

1 **USE OF WINEMAKING BY-PRODUCTS AS AN INGREDIENT FOR TOMATO PUREE: THE**  
2 **EFFECT OF PARTICLE SIZE ON PRODUCT QUALITY**

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5 Vera Lavelli<sup>a\*</sup>, P.S.C. Sri Harsha<sup>a</sup>, Luisa Torri<sup>b</sup>, Giuseppe Zeppa<sup>c</sup>

6 <sup>a</sup>DeFENS, Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano,  
7 via Celoria 2, 20133 Milano, Italy

8 <sup>b</sup>University of Gastronomic Sciences,, Piazza Vittorio Emanuele 9, 12060 Bra, Italy

9 <sup>c</sup>DISAFA, , Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Via L.  
10 da Vinci 44, 10095 Grugliasco, Italy

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13 **Running title:** Grape skin as ingredient for tomato puree

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15 <sup>\*</sup>Corresponding author (Tel: +39 2 50319172; Fax: +39 2 50316632; E-mail address: [vera.lavelli@unimi.it](mailto:vera.lavelli@unimi.it))

16 ABSTRACT

17 Formulations of tomato puree with grape skin fibres (Chardonnay variety) having varying particle sizes were  
18 studied. The contents of flavonoids (by HPLC-DAD) and proanthocyanidins (*n*-butanol/HCl assay), reducing  
19 capacity (ferric ion reducing antioxidant power, FRAP) and anti-glycation activity by a bovine serum  
20 albumin (BSA)/fructose model system were analysed *in vitro*. A liking test was performed with consumers.  
21 Stabilization was carried out by either an intensive autoclave treatment or an optimized microwave-treatment  
22 achieving 6D-reduction of the target microorganism (*Alicyclobacillus acidoterrestris*). In the fortified tomato  
23 purees, proanthocyanidins' solubility decreased, but it was partly restored by autoclave treatment, which also  
24 caused deglycosylation of flavonol glycosides. Microwave treatment did not show any effect on phenolics.  
25 The reducing capacity and ability to inhibit protein glycation greatly increased in the fortified purees. The  
26 particle sizes of solids in the formulations played a major role with respect to the consumers' liking, with the  
27 smallest ones showing maximum ratings.

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29 KEYWORDS

30 Tomato, grape skins, reducing capacity, *in vitro* anti-glycation activity, liking

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## 32 **1. Introduction**

33 The food industry is facing the challenge of developing new foods having increased health benefits and  
34 meeting consumers' appreciation. In fact, with the surge in the incidence of cardiovascular diseases, cancer  
35 and type-2 diabetes, there is a need to develop new dietary strategies, especially with reference to the  
36 potential health properties of underutilized by-products of food processing (Schieber, Stintzing, & Carle,  
37 2001; Hokayem et al., 2013).

38 Grape (*Vitis vinifera*) pomace, the by-product of winemaking, is a bioresource available on large-scale as  
39 grape constitutes one of the main fruit crops in the world. Grape pomace contains both phenolics and dietary  
40 fibres, thus it can be referred to as "antioxidant dietary fibre". Because of the close relationship between  
41 antioxidant and dietary fibre and their common fate in the gut, it has been proposed that these food  
42 components have a joint role in prevention of human diseases (Perez-Jimenez et al., 2008). *In vivo* studies on  
43 human adults have demonstrated that grape pomace has a positive effect in the prevention of cardiovascular  
44 diseases (Perez-Jimenez et al., 2008). The anti-diabetic efficiency of grape polyphenols was tested in type-2  
45 diabetic patients, resulting in improved insulin resistance and suppressed oxidative stress (Hokayem et al.,  
46 2013).

47 These results have boosted the use of grape pomace as an ingredient for new functional foods, such as bread  
48 (Mildner-Szkudlarz, Zawirska-Wojtasiak, Szwengiel, & Pacynski, 2011), fish products (PazosTorres,  
49 Medina, 2005; Ribeiro, Cardoso, Silva, Serrano, Ramos, & Santos, 2013), meat products (Sayago-Ayerdi,  
50 Brenes, & Goni, 2009) and yogurt (Tseng & Zhao, 2013). The development of foods that provide additional  
51 health benefits beyond basic nutrients is also a trend in the fruit processing industry (Augusto, Falguera,  
52 Cristianini, & Ibarz, 2011).

53 The aim of the present study was to assess the prospective use of a phytochemical- and fibre-rich ingredient  
54 recovered from winemaking by-products for the development of a new tomato-based product. Technological  
55 challenges raised by fortification were studied, such as: the choice of the particle size of the suspension, the  
56 incorporation of an adequate level of the new ingredient, the choice of pasteurization conditions, the  
57 processing effect on phenolic stability and the need to address consumers' liking.

## 58 **2. Materials and methods**

59 *2.1. Chemicals*

60 Standards of catechin, quercetin 3-O-rutinoside (rutin), quercetin 3-O-glucuronide, quercetin 3-O-glucoside,  
61 kaempferol 3-O galactoside, kaempferol 3-O glucuronide, kaempferol 3-O glucoside, quercetin, kaempferol  
62 and naringenin were purchased from Extrasynthese (Lyon, France). The integrated total dietary fibre assay  
63 procedure kit was purchased from Megazyme International Ireland Ltd (Bray, Ireland). All other chemicals  
64 were purchased from Sigma Aldrich Italia (Milan, Italy).

65 *2.2. Grape skins*

66 Grape pomace samples of the Chardonnay (Ch) variety were kindly provided by a winery located in  
67 Northern Italy. At the winery, Ch grapes were pressed with separation of grape solids and must. Then grape  
68 stalks were separated with a mechanical destemming and the remaining material was sieved (with a 5 mm  
69 sieve) to separate the skins from the seeds and frozen to inhibit microbial growth. The skins were transported  
70 frozen to the lab, dried at 50 °C for about 8 h. The powders obtained were sieved by using the Octagon  
71 Digital sieve shaker (Endecotts L.t.d., United Kingdom), with three certified sieves (openings: 125, 250 and  
72 500µm), under continuous sieving for 10 min at amplitude 8. Three fibrous fractions having different  
73 particle sizes were collected, namely: ChL (250µm < ChL ≤ 500µm), ChM (125µm < ChM ≤ 250µm) and  
74 ChS (ChS ≤ 125µm). These fractions were stored under vacuum, in the dark, at 4 °C.

75 *2.3. Tomato puree*

76 Two tomato puree samples, namely PV and PR were provided by Conserve Italia Soc. Coop. (San Lazzaro di  
77 Savena, Italy). At the industrial plant, tomatoes were homogenized and heated to approximately 95 °C by  
78 steam injection to inactivate endogenous enzymes (hot-break). The homogenate was then passed hot through  
79 a 0.5 mm-screen (PV) or a 1 mm-screen (PR) pulper/finisher to remove seeds and skin fragments and  
80 deaerated under vacuum. The finished purees were then concentrated at 80 °C and under reduced  
81 atmospheric pressure using a tubular heat exchanger (the final moisture contents were 89.1 ± 0.2 and 89.8 ±  
82 0.2 for PV and PR, respectively). The purees were then aseptically stored in tank under nitrogen for 6 months  
83 before bottling. After bottling, the purees were autoclaved at 115 °C for 5.5 min.

