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ANTIOXIDANT ACTIVITY OF POLYPHENOL-ENRICHED APPLE JUICE

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This paper shows that it is possible to improve antioxidant activity of apple juice by extraction of polyphenolic compounds from apple pomace, as waste, and their addition to the apple juice. Raw apple juice was prepared by pressing of apple mash. After thermal treatment of raw apple juice, depectinisation, additional clarification and filtration, the clarified juice was obtained. In raw and clarified apple juice soluble solids, acidity, reducing sugar, total sugars and brown component content were determined, as well as total dry matter, ash, acidity, reducing sugar, total sugars, total pectins, cellulose and starch content in apple mash and pomace. The total cotent of phenolics in clarified apple juice and apple pomace extract, determined spectrophotometrically using the Folin-Ciocalteu reagent, was 0.496 mg/ml and 6.505 mg/g, respectively. The antioxidant activity of clarified and polyphenol-enriched clarified juice (with addition of apple pomace extract in the concentrations 0.05 g, 0.1 g, 0.5 g and 1 g of phenolic compounds per liter of clarified apple juice) was examined on stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Based on the obtained results it can be concluded that polyphenol-enriched clarified juice was more effective on DPPH radicals than the clarified apple juice.

KEY WORDS: Apple, apple juice, apple pomace, phenolic compounds, antioxidant activity

INRODUCTION

Different studies have shown that free radicals present in the human organism cause oxidative damage to various biomolecules, such as lipids, proteins and nucleic acids, and thus are involved in the initiation phase of degenerative disease. Phenolic and other phytochemical antioxidants found in fruits and vegetables are bioactive compounds capable of neutralizing free radicals and may play a major role in the prevention of certain diseases (1). Also, dietary supplements and food fortification may be an alternative route to the consumption of minor plant components that may have health benefits.

Apple (*Malus domestica*) has been the leading fruit variety according to its world production. The most important industrial utilization of apple is the juice production. Apples contain 85% of water, 12-14% of carbohydrates, about 0.3% of proteins, minor quantity

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of lipids (<0.1%), minerals and vitamins (2). The differences in chemical composition of apples are due to the region of growth, variety, ripening state during harvesting, agronomic and environmental conditions. About 80% of apple carbohydrates are soluble sugars: sucrose (~ 2.1%), glucose (2.4%) and fructose (5.9%). Apples contain about 2.4% of total dietary fibers, and it is proved that they contain sorbitol (2). Malic acid is the predominant organic acid in apples (80-90% of total acids), and its content varies depending on the variety, ripeness, and environmental conditions during growing and storage. The phenolic compounds content in apples is 36 ± 19 mg/kg fresh weight (3). The most important groups of phenolic antioxidants present are flavonols (with quercetin glycosides as the main representative), monomeric and oligomeric flavanols, dihydrochalcones (e.g., phloridzin), anthocyanins, *p*-hydroxycinnamic and *p*-hydroxybenzoic acids (3, 4). Apples are not rich in vitamin C (its content in fresh fruit can be only a few miligrams) (5).

The technological process of apple juice production at industrial scale include raw material preparation for processing, mashing, pressing, thermal treatment, depectinization, clarification, filtration, pasteurization and packing. The raw material preparation for further processing consists of washing and inspection. During processing, losses of antioxidant components occur, especially due to the exposure of raw material to oxygen and thermal treatment of raw material and juice. The conventional apple juice production (direct pressing of apple mash or pressing after mash depectinization) results in a juice poor in phenolics and with only 3-10% of the antioxidant activity of fresh apples. The fact that in conventional apple juice production techniques most of the phenolics do not get deteriorated or lost during juice manufacture, but remain in the pomace, suggests that pomace can be considered as a source of phenolic antioxidants.

