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Note

Subcritical Water Extraction of Barley to Produce a Functional Drink

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We investigated the effects of various temperatures between 150 and 280 °C during subcritical water extraction of barley to make a barley tea-like extract, a popular summer beverage in Japan. Each barley extract was analyzed for sensory properties, antioxidative activity, and the amount of residual matter, which revealed 205 °C to be the best extraction parameter. 5-Hydroxymethyl-2-furaldehyde was found to be the major antioxidative component in the 205 °C extract, along with the formation of several important amino acids.

Key words: barley (Hordeum vulgare); subcritical water; peroxynitrite; antioxidative compound; 5-hydroxymethyl-2-furaldehyde

The rapid development of food science recently indicates the need for effective processing parameters and technology in food production. Barley is a widely consumed cereal among the most ancient cereal crops. Almost 80–90% of barley production is for animal feed and malt, but now barley is gaining renewed interest as an ingredient in the production of functional foods due to its concentration of bioactive compounds. Barley tea is a common drink in Japan, especially during the summer. This non-caffeinated, non-tannin drink is valued for its high percentage of β-glucans (polysaccharides) and the presence of antioxidant compounds.[1,2] The present method of making barley tea involves roasting barley seeds at 280 °C and then processing with hot water to yield barley tea. The demerits of this method are the possible loss of important volatile components due to constant exposure to the air during roasting and difficulties in the disposal of the large quantities of residues after the extraction process.

Subcritical water is water in a temperature range of 100 °C to 374 °C at pressures between 1 and 22 MPa. Under these conditions, the dielectric constant of water decreases, imparting changed polarity and thereby efficient extraction abilities.[3] Worldwide, subcritical water extraction (SWE) has received much attention recently in the extraction of natural products, since this technique is environment friendly, quick, and inexpensive compared to various other solvent extraction techniques. Furthermore, the extraction occurs in a closed chamber in water, preventing the loss of volatile compounds. SWE presents virtually no disposal costs and can be considered a clean alternative to conventional organic solvents.

Considering the above merits of the technique, we investigated the utility of SWE in preparing barley extracts. We extracted barley at various temperatures during SWE and further fine-tuned the subcritical conditions with respect to temperature, for a better yield of barley extracts. Each extract was rated for its sensory properties, including taste, odor, and overall value as a beverage on a scale of 1 to 5 by a panel of eight. Furthermore, the pH, percentage of residual matter, and peroxynitrite antioxidant activity was determined for each extract.

Powdered barley samples (particle size, between 250 and 500 μm) were received from Yagisho Corporation, Shizuoka, Japan. For antioxidant activity analysis, peroxynitrite was prepared according to a previously described method.[4] All other chemicals used were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Powdered barley (60 g) was extracted with 200 ml of water following a procedure described in our previous studies.[5] The extracts were cooled to room temperature and then stored in the dark at 4 °C until analysis.

Table 1A shows the results of the analysis of the sensory properties of the barley samples obtained under preliminary extraction conditions, which are also indicated in the table. The analysis revealed that extraction at about 200 °C produced good barley extract.

Moreover, as Table 1A indicates, the quantity of residual matter decreased as the extraction temperature increased. While the reduction of residue by the currently used roasting method is about 15%, a reduction of

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Table 1. Preliminary and Secondary Extracted Barley Samples and Analysis of Sensory Properties

(A)	Preliminary extracted barley samples and analysis of sensory properties							
Sample no.	Temp (°C)	Press (MPa)	Aroma*[1)]	Taste*[1)]	Concentration*[1)]	pH[2)]	Residue (%)	Rank*
1	150	3.90	2.7	3.0	1.5	6.0	94.6	3
2	180	4.30	2.3	3.2	1.9	6.0	82.0	2
3	200	4.91	3.8	4.2	2.3	6.0	71.2	1
4	230	5.55	2.7	1.4	3.8	4.0	58.3	4
5	280	6.48	2.0	1.4	3.2	5.0	47.2	5
(B)	Secondary extracted barley samples and analysis of sensory properties							
Sample no.	Temp (°C)	Press (MPa)	Aroma*[1)]	Taste*[1)]	Concentration*[1)]	pH[2)]	Residue (%)	Rank*
6	175	4.10	2.7	2.8	1.9	6.0	81.1	5
7	185	4.34	2.5	3.0	2.0	6.0	77.0	4
8	195	4.72	3.0	3.5	2.3	6.0	76.6	2
9	205	5.00	3.3	3.1	4.0	5.8	70.5	1
10	215	5.18	3.0	2.3	3.9	5.5	64.5	3