84 *2.4. Preparation of the fortified tomato purees*

85 An amount of 3.2 g of the ChL, ChM and ChS fractions was added to 96.8 g of the PV and PR tomato  
86 purees. Each puree was filled into different glass bottles (250 mL capacity). A set of the bottled fortified  
87 purees was then submitted to microwave heating (8 min at 900 watt). During heating, the temperature of the  
88 tomato puree was monitored continuously by using a thermocouple set in the geometric centre of one of the  
89 bottles (the slowest heating point).

90 To calculate the pasteurization effectiveness during microwave heating, *Alicyclobacillus acidoterrestris* was  
91 used as a target (Silva & Gibbs, 2004). Different heating conditions were tried and the resulting  
92 time/temperature curves were obtained. D values for the target microorganism were calculated as a function  
93 of temperature using the Bigelow's model, as reported below:

$$94 D = D_{\text{ref}} * 10^{(T_{\text{ref}} - T)/z}$$

95 where for the target microorganism,  $D_{\text{ref}} = 1.5$  min,  $T_{\text{ref}} = 95$  °C and  $z = 7$  °C (Bevilacqua & Corvo, 2011).

96 The 1/D values were then plotted as a function of time and the resulting curves were then integrated to  
97 evaluate the total decimal reductions (Silva & Gibbs, 2004). Microwave conditions were then chosen in  
98 order to achieve 6D for the target microorganism.

99 Another set of bottled fortified purees was submitted to autoclave treatment (100 °C, 30 min).

#### 100 2.5. Moisture, fibre, protein, carbohydrates, fat and ash contents

101 Moisture content was determined by drying in a vacuum oven at 70 °C and 50 Torr for 18 h. Protein, fat, and  
102 ash contents were measured according to AOAC official methods of analysis (Tseng & Zhao, 2013).  
103 Glucose and fructose were determined as described in Lavelli, Pagliarini, Ambrosoli, Minati, & Zanoni  
104 (2006). Fibre contents were determined by the Megazyme total dietary fibre assay procedure (based on  
105 AOAC 991.43).

#### 106 2.6. Sample extraction

107 For grape skin powder extraction, an aliquot of 1 g was weighed in duplicate, added with 20 mL  
108 methanol:water:formic acid (70:29.9:0.1, v/v/v) and extracted for 2 h at 60 °C with continuous stirring. The  
109 mixture was centrifuged at 10000g for 10 min, the supernatant recovered and the solid residue was re-  
110 extracted using 10 mL of the same solvent. The supernatants were pooled.

111 For tomato puree extraction, 3.75 g was weighed in duplicate and added to 1.9 mL of water, 7 mL of  
112 methanol and 0.3 mL of formic acid (in order to use the same medium as for the grape skin fractions, taking  
113 into account the amount of water present in the puree). Extraction was performed as that of grape skin  
114 fractions. Extracts were stored at -20°C until analytical characterization.

#### 115 *2.7. Polyphenol analysis by HPLC-DAD*

116 The HPLC equipment consisted of a model 600 HPLC pump coupled with a Waters model 2996 photodiode  
117 array detector, operated by Empower software (Waters, Vimodrone, Italy). A 2.6 µm Kinetex C<sub>18</sub> column  
118 (150 x 4.6 mm) equipped with a C<sub>18</sub> precolumn (Phenomenex, Castel Maggiore, Italy) was used for the  
119 separation at a flow-rate of 1.8 mL/min. The injection volume was 50 µL. The column was maintained at  
120 60°C and the separation was performed by means of a gradient elution using (A): 0.1% formic acid and (B):  
121 acetonitrile. The gradient was as follows: from 5% B to 15% B in 15 min, from 15% B to 20% B in 2 min,  
122 from 20% B to 90% B in 4 min; 90% B for 5 min and 5% B for 3 min. DAD analysis was carried out in the  
123 range of 200-600 nm. Standard compounds were used to identify peaks by retention times and UV-vis  
124 spectra. Calibration curves were built with catechin (280 nm), quercetin 3-O glucoside (reference compound  
125 for all flavonols, at 353 nm) and naringenin (at 288 nm). Concentrations of phenolic compounds were  
126 expressed as milligrams per kilogram of dry product.

#### 127 *2.8. Proanthocyanidin content*

128 Proanthocyanidin content was analysed as described previously (Porter, Hrstich, & Chan, 1986). Briefly, for  
129 evaluation of soluble proanthocyanidins 1 mL of the sample extract (opportunistically diluted with  
130 methanol:water:formic acid (70:29.9:0.1, v/v/v) was added to 6 mL of *n*-butanol:HCl (95:5, v/v) and 0.2 mL  
131 of 2% NH<sub>4</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·12 H<sub>2</sub>O in 2M HCl. For evaluation of insoluble proanthocyanidins, 10 mg of the  
132 extraction residue was weighted in quadruplicate and added to 20 mL methanol, 120 mL *n*-butanol:HCl  
133 (95:5, v/v) and 4 mL of 2% NH<sub>4</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·12 H<sub>2</sub>O in 2M HCl. Hydrolysis was carried out at 95 °C for 40  
134 min. The reaction mixtures were cooled and the absorbance was recorded at 550 nm on a Jasco UVDEC-610  
135 spectrophotometer (Jasco Europe, Cremella, Italy) against a blank made as for the sample but incubated at  
136 room temperature. For each sample extract, 2 - 4 dilutions were assessed in duplicate. Proanthocyanidin

137 amount was determined using 0.1736 (mg/mL) as conversion factor (Sri Harsha, Gardana, Simonetti,  
138 Spigno, & Lavelli, 2013) and expressed as grams per kilogram of dry product.

### 139 2.9. Ferric ion reducing antioxidant power (FRAP) assay

140 The FRAP assay was performed as described previously (Sri Harsha et al., 2013). Briefly, FRAP reagent was  
141 prepared by adding 25 mL of 300 mM acetate buffer, pH 3.6; 2.5 mL of 10 mM 2,4,6-Tripyridyl-*s*-Triazine  
142 in 40 mM HCl and 2.5 mL of 20 mM FeCl<sub>3</sub>. The reaction mixture contained 0.4 mL of sample extracts  
143 opportunely diluted with methanol:water:formic acid (70:29.9:0.1, v/v/v) and 3 mL of FRAP reagent. The  
144 absorbance at 593 nm was evaluated on a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremella,  
145 Italy) after 4 min of incubation at 37 °C against a blank with no extract addition. For each sample extract, 2 -  
146 4 dilutions were assessed in duplicate. A methanolic solution of FeSO<sub>4</sub>·7H<sub>2</sub>O was used for calibration.  
147 Results were expressed as millimoles of Fe(II) sulfate equivalents per kilogram of dry product.

