INFLUENCE OF HEAT TREATMENT ON POMEGRANATE BIOACTIVE COMPOUNDS (*PUNNICA GRANATUM L.)*

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RESEARCH ARTICLE (REVIEW ARTICLE)

Abstract
An important source of bioactive compounds, the pomegranate (*Punnica granatum L.*), has demonstrated its qualities by the multitude of researches accomplished over the years. The pomegranate juice is rich in bioactive compounds of the polyphenols type, with antioxidant activity. The object of the study was the determination of the antioxidant activity and the content of total polyphenols and anthocyanin pigments from 6 samples of pomegranate (seeds and pulp) submitted to thermal treatment gradually from 40-90°C. The purpose of this study is classified among the present tendencies of strengthening of the food products thermally treated with fruits. Moreover, the utilization of the waste resulting from the food industry for the purpose of the recovery of the compounds of interest is another approach of this paper. The results obtained, have underlined that the thermal moderate treatment doesn't affect significantly the content of total polyphenols and of anthocyanin and by default their antioxidant activity. To a small extent the antioxidant activity of the pomegranate fruits, their content of polyphenols is similar in all the analyzed samples and the anthocyanins were present in all the samples no matter the method used.

Keywords: pomegranate, antioxidant activity, polyphenols
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INTRODUCTION

The pomegranate (*Punnica granatum L.*), one of the most famous and sold fruit, it gained along time an important place in the daily consumption of the people due to its content of bioactive compounds, being used in the popular medicine for thousands of years. Due to its content of antioxidants and nutritive compounds it is labelled today as being a super food for health. (Tezcan and collab. 2009, T and collab., 2009; Tehranifar 2010, Adidaz 2014, Gadze 2012, Zunfung Li 2006).

The main classes of substances identified in the pomegranate are: simple sugars, aliphatic organic acids, phenolic or enolic acids all with antioxidant properties. The organic compounds identified in the pomegranate are: flavonoids, anthocyanin glycosides and anthocyanins. Also in different parts of the pomegranate we found: ellagitannins, amino acids, indolamines, alkaloids, fatty acids and sterols in the seeds, triterpenoids in the flower and pomegranate seeds, glycolipids and cerebrosides and phenyl aliphatic glycosides in the seeds (Arzu, 2012).

Other studies have demonstrated that the pomegranate juice, by the properties it has, can prevent atherosclerosis (Zunfung li 2006), it can help to prevent or treat different factors of risk of disease, including the arterial hypertension, the high cholesterol, the oxidative stress, the hyperglycaemia and the inflammatory activities (Aidaz, 2014). Also, epidemiological studies have showed that the consumption of fruits and vegetables is negatively associated with the morbidity and mortality of the cardio- and cerebrovascular diseases and of some types of cancer (Johnsen and collab., 2003, Rissanen and collab., 2003, Temple and Gladwin, 2003), and the antioxidants from the fruits and vegetables, including the ascorbic acid, carotenoids, flavonoids, hydrolyzable tannins are presupposed to play an important role in the prevention of these diseases (Huxley and Neil, 2003, Knekt et al., 2002, Lampe, 1999).

The quality of the pomegranate fruits is influenced by the variety, region of growth, pedoclimatic factors, maturity and technology of growth (Poyrazoglu and collab., 2002; T and collab., 2002; Tehranfiar Ehranfiar and collab., 2010). The period of maturity of the pomegranate fruits is determined usually based on the colour of the peel, the dimension of the fruits, the content of sugar and the acidity (Ercisli and collab., 2007; Al Said and collab., 2009, Gadze,2012). The colour and dimension of the fruits affect the behaviour of the consumers, being considered the most important external parameters of quality (Alighourchi and Barzegar, 2009; Celik and Ercisli, 2009). All the factors mentioned

influence the content in nutritive compounds of the fruits (Gadze,2012).

The pomegranate being very often used for the obtaining of the food products (juices, alcoholic drinks, bakery, pastry and confectionery), our attention was directed on the bioactive compounds after the thermal treatment of the pomegranate seeds.

The main objective of the paper was to underline the effect of the thermal treatment, on a limit of 50 to 90°C on the content of total phenols, of anthocyanin from the pomegranate seeds. The evaluation of the antioxidant capacity by the FRAP method of the samples submitted to the thermal treatment has represented another objective of the paper.

