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Research paper

The disappearance of vitexin from Tartary buckwheat flour-water mixtures after the hydrothermal treatment

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ABSTRACT

Vitexin is an apigenin flavone glucoside with known biological functions. This research reported the effects of initial temperature treatments of Tartary buckwheat flour mixture with water and time of methanol extraction from the cooked doughs on the amount of extractable vitexin. The mixtures of flour and water were initially hydrothermally treated at temperatures from 25 °C to 95 °C. Afterward the mixtures were cooked at 95 °C for 20 min, and vitexin extracted at room temperature with 80% aqueous methanol for 20 min, 2 h and 8 h. The extractable vitexin was extracted during the same extraction times for the control in the nonhydrothermally treated Tartary buckwheat flour-water mixture samples. For the cooked dough samples, the hydrothermal treatments were important in terms of the extractability of vitexin. The extractable vitexin persisted in the control samples during the extraction time up to 8 hours, while in the hydrothermally treated and cooked dough samples, there remained none detectable vitexin. The high-temperature initial treatments during dough preparation appeared not to prevent the degradation of vitexin in Tartary buckwheat flour.

INTRODUCTION

Vitexin is a vital nutraceutical known for suppressing renal cell carcinoma and wound healing effects (Kim et al., 2005; He et al., 2016; Li et al., 2020; Bektas et al., 2020; Peng et al., 2021). Abbasi et al. (2021) reported the neuroprotective effects of vitexin, the experiments with rats have shown that vitexin has anticonvulsant effects in the brain.

Ganesan and Xu (2017) reviewed comprehensive information on various cancers and therapeutic possibilities of vitexin and isovitexin. According to Ganesan and Xu (2017), both, in vitro and in vivo studies are suggesting that vitexin and its isomere isovitexin are compounds with chemopreventive activity against various cancers, as they are included in proapoptotic events and/or autophagy. Li et al. (2020) reported the suppression of renal cell carcinoma by vitexin. Peng et al. (2020) reviewed recent advances in studies of absorption, metabolism, and bioactivity of vitexin. Bektas et al. (2020) recently evaluated the wound healing effect of chitosan-based gel formulation containing vitexin.

This activity is possibly through interaction at the benzodiazepine site of the gamma-aminobutyric acid type A receptor complex (Abbasi et al., 2012). Vitexin has shown free radical scavenging activity in ultraviolet B-irradiated cultured human dermal fibroblasts (Dong et al., 2011).

Due to important effects on human health, Tartary buckwheat has become more widely used for preparing gluten-free foods (Kreft 2016, Costantini *et al.* 2014, Capraro *et al.* 2018). Tartary buckwheat contains phenolic compounds that are reported to have antioxidant properties (Holasova *et al.* 2002;).

Hydrothermal treatments comprised heating with steam or hot water and followed by slow cooling and drying. The present study aimed to define the effects of temperature and moisture in Tartary buckwheat dough preparation on the concentration of extractable vitexin.

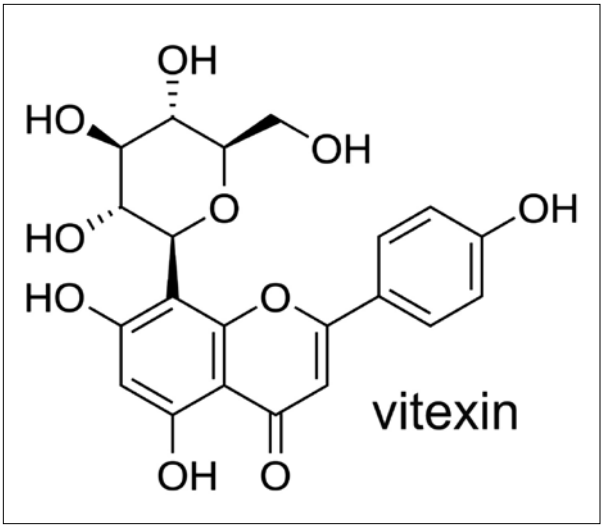

Figure 1. *Vitexin molecule. The upper part of the vitexin molecule is glucose, the lower part of the molecule is apigenin.*

MATERIAL AND METHODS

The Tartary buckwheat flour (cv. 'Zlata') was obtained from Rangus Mill (Šentjernej, Slovenia). Flour (100 g) containing 8% moisture was mixed for 1 min with 200 g water at 25 °C, 45 °C, 60 °C, 80 °C, or 95 ° C. The resulting doughs were kept in a chamber for 20 min at their respective temperatures. After this treatment, each dough sample was heated to 95 °C for a further 20 min. After cooling these cooked dough samples to room temperature, they were frozen (-18 °C) until they were lyophilized. The complete procedures were performed independently as three repetitions. The freeze-dried samples were milled and used in the methanol extractions for HPLC analyses. Extraction and HPLC analyses were performed according to the methods described by Germ et al. (2019).