### 148 2.10. Determination of fructose-induced glycation of bovine serum albumin (BSA)

149 The inhibition of fructose-induced glycation of BSA was conducted as described in Lavelli & Scarafoni  
150 (2012). The reaction mixture consisted of 100 µL of sample extracts or standard (catechin) opportunely  
151 diluted with methanol:water:formic acid (70:29.9:0.1, v/v/v), 900 µL of phosphate buffer (200 mM  
152 potassium phosphate buffer, pH 7.4 with 0.02% sodium azide), 300 µL of BSA solution (50 mg/mL of BSA  
153 in phosphate buffer), and 300 µL of fructose solution (1.25 M fructose in phosphate buffer). A BSA solution  
154 (blank sample) and control reaction without sample addition were prepared in parallel. The reaction mixtures  
155 were incubated at 37 °C for 72 h. Following incubation, 1.6 mL of 20% trichloroacetic acid was added to the  
156 reaction mixture before centrifugation at 10000g for 10 min. The supernatant was discarded and the  
157 precipitate was re-dissolved in 1.6 mL of phosphate buffer and analyzed for fluorescence on a Perkin-Elmer  
158 LS 55 Luminescence Spectrometer (Perkin-Elmer Italia, Monza, Italy) with an excitation/emission  
159 wavelength pair  $\lambda = 370/440$  nm, 5 nm slit width, against phosphate buffer. For each sample extract, 3 - 4  
160 dilutions were assessed in duplicate. Catechin was analysed at six dilutions to build a calibration curve.  
161 Dose-response curves were built reporting % inhibition of fructose-induced glycation of BSA as a function  
162 of sample or catechin concentration. % Inhibition was calculated as:  $100-100*(FL_s-FL_b)/(FL_c-FL_b)$ ,

163 where  $FL_s$  is the fluorescence intensity of the mixture with the sample extract or with catechin,  $FL_b$  is the  
164 fluorescence intensity of the blank (BSA alone) and  $FL_c$  is the fluorescence intensity of the control mixture.  
165 Results were expressed as millimoles of catechin equivalents (CE) per kilogram of product.

#### 166 *2.11. Liking test*

167 Eighty-six consumers (44 males, 42 females, 19–68 years, mean age 28) participated in the study. They had  
168 seen or received an invitation and volunteered based on their interest and availability. All tests were  
169 conducted individually and social interaction was not permitted. The experimenter verbally introduced the  
170 consumers to the computerised data collection procedure (FIZZ Acquisition software, version 2.46A,  
171 Biosystèmes, Courtenon, France). The consumers' test was organized in two sub-sessions. In the first sub-  
172 session, participants evaluated a set of six fortified tomato purees. In the second sub-session, a set of the  
173 control unfortified purees was tested. Fortified and control purees were analyzed in different sub-sessions to  
174 limit the contrast effect (Meilgaard, Civille, & Carr, 2006).

175 The samples (20 g) were offered to the consumers in completely randomized order within the two sessions,  
176 at  $50 \pm 1$  °C in coded, opaque white plastic cup (38 mL) hermetically sealed with a clear plastic lid. For each  
177 sample, consumers stirred accurately the tomato puree using a plastic teaspoon, observed its appearance and  
178 tasted a full teaspoon of product. Then, consumers rated overall liking, liking for colour and texture on a  
179 nine-point hedonic scale ranging from 'dislike extremely' (1) to 'like extremely' (9). A 30 s gap between  
180 each sample was enforced by the computerised system. Consumers were required to eat unsalted crackers  
181 and rinse their mouth with still water during the gap interval. A 10 min gap was enforced between the two  
182 sub-sessions. Preference tests were performed in individual booths under white light. Consumers took  
183 between 25 and 35 min to complete their evaluation.

#### 184 *2.12. Statistical analysis of data*

185 Experimental data were analyzed by one-way ANOVA using the least significant difference (LSD,  $p \leq 0.05$ )  
186 as a multiple range test, and by linear regression analyses using Statgraphics 5.1 (STCC Inc.; Rockville,  
187 MD). Results are reported as average  $\pm$  SD.

188 Liking data (overall liking, liking for colour and texture) from consumers were independently submitted to a  
189 two-way ANOVA model, assuming sample and subject as main effects, by performing LSD ( $p < 0.05$ ).



190 Overall liking data expressed by all 86 subjects were analysed by means of an Internal Preference Map for  
191 explorative purposes. A visually oriented approach, based on the inspection of loading plot, was used for  
192 subject clustering and Y-axis was set as limit between consumer segments. Liking data expressed by Cluster  
193 1 and Cluster 2 were independently treated with a two-way ANOVA model, with LDS ( $p \leq 0.05$ ). Liking  
194 data were analyzed using FIZZ Calculations software, version 2.46A (Biosystèmes, Courtenon, France).

### 195 **3. Results and discussion**

#### 196 *3.1. Product and process design*

197 The increase in fibre content of food generally has a negative impact on texture, which could be greatly  
198 affected by the particle size of the fibrous material. For a fruit puree, particle concentration, size and type  
199 have been found to be key structural parameters controlling the rheological properties (Moelants et al.,  
200 2013). Hence, in this study three granulometric fractions of Ch grape skins (in the range 125 – 500  $\mu\text{m}$ ) and  
201 two tomato purees of different particle sizes (0.5 and 1 mm) were used in combined formulations. In studies  
202 focused on the incorporation of grape skins or pomace into various foods, the selected particle sizes were  
203 less than 1 mm for addition in fish products (Riberio et al, 2012), less than 0.5 in meat products (Sayago-  
204 Ayerdi et al., 2009) less than 0.18 mm for addition in yogurt (Tseng & Zhao, 2013), while in other  
205 incorporation studies the particle size of this ingredient was not specified (Mildner-Szkudlarz et al., 2011).

