PRELIMINARY RESULTS OF THE EFFECT OF STORAGE ON FUNCTIONAL PROPERTIES OF BUCKWHEAT

PRELIMINARNI REZULTATI O UTICAJU SKLADIŠTENJA NA FUNKCIONALNE OSOBINE HELJDE

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ABSTRACT

Cereal grains mostly belong to warm-season crops, but the processing of and demand for cereal grains are notable throughout the year, which emphasizes the paramount importance of storage in maintaining a sufficient supply of cereals in all seasons. However, storage facilities, grain condition and storage period are critical factors for preserving the quality of cereal grains. Furthermore, different cereals require different storage conditions for grain quality preservation.

The present study provides the preliminary results of the effect of storage in shopping centres on the phenolic compounds content of buckwheat whole grains. A total of fifteen free and bound phenolic compounds were identified and quantified in fresh buckwheat grains and those stored for one year using the high-performance liquid chromatography (HPLC). The results obtained indicate that storage exerted detrimental effects on the free phenolic content examined, while the content of bound phenolic compounds significantly increased during the storage period under consideration.

Keywords: phenolic acids, flavonoids, rutin, quercetin, whole grain buckwheat flour.

REZIME

Žetva većine žita rica odvija se u letnjem periodu, ipak, potreba za njima kao i njihova prerada neophodni su tokom čitave godine. Iz navedenih razloga skladištenje žitarica jedini je način da se omoguće njegove zalihe tokom cele godine. Međutim, za očuvanje biološkog, hemijskog i fizičkog kvaliteta žita, ključnu ulogu imaju uslovnost objekata za skladištenje, kvalitet zrna pre skladištenja i dužina trajanja skladištenja. Nadalje, različite sorte žita i pseudožita imaju različite zahteve u pogledu uslova skladištenja kako bi se očuvao njihov kvalitet.

Cilj ovog rada je da pruži uvid u uticaj skladištenja heljde u uslovima koji se obično javljaju u prodajnim objektima na sadržaj različitih polifenolnih jedinjenja. Primenom tačne hromatografije visoke rezolucije, identifikovano je i kvantifikovano petnaest polifenolnih jedinjenja, fenolnih kiselina i flavonoida, u slobodnom i u vezanom obliku, u uzorcima zrna heljde pre i nakon skladišnog perioda od godinu dana.

Dobijeni rezultati ukazali su da skladištenje zrna heljde u ispitivanim uslovima, sa jedne strane, ima nepovoljan uticaj na sadržaj slobodne frakcije polifenolnih jedinjenja, dok sa druge strane, pozitivno deluje na sadržaj vezane frakcije izazivajući njeno povećanje.

Ključne reči: fenolne kiseline, flavonoidi, rutin, kvercetin, heljda.

INTRODUCTION

Cereal crops are among the most important staple foods in the world, playing a decisive and irreplaceable role in both agricultural production and feeding the world's population (Lang *et al.*, 2014). Cereals are extensively used not only because of their exceptional nutritional properties, but also due to easy storage, convenient shipment and their adaptability to different climatic conditions. Therefore, the objective of adequate grain transportation and storage is to preserve the biological, chemical and physical qualities of grains as long as possible after harvest. However, storage conditions and postharvest technologies are not always adequate and cannot be always accurately monitored. The postharvest conditions in food factories, supermarkets and homes can prove inadequate to preserve the grain quality during the storage period. Considering consumer habits and demands for healthier and more diverse foods, producers and researchers alike have been searching for new crops, or have placed a new and stronger emphasis on those previously used but neglected at present. Therefore, assiduous attention is paid to ancient crops such as buckwheat (Filipčev *et al.*, 2013; Jambrec *et al.*, 2013). Buckwheat is a dicotyledonous pseudocereal which belongs to the family *Polygonaceae*, the genus *Fagopyrum*. Although ubiquitous around the world, it is predominantly found in the Northern Hemisphere (Li and Zhang, 2001). Over the past years, the production of buckwheat in the world has averaged about 1 million tonnes, with Russia and China as major producers

