Study of a gluten–free bread made from Gagome kelp (*Kjellmaniella crassifolia Miyabe*).

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Abstract

Gluten-free bread was made from Gagome kelp, wheat starch, sugar, compressed yeast, and water. When the Gagome kelp was digested with pepsin or treated with ethyl ether, bread baked with the deproteinized or defatted Gagome kelp did not display worse properties. However, when the Gagome kelp was autoclaved at 120 °C for 100 min, its bread making properties deteriorated markedly. A mixture of Gagome kelp and water was homogenized and centrifuged at 1,700 g. The supernatant and precipitate were subjected to bread making tests. The results indicated that the supernatant fraction had good breadmaking properties. The supernatant was further dialyzed against a large amount of water and subjected to bread making tests. The undialyzable fraction display good bread making properties. The supernatant was divided into an upper transparent layer and a lower dark and viscous layer, and bread making tests were conducted. The upper transparent layer demonstrated better bread making properties than the lower dark and viscous layer.

Keywords: Gluten free bread, *Kjellmaniella crassifolia Miyabe*, Gagome kelp, Celiac disease (CD).

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Introduction

Celiac disease (CD) is an autoimmune-type of gastrointestinal disorder that is triggered by gluten-containing grains, such as wheat, rye, barley, and oats [1]. With the advent of multiple serological tests for CD, a substantial number of previously undiagnosed cases have been identified, yielding a prevalence of approximately 1% among the Caucasian population in the US and Europe [1,2]. Nakazawa et al. [3] used serological tests based on tissue transglutaminase-IgA (TTG-IgA) and histological examination to identify CD among the Japanese population. Their screening program among 710 patients identified seven cases (0.98%) with both positive TTG-IgA and the pertinent mucosal changes, compatible with celiac disease, which study would shed light firstly on the discussion of possible diagnosis of celiac disease among East Asian population.

The only known treatment of celiac disease is the complete avoidance of wheat, rye, and barley [4]. In practice, commercial gluten-free products are formulated with a relatively short list of wheat-flour-replacing food-approved ingredients. Most commonly used are tapioca starch, rice flour, rice starch, potato starch, corn starch, egg whites, xanthan gum, guar gum, and HPMC (Hydroxyl Propyl Methyl Cellulose). Since commercial gluten-free bread formulas are highly proprietary, more useful and safety gluten-free bread formulas are searched [5]. Japanese people like sticky foods such as yam flour, gagome kelp, Natto (fermented soybean), Okura (*Abelmoschus esculentus*), Nameko (*Pholiota microspore (Berk.) Sacc*.), and Mozuku (*Nemacystus decipiens*) which are all low cost diet and will be accepted by people in all over the world, so we would like to use these viscous food materials in gluten-free diets.

Gagome kelp (*Kjellmaniella crassifolia Miyabe*), which is more viscous than wheat gluten can be used to make gluten-free bread. Gagome kelp is grown in the Hakodate area of Hokkaido in Japan and is considered to have beneficial health effects [6]. Gagome kelp contains water-soluble polysaccharides, which are widely used as a thickener in the low-calorie food sector and as a hemostatic agent or a humectant in the medical sector. It is commercially reported that Gagome kelp contains alginate (19.0-22.0 g), laminarin (4.0-4.5 g), and fucoidan (3.5-4.5 g per 100 g), and absorbs water in the stomach to produce a sticky substance. The chemical structures of alginate, laminarin, and fucoidan molecules found in kelp were reported by Teruya et al., Kadam et al. and Tako et al. [7-9] respectively. Alginate is a linear, 1,4-linked copolymer of α-L-gluronate and β-D-mannuronate, and has been reported to protect cells from apoptosis, induce cytokine secretion, and regulate the uptake of cholesterol and glucose [10]. Laminarin is a storage β-glucan composed of 1,3-β-D-glucan and β-1,6-linked branches, and it has many biofunctional activities including antitumour, anti-apoptotic, anti-inflammatory, anticoagulant, and antioxidant activity [8]. Fucoidan is a fucose-containing sulfated polysaccharide and exhibits anti-allergic activity, anti-infectious effects against Helicobacter pylori and prions, and anti-tumor activity [6].

