Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2022; 7(1): 51-59 Website: jnfs.ssu.ac.ir

The Effect of Superheated Water Extraction Conditions on Properties of Quinoa-Barley Malt Extract

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ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received:5 Mar 2021
Revised: 26 Jun 2021
Accepted: 26 Jun 2021

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ABSTRACT

Background: Superheated water extraction (SWE), is a novel method, used to extract proteins or other bioactive component from agriculture products**. Methods:** In this study, the extract from quinoa-barley malt (in a proportion of 30:70) was treated by SWE method. The effect of extraction time (15, 30, and 45 min) on physicochemical and functional properties of the extract was determined at 120 ºC. **Results:** The result showed that by increasing the extraction time the amount of protein increased. The maximum protein (%) was obtained in 45 min of SWE. The results revealed that increasing the extraction time had a significant effect on the turbidity and pH. In addition, increasing the extraction time had a positive effect on the functional properties. The highest protein isolate (PI) solubility was at 120 °C. **Conclusions:** Although SWE method appears to be a useful extraction method for producing valuable materials from cereal and pseudocereal, this method has only been used at pre-commercial scale and more investigation is required to study the quantity, quality, and stability of the extracted valuable materials to scale it up for industrial means.

Keywords: *Quinoa; Barley malt; Extraction; Superheated water extraction*

Introduction

Quinoa (*Chenopodium quinoa Willd.*) plant belonging to the Chenopodiaceae family is a pseudocereal and its consumption by human dates back 7000 years ago (Abugoch *et al.*, 2009, Guerreo-Ochoa *et al.*, 2015).

These seeds have encouraged food and agriculture organization (FAO) to determine potential cultivation areas in Europe, Asia, Africa, Australia, and North America in order to expand its cultivation to different geographical regions. (Guerreo-Ochoa *et al.*, 2015). Quinoa is a good source of riboflavin, thiamine and folic acid and contain compounds such as calcium, magnesium, iron and other important minerals, and adding these seeds to the diet of celiac patients can improve their nutritional value (Nisar *et al.*, 2017, Pereira *et al.*, 2019). (Table 1)

This paper should be cited as: Sabah S, Sharifan A, Akhonzadeh Basti A, Jannat B, TajAbadi Ebrahimi M**.** The***ation of The Effect of Superheated Water Extraction Conditions on Properties of Quinoa-Barley Malt Extract.*** *Journal of Nutrition and Food Security (JNFS)*, 2022; 7 (1): 51-59.

In addition to the nutritional value and gluten-free properties of this seed, quinoa has been reported to have beneficial effects on consumers of high-risk groups such as children, especially children suffering from malnutrition, the elderly, lactose intolerant people and people with anemia, obesity. And people with celiac disease (gluten sensitive) (López *et al.*, 2018, Navruz-Varli and Sanlier, 2016).

Quinoa is one of the rich grain proteins having 12% to 24% of this nutrient (Ahmed *et al.*, 2018). Quinoa proteins are one of the valuable food ingredients, the main proteins of which are globulin and albumins. This high nutritional protein can be used as food ingredients in the form of the protein isolates (PI) due to having high quality amino acids) Salak Asghari and Yoshida, 2006(. Due to its high nutritive potential and genetic diversity, quinoa is classified by FAO as one of promising crops that can contribute to food security in the 21st century (Guerreo-Ochoa *et al.*, 2015). The recommended dietary allowance for protein is currently set at 0.8 g protein/kg/day, representing a daily protein intake level to meet the needs of nearly all individuals.