*rated on a scale of 1 to 5 by a panel of eight
[1)]dilution factor ×30 in water
[2)]dilution factor ×3 in water

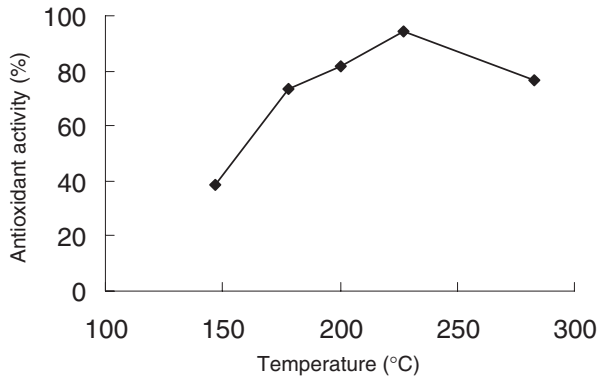

Fig. 1. Peroxynitrite Antioxidant Activity for Preliminary Extractions.
Data given as mean ± standard deviation; $n = 3$.

up to 29% can be achieved by SWE under the best extraction conditions (*viz.*, 200 °C). This fact should be useful when processing barley on a larger scale.

The peroxynitrite antioxidant activity of the preliminary extractions is shown in Fig. 1. The parabolic graph of antioxidant activity with respect to the extraction temperature indicates that the antioxidant activity increased linearly with temperature. The decline at higher temperatures was possibly due to degradation of compounds.

We further fine-tuned the extraction of barley samples with respect to temperatures near 200 °C based on preliminary extraction results. Table 1B shows the secondary precise SWE conditions applied in barley extraction and their respective analysis results. The results of analysis of sensory properties revealed that SWE at 205 °C produced a better barley extract.

In order to investigate the reasons for the comparatively high antioxidant activity, and better taste, aroma, and overall good value, we carried out further analysis of the 205 °C extract. The extract was filtered (Advantec 2, 185 mm), and the aqueous filtrate obtained (140 ml) was successively extracted with 150 ml of hexane, chloroform, ethyl acetate, and butanol three times each to separate the extracted components according to their polarities. Each of the organic extracts thus separated was dehydrated (anhydrous Na_2SO_4), vacuum concentrated, weighed, and finally preserved at −20 °C until analysis. The yields of the organic extracts were found to be 6 mg (hexane), 17 mg (chloroform), 26 mg (ethyl acetate), and 158 mg (butanol).

Next, the peroxynitrite antioxidant activities of all the isolated extracts were measured to determine the most effective organic extract. The concentration of all the extracts was maintained at 1 mg/ml. Butanol extract exhibited the highest peroxynitrite antioxidant activity (77.17 ± 0.71%), followed by ethyl acetate (45.78 ± 1.52%), chloroform (42.42 ± 1.44%), and hexane (25.48 ± 1.56%) (std. mean values of three tests). Due to its high extraction yield and strong antioxidant activity, butanol extract was selected for further investigation.

TLC and silica gel column chromatography were used for analysis and separation of the concentrated butanol extract. TLC analysis (Silica gel $60F_{254}$ plate, $CHCl_3$: MeOH::5:1), showed a concentrated dark spot at *Rf* 0.30 when observed under UV radiation (254 nm) and visualized with vanilline sulphate. This major spot was isolated by silica gel column chromatography ($CHCl_3$: MeOH::15:1). After chromatographic separation, fraction 2 (111 mg) was obtained as a major fraction of the butanol extract. Structure elucidation of the isolated fraction by ^1H, ^{13}C NMR spectroscopy and comparison with an authentic sample revealed fraction 2 to be 5-hydroxymethyl-2-furaldehyde (HMF).

