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Development of Novel Bread by Combining Seaweed *Kappaphycus alvarezii* from Sri Lanka and *Saccharomyces cerevisiae* Isolated from Nectarine

Noriko Komatsuzaki[1], Sayoko Arai[1], Shinobu Fujihara[1], Jun Shima[2], Rathnayake Saman Wijesekara[3] and M. Dileepa S. T. de Croos[3]

1. Department of Human Nutrition, Seitoku University, 550 Iwase Matsudo, Chiba 271-8555, Japan

2. Faculty of Agriculture, Ryukoku University, 1-5 Yokototani, Seta Oe-Cho, Otsu, Shiga 520-2194, Japan

3. Department of Aquaculture and Fisheries, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Gonawila (NWP) 60170, Sri Lanka

Abstract: Seaweeds are not used as much for edible use although many kinds of seaweed are collected in Sri Lanka. The objective of this study was to develop high quality bread using seaweed, *Kappaphycus alvarezii* collected from Sri Lanka, and wild yeast, *Saccharomyces cerevisiae* isolated from nectarine in Japan. Dietary fiber in *K. alvarezii*, Japanese sea weeds, Wakame and Aosa contained 48.1, 31.7 and 29.1 g/100 g, respectively. The amount of total organic acid increased in garlic bread compared to bread without garlic. The best swelling properties were evident in the bread with *K. alvarezii* (0.5%) and garlic. The bread including *K. alvarezii* increased the fermentative power of wild yeast while the addition of garlic increased the degree of swelling of the dough. In the sensory testing, the bread with *K. alvarezii* (0.5%) and garlic was favored for the fineness of its texture and had the most preferred texture. It is possible to develop seaweed bread that is rich in dietary fiber and has excellent flavor by adding a suitable amount of garlic during the fermentation process.

Key words: *Kappaphycus alvarezii*, *Saccharomyces cerevisiae*, organic acids, bread making.

1. Introduction

In 2016, the consumption of seaweeds per capita in Japan was 10.9 g/d [1]. Seaweeds have been eaten since ancient times throughout Japan. However, seaweeds are not eaten as frequently outside of Japan. Seaweeds are not used as much for edible use although many kinds of seaweeds are collected in Sri Lanka. *Kappaphycus alvarezii* is red algae, used as the major commercial source of carrageenan, which is widely utilized as a gelling and stabilizing agent for some food products [2]. It was reported that this seaweed, when used as a food supplement, prevents diet-induced metabolic syndrome in rats [3]. A study was conducted to develop an herbal porridge and a bun that incorporated seaweed using *K. alvarezii* because there has been a tradition of consuming seaweeds as sea vegetables since ancient times in Sri Lanka [4, 5].

Baker's yeast, or *Saccharomyces cerevisiae*, is an essential ingredient in bakery products produced by dough fermentation [6]. The main function of baker's yeast in bread baking is to increase dough volume through the production of gas (CO_2) during fermentation [7]. Previously, it was showed that seven wild yeasts isolated from leaves of apple (10-2), seed of nectarine (9-3) and humus (1-2) had fermentation abilities suitable for bread making [8]. The ethanol and CO_2 production by *S. cerevisiae* isolated from the leaves of apple (10-2) were the highest among the strains. The total acid contents of *S. cerevisiae* isolated the seed of nectarine (9-3).

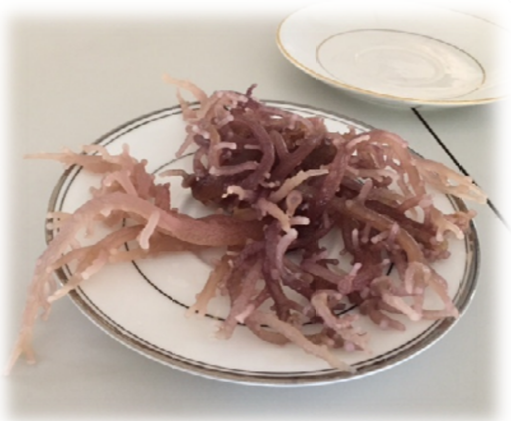
Corresponding author: Noriko Komatsuzaki, Ph.D., research field: food microbiology.

The aim of this study was to develop unique bread having a high nutritive value using *K. alvarezii* and wild yeast. In fermentation process, yeast generates organic acids such as succinic acid and malic acid [8]. These organic acids are associated with the flavor and taste of bread made with yeast [9, 10]. The amount of organic acid contained in the bread was analyzed by using high-performance liquid chromatography (HPLC) analysis.

2. Materials and Methods

2.1 Sample Collection and Pretreatment

K. alvarezii was harvested from the Jaffna area in Sri Lanka, in March 2016. It was dried in solar dryer for 3-5 d without direct sunlight. Drying temperature of the solar dryer was 50-56 °C. Relative humidity (RH) inside the drying chamber was 40%-60%. Weather condition outside the dryer was 30 °C and RH 85%. Drying sample was grounded using a blender prior to further analysis (Fig. 1). The composition analysis of *K. alvarezii* was conducted following the Association of Official Analytical Chemists (AOAC) method at the Japan Chemical Analysis Center in Tokyo, Japan. Dietary fiber was analyzed using AOAC 991.43 [11]. To determine the total dietary fiber (TDF), *K. alvarezii* was digested with three enzymes (protease, amylase, and amyloglucosidase) to break down starch and protein. TDF residue was washed with 95% ethanol and acetone, dried, and weighed. TDF residue values were



(a)

(b)

Fig. 1 The seaweed from Sri Lanka: (a) *K. alvarezii*; (b) drid *K. alvarezii* and powder.

corrected for protein, ash and blank. TDF was the sum of these two amounts.

Commercially sold dried Wakame, *Undaria pinnatifida* (brown algae) and dried Aosa, *Ulva linnaeus* (green algae) were purchased from Riken Food Co., Ltd, and Isekanbutsu Co., Ltd in Japan, in April 2016. Wakame and Aosa were ground using a blender for bread-making.

2.2 Media and Cultivation Conditions

The molasses medium contained 30 g of sugar (calculated as sucrose), 2.25 g of urea and 0.46 g of KH_2PO_4. The yeast extract peptone dextrose (YPD) medium contained 10 g of yeast extract (Difco), 20 g of peptone (Difco) and 20 g of glucose (per liter). Wild yeast 9-3 (*S. cerevisiae*) isolated from nectarine was used [8]. The preparation of compressed yeast was carried out according to the method of Nishida *et al.* [7]. A 50-mL volume of YPD medium was placed in a 100-mL flask and adjusted to pH 5.5-6.0 with 1.0 N HCl prior to autoclaving. Wild yeast was grown in the flask for 24 h at 30 °C. The culture was transferred by means of sterile inoculation loops to 450 mL of the molasses medium in a flask. The flask was incubated at 30 °C for 2 d with 130 rpm shaking rate. Fermentation samples were centrifuged at 3,000 rpm for 10 min. The supernatant was removed, and the collected wild yeasts were used in the bread-making.

2.3 Bread-Making

The bread-making was performed three times. The seaweed and garlic used for bread making are shown in Table 1. