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Front page photo: A prototype of commercial product of buckwheat noodles with added blue-green alga, ishi-kurage (*Nostoc commune* Vauch.) (See paper of Asami et al.).

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Research paper

Mechanical characteristics of buckwheat noodles made with blue-green alga, *ishi-kurage* (*Nostoc commune* Vauch.)

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ABSTRACT

The present study was conducted to clarify the effect of a kind of blue-green algae, i.e., *ishi-kurage*, on the mechanical characteristics of buckwheat noodles. Mechanical analysis of buckwheat noodles with *ishi-kurage* showed that incorporation of *ishi-kurage* into buckwheat noodles enhanced breaking stress and energy. Sensory evaluation with human panels showed that buckwheat noodles with *ishi-kurage* were more preferred when compared with noodles without *ishi-kurage*. On the other hand, incorporation of *ishi-kurage* into buckwheat noodles enhanced decreased solubility of the albumin plus globulin fraction. The present study finding suggests that the endogenous protein may be an important factor responsible for the mechanical characteristic of buckwheat noodles with *ishi-kurage*.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an important crop in some regions of the world (Kreft et al., 2003; Ikeda, 2002). Buckwheat flour contains high levels of essential nutrients such as protein (Ikeda et al., 1991) and minerals (Ikeda and Yamashita, 1994). Thus buckwheat flour is an important dietary source of these essential nutrients. On the other hand, components in buckwheat flour have still not been well characterized from the viewpoints of both nutrition and food-functionality. Careful characterization of the components is needed to better understand their nutritional and food-functional properties.

There is a variety of buckwheat foods, such as bread, pancake, crepe, galettes, pasta, blini, kasha etc., around the world (Ikeda, 2002). In view of their processing and cooking, increasing attention has been paid to the palatability and acceptability of buckwheat products. Clarifying the mechanical characteristics of buckwheat products, including noodles and pasta, is a subject of great interest. Noodles made from buckwheat flour-water dough are popular in some regions including Japan (Ikeda, 2002). In Japan, buckwheat noodles are a popular, traditional food. Traditional processing methods for buckwheat noodles have been long recorded in Japanese history for approximately four hundred years or more (Zen-men-kyo, 2014). As buckwheat flour has low cohesiveness, dough-binders, such as wheat flour, egg, seaweed, Japanese yam flour, are often added in preparing buckwheat noodles (Zen-men-kyo, 2014). A variety of buckwheat noodles with various dough-binders has been traditionally available in Japan. We reported mechanical effects by addition of various dough-binders to buckwheat noodles (Ikeda et al., 2005; Asami et al., 2019). However, further systematic analysis is needed to understand the exact mechanical effects of various dough-binders to buckwheat products.

Japanese people prefer to eat edible algae including marine algae. As Japan is surrounded by the sea on all sides, there is a variety of edible marine algae in Japan. In view of their color, algae are classified into four groups, i.e., brown algae, red algae, blue green algae, and indigo algae. In relation to buckwheat processing, various edible marine algae are traditionally utilized as dough-binders (Zen-men-kyo, 2014). They include buckwheat noodles with red algae, called *hegi-soba* in Niigata, the central region of Japan, and soba with agar-agar in several regions (Zen-men-kyo, 2014). These buckwheat noodles with red algae or agar-agar have a unique preferred texture.

In Japan, there is a kind of blue-green algae, i.e., *Nostoc commune* Vauch., called *ishi-kurage* in Japanese (Fig.1). *Ishi-kurage* belongs to a cyanobacterium phyrum and a Nostoc genus (Itoh, 2015). *Ishi-kurage* grows naturally on some conditions such as a surface of soil, but is fragile to drying (Fig. 1-(A)) and wetting, i.e., this algae becomes swollen (Fig. 1-(B)) when wetting (Itoh, 2015). In Japan, *ishi-kurage* is traditionally utilized as an edible algae in some limited areas such as Ane-gawa River in Shiga Prefecture, Western region of Japan, and Miyakojima Island in Okinawa Prefecture. *Ishi-kurage* contains functional components such as effect of reducing serum and liver cholesterol concentrations that may exhibit beneficial effects to humans (Hori at al., 1990; Ishibashi et al., 1994; Itoh, 2015). Therefore, the nutritional value as a functional food of *ishi-kurage* has been increasing in recent years. From the nutritional importance of buckwheat and *ishi-kurage*, it is an interesting subject to utilize *ishi-kurage* as their dough-binder to buckwheat noodles. There are up to now no buckwheat noodles prepared with *ishi-kurage*. If buckwheat noodles with high palatability can be prepared with *ishi-kurage*, much attention to such buckwheat noodles will be attracted.

This study aimed to prepare buckwheat noodles prepared with *ishi-kurage* and to clarify mechanical characteristics of buckwheat noodles with added *ishi-kurage*.

MATERIALS AND METHODS

Materials

Mechanical characteristics of buckwheat noodles were analyzed in the present study. Two mechanical analysis, I and II, were conducted in this study.

Mechanical analysis I was conducted to clarify the effects of the *ishi-kurage* on the buckwheat noodles. Buckwheat flour (*Fagopyrum esculentum* Moench, var. Kitawase-soba), which was harvested in Hokkaido (in 2018), was used in this research. Buckwheat flour was kindly provided prepared from Terao Milling Co. (Hyogo, Japan) and stored at -80ºC until use. Ground blue-green algae, i.e., *ishi-kurage* in Japanese (*Nostoc commune* Vauch.) (Fig. 1-(C)) used in this study was a commercial product (Micro Algae Co., Gifu, Japan).

