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

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Running title: Grape skin as ingredient for tomato puree

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ABSTRACT

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

KEYWORDS

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

1. Introduction

The food industry is facing the challenge of developing new foods having increased health benefits and meeting consumers' appreciation. In fact, with the surge in the incidence of cardiovascular diseases, cancer and type-2 diabetes, there is a need to develop new dietary strategies, especially with reference to the potential health properties of underutilized by-products of food processing (Schieber, Stintzing, & Carle, 2001; Hokayem et al., 2013~~Rockenbach, Rodrigues, Gonzaga, Caliari, Genovese, Gonçalves, & Fett, 2011~~). Grape (*Vitis vinifera*) pomace, the by-product of winemaking, is a bioresource available on large-scale as grape constitutes one of the main fruit crops in the world. Grape pomace contains both phenolics and dietary fibres, thus it can be referred to as "antioxidant dietary fibre". Because of the close relationship between antioxidant and dietary fibre and their common fate in the gut, it has been proposed that these food components have a joint role in prevention of human diseases (Perez-Jimenez et al., 2008). ~~Grape and wine phenolics have been demonstrated to inhibit human low-density lipoprotein oxidation *in vitro*.~~ *In vivo* studies on human adults have demonstrated that grape pomace has a positive effect in the prevention of cardiovascular diseases (Perez-Jimenez et al., 2008~~; Saura-Calixto et al., 2010~~). ~~Grape skin extracts from *Vitis rotundifolia* and *Vitis vinifera* can also inhibit fructose- and methylglyoxal-mediated protein glycation *in vitro*, thus having a potential role in preventing hyperglycaemia's complications (Farrar, Hartle, Hargrove, Greenspan, 2007; Sri Harsha, Gardana, Simonetti, Spigno, & Lavelli, 2013).~~ The anti-diabetic efficiency of ~~An anti-diabetes effect has been demonstrated when g~~grape polyphenols ~~derived extracts with high amounts of proanthocyanidins~~ was~~ere~~ tested ~~supplemented~~ in type-2 diabetic patients~~to the diet of high fructose fed rats~~, resulting in improved insulin resistance and suppressed oxidative stress (Hokayem et al., ~~Dandona, Aljada, Chaudhuri, Mohanty, & Garg,~~ 2013~~05~~).

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

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

94 **2. Materials and methods**

95 *2.1. Chemicals*

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

101 *2.2. Grape skins*

102 Grape pomace samples of the Chardonnay (Ch) variety were kindly provided by a winery located in
103 Northern Italy. At the winery, Ch grapes were pressed with separation of grape solids and must. Then grape
104 stalks were separated with a mechanical destemming and the remaining material was sieved (with a 5 mm
105 sieve) to separate the skins from the seeds and frozen to inhibit microbial growth. The skins were transported
106 frozen to the lab, dried at 50 °C for about 8 h. The powders obtained were sieved by using the Octagon
107 Digital sieve shaker (Endecotts L.t.d., United Kingdom), with three certified sieves (openings: 125, 250 and
108 500μm), under continuous sieving for 10 min at amplitude 8. Three fibrous fractions having different
109 particle sizes were collected, namely: ChL (250μm < ChL ≤ 500μm), ChM (125μm < ChM ≤ 250μm) and
110 ChS (ChS ≤ 125μm). These fractions were stored under vacuum, in the dark, at 4 °C.

111 *2.3. Tomato puree*

112 Two tomato puree samples, namely PV and PR were provided by Conserve Italia Soc. Coop. (San Lazzaro di
113 Savena, Italy). At the industrial plant, tomatoes were homogenized and heated to approximately 95 °C by
114 steam injection to inactivate endogenous enzymes (hot-break). The homogenate was then passed hot through
115 a 0.5 mm-screen (PV) or a 1 mm-screen (PR) pulper/finisher to remove seeds and skin fragments and

deareated under vacuum. The finished purees were then concentrated at 80 °C and under reduced atmospheric pressure using a tubular heat exchanger (the final moisture contents were 89.1 ± 0.2 and 89.8 ± 0.2 for PV and PR, respectively). The purees were then aseptically stored in tank under nitrogen for 6 months before bottling. After bottling, the purees were autoclaved at 115 °C for 5.5 min.