206 The composition of Ch skins and tomato purees were first characterized in order to choose the level of  
207 addition. In Ch skins, dietary fibre content was 50.5%. Protein, carbohydrate (fructose and glucose), fat, ash  
208 and moisture contents were:  $10.0 \pm 0.6$ ,  $16.2 \pm 0.2$ ,  $5.7 \pm 1.6$ ,  $4.1 \pm 0.7$  and  $4.0 \pm 0.1$  g/100g, respectively.  
209 Insoluble proanthocyanidin contents, analysed after depolymerisation with *n*-butanol/HCl, were  $10.6 \pm 2$  in  
210 the ChL fraction and  $13.9 \pm 1$  in both the ChM and Ch S fractions, respectively. This could be due to a lower  
211 hydrolysis yield in the ChL fraction. The total amount of flavonols, namely: quercetin 3-O glucuronide,  
212 quercetin 3-O glucoside, quercetin, kaempferol 3-O galactoside, kaempferol 3-O glucuronide, kaempferol 3-  
213 O glucoside and kaempferol was about 600 mg/kg (Tables 1, 2). Soluble proanthocyanidin content of the  
214 ChL fraction was  $20700 \pm 42$  mg/kg (Table 3). Higher proanthocyanidin contents were observed in the ChM  
215 and ChS fractions. The increased surface/solvent ratio likely increased extraction efficiency of these  
216 compounds, which are strongly associated with the fibre (Perez-Jimenez et al., 2008). FRAP values were >

217 170 ± 26 mmolFe eq. (II)/kg, which is two order of magnitude higher than that observed in tomato products  
218 (García-Valverde, Navarro-González, García-Alonso, & Jesús Periago, 2013). The highest FRAP value was  
219 observed in the ChS fraction. The ability of the Ch fractions to inhibit protein glycation was analysed by an  
220 *in vitro* BSA/fructose model system (Figure 1). This system was used to simulate protein glycation that  
221 occurs at an accelerated rate *in vivo* under non-physiological conditions, accounting for some of the  
222 complications of hyperglycaemia and diabetes (Saraswat, Reddy, Muthenna, & Reddy, 2009). There is a  
223 continuous search for novel inhibitors of protein glycation that could be helpful to prevent advanced-  
224 glycation-endproduct-associated diseases and with the potential to be used as functional food ingredients  
225 (Farrar, Hartle, Hargrove, & Greenspan, 2007; Saraswat et al., 2009; Sri Harsha et al., 2013; Wu et al.,  
226 2013). In this study, a dose-response effect was observed *in vitro* for the anti-glycation activity of the Ch  
227 fractions. Phenolics are known to inhibit protein glycation by acting as radical scavengers, metal chelators  
228 and carbonyl trapping agents (Dearlove, Greenspan, Hartle, Swanson, & Hargrove, 2008; Wu et al., 2013).  
229 Hence, in terms of catechin equivalents, the anti-glycation effectiveness was 100 ± 15 mmol/kg for all the Ch  
230 fractions.

231 In PV and PR tomato purees percent contents of major components were: 4.9 ± 0.1 and 5.7 ± 0.1 for  
232 carbohydrates, 1.5 ± 0.1 and 1.5 ± 0.1 for fibres; 1.2 ± 0.1 and 1.6 ± 0.1 for proteins; 0.1 ± 0.02 and 0.20 ±  
233 0.02 for fat, respectively. The main flavonoids in tomato purees were rutin and naringenin (Tables 1, 2).  
234 Before heat treatments, flavonol contents (sum of quercetin derivatives) were in the range of 52 - 72 mg/kg  
235 and flavanone contents (naringenin) were in the range of 14 - 51 mg/kg. The PV and PR purees had a  
236 medium-high flavonol and flavanone contents in comparison with previous results obtained on twenty  
237 cultivars of fresh tomatoes extracted with an optimized procedure (Li, Deng, Wuc, Liu, Loewen, & Tsao,  
238 2012). FRAP values of the PR and PV purees were 1.97 ± 0.14 and 2.68 ± 0.22 mmol Fe(II) eq./kg,  
239 respectively (Table 3). Similar values were observed by Garcia-Valverde et al. (2013) in various cultivars of  
240 tomatoes destined to industrial processing. The unfortified tomato purees showed a dose-dependent anti-  
241 glycation activity *in vitro*, with anti-glycation effectiveness of 2.97 ± 0.15 and 2.82 ± 0.40 mmol catechin  
242 eq./kg for PV and PR, respectively. These values were much lower than that of the Ch fractions (Figure 1).

243 The level of Ch/tomato addition was then chosen to have 3% fibre content in the final products (3.2 g of  
244 grape skins added to 96.8 g of tomato puree). Hence, the purees can be labelled as “fibre-source” according  
245 to the EC Regulation 1924/2006. Furthermore, in a human study, Pérez-Jiménez et al. (2008) have  
246 demonstrated that the intake of grape antioxidant dietary fibre (5.25 g of dietary fibre and 1.06 g of  
247 proanthocyanidins in the supplemented dose) significantly reduces the biomarkers of cardiovascular risk.  
248 Based on Ch fibre and proanthocyanidin contents, a 175 g-dose of the fortified purees (that could be a daily  
249 dose in the Mediterranean diet) can provide 5.25 g of dietary fibres and around 1 g of proanthocyanidins  
250 (soluble + insoluble). Hence, positive *in vivo* effects of these purees can be hypothesised. However, the food  
251 matrix is more complicated than grape skins, therefore an effect of the matrix on food components’  
252 bioavailability cannot be ruled out.

253 The incorporation of grape skin derived fractions into a liquid food, such as tomato puree, requires the design  
254 of an effective heat treatment. The pH values of these products were in the range 4.1 – 4.3. To achieve  
255 pasteurization of low-pH foods, *Alicyclobacillus acidoterrestris* has been proposed as a process target. It is a  
256 thermoacidophilic non-pathogenic and sporeforming bacterium, which has been found in fruit juices,  
257 including tomato puree and white grape juice (Silva & Gibbs, 2004). It is often the most heat resistant  
258 microorganism among the most common spoilage microorganisms found in these foods. The heating  
259 conditions were then selected to achieve 6D-reduction of the target microorganism (Figure 2), which is  
260 considered effective (Silva & Gibbs, 2004). This treatment is representative for an optimized continuous  
261 industrial treatment. In parallel, tomato purees were also autoclaved to study the effects of an intensive heat-  
262 treatment on the antioxidant components.

### 263 3.2. Processing effects on antioxidant components

264 Flavonols and naringenin were not affected by microwave treatment (not shown). Similarly, Capanoglu,  
265 Beekwilder, Boyacioglu, Hall, & De Vos (2008) found that pasteurization at 98 °C does not change rutin and  
266 naringenin contents of tomato. Upon autoclave treatment, quercetin and kaempferol glycosides and  
267 glucuronides decreased by less than 30% (Tables 2-3). Conversely, the corresponding aglycones increased.  
268 The recovery was ~100% when the sum of quercetin derivatives was considered and ~90% for the sum of  
269 kaempferol derivatives. This means that the prevalent modification occurring during autoclave treatment was

270 deglycosylation. Interestingly, Stewart, Bozonnet, Mullen, Jenkins, Lean, & Crozier (2000) found that in  
271 contrast to fresh tomatoes, most tomato-based products contained significant amounts of free flavonols and  
272 concluded that the accumulation of quercetin in juices, purees, and paste may be a consequence of enzymatic  
273 hydrolysis of rutin and other quercetin conjugates during pasteurization. Instead, enzymatic activities can be  
274 ruled out in this study, due to the intense heating during autoclave treatment. Rohn, Buchner, Driemel,  
275 Rauser, & Kroh (2007) found that during the roasting process of model flavonols (180°C, 60 min), quercetin  
276 glycosides are degraded and produce quercetin as the major degradation product. Quercetin is not sensitive  
277 to degradation under such conditions and therefore it has to be regarded as a stable end-product. Naringenin  
278 content was above 88%, with lower retention for the unfortified purees than for the fortified purees.