Mechanical analysis II, buckwheat noodles with *ishi-kurage* as a commercial dried noodle product as were prototyped and their mechanical characteristics were measured. Production of buckwheat noodles with *ishi-kurage* was outsourced to the Tanaka Seimenjyo Co. Ltd., Japan. Buckwheat flour used was that harvested in Japan, and wheat flour used was that harvested in the USA, Canada, and Australia. The same *ishi-kurage* sample, as used in mechanical analysis I, was used in mechanical analysis II.

Mechanical measurements

Mechanical analysis I:

Mechanical characteristics of buckwheat noodles were evaluated by breaking analysis. Prior to mechanical analysis, buckwheat flour, which had been stored at -80°C, was placed in a desiccator at room temperature until the flour exhibited a constant moisture content. The moisture of the flour was measured with a moisture analyzer (ML-50, A&D Co. Ltd., Japan). *Ishi-kurage* was boiled, and then sticky gel of *ishi-kurage* obtained by boiling was added to buckwheat flour. The buckwheat dough was prepared just prior to mechanical analysis to have a moisture content of about 42% (w/w) by adding an appropriate amount of distilled water. Then buckwheat noodles were made from the buckwheat dough using a hand-made pasta machine (SP-150, Imperta Co., Torino,

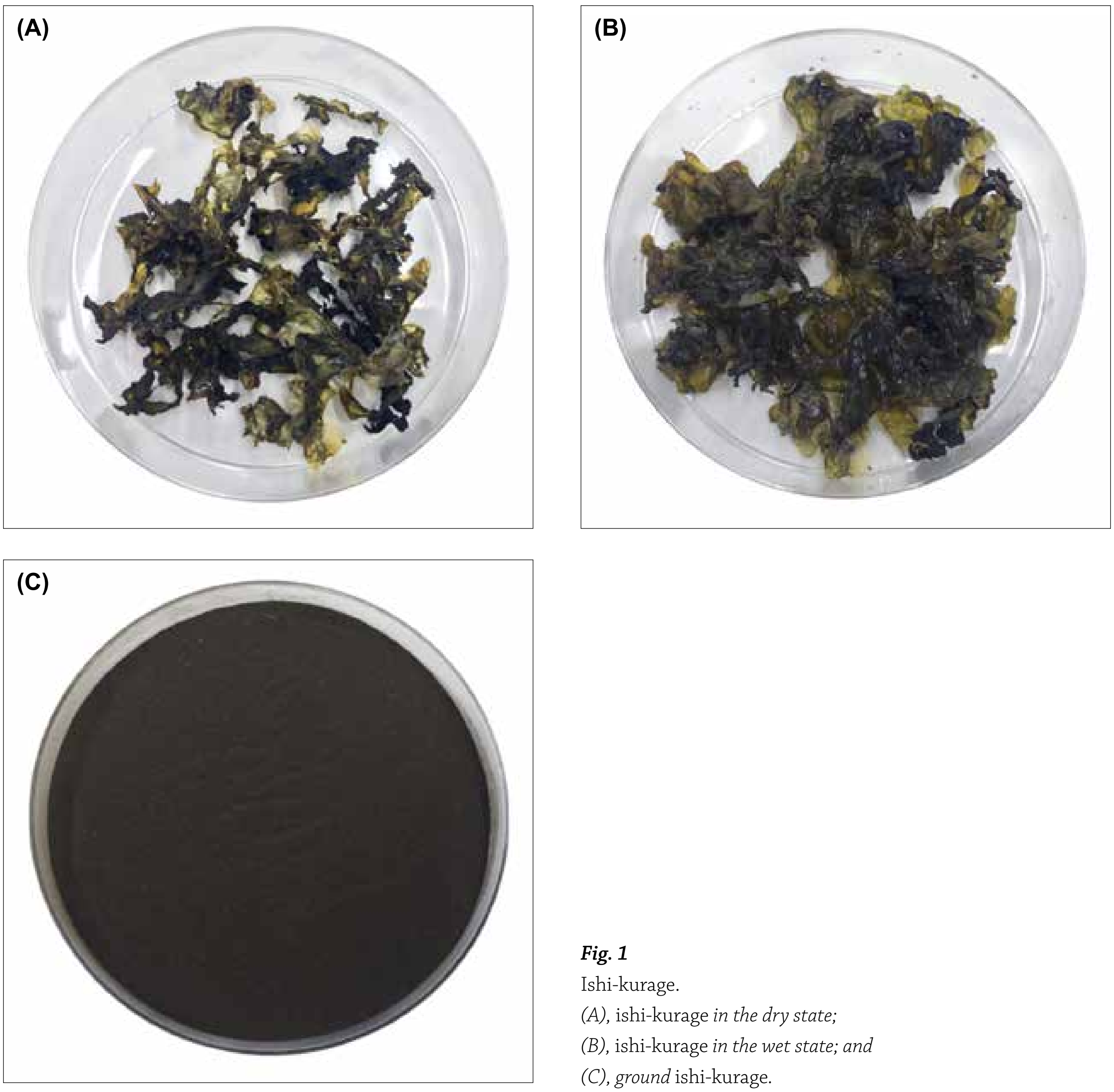

Fig. 1
Ishi-kurage.
(A), ishi-kurage *in the dry state;*
(B), ishi-kurage *in the wet state; and*
(C), ground ishi-kurage.