Several possibilities exist in the juice production chain to enhance the phenolic content of apple juice, by the choice of cultivation methods, raw material, production methods, processing, storage and distribution conditions (3). Also, the juice with a high content of phenolic bioactive components can be produced by enrichment with phenolics from other sources. For this reasons, the possibility of utilization of apple pomace antioxidant compounds by their extraction, and enrichment of clarified juice, was examined in this work. The objectives of this study were: (I) to obtain raw and clarified apple juice; (II) to determine soluble solids, acidity, sugar and brown component content in obtained juices; (III) to determine chemical compositions of apple mash and apple pomace; (IV) to perform an extraction of pomace obtained after direct pressing of apple mash; (V) to determine phenolic content of apple pomace and clarified apple juice spectrophotometrically using the Folin-Ciocalteu reagent; (VI) to obtain polyphenol-enriched apple juice, by adding pomace extract to the previously produced clarified juice, and (VII) to compare the antioxidant activity of clarified and polyphenol-enriched clarified juice on stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals.

EXPERIMENTAL

Chemicals and samples

Methanol and sodium carbonate were obtained from „Zorka” Šabac (Serbia). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and gallic acid were purchased from Sigma Chemical Co. (USA). These chemicals were of analytical reagent grade. Other chemicals and solvents used were of the highest analytical grade.

For apple juice production in laboratory conditions Red Delicious variety (from a local market) was used.

Apple juice production

Apples were washed and mashed, and the mash was pressed using laboratory cider press. The obtained raw apple juice was heated to 85-90°C and treated with 0.04 g/l Rapidase Pro (DSM) at 45-50°C, during 1.5 hours. After depectinization, enzyme was inactivated by heating the raw juice up to 85-90°C/2-3 min., cooled and treated with gelatine and bentonite solutions (0.005% and 0.05%, respectively). The clarified juice was filtered through Büchner funnel using vacuum, filled into PET bottles and stored at -18°C. Apple pomace that remained after juice production was stored in PE bags at -18°C.

Determination of soluble solids, acidity, sugar and brown component content in raw and clarified apple juice

In raw and clarified apple juice soluble solids, acidity and sugar content were determined according to valid Regulations (6). Brown component was assayed by measuring absorbance of the sample extract at 380 nm (7).

Determination of chemical compositions of apple mash and apple pomace

In apple mash and pomace total dry matter, ash, acidity, reducing sugar, total sugars, total pectins, cellulose and starch content were determined according to valid Regulations (6). Cellulose content was determined according to Kirschner-Gannak (7).

Extraction procedure

Sample of apple pomace (20 g) was extracted at room temperature using an ultrasonic bath, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with different amounts of 80% methanol: 160 ml in 60 min, 80 ml in 60 min, 80 ml in 30 min. The total extraction time was 150 min. The obtained three extracts were combined and evaporated to dryness under reduced pressure. The yield of extract was 2.65 g.

Total phenolics

Total phenolics in apple pomace extract and clarified apple juice were determined using the Folin-Ciocalteu reagent (8). The reaction mixture was prepared by mixing 0.1 ml of water solution of extract (concentration 50 mg/ml) or 0.1 ml juice, 7.9 ml of distilled water, 0.5 ml of the Folin-Ciocalteu's reagent and 1.5 ml of 20% sodium carbonate. After 2 h, the absorbance at 750 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) was obtained against blank prepared in a similar manner, by replacing the extract

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with distilled water. The total phenolic content, expressed as mg of gallic acid equivalents per g of apple pomace extract or per ml of apple juice, was determined using calibration curve of gallic acid standard.

Apple juice enrichment

Apple pomace extract was added to the previously produced clarified juice in the concentrations 0.05 g, 0.1 g, 0.5 g and 1g of phenolic compounds per liter of apple juice.

DPPH radical-scavenging spectrophotometric assay

The potential antioxidant activity of apple juice was assessed on the basis of the scavenging activity of the stable DPPH free radicals according to the method of Yen and Chen (9). The juice (1 ml) was diluted with distilled water. The range of the investigated juice concentration was 5-50%. An aliquot (1 ml) of diluted juice was added to 3 ml of absolute methanol and 1 ml of methanolic DPPH solution (concentration 0.3 mmol/l). The mixture was shaken and left at room temperature for 10 min, then the absorbance was measured at 517 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The blank probe contained all components except the radicals. The antioxidant activity on the basis of the capability to scavenge the DPPH radicals (AA_{DPPH}) was estimated from the differences in absorbance of DPPH solution with or without juice (control) and the inhibition percent was calculated using the following equation:

$$AA_{DPPH} (\%) = (A_{Control} - A_{Sample})/A_{Control} \times 100$$

where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the juice) and A_{Sample} is the absorbance in the presence of the juice. The values of antioxidant activity were investigated for the various concentrations of the juice.

