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Papers

EVALUATION OF ANTIOXIDANT CAPACITIES OF ORANGE, LEMON, APPLE AND BANANA PEEL EXTRACTS BY FRAP AND ABTS METHODS

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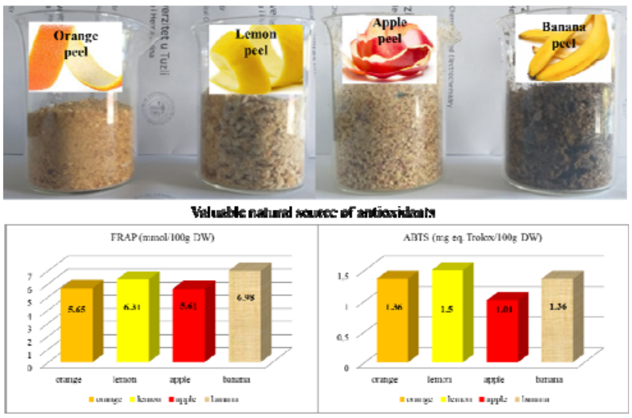
In this paper were studied the antioxidant capacity of orange, lemon, apple and banana fruit peel. The fruit peels were collected, air dried at room temperature for seven days and homogenized by grinding. The homogenization of the sample was done for 2 hours at 200 rpm in the shaker in 80% methanol. The antioxidant capacities of the obtained fruit peel extracts were quantified by FRAP and ABTS methods. The results showed that apple contained lowest antioxidant capacity, using both method of analysis. Generally, the antioxidative capacities obtained by FRAP method ranged from 5.61 to 6.98 mmol Fe^{2+}/100g DW with the highest antioxidative capacity in banana peel (6.98 mmol Fe^{2+}/100g DW). Antioxidative capacity determined by ABTS method decreased in the following order: lemon > banana > orange > apple and ranged between 1.01 to 1.50 mg TE/100 g DW.



INTRODUCTION

Fruit peels are generally considered as environmental burden and waste. Orange, lemon, banana and apple peel contain high levels of cellulose, hemicellulose, and phenolic compounds.[1] The orange peels are rapidly becoming a superpower in the sustainability sphere. The trend towards reuse rather than new design to address problems is a flourishing one in the world of eco-conscious design particularly with regard to finding creative ways for repurposing food waste. Orange peels have also shown immense promise in their ability to be transformed into other materials, particularly textiles for the fashion industry.[2] Lemon peel have several antimicrobial and antifungal properties.[3] Four compounds identified in lemon peel have powerful antibacterial properties and effectively fight common oral-disease-causing bacteria.[4] Lemon peel is rich in antioxidants, including D-limonene and vitamin C.[5,6] Intake of flavonoid antioxidants like D-limonene is linked to a reduced risk of certain conditions, such as heart disease and type 2 diabetes.[7,8] One test-tube study determined that lemon peel had stronger antioxidant activity than grapefruit or tangerine peels.[9] Notably, in a test-tube study, this peel significantly harmed and reduced the growth of antibiotic-resistant bacteria.[10] A review of 14 studies on 344,488

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people found that an average increase of 10 mg of flavonoids per day reduced heart disease risk by 5%.[11] A 4-week study that evaluated 60 children with excess weight showed that supplementing with lemon powder (containing peel) led to reductions in blood pressure and LDL cholesterol.[12] Lemon peel may have several cancer-fighting properties. For example, flavonoid intake is associated with a reduced risk of several types of cancer, and vitamin C may bolster the growth of white blood cells, which help eliminate mutated cancer cells.[13]

Apple peel is a good source of natural antioxidants and almost all types of vitamins, especially vitamin A, C and K. Apple peel contain high amounts of calcium and phosphorous. In addition, there is an adequate amount of zinc, sodium, and magnesium present in the apple peel. Iron quantity in apple is satisfactorily high, and therefore makes a healthy food option for anaemic patients. The fibre in apple skin is present in both soluble and insoluble form. About two-third of the apple's fibre is present in the peel alone. Triterpenoid is also found in the apple peel which has the ability to fight with harmful cancer creating cells. Apple peel efficiently reduces the risk of liver, breast, and colon cancer.[14]

Banana peel is full of anti-aging compounds, vitamins C and E that are perfect for brightening teeth and reducing wrinkles. Because of these properties, its helps skin with acne, hydration, and reducing dark spots. Banana peels also contain potassium, zinc, iron and manganese. These nutrients can calm inflamed skin and reduce acne outbreaks. Furthermore, banana peels contain lutein and carotenoids which are fat-soluble compounds that help fight inflammation and acne breakouts.

