Antioxidant profile and stability of spread made of watermelon seed kernels

[1]Bojan Antonić, [1]Dani Dordević

[1]Department of Plant Origin Foodstuffs Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno

Abstract

A large amount of by-product is created annually in the processing of watermelons and are being disposed of in most of the cases. The present study aimed to create a non-traditional product made of watermelon seed kernels and to analyse its stability and antioxidant potential during the 28 days of refrigerated storage. The obtained initial value for TPC was 0.94 mg GAE/g, for FRAP 3.79 μmol/g Trolox and DPPH inhibition was 28 %. The MDA was found at the level of 2.08 μg/g. After 28 days of storage, TPC value dropped to 0.57 mg GAE/g, FRAP to 2.52 μmol/g Trolox, while no statistically significant change was observed for parameters of DPPH and MDA. It can be concluded that even though the obtained product was contained in a large extent with oil rich in unsaturated fatty acids that are susceptible to oxidation, no increase in MDA as an indicator of lipid oxidation was found. The created spread represents a product with great potential for development for human consumption.

Keywords: total phenolic content, novel spread product, lipid oxidation, watermelon by-products, DPPH

Introduction

In the processing of watermelon in the production of jams, juices, fresh salads and others, the seeds are generated as a waste by-product (Rico et al. 2020). According to the Food and Agriculture Organization (FAO) of the United Nations, the estimated annual production of watermelon in the World was more than 100 millions of tons FAOSTAT (2018). The seeds make 1 - 4 % of total watermelon weight and in most cases, it's disposed of which makes ecological problems (Seidu & Otutu, 2016; Falade et al. 2020). The seeds contained in watermelon have great potential as a source of high-value nutrition, making them a source of proteins, vitamin B, minerals and oils (Gwana et al. 2014). Fat and protein content makes approximately 75 % of seed weight (Kaul et al. 2011). Because of low free fatty acids content and a high percentage of unsaturation in the lipid structure, and also the presence of essential fatty acids like linoleic acid, watermelon seed oil can be classified into high-quality groups of oils for human consumption (Milovanovic et al. 2005; Acar et al., 2012; Ramaiya et al. 2019). Also, substances such as lycopene and polyphenols give them antioxidant properties (Perkins-Veazie & Davis, 2004; Tabiri et al. 2016).

Some of the use of watermelon seeds includes preparation of snacks, usage in form of flour for the preparation of sauces, use of oil for cooking and even in the preparation of cosmetics (Tabiri et al., 2016).

The present study aimed to create the non-traditional product made of watermelon seed kernels, to test its stability and to show its potential as a novel product without using any of the food preservation substances.

Materials and methods

The sample was prepared by milling dry melon seed kernels from producer "TRS" India. The picture of milled dry melon seed kernels is shown in (Plate VII, Fig. 1) . The obtained sample was spread like consistency and was tested during 28 days (on day 0, 4, 21 and 28) of refrigerated storage at 6 °C in vacuum-sealed bags. The tested parameters were MDA (malondialdehyde), TPC (total phenolic content), FRAP (Ferric reducing antioxidant power)

Address for correspondence:
Bojan Antonić
Department of Plant Origin Foodstuffs Hygiene and Technology,
Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého tř. 1946/1, 612 42 Brno
E-mail: ANTONICB@vfu.cz
www.maso-international.cz

and DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging activity. All optical measurements were done on UV/VIS spectrophotometer CE7210, Cecil Instruments.

The MDA evaluation using TBA (thiobarbituric acid) method.

The sample of 1.5 g was placed into a centrifugal tube with the addition of 1 mL of EDTA and mixed, after which is added 5 mL of 0.8 % BHT and again mixed. 10 % of TCA is added to the mixture and homogenized for 30 seconds at 10000 rpm. After homogenization, the tube is centrifuged for 5 minutes at 3500 x g at 4°C. The upper layer is then taken from the tube and filtration is done on the lower layer. The filtrate is added to the 10mL volumetric flask and then added 10 % TCA to the mark. From this flask, 4 mL was taken and 1 mL of TBA is added to it. These, same as standard solutions of MDA, are incubated for 90 minutes at 70°C. After incubation, all is carried to the ice bath for 2-3 minutes and then rested at room temperature for 45 minutes. Absorption is measured on a spectrophotometer at 532 nm and results are calculated using the standard calibration curve.