2.4. Preparation of the fortified tomato purees

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 purees. Each puree was filled into different glass bottles (250 mL capacity). A set of the bottled fortified purees was then submitted to microwave heating (8 min at 900 watt). During heating, the temperature of the tomato puree was monitored continuously by using a thermocouple set in the geometric centre of one of the bottles (the slowest heating point).

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

$$D = D_{ref} * 10^{(Tref-T)/z}$$

where for the target microorganism, D_{ref} = 1.5 min, T_{ref} = 95 °C and z = 7°C (Bevilacqua & Corvo, 2011). The 1/D values were then plotted as a function of time and the resulting curves were then integrated to evaluate the total decimal reductions (Silva & Gibbs, 2004). Microwave conditions were then chosen in order to achieve 6D for the target microorganism.

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

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

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

2.6. Sample extraction

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

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

2.7. Polyphenol analysis by HPLC-DAD

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

2.8. Proanthocyanidin content

Proanthocyanidin content was analysed as described previously (Porter, Hrstich, & Chan, 1986~~Sri Harsha et al., 2013~~). Briefly, for evaluation of soluble proanthocyanidins 1 mL of the sample extract (opportunely diluted with 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 of 2% $NH_4Fe(SO_4)_2.12\ H_2O$ in 2M HCl. For evaluation of insoluble proanthocyanidins, 10 mg of the extraction residue was weighted in quadruplicate and added to 20 mL methanol, 120 mL *n*-butanol:HCl (95:5, v/v) and 4 mL of 2% $NH_4Fe(SO_4)_2.12\ H_2O$ in 2M HCl. Hydrolysis was carried out at 95 °C for 40

min. The reaction mixtures were cooled and the absorbance was recorded at 550 nm on a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremella, Italy) against a blank made as for the sample but incubated at room temperature. For each sample extract, 2 - 4 dilutions were assessed in duplicate. Proanthocyanidin amount was determined using 0.1736 (mg/mL) as conversion factor (Sri Harsha, Gardana, Simonetti, Spigno, & Lavelli, 2013) and expressed as grams per kilogram of dry product.

2.9. Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed as described previously (Sri Harsha et al., 2013). Briefly, FRAP reagent was 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 in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3$. The reaction mixture contained 0.4 mL of sample extracts opportunely diluted with methanol:water:formic acid (70:29.9:0.1, v/v/v) and 3 mL of FRAP reagent. The absorbance at 593 nm was evaluated on a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremella, Italy) after 4 min of incubation at 37 °C against a blank with no extract addition. For each sample extract, 2 - 4 dilutions were assessed in duplicate. A methanolic solution of $FeSO_4 \cdot 7H_2O$ was used for calibration. Results were expressed as millimoles of Fe(II) sulfate equivalents per kilogram of dry product.

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

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

197 Dose-response curves were built reporting % inhibition of fructose-induced glycation of BSA as a function
198 of sample or catechin concentration. % Inhibition was calculated as: $100-100*(FL_s-FL_b)/(FL_c-FL_b)$,
199 where FL_s is the fluorescence intensity of the mixture with the sample extract or with catechin, FL_b is the
200 fluorescence intensity of the blank (BSA alone) and FL_c is the fluorescence intensity of the control mixture.
201 Results were expressed as millimoles of catechin equivalents (CE) per kilogram of product.

202 *2.11. Liking test*

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

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

220 *2.12. Statistical analysis of data*

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

Liking data (overall liking, liking for colour and texture) from consumers were independently submitted to a two-way ANOVA model, assuming sample and subject as main effects, by performing LSD ($p < 0.05$). Overall liking data expressed by all 86 subjects were analysed by means of an Internal Preference Map for explorative purposes. A visually oriented approach, based on the inspection of loading plot, was used for subject clustering and Y-axis was set as limit between consumer segments. Liking data expressed by Cluster 1 and Cluster 2 were independently treated with a two-way ANOVA model, with LDS ($p \leq 0.05$). Liking data were analyzed using FIZZ Calculations software, version 2.46A (Biosystèmes, Courtenon, France).