279 After mixing of the purees with the ChL, ChM and ChS skin fractions at room temperature soluble  
280 proanthocyanidin contents were lower in the puree added with the ChL fraction. For all the purees,  
281 proanthocyanidin content was lower than that calculated based on the proanthocyanidin content of grape  
282 skins, with 53-56% recovery percentages (Table 3). These data can be explained with the hypothesis that  
283 proanthocyanidins interacted with tomato components, such as proteins or polysaccharides, to produce high  
284 molecular weight aggregates, through hydrogen bonding or hydrophobic interactions (Pinelo, Arnous, &  
285 Meyer, 2006). These aggregates could not be extracted by the solvents used in this experiment. Similar to  
286 these results, Peng, Maa, Cheng, Jiang, Chen & Wang (2010) found that in a bread added with a  
287 proanthocyanidin-rich grape seed extract, the observed antioxidant activity increases less than what is  
288 expected. They did not analyse the unheated samples and concluded that the decreases could be either due to  
289 the interactions of proanthocyanidins with food components to produce insoluble molecules, or due to  
290 thermal degradation.

291 Similarly, FRAP values of the mixtures increased approximately by twofold, probably due to the high  
292 proanthocyanidin contents of the Ch fractions (Table 3). The lowest value was found in the puree added with  
293 the ChL fraction. However, as observed for proanthocyanidins the increase in FRAP values were only 61-  
294 66% of that calculated considering the values of the ChL, ChM and ChS skin fractions.

295 Microwave treatment had no effect on the proanthocyanidin contents and FRAP values of any of the  
296 mixtures considered. On the contrary, upon autoclave treatment, proanthocyanidin contents increased in the

297 fortified puree with respect to the raw mixtures. The parallel increased FRAP values in the fortified purees  
298 can be related to the rise in the content of proanthocyanidins. The intense thermal treatment could have  
299 weakened the binding between proanthocyanins and other food components (Pinelo et al., 2006), or it could  
300 have promoted proanthocyanidin depolymerisation (Chamorro, Goni, Viveros, Hervert-Hernandez, &  
301 Brenes, 2012) and thus increased proanthocyanidins' solubility.

302 The dose-dependent anti-glycation activity *in vitro* of the fortified purees showed much higher effectiveness  
303 than the controls, corresponding to  $8.1 \pm 0.1$  and  $7.2 \pm 0.1$  mmol catechin eq./kg for PV and PR, respectively  
304 (Figure 1). These new purees have the potential ability to act as dietary factors in the prevention of  
305 hyperglycaemia's complications.

### 306 3.3. Consumers' preferences

307 The prospective use of fibrous fractions in developing new functional tomato purees needs to be evaluated  
308 not only from an analytical point of view but also exploring the sensory acceptability of the formulations.  
309 Several works have shown that functional benefits may provide added value to consumers but cannot  
310 outweigh the sensory properties of foods. In fact, consumers base their choices more on pleasantness than  
311 perceived healthiness (Lähteenmäki, 2006). For this reason, a liking test was performed in order to  
312 estimate the consumer overall acceptability of the fortified purees. Since variations in particle sizes of fruit  
313 puree influences the texture (Moelants et al., 2013) and processing of fruit puree can affect colour (Lavelli  
314 & Torresani, 2011), liking ratings for texture and colour were also investigated.

315 The average liking ratings expressed by all 86 consumers for overall acceptability, colour and texture of the  
316 analysed tomato purees are reported in Table 4. Consumers highly rated the unfortified purees in terms of  
317 overall acceptability ( $6.9 \pm 1.8$  for PR;  $6.7 \pm 1.9$  for PV), liking for colour ( $7.4 \pm 1.7$  for PR;  $7.2 \pm 1.7$  for  
318 PV) and texture ( $7.0 \pm 1.8$  for PR;  $6.8 \pm 1.7$  for PV). The addition of the Ch fractions to the tomato purees  
319 decreased the ratings for all the sensory parameters ( $p < 0.05$ ). This effect could be explained taking into  
320 account that consumers were familiar with the unfortified samples (commercially available regular tomato  
321 purees), but they had not been previously exposed to the fortified samples. As it is known, the level of  
322 familiarity for a food influences powerfully its acceptability by the consumer and repeated exposure to the  
323 taste of a food can increase liking for it (Wardle & Cooke, 2008).

324 Regarding the overall liking, average ratings of the fortified samples corresponded approximately to the  
325 central value of the scale (5 = neither like nor dislike). PVChL, PVChM and PVChS were significantly  
326 preferred ( $5.3 \pm 1.9$ ) than PRChL ( $4.6 \pm 2.1$ ) ( $p < 0.05$ ). Concerning the texture, as the particle size  
327 decreased, liking increased. This tendency was more evident for the PV formulations. Average ratings of  
328 liking for colour were all above the central value (5). The only significant difference in colour was observed  
329 for PVChS, which was rated higher than the PR formulations.

330 The overall liking data expressed by all 86 subjects for the fortified samples were then submitted to the  
331 principal component analysis in order to obtain an internal preference map (data not shown). The first two  
332 principal components of the model explained the 48% of the total variance, 28% and 21% the first and the  
333 second dimensions, respectively. A visually oriented approach, based on the inspection of loading plot, was  
334 used for subject clustering and segmentation was performed according to whether consumer loadings lie on  
335 the left or right side of the Y-axis set as limit (Næs et al., 2010). Two groups of consumers were obtained:  
336 the first consisting of 46 subjects (53.5%) positioned on the left side of the map (Cluster 1); the second  
337 consisting of 40 subjects (46.5%) positioned on the right side of the map (Cluster 2). Liking data expressed  
338 by subjects belonging to Cluster 1 and Cluster 2 for all samples were independently treated with a two-way  
339 ANOVA model (samples and subjects as factors), with Fisher's LDS post hoc test considered significant for  
340  $p \leq 0.05$  (Table 4). As expected, both clusters provided similar average ratings of the three sensory  
341 parameters evaluated for the unfortified PR and PV purees, confirming the results obtained by the total of  
342 subjects (Table 4). Focusing on the fortified purees, different results were obtained by the two clusters. In  
343 terms of overall acceptability, Cluster 1 preferred the purees fortified with the ChM and ChS fibrous  
344 fractions both for the PR and PV formulations. The highest rating was observed for PVChS ( $6.4 \pm 1.5$ ),  
345 which was not significantly different to that of the PV puree ( $7.0 \pm 1.8$ ). For Cluster 1, liking for texture  
346 decreased as the particle size of the added fibrous fraction increased, as noticed by the preference of all  
347 consumers. Again, in terms of texture PVChS reached the highest average value among the fortified purees,  
348 which was the same as that observed for PV. The good ratings given for the ChS fraction were confirmed  
349 also in terms of liking for colour.