We have published reports about the production of gluten-free bread using yam flour [11] and banana flour [12], but neither of

these flours was as viscous as Gagome kelp, so it is possible to make bread with a smaller amount of Gagome kelp than yam or banana flour. The bread making properties of Gagome kelp, such as the height (mm) and specific volume (cm^3/g) of the bread it produces were comparable with those of wheat bread [13]. To know clear contribution of Gagome kelp in this gluten- free bread making experiment we used a similar formula to wheat sweet dough, which composed of 14-22 g sugar, 1.5-2.0 g NaCl, 1.5-4.5 g of dry-yeast, 100 g wheat flour and 40-50 g water.

Materials and Methods

Materials

Gagome kelp was purchased from commercial sources. The Gagome kelp was lightly washed with water, cut into small pieces, crushed in a Waring blender (Hamilton Beach/Proctor-Silex, Inc., Southern Pines, NC, USA), and freeze-dried at -15 °C for 70 h in a Kyowa RLE-120 freeze-drier (Kyowa Vacuum Engineering Co., Ltd., Tokyo, Japan). A general compositional analysis of the Gagome kelp obtained the following findings: moisture, 10.13%; protein, 11.79%; fat, 0.58%; and ash content, 17.11%; respectively. Protein conversion calculations were carried out using the formula Nx5.7 [14]. Ash content was determined using the AACC International Method (08-01, 2000) at a moisture content of 14.0%. Moisture content was determined using the method of Tsutsumi and Nagahara [15]. Pepsin from the porcine stomach mucosa was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Pepsin digestion of Gagome kelp

The treatment of Gagome kelp with pepsin was performed according to the method of Seguchi [16]. The Gagome kelp (3.0 g) was mixed in a 120 ml (pH 2.0) HCl suspension of pepsin (3 mg) and then incubated at 37˚C for 60 min with stirring. Then, the suspension was centrifuged at 1,700 g for 10 min, and the precipitated Gagome kelp was washed with water (120 ml) 10 times and dried at room temperature.

Defatting of Gagome kelp with ethyl ether

Gagome kelp (10 g) was subjected to ethyl ether extraction (at 65 ˚C) in a Soxhlet apparatus for 8 hs.

Autoclaving treatment of Gagome kelp

Gagome kelp (30 g) was suspended in 40 ml of water, heated at 127 °C for 100 min in an autoclave (Shimadzu, Kyoto, Japan), and then cooled until use [12]. Although the pH in Gagome kelp suspension was not determined, it may be neutral.

Bread making

Bread making was made in the following manner: 300 mg Gagome kelp, 30.5 g wheat starch, 8.86 g sugar, 10 g compressed yeast and 50 ml water were mixed in a 3.6-L bowl using a kitchen aid mixer (Kenmix Chef Aicoh Mixers and Aicoh Systems Co., Ltd., Japan) for 9 min at 116 rpm. The homogenized dough was placed into a pan (5.5x9.5x6.6 cm^3), proofed in an oven at 40 °C for 20 min, and baked at 210 °C for 10 min (Sanyo Drying Oven MOV-212, Sanyo Co. Ltd., Japan). After being baked, the bread was removed from the pan and cooled for 1h at room temperature (26 °C) at a relative humidity of 43%. The height (mm), weight (g), and volume (cm^3) of the bread were measured, and bread crumbs were evaluated visually. Bread volume was measured using the rapeseed displacement method. The color (*L, a*, and *b* values) of the bread crumbs was evaluated using an *L*, *a*, and *b* color specification system based on a chromaticity diagram and a Hunter Lab Color Meter NE 2000 (Nippon Denshoku Co., Ltd., Tokyo, Japan). Positive values for *L*, *a*, and *b* indicate white, red, and yellow, respectively. Negative values for *a* indicate green.

Fractionation of Gagome kelp/water into supernatant and precipitate fractions by centrifugation

Gagome kelp (3.6 g)/ water (264 ml) was homogenized in a Waring blender with vigorous shaking (10,000 rpm for 2 min) and centrifuged at 1,700 g for 10 min. The supernatant and precipitate (Figure 1) were collected, freeze-dried, and subjected to bread making tests.