Italy). The buckwheat noodles obtained were subjected to mechanical analysis. Before the mechanical analysis, buckwheat noodles prepared were heated in boiling water for a period of 150 sec and subsequently were cooled for a period of 150 sec at 4ºC. Immediately after cooling, mechanical measurements of the noodles were performed. Breaking analysis of buckwheat noodles was performed with Rheoner RE2-3305C (Yamaden Co. Ltd., Japan). Measurements of breaking analysis were performed with a load cell of 200N and measurement speed of 0.50 mm/sec. A wedge-style plunger (No.49: W 13mm, D 30mm, H 25mm) was used in measurements with the Rheoner RE2-3305C. Mechanical measurements were replicated twenty times for each sample.

Mechanical analysis II:

Noodles were prepared with a buckwheat flour-to-wheat flour ratio of 1:4. Two types of buckwheat noodles were prepared. Two types of buckwheat noodles were prepared with or without addition of *ishi-kurage*. In the case of buckwheat noodles with added *ishi-kurage*, the amount of *ishi-kurage* added was 2% of the flour weight. Mechanical analysis of the buckwheat noodles was measured in the same as in mechanical analysis I, except that the noodle boiling time was 5 minutes. Figure 2 shows buckwheat noodles with and without addition of *ishi-kurage*.

Sensory evaluation

Sensory evaluation was conducted by a scoring-scale method (Toda, 1994) with volunteer panels (n=26). The evaluation criteria which was selected consisted of six items, i.e., overall evaluation, hardness, springiness, easiness to bite through, smoothness and color. The scoring scales consisted of seven points: +3, the most prefer; +2, moderately prefer; +1, slightly prefer; 0, medium prefer; -1, slightly less prefer; -2, moderately less prefer; and -3, the least prefer. The buckwheat noodle samples in soy-sauce soup were presented to the panels immediately after cooking and were immediately evaluated. This study was implemented after the permission from the Ryukoku University Ethics Committee. The panels in this study gave their consent regarding the purpose of the study, study methodology and publication of the study results.

Protein determination

For chemical analysis of the combined fractions of buckwheat albumin plus globulin in the heated noodle samples which had been subjected to the mechanical measurements, the noodle samples were lyophilized and

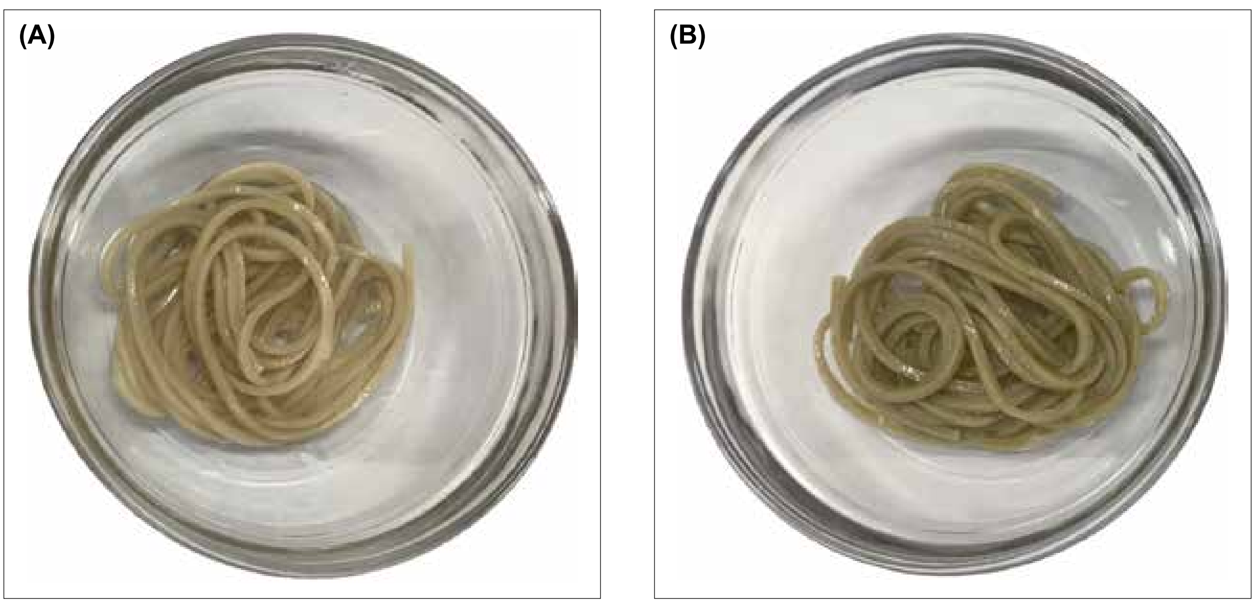

Fig. 2

A prototype of commercial product of buckwheat noodles with added ishi-kurage.
(A), non added ishi-kurage and (B), added ishi-kurage.

then ground into flour. The flours obtained were extracted with a ten-fold (v/w) volume of 0.2M NaCl for 1hr at 4ºC. After the extraction, the suspensions were centrifuged at 17,000 X g for 20 min. Protein concentration was determined using the Bradford method (Bradford, 1976) with bovine serum albumin as a standard protein.