3. Results and discussion

3.1. Product and process design

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

The composition of Ch skins and tomato purees were first characterized in order to choose the level of addition. In Ch skins, dietary fibre content was 50.5%. Protein, carbohydrate (fructose and glucose), fat, ash and moisture contents were: 10.0 ± 0.6, 16.2 ± 0.2, 5.7 ± 1.6, 4.1 ± 0.7 and 4.0 ± 0.1 g/100g, respectively. Insoluble proanthocyanidin contents, analysed after depolymerisation with *n*-butanol/HCl, were 10.6 ± 2 in the ChL fraction and 13.9 ± 1 in both the ChM and Ch S fractions, respectively. This could be due to a lower hydrolysis yield in the ChL fraction. The total amount of flavonols, namely: quercetin 3-O glucuronide, quercetin 3-O glucoside, quercetin, kaempferol 3-O galactoside, kaempferol 3-O glucuronide, kaempferol 3-O glucoside and kaempferol was about 600 mg/kg (Tables 1, 2). Soluble proanthocyanidin content of the ChL fraction was 20700 ± 42 mg/kg (Table 3). Higher proanthocyanidin contents were observed in the ChM

and ChS fractions. The increased surface/solvent ratio likely increased extraction efficiency of these compounds, which are strongly associated with the fibre (Perez-Jimenez et al., 2008). FRAP values were > 170 ± 26 mmolFe eq. (II)/kg, which is two order of magnitude higher than that observed in tomato products (García-Valverde, Navarro-González, García-Alonso, & Jesús Periago, 2013). The highest FRAP value was observed in the ChS fraction.

The ability of the Ch fractions to inhibit protein glycation was analysed by an *in vitro* BSA/fructose model system (Figure 1). This system was used to simulate protein glycation that occurs at an accelerated rate *in vivo* under non-physiological conditions, accounting for some of the complications of hyperglycaemia and diabetes (Saraswat, Reddy, Muthenna, & Reddy, 2009). There is a continuous search for novel inhibitors of protein glycation that could be helpful to prevent advanced-glycation-endproducts~~AGEs~~-associated diseases and with the potential to be used as functional food ingredients (Farrar, Hartle, Hargrove, & Greenspan, 2007; Saraswat et al., 2009; Sri Harsha et al., 2013; Wu et al., 2013).

In this study, ~~Grape phenolics have been shown to effectively inhibit protein glycation in vitro (Sri Harsha et al., 2013), most likely by acting both as radical scavengers, metal chelators, and carbonyl trapping agents. This process occurs at an accelerated rate in vivo under non-physiological conditions, accounting for some of the complications of hyperglycaemia and diabetes (Dearlove, Greenspan, Hartle, Swanson, & Hargrove, 2008). In fact, the amino groups of some mammalian proteins react non-enzymatically with both glucose and fructose, in vivo. Subsequent reactions may result in the formation of cross-linked, fluorescent, protein derivatives (AGE) which damage their functionality. Hence, in this study the anti-glycation activity of the Ch fractions was analysed (Figure 1).~~ a~~A~~ dose-response effect was observed *in vitro* for the anti-glycation activity of the Ch fractions. Phenolics are known to ~~ean~~ inhibit protein glycation by acting as radical scavengers, metal chelators and carbonyl trapping agents (Dearlove, Gre~~r~~enspan, Hartle, Swanson, & Hargrove, 2008; Wu et al., 2013). Hence, i~~I~~n terms of catechin equivalents, the anti-glycation effectiveness was 100 ± 15 mmol/kg for all the Ch fractions.

