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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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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 optimised microwave-treatment achieving 6D-reduction of the target microorganism (*Alicylobacillus acidoterrestris*). In the fortified tomato purees, the solubility of proanthocyanidins decreased, but 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 fraction showing maximum ratings.

1. Introduction

The food industry is facing the challenge of developing new foods with 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 (Hokayem et al., 2013; Schieber, Stintzing, & Carle, 2001).

Grape (*Vitis vinifera*) pomace, the by-product of winemaking, is a bioresource available on a 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". Due to 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). *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). The anti-diabetic efficiency of grape polyphenols was tested in type-2 diabetic patients, resulting in improved insulin resistance and suppressed oxidative stress (Hokayem et al., 2013).

These results have boosted the use of grape pomace as an ingredient in new functional foods, such as bread (Mildner-Szkudlarz, Zawirska-Wojtasiak, Szwengiel, & Pacynski, 2011), fish products (Pazos, Gallardo, Torres, & Medina, 2005; Ribeiro et al., 2013), meat products (Sayago-Ayerdi, Brenes, & Goni, 2009) and yoghurt (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).

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

2. Materials and methods

2.1. 1. Chemicals

Standards of catechin, quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-glucuronide, quercetin 3-*O*-glucoside, kaempferol 3-*O*-galactoside, kaempferol 3-*O*-glucuronide, kaempferol 3-*O*-glucoside, quercetin, kaempferol and naringenin were purchased from Extrasynthese (Lyon, France). The integrated total dietary fibre

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assay procedure kit was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All other chemicals were purchased from Sigma–Aldrich Italia (Milan, Italy).

2.2. Grape skins

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

2.3. Tomato puree

Two tomato puree samples, namely PV and PR were provided by Conserve Italia Soc. Coop. (San Lazzaro di Savena, Italy). At the industrial plant, tomatoes were homogenised and heated to approximately 95 °C by steam injection to inactivate endogenous enzymes (hot-break). The homogenate was then passed hot through 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 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 a 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

A 3.2 g sample 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 W). 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 pasteurisation 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:

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

where for the target microorganism, D_{ref} = 1.5 min, T_{ref} = 95 °C and z = 7 °C (Bevilacqua & Corbo, 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 6*D* 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 by Lavelli, Pagliarini, Ambrosoli, Minati, and 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 10,000*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). The same extraction method for the grape skins was used for the tomato purees. Extracts were stored at −20 °C until analytical characterisation.

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 × 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 separation was performed by 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 product.

2.8. Proanthocyanidin content

Proanthocyanidin content was analysed as described previously (Porter, Hrstich, & Chan, 1986). 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% $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2 M HCl. For the 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% $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2 M 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 UV-DEC-610 spectrophotometer (Jasco Europe, Cremella, Italy) against a blank, which was made the same as the samples 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 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) equivalents per kilogram of 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 by Lavelli and 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 10,000*g* for 10 min. The supernatant was discarded and the precipitate was re-dissolved in 1.6 ml of phosphate buffer and analysed for fluorescence on a Perkin-Elmer LS 55 Luminescence Spectrometer (Perkin-Elmer Italia, Monza, Italy) with an excitation/emission wavelength pair $\lambda = 370/440$ 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. Dose–response curves were built reporting % inhibition of fructose-induced glycation of BSA as a function of sample or catechin concentration. % Inhibition was calculated as: $100 - 100 * (FL_s - FL_b)/(FL_c - FL_b)$,where FL_s is the fluorescence intensity of the mixture with the sample extract or with catechin, FL_b is the fluorescence intensity of the blank (BSA alone) and FL_c is the fluorescence intensity of the control mixture. Results were expressed as millimoles of catechin equivalents (CE) per kilogram of product.

2.11. Liking test

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

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

2.12. Statistical analysis of data

Experimental data were analysed by one-way ANOVA using the least significant difference (LSD, $p \leqslant 0.05$) as a multiple range test and by linear regression analyses using Statgraphics 5.1 (STCC Inc.; Rockville, 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 ($p \leqslant 0.05$). Liking data were analysed using FIZZ Calculations software, version 2.46A (Biosystèmes, Courtenon, France).

3. Results and discussion

3.1. Product and process design

The increase in the 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 (Ribeiro et al., 2013), less than 0.5 in meat products (Sayago-Ayerdi et al., 2009) and less than 0.18 mm for addition in yoghurt (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 characterised 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/100 g, respectively. Insoluble proanthocyanidin contents, analysed after depolymerisation with *n*-butanol/HCl, were 10.6 ± 2 in the ChL fraction and 13.9 ± 1 g/100 g in both the ChM and ChS 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 and 2). Soluble proanthocyanidin content of the ChL fraction was 20,700 ± 42 mg/kg

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			$111^{e} \pm 2$	$98^{b} \pm 5$	$13.8^{e} \pm 0.6$	$223^{c} \pm 8$
ChM			$114^{e} \pm 4$	$92^{b} \pm 1$	$13.6^{e} \pm 0.6$	$220^{c} \pm 5$
ChS			$115^{e} \pm 1$	$97^{b} \pm 1$	$12.8^{e} \pm 0.8$	$225^{c} \pm 3$
PR	$3.28^{a} \pm 0.01$ (72)	$42.10^{b} \pm 0.09$ (91)			$0.35^{a} \pm 0.01$	$45.73^{a} \pm 0.12$ (88)
PRChL	$3.10^{a} \pm 0.03$ (76)	$36.30^{a} \pm 1.52$ (87)	$2.50^{ab} \pm 0.03$ (73)	$2.50^{a} \pm 0.01$ (87)	$4.52^{b} \pm 0.16$ (1139)	$49.12^{a} \pm 1.76$ (100)
PRChM	$2.92^{a} \pm 0.08$ (71)	$36.10^{a} \pm 0.05$ (86)	$2.27^{a} \pm 0.03$ (67)	$2.78^{a} \pm 0.03$ (97)	$5.41^{bc} \pm 0.42$ (1364)	$49.48^{a} \pm 0.61$ (103)
PRChS	$3.80^{a} \pm 0.28$ (91)	$39.00^{a} \pm 0.01$ (93)	$2.64^{bc} \pm 0.31$ (78)	$2.81^{a} \pm 0.08$ (98)	$4.40^{b} \pm 0.78$ (1109)	$52.65^{a} \pm 1.45$ (102)
PV	$10.71^{b} \pm 0.44$ (81)	$55.89^{d} \pm 0.34$ (95)			$0.85^{a} \pm 0.01$	$67.45^{b} \pm 0.79$ (93)
PVChL	$10.92^{b} \pm 1.91$ (85)	$53.59^{c} \pm 0.05$ (94)	$2.93^{cd} \pm 0.18$ (80)	$2.97^{a} \pm 0.96$ (97)	$6.77^{cd} \pm 0.04$ (1590)	$77.17^{b} \pm 3.14$ (100)
PVChM	$10.59^{b} \pm 0.62$ (82)	$52.42^{c} \pm 1.07$ (92)	$3.05^{d} \pm 0.29$ (84)	$2.88^{a} \pm 0.74$ (94)	$6.67^{cd} \pm 0.85$ (1567)	$75.60^{b} \pm 3.57$ (98)
PVChS	$10.49^{b} \pm 0.96$ (82)	$53.61^{c} \pm 0.98$ (94)	$3.05^{d} \pm 0.03$ (84)	$3.03^{a} \pm 0.18$ (99)	$7.10^{d} \pm 0.99$ (1669)	$77.28^{b} \pm 3.15$ (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	$77^{b} \pm 7$	$313^{b} \pm 6$	$16.9^{b} \pm 1.5$	$407^{b} \pm 12$	
ChM	$70^{b} \pm 2$	$304^{b} \pm 5$	$16.7^{b} \pm 0.4$	$391^{b} \pm 8$	
ChS	$67^{b} \pm 7$	$297^{b} \pm 20$	$18.2^{b} \pm 1.3$	$382^{b} \pm 28$	
PR					$11.37^{a} \pm 0.64$ (81)
PRChL	$1.58^{a} \pm 0.03$ (77)	$6.93^{a} \pm 0.16$ (76)	$2.15^{a} \pm 0.08$ (418)	$10.66^{a} \pm 0.07$ (91)	$11.13^{a} \pm 0.03$ (88)
PRChM	$1.74^{a} \pm 0.02$ (84)	$6.64^{a} \pm 0.21$ (73)	$2.04^{a} \pm 0.14$ (397)	$10.41^{a} \pm 0.10$ (89)	$10.61^{a} \pm 0.70$ (84)
PRChS	$1.66^{a} \pm 0.03$ (81)	$6.38^{a} \pm 0.02$ (70)	$1.81^{a} \pm 0.01$ (352)	$9.84^{a} \pm 0.01$ (85)	$11.72^{a} \pm 0.23$ (93)
PV					$45.53^{b} \pm 0.72$ (90)
PVChL	$2.10^{a} \pm 0.49$ (95)	$6.81^{a} \pm 1.45$ (70)	$1.79^{a} \pm 0.05$ (325)	$10.70^{a} \pm 0.71$ (86)	$45.99^{b} \pm 0.89$ (94)
PVChM	$2.02^{a} \pm 0.27$ (91)	$7.22^{a} \pm 0.46$ (74)	$2.23^{a} \pm 0.02$ (404)	$11.46^{a} \pm 0.22$ (92)	$44.60^{b} \pm 0.36$ (91)
PVChS	$1.97^{a} \pm 0.12$ (89)	$7.23^{a} \pm 0.36$ (74)	$1.95^{a} \pm 0.04$ (354)	$11.15^{a} \pm 0.17$ (89)	$44.63^{b} \pm 0.01$ (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	$20700^{c} \pm 42$			$170^{d} \pm 25$		
ChM	$25300^{d} \pm 28$			$207^{e} \pm 26$		
ChS	$27000^{e} \pm 14$			$217^{f} \pm 24$		
PR				$1.97^{a\ x} \pm 0.14$	$2.29^{a\ x} \pm 0.14$	$2.15^{a\ x} \pm 0.11$
PRChL	$352^{a\ x} \pm 63$ (53)	$353^{a\ x} \pm 3$ (53)	$406^{a\ y} \pm 1$ (61)	$4.74^{abc\ x} \pm 0.04$ (64)	$4.55^{c\ x} \pm 0.03$ (61)	$5.34^{b\ y} \pm 0.27$ (72)
PRChM	$445^{b\ x} \pm 23$ (55)	$399^{ab\ x} \pm 4$ (49)	$506^{bc\ y} \pm 10$ (62)	$5.25^{bc\ x} \pm 0.55$ (61)	$5.30^{d\ x} \pm 0.09$ (62)	$6.25^{c\ y} \pm 0.35$ (73)
PRChS	$482^{b\ x} \pm 14$ (56)	$450^{bc\ x} \pm 11$ (52)	$555^{cd\ y} \pm 3$ (64)	$5.82^{c\ x} \pm 0.12$ (65)	$6.04^{e\ x} \pm 0.09$ (68)	$6.91^{de\ y} \pm 0.10$ (78)
PV				$2.68^{ab\ x} \pm 0.22$	$2.60^{b\ x} \pm 0.18$	$2.75^{a\ x} \pm 0.15$
PVChL	$355^{a\ x} \pm 6$ (54)	$348^{a\ x} \pm 1$ (53)	$455^{ab\ xy} \pm 81$ (69)	$5.16^{bc\ x} \pm 0.04$ (64)	$5.35^{d\ x} \pm 0.15$ (66)	$6.27^{cd\ y} \pm 0.38$ (77)
PVChM	$446^{b\ x} \pm 17$ (55)	$411^{abc\ x} \pm 45$ (51)	$629^{dc\ y} \pm 65$ (78)	$5.89^{c\ x} \pm 0.07$ (63)	$5.93^{e\ x} \pm 0.04$ (64)	$6.95^{e\ y} \pm 0.23$ (75)
PVChS	$487^{b\ x} \pm 35$ (56)	$468^{c\ x} \pm 44$ (54)	$668^{e\ y} \pm 19$ (77)	$6.35^{c\ x} \pm 0.30$ (66)	$6.02^{e\ x} \pm 0.18$ (63)	$7.50^{e\ y} \pm 0.45$ (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 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 $\geq 170 \pm 26$ mmolFe(II) eq./kg, which is two orders of magnitude higher than that observed in tomato products (García-Valverde, Navarro-González, García-Alonso, & 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 (Fig. 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-endproduct-associated

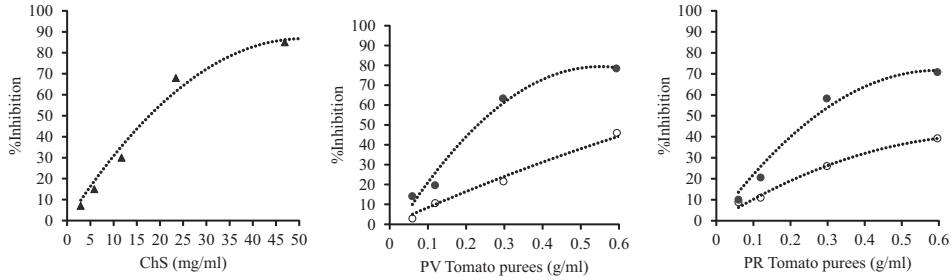

Fig. 1. Dose–response curves for the inhibition of protein glycation by the ChS fraction, autoclaved PR and PV purees (○) and their formulation with the ChS fraction (●). The ChM and ChL fractions behaved similarly to the ChS fraction.

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, a dose–response effect was observed *in vitro* for the anti-glycation activity of the Ch fractions. Phenolics are known to inhibit protein glycation by acting as radical scavengers, metal chelators and carbonyl trapping agents (Dearlove, Greenspan, Hartle, Swanson, & Hargrove, 2008; Wu et al., 2013). Hence, in terms of catechin equivalents, the anti-glycation effectiveness was 100 ± 15 mmol/kg for all the Ch fractions.

In PV and PR tomato purees, the percentage content 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 and 2). Before heat treatments, flavonol contents (sum of quercetin derivatives) were in the range of 52–72 mg/kg and flavanone contents (naringenin) were in the range of 14–51 mg/kg. The PV and PR purees had a medium–high flavonol and flavonone content when compared with previous results obtained on twenty cultivars of fresh tomatoes extracted with an optimised procedure (Li et al., 2012). FRAP values of the PR and PV purees were 1.97 ± 0.14 and 2.68 ± 0.22 mmol Fe(II) eq./kg, respectively (Table 3). Similar values were observed by García-Valverde et al. (2013) in various cultivars of tomatoes destined for industrial processing. The unfortified tomato purees showed a dose-dependent anti-glycation activity *in vitro* (Fig. 1), with anti-glycation effectiveness of 2.97 ± 0.15 and 2.82 ± 0.40 mmol catechin eq./kg for PV and PR, respectively. These values were much lower than that of the Ch fractions.

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

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

3.2. Processing effects on antioxidant components

Flavonols and naringenin were not affected by microwave treatment (data not shown). Similarly, Capanoglu, Beekwilder, Boyacioglu, Hall, and De Vos (2008) found that pasteurisation at 98 °C does not change rutin and naringenin contents in tomatoes. Upon autoclave treatment, quercetin and kaempferol glycosides and glucuronides decreased by less than 30% (Tables 2 and 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 et al. (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 pasteurisation. Instead, enzymatic activities can be ruled out in this study, due to the intense heating during autoclave treatment. Rohn, Buchner, Driemel, Rauser, and Kroh (2007) found that during the roasting process of model flavonols (180 °C, 60 min), quercetin glycosides were degraded and produced 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

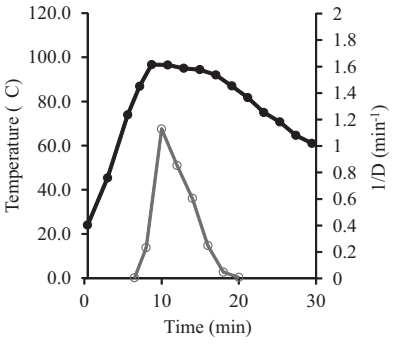

Fig. 2. Temperature (●) and 1/*D* values (○) for the target microorganism *Alicyclobacillus acidoterrrestris* of tomato puree during microwave treatment.