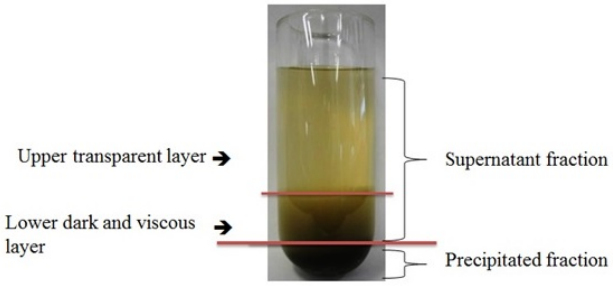

Figure 1. Separation of the supernatant and precipitate and the subsequent separation of the supernatant into an upper transparent layer and a lower dark and viscous layer.

Separation of the supernatant into high molecular weight (HMW) and low molecular weight (LMW) fractions by dialysis

A dialysis tube having MWCO (molecular weight cutoff), 12,000-14,000 Daltons and pore size 24 A° (Nihon Medical Science Co., Ltd., Takasaki, Japan) was used to dialyze. The supernatant was dialyzed against 10 L of water overnight in a cold room (4 °C) and separated into undialyzable (HMW) and dialyzable (LMW) fractions.

The undialyzable fraction (in the dialysis tube) was freeze-dried at -15 °C for 72 h and crushed; this was termed the HMW fraction (its moisture content was not determined). The dialyzable fraction was concentrated to syrup at 65 °C using a rotary evaporator (RE-51, Yamato Scientific Co., Ltd., Tokyo, Japan) and was termed the LMW fraction.

Determination of the viscosity of the Gagome kelp supernatant

The viscosity of the Gagome kelp supernatant was measured at room temperature using a B-type viscometer (Brookfield Engineering Labs Inc., MA, USA).

Fractionation of the supernatant into upper transparent and lower dark and viscous layers by centrifugation

The supernatant was composed of an upper transparent layer and a lower dark and viscous layer (Figure 1). The two layers were collected, freeze-dried, and used for bread making.

Statistical analysis

A statistical software package (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Three or more loaves of bread were baked for each experiment. Bread height (mm) and specific volume (cm^3/g) were measured three or more for each sample and averaged. Data with significant F values were subjected to analysis of variance, followed by Duncan's multiple range tests for comparison of means.

Results and Discussion

Optimal amounts of ingredients for bread making with Gagome kelp

To determine the optimal amounts of ingredients for bread making with Gagome kelp, bread making tests were performed using various combinations of 50-700 mg Gagome kelp, 29.80-30.45 g wheat starch, 8.86 g sugar, 10 g compressed yeast, and 18-30 ml water. In bread making tests involving 300 mg Gagome kelp, 30.2 g wheat starch, 8.86 g sugar, 10 g compressed yeast, and 18 to 30 ml of water (Table 1), the maximum bread height was obtained when 22-24 ml water were used.

The resulting breads exhibited the following properties: 22 ml water: bread height; 79.56 mm, specific volume; 6.89 cm^3/g: 24 ml water: bread height; 84.04 mm, specific volume; 6.67 cm^3/g. Those results were higher than bread making properties as Haruyutaka wheat flour (bread height, 69.4 mm and specific volume, 3.45 cm^3/g) [13].

Thus, it was determined that the best Gagome kelp bread was made with 300 mg Gagome kelp, 30.2 g wheat starch, 8.86 g sugar, 10 g compressed yeast, and 22-24 ml water. It was calculated that flour (Gagome kelp+wheat starch) 30.5 g (100%), sugar 8.86 g (29.1%), and dry yeast (compressed yeast 10 g = around 9 g water and 1g dry yeast) 3.3% were obtained.

This was a similar formula to wheat sweet dough for coffee sweet bread. Seguchi et al. [11] reported that they could obtain a nice bread making properties such as bread height and specific volume (68.4 mm and 3.95 cm^3/g) using yam flour (10 g). Seguchi et al. [12] also obtained same nice bread height and specific volume, 69.4 mm and 3.45 cm^3/g, respectively in banana flour (30 g).

The amount of Gagome kelp flour (300 mg) was extensively small amount than that of yam flour or banana flour to obtain maximum bread making properties, which was due to higher viscosity of Gagome kelp flour, and which is effectively convenient to the bread flavor and bread color. The gluten-free bread indicated whitish bread and lower flavor by the addition of Gagome kelp, which may be suitable for consumer.

Table 1. Bread making properties baked with Gagome kelp (300 mg) and various water (Values represent means of three or more replicates with SD in parentheses. Means followed by different letters in columns are significantly at $p<0.05$ according to Duncan's multiple range test).