Statistical analysis

Statistical analysis was conducted using a personal computer with the program Excel (Microsoft Co., USA), Ekuseru-Toukei (Social Survey Research Information Co., Japan) and SPSS Ver.23.0 (IBM, USA).

RESULTS AND DISCUSSION

***Mechanical analysis I:** mechanical characteristics of buckwheat noodles made with ishi-kurage*

Figure 3 shows breaking characteristics of buckwheat noodles prepared without or with *ishi-kurage*. As amounts of *ishi-kurage* added into buckwheat noodles increased, breaking stress and energy of the buckwheat noodles concomitantly increased (Fig. 3 (A and B)). A significant high breaking stress and breaking energy was found with buckwheat noodles with a concentration of *ishi-kurage* with 1.8% or over as compared with buckwheat noodles without *ishi-kurage* ($P<0.05$) (Fig. 3 (A and B)). These findings showed unique mastication buckwheat noodles prepared with *ishi-kurage* noodles.

***Mecanical analysis II:** mechanical characteristics of prototype of buckwheat product with ishi-kurage*

Figure 4 shows the comparison of breaking characteristics between prototype noodles made without and with *ishi-kurage*. There was a significant ($P<0.05$) difference in breaking stress and breaking energy between the two different buckwheat noodles examined (Fig. 4). The effect of addition of *ishi-kurage* could be shown as in the results of mechanical analysis I (Fig. 3).

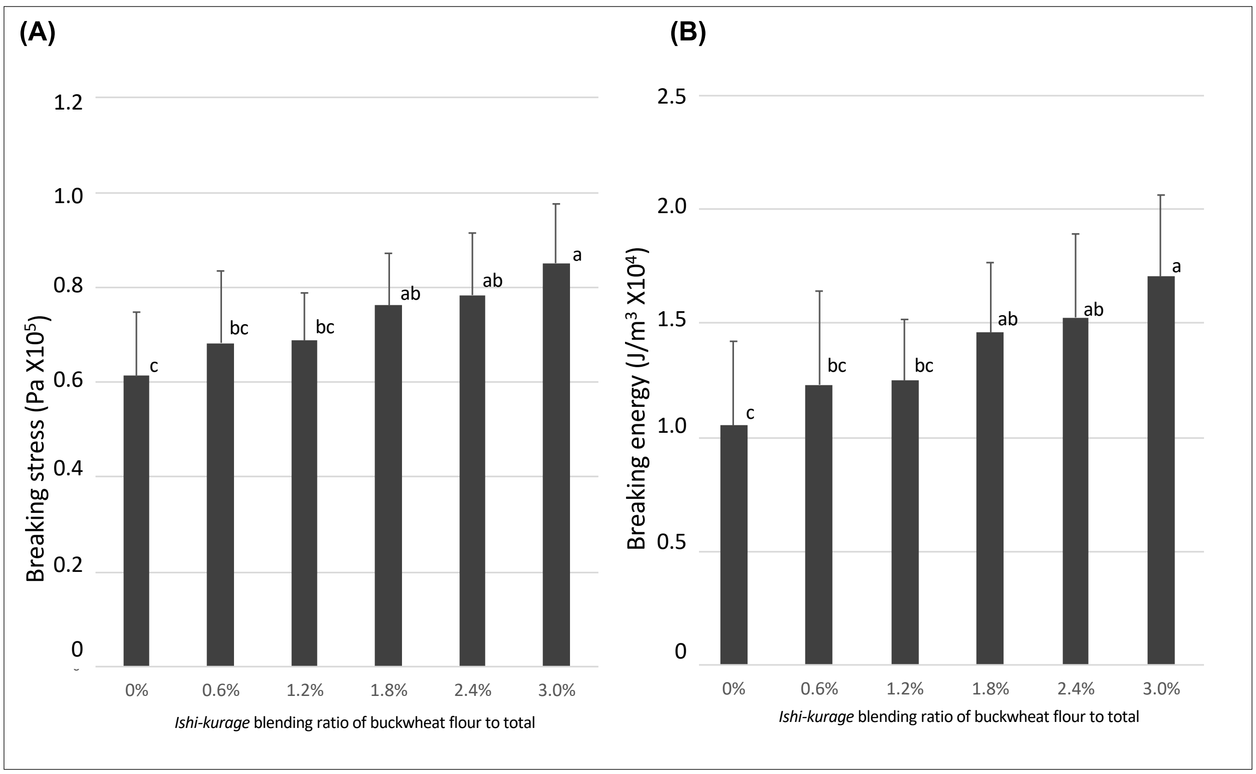

Fig. 3

Breaking characteristics of buckwheat noodles made with ishi-kurage.

(A), breaking stress; and (B), breaking energy. Vertical bars in the figure show the standard deviations.

Values within the same row that are not followed by the same letter are significantly different at P<0.05.