The normal distribution of the data was tested using Shapiro-Wilk tests. Differences between treatments were tested using one-way analysis of variance followed by Duncan multiple range test. The level of significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The extractability of vitexin using 80% aqueous methanol from the control Tartary buckwheat flour samples and following the cooking (95 °C, 20 min) of the hydrothermally treated (25, 45, 60, 80, 95 °C) Tartary buckwheat dough samples are illustrated in Table 1. In the controls, all of the extractable vitexin was extracted within the first 20 min, which remained similar after the extraction times of 2 h and 8 h. Thus, vitexin was easily extractable from the untreated Tartary buckwheat flour using 80% aqueous methanol.

For the hydrothermal treatments of the doughs at 25, 40, and 60 °C, the vitexin extracted from the cooked doughs using 20% aqueous methanol was lower than detection limits. Control samples contained about 5.4 to

Table 1. *Time-courses of vitexin extraction (mg vitexin·kg-1 dry matter) with 20% (v/v) aqueous methanol for Tartary buckwheat flour (C; control) and following cooking (95 °C, 20 min) of the hydrothermally treated (25, 45, 60, 80, 95 °C) Tartary buckwheat dough samples. Data are means ± standard deviation (n = 3), except for treatments where vitexin concentration was under the limit of detection. <LOD means under the limit of detection*

Extraction time	Control	25°C	40°C	60°C	80°C	95°C
20 min	5.4 + 1.2 [a]	<LOD	<LOD	<LOD	<LOD	<LOD
2 hours	6.3 + 0.4 [a]	<LOD	<LOD	<LOD	<LOD	<LOD
8 hours	6.4 + 0.4 [a]	<LOD	<LOD	<LOD	<LOD	<LOD

Means followed by the same superscript letters are not significantly different at p < 0.05 (n = 3). Legend: LOD – under detection limit

6.4 mg vitexin·kg-1 dry matter (Table 1). Also, after all of the dough hydrothermal treatments (i.e., 25, 40, 60, 80, 95 °C), there were no more detectable concentrations of vitexin extracted from the cooked doughs in any of the times of extraction. Thus, in one scenario vitexin might be degraded by the conditions of hydrothermal treatment of the Tartary buckwheat doughs. Alternatively, vitexin might form insoluble complexes.

In nonhydrothermally treated Tartary buckwheat flour samples, extraction of vitexin with 80% aqueous methanol was complete within 20 min of extraction.

The sugar part of vitexin is glucose (Figure 1). The aglicone part is apigenin.

For the difference to rutin, which is O glucozide (sugar part and the flavonoid part of the molecule are connected by oxygen), is vitexin C glicoside (sugar and flavonoid part are connected by carbon-carbon bond). C glycosides are more stable than O glycosides, and they are less likely to be split apart. However, it is possible.

One possibility for disappearance of vitexin after hydrothermal treatment is its oxidation, and by additional –OH group there could appeared orientin. But no orientin was detected in the samples.

According to our knowledge, this is the first report that Tartary buckwheat contains in grain and flour a flavonoid vitexin, which is stable during the prolong extraction by methanolic extraction medium, but not stable when the mixture of flour with water is exposed to hydrothermal condition. Regarding importance of vitexin in health-preserving nutrition it is a challenge to study further the appearance and disappearance of vitexin and related metabolites in Tartary buckwheat.

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IZVLEČEK

Vpliv hidrotermičnega tretiranja na vsebnost viteksina v testu iz tatarske ajdove moke

Viteksin je flavonski glukozid z apigeninom. Viteksinu pripisujejo pomembne biološke učinke. V članku avtorji poročajo o vplivu predhodnega tretiranja mešanice moke tatarske ajde in vode pri različnih temperaturah na ekstrakcijo viteksina iz tako pripravljenega testa. Mešanica tatarske ajdove moke in vode je bila 20 minut tretirana pri temperaturah od 25 °C do 95 °C in nato še 20 minut pri 95 °C. Rkstrakcija viteksina je potekala pri sobni temperaturi (80% vodnim metanolom) 20 minut, 2 uri oziroma 8 ur. Na enak način je bil termično netretiran viteksin ekstrahiran tudi iz kontrolne mešanice tatarske ajdove moke. Na kuhane vzorce testa (mešanice moke in vode) je imelo hidrotermično tretiranje pomemben vpliv. Medtem ko je bila pri kontrolnih vzorcih količina viteksina nespremenjena še po 8 urah ekstrakcije, je bil pri hidrotermično tretiranih vzorcih viteksina pod mejo detekcije. Hidrotermično tretiranje testa tatarske ajde torej ni preprečilo degradacije viteksina v tatarski ajdovi moki.