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	$6.9^{a} \pm 1.8$	$6.9^{a} \pm 1.5$	$7.0^{a} \pm 2.1$	$7.0^{a} \pm 1.8$	$6.8^{a} \pm 2.0$	$7.1^{a} \pm 1.6$	$7.4^{a} \pm 1.7$	$7.4^{a} \pm 1.8$	$7.5^{a} \pm 1.6$
PRChL	$4.6^{d} \pm 2.1$	$3.6^{d} \pm 1.7$	$5.7^{bc} \pm 2.0$	$4.3^{e} \pm 2.3$	$3.5^{d} \pm 1.9$	$5.3^{b} \pm 2.4$	$5.3^{c} \pm 1.8$	$4.7^{d} \pm 1.7$	$6.0^{b} \pm 1.7$
PRChM	$4.8^{cd} \pm 2.1$	$4.7^{c} \pm 1.9$	$5.0^{cd} \pm 2.4$	$4.9^{cd} \pm 2.1$	$4.7^{c} \pm 1.9$	$5.3^{b} \pm 2.3$	$5.3^{c} \pm 1.7$	$5.1^{cd} \pm 1.5$	$5.7^{b} \pm 1.8$
PRChS	$5.0^{bcd} \pm 2.1$	$5.1^{c} \pm 1.9$	$5.0^{cd} \pm 2.3$	$5.0^{cd} \pm 2.1$	$4.9^{bc} \pm 1.8$	$5.1^{b} \pm 2.4$	$5.3^{c} \pm 1.7$	$5.1^{cd} \pm 1.6$	$5.6^{b} \pm 1.8$
PV	$6.7^{a} \pm 1.9$	$7.0^{a} \pm 1.8$	$6.3^{ab} \pm 1.9$	$6.8^{a} \pm 1.7$	$7.0^{a} \pm 1.6$	$6.7^{a} \pm 1.7$	$7.2^{a} \pm 1.7$	$7.4^{a} \pm 1.8$	$7.1^{a} \pm 1.7$
PVChL	$5.3^{b} \pm 1.9$	$5.2^{c} \pm 1.9$	$5.5^{c} \pm 2.0$	$4.7^{de} \pm 2.3$	$4.6^{c} \pm 2.3$	$4.8^{b} \pm 2.4$	$5.6^{bc} \pm 1.8$	$5.4^{c} \pm 1.8$	$5.9^{b} \pm 1.7$
PVChM	$5.3^{bc} \pm 2.1$	$6.0^{b} \pm 1.5$	$4.5^{d} \pm 2.5$	$5.3^{c} \pm 2.0$	$5.4^{b} \pm 1.7$	$5.2^{b} \pm 2.3$	$5.5^{bc} \pm 1.8$	$5.5^{bc} \pm 1.6$	$5.6^{b} \pm 2.0$
PVChS	$5.5^{b} \pm 2.1$	$6.4^{ab} \pm 1.5$	$4.5^{d} \pm 2.2$	$5.9^{b} \pm 1.9$	$6.6^{a} \pm 1.3$	$5.2^{b} \pm 2.2$	$5.8^{b} \pm 1.8$	$6.1^{b} \pm 1.7$	$5.5^{b} \pm 1.8$

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

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 et al. (2010) found that in bread made with a proanthocyanidin-rich grape seed extract, the observed antioxidant activity increases less than what was 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 or FRAP values for 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 was higher than the controls, corresponding to 8.1 ± 0.1 and 7.2 ± 0.1 mmol catechin eq./kg for PV and PR, respectively (Fig. 1). These new purees have the potential ability to act as dietary factors in the prevention of complications relating to hyperglycaemia.

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 by exploring the sensory acceptability of the formulations. Several studies 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 overall consumer 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.

The average liking ratings expressed by all 86 consumers for overall acceptability, colour and texture of the analysed tomato purees are reported in Table 4. Consumers rated the unfortified purees highly in terms of 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 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 decreased the ratings for all the sensory parameters ($p < 0.05$). This effect could be explained taking into account that consumers were familiar with the unfortified samples (commercially available regular tomato purees), but they had not been previously exposed to the fortified samples. As it is known, the level of familiarity for a food strongly influences its acceptability by the consumer and repeated exposure to the taste of a food can increase liking for it (Wardle & Cooke, 2008).

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

The overall liking data expressed by all 86 subjects for the fortified samples were then submitted to the principal component analysis in order to obtain an internal preference map (data not shown). The first two principal components of the model explained 48% of the total variance, 28% of the first and 21% of the second dimensions. A visually oriented approach, based on the inspection of loading plot, was used for subject clustering and segmentation was performed according to whether consumer loadings lie on the left or right side of the *Y*-axis set as limit (Næs, Brochkhoff, & Tomic, 2010). Two groups of consumers were obtained: the first consisting of 46 subjects (53.5%) positioned on the left side of the map (Cluster 1); the second consisting of 40 subjects (46.5%) positioned on the right side of the map (Cluster 2). Liking data expressed by subjects belonging to Cluster 1 and Cluster 2 for all samples were independently treated with a two-way ANOVA model (samples and subjects as factors) and with Fisher's LDS post hoc test considered significant for $p \leqslant 0.05$ (Table 4). As expected,

both clusters provided similar average ratings for the three sensory 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 considered more beneficial to improving health than the conventional purees. Indeed, tomato is rich in lycopene but it does not contain proanthocyanidins and hence the addition of grape pomace ingredients could 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.

The varying particle sizes of puree formulations had a moderate effect on proanthocyanidin 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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