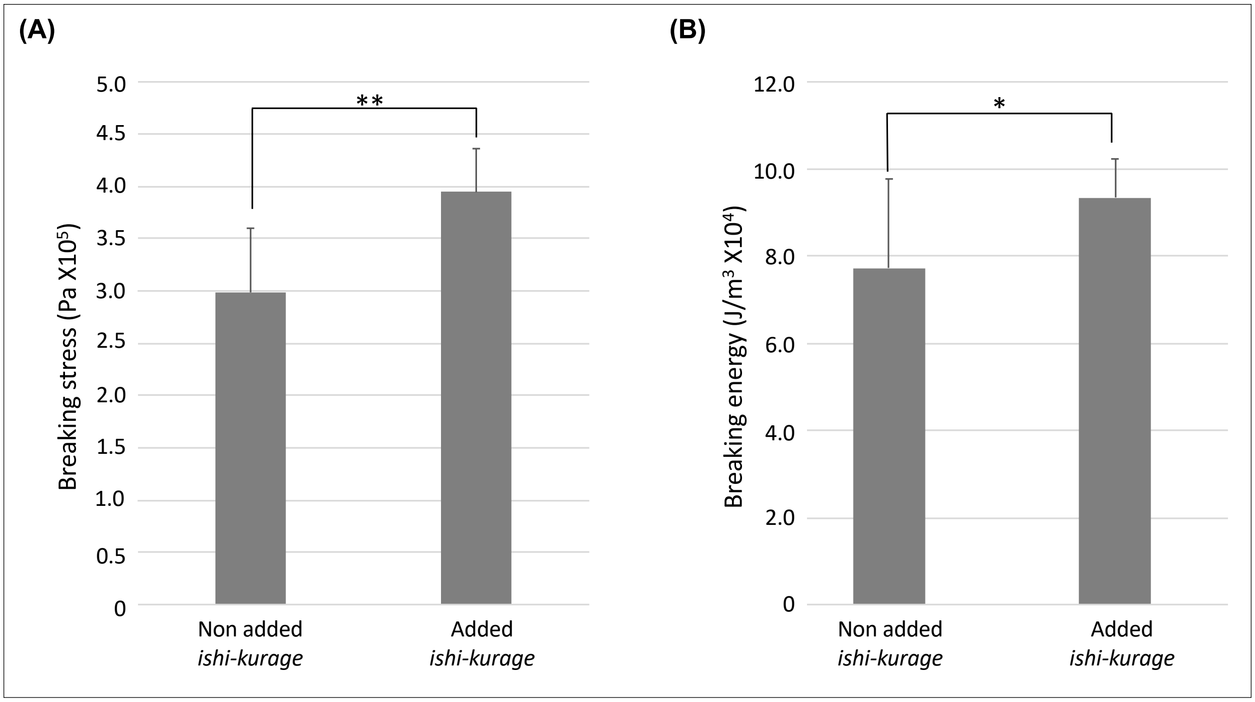

Fig. 4

Comparison of breaking characteristics between prototype noodles made without and with ishi-kurage.
(A), breaking stress; and (B), breaking energy. Vertical bars in the figure show the standard deviations.
*Significant difference between the two buckwheat noodles: *P<0.05, **P<0.01.*

Sensory evaluation of buckwheat noodles with *ishi-kurage*

Figure 5 shows the comparison of sensory evaluation between noodles made without and with *ishi-kurage*. Significant differences (P<0.05) between two types of buckwheat noodles were found for springiness, smoothness and color (Fig. 5), respectively. Springiness and color of buckwheat noodles with *ishi-kurage* were significantly higher than without *ishi-kurage* noodles (Fig. 5). On the other hand, smoothness of buckwheat noodles with *ishi-kurage* was significantly lower than without *ishi-kurage* noodles (Fig. 5). The present findings (Figs. 3, 4 and 5) suggest that incorporating *ishi-kurage* as a dough-improver into buckwheat noodles can produce buckwheat noodles with stable masticatory characteristics together with high palatability and acceptability.

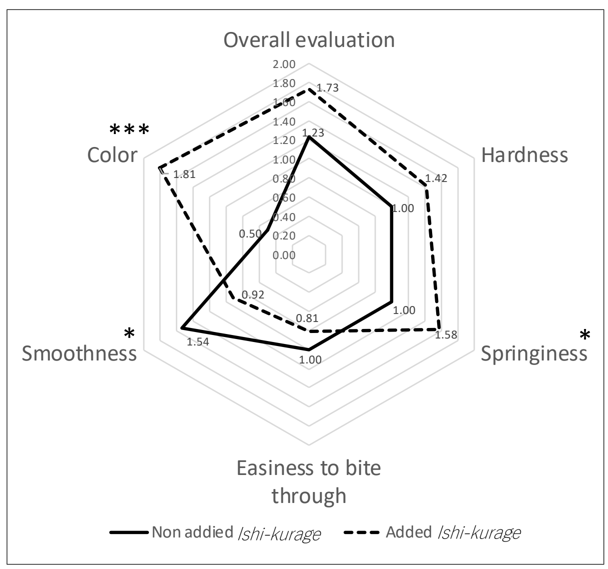

Fig. 5

Sensory evaluation of prototype noodles made without and with ishi-kurage. *Significant difference between the two buckwheat noodles: *P<0.05, ***P<0.001.*