Water (ml)	Breadheight (mm)	Specificvolume (cm^3/g)	*L*	*a*	*b*
18	65.44^a (2.22)	5.56^a (0.28)	61.82	-0.94	8.1
20	72.38^b (4.03)	5.88^a (0.42)	65.99	-1.74	8.64
22	79.56^c (3.05)	6.89^b (0.39)	65.7	-0.9	8.21
24	84.04^c (3.37)	6.67^b (0.30)	66.09	-1.13	7.34
26	78.74^c (1.92)	5.44^c (0.15)	68.59	-0.51	8.26
28	64.01^d (4.17)	3.96^d (0.11)	71.99	-0.53	8.03
30	63.81^d (9.63)	3.96^d (0.57)	67.78	-1.41	8.96

Effects of subjecting Gagome kelp to various treatments on its bread making properties

To clarify the key components in Gagome kelp for bread making, Gagome kelp was subjected to pepsin treatment, ethyl ether extraction, or autoclaving treatment before being used for bread making. The results of these experiments are shown in Table 2. Pepsin was able to digest the proteins in Gagome kelp, and ethyl ether was able to defat Gagome kelp.

Table 2. Effects of various treatments of Gagome kelp on bread making properties (Values represent means of three or more replicates with SD in parentheses. Means followed by different letters in columns are significantly at $p<0.05$ according to Duncan's multiple range tests).

Treatments	Bread height		Specific volume		*L*	*a*	*b*
	(mm)	(%)	(cm^3/g)	(%)			
None	79.56^a (3.05)	100	6.89^a (0.39)	100	65.7	-0.9	8.21
Pepsin	81.68^a (3.82)	103	6.12^a (0.42)	89	71.82	-0.77	8.09
Ethyl ether	83.95^a (6.24)	106	6.63^a (0.70)	96	64.71	-0.74	8.03
Autoclave	23.81^b (0.58)	30	1.54^b (0.05)	22	61.55	-0.96	13.38

However, neither treatment affected the bread making properties of Gagome kelp (after pepsin treatment: bread height: 81.68 mm (103%), specific volume: 6.12 cm^3/g (89%): after ethyl ether treatment: bread height: 83.95mm (106%), specific volume: 6.63 cm^3/g (96%)). Thus, it was clear that

protein and fat in Gagome kelp do not influence its bread making properties.

Conversely, subjecting Gagome kelp to autoclaving treatment (127°C, 100 min) caused a marked reduction in its bread making properties (bread height: 23.81 mm (30%), specific volume: 1.54 cm^3/g (22%)). It was suggesting that the good bread making properties of Gagome kelp are caused by its heat labile components: i. e., components other than enzymes.

Purification of the key bread making components of Gagome kelp

Next, we tried to purify the key bread making components of Gagome kelp. After homogenizing Gagome kelp with water and centrifuging it at 1,700 g for 10 min. the precipitate (1.19 g, 33.1%) and supernatant (2.11 g, 58.6%) from Gagome kelp (3.60 g, 100%) were obtained, which were then freeze-dried and subjected to bread making tests (Table 3).

The bread making tests were performed according to viscosity of bread dough using various amounts of the supernatant or the precipitate, and finally baked with 180 mg of the supernatant and 22 ml of water, or 110 mg of the precipitate and 22 ml of water to their optimal bread making properties. The precipitate exhibited poor bread making properties (bread height: 26.34 mm, specific volume: 1.48 cm^3/g), whereas the supernatant displayed good bread making properties (bread height: 83.48 mm, specific volume: 6.59 cm^3/g).

Therefore, it was shown that the key components for bread making are present in the water soluble fraction of Gagome kelp.

Table 3. Bread baked with supernatant and precipitation fractions (Values represent means of three or more replicates with SD in parentheses. Means followed by different letters in columns are significantly at $p<0.05$ according to Duncan's multiple range test).