Folin-Ciocalteu TPC (total phenolic content) method

1g of the sample was extracted in 10 mL of water by shaking for 10 minutes. 1 mL of obtained extract was then mixed with 5 mL of Folin-Ciocalteu/water solution 1/10 and 4 mL Na2CO3 (7.5 %) in 25 mL volumetric flask and left in the dark for 30 minutes incubation. The flask is then filled to the mark with distilled water and solution measured at 765 nm on a spectrophotometer. In parallel, the standard calibration curve was done using the gallic acid standard solutions and the results are expressed as mg/g of gallic acid equivalent (GAE).

FRAP (Ferric reducing antioxidant power) method

Extraction of 0.1 g of homogenized sample was done in an ultrasonic bath in 20 mL of 75% methanol during 30 minutes. 180 µL of the filtered extract was supplemented with 300 µL of distilled water and incubated with 3.6 mL of working solution (acetate buffer + TPTZ + FeCl3 x 6H2O in ratio 10:1:1) for 8 minutes in the dark. The absorbance was then measured on the spectrophotometer at 593 nm. The calibration curve was made with Trolox standard solutions and the results are expressed as µmol/g Trolox.

DPPH (diphenyl picrylhydrazyl) radical scavenging method

Extract for analysis was made by 30 minutes ultrasonic extraction of 0.1 g of homogenized sample in 20 mL of ethanol. After filtration 3 mL of extract and in parallel 3 mL of ethanol as a blank were mixed with 1 mL of 0.1 mM DPPH in ethanol and incubated in the dark for 30 minutes. After incubation, the measurement of absorbance of both solutions was done on a spectrophotometer at 517 nm and calculated a percentage of inhibition of free radical DPPH by the sample against the blank.

Results and discussion

Table 1. Obtained values of measured parameters for selected days of storage period

Storage period	MDA (µg/g)	FRAP (µmol/g Trolox)	DPPH (%)	TPC mg GAE/g
Day 0	2.08 ± 0.07^{a}	3.79 ± 0.41^{a}	28.00 ± 1.29	0.94 ± 0.02^{a}
Day 4	1.80 ± 0.18	3.13 ± 0.15	28.62 ± 6.65	0.73 ± 0.02^{b}
Day 21	1.68 ± 0.03^{b}	3.37 ± 0.22	26.95 ± 2.19	0.57 ± 0.02^{c}
Day 28	1.82 ± 0.12	2.52 ± 0.49^{b}	19.83 ± 8.92	0.57 ± 0.02^{c}

*indicated letters (a, b, c) show a statistically significant difference ($p < 0.05$)

The lipid content of watermelon seeds is high and can range from 40 - 50 % (Hannah, 2015; Milovanović 2005). The study of Milovanovic (2005) revealed that oil contained in watermelon seeds had unsaturated fatty acids at the level of 77.4%. This makes it very susceptible to the lipid oxidation, which alters the sensory properties, leads to loss of essential fatty acids and vitamins and forms toxic compounds as secondary products (Barden et al. 2016; Jacobsen et al. 2016). One of the main secondary products of lipid peroxidation is malondialdehyde (Gaweł et al., 2004). In Table 1. obtained values were presented for MDA, FRAP, DPPH and TPC for selected days of storage period. Although the growth in MDA content in the sample was expected, there was no significant ($p<0.05$) difference in its levels when comparing the means of Day 0 and Day 28 of the refrigerated storage.

TPC value at Day 0 was 0.94 mg GAE/g of the sample and it was lower than values obtained by Tabiri et al. (2016). Their experiments resulted in 14.94, 39.49 and 54.16 mg GAE/g of total phenolics for three watermelon varieties they investigated. The reason for higher values might be that those studies involved whole seed, while the present study used the kernels only. The kernels usually contain a lower percentage of whole seed polyphenolic content which was confirmed in studies of pumpkin seeds and pistachio (Krimer-Malešević et al., 2011; Nadernejad et al., 2013). The study of Acar et al. (2012) resulted in lower TPC values which were 0.13 and 0.28 mg GAE/g for kernel and whole seed respectively. This could also mean that TPC content in watermelon seeds is highly dependable on plant variety.

