

Communication

Hydrothermal Treatments Affecting the Concentration of Neochlorogenic Acid in Dough of Tartary Buckwheat

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Abstract: This study investigated the effects of initial temperature treatments of gluten-free doughs made from Tartary buckwheat flour, and time of methanol extraction from the cooked doughs of neochlorogenic acid, an important polyphenol metabolite. The doughs were hydrothermally treated from 25 °C to 95 °C, cooked at 95 °C for 20 min, and extracted using 80% aqueous methanol at the room temperature for 20 min, 2 h, and 8 h. For the control, nonhydrothermally treated Tartary buckwheat flour samples, the extractable neochlorogenic acid was similar for these extraction times. For the cooked dough samples, the hydrothermal treatments were important in terms of extractability of neochlorogenic acid. The extractable neochlorogenic acid was higher for the control samples in comparison to the hydrothermally treated and cooked dough samples. Among these hydrothermally treated dough samples, the high extractable neochlorogenic acid concentrations were maintained for temperatures of at least 80 °C. These high-temperature initial treatments during dough preparation appear to prevent degradation of the neochlorogenic acid in Tartary buckwheat flour. During hydrothermal treatment, neochlorogenic acid is bound to grain structures in such a way that prolonged extraction time is needed to extract it.

Keywords: hydrothermal treatment; neochlorogenic acid; buckwheat dough; Tartary buckwheat (*Fagopyrum tataricum*)

1. Introduction

Due to important effects on human health, Tartary buckwheat (*Fagopyrum tataricum*) is recently widespread in the preparation of various gluten-free foods [1]. It is cultivated or was recently cultivated in the mountains of south-west China, and in Nepal, northern India, Bhutan, Luxemburg, Slovenia, Italy, Sweden, and Bosnia and Herzegovina [2–4]. The high attention in common buckwheat and Tartary buckwheat breeding was given to reach high yields of grain in different ecological and agricultural environments. Much care has been given on the breeding, to facilitate husking and ensure nice taste [5]. Buckwheat is an annually growing plant rich in flavonoids, especially rutin. In the study by Dziedzic et al. [6], they found out that in Tartary buckwheat, among the investigated polyphenolic substances the contents of rutin and chlorogenic acid were the highest. Other polyphenolic substances, determined in Tartary buckwheat were: 2,6-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, caffeic acid, catechin, chlorogenic acid, fagopyrin, ferulic acid, myricetin, gallic acid, isovanillic acid, isovitexin, kaempferol, luteolin, p-coumaric acid,

procyanidin B2, quercetin, quercetin 3-D galactoside, rutin, syringic acid, and vitexin. Since this characteristic is an important factor in the usefulness of the plant for the pharmaceutical industry, buckwheat herb is therefore included in European Pharmacopoeia [7]. Whole buckwheat contains 2–5 times more phenolic compounds than barley and oats, while buckwheat bran and hulls have 2–7 times higher antioxidant activity than barley, triticale, and oats [8]. Buckwheat plants contain three classes of flavonoids: flavonols, anthocyanins and C-glucosyl flavones, which have been attributed beneficial properties as food constituents [6]. Buckwheat flour can be used in making gluten free food products, such as cakes, bread, wafers, and even tulumba dessert [9–13]. Gluten free products are very important in the diet of people with celiac disease [14]. Tartary buckwheat contains phenolic compounds that are reported to have anti-inflammatory, anticancer, antithrombotic and antioxidant activity [15–19], whereby the overall antioxidative activity of Tartary buckwheat will depend on the combination of the identified phenolics and the unidentified (minor) components. Phenolic compounds have variety of roles in plant life ranging from structural to protection [20,21]. They consist of an aromatic ring with one or more hydroxyl groups [21]. Robbins [21] stated that plant phenolic acids are divided into two groups: hydroxycinnamic acids and hydroxybenzoic acids. An important conjugated phenolic acid described is the chlorogenic acid (3-O-caffeoylquinic acid, CQA) which is essentially ubiquitous in the plant kingdom [22,23]. Clifford [22] reported in his paper that many of biological effects has been assigned to C-QA that attributed it into a popular compound, which has been often reported as a health promoting agent.

