52 ± 0.61	b	B	28.01 ± 1.61	c	**	
Significance		**			***			***			***					
Procyanidin B1	Georgia	3	A,B	40.31 ± 4.70		C	47.71 ± 2.21		C	45.71 ± 0.30		B	47.74 ± 9.83		ns	
		6	B	63.82 ± 17.71		D	70.10 ± 5.01		D	70.20 ± 1.80		C	66.72 ± 2.01		ns	
	San Giovanni	3	A	17.11 ± 0.51		A	19.54 ± 1.32		A	16.83 ± 0.61		A	18.33 ± 1.21		ns	
		6	A	25.11 ± 4.12		B	32.12 ± 1.81		A,B	25.31 ± 1.50		A	26.01 ± 1.61		ns	
	TGT	3	A	30.90 ± 2.81		B	33.33 ± 3.72		B,C	35.04 ± 0.00		A	28.04 ± 1.93		ns	
		6	A,B	44.01 ± 2.60		C,D	58.50 ± 2.50		C	46.91 ± 10.20		C	66.32 ± 1.80		ns	
Significance		**			***			***			***					
Gallocatechingallate	Georgia	3		4.10 ± 0.30		A	3.93 ± 0.11			3.71 ± 0.00			3.51 ± 0.00		ns	
		6		4.72 ± 0.11		A,B	4.50 ± 0.00			4.42 ± 0.71			4.02 ± 0.00		ns	
	San Giovanni	3		4.73 ± 0.00		B	4.84 ± 0.23			4.54 ± 0.21			3.84 ± 0.52		ns	
		6		5.01 ± 0.42		B	5.02 ± 0.00			4.51 ± 0.40			4.11 ± 0.00		ns	
	TGT	3		4.83 ± 0.21		B	4.82 ± 0.11			5.63 ± 1.40			4.22 ± 0.31		ns	
		6		4.89 ± 0.21		B	5.01 ± 0.30			4.52 ± 0.21			4.62 ± 0.31		ns	
Significance		NS			**			NS			NS					
3-Coumaric acid	Georgia	3		0.10 ± 0.00			1.90 ± 0.00			0.17 ± 0.00			0.19 ± 0.00		ns	
		6		0.10 ± 0.00			1.90 ± 0.00			0.18 ± 0.00			0.10 ± 0.00		ns	
	San Giovanni	3		0.19 ± 0.00			1.80 ± 0.00			0.19 ± 0.00			0.17 ± 0.00		ns	
		6		0.22 ± 0.00			3.00 ± 0.00			0.10 ± 0.00			0.22 ± 0.11		ns	
	TGT	3		0.10 ± 0.00			2.00 ± 0.00			0.20 ± 0.00			0.29 ± 0.00		ns	
		6		0.21 ± 0.00			1.00 ± 0.00			0.1 ± 0.00			0.1 ± 0.00		ns	
Significance		NS			NS			NS			NS					
2-Coumaric acid	Georgia	3		< LOQ			< LOQ			< LOQ			< LOQ			
		6		< LOQ			< LOQ			< LOQ			< LOQ			
	San Giovanni	3		ND			ND			ND			< LOQ			
		6		< LOQ			ND			ND			ND			
	TGT	3		ND			ND			ND			ND			
		6		ND			ND			ND			ND			
Significance																
Rutin	Georgia	3	A	0.10 ± 0.00		A	0.10 ± 0.00		A,B	0.29 ± 0.00		A	0.39 ± 0.00		ns	
		6	B	0.80 ± 0.10		B,C	0.89 ± 0.00		B	0.71 ± 0.10		A,B	0.61 ± 0.10		ns	
	San Giovanni	3		< LOQ		A	0.10 ± 0.00	a	A	0.10 ± 0.00	a	A	0.32 ± 0.00	b	*	
		6	A,B	0.51 ± 0.20		C	1.22 ± 0.22		A,B	0.59 ± 0.30		C	1.21 ± 0.20		ns	
	TGT	3		< LOQ		A	0.10 ± 0.00		A	0.11 ± 0.00		A	0.31 ± 0.00		ns	
		6	A,B	0.31 ± 0.10	a	B	0.51 ± 0.11	a	A,B	0.51 ± 0.10	a	B,C	1.11 ± 0.20	b	*	
Significance		**			***			*			**					

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^W Data are expressed as mean ± SD (n = 3).

Abbreviations: HS % = hazelnut skin content (%), TGT = Tonda Gentile Trilobata, LOQ = limit of quantification.