In PV and PR tomato purees percent contents of major components were: 4.9 ± 0.1 and 5.7 ± 0.1 for 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 ± 0.02 for fat, respectively. The main flavonoids in tomato purees were rutin and naringenin (Tables 1, 2).

278 Before heat treatments, flavonol contents (sum of quercetin derivatives) were in the range of 52 - 72 mg/kg
279 and flavanone contents (naringenin) were in the range of 14 - 51 mg/kg. The PV and PR purees had a
280 medium-high flavonol and flavonone contents in comparison with previous results obtained on twenty
281 cultivars of fresh tomatoes extracted with an optimized procedure (Li, Deng, Wuc, Liu, Loewen, & Tsao,
282 2012). FRAP values of the PR and PV purees were 1.97 ± 0.14 and 2.68 ± 0.22 mmol Fe(II) eq./kg,
283 respectively (Table 3). Similar values were observed by Garcia-Valverde et al. (2013) in various cultivars of
284 tomatoes destined to industrial processing. The unfortified tomato purees showed a dose-dependent anti-
285 glycation activity *in vitro*, with anti-glycation effectiveness of 2.97 ± 0.15 and 2.82 ± 0.40 mmol catechin
286 eq./kg for PV and PR, respectively. These values were much lower than that of the Ch fractions (Figure 1).
287 The level of Ch/tomato addition was then chosen to have 3% fibre content in the final products (3.2 g of
288 grape skins added to 96.8 g of tomato puree). Hence, the purees can be labelled as "fibre-source" according
289 to the EC Regulation 1924/2006. Furthermore, in a human study, Pérez-Jiménez et al. (2008) have
290 demonstrated that the intake of grape antioxidant dietary fibre (5.25 g of dietary fibre and 1.06 g of
291 proanthocyanidins in the supplemented dose) significantly reduces the biomarkers of cardiovascular risk.
292 Based on Ch fibre and proanthocyanidin contents, a 175 g-dose of the fortified purees (that could be a daily
293 dose in the Mediterranean diet) can provide 5.25 g of dietary fibres and around 1 g of proanthocyanidins
294 (soluble + insoluble). Hence, positive *in vivo* effects of these purees can be hypothesised. However, the food
295 matrix is more complicated than grape skins, therefore an effect of the matrix on food components'
296 bioavailability cannot be ruled out.
297 The incorporation of grape skin derived fractions into a liquid food, such as tomato puree, requires the design
298 of an effective heat treatment. The pH values of these products were in the range 4.1 – 4.3. To achieve
299 pasteurization of low-pH foods, *Alicyclobacillus acidoterrestris* has been proposed as a process target. It is a
300 thermoacidophilic non-pathogenic and sporeforming bacterium, which has been found in fruit juices,
301 including tomato puree and white grape juice (Silva & Gibbs, 2004). It is often the most heat resistant
302 microorganism among the most common spoilage microorganisms found in these foods. The heating
303 conditions were then selected to achieve 6D-reduction of the target microorganism (Figure 2), which is
304 considered effective (Silva & Gibbs, 2004). This treatment is representative for an optimized continuous

industrial treatment. In parallel, tomato purees were also autoclaved to study the effects of an intensive heat-treatment on the antioxidant components.

3.2. Processing effects on antioxidant components

Flavonols and naringenin were not affected by microwave treatment (not shown). Similarly, Capanoglu, Beekwilder, Boyacioglu, Hall, & De Vos (2008) found that pasteurization at 98 °C does not change rutin and naringenin contents of tomato. Upon autoclave treatment, quercetin and kaempferol glycosides and glucuronides decreased by less than 30% (Tables 2-3). Conversely, the corresponding aglycones increased. The recovery was ~100% when the sum of quercetin derivatives was considered and ~90% for the sum of kaempferol derivatives. This means that the prevalent modification occurring during autoclave treatment was deglycosylation. Interestingly, Stewart, Bozonnet, Mullen, Jenkins, Lean, & Crozier (2000) found that in contrast to fresh tomatoes, most tomato-based products contained significant amounts of free flavonols and concluded that the accumulation of quercetin in juices, purees, and paste may be a consequence of enzymatic hydrolysis of rutin and other quercetin conjugates during pasteurization. Instead, enzymatic activities can be ruled out in this study, due to the intense heating during autoclave treatment. Rohn, Buchner, Driemel, Rauser, & Kroh (2007) found that during the roasting process of model flavonols (180°C, 60 min), quercetin glycosides are degraded and produce quercetin as the major degradation product. Quercetin is not sensitive to degradation under such conditions and therefore it has to be regarded as a stable end-product. Naringenin content was above 88%, with lower retention for the unfortified purees than for the fortified purees.