Neochlorogenic acid (Figure 1) is the ester formed by caffeic acid and (–)-quinic acid, and it functions as an intermediate in lignin biosynthesis. It is found in some types of dried fruit and a variety of other plant sources, such as peaches and herbs. According to Noratto et al. [24], neochlorogenic and chlorogenic acids have potential as chemopreventive dietary compounds, because they have shown relatively high growth inhibition on an estrogen-independent breast cancer cell line and on the other hand low toxicity against normal cells. In fact, neochlorogenic acid might be a colon cancer suppressive component of prunes [25].

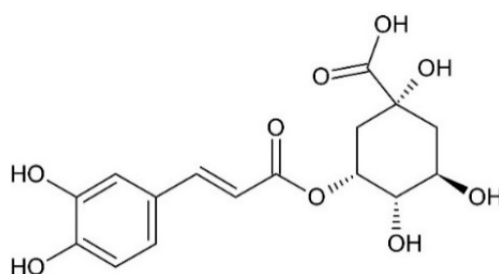


Figure 1. The structure of neochlorogenic acid (5-O-caffeoylquinic acid), an isomer of chlorogenic acid and a natural polyphenolic compound.

For Tartary buckwheat, Vogrinčič et al. [26] investigated the protective effects against DNA damage of complex mixtures of polyphenols, together with the other constituents that are present in whole plant material. Here, there were greater benefits for these complex mixtures than for the pure individual metabolites alone. Based on this, there is a need for further research into the activities of these and other compounds in Tartary buckwheat extracts. Although these are not as abundant as the main flavonoids, they have potential synergistic effects or can have significant contributions toward antigenotoxic properties. Thus, Vogrinčič et al. [26] suggested that Tartary buckwheat can be recommended to be included in the diet more often. Kurita et al. [27] reported about the identification of the predominant antioxidant compound in *Polygonum cuspidatum*, which belongs to the same family as *Fagopyrum tataricum*. The main active compound in the methanol extract of fresh samples was identified as neochlorogenic acid. Given the reports of health-promoting effects of neochlorogenic acid, their result may increase the utility of *P. cuspidatum*.

Hydrothermal treatments comprise heating with hot water or also steam, followed by slow cooling and drying. Such hydrothermal treatments are used with buckwheat to produce buckwheat groats, according to traditional technologies in Slovenia, Croatia, Czech Republic, Slovakia, Poland, Ukraine, and Russia [28]. The purpose of this study was to define the impact of temperature in Tartary buckwheat dough preparation on the concentration of extractable neochlorogenic acid.

2. Materials and Methods

2.1. Plant Material and Chemicals

Cv. Zlata Tartary buckwheat flour was received from Rangus Mill (Dolenje Vrhopolje, Šentjernej, Slovenia).

The neochlorogenic acid standard ((1R,3R,4S,5R)-3-[[[(2E)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid), methanol (CH₃CH₂OH, HPLC grade), acetonitrile (CH₃CN, gradient HPLC grade), and orthophosphoric acid (H₃PO₄, ACS grade) were obtained from Sigma-Aldrich (Steinheim, Germany). Double deionized water (ddH₂O) was produced using a water purification system (Simplicity 185; Millipore SAS, Molsheim, France), to an electrical resistance of 0.054 µS cm⁻¹.