Protein compositions of buckwheat noodles made with *ishi-kurage*

Figure 6 shows the NaCl-soluble protein content of buckwheat noodles made with *ishi-kurage*, i.e., noodles evaluated in mechanical analysis I (Fig. 3). The NaCl-soluble protein exhibits the combined fraction of the two major buckwheat proteins, i.e., albumin plus globulin (Ikeda, 2002), designated the AG fraction below. Changes by the addition of the *ishi-kurage* in solubility of the AG fraction were found (Fig. 6). Addition of *ishi-kurage* reduced the solubility of the AG fraction in buckwheat noodles as the *ishi-kurage* added into buckwheat noodles increased (Fig. 6). *Ishi-kurage* is reported to contain high levels of dietary fiber such as pectin (Hori et al., 1992). This observed phenomenon (Fig. 6) suggests a possibility indicating that buckwheat protein may be precipitated arisen by addition of dietary fiber present in *ishi-kurage*, as we have suggested in our previous findings also suggested that buckwheat protein may be precipitated by addition of some seaweeds (Asami et al., 2019). Our studies suggest that precipitation, if any, of buckwheat proteins in buckwheat products may lead to large alterations in the mechanical properties of buckwheat proteins (Ikeda, et al., 1999; Asami et al., 2008). Actually, statistical analysis showed that the AG fraction content (Fig. 6) negatively correlated to their observed breaking stress (Fig. 3 (A)) with $r = -0.869$ ($P<0.05$), breaking energy (Fig. 3 (B)) with $r = -0.865$ ($P<0.05$). These statistical findings suggest that the proteins of AG fraction (Fig. 6) may be associated with the observed mechanical characteristics (Fig. 3) of buckwheat noodles made with *ishi-kurage*.

Finally, the present study shows clear alterations in mechanical characteristics of buckwheat noodles made with *ishi-kurage*. The present study suggests that changes in the protein of AG fraction in buckwheat noodles with *ishi-kurage* may be an important factor affecting the mechanical characteristics of buckwheat noodles, although the exact mechanism remains uncertain. In this study, *ishi-kurage*, which was reported to have high functionality (Hori at al., 1990; Ishibashi et al., 1994; Itoh, 2015), was added to buckwheat noodles. The findings of the present study will hopefully stimulate further development of new buckwheat products.

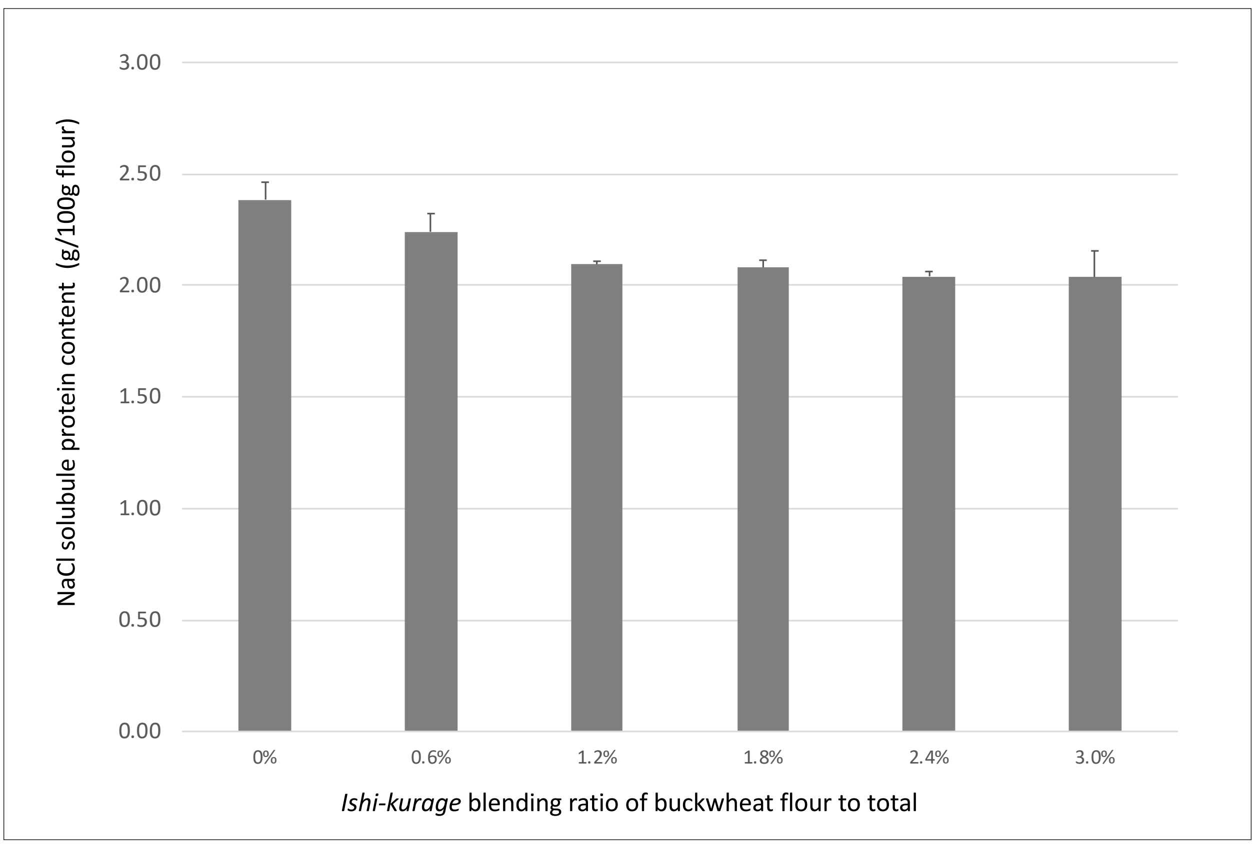

Fig. 6

NaCl-soluble protein content of buckwheat noodles made with ishi-kurage. *Vertical bars in the figure show the standard deviations.*

ACKNOWLEDGMENT

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IZVLEČEK

Namen te raziskave je bil ugotoviti vpliv modro-zelene alge *ishi-kurage* (*Nostoc commune* Vauch.) na mehanične lastnosti ajdovih testenin. Raziskava je pokazala, da je vključitev *ishi-kurage* v ajdove testenine povečala odpornost testenin na lomljenje. Senzorični preizkus je pokazal, da so bile ajdove testenine z algo *ishi-kurage* boljše v primerjavi s kontrolo brez te alge. Dodatek alge v ajdove testenine je povezan z zmanjšanjem topnosti albuminske in globulinske frakcije beljakovin testenin. Na osnovi rezultatov te raziskave lahko sklepamo, da so beljakovine ajde pomembne za mehanične lastnosti ajdovih testenin z dodatkom alge *ishi-kurage*.