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

expected. They did not analyse the unheated samples and concluded that the decreases could be either due to the interactions of proanthocyanidins with food components to produce insoluble molecules, or due to thermal degradation.

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

Microwave treatment had no effect on the proanthocyanidin contents and FRAP values of any of the mixtures considered. On the contrary, upon autoclave treatment, proanthocyanidin contents increased in the fortified puree with respect to the raw mixtures. The parallel increased FRAP values in the fortified purees can be related to the rise in the content of proanthocyanidins. The intense thermal treatment could have weakened the binding between proanthocyanins and other food components (Pinelo et al., 2006), or it could have promoted proanthocyanidin depolymerisation (Chamorro, Goni, Viveros, Hervert-Hernandez, & Brenes, 2012) and thus increased proanthocyanidins' solubility.

The dose-dependent anti-glycation activity *in vitro* of the fortified purees showed much higher effectiveness than the controls, corresponding to 8.1 ± 0.1 and 7.2 ± 0.1 mmol catechin eq./kg for PV and PR, respectively (Figure 1). These new purees have the potential ability to act ~~could therefore play a role~~ as dietary factors in the prevention of hyperglycaemia's complications.

3.3. Consumers' preferences

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

359 The average liking ratings expressed by all 86 consumers for overall acceptability, colour and texture of the
360 analysed tomato purees are reported in Table 4. Consumers highly rated the unfortified purees in terms of
361 overall acceptability (6.9 ± 1.8 for PR; 6.7 ± 1.9 for PV), liking for colour (7.4 ± 1.7 for PR; 7.2 ± 1.7 for
362 PV) and texture (7.0 ± 1.8 for PR; 6.8 ± 1.7 for PV). The addition of the Ch fractions to the tomato purees
363 decreased the ratings for all the sensory parameters ($p < 0.05$). This effect could be explained taking into
364 account that consumers were familiar with the unfortified samples (commercially available regular tomato
365 purees), but they had not been previously exposed to the fortified samples. As it is known, the level of
366 familiarity for a food influences powerfully its acceptability by the consumer and repeated exposure to the
367 taste of a food can increase liking for it (Wardle & Cooke, 2008).
368 Regarding the overall liking, average ratings of the fortified samples corresponded approximately to the
369 central value of the scale (5 = neither like nor dislike). PVChL, PVChM and PVChS were significantly
370 preferred (5.3 ± 1.9) than PRChL (4.6 ± 2.1) ($p < 0.05$). Concerning the texture, as the particle size
371 decreased, liking increased. This tendency was more evident for the PV formulations. Average ratings of
372 liking for colour were all above the central value (5). The only significant difference in colour was observed
373 for PVChS, which was rated higher than the PR formulations.
374 The overall liking data expressed by all 86 subjects for the fortified samples were then submitted to the
375 principal component analysis in order to obtain an internal preference map (data not shown). The first two
376 principal components of the model explained the 48% of the total variance, 28% and 21% the first and the
377 second dimensions, respectively. A visually oriented approach, based on the inspection of loading plot, was
378 used for subject clustering and segmentation was performed according to whether consumer loadings lie on
379 the left or right side of the Y-axis set as limit (Næs et al., 2010). Two groups of consumers were obtained:
380 the first consisting of 46 subjects (53.5%) positioned on the left side of the map (Cluster 1); the second
381 consisting of 40 subjects (46.5%) positioned on the right side of the map (Cluster 2). Liking data expressed
382 by subjects belonging to Cluster 1 and Cluster 2 for all samples were independently treated with a two-way
383 ANOVA model (samples and subjects as factors), with Fisher's LDS post hoc test considered significant for
384 $p \leq 0.05$ (Table 4). As expected, both clusters provided similar average ratings of the three sensory
385 parameters evaluated for the unfortified PR and PV purees, confirming the results obtained by the total of