2.2. Determination of Neochlorogenic Acid

A standard solution of neochlorogenic acid was prepared by dissolving 0.5 mg neochlorogenic acid in 10 mL methanol. The lyophilized cooked dough samples (2 g) were homogenized using a pestle and mortar and then extracted with 20 mL 80% aqueous methanol (*v/v*) at room temperature for 20 min, 2 h and 8 h, with shaking on a shaker (horizontal, 250 rpm). The methanol extracts were then filtered (Munktell No 390 paper; Munktell & Filtrac, GmbH, Bärenstein, Germany) and closed and stored in plastic 20-mL tubes. Prior to injection into the HPLC system (see below), the extracts and standard solutions were cleaned by filtering through syringe filters (Q-Max, 25 mm, 0.22 µm; Frisette ApS, Knebel, Denmark).

The neochlorogenic acid concentrations in the cooked dough samples and control flour were determined using a high performance liquid chromatography system (1260 Infinity; Agilent Technologies, Waldbronn, Germany) with a quaternary solvent manager coupled to a degasser (Cat N° G1311B), sample manager (Cat N° G1329B), column manager (Cat N° G1316A) and diode array detector (Cat N° G1315C; all from Agilent Technologies). All of the HPLC analyses were performed on a reverse phase C18 column Purosphere® 4 mm × 250 mm × 5 µm (Merck, KGaA, Darmstadt, Germany).

The mobile phase for the HPLC analyses was comprised of acetonitrile (A) and 0.1% orthophosphoric acid in ddH₂O (B). The gradient of elution was following: 0→1 min, 20% A; 1→5 min 20%→25% A; 5→15 min, 25%→30% A; 15→25 min, 30%→40% A. The injection volume was 10 µL and the flow rate 1 mL min⁻¹. The temperature of the column oven was adjusted to 30 °C, the samples were kept in the sample manager at 6 °C. The detection wavelength was 320 nm. The results were collected and processed further using the OpenLab ChemStation software for LC 3D systems (Agilent Technologies). The limit of detection for neochlorogenic acid was 1.06 mg kg⁻¹ sample, and the limit of quantification was 3.50 mg kg⁻¹ sample.

2.3. Hydrothermal Treatments

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 (TurboFixx 400 W, speed 3; Robert Bosch GmbH, Gerlingen-Schillerhöhe, Germany). 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 additional 20 min. After the dough samples were cooled to room temperature, they were frozen (−18 °C) until they were lyophilized. The complete procedures were performed independently as three repetitions. The samples were freeze-dried, milled and used in the methanol extractions for HPLC analyses. Chromatograms for the samples analyzed were for control flour and for all mixtures with water presented in the way as it is shown as examples in Figures 2–6.

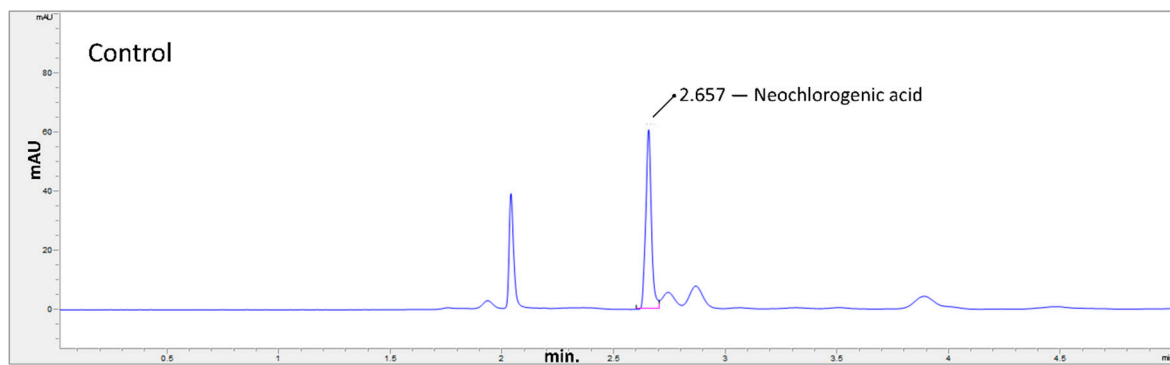


Figure 2. The chromatogram of neochlorogenic acid result in control flour, after 8 h of extraction.

