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Juices Enriched with Phenolic Extracts from Grapes

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Abstract

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The paper describes the preparation and evaluation of phenolic extracts from waste materials – pomace (grape marc), seeds and press oil cake of the white grape variety Irsai Oliver (*Vitis vinifera* L.) and their addition to apple and grape juices to increase the nutritional properties. The waste samples were extracted using 50 or 80% ethanol (v/v). Some of the samples were extracted for 60 min at boiling temperature under reflux; the remainder were processed for 24 h on a shaker at room temperature. The highest antioxidant capacity (as measured using DPPH (758 ± 28 mM Trolox/kg of extracted matter)) and content of total polyphenols (74 ± 0.7 g gallic acid/kg of extracted matter) were found in the extract of the seeds obtained through extraction using 50% ethanol (v/v) at boiling temperature for 60 minutes. The press oil cake extract obtained by means of 80% ethanol (v/v) at boiling temperature for 60 min was evaluated as the best for enriching the sensory quality of apple and grape juices. The addition of 1 g of freeze-dried press oil cake extract to 1 l of juice increased the antioxidant capacity and total polyphenol content two-fold

Keywords: antioxidant capacity; catechin; epicatechin; grape pomace; grape seeds; press oil cake

Phenols are a group comprising several thousand substances normally encountered in the plant kingdom. These substances are secondary plant metabolites containing one or multiple aromatic cores that are substituted by hydroxyl groups. In general, they are considered antioxidants; some have vasorelaxation effects, reduce blood pressure and act against cardiovascular diseases, cancer proliferation and inflammation (Manach *et al*. 2004; Watson *et al*. 2014). The concentration of phenols in a plant is dependent on the type, variety, degree of ripeness and health condition (Watson 2014).

Grape skins and seeds are important sources of plant phenols. During the vinification of 1 t of grapes about 70–120 kg grape pomace (grape marc) are obtained on a dry matter basis. The seeds constitute a considerable proportion of the pomace, amounting to 38–52% on a dry matter basis (Maier *et al*. 2009). The content of phenolic compounds in grape berries ranges from 3.3 to 5.5 g/kg with catechin, epicatechin, gallic acid and caftaric acids determined to be the key substances (Balík *et al*. 2008). Nechita *et al*. (2012) studied the extracts of seeds, skins and pomace produced during the making of wine and treated cancer cells with the extracts. They found the bioactive substances contained in the extracts to reduce the viability of these cells. The positive action against cancer cells was also confirmed in the research

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of Raina *et al*. (2013) who studied the influence of the extract of grape seeds on cancer of the urinary bladder and in the treatment of leukaemia. Draijer *et al*. (2009) tested adding a mixture of red wine and polyphenols from grapes into soya drinks. The resulted beverage was administered to evaluators on a daily basis and was found to have a positive effect on blood pressure. In view of the above findings, grape seeds, press oil cake and grape pomace produced during the processing of common grape vine represent a valuable source of substances beneficial to health that can be added to foodstuffs in the form of a liquid extract or powder. The aim of this study was to obtain phenolic extracts from waste materials – pomace (grape marc), seeds and press oil cake of grapes – and their addition to fruit juice preparations in order to increase their nutritional properties.

MATERIAL AND METHODS

Chemicals and solvents. Ethanol was used for extraction, while 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-s-triazine; hydrochloric acid, acetic acid, iron trichloride, sodium acetate trihydrate, Folin-Ciocalteu reagent, gallic acid monohydrate and sodium carbonate were used for analysis.

For HPLC analysis, methanol, acetonitrile, orthophosphoric acid, chlorogenic acid, catechin, epicatechin, and caftaric acid were used (Sigma-Aldrich, Czech Republic).