350 Cluster 2 did not discriminate among the three PR formulations in terms of overall acceptability, while  
351 among the PV formulations PVChL was preferred. This cluster did not discriminate among the fortified  
352 samples for both texture and colour, but ratings were higher for the control purees than those of the fortified  
353 purees.

#### 354 **4. Conclusions**

355 Tomato purees fortified with Ch fractions could be positioned noticeably above with respect to the  
356 conventional purees in terms of potential health benefits. Indeed, tomato is rich in lycopene but it does not  
357 contain proanthocyanidins and hence the addition of grape pomace ingredients could overall improve its  
358 antioxidant and anti-glycation properties *in vitro*. Upon heat-stabilization, phenolic contents and reducing  
359 capacity remained much higher in all the fortified purees than in the controls. Increase in anti-glycation  
360 activity was also observed in the fortified formulations.

361 The varying particle sizes of puree formulations had a moderate effect on proanthocyanidins' solubility and a  
362 marked influence on consumers' preference. PVChS, having the smallest particle sizes, had the maximum  
363 appreciation by a cluster of consumers, with similar liking ratings to those of the control puree. Thus, this  
364 innovative functional puree can have a positive feedback by a relevant segment of consumers.

365 The overall results indicate that grape skins could be used as ingredients for the development of new tomato  
366 purees, contributing to a sustainable process innovation.

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**Table 1.** Contents of Quercetin Derivatives and Quercetin Aglycone (mg quercetin 3-O glucoside eq./kg) in the ChL, ChM and ChS Fractions, PV and PR Tomato Purees and their Combined Formulations, after Autoclave Treatment.

Sample	Quercetin derivatives					
	Q-ud	Q-rut	Q-gln	Q-glc	Q	tot Q-der
<b>ChL</b>			111 <sup>c</sup> ± 2	98 <sup>b</sup> ± 5	13.8 <sup>e</sup> ± 0.6	223 <sup>c</sup> ± 8
<b>ChM</b>			114 <sup>c</sup> ± 4	92 <sup>b</sup> ± 1	13.6 <sup>e</sup> ± 0.6	220 <sup>c</sup> ± 5
<b>ChS</b>			115 <sup>c</sup> ± 1	97 <sup>b</sup> ± 1	12.8 <sup>e</sup> ± 0.8	225 <sup>c</sup> ± 3
<b>PR</b>	3.28 <sup>a</sup> ± 0.01 (72)	42.10 <sup>b</sup> ± 0.09 (91)			0.35 <sup>a</sup> ± 0.01	45.73 <sup>a</sup> ± 0.12 (88)
<b>PRChL</b>	3.10 <sup>a</sup> ± 0.03 (76)	36.30 <sup>a</sup> ± 1.52 (87)	2.50 <sup>ab</sup> ± 0.03 (73)	2.50 <sup>a</sup> ± 0.01 (87)	4.52 <sup>b</sup> ± 0.16 (1139)	49.12 <sup>a</sup> ± 1.76 (100)
<b>PRChM</b>	2.92 <sup>a</sup> ± 0.08 (71)	36.10 <sup>a</sup> ± 0.05 (86)	2.27 <sup>a</sup> ± 0.03 (67)	2.78 <sup>a</sup> ± 0.03 (97)	5.41 <sup>bc</sup> ± 0.42 (1364)	49.48 <sup>a</sup> ± 0.61 (103)
<b>PRChS</b>	3.80 <sup>a</sup> ± 0.28 (91)	39.00 <sup>a</sup> ± 0.00 (93)	2.64 <sup>bc</sup> ± 0.31 (78)	2.81 <sup>a</sup> ± 0.08 (98)	4.40 <sup>b</sup> ± 0.78 (1109)	52.65 <sup>a</sup> ± 1.45 (102)
<b>PV</b>	10.71 <sup>b</sup> ± 0.44 (81)	55.89 <sup>d</sup> ± 0.34 (95)			0.85 <sup>a</sup> ± 0.01	67.45 <sup>b</sup> ± 0.79 (93)
<b>PVChL</b>	10.92 <sup>b</sup> ± 1.91 (85)	53.59 <sup>c</sup> ± 0.05 (94)	2.93 <sup>cd</sup> ± 0.18 (80)	2.97 <sup>a</sup> ± 0.96 (97)	6.77 <sup>cd</sup> ± 0.04 (1590)	77.17 <sup>b</sup> ± 3.14 (100)
<b>PVChM</b>	10.59 <sup>b</sup> ± 0.62 (82)	52.42 <sup>c</sup> ± 1.07 (92)	3.05 <sup>d</sup> ± 0.29 (84)	2.88 <sup>a</sup> ± 0.74 (94)	6.67 <sup>cd</sup> ± 0.85 (1567)	75.60 <sup>b</sup> ± 3.57 (98)
<b>PVChS</b>	10.49 <sup>b</sup> ± 0.96 (82)	53.61 <sup>c</sup> ± 0.98 (94)	3.05 <sup>d</sup> ± 0.03 (84)	3.03 <sup>a</sup> ± 0.18 (99)	7.10 <sup>d</sup> ± 0.99 (1669)	77.28 <sup>b</sup> ± 3.15 (100)

Data are average ± SD. Percent recovery after autoclave treatment is indicated in parenthesis. *Q-ud*, unidentified quercetin derivative; *Q-rut*, rutin; *Q-gln*, quercetin 3-O glucuronide; *Q-glc*, quercetin 3-O glucoside; *Q*, quercetin; *tot Q-der*, sum of quercetin derivatives. Values in the same column with differing superscripts are significantly different (LSD,  $p < 0.05$ ).

**Table 2.** Contents of Kaempferol Derivatives, Kaempferol Aglycone (mg Kaempferol 3-O glucoside eq./kg) and Naringenin (mg/kg) in the ChL, ChM and ChS Fractions, PV and PR Tomato Purees and their Combined Formulations, after Autoclave Treatment.