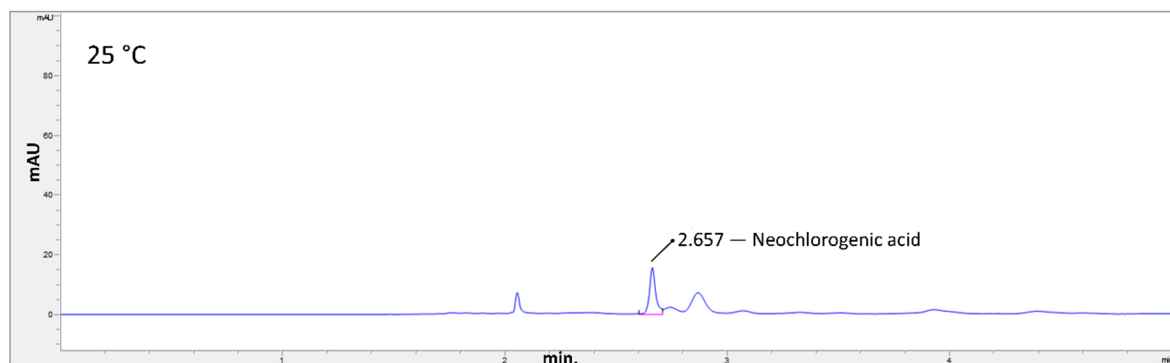


Figure 3. The chromatogram of neochlorogenic acid result for the water–flour mixture at 25 °C, after 8 h of extraction.

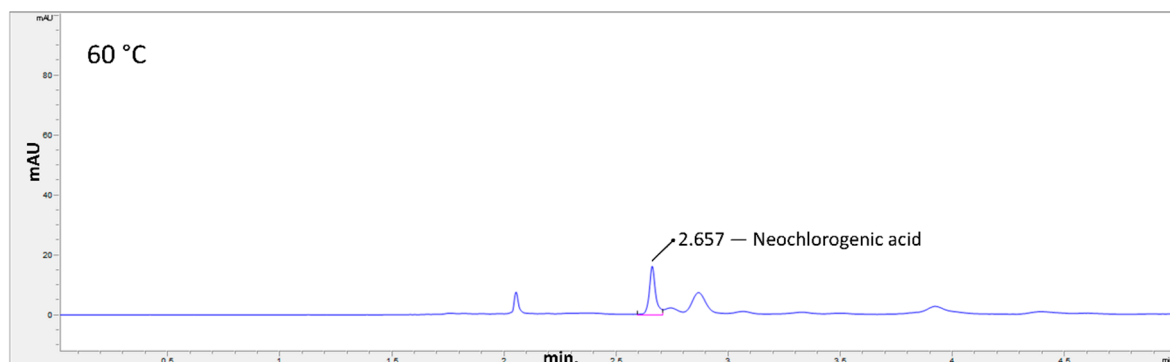


Figure 4. The chromatogram of neochlorogenic acid result for the water–flour mixture at 60 °C, after 8 h of extraction.