(FAOSTAT, 2013). Buckwheat has received increasing attention from food scientists and consumers as one of the best plant sources of proteins, minerals, antioxidants and dietary fibres, which is also suitable for people suffering from celiac disease (Sedej *et al.*, 2011). The antioxidant components in buckwheat, phenolic compounds, tannins, phytosterols and tocopherols exert anti-inflammatory and anti-carcinogenic effects (Kreft, *et al.*, 2006). Rutin and quercetin, some of the most important flavonoids found in buckwheat (but not in other cereals or pseudocereals) (Sakač *et al.*, 2011), are associated with the healing impact on chronic diseases (Zhang *et al.*, 2012). In addition to these compounds, catechin is also a very valuable buckwheat constituent, featuring a high antioxidant capacity accompanied with cardioprotective, diuretic and hypotensive actions (Yetuk *et al.*, 2014). Buckwheat, as well as other grains, is generally stored prior to consumption or processing. Consequently, grains often remain in market and retail centre storage facilities for a long period of time. Conversely, buckwheat grains are usually processed into groats (i.e. residues found after seed hulling) or flour before storage. However, buckwheat contains volatile essential fats that are not well protected after the air-tight hull is removed, and, under unfavourable humidity and temperature conditions, the presence of oxygen leads to rancidness, which adversely affects the taste, phenolic compounds content and expected health benefits of buckwheat grains. Although some previous studies (Ziegler *et al.*, 2016) suggest that the storing process modifies the content of phenolic compounds in plant materials, those studies were

mainly focused on changes in technological and nutritional qualities of grains and seeds. Moreover, there is a lack of information relevant to the storage of buckwheat, and particularly to changes in the phenolic compounds content caused by storage. With regard to buckwheat health benefits (stemming from the rich phenolic content), the purpose of this paper is to provide the preliminary results of the change in the phenolic compounds content induced by the effect of storage on unhulled buckwheat seeds under conditions comparable to those in shopping centres.

MATERIALS AND METHODS

Raw materials

Common buckwheat (the proximate composition: moisture 11.81 %, protein 15.24 %, fat 3.24 %, ash 2.65 %, reducing sugars 2.30 % and starch 71.60 %) was purchased from Hemija Commerce, Novi Sad, Serbia, placed in paper bags and stored on shelves at room temperature (25 °C) and a relative humidity of 50–60 % for a year. The room was periodically ventilated. Shortly before the analysis, grains were milled on a stone mill and sieved through a 100-mesh screen into flour containing the aleurone layer, germ and hulls which passed through the sieve.

Extraction of phenolic compounds and the HPLC/DAD analysis

The extraction of phenolic compounds from the flour was performed in accordance with the method described in Jambrec et al. (2015), while the HPLC/DAD analysis was consistent with the procedure argued by Mišan et al. (2011). The procedure is shown in Figure 1.

Statistical analysis

All analyses were performed in triplicate. Dependent t-tests were performed to determine the significance ($p < 0.05$) of difference in the content of phenolic compounds in buckwheat grains before and after the storage period of one year, using the statistical software STATISTICA (version 12., StatSoft, Inc, Tulsa, OK, USA (2013)).

RESULTS AND DISCUSSION

In the present study, five hydroxybenzoic acids (gallic acid (GA), protocatechuic acid (PCA), p-hydroxybenzoic acid (p-OH-BA), vanillic acid (VA) and syringic acid (SRA)), six hydroxycinnamic acids (p-coumaric acid (p-CoA), ferulic acid (FA), caffeic acid (CfA), sinapic acid (SIA), chlorogenic acid (CHA) and cinnamic acid (CA)), and four flavonoids (flavonols: rutin (RU), and quercetin (QU), flavan-3-ols: catechin (CAT), and epicatechin (ECAT)) were identified and quantified, both in free and bound forms. The fresh and stored buckwheat grain samples indicated the same phenolic compounds profile, whereas the stored samples contained less phenolic acids in the free form and more in the bound form in comparison with fresh grains. These changes can be accounted for by the activity of enzymes which facilitate the extraction of phenolics from the stored grains. Conversely, the observed reduction of free phenolics may be a result of the oxidation processes prompted by the storage conditions, or a consequence of converting one compound into another. The highest reduction in the content of phenolic acids during storage was observed in the free FA, GA and SIA, which is in agreement with the results of Sosulski et al. (1982), who observed a decrease in the ferulic acid content of wheat flour during storage. The authors suggested that destructive oxidation reactions of ferulic acid during the grain storage period can be accounted for the results obtained. With regard to the content of flavonoids, the research results showed that they were not found in the bound form neither in fresh nor in stored grain samples, with the exception of catechin. The ratio between free and bound phenolic forms was approximately 97:3, with rutin and catechin

as the most abundant free flavonoids (Table 2). However, the rutin content was significantly ($P < 0.05$) reduced during storage.