Sample (mg)	Water (ml)	Bread height (mm)	Specific volume (cm^3/g)	*L*	*a*	*b*
Supernatant 180 mg	20	55.36^a (7.43)	4.50^a (0.43)	65.92	-1.21	9.36
	22	83.48^b (9.22)	6.59^b (0.70)	70.16	-0.91	7.57
	24	53.69^a (2.52)	3.67^c (0.17)	65.16	-0.91	7.6
	26	51.56^a (2.63)	3.46^c (0.39)	71.85	-1	9.88
Precipitation 110 mg	20	26.13^a (1.12)	1.42^a (0.07)	71.81	-0.48	14.7
	22	26.34^a (0.82)	1.48^a (0.04)	72.99	-0.91	14.94
	24	26.16^a (0.76)	1.32^b (0.08)	67.94	-1.58	13.83
	26	27.95^b (0.71)	1.33^b (0.05)	62.42	-1.87	13.18

Separation of the supernatant into HMW and LMW fractions by dialysis

Furthermore, the supernatant obtained from Gagome kelp (3.32 g) was dialyzed against 10 L water, and the undialyzable (HMW, 1.34 g, 61.8%) and dialyzable (LMW, 0.83 g, 38.3%) fractions were separated and freeze-dried.

The optimal bread making properties were obtained with 110 mg of the HMW fraction and less than 20 ml of water, or 70 mg of the LMW fraction and 24-26 ml of water (HMW fraction: bread height; more than 79.10 mm, specific volume: 5.88 cm^3/g.

Those data may be higher if addition of water was smaller: LMW fraction: bread height: 29.28 mm, specific volume: 1.33 cm^3/g) (Figure 2 and Table 4).

It was clear that the key components for bread making were present in the HMW fraction.

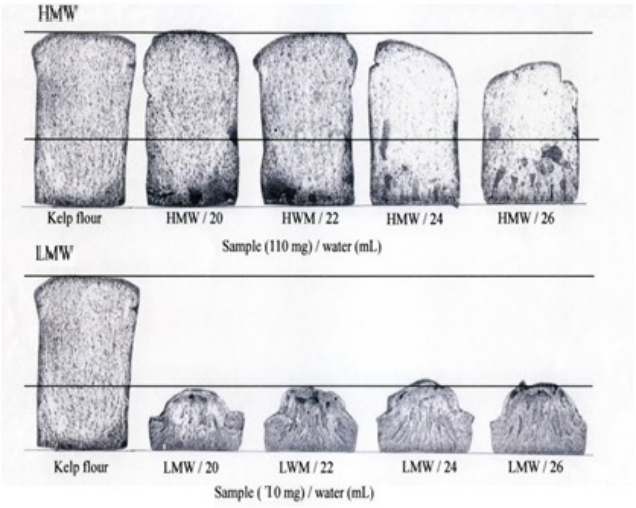

Figure 2. Cross-sections of bread baked with the HMW or LMW fraction Bread making conditions: 110 mg HMW fraction or 70 mg LMW fraction were used.

The dialyzable LMW fraction was spotted on paper, dried and sprayed with two reagents (ninhydrin and AHP (Aniline Hydrogen Phthalate)), respectively, and it was realized that LMW fraction only contained ninhydrin-positive materials,

such as amino acids and peptides; i.e., it did not contain AHP-positive materials of carbohydrates (data were not shown here).

Table 4. Bread baked with HMW or LMW fraction in Gagome kelp (Values represent means of three or more replicates with SD in parentheses. Means followed by different letters in columns are significantly at $p<0.05$ according to Duncan's multiple range test).

Sample (mg)	Water (ml)	Bread height (mm)	Specific volume (cm^3/g)	*L*	*a*	*b*
HMW 110 mg	20	79.10^a (3.55)	5.88^a (0.26)	64.85	-0.84	8.51
	22	70.60^a (6.19)	5.20^b (0.32)	65.82	-0.94	8.04
	24	68.22^a (3.73)	4.72^b (0.26)	68.92	-0.52	7.2
	26	51.90^b (7.42)	3.46^c (0.30)	68.04	-0.09	9.56
LMW 70 mg	20	25.29^a (1.45)	1.24^a (0.02)	68.46	-0.34	11.51
	22	26.98^a (0.58)	1.29^a (0.04)	65.81	-0.78	12.64
	24	29.28^a (1.43)	1.33^a (0.03)	68.35	-0.59	11.34
	26	27.33^c (1.21)	1.33^a (0.07)	62.7	-1.16	10.41

Autoclaving-induced changes in the viscosity of the supernatant and the effects on the supernatant's bread making properties

The supernatant of Gagome kelp was subjected to autoclaving treatment (127 °C, 100 min), and its viscosity was measured.