Barley is the fourth grain in the world that has a lot of nutrients Barley has a long history of use as a source of human nutrition. Barley has a higher nutritional value and properties than wheat such as beta-glucan and arabinoxylan, oligosaccharides (galacto oligosaccharide, fructo oligosaccharide, isomalt oligosaccharide), lactulose, inulin, starch resistant, polyphenols, tocopherols, tocotrienols. Barley, due to the presence of various B vitamins and other valuable compositions, is recommended for those who suffer from certain diseases such as high blood pressure, diabetes, obesity and colorectal cancer. (Arendt and Zannini, 2013, Chappell *et al.*, 2017, De Arcangelis *et al.*, 2019, Suriano *et al.*, 2018). Barley can also reduce plasma cholesterol levels and the risk of heart Disease (Cardinali *et al.*, 2021).

PI or protein concentrates are used in many food processes because of their good functional properties, such as solubility, emulsifying capacity, emulsion stability, foaming capacity and stability. Solubility is one of the most important functional properties of proteins because other properties of protein depend on this property. Many factors affect the solubility of protein, including temperature, time, salt concentration and pH. (Valero-Cases *et al.*, 2020).

There are some common extraction methods for extracting functional compounds from cereal or pseudocereal, using high temperature, enzymatic hydrolysis or modification which are time-consuming, expensive, and some of the solvents are toxic (Yeom *et al.*, 2010).

Recently, advanced extraction technologies, such as superheated water extraction (SWE), a useful method for proteins and bioactive components, have been developed to overcome these problems (Narita and Inouye, 2012). The extraction yield would increase and the extraction time cost would decrease using the SWE (Alboofetileh *et al.*, 2019). In this study, the SWE was applied as environmentally friendly method for producing quinoa-barley malt extract and for investigating the effect of extraction condition on functional properties of the samples.

Materials and Methods

Quinoa seeds of Sajama variety were obtained from The Seed and Plant Improvement Institute, Karaj, Iran, in September 2018. Barley malt was purchased from Beh malt Co., Iran, and all chemicals used were of reagent grade obtained from Sigma (St Louis, Missouri) and Merck (Darmstadt, Germany).

Sample preparation: Quinoa seeds were cleaned and soaked in water for 24 h and washed thoroughly to remove saponins, then dried in ambient temperature. After that, quinoa seeds were ground into powder by an electric grinder (IKA 1603600 M 20 Universal Mill, 230V, U.S.A.). The milled quinoa flour and barley malt flour were defatted with hexane as solvent in a ratio of 1:5 in 24 h by the help of a shaker (Fisher Scientific Ltd, No.14-285-729). Afterward, the fat-free flours were placed in an oven at 40 °C for 24 h to isolate the residues of solvent. Then, all the flours were sieved, using 40 mesh sieve for obtaining fractions

[DOR: 20.1001.1.24767417.2022.7.1.8.7]
[DOI: 10.18502/jnfs.v7i1.8536]

[DOR: 20.1001.1.24767417.2022.7.1.8.7]
[DOI: 10.18502/jnfs.v7i1.8536]

<0.420 mm mesh N° (ASTM E11) 40, sieve size 420 µm (sarvazma Co, Iran). The sample was stored at -18 ºC until use. The SWE was performed using Synth wave apparatus (Milestone, Bergamo Italy) with some modification. For extraction first, quinoa-barley malt was mixed with tap water, the mixture was stirred by an industrial blender at room temperature and homogenized at 700 rpm for 15 min, yielding homogenous mixture. The obtained substrates were subjected to superheated water extraction apparatus with time-temperature treatments (120 ºC for 15-45 min) (Alboofetileh *et al.*, 2019). After SWE in autoclave, the sample was mixed in a blender (IKA MYP2011100, German) at high speed for 10 min. pH was measured using a 4-7pH meter Metrohm model (Metrohm, Herisau, Switzerland) at 20 ºC.

Then, the mixture pH was adjusted to 9.0 with 1 N NaOH, stirred for 2 h at room temperature to extract the protein, and then centrifuged at 9,000×g for 20 min at 4 ºC to remove the insoluble materials. Supernatant was collected and adjusted to pH 4.0 with 1 N HCl and centrifuged at 8,000×g for 15 min at 4 ºC to recover protein precipitate. The precipitate was washed twice in distilled water for 30 min to remove the soluble materials. The precipitate was then suspended in distilled water (1:1, w/v), neutralized by adjusting the pH to 6.5, and freeze-dried.