subjects (Table 4). Focusing on the fortified purees, different results were obtained by the two clusters. In terms of overall acceptability, Cluster 1 preferred the purees fortified with the ChM and ChS fibrous fractions both for the PR and PV formulations. The highest rating was observed for PVChS (6.4 ± 1.5), which was not significantly different to that of the PV puree (7.0 ± 1.8). For Cluster 1, liking for texture decreased as the particle size of the added fibrous fraction increased, as noticed by the preference of all consumers. Again, in terms of texture PVChS reached the highest average value among the fortified purees, which was the same as that observed for PV. The good ratings given for the ChS fraction were confirmed also in terms of liking for colour.

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

4. Conclusions

Tomato purees fortified with Ch fractions could be positioned noticeably above with respect to the conventional purees in terms of potential health benefits. Indeed, tomato is rich in lycopene but it does not contain proanthocyanidins and hence the addition of grape pomace ingredients could overall improve its antioxidant and anti-glycation properties *in vitro*. ~~–~~Upon heat-stabilization, phenolic contents and reducing capacity remained much higher ~~–~~in all the fortified purees than in the controls. Increase in anti-glycation activity was also observed in the fortified formulations~~.~~, ~~leading to the potential use of these food products in prevention of hyperglyceamia's complications.~~

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

The overall results indicate that grape skins could be used as ingredients for the development of new tomato 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		
ChL							111^{e}	±	2	98^{b}	±	5	13.8^{e}	±	0.6	223^{c}	±	8
ChM							114^{e}	±	4	92^{b}	±	1	13.6^{e}	±	0.6	220^{c}	±	5
ChS							115^{e}	±	1	97^{b}	±	1	12.8^{e}	±	0.8	225^{c}	±	3
PR	3.28^{a}	±	0.01 (72)	42.10^{b}	±	0.09 (91)							0.35^{a}	±	0.01	45.73^{a}	±	0.12 (88)
PRChL	3.10^{a}	±	0.03 (76)	36.30^{a}	±	1.52 (87)	2.50^{ab}	±	0.03 (73)	2.50^{a}	±	0.01 (87)	4.52^{b}	±	0.16 (1139)	49.12^{a}	±	1.76 (100)