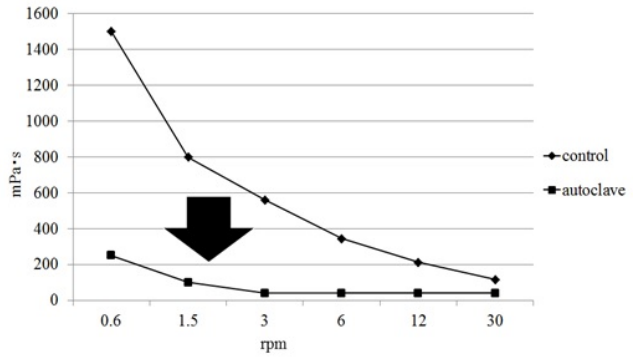

Figure 3. Autoclaving-induced changes in the viscosity of the Gagome kelp supernatant.

The viscosity of the supernatant decreased after the autoclaving treatment (Figure 3), and its bread making properties in baking tests with 180 mg sup and 22 ml water deteriorated: autoclaved supernatant: bread height; 27.14 mm, specific volume: 1.41 cm^3/g: unautoclaved supernatant fraction: bread height: 83.48 mm, specific volume: 6.59 cm^3/g (Figure 4).

It was clear that the reduction in the viscosity of the autoclaved supernatant was related to the deterioration of its bread making properties.

Although the viscous materials in Gagome kelp, such as alginic acid, laminarin, and fucoidan, are all water-soluble HMW polysaccharides, it remains unclear whether they are heat labile.

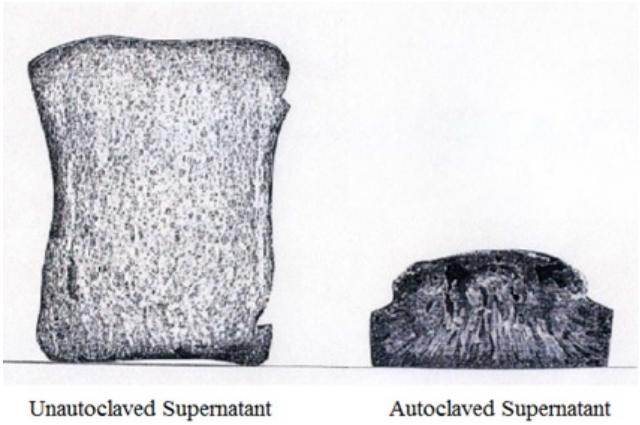

Figure 4. Cross-sections of bread baked with the autoclaved supernatant.

Fractionation of the supernatant into upper transparent and lower dark and viscous layers by centrifugation

The supernatant of Gagome kelp (14.4 g, 100 %) contained two layers (Figure 1), an upper transparent layer (2.10 g, 14.6 %) and a lower dark and viscous layer(5.86 g, 40.7%). These layers were separated, freeze-dried, and subjected to bread making tests. According to viscosity of bread dough, sample 150 mg and water 22 ml were selected. The results (Table 5) indicated that the bread making properties of two layers were as follows: upper transparent layer: bread height: 62.56 mm, specific volume: 4.84 cm^3/g: lower dark and viscous layer: bread height: 57.61 mm, specific volume: 3.64 cm^3/g. Thus, it was found that the upper transparent layer had better bread making properties.

Table 5. Bread baked with upper or lower layer in supernatant fraction of Gagome kelp (Values represent means of three or more replicates with SD in parentheses).

Sample (150mg)	Water (ml)	Bread height (mm)	Specific volume (cm^3/g)	*L*	*a*	*b*
Upper layer	22	62.56 (4.61)	4.84 (0.54)	70.39	-0.58	8.77
Lower layer	22	57.61 (7.74)	3.64 (0.45)	52.67	-1.21	7.16

However, the amount of the lower dark and viscous layer (73.6%) was larger than that of the upper transparent layer (26.4%). In this study, we did not extract alginate, laminarin, and fucoidan from Gagome kelp: however, the upper transparent layer or the lower dark viscous layer of the supernatant might have contained these polysaccharides.

Conclusion

It was shown that gluten-free bread can be made with 300 mg, Gagome kelp, 30.2 g wheat starch, 8.86 g sugar, 10 g compressed yeast, and 22.0 ml water. The water-soluble fraction of Gagome kelp exhibited good bread making properties; however, its bread making properties deteriorated

after autoclaving treatment, suggesting that alginate, laminarin, and fucoidan are important for making bread from Gagome kelp.

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