Physicochemical and functional analysis: Total nitrogen content of the samples was determined according to the Kjeldahl method and crude protein content by using the 6.25 conversion factor (Helric, 1990). Soluble protein was determined by the Bradford procedure (Bradford, 1976) using Coomassie Brilliant G-250 dye binding and bovine serum albumin as the standard (Lin, 2011). Turbidity of the samples was measured using a turbidometer (2100N Turbidimeter, HACH, CO, USA) and reported in terms of nephalometric turbidity units (NTU). A digital pH meter was used to measure pH at 20 ºC.

Solubility: The protein solubility (PS) of the samples was determined with some modification as follows(Hu *et al.*, 2013). First the protein sample was dispersed in deionized water and the pH was adjusted to a range of 3 to 10 using 0.1 mol/l HCl or NaOH, and magnetically stirred at room temperature for 30 min. After pH adjustment, the samples were centrifuged at 10000 g for 20 min at 20 °C. Then each supernatant was filtered with Whatman filter paper (No. 1) (Narita and Inouye, 2012). The protein solubility of the samples was determined by modifying the methods described by Shimada and Cheftel (Shimada and Cheftel, 1989), (Yin *et al.*, 2007), (Manoi and Rizvi, 2009), and AOAC Official Method 930.29 (Association of Official Analytical Chemists International (AOAC), 2005) based on **Equation 1**.

PS (%) = Cs/Ci ×100 (1)

CS: The protein concentration in the supernatant (mg/ml);Ci: The protein concentration in the initial suspension (mg/ml)

Degree of hydrolysis: Degree of hydrolysis (DH) was measured by determining the soluble nitrogen content. Ten ml of PI samples was mixed with trichloroacetic acid (TCA) (20%) and centrifuged in 8900×g for 20 min at 4 °C (Yoon *et al.*, 2009). The soluble nitrogen of supernatant was measured by the Kjeldahl method (Association of Official Analytical Chemists International (AOAC), 2000). The DH (%) was calculated based on **Equation 2**.

DH (%) = Soluble nitrogen in 10% TCA solution (mg)/Total nitrogen (mg) ×100 (2)

Data analysis: The statistical analysis was performed by SPSS 24 software (IBM Corp., USA). Normality of data and homogeneity of data were conducted by Kolmogorov-Smirnov and levene's tests, respectively. To examine the statistical significance of treatments effect One-way ANOVA and for statistical comparison of data Duncan's test were performed. Statistical analysis of the variance was performed with the statistical analysis system software 8.2 (SAS, USA). In all statistical analyses, P-value < 0.05 was considered to be significant. Plots were plotted in Excel 2016**.**

Results

Protein analysis: **Figure 1** shows the effect of extraction time on the protein content**.** The

percentage of protein increased by increasing the extraction time as a processing factor of superheated water extraction. The maximum protein was extracted when this factor was at high level (Time = 45min).

Turbidity: According to **Figure 2**, by increasing the extraction time, leading to an increase in the amount of protein in the extract, the turbidity increased, although the other factors, such as polysaccharide and polyphenol have been implicated in haze formation in many beverages. **Figure 2** also indicates that the maximum turbidity was produced when the extraction time was at high level (Time = 45min).

pH: As shown in **Figure 3**, by increasing the extraction time, pH decreased. The minimum pH was obtained when the extraction was time at high level (time = 45min). Decomposition of biomass, carbohydrates, and amino acids under superheated water extraction produces acidic components, such as organic acid.

Solubility: The effect of extraction time and the temperature on the solubility of superheated water extract were studied. **Figure 4** reveals that by increasing time from 15 to 45 min, the protein solubility increased significantly ($P < 0.05$). **Figure 5** shows the effect of SWE extraction temperature (110–130 °C) on the solubility of PI.