Short communication

Extraction of rutin and quercetin from Tartary buckwheat grains, hydrothermally treated at different temperatures

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ABSTRACT

Tartary buckwheat grains were hydrothermally treated to establish the conditions under which rutin remains in the grain. Tartary buckwheat grains were soaked in water at the temperatures 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, and a control group at 21 °C. During 20 minutes soaking at 51 °C or 61 °C the concentration of rutin decreased. This effect was mostly pronounced by soaking at 70 °C and 75 °C, where instead of missing amount of rutin, some quercetin appeared. After soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly different in comparison to the concentration of rutin after soaking 20 minutes at 21°C. It is suggested that exposure to water at 21°C is similar to natural conditions, where rutin degrading enzymes remain mainly inactive and in grain separated from its potential substrate. Further is suggested that at the soaking temperatures 51 °C, 61 °C, 70 °C and 75 °C, grain structures are partly degraded and rutin degrading enzymes got contact to the substrate. By soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, rutin degrading enzymes lose their activity. Thus wet treatment of Tartary buckwheat grains for 20 minutes at temperature at 80 °C or above, this threshold is enough to preserve the content of rutin in the samples. This is of importance for nutritional quality of Tartary buckwheat food products.

INTRODUCTION

Flavonoid rutin and rutin degrading enzymes are in different structures of buckwheat grain. Rutin is in embryo, in the middle of the grain, while rutin degrading enzymes are located in the peripheral part of the grain. After crushing and milling of the grain, in the wet environment, rutin degrading enzymes are in the direct contact with their substrat, and the concentration of rutin may begin to decrease (MetaCyc, 2011). Harvested buckwheat grain contain among flavonoids mainly rutin, and just a low concentration of quercetin. During dough and bread making, due to transforming of rutin to quercetin, the concentration of rutin decreases and quercetin concentration increases (MetaCyc, 2011; Lukšič et al., 2016).

Quercetin is a flavonoid, frequently present in foods and drinks, with bitter taste (Anand et al., 2016).

Tartary buckwheat contains in its leaves and grain higher concentrations of rutin in comparison to common buckwheat. Bitter taste of Tartary buckwheat is due to the high concentration of rutin and quercetin (Bonafaccia et al., 2003). Buckwheat cultivation decreased for years, but recently it is coming back because of knowledge of health promoting properties (Li et al., 2001).

The aim of this investigation was to establish the temperatures, at which the activity of rutin degrading enzymes are hindered.

MATERIAL AND METHODS

Preparation of samples

Tartary buckwheat (*Fagopyrum tataricum*, cv. Zlata) grains were obtained from Mill Rangus, Šentjernej, Slovenia. Grains were soaked in water for 20 minutes at temperatures 21 °C, 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C. After hydrothermal treatment samples were dried for 24 hours at 40 °C. Dry samples were milled in a coffee grinder (Gorenje, SMK 202, Velenje). Samples (1 g per 25 mL) were extracted for 20 min in a 80 % (v/v) methanol in horizontal shaker (Phoenix Instrument, RS-OS 5, Garbsen, Germany). After the extraction solutions were filtered (Agilent Econofilters, PTFE 25 mm 0.2 μm, Santa Clara, USA). Three independent samples were analyzed for each hydrothermal treatment.

Determination of rutin and quercetin by HPLC

Standard chemicals (rutin and quercetin), methanol (Chromasolv for HPLC), acetonitrile (LC-MS Chromasolv) and phosphoric acid (ACS grade) were purchased by Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany). Deionized water (dH_2O) was treated in a deionization system DI 425 TK-0.10425 (Thermo Scientific, Waltham, USA).

Preparation of calibration solutions and samples solutions were described by Lukšič et al. (2016).

Rutin and quercetin were determined using an Agilent 1100 Series high performance liquid chromatograph (Agilent Technologies, Santa Clara, USA) with quaternary solvent pump (G1311A) coupled with degasser (G1379A), sample manager (G1329A), column manager (G1316A), autosampler (G1329A) and DAD detector (G1315B). All HPLC analyses were performed on a Zorbax Eclipse XDB-C18 column (4.6 mm x 250 mm x 5 μm) (Agilent Technologies, Santa Clara, USA).

The mobile phase consisted of acetonitrile (gradient) (A) and 0.1% phosphoric acid in dH_2O (B). The gradient elution was as follows: 0-1 min isocratic elution (20% A and 80% B), 1-5 min linear gradient elution (25% A and 75% B), 5-15 min (30% A and 70% B) and 20-25 min (40% A and 60% B). The initial flow rate was 1 mL min^{-1} and the injection volume was 10 μL. Column oven temperature was set up to 25 °C and the samples were kept at 4 °C in the sample manager. The detection wavelengths were conducted at 265 nm (rutin) and 372 nm (quercetin). The data were collected and proceed using Agilent Chemstation 9.01 software.