Sample	Kaempferol derivatives				Naringenin
	K-gal	K-gln+K-glc	K	tot K-der	
<b>ChL</b>	77 <sup>b</sup> ± 7	313 <sup>b</sup> ± 6	16.9 <sup>b</sup> ± 1.5	407 <sup>b</sup> ± 12	
<b>ChM</b>	70 <sup>b</sup> ± 2	304 <sup>b</sup> ± 5	16.7 <sup>b</sup> ± 0.4	391 <sup>b</sup> ± 8	
<b>ChS</b>	67 <sup>b</sup> ± 7	297 <sup>b</sup> ± 20	18.2 <sup>b</sup> ± 1.3	382 <sup>b</sup> ± 28	
<b>PR</b>					11.37 <sup>a</sup> ± 0.64 (81)
<b>PRChL</b>	1.58 <sup>a</sup> ± 0.03 (77)	6.93 <sup>a</sup> ± 0.16 (76)	2.15 <sup>a</sup> ± 0.08 (418)	10.66 <sup>a</sup> ± 0.07 (91)	11.13 <sup>a</sup> ± 0.03 (88)
<b>PRChM</b>	1.74 <sup>a</sup> ± 0.02 (84)	6.64 <sup>a</sup> ± 0.21 (73)	2.04 <sup>a</sup> ± 0.14 (397)	10.41 <sup>a</sup> ± 0.10 (89)	10.61 <sup>a</sup> ± 0.70 (84)
<b>PRChS</b>	1.66 <sup>a</sup> ± 0.03 (81)	6.38 <sup>a</sup> ± 0.02 (70)	1.81 <sup>a</sup> ± 0.01 (352)	9.84 <sup>a</sup> ± 0.01 (85)	11.72 <sup>a</sup> ± 0.23 (93)
<b>PV</b>					45.53 <sup>b</sup> ± 0.72 (90)
<b>PVChL</b>	2.10 <sup>a</sup> ± 0.49 (95)	6.81 <sup>a</sup> ± 1.45 (70)	1.79 <sup>a</sup> ± 0.05 (325)	10.70 <sup>a</sup> ± 0.71 (86)	45.99 <sup>b</sup> ± 0.89 (94)
<b>PVChM</b>	2.02 <sup>a</sup> ± 0.27 (91)	7.22 <sup>a</sup> ± 0.46 (74)	2.23 <sup>a</sup> ± 0.02 (404)	11.46 <sup>a</sup> ± 0.22 (92)	44.60 <sup>b</sup> ± 0.36 (91)
<b>PVChS</b>	1.97 <sup>a</sup> ± 0.12 (89)	7.23 <sup>a</sup> ± 0.36 (74)	1.95 <sup>a</sup> ± 0.04 (354)	11.15 <sup>a</sup> ± 0.17 (89)	44.63 <sup>b</sup> ± 0.01 (91)

Data are average ± SD. Percent recovery after autoclave treatment is indicated in parenthesis. K-gal, kaempferol 3-O galactoside; K-gln, kaempferol 3-O glucuronide; K-glc, kaempferol 3-O glucoside; K, kaempferol, tot K-der, sum of total kaempferol derivatives. Values in the same column with differing superscripts are significantly different (LSD,  $p < 0.05$ ).

**Table 3.** Soluble Proanthocyanin Contents ( $PCy_{soluble}$ , mg/kg) and FRAP Values (mmolFe(II) eq./kg) of the ChL, ChM and ChS Fractions, PV and PR Tomato Purees and their Combined Formulations, after Mixing (raw), Microwave Treatment and Autoclave Treatment.

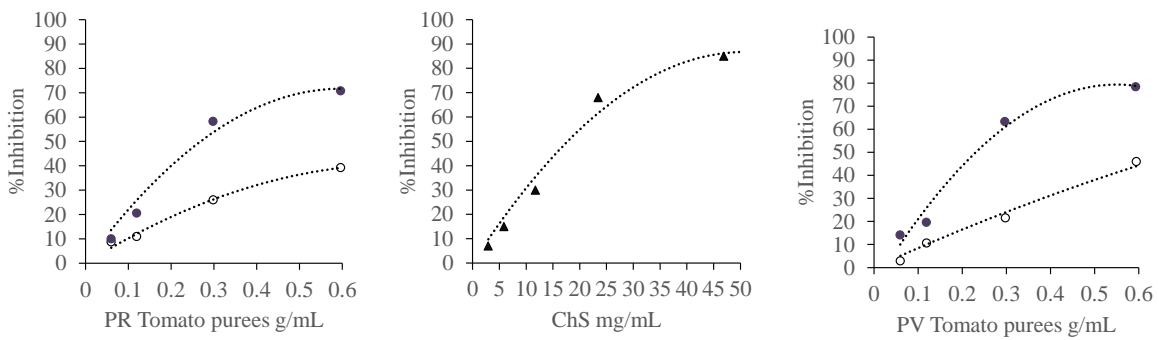
Puree	$PCy_{soluble}$			FRAP		
	Raw	Microwaved	Autoclaved	Raw	Microwaved	Autoclaved
<b>ChL</b>	20700 <sup>c</sup> ± 42			170 <sup>d</sup> ± 25		
<b>ChM</b>	25300 <sup>d</sup> ± 28			207 <sup>e</sup> ± 26		
<b>ChS</b>	27000 <sup>e</sup> ± 14			217 <sup>f</sup> ± 24		
<b>PR</b>				1.97 <sup>a x</sup> ± 0.14	2.29 <sup>a x</sup> ± 0.14	2.15 <sup>a x</sup> ± 0.11
<b>PRChL</b>	352 <sup>a x</sup> ± 63 (53)	353 <sup>a x</sup> ± 3 (53)	406 <sup>a y</sup> ± 1 (61)	4.74 <sup>abc x</sup> ± 0.04 (64)	4.55 <sup>c x</sup> ± 0.03 (61)	5.34 <sup>b y</sup> ± 0.27 (72)
<b>PRChM</b>	445 <sup>b x</sup> ± 23 (55)	399 <sup>ab x</sup> ± 4 (49)	506 <sup>bc y</sup> ± 10 (62)	5.25 <sup>bc x</sup> ± 0.55 (61)	5.30 <sup>d x</sup> ± 0.09 (62)	6.25 <sup>c y</sup> ± 0.35 (73)
<b>PRChS</b>	482 <sup>b x</sup> ± 14 (56)	450 <sup>bc x</sup> ± 11 (52)	555 <sup>cd y</sup> ± 3 (64)	5.82 <sup>c x</sup> ± 0.12 (65)	6.04 <sup>e x</sup> ± 0.09 (68)	6.91 <sup>de y</sup> ± 0.10 (78)
<b>PV</b>				2.68 <sup>ab x</sup> ± 0.22	2.60 <sup>b x</sup> ± 0.18	2.75 <sup>a x</sup> ± 0.15
<b>PVChL</b>	355 <sup>a x</sup> ± 6 (54)	348 <sup>a x</sup> ± 1 (53)	455 <sup>ab xy</sup> ± 81 (69)	5.16 <sup>bc x</sup> ± 0.04 (64)	5.35 <sup>d x</sup> ± 0.15 (66)	6.27 <sup>cd y</sup> ± 0.38 (77)
<b>PVChM</b>	446 <sup>b x</sup> ± 17 (55)	411 <sup>abc x</sup> ± 45 (51)	629 <sup>dc y</sup> ± 65 (78)	5.89 <sup>c x</sup> ± 0.07 (63)	5.93 <sup>e x</sup> ± 0.04 (64)	6.95 <sup>e y</sup> ± 0.23 (75)
<b>PVChS</b>	487 <sup>b x</sup> ± 35 (56)	468 <sup>c x</sup> ± 44 (54)	668 <sup>e y</sup> ± 19 (77)	6.35 <sup>c x</sup> ± 0.30 (66)	6.02 <sup>e x</sup> ± 0.18 (63)	7.50 <sup>e y</sup> ± 0.45 (78)

Data are average ± SD. Percent recovery is indicated in parenthesis. Values in the same column with differing superscripts (a-f) are significantly different (LSD,  $p < 0.05$ ). Values in the same row with differing superscripts (x-z) are significantly different (LSD,  $p < 0.05$ ).