Recent research carried out by Bratovcic [15] has revealed that orange and lemon water peel extracts 5 % (w/v) could be a very valuable source of potassium (K) and calcium (Ca), needed micronutrients to ensure the water and electrolyte balance. Potassium and calcium build and maintain strong bones, proper function of muscles and nerves. K concentrations determined by flame photometric method were 308 mg/L at 62ºC and 361 mg/L at 92ºC in lemon extracts, while in orange extracts the concentration of K was 476 mg/L at 62ºC and 483 mg/L at 92ºC. The concentration of Ca was 70.8 mg/L at 62ºC and 71.9 mg/L at 92ºC in lemon extracts, while in orange extracts the concentration of Ca was 91 mg/L at 62ºC and 93.6 mg/L at 92ºC.

In order to contribute to sustainability, we have investigated the antioxidant capacity of orange, lemon, apple and banana peels since fruit peel extracts may be encapsulated. Microencapsulation can improve the retention time of the nutrient in the food and allow controlled release at specific times during food consumption or in the intestinal gut (microencapsulation of vitamin).[16] Nano-encapsulation has the potential to protect sensitive bioactive food ingredients from unfavourable environmental conditions, enhance solubilisation, improve taste and odour masking, and enhance bioavailability of poorly absorbable function ingredients.[17]

The objective of this research was to study the possibility to use lemon, orange, banana and apple peels extracts as a source of natural antioxidants and to quantify the antioxidative capacity of the extracts. Several antioxidant capacity methods have been used or developed in recent years to evaluate antioxidant capacity of fruits and vegetables. Antioxidant capacity values may greatly vary depending on the experimental conditions and the specificity of the free radical used.[18] ABTS radical cation decolorization assay and the ferric reducing antioxidant power (FRAP) method were used.

MATERIALS AND METHODS

Plant material

Fresh orange (*Citrus sinensis*), fresh lemon (*Citrus limon*), apple (*Malus domestica*) and banana (*Musa acuminata*) fruit peels were collected in 2019. The fruits were purchased from local supermarket in Tuzla in Bosnia and Herzegovina. Fruit was fully developed in size and ready for immediate consumption.

The fruit was washed with tap water and manually pealed. To prepare laboratory samples, each fruit peel was cut in small pieces, distributed on the paper and dried at ambient conditions for seven days. Afterward the peels were grinded in a grinder AD 443, Adler Europe, 150 W. In Figure 1 the appearance of the samples is presented.

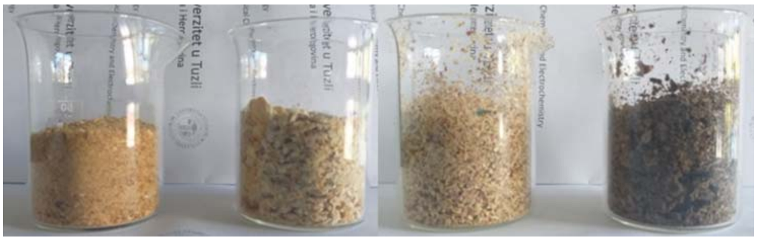

Fig. 1 – Air dried and grinded samples of orange, lemon, apple and banana peels.

Chemicals

All chemicals used were of analytical grade and obtained from the manufacturer Semikem, Sarajevo. The following chemicals were used: sodium acetate trihydrate, CH_3COONa x $3H_2O$; Acetic acid, CH_3COOH; Concentrated hydrochloric acid, HCl; 2,4,6- Tris(2-pyridyl)-s-triazine (TPTZ); Iron (III) chloride hexahydrate, $FeCl_3$ x $6H_2O$; Iron (II) sulfate–heptahydrate, $FeSO_4$ x $7H_2O$; Potassium peroxodisulfate, $K_2S_2O_8$; 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Trolox); Ethanol, C_2H_5OH; Methanol, CH_3OH.

Extraction

0.5 g of the sample were weight on an analytical balance (± 0.0001 g) and homogenized with 25 mL of extraction solvent (80% methanol) in a 100 mL Erlenmeyer flask. The homogeneous mixture was extracted on orbital shaker for 2 hours at 200 rpm. The extract was filtered and stored at -18 ° C until analysis. Before analysis extracts were diluted two times.

