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Use of winemaking by-products as an ingredient for tomato puree: The effect of particle size on product quality

Vera Lavelli [a,*], P.S.C. Sri Harsha [a], Luisa Torri [b], Giuseppe Zeppa [c]

[a] DeFENS – Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
[b] University of Gastronomic Sciences, Piazza Vittorio Emanuele 9, 12060 Bra, Italy
[c] DISAFA – Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy

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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.

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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

* Corresponding author. Tel.: +39 2 50319172; fax: +39 2 50316632.
E-mail address: vera.lavelli@unimi.it (V. Lavelli).

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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:

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

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 $6D$ 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 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 10,000g 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.

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