PRChM	2.92^{a}	±	0.08 (71)	36.10^{a}	±	0.05 (86)	2.27^{a}	±	0.03 (67)	2.78^{a}	±	0.03 (97)	5.41^{bc}	±	0.42 (1364)	49.48^{a}	±	0.61 (103)
PRChS	3.80^{a}	±	0.28 (91)	39.00^{a}	±	0.00 (93)	2.64^{bc}	±	0.31 (78)	2.81^{a}	±	0.08 (98)	4.40^{b}	±	0.78 (1109)	52.65^{a}	±	1.45 (102)
PV	10.71^{b}	±	0.44 (81)	55.89^{d}	±	0.34 (95)							0.85^{a}	±	0.01	67.45^{b}	±	0.79 (93)
PVChL	10.92^{b}	±	1.91 (85)	53.59^{c}	±	0.05 (94)	2.93^{cd}	±	0.18 (80)	2.97^{a}	±	0.96 (97)	6.77^{cd}	±	0.04 (1590)	77.17^{b}	±	3.14 (100)
PVChM	10.59^{b}	±	0.62 (82)	52.42^{c}	±	1.07 (92)	3.05^{d}	±	0.29 (84)	2.88^{a}	±	0.74 (94)	6.67^{cd}	±	0.85 (1567)	75.60^{b}	±	3.57 (98)
PVChS	10.49^{b}	±	0.96 (82)	53.61^{c}	±	0.98 (94)	3.05^{d}	±	0.03 (84)	3.03^{a}	±	0.18 (99)	7.10^{d}	±	0.99 (1669)	77.28^{b}	±	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		
ChL	77^{b} ± 7	313^{b} ± 6	16.9^{b} ± 1.5	407^{b} ± 12		
ChM	70^{b} ± 2	304^{b} ± 5	16.7^{b} ± 0.4	391^{b} ± 8		
ChS	67^{b} ± 7	297^{b} ± 20	18.2^{b} ± 1.3	382^{b} ± 28		
PR					11.37^{a} ± 0.64	(81)
PRChL	1.58^{a} ± 0.03 (77)	6.93^{a} ± 0.16 (76)	2.15^{a} ± 0.08 (418)	10.66^{a} ± 0.07 (91)	11.13^{a} ± 0.03	(88)
PRChM	1.74^{a} ± 0.02 (84)	6.64^{a} ± 0.21 (73)	2.04^{a} ± 0.14 (397)	10.41^{a} ± 0.10 (89)	10.61^{a} ± 0.70	(84)
PRChS	1.66^{a} ± 0.03 (81)	6.38^{a} ± 0.02 (70)	1.81^{a} ± 0.01 (352)	9.84^{a} ± 0.01 (85)	11.72^{a} ± 0.23	(93)
PV					45.53^{b} ± 0.72	(90)
PVChL	2.10^{a} ± 0.49 (95)	6.81^{a} ± 1.45 (70)	1.79^{a} ± 0.05 (325)	10.70^{a} ± 0.71 (86)	45.99^{b} ± 0.89	(94)
PVChM	2.02^{a} ± 0.27 (91)	7.22^{a} ± 0.46 (74)	2.23^{a} ± 0.02 (404)	11.46^{a} ± 0.22 (92)	44.60^{b} ± 0.36	(91)
PVChS	1.97^{a} ± 0.12 (89)	7.23^{a} ± 0.36 (74)	1.95^{a} ± 0.04 (354)	11.15^{a} ± 0.17 (89)	44.63^{b} ± 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
ChL	20700^{c} ± 42			170^{d} ± 25		
ChM	25300^{d} ± 28			207^{e} ± 26		
ChS	27000^{e} ± 14			217^{f} ± 24		
PR				$1.97^{a\ x}$ ± 0.14	$2.29^{a\ x}$ ± 0.14	$2.15^{a\ x}$ ± 0.11
PRChL	$352^{a\ x}$ ± 63 (53)	$353^{a\ x}$ ± 3 (53)	$406^{a\ y}$ ± 1 (61)	$4.74^{abc\ x}$ ± 0.04 (64)	$4.55^{c\ x}$ ± 0.03 (61)	$5.34^{b\ y}$ ± 0.27 (72)