Extracted waste materials. Waste pomace (grape marc) was what remained (25%) after pressing of destemmed and crushed berries of the Irsai Oliver white grape variety during the process of wine making (vintage 2016). Pomace (grape skins and seeds) was dried for 24 h at 50–60°C. Press oil cake was the waste (8.3%) that resulted from the pressing of separated seeds to obtain grape seed oil. Dry seeds were pressed using a screw extrusion press (UNO FM 3F; Farmet Company, Czech Republic).

Enriched beverages. The tested enriched juices were Lažanský rubín apple juice (pH 3.5, soluble solids 9.8 Brix, titratable acidity 0.6% as citric acid) and juice from grapes of the Irsai Oliver variety (pH 3, soluble solids 16.7 Brix, titratable acidity 1.05% as tartaric acid). Both juices were purchased in the trade network.

Preparation of liquid phenolic extracts. Waste samples were ground to obtain powder using a laboratory mill (IKA MF 10 Basic) and a 1-mm sieve mesh. Four types of extraction were compared: 50 or 80% ethanol (v/v) and boiling temperature (84 or 81°C, respectively) or room temperature (22°C). A quantity of 20 g per ground sample was removed and weighed and put into a 100 ml graduated flask and ethanol of appropriate concentration was added up to the level of 100 ml. A batch of the samples was transferred into Erlenmeyer flasks and extracted for 60 min at boiling temperature under a reflux condenser. The remaining samples were placed in the rotary shaker for 24 h at 22°C. Then, the samples were passed through a sintered glass filter (Simax, porosity S4/P16; Kavalierglass, Czech Republic). The solids were not pressed. Total antioxidant activity was measured in the obtained extracts (using the FRAP and DPPH methods) along with the content of total polyphenols and contents of catechin and epicatechin.

Enrichment of grape and apple juices with freeze-dried phenolic extract. Extract was added to apple and grape juices of the press oil cake extracted for 60 min with 80% ethanol at boiling temperature (81°C). This filtered liquid extract was freeze-dried (Heto Power Dry PL 3000 Freeze Dryer) until dry and then dissolved in food-grade ethanol (50% v/v) so that 10 ml was equivalent to 1 g of freeze-dried extract. Four doses (0, 0.25, 0.5, and 1 g) of the freeze-dried extract per litre of beverage were applied into the juice. After adding the extract at the appropriate dose, the beverage was pasteurised for 20 min at 80°C in closed glass bottles. After one month of incubation at 22°C, the enriched juices were evaluated for the specified analytical and sensory parameters.

Antioxidant capacity. Antioxidant capacity was determined using the modified FRAP and DPPH methods (Brand-Williams *et al*. 1995; Benzie & Strain 1996; Pellegrini *et al*. 2003). The ferric reducing antioxidant power (FRAP) method was carried out in a pH 3.6 acetate buffer (23 mmol/l sodium acetate trihydrate in a solution of 34 mmol/l acetic acid). The reaction mixture contained 12 mmol/l $FeCl_3$ solution, 10 mmol/l 2,4,6-tris(2-pyridyl)-s-triazin in 40 mmol/l HCl solution and buffer in a ratio of 1 : 1 : 10. Two millilitres of the reaction mixture were mixed with 25 µl of diluted sample with deionised water in a disposable plastic cuvette (10 mm) and the obtained solution was measured using a Helios β spectrophotometer (Unicam, UK) after 10 min at a wavelength of 593 nm. The antioxidant capacity was calculated from the calibration curve using Trolox. For the DPPH method, 1.9 ml of 2,2-diphe-

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nyl-1-picrylhydrazyl radical solution in methanol (0.1 mmol/l) was mixed with 0.1 ml of diluted sample with deionised water in a disposable plastic cuvette (10 mm). Absorbance at 515 nm was measured after 30 min using the Helios β spectrophotometer (Unicam, UK). The antioxidant capacity was calculated from the calibration curve using Trolox standard and expressed in mmol Trolox (mM Trolox).

Total polyphenols. Total polyphenol (TP) content was determined with a modified method using the Folin-Ciocalteau reagent (Singleton & Rossi 1965). Using a 50-ml volumetric flask, 0.5 ml of the sample were mixed with 20 ml of distilled water and 1 ml of the Folin-Ciocalteau reagent. The flask was shaken and after 3 min 5 ml of 20% Na_2CO_3 were added. After mixing, the flask was filled to the mark with distilled water. After 30 min the sample was measured at a wavelength of 700 nm using the Helios β spectrophotometer (Unicam, UK). Total polyphenol content was calculated from a calibration curve for gallic acid.

Determination of selected phenolic compounds. Catechin and epicatechin contents were determined in all experimental samples along with caftaric acid (in grape juice) and chlorogenic acid (in apple juice). The samples were separated using a HP 1050 HPLC instrument (Hewlett Packard, USA) on 3 µm, 150 mm × 2 mm, Luna C18(2) columns (Phenomenex, USA) with water-acetonitrile-*o*-phosphoric acid as the mobile phase. We used a G1315B diode array detector (DAD; Agilent, Czech Republic) and the following conditions: mobile phase A: 5% acetonitrile + 0.1% *o*-phosphoric acid; mobile phase B: 80% acetonitrile + 0.1% *o*-phosphoric acid. For separation, a gradient from 0% B to 45% B within 55 min was used. The flow rate was 0.25 ml/min and temperature 25°C (Balík *et al*. 2008).

Sensory analysis. Ten trained assessors took part in the sensory analysis within the scope of ISO 8586 requirements; the procedure was undertaken in the sensory laboratory of the Faculty of Horticulture, Lednice. The method used for assessment was one according to the graphic scale. The results were recorded on an unstructured graphic scale using a length of 100 millimetres. The assessors were asked to taste the samples and evaluate the consumer acceptability using the graphic scale. When evaluating, the distance was measured between the marks assigned to the sample by the assessor and the beginning of the scale, with 1 mm = 1 score, meaning that the person was able to give a minimum of 0 and a maximum of 100 scores.

Statistical analysis. All samples were measured three times. Homogeneity was tested and was followed by one-way parametric analysis of variance (ANOVA). Tukey's LSD post hoc test with a level of significance of $P < 0.05$ was carried out with Statistica CZ 12 (StatSoft, USA) and MS Excel 2010 (Microsoft, USA) software.

RESULTS AND DISCUSSION

Antioxidant capacity of liquid phenolic extracts. Antioxidant capacity was statistically significantly ($P < 0.05$) influenced by exposure to boiling temperature during the extraction process. It was always higher for samples that had been boiled for 60 min compared with those not boiled (22°C) for 24 hours. The seed extract obtained at boiling temperature (84°C) for 60 min using 50% ethanol (v/v) was found to have the highest antioxidant capacity using the DPPH method (758 ± 28 mM Trolox/kg of extracted matter). Samples that were extracted with 50% ethanol (v/v) at room temperature reached statistically significant ($P < 0.05$) higher antioxidant capacity values than samples extracted with 80% ethanol (v/v) (Figure 1). Duba *et al*. (2015) extracted polyphenols from grape skins and seeds using water. The extraction was carried out at various temperatures (80, 100 and 120°C). In the case of both skins and the seeds, the quantities of total polyphenols obtained increased with rising temperature. Drosou *et al*. (2015) extracted polyphenols from grape pomace with water, ethanol and a water-ethanol mixture (1 : 1) using the microwave and ultrasonic extraction methods and extraction using Soxhlet. The results showed that the ultrasonic

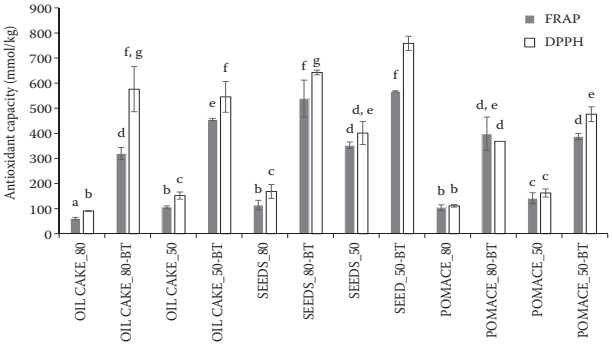

Figure 1. Antioxidant capacity in extracts of press oil cake, seeds and pomace of vine

BT – boiling temperature of the mixture; 50 – 50% ethanol (v/v) (84°C); 80 – 80% ethanol (v/v) (81°C); different letters indicate significant differences ($P < 0.05$)

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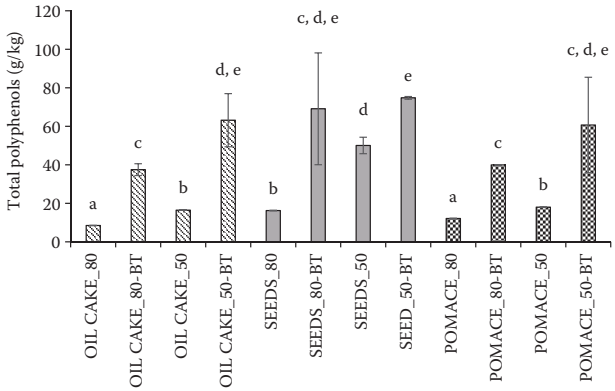

Figure 2. Total polyphenols in extracts of press oil cake, seeds and pomace of vine

method of extraction with water and ethanol (1 : 1) can be used to obtain extracts which are very rich in polyphenolic substances, mainly catechin, epicatechin, phenolic acids, procyanidins and stilbenes.

Content of total polyphenols in liquid phenolic extracts. The content of total polyphenols was statistically significantly ($P < 0.05$) influenced by the temperature of the extraction (Figure 2). Antioxidant capacities determined in all prepared extracts significantly correlated with the content of total polyphenols ($R = 0.9331$). The samples that were extracted for 60 min using boiling temperature reach higher values of total polyphenols than those extracted only by agitation at 22°C. Statistically significant differences were found between the samples extracted from press cake using 50 and 80% ethanol (v/v). A statistically significant difference was also found between the extracts of seeds extracted with 80 and 50% ethanol (v/v) without using boiling temperature; the same was true for the extract from pomace. Samples extracted by boiling and using varying ethanol concentrations did not show statistically significant differences. The highest content of polyphenols (74 ± 0.7 g gallic acid per kg of extracted matter) was reached for the extract of seeds obtained by boiling the mixture for 60 min using 50% ethanol (v/v). All samples boiled for 60 min reached higher values of total polyphenols than those for which boiling was not applied. However, this boiling step resulted in a higher variability in extraction products, i.e., higher standard deviations (Figure 2). Yilmaza and Toledo (2006) and Maier *et al*. (2009) evaluated various wine industry by-products as significant sources of phenolic antioxidants. Nevertheless, the kind of by-product and extraction procedure influence the contents of phenolic substances and antioxidant capacity.

Content of catechin and epicatechin in liquid phenolic extracts. The rate of yield of catechin and epicatechin from the solid waste was also significantly ($P < 0.05$) influenced by boiling (Figure 3). The highest amount of catechin was present in the extract of grape seeds that was extracted with 50% ethanol and boiling for 60 minutes. In this case, the catechin value was 5215 ± 158 mg/kg of extracted matter. The quantity of epicatechin was also the highest in this extract: 2697 ± 90 mg/kg of extracted matter. On the other hand, the extract of grape seeds obtained using 50% ethanol without boiling for 60 min contained only 1271 ± 39 mg/kg catechin and 779 ± 26 mg/kg epicatechin. The extract produced from grape seeds using 80% ethanol and boiling for 60 min also showed high concentrations: 4646 ± 140 mg/kg of catechin and 2500 ± 83 mg/kg of epicatechin. The extract produced using 80% ethanol (v/v) but without the 60-minute boiling step reached only 432 ± 13 mg/kg of catechin and 299 ± 10 mg/kg of epicatechin. Figure 3 shows that the 60-min boiling step statistically significantly influences the yield of catechin and epicatechin from the extracted matter. The concentration of ethanol used for the extraction along with the boiling step did not have a statistically significant influence on the quantity of catechin and epicatechin in the resulting extract. Balík *et al*. (2008) investigated the concentrations of catechin and epicatechin in grape berries by means of 80% (v/v) methanol extraction. The content of catechin ranged from 296 to 856 mg/kg and content of epicatechin ranged from 150 to 591 mg/kg.

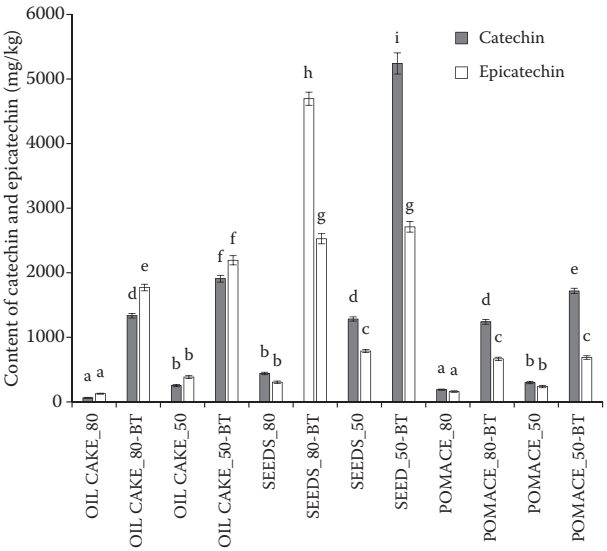

Figure 3. Catechin and epicatechin content

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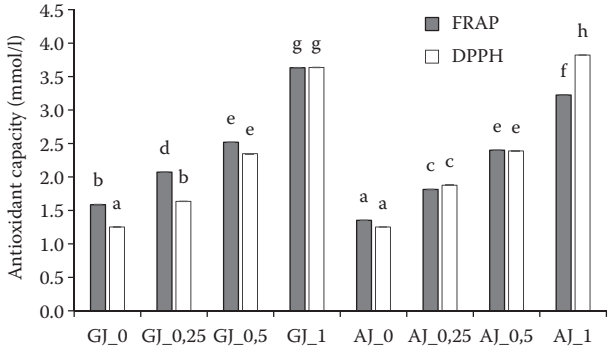

Figure 4. Antioxidant capacity in grape and apple juices enriched with press oil cake freeze-dried extract

GJ – grape juice; AJ – apple juice; 0 – 0.25; 0.5 – 1 g of freeze-dried extract per 1 l of beverage

Antioxidant capacity of enriched juices with freeze-dried phenolic extract. The highest antioxidant capacity identified using the FRAP method was observed for the grape juice enriched with a dose of 1 g of the freeze-dried extract (Figure 4); apple juice that was also enriched with a dose of 1 g of freeze-dried extract reached nearly the same value. A dose-dependent relationship was observed between extract dose and antioxidant capacity: the lower the dose was, the lower the antioxidant capacity. In juices enriched with extracts, the antioxidant capacity was statistically significantly higher than in control samples lacking freeze-dried extract from press oil cake. The addition of the smallest dose caused about a 1.3-fold increase in the antioxidant capacity, while the increase elicited by the highest dose was 1.7-fold higher than the control sample (Figure 4). Novotná *et al*. (2016) studied the increase in antioxidant capacity in beverages. They added spruce wood chips to the beverages in order to increase the content of 7-hydroxymatairesinol and α-conidendrin. This caused both the antioxidant capacity and contents of total polyphenols to increase in the enriched beverages. A similar experiment was carried out by Liu *et al*. (2016), who added oak wood chips to a blueberry wine during ripening. The results revealed a significant increase in the contents of phenolic substances.

Total polyphenol content of juices enriched with freeze-dried phenolic extract. The highest contents of polyphenols were achieved for the grape and apple juices enriched with a dose of 1 g of extracted press cake per litre of beverage. The addition of extract elicited an increase in total polyphenols a dose-dependent manner. The addition of 0.25 g of freeze-dried extract elicited a 1.4-fold increase in total polyphenols and the addition of 1 g of freeze-dried extract yielded a 2.2-fold increase in comparison with the control. For apple juices, there was a statistically significant difference ($P < 0.05$) after adding 0.5 and 1 g of the freeze-dried extract (Figure 5). The addition of 1 g of the freeze-dried extract caused total polyphenols to increase two-fold relative to the control. A positive effect of adding the polyphenolic extract from the *Syzygium cumini* plant to pear juice was observed by Kapoor and Ranote (2016). The enriched pear juice with 4% jamun extract exhibited the highest sensory evaluation; the content of total polyphenols was also 9.24% higher than for the juice that received no extract. The antioxidant capacity increased by 18.13%.

Content of catechin and epicatechin in juices enriched with freeze-dried phenolic extract. The content of catechin and epicatechin in juices increased together with increasing dose of extract, reaching values of 6.9–13.2 and 6.4–24.5 mg/l for catechin and epicatechin, respectively (Figure 6). Nevertheless, the different doses of extract did not significantly change the already high concentrations of chlorogenic acid (128.2 ± 1.4 mg/l) in apple juices and caftaric acid (47.1 ± 1.8 mg/l) in grape juices. The increases in the selected polyphenols (lignans, 7-hydroxymatairesinol and α-conidendrin) after addition of an extract of spruce wood chips was studied by Balík *et al*. (2016). The addition of the extract increased the content of the lignans in the beverages, while the antioxidant capacity and the content of total polyphenols was not significantly affected. Massini *et al*. (2016) successfully increased the antioxidant capacity and the content of total polyphenols in vegetable juices. They obtained an extract from apple skins. Juices enriched using this extract exhibited

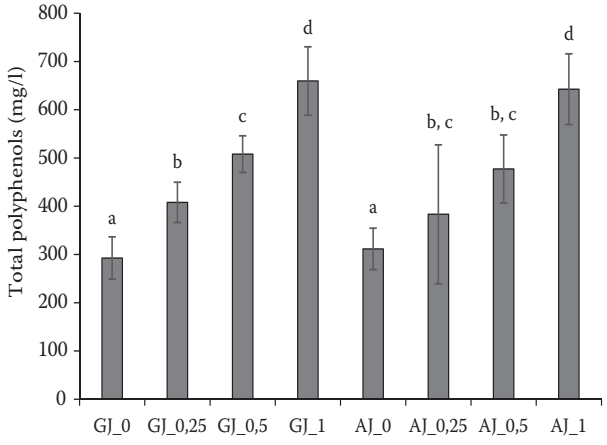

Figure 5. Polyphenol content in grape and apple juices enriched with press oil cake freeze-dried extract

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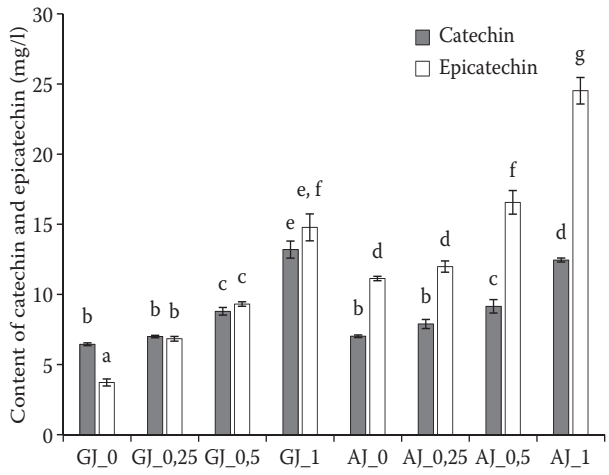

Figure 6. Catechin and epicatechin content in grape and apple juices enriched with press oil cake freeze-dried extract

an increased content of polyphenols and increased ability to protect against oxidation of lipids.

Sensory analysis of juices enriched with freeze-dried phenolic extract. A sensory evaluation was carried out by ten appointed assessors; their task involving evaluating overall consumer acceptability. The results are displayed in Figure 7. The addition of the freeze-dried extract (at doses of 0.25 and 0.5 g/l of beverage) did not have a statistically significant effect on the overall consumer acceptability of the apple and grape juices. Juices with 1 g/l freeze-dried extract were significantly less acceptable in terms of consumer experience because of increased astringent taste. In their studies on the antioxidant activity and sensory acceptability of enriched beverages, Perlman and Ramones (2008) added various concentrations of a grape press oil cake extract to grape juice. At a concentration of 4% in the

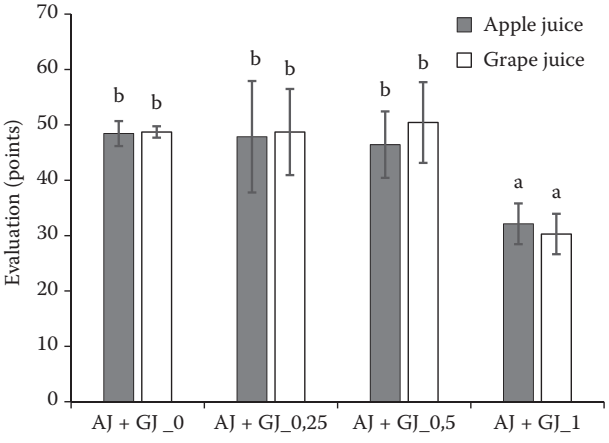

Figure 7. Sensory evaluation of consumer acceptability of grape and apple juices enriched with a press oil cake freeze-dried extract

juice, the beverage was evaluated to be sensorially unacceptable; the antioxidant activity was increased three-fold at this concentration.

CONCLUSIONS

Waste materials such as pomace (grape marc), seeds and press oil cake of grapes represent rich sources of phenolic substances, particularly catechin and epicatechin; it is possible to extract these substances using ethanol and water. The best extraction results were achieved when the mixture was boiled for 60 minutes. The greatest amounts of catechin (5215 ± 158 mg/kg of extracted matter) and epicatechin (2697 ± 90 mg/kg of extracted matter) were obtained with the extraction protocol from grape seeds using 50% ethanol (v/v) and boiling (84°C) for 60 minutes. This extract also exhibited the highest antioxidant capacity as measured by DPPH (758 ± 28 mM Trolox/kg of extracted matter) and the highest content of total polyphenols (74 ± 0.7 g gallic acid per kg of extracted matter). The lowest antioxidant capacity (90 ± 2.4 mmol Trolox/kg of extracted matter) was observed for the sample of press cake extracted with 80% ethanol and agitated for 24 h at 22°C. This sample was also found to have the lowest content of total polyphenols and of catechin and epicatechin. The obtained extracts can be used to enhance the nutritional properties of beverages. The addition of 1 g of press oil cake freeze-dried extract resulted in a two-fold increase in the antioxidant capacity and the content of total polyphenols compared with the control sample without any enrichment. In terms of sensory characteristics, the addition of 0.5 g of press oil cake freeze-dried extract per litre of beverage gave the best results: there was no effect on the consumer acceptability of the beverage and both the antioxidant capacity and total polyphenols in the beverage were 1.5-fold higher compared with the control sample. Further, there was no 1.5-fold reduction in consumer acceptability compared with the control.

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