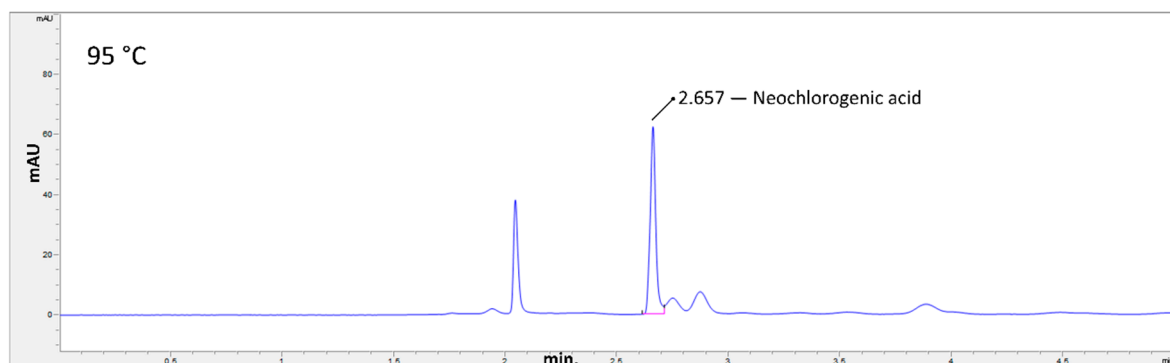


Figure 5. The chromatogram of neochlorogenic acid result for the water–flour mixture at 95 °C, after 8 h of extraction.

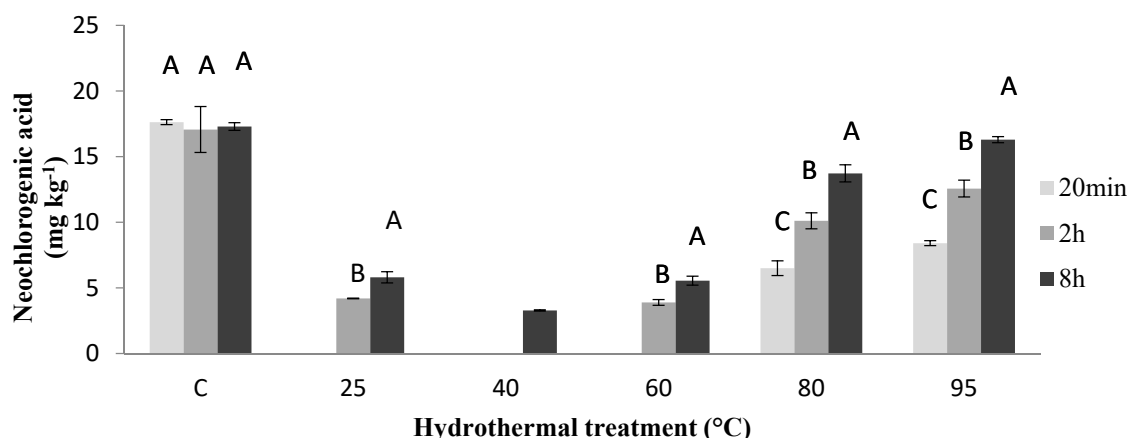


Figure 6. Time-courses of neochlorogenic acid extraction (mg neochlorogenic acid·kg⁻¹ dry matter) with 20% (*v/v*) aqueous methanol for Tartary buckwheat flour (C; control) and following cooking (95 °C, 20 min) of the hydrothermally treated (25, 45, 60, 80, 95 °C) Tartary buckwheat dough samples. Results are means ± standard deviation (*n* = 3), except for treatments where neochlorogenic acid was under the limit of detection. Different letters above the columns (A, B, C), indicate statistically significant differences between the extraction times within control (flour), respectively within each temperature condition (*p* < 0.05).

2.4. Statistical Analysis

The results are given as means ± standard deviation from three independently processed samples. ANOVA was performed, followed by Tukey post-hoc tests (for controls and hydrothermally treated samples at 80 °C and 95 °C), or Student's *t*-tests (for hydrothermally treated samples at 25 °C, 40 °C and 60 °C). The results were considered as significantly different at *p* < 0.05.

3. Results and Discussion

The extractability of neochlorogenic acid 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 Figure 6. In the controls, all of the extractable neochlorogenic acid was extracted within the first 20 min, which remained similar after the extraction times of 2 h and 8 h. Thus, the neochlorogenic acid was easily extractable from the untreated Tartary buckwheat flour with 80% *v/v* aqueous methanol.