Fig. 1. Extraction of free and bound phenolic compounds and the HPLC/DAD analyses

Table 1. Content of phenolic acids (mg/kg dw) in buckwheat grains before and after the storage period of one year

		Initial	After storage
Gallic acid	Free	1.81 ± 0.06*	0.16 ± 0.00
	Bound	1.94 ± 0.51	14.59 ± 3.41*
Protocatechuic acid	Free	26.08 ± 0.78*	14.65 ± 0.89
	Bound	2.09 ± 0.34	14.14 ± 0.41*
p-Hydroxybenzoic acid	Free	2.31 ± 0.05*	0.98 ± 0.04
	Bound	7.12 ± 1.07	14.65 ± 1.47*
Caffeic acid	Free	42.73 ± 1.68*	14.94 ± 1.46
	Bound	5.35 ± 1.33	40.42 ± 1.78*
Vanillic acid	Free	15.53 ± 0.20*	6.29 ± 0.70
	Bound	nd	12.40 ± 1.50
Chlorogenic acid	Free	1.86 ± 0.01	6.36 ± 0.62*
	Bound	nd	nd
Syringic acid	Free	1.85 ± 0.09	2.88 ± 0.26*
	Bound	0.27 ± 0.04	6.46 ± 0.82*
p-Coumaric acid	Free	1.49 ± 0.03*	0.88 ± 0.06
	Bound	0.85 ± 0.02	2.75 ± 0.53*
Ferulic acid	Free	3.86 ± 0.01*	nd
	Bound	0.95 ± 0.43	3.34 ± 0.55*
Sinapic acid	Free	9.81 ± 0.03*	2.70 ± 0.23
	Bound	nd	nd
Cinnamic acid	Free	0.23 ± 0.01	0.31 ± 0.01
	Bound	nd	nd

* indicates significant differences between the samples in the same row

nd – non-detected

The stored samples contained 50% less rutin than the fresh samples. Furthermore, this increase was accompanied by an increase in the quercetin content, which can be explained by the conversion of rutin into quercetin in a reaction catalysed by rutin degrading enzymes (Vogrinčič et al., 2010). The total phenolic compounds content (Table 3) increased during storage due to a considerable increase in the bound phenolic content, primarily phenolic acids. Namely, the total content of bound phenolic acids was about six times higher, whereas the total content of bound flavonoids was about seven times higher in the stored

sample compared to the fresh sample. Similar results were observed by Dimberg et al. (1996) and Ziegler et al. (2016), who reported increased levels of phenolic acids in stored oat groats and soybean.

Table 2. Content of flavonoids (mg/kg dw) in wholegrain buckwheat grains before and after the storage period of one year

		Initial	After storage
Catechin	Free	68.99 ± 1.46	61.49 ± 3.78
	Bound	7.77 ± 1.33	53.61 ± 2.81*
Epicatechin	Free	19.56 ± 0.11	26.50 ± 1.41*
	Bound	nd	nd
Rutin	Free	168.90 ± 2.83*	93.40 ± 4.23
	Bound	nd	nd
Quercetin	Free	2.39 ± 0.03	3.75 ± 0.13*
	Bound	nd	nd

* indicates significant differences between the samples in the same row

nd – non-detected

Table 3. Total phenolic acids content (mg/kg dw) (TPAC) and total flavonoids content (TFC)

		Initial	After storage
Total TPAC	Free	107.56	50.15
	Bound	18.57	108.75
		126.13	158.90
Total TFC	Free	259.84	185.14
	Bound	7.77	53.61
		267.61	238.75
TOTAL		393.74	397.65

CONCLUSION

On balance, the total content of phenolic compounds in buckwheat grains was not significantly changed in the storage period under consideration. However, with regard to the content of specific compounds, the storage period was shown to exert significant impacts on the phenolic content. Namely, the highest reduction in the total content of phenolics during storage was observed in ferulic (30.56 %) and sinapic acids (72.48 %), as well as in the rutin content (44.70 %). Bearing in mind distinct biological activities of every phenolic compound and their impact on the functionality and health benefits of buckwheat, there is an overriding need for future research of the influence of storage conditions (i.e. varying air temperatures and humidity levels) and duration on the phenolic compounds content of buckwheat.

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