RESULTS AND DISCUSSION

Chemical composition of raw and clarified apple juice is given in Table 1. As previously said, the chemical composition of apples depends on the number of parameters.

The yield of raw apple juice was 55.6%.

The differences in chemical composition of raw and clarified apple juice are due to the influence of different treatments during juice production.

Table 1. Chemical composition of raw and clarified apple juice

Component	Raw juice	Clarified juice
Soluble solids (g/100g)	15.15	15.25
Acidity (g malic acid/100g)	0.21	0.22
Reducing sugars (g/100g)	11.41	14.14
Total sugars (g/100g)	13.67	14.42
Brown component (μg $K_2Cr_2O_7$/ml)	117.5	98.0

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As can be seen from Table 1, soluble solids of raw apple juice were somewhat lower than in clarified juice (15.15% and 15.25%, respectively). Acidity of raw juice was also lower than that of clarified juice (0.21 g/100g and 0.22 g/100g of dry matter, respectively). Results of chemical composition of raw and clarified apple juice are in accordance with the available literature (5). According to Hui et al. (5), dry matter content in apple is somewhat lower than obtained in this research.

Chemical compositions of apple mash and apple pomace are given in Table 2.

Table 2. Chemical composition of apple mash and pomace

Component	Apple mash	Apple pomace
Total dry matter (%)	14.95	18.65
Ash (g/100g)	0.29	0.35
Acidity (% malic acid)	0.17	0.15
Reducing sugar (g/100g)	11.68	11.5
Total sugars (g/100g)	11.85	13.3
Total pectins (g/100g Ca-pectate)	0.54	0.19
Cellulose (g/100g)	0.71	1.58
Starch (g/100g)	0.87	2.11

The Folin-Ciocalteu method is a rapid and widely-used assay to investigate the total phenolic content. The content of total soluble phenolics of clarified apple juice and apple pomace extract was expressed as gallic acid equivalent and it was 0.496 mg/ml and 6.505 mg/g, respectively.

The potential antioxidant activity of apple juice was assessed on the basis of the scavenging activity of the stable DPPH free radicals. Antioxidant activity of clarified apple juice without and with addition of apple pomace extract in the concentrations 0.05 g, 0.1 g, 0.5 g and 1g of phenolic compounds per liter of apple juice is shown in Fig. 1.

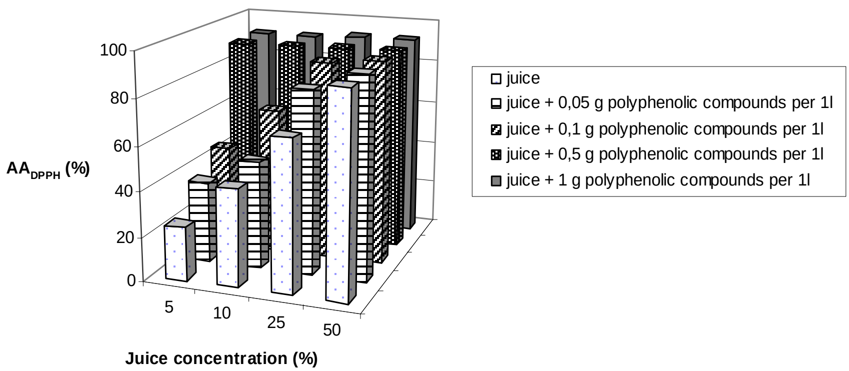

Fig. 1. Antioxidant activity of clarified apple juice without and with addition of apple pomace extract in the concentrations 0.05 g, 0.1 g, 0.5 g and 1g of phenolic compounds per liter of apple juice