Means followed by different lowercase letters in same row within each concentration were significantly different at

$p < 0.05$; means followed by different capital letters in same column within each storage time were significantly different at $p < 0.05$.

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns or NS = not significant.

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Table 6: Sugar and organic acid concentrations (g/kg) of yogurt during 3 week of storage at 4 °C^W.

Parameter	Hazelnut varieties	HS %	Storage period (days)				Significance
			1	7	14	21	
Lactose	Control	0	48.9 ± 0.04 b	47.05 ± 0.19 a	46.24 ± 0.39 a	45.83 ± 0.89 a	*
	Georgia	3	48.02 ± 0.27 b	47.62 ± 2.00 b	45.17 ± 0.48 a,b	44.05 ± 0.11 a	*
		6	47.28 ± 0.73 b	45.76 ± 0.05 a,b	44.70 ± 0.32 a	44.11 ± 1.12 a	*
	San Giovanni	3	47.90 ± 0.30 c	46.32 ± 0.37 b	45.12 ± 0.33 a	44.80 ± 0.28 a	**
		6	46.29 ± 1.05	46.14 ± 0.73	44.70 ± 0.24	44.91 ± 2.48	ns
	TGT	3	49.52 ± 2.69	46.81 ± 1.10	45.44 ± 0.35	44.77 ± 0.07	ns
		6	46.89 ± 0.10	46.37 ± 0.67	45.32 ± 0.18	46.29 ± 1.59	ns
Significance		NS		NS	NS	NS	
Glucose	Control	0	A,B,C 0.37 ± 0.04 a	B 0.37 ± 0.04 b	C 0.92 ± 0.00 c	B 0.96 ± 0.00 c	***
	Georgia	3	A,B 0.35 ± 0.05 a	B 0.69 ± 0.04 b	B,C 0.81 ± 0.03 b	A 0.23 ± 0.06 a	***
		6	B,C 0.40 ± 0.04 b	C 0.87 ± 0.00 d	A 0.49 ± 0.01 c	A 0.17 ± 0.02 a	***
	San Giovanni	3	A 0.31 ± 0.02 a	A,B 0.63 ± 0.05 b	B,C 0.81 ± 0.09 c	A 0.33 ± 0.06 a	**
		6	C 0.43 ± 0.01	C 0.82 ± 0.00	B 0.67 ± 0.01	A 0.53 ± 0.41	ns
	TGT	3	A 0.31 ± 0.00 a	A 0.59 ± 0.01 b	B,C 0.80 ± 0.13 c	A 0.23 ± 0.01 a	*
		6	C 0.43 ± 0.02 b	C 0.83 ± 0.67 c	A 0.37 ± 0.07 b	A 0.21 ± 0.01 a	***
Significance		*	***	***	*		
Galactose	Control	0	C 11.97 ± 0.24 a	D 11.97 ± 0.24 a,b	C 12.91 ± 0.11 b,c	C 13.33 ± 0.29 c	*
	Georgia	3	B,C 11.46 ± 0.20 b	C,D 12.27 ± 0.20 b	A 11.09 ± 0.03 b	A,B 9.42 ± 1.15 a	*
		6	A,B 10.90 ± 0.10 b	A,B 11.25 ± 0.05 b,c	A 11.44 ± 0.24 c	A 8.41 ± 0.05 a	***
	San Giovanni	3	B,C 11.51 ± 0.30 a,b	C 12.09 ± 0.02 b	B,C 12.42 ± 0.13 b	B 10.17 ± 0.96 a	*
		6	A,B 10.89 ± 0.01	B 11.47 ± 0.04	B 12.18 ± 0.02	B 10.81 ± 0.98	ns
	TGT	3	B,C 11.63 ± 0.78 b	C 12.07 ± 0.11 b	A 11.31 ± 0.71 b	A,B 9.48 ± 0.20 a	*
		6	A 10.49 ± 0.22 b	A 11.13 ± 0.01 c	A 11.23 ± 0.01 c	A 7.97 ± 0.12 a	***
Significance		*	***	**	*		
Pyruvic acid	Control	0	B 0.89 ± 0.00	C 0.89 ± 0.00	0.91 ± 0.00	0.91 ± 0.02	ns
	Georgia	3	B 0.88 ± 0.02	C 0.91 ± 0.02	0.87 ± 0.01	0.89 ± 0.02	ns