There was a statistically significant ($p<0.05$) decrease of total phenolic content from Day 0 to Day 21, but there was no difference in values obtained for Day 21 and Day 28. The value at the end of the tested period was 0.57 mg GAE/g which means that 60 % of the initial TPC value has been preserved.

DPPH inhibition on Day 0 was 28.0 %, which can be graded as good when taking into consideration relatively low TPC value. The three watermelon seed extracts from the work of Tabiri (2016), exhibited 59.88, 70.06 and 94.66 % of DPPH inhibition and those values were in correlation with their total phenolic content. Although there was a change in phenolic content during the experiment period, no statistically significant ($p<0.05$) difference was obtained for DPPH inhibition in all tested samples.

The results for FRAP on Day 0 and Day 28 were 3.79 and 2.52 μmol/g Trolox respectively and this decrease was statistically ($p<0.05$) significant. The values for the first 21 days of storage were not significantly different which is one more indicator of good stability of the tested product.

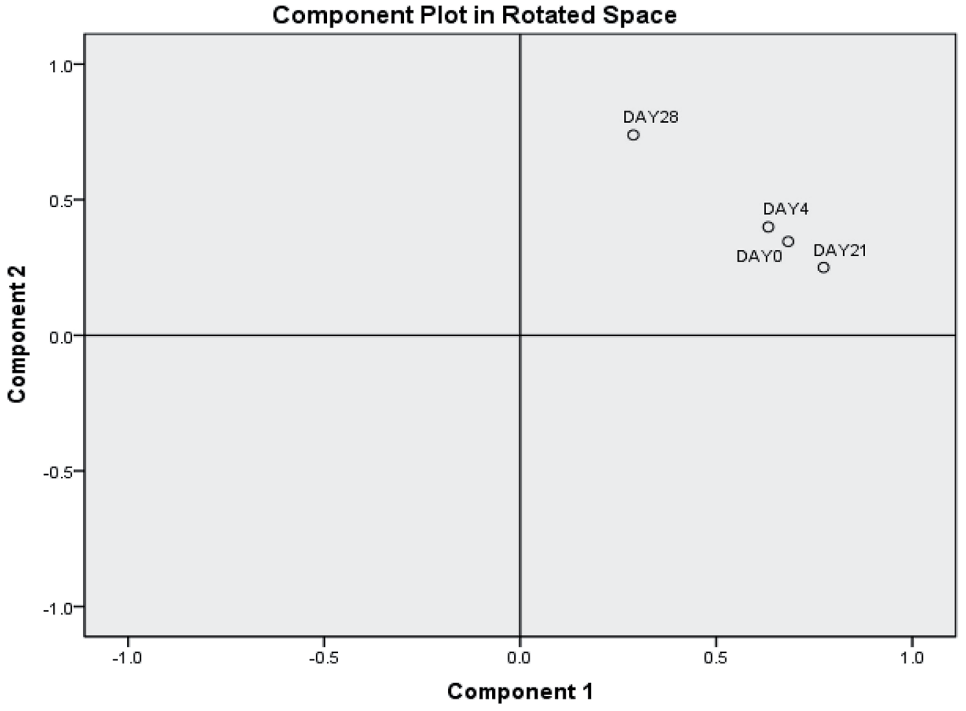

Fig. 2. Principal component analysis (PCA) of all measured parameters in 0, 4, 21 and 28 days of storage

The results obtained after each selected day of storage were compared with principal component analysis (PCA) (Fig. 2.). All results showed significant ($p < 0.05$) difference, with the most noticeable deviation of the results of the sample from Day 28. This can mean that starting from the Day 28, the considerable changes occur in the product regarding its quality.

Conclusion

In the present study, the spread was made out of watermelon seed kernels and tested the stability during the 28 days while being stored in a refrigerator at 6 °C. Produced spread potentially contains a high amount of oil rich in unsaturated fatty acids which easily undergoes the lipid oxidation. Although the spread was not packed into the protective atmosphere, neither was supplemented with any kind of preservation substances, the obtained results for malondialdehyde as the secondary lipid oxidation product revealed no increase during the storage. This implies that the watermelon seeds are very rich in natural antioxidants which in this case have the role of preservation agents against the lipid spoilage. The level of total polyphenolic compounds was reduced to the acceptable 60 % of the initial content, which didn't have an impact on DPPH radical scavenging activity. It can be concluded that watermelon seed spread with high nutritive value and good stability represent great potential for utilization as a product for human consumption. For future experiments, the sensory analysis is needed to check the acceptance among the potential consumers.