Based on the DPPH radical-scavenging spectrophotometric measurments, it can be observed that apple juice showed dose-dependent antioxidant activity (AA_{DPPH}). Also, it can be concluded that apple juice without addition of apple pomace extract was less effective on DPPH radicals than with addition of apple pomace extract. With increasing concentrations of added phenolic compounds ranged from 0.05 g to 1 g per liter of apple juice, AA_{DPPH} increased from 36.42% to 90.94%, at juice concentration of 5%. This indicates that enriched apple juice, with addition of 0.5 g of phenolic compounds per liter of apple juice, had an antioxidant activity that was 3.8 times higher than that of the corresponding clarified juice.

The IC_{50} value is a parameter used to measure antioxidative activity and it is defined as the juice concentration required for 50% scavenging of DPPH radicals under experimental condition employed. A smaller IC_{50} value corresponds to a higher antioxidant activity. The IC_{50} values of apple juice without and with addition of apple pomace extract in the concentrations 0.05 g and 0.1 g of phenolic compounds per liter of apple juice, determined based on antioxidant activities, were 13.89%, 10.77% and 8.16%, respectively. It was observed that with increasing concentrations of added phenolic compounds to the juice antioxidant activity increased.

The fact that phenolic compounds that contribute to antioxidant activity preferentially remain in the pomace offers a possibility for apple juice optimization with respect to phenolic content and antioxidant activity. Thus, it is a challenging option to search for methods in which the phenolics may be extracted from the pomace and later added to the final apple juice.

CONCLUSION

- The differences in chemical composition of raw and clarified apple juice are due to the influence of different treatments during juice production;
- Soluble solids of raw apple juice were somewhat lower than in clarified juice (15.15% and 15.25%, respectively);
- Raw juice acidity was also lower than in clarified juice (0.21 g/100g and 0.22 g/100g of dry matter, respectively);
- The content of total soluble phenolics of clarified apple juice and apple pomace extract was expressed as gallic acid equivalent and it was 0.496 mg/ml and 6.505 mg/g, respectively;
- Apple juice without addition of apple pomace extract was less effective on DPPH radicals than with addition of apple pomace extract;
- With increasing concentrations of added phenolic compounds, ranging from 0.05 g to 1 g per liter of apple juice, AA_{DPPH} increased from 36.42% to 90.94%, at juice concentration 5%;
- The enriched apple juice by adding apple pomace extract in the concentration of 0.5 g of phenolic compounds per liter of apple juice, had an antioxidant activity that was 3.8 times higher than that of the corresponding clarified juice, at the juice concentration of 5%.

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АНТИОКСИДАТИВНА АКТИВНОСТ СОКА ОД ЈАБУКА ОБОГАЋЕНОГ ПОЛИФЕНОЛНИМ ЈЕДИЊЕЊИМА

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У овом раду испитана је могућност побољшања антиоксидативне активности сока од јабука додатком полифенолних једињења екстрахованих из тропа, споредног производа насталог приликом добијања сока од јабука. Матични сок је произведен стандардним поступком производње – пресовањем каше од јабука. Након термичке обраде матичног сока, депектинизације, бистрења и филтрирања добијен је бистри сок. У матичном и бистром соку одређен је садржај киселина, суве материје, шећера и смеђе компоненте. Такође, одређен је садржај суве материје, киселина, шећера, скроба, целулозе, пектина и пепела у каши и тропу јабука. Садржај укупних полифенолних једињења у бистром соку од јабука и екстракту тропа, одређен спектрофотометријски, методом по Folin-Ciocalteu, износи 0.496 mg/ml и 6.505 mg/g. Антиоксидативна активност бистрог сока, као и бистрог сока од јабука обогаћеног полифенолним једињењима (са додатком екстракта тропа у концентрацијама 0.05 g, 0.1 g, 0.5 g и 1 g полифенолних једињења po 1l бистрог сока) испитана је на стабилне 1,1-дифенил-2-пикрилхидразил (DPPH) радикале. Обогаћени сок од јабука показао је израженију антиоксидативну активност на DPPH радикале од сока без додатка екстракта тропа.

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