		6	B 0.87 ± 0.00 a	B 0.87 ± 0.00 a,b	0.88 ± 0.01 b	0.90 ± 0.00 c	*
	San Giovanni	3	B 0.86 ± 0.01 a	B,C 0.89 ± 0.00 b,c	0.88 ± 0.00 a,b	0.90 ± 0.00 c	**
		6	A 0.81 ± 0.01	A 0.85 ± 0.00	0.86 ± 0.00	0.86 ± 0.02	ns
	TGT	3	B 0.88 ± 0.04	B,C 0.89 ± 0.01	0.86 ± 0.03	0.89 ± 0.01	ns
		6	A 0.79 ± 0.00 a	A 0.84 ± 0.00 b	0.85 ± 0.01 b	0.92 ± 0.02 c	**
Significance		**	***	NS	NS		
Lactic acid	Control	0	C 18.15 ± 0.44 a	D 18.15 ± 0.44 a,b	D 19.52 ± 0.18 b,c	C 20.39 ± 0.53 c	*
	Georgia	3	B,C 17.38 ± 0.46 a	C,D 18.43 ± 0.34 b	A,B,C 18.21 ± 0.19 a,b	B 18.98 ± 0.13 b	*
		6	A,B 16.31 ± 0.32 a	A,B 16.37 ± 0.13 a,b	A,B 17.78 ± 0.38 b	A,B 18.5 ± 0.07 b	**
	San Giovanni	3	B,C 17.51 ± 0.63 a	C,D 18.29 ± 0.04 a,b	C 18.68 ± 0.03 b,c	B,C 19.4 ± 0.20 c	*
		6	A,B 16.32 ± 0.19 a	B 16.76 ± 0.01 a,b	B,C 18.36 ± 0.04 a,b	A 17.56 ± 1.13 b	*
	TGT	3	B,C 17.61 ± 1.17 a	C 18.23 ± 0.21 a	A 17.72 ± 0.47 b	B,C 19.37 ± 0.06 c	*
		6	A 15.61 ± 0.51 a	A 16.11 ± 0.00 a	A,B,C 18.18 ± 0.00 b	B 19.06 ± 0.22 c	***
Significance		*	***	**	*		
Malic acid	Control	0	A - ± -	A - ± -	A - ± -	A - ± -	
	Georgia	3	B 0.08 ± 0.01	C 0.07 ± 0.01	B 0.08 ± 0.00	B 0.08 ± 0.00	ns
		6	B 0.07 ± 0.00	B 0.05 ± 0.00	C 0.10 ± 0.01	B 0.07 ± 0.00	ns
	San Giovanni	3	C 0.17 ± 0.01	E 0.17 ± 0.00	D 0.16 ± 0.00	C 0.16 ± 0.00	ns
		6	D 0.40 ± 0.05	F 0.33 ± 0.01	E 0.32 ± 0.01	C 0.33 ± 0.01	ns
	TGT	3	B 0.07 ± 0.00	B 0.07 ± 0.01	B 0.07 ± 0.01	B 0.07 ± 0.00	ns
		6	C 0.21 ± 0.00	D 0.15 ± 0.00	D 0.15 ± 0.00	B 0.12 ± 0.05	ns
Significance		***	***	***	***	***	
Citric acid	Control	0	2.72 ± 0.01	2.72 ± 0.01	2.74 ± 0.01	2.77 ± 0.07	ns
	Georgia	3	2.67 ± 0.02	2.75 ± 0.08	2.68 ± 0.01	2.68 ± 0.03	ns
		6	2.64 ± 0.00	2.63 ± 0.02	2.66 ± 0.03	2.70 ± 0.01	ns
	San Giovanni	3	2.72 ± 0.02	2.73 ± 0.00	2.73 ± 0.01	2.68 ± 0.11	ns
		6	2.71 ± 0.03	2.74 ± 0.01	2.75 ± 0.03	2.75 ± 0.00	ns
	TGT	3	2.76 ± 0.14	2.71 ± 0.03	2.70 ± 0.02	2.73 ± 0.01	ns
		6	2.66 ± 0.00	2.67 ± 0.01	2.71 ± 0.02	2.85 ± 0.08	ns
Significance		NS	NS	NS	NS	NS	

^W Data are expressed as mean ± SD (n = 3).

Abbreviations: HS % = hazelnut skin content (%), TGT = Tonda Gentile Trilobata.

Means followed by different lowercase letters in same row within each concentration were significantly different at $p < 0.05$; means followed by different capital letters in same column within each storage time were significantly different at $p < 0.05$.

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns or NS = not significant.

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