References

Acar, R., Özcan, M. M., Kanbur, G., & Dursun, N, 2012: Some Physico-Chemical Properties of Edible and Forage Watermelon Seeds. Iran. J. Chem. Chem. Eng. Vol, **31**

Barden, L. and Decker, E. A. 2016: Lipid oxidation in low-moisture food: a review. Critical reviews in food science and nutrition, **56**, 2467-2482.

Falade, O. S., Otemuyiwa, I. O., Adekunle, A. S., Adewusi, S. A. and Oluwasefunmi, O. 2020: Nutrient composition of watermelon (Citrullis lanatus (Thunb.) Matsum. &Nakai) and egusi melon (Citrullus colocynthis (L.) Schrad.) seeds. Agriculturae Conspectus Scientificus, **85**, 43-49.

Food and Agriculture Organization of the United Nations. FAOSTAT Database. Rome, Italy:

FAO, 2018: http://faostat3.fao.org/home/E Accessed May 7, 2020

Gaweł, S., Wardas, M., Niedworok, E. and Wardas, P., 2004: Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960), **57**, 453-455

Gwana, A. M., Bako, M. M., Bagudu, B. Y., Sadiq, A. B. and Abdullahi, M. M. 2014: Determinations of phytochemical, vitamin, mineral and proximate compositions of varieties of watermelon seeds cultivated in Borno State, North-Eastern Nigeria. International Journal of Nutrition and food sciences, **3**, 238-245

Hannah, M. A. C., & Krishnakumari, S. 2015: Profiling of Lipid and Vitamin contents in the extract of Watermelon (Citrullus vulgaris Schrad.) seeds. *Journal of Pharmacognosy and Phytochemistry*, **4**, 247.

Jacobsen, C., 2016: Oxidative stability and shelf life of food emulsions. In Oxidative stability and shelf life of foods containing oils and fats AOCS Press., 287-312

Kaul, P., 2011: Nutritional potential, bioaccessibility of minerals and functionality of watermelon (Citrullus vulgaris) seeds. LWT-Food Science and Technology, **44**, 1821-1826

Krimer-Malešević, V., Mađarev-Popović, S., Vaštag, Ž., Radulović, L., & Peričin, D., 2011: Phenolic acids in pumpkin (Cucurbita pepo L.) seeds. In Nuts and Seeds in Health and Disease Prevention, Academic Press., 925-932)

Milovanović, M., & Pićurić-Jovanović, K., 2005: Characteristics and composition of melon seed oil. *Journal of Agricultural Sciences (Belgrade)*, **50**, 41-47

Nadernejad, N., Ahmadimoghadam, A., Hossyinifard, J. and Poorseyedi, S., 2013: Effect of different rootstocks on PAL activity and phenolic compounds in flowers, leaves, hulls and kernels of three pistachio (Pistacia vera L.) cultivars. Trees, **27**, 1681-1689

Perkins-Veazie, P., & Davis, A. R., 2004: In search of high lycopene watermelon. REPORT-CUCURBIT GENETICS COOPERATIVE, **27**, 51

Ramaiya, S. D., Bujang, J. S., & Zakaria, M. H., 2019: Physicochemical, Fatty Acid and Antioxidant Properties of Passion Fruit (Passiflora Species) Seed Oil. Pakistan Journal of Nutrition, **18**, 421-429

Rico, X., Gullón, B., Alonso, J. L. and Yáñez, R., 2020: Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: an overview. Food Research International, 109086

Seidu, K. T. and Otutu, O. L., 2016: Phytochemical composition and radical scavenging activities of watermelon (Citrullus lanatus) seed constituents. Croatian journal of food science and technology, **8**, 83-89
Tabiri, B., Agbenorhevi, J. K., Wireko-Manu, F. D. and Ompouma, E. I., 2016: Watermelon seeds as food: nutrient composition, phytochemicals and antioxidant activity.