Determination of antioxidative capacity by FRAP method

This method is based on the reduction of the colourless iron (III)-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous form (Fe^{2+}) of intense blue color.[19] The antioxidant capacity of the tested samples was determined spectrophotometrically by measuring the absorbance at 593 nm as described in previous work by Kazazic *et al.*[20] Briefly, FRAP reagent was prepared with 200 mL sodium acetate buffer solution (300 mmol L−1, pH 3.6), a 20 ml tripyridyltriazine (TPTZ) solution (10 mmol L−1in 40 mmol L−1 HCl), 20mL $FeCl_3$ solution (20 mmol L−1) and 24 mL distilled water. 0.200 ml of fruit peels extracts prepared in methanol was added to 3.8 mL of FRAP reagent. The mixture was incubated at room temperature for 4 minutes and its absorbance was measured at 593nm. The control solution was made of 0.20 mL distilled water and 3.8ml FRAP reagent. The antioxidant capacity was determined using the calibration curve and represented as mmol $FeSO_4$ equivalents per 100 g-1 of sample in dry weight.

Determination of antioxidant capacity by ABTS method

This method is based on the “quenching” of the blue-green radical cation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation). The addition of antioxidants results in the reduction of the previously generated ABTS radical which is measured by monitoring the decrease in the absorption of ABTS radicals and is compared with the decrease in absorbance caused by the addition of a certain amount of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue, under the same conditions.[21] Determination of the antioxidant capacity with ABTS radical was done as described in work by Kazazic *et al*.[20]

RESULTS AND DISCUSSION

The industrial processing of fruit results in high amounts of waste materials. In order to solve problem of waste, fruit waste can be utilized as animal feed or fertilizer.[22] Last decade valorisation of agricultural co-products is in research focus since it has been reported that these co-products contain valuable substances such as pigments, sugars, organic acids, flavours, bioactive compounds with antioxidant activity.[23] In this study we used two different methods to evaluate antioxidant capacity of fruit peels. The results presented in Table 1 demonstrated that methanol extracts of all investigated samples have the ability to prevent potential damage from free radicals.

Table 1

Antioxidant capacity of analysed fruit peels determined by FRAP and ABTS method

Sample	FRAP (mmol Fe^{2+}/100g DW)	ABTS (mg TE/100g DW)
Orange peel	5.65±0.12	1.36±0.09
Lemon peel	6.31±0.14	1.50±0.06
Apple peel	5.61±0.11	1.01±0.07
Banana peel	6.98±0.34	1.36±0.34

Values are expressed as mean ± standard deviation (n = 3).

Reductive capacity of methanol fruit peel extracts determined by the FRAP method is in the range of 15.61±0.11 mmol Fe^{2+}/100g DW, which is detected for apple peel to 6.98±0.34 mmol Fe^{2+}/100g DW for banana peel.

From results obtained by ABTS assay it is possible to notice that lemon has the highest antioxidant capacity, 1.50 mg TE/100 g DW, followed by banana and orange with 1.36 mg TE/100 g DW and apple, 1.01 mg TE/100 g DW. Generally, it is possible to conclude that apple has the smallest antioxidant capacity whether it is determined by FRAP or ABTS method.

Antioxidant capacity of fruit peels was reported by others.[24-26] Higher antioxidant capacity was determined in orange, apple and banana peel by Afsharnezhad *et al.*[25] These differences can be due to the complexity of phenols and the methods of extraction and analysis used.[27] It has been reported that extracts obtained using more-polar solvents had greater amount of antioxidants than those obtained using less polar solvents.[28,29] For phenolic compounds, flavonoids and flavonols, methanol was found to be better solvent than ethanol.

The antioxidant capacity depends on the content of total polyphenols and some phenolic acids.[30,31] Fruit and vegetables peels contain higher amounts of total phenolics compared to the edible portions. It has been reported that phenolic content and antioxidant capacities in different vegetable tissues decreased from peel, phloem to xylem. [32, 33] Someya *et al.* [34] reported that total phenolics were more abundant in banana peel than in pulp.

CONCLUSION

Fruit peels, generated as agricultural co-products, can be considered valuable natural source of antioxidants. The results of this research show that orange, lemon, apple and banana peel contain bioactive compounds with antioxidant capacity. Food industries should be encouraged to use them as raw ingredients for production of dietary supplements and in value-added products. In the future, extensive work in isolation and characterization of the active biomolecules in these fruit peels is required.

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