The highest PI solubility was at 120 °C. The solubility of PI increased by increasing temperature up to 120 °C due to the hydrolysis reaction, but at 130 °C it decreased, since at higher temperatures (more than 120 °C) the aggregation was started and affected the solubility, which is in line with previous studies (Teo *et al.*, 2010) .

Degree of protein hydrolysis (DH): DH of the protein increased by increasing time. The highest level was in 45 min (**Figure 6)**.

Table 1. The proximate composition (mean ± SD) of quinoa and barley malt flour (g/100 g d. W. Basis)

The proximate composition	Quinoa flour	Barley malt flour
Protein	15.76 ± 0.20	11.13 ± 0.20
Carbohydrates	68.71 ± 0.50	69.80 ± 0.40
Starch	54.40 ± 0.20	60.24 ± 0.50
Fat	5.32 ± 0.30	4.46 ± 0.09
Ash	2.84 ± 0.20	2.04 ± 0.08
pH	6.40 ± 0.08	6.07 ± 0.06

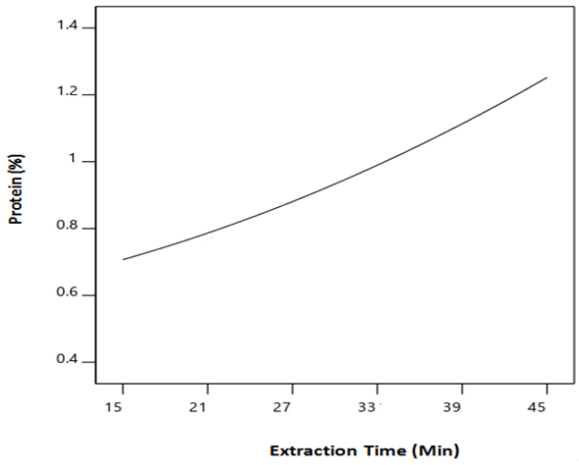

Figure 1. The effect of superheated water extraction time on protein % (at 120 °C)

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[DOI: 10.18502/jnfs.v7i1.8536]

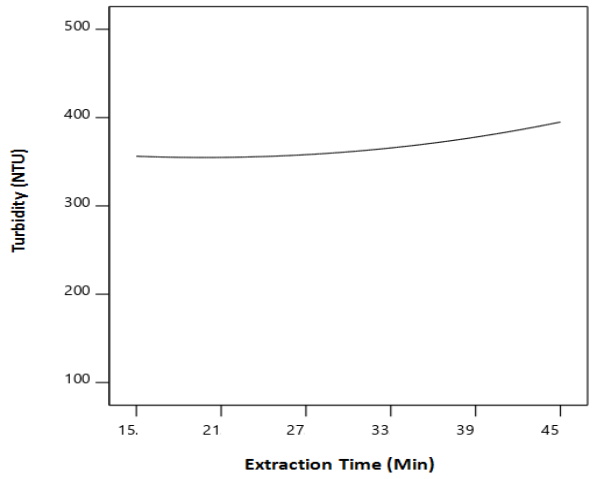

Figure 2. The effect of superheated water extraction time on turbidity (at 120 °C)

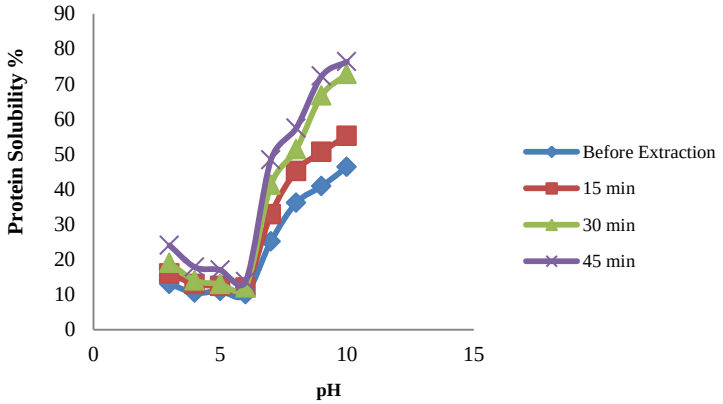

Figure 3. The effect of superheated water extraction time on pH (at 120 °C)