Statistical evaluation

The data are expressed as means, and standard deviation, from three independently prepared samples. ANOVA was performed, the data were considered to be significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Results are presented in Fig. 1.

During 20 minutes soaking at 51 °C or 61 °C the concentration of rutin decreased. This effect was most pronounced by soaking at 70 °C and 75 °C, where instead of missing amount of rutin, some quercetin appeared. After soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly different in comparison to the concentration of rutin after soaking 20 minutes at 21°C. It is suggested that exposure to water at 21°C is similar to natural conditions, where rutin degrading enzymes remain mainly inactive and in grains separated from its potential substrate. Further is suggested that at the soaking temperatures 51 °C, 61 °C, 70 °C and 75 °C, grain structures are partly degraded and rutin degrading enzymes got contact to the substrate. By soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, rutin degrading enzymes lose their activity. Thus wet treatment of Tartary buckwheat grains for 20 minutes at temperature at 80 °C or above this threshold is enough to preserve the content of rutin in the samples. This is of importance for nutritional quality of Tartary buckwheat food products.

CONCLUSION

Rutin degrading enzymes were most active during soaking Tartary buckwheat grain at the temperatures 70 °C and 75. At temperatures 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly decreased, and no quercetin appeared, meaning that the rutin degrading enzymes were under this condition inactivated.

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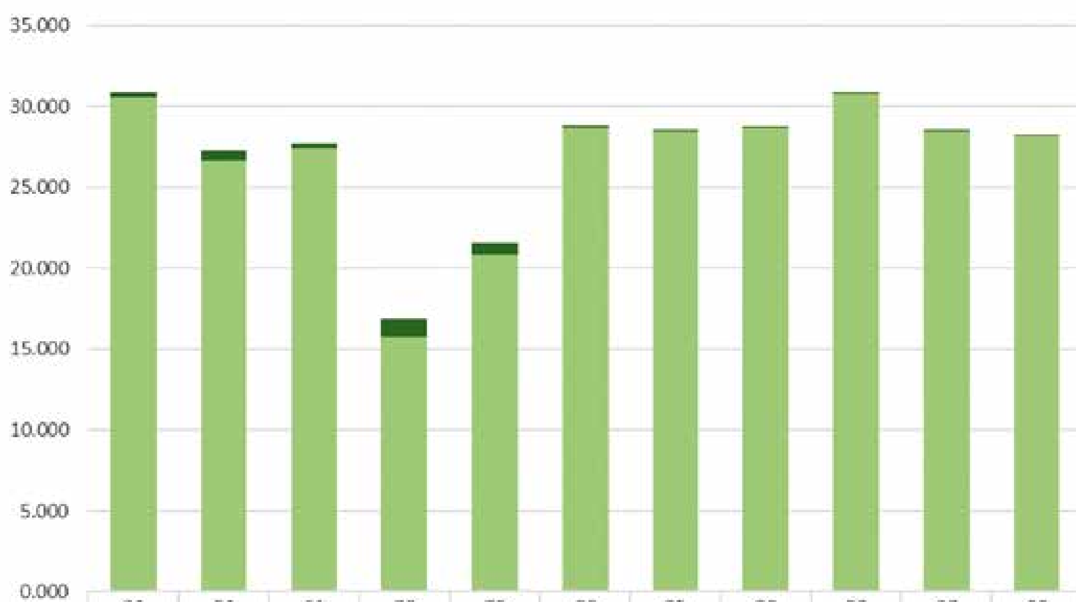
Fig. 1

Concentration of rutin and quercetin in Tartary buckwheat samples (in mg/g dry mass), treated at given temperatures



	21	51	61	70	75	80	85	90	93	97	99
QUERCETIN	0.236	0.620	0.255	1.124	0.715	0.152	0.073	0.072	0.049	0.058	0.042
RUTIN	30.602	26.666	27.440	15.751	20.858	28.678	28.476	28.716	30.768	28.457	28.209
QUERCETIN STDEV	0.027	0.014	0.003	0.048	0.010	0.0040	0.0022	0.0013	0.0006	0.0005	0.0003
RUTIN STDEV	3.526	0.842	0.211	0.961	0.267	0.919	1.245	1.409	1.303	0.641	0.656

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IZVLEČEK

Ekstrakcija rutina in kvercetina iz hidrotermično obdelanih zrn tatarske ajde pri različnih temperaturah

Namen raziskave je bil določiti pogoje, pod katerimi se rutin ohrani v ajdi pri hidrotermični obdelavi zrn tatarske ajde. Zrna tatarske ajde so bila hidrotermično obdelana v vodi pri temperaturah 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C in 99 °C s kontrolno skupino pri 21 °C. Pri 51 °C in 61 °C se je koncentracija rutina znižala. Pri 70 °C in 75 °C se je znaten del rutina pretvoril v kvercetin. Pri 80 °C, 85 °C in 90 °C se je pretvorba rutina v kvercetin zmanjšala predvidoma zaradi denaturacije encimov, ki razgrajujejo rutin. Pri višjih temperaturah 93 °C, 95 °C in 99 °C je bila koncentracija kvercetina izrazito manjša, ohranil pa se je velik delež rutina. Glede na ugotovljeno imajo hidrotermično obdelana zrna tatarske ajde višjo koncentracijo rutina, kar je pomembno s stališča prehrane.

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