PRChM	$445^{b\ x}$ ± 23 (55)	$399^{ab\ x}$ ± 4 (49)	$506^{bc\ y}$ ± 10 (62)	$5.25^{bc\ x}$ ± 0.55 (61)	$5.30^{d\ x}$ ± 0.09 (62)	$6.25^{c\ y}$ ± 0.35 (73)
PRChS	$482^{b\ x}$ ± 14 (56)	$450^{bc\ x}$ ± 11 (52)	$555^{cd\ y}$ ± 3 (64)	$5.82^{c\ x}$ ± 0.12 (65)	$6.04^{e\ x}$ ± 0.09 (68)	$6.91^{de\ y}$ ± 0.10 (78)
PV				$2.68^{ab\ x}$ ± 0.22	$2.60^{b\ x}$ ± 0.18	$2.75^{a\ x}$ ± 0.15
PVChL	$355^{a\ x}$ ± 6 (54)	$348^{a\ x}$ ± 1 (53)	$455^{ab\ xy}$ ± 81 (69)	$5.16^{bc\ x}$ ± 0.04 (64)	$5.35^{d\ x}$ ± 0.15 (66)	$6.27^{cd\ y}$ ± 0.38 (77)
PVChM	$446^{b\ x}$ ± 17 (55)	$411^{abc\ x}$ ± 45 (51)	$629^{dc\ y}$ ± 65 (78)	$5.89^{c\ x}$ ± 0.07 (63)	$5.93^{e\ x}$ ± 0.04 (64)	$6.95^{e\ y}$ ± 0.23 (75)
PVChS	$487^{b\ x}$ ± 35 (56)	$468^{c\ x}$ ± 44 (54)	$668^{e\ y}$ ± 19 (77)	$6.35^{c\ x}$ ± 0.30 (66)	$6.02^{e\ x}$ ± 0.18 (63)	$7.50^{e\ y}$ ± 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
PR	6.9 ^{a} ± 1.8	6.9 ^{a} ± 1.5	7.0 ^{a} ± 2.1	7.0 ^{a} ± 1.8	6.8 ^{a} ± 2.0	7.1 ^{a} ± 1.6	7.4 ^{a} ± 1.7	7.4 ^{a} ± 1.8	7.5 ^{a} ± 1.6
PRChL	4.6 ^{d} ± 2.1	3.6 ^{d} ± 1.7	5.7 ^{bc} ± 2.0	4.3 ^{e} ± 2.3	3.5 ^{d} ± 1.9	5.3 ^{b} ± 2.4	5.3 ^{c} ± 1.8	4.7 ^{d} ± 1.7	6.0 ^{b} ± 1.7
PRChM	4.8 ^{cd} ± 2.1	4.7 ^{c} ± 1.9	5.0 ^{cd} ± 2.4	4.9 ^{cd} ± 2.1	4.7 ^{c} ± 1.9	5.3 ^{b} ± 2.3	5.3 ^{c} ± 1.7	5.1 ^{cd} ± 1.5	5.7 ^{b} ± 1.8
PRChS	5.0 ^{bcd} ± 2.1	5.1 ^{c} ± 1.9	5.0 ^{cd} ± 2.3	5.0 ^{cd} ± 2.1	4.9 ^{bc} ± 1.8	5.1 ^{b} ± 2.4	5.3 ^{c} ± 1.7	5.1 ^{cd} ± 1.6	5.6 ^{b} ± 1.8
PV	6.7 ^{a} ± 1.9	7.0 ^{a} ± 1.8	6.3 ^{ab} ± 1.9	6.8 ^{a} ± 1.7	7.0 ^{a} ± 1.6	6.7 ^{a} ± 1.7	7.2 ^{a} ± 1.7	7.4 ^{a} ± 1.8	7.1 ^{a} ± 1.7
PVChL	5.3 ^{b} ± 1.9	5.2 ^{c} ± 1.9	5.5 ^{c} ± 2.0	4.7 ^{de} ± 2.3	4.6 ^{c} ± 2.3	4.8 ^{b} ± 2.4	5.6 ^{bc} ± 1.8	5.4 ^{c} ± 1.8	5.9 ^{b} ± 1.7
PVChM	5.3 ^{bc} ± 2.1	6.0 ^{b} ± 1.5	4.5 ^{d} ± 2.5	5.3 ^{c} ± 2.0	5.4 ^{b} ± 1.7	5.2 ^{b} ± 2.3	5.5 ^{bc} ± 1.8	5.5 ^{bc} ± 1.6	5.6 ^{b} ± 2.0
PVChS	5.5 ^{b} ± 2.1	6.4 ^{ab} ± 1.5	4.5 ^{d} ± 2.2	5.9 ^{b} ± 1.9	6.6 ^{a} ± 1.3	5.2 ^{b} ± 2.2	5.8 ^{b} ± 1.8	6.1 ^{b} ± 1.7	5.5 ^{b} ± 1.8

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