MATERIAL AND METHOD

The pomegranate fruits (*P. granatum* L.) were purchased from the business network (Oradea, România), origin Croatia, in the spring of 2021. The fruits were washed, the kernels were separated together with the pulp from the peel, were weighed and left to dry for 11 days at 40°C, until they reached 17% humidity. After the reaching of the relative humidity of 17%, the pomegranate was submitted gradually to a thermal treatment from 10 to 10 degrees up to 90°C, for 2 hours in each thermal point, sampling 10g of sample at each period of time resulting 6 versions (noted V1-40°C, V2-50°C, V3-60°C, V5-70°C, V6-80°C and V6-90°C). In the 6 samples it was determined: the total content of polyphenols by the Folin Ciocalteu method, it was determined the quality of anthocyanins and it was determined the antioxidant activity by the FRAP method.

⇒ *The obtaining of the hydro alcoholic extract*

The pomegranate hydroalcoholic extracts were obtained passing through the phases presented in figure 1. For this, it was weighed 5 g of pomegranate from each experimental version, over which was added 45 ml of ethyl alcohol 70%, they were milled for 30 seconds, afterwards they were left to soak for 24 hours in the dark. Afterwards the samples were filtered and centrifuged at 5000 rpm, for 15 minutes, and the supernatant was used for analysis.

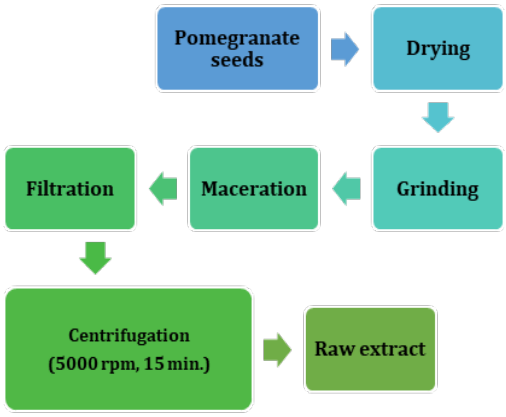

Figure 1. Diagram for obtaining the hydroalcoholic extracts

⇒ *Determinarea conținutului de polifenoli totali prin metoda Folin Ciocalteu*

Total phenolics content from samples was determined using the Folin-Ciocalteu method, according to Memete et al., 2021. Briefly, the hydroalcoholic extract of pomegranate seeds, both (100 µL) was mixed with 1700 µL of distilled water, 200 µL of Folin-Ciocalteu reagent (freshly prepared, dilution 1:10, v/v) and 1000 µL of 7.5% Na_2CO_3 solution. Then, the samples was incubated at room temperature, in the dark, for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu 1240 mini UV-Vis). The results were expressed in mg gallic acid equivalents (GAE)/100g dry weight (dw). *Qualitative ident of anthocyanin pigment.* The anthocyanin pigments were highlighted based on their property to change the colour depending on pH [***].

⇒ *Detrminarea activității antioxidante prin metoda FRAP (ferric reducing antioxidant power)*

The antioxidant capacity was determined using FRAP assay (Benzie, & Strain, 1996; Memete at al., 2021). The working FRAP solution was freshly prepared by mixing 300 mM acetate buffer, $FeCl_3 \cdot 6\ H_2O$ solution (20 mM) and TPTZ solution (10 mM) in ratio of 10:1:1, v/v/v. The hydroalcoholic extract of pomegranate seeds (100 µL) was combinated with 500 µL working FRAP solution and 2 ml distilled water. The mixture was incubated at room temperature, in the dark, for 1 h. The absorbance was recorded at 595 nm. The results were expressed in mmol Trolox equivalent (TE)/100 g fw.

RESULTS AND DISCUSSIONS

The content of total polyphenols determined by the Folin-Ciocalteu method has registered values between 43,2-55,9 mg GAE/100g, values (figure 2). From the data obtained we can say that the gradual applying of a thermal treatment on the pomegranate seeds doesn't cause significant effects on the polyphenol content.