5-Hydroxymethyl-2-furaldehyde (HMF): ^1H-NMR δ (CD_3OD): 9.60 (1H, s, 1-CHO), 7.22 (1H, d, 3-CH), 6.52 (1H, d, 4-CH), 4.72 (2H, s, 6-CH_2). ^{13}C-NMR δ

(CD_3OD): 177.6 (1-C), 152.4 (2-C), 122.6 (3-C), 110.0 (4-C), 160.5 (5-C), 57.6 (6-C).

The mechanism of formation of HMF in 205 °C-SWE is proposed to be as follows: Barley contains a very high percentage of polysaccharides known as β-glucan (3 to 6%).[2] At high temperatures in SWE, β-glucan undergoes a thermal degradation reaction to form glucose, which on further dissociation forms HMF.[6] The HMF concentration in the roasted barley extract was found to be as low as 0.7 mg/60 g. The low concentration of HMF can be attributed to the higher temperature (280 °C) of roasting, at which degradation of HMF took place. Research on roasted coffee also shows that HMF is formed during the roasting of coffee, with the highest concentration, of 909 µg/g, at 240 °C.[7] The very high concentration of HMF in the 205 °C extract can be attributed to the high amounts of β-glucan found in barley. The acute toxicity of HMF is relatively low, and the LD_{50} was found to be 3.1 g/kg bw in rats.[8] HMF has also been identified in mushroom (*D. indusiata*), and was reported to exhibit effective anti-tyrosinase activity.[9] The genotoxic and carcinogenic potential was reviewed by Janzowski *et al.*,[10] who found that HMF is not a highly dangerous compound. The known biological effects of HMF and related compounds were recently summarized by Glatt and Sommer.[11]

The individual antioxidant activity of HMF is moderate,[5] but, the stronger antioxidant activity exhibited by butanol extract can be attributed to its very high yield. From HPLC analysis (data not shown), it was furthermore found that HMF was formed in the barley extracts only near 205 °C.

Additionally, amino acid analysis of aqueous filtrate of 205 °C extract was carried out in order to identify the amino acids contributing to the overall good value of the extract.

One ml of 205 °C extract was mixed with 1 ml of 5% trichloroacetic acid, and this mixture was centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through a 0.2-µl filter and subjected to amino acid analysis (Hitachi L-8900 automatic amino acid analyzer). The amino acids were identified by comparison of their retention times and were quantified by the calibration curve method. Table 2 shows the major amino acids and their respective quantities in the 205 °C extract. The results indicate that asparagine, glutamine, alanine, and isoleucine were some of the major amino acids in the 205 °C extract. In this study, we studied only the 205 °C extract, but it can be assumed that extracts near 205 °C also exhibit similar characteristics.

In conclusion, we determined the best extraction conditions for the extraction of barley by subcritical water extraction, as far as sensory properties, antioxidant activity, and amount of residual matter are concerned. 5-Hydroxymethyl-2-furaldehyde (HMF) was found to be the component of the 205 °C extract most responsible for its high antioxidant activity, taste, and odor. Furthermore, amino acid analysis revealed that asparagine, glutamine, alanine, and isoleucine were among the main amino acids in the 205 °C extract. These results should be useful in processing barley tea on an industrial scale in the future. Further studies are presently in progress at the Yagisho Corporation to check the applicability of our laboratory scale results for production at the industrial level. Additionally, research on minor compounds present in the 205 °C extract is also currently underway in our laboratory.

Table 2. Amino Acid Analysis of 205 °C Extract

Amino acid	Amount (n mol/ml)
Asp	162.1
Thr	19.7
Ser	3.0
Glu	110.8
Gly	71.5
Ala	97.2
Val	34.1
Ile	87.2
Leu	32.4
Tyr	12.7
Phe	21.0
Lys	19.6
His	10.6
Arg	31.1
Pro	46.5

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