During the hydrothermal treatments of the doughs at 25, 40, and 60 °C, the neochlorogenic acid extracted from the cooked doughs using 20% aqueous methanol was lower than for the control samples (at about 30%; Figure 6). Moreover, after all of the dough hydrothermal treatments (i.e., 25, 40, 60, 80, 95 °C), the concentrations of neochlorogenic acid extracted from the cooked doughs were dependent on the times of extraction. Indeed, in the dough samples that had been hydrothermally treated at 25, 40 and 60 °C, no neochlorogenic acid was extracted from the cooked doughs after 20 min, indicating that compared to the flour controls, the extraction of neochlorogenic acid from these cooked doughs requires a relatively longer time. Hydrothermal treatment at 25 °C, 40 °C and 60 °C affected the degradation process of neochlorogenic acid only partly. So, some neochlorogenic acid persisted in the dough after treatment at 25 °C, 40 °C and 60 °C, up to the 8 h extraction.

For these hydrothermal treatments at 25, 40, and 60 °C where there was relatively little extraction of neochlorogenic acid, two processes might be involved that would account for this lower extractability. In one scenario, neochlorogenic acid might be degraded by the enzymes in the Tartary buckwheat doughs, which would be functional during the hydrothermal treatments at 25, 40, and 60 °C, but not during the hydrothermal treatments at 80 and 95 °C. Alternatively, at the lower temperatures of the doughs, the neochlorogenic acid might form insoluble complexes. Here, while these low concentrations of neochlorogenic acid extracted from the cooked doughs might support the first process, the delayed

extraction of neochlorogenic acid across all of the hydrothermal treatments used would support the second process. However, at this stage, it can only be said that both types of metabolic events might be involved.

It is also interesting that the highest concentrations of neochlorogenic acid extracted were from the control flour after 20 min and 2 h of extraction, while after 8 h of extraction, the neochlorogenic acid extracted was similar in the control and 95 °C hydrothermally treated dough samples (Figure 6). Dawidowicz and Typek [29] identified 14 compounds (i.e., chlorogenic acid derivatives and its hydrolysis products) that were formed from 5-O-caffeoylquinic acid by heating its aqueous solutions at different pHs. The levels of the components formed depended on the heating times and the pH. Moreover, data from Dawidowicz and Typek [30] showed that high temperature cooking of blueberries caused 5-O-caffeoylquinic acid transformation and isomerization, with 11 compounds formed from 5-O-caffeoylquinic acid during the treatment of blueberries.

Apart from the controls, the highest concentrations of neochlorogenic acid extracted here were from the dough treated at 95 °C by ‘scalding’ the Tartary buckwheat flour and maintaining the dough formed at the same temperature for 20 min, followed by cooking (95 °C for 20 min). However, the efficient extraction of neochlorogenic acid from these samples using 20% aqueous methanol required 8 h of extraction. This indicates that during the high-temperature treatment of the dough, slowly extractable complexes of neochlorogenic acid appear to have been formed. It is possible that neochlorogenic acid formed complexes with amylose, or with some of the other components of the Tartary buckwheat flour; however, this remains to be determined through further studies.

4. Conclusions

In nonhydrothermally treated Tartary buckwheat flour samples, extraction of neochlorogenic acid with 80% aqueous methanol was complete within 20 min of extraction. The hydrothermal treatments of the dough samples were important for the extractability of neochlorogenic acid from the cooked doughs. For successful extraction of neochlorogenic acid from these cooked doughs, at least a prior 80 °C or more hydrothermal treatment of the dough and an 8 h extraction with 80% aqueous methanol are needed. These data thus indicate that high temperature (>80 °C) hydrothermal treatment is needed for Tartary buckwheat flour during dough preparation to preserve the valuable extractable neochlorogenic acid in such Tartary buckwheat food products. Hydrothermal treatment is a part of procedure for making Tartary buckwheat food products and foods. During this treatment small molecules, like neochlorogenic acid, leave their original place and with the water flow they enter into starch grains and other grain structures. Therefore, for extraction medium (20% (v/v) aqueous methanol), it takes a longer time to dissolve neochlorogenic acid from starch and other grain structures.

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