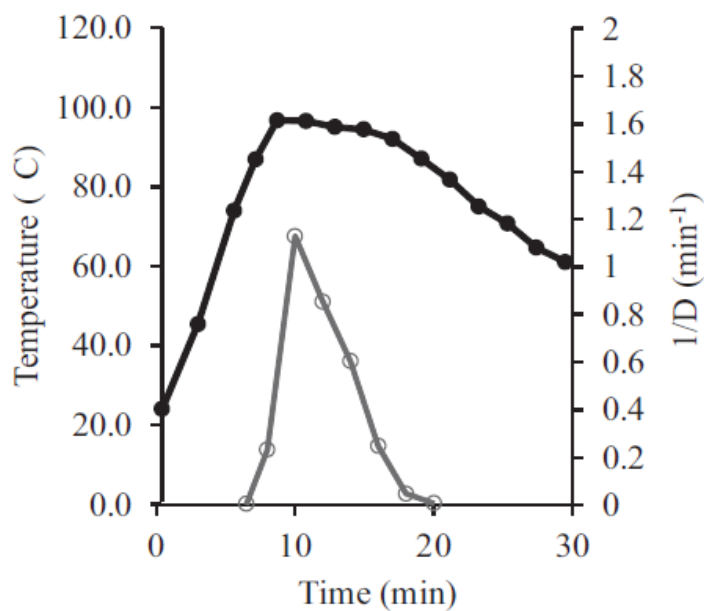
**Table 4.** Overall Liking and Liking for Texture and Colour of the PV and PR Tomato Purees and their Formulations with ChL, ChM and ChS Fractions Expressed by All Consumers (n=86), Cluster 1 (n=46) and Cluster 2 (n=40).

Puree	Overall			Texture			Colour		
	All	Cluster 1	Cluster 2	All	Cluster 1	Cluster 2	All	Cluster 1	Cluster 2
<b>PR</b>	6.9 <sup>a</sup> ± 1.8	6.9 <sup>a</sup> ± 1.5	7.0 <sup>a</sup> ± 2.1	7.0 <sup>a</sup> ± 1.8	6.8 <sup>a</sup> ± 2.0	7.1 <sup>a</sup> ± 1.6	7.4 <sup>a</sup> ± 1.7	7.4 <sup>a</sup> ± 1.8	7.5 <sup>a</sup> ± 1.6
<b>PRChL</b>	4.6 <sup>d</sup> ± 2.1	3.6 <sup>d</sup> ± 1.7	5.7 <sup>bc</sup> ± 2.0	4.3 <sup>e</sup> ± 2.3	3.5 <sup>d</sup> ± 1.9	5.3 <sup>b</sup> ± 2.4	5.3 <sup>c</sup> ± 1.8	4.7 <sup>d</sup> ± 1.7	6.0 <sup>b</sup> ± 1.7
<b>PRChM</b>	4.8 <sup>cd</sup> ± 2.1	4.7 <sup>c</sup> ± 1.9	5.0 <sup>cd</sup> ± 2.4	4.9 <sup>cd</sup> ± 2.1	4.7 <sup>c</sup> ± 1.9	5.3 <sup>b</sup> ± 2.3	5.3 <sup>c</sup> ± 1.7	5.1 <sup>cd</sup> ± 1.5	5.7 <sup>b</sup> ± 1.8
<b>PRChS</b>	5.0 <sup>bcd</sup> ± 2.1	5.1 <sup>c</sup> ± 1.9	5.0 <sup>cd</sup> ± 2.3	5.0 <sup>cd</sup> ± 2.1	4.9 <sup>bc</sup> ± 1.8	5.1 <sup>b</sup> ± 2.4	5.3 <sup>c</sup> ± 1.7	5.1 <sup>cd</sup> ± 1.6	5.6 <sup>b</sup> ± 1.8
<b>PV</b>	6.7 <sup>a</sup> ± 1.9	7.0 <sup>a</sup> ± 1.8	6.3 <sup>ab</sup> ± 1.9	6.8 <sup>a</sup> ± 1.7	7.0 <sup>a</sup> ± 1.6	6.7 <sup>a</sup> ± 1.7	7.2 <sup>a</sup> ± 1.7	7.4 <sup>a</sup> ± 1.8	7.1 <sup>a</sup> ± 1.7
<b>PVChL</b>	5.3 <sup>b</sup> ± 1.9	5.2 <sup>c</sup> ± 1.9	5.5 <sup>c</sup> ± 2.0	4.7 <sup>de</sup> ± 2.3	4.6 <sup>c</sup> ± 2.3	4.8 <sup>b</sup> ± 2.4	5.6 <sup>bc</sup> ± 1.8	5.4 <sup>c</sup> ± 1.8	5.9 <sup>b</sup> ± 1.7
<b>PVChM</b>	5.3 <sup>bc</sup> ± 2.1	6.0 <sup>b</sup> ± 1.5	4.5 <sup>d</sup> ± 2.5	5.3 <sup>c</sup> ± 2.0	5.4 <sup>b</sup> ± 1.7	5.2 <sup>b</sup> ± 2.3	5.5 <sup>bc</sup> ± 1.8	5.5 <sup>bc</sup> ± 1.6	5.6 <sup>b</sup> ± 2.0
<b>PVChS</b>	5.5 <sup>b</sup> ± 2.1	6.4 <sup>ab</sup> ± 1.5	4.5 <sup>d</sup> ± 2.2	5.9 <sup>b</sup> ± 1.9	6.6 <sup>a</sup> ± 1.3	5.2 <sup>b</sup> ± 2.2	5.8 <sup>b</sup> ± 1.8	6.1 <sup>b</sup> ± 1.7	5.5 <sup>b</sup> ± 1.8

Data are average ± SD. Values in the same column with differing superscripts are significantly different (LSD, p < 0.05).



**Figure 1.** Dose-response curves for the inhibition of protein glycation by the ChS fraction, autoclaved PR and PV purees (○) and their formulation with the ChS fraction (●). The ChM and ChL fractions behaved similarly to the ChS fraction.



**Figure 2.** Temperature (●) and 1/D values (○) for the target microorganism *Alicyclobacillus acidoterrestris* of tomato puree during microwave treatment