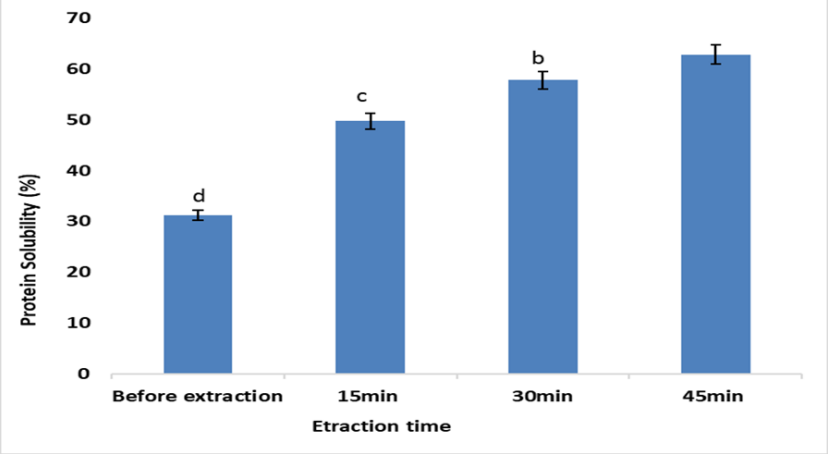

Figure 4. Solubility of protein at different time at 120 °C. Different letters on the top of the bars denote significant difference ($P < 0.05$). Control: sample before extraction.

[DOR: 20.1001.1.24767417.2022.7.1.8.7]
[DOI: 10.18502/jnfs.v7i1.8536]

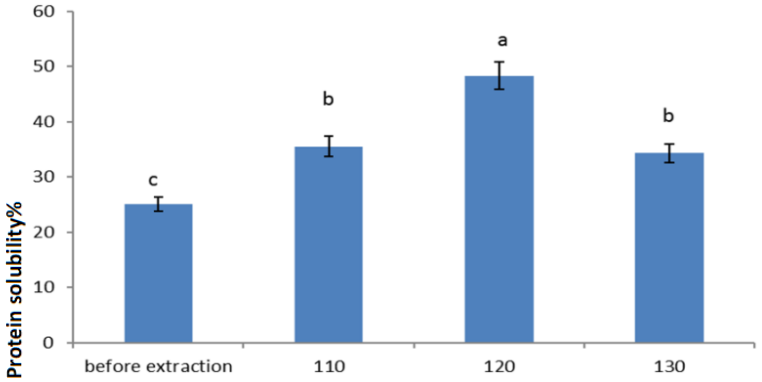

Figure 5. Solubility of protein at different temperature. Different letters on the top of the bars denote significant difference (P <0.05). Control: sample before extraction