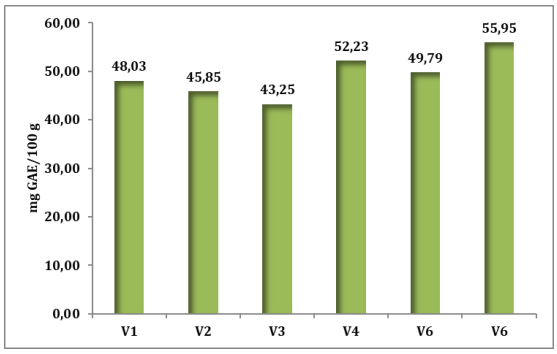

Figura 2. Rezultate privind conţinutul în polifenoli - metoda Folin-Ciocalteu

The presence of anthocyanins was highlighted in the acid medium based on the anthocyanins properties to change colour depending on pH. The results obtained highlighted the presence of anthocyanins in all the samples submitted to examination, by the appearance of pink colour (figure 3).

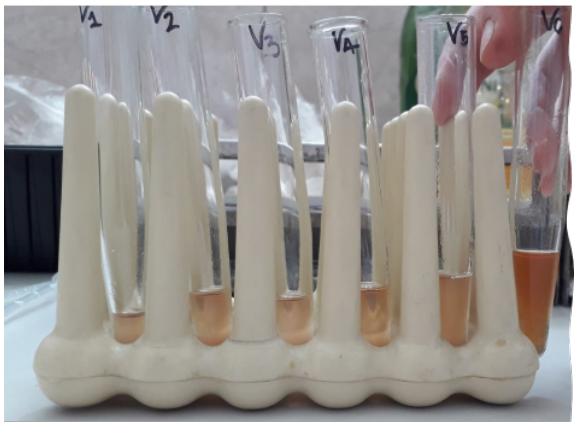

Figure 3. Highlighting the presence of anthocyanins

The results of the spectrophotometric analysis regarding the antioxidant activity determined by the FRAP method, shows that the greatest antioxidant activity was found in V1 (0,590 mmol TE / 100 g). Also in figure 4 we can observe that together with the increase of drying temperature the antioxidant activity decreases to 50% in the analysed samples.

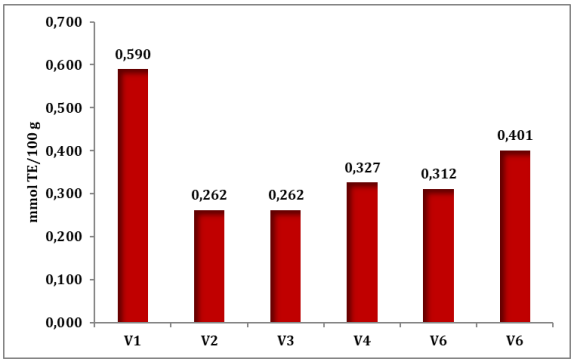

Figure 4. The results regarding the determined antioxidant activity – FRAP method

Some studies highlight the results in which are presented the implications of the utilization of pomegranate seeds in obtaining bakery products and the behaviour of the flour obtained from kernel on the rheological structure of the dough, on the organoleptic properties, on the total quality of bread. H. Gül, H. Şen in 2017 have demonstrated the effects of the admixture of flour from pomegranate kernels up to 10% in the bread.

The results obtained have demonstrated that the substitution of the wheat flour in a percentage of 5% doesn't produce modifications on the sensorial and technological quality but, once the percentage increases there are modifications appearing, as would be the decrease of volume, the widening of the products, the shining of the bread, modifications in the texture properties as would be the toughness and mastication. Also, Bhol S. and collab. in 2015 have demonstrated that the admixture of 5% and 15% of flour obtained from the whole pomegranate in the bread increases significantly the antioxidant potential of the products, directly proportional to the increase of the percentage of pomegranate flour introduced.

CONCLUSIONS

The content of bioactive compounds from the pomegranate kernels thermally treated at ≲90°C is kept also after the thermal treatment. The obtained results regarding the antioxidant capacity of the pomegranate seeds indicate the fact that, it decreases once the temperature increases, the significant differences being registered between the sample V1(0,590 mmol TE/100g) and V2 (0,262 mmol TE/100g), afterwards the results being close.

The content of polyphenols from the analyzed samples is maintained at close values,

between 42.2-55.9 mg GAE/100g no matter the applied temperature. The presence of the anthocyanins was highlighted in all the analyzed experimental versions.

In the context of the present tendencies of strengthening the thermally processed food products with fruits, following the data obtained, from which it can be observed that the thermal treatment applied to the pomegranate kernels doesn't deteriorate but only a small part of bioactive compounds, we recommend the utilization of the pomegranate kernels as auxiliary material for the obtaining of the thermally treated food products.

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