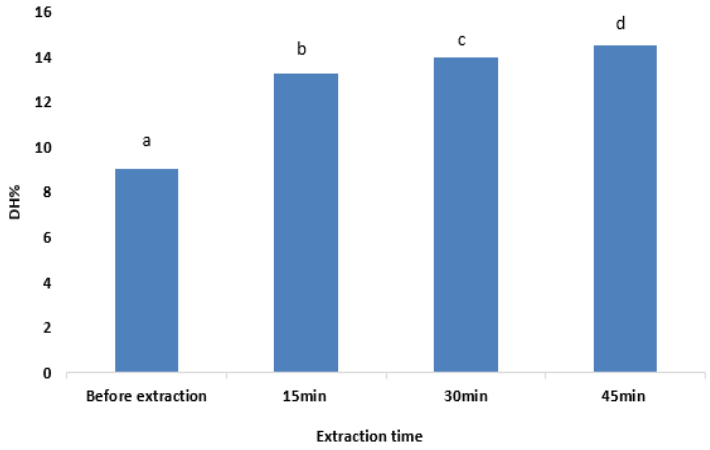

Figure 6. The degree of hydrolysis at different time (15, 30, and 45 min) at 120 °C. Different letters on the top of the bars denote significant difference (P < 0.05). Control: sample before extraction.

Discussion

According to results, the extraction time had a positive effect on the protein content. Protein is one of the important factor that affects turbidity in beverages (Siebert and Lynn, 2000). However, several studies have been carried out on the interaction of protein–polysaccharide (Duran *et al.*, 2018, Sommer *et al.*, 2019, Tavernier *et al.*, 2017); however, polysaccharide–protein interactions of quinoa in beverage have not been widely investigated. The knowledge of these properties can contribute to the understanding the effect of polysaccharides.

Due to the formation of water soluble organic acids, the pH of the treatment medium may decrease (Lamoolphak *et al.*, 2006, Pourali *et al.*, 2009, Salak Asghari and Yoshida, 2006).

Increasing protein solubility was due to the hydrolysis reaction. In addition to the extraction time, other processing factors, such as extraction temperature affected functional properties of protein, such as solubility. Teo *et al.* found that the solubility increases by increasing temperature up to 120 °C due to the hydrolysis reaction, but at 130 °C it decreases, since at higher temperatures (more than 120 °C) the aggregation starts, affecting solubility, which is in line with previous studies (Teo *et al.*, 2010) . Therefore, the optimization of the SWE is important for extraction of by-product of agriculture products. The same results for hydrolysis degree were observed by Yoon *et al.* (Yoon *et al.*, 2009). The number of available hydrophilic groups increases and the protein's molecular weight decreases during hydrolysis

[DOR: 20.1001.1.24767417.2022.7.1.8.7]
[DOI: 10.18502/jnfs.v7i1.8536]

process and all these changes alter the functional properties of sample and therefore terminate with increasing the extraction time (Betancur-Ancona *et al.*, 2009, Song *et al.*, 2018).

Protein is one of the most important factors that can affect the turbidity of beverages. The proteins in beer, red and white wine, apple juice, grape juice and kiwifruit juice cause turbidity. Pectin, arabinogalactan and poly-galacturonic acid increase turbidity while free amino acids and other carbohydrates have no effect on turbidity (Siebert and Lynn, 2000). According to, the protein content of quinoa was higher than barley malt and by increasing the amount of protein in the extract the turbidity increased. However, other factors such as polysaccharides and polyphenols have been implicated in the turbidity of many beverages. A number of polysaccharides, including beta-glucans, starches, and mannans, have been linked to turbidity in beer. (Stounbjerg *et al.*, 2018). Therefore, beta-glucan, starch of barley malt, and the starch of quinoa may affect turbidity of quinoa-barley malt extract.

Conclusion

In this study, the PI was obtained by SWE technology. SWE is a great extraction method for producing PI from different raw plants. The SWE conditions were optimized and the optimal SWE condition for producing PI from the quinoa and barley malt (20:80) was at 120 °C and pH=6. The results showed the improvement of the functional properties (solubility and degree of hydrolysis) of PI by passing time (from 15 to 45 min). Consequently, this combination would be a good source of PI as a functional food or for use in food processing.

Acknowledgment

Thanks are owed to Halal Research Center Islamic Republic of Iran, Tehran, for financial support.

Authors' contribution

All of the authors involved in conceptualization, data gathering, formal analysis and writing the original draft of manuscript ande finally approved manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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[DOI: 10.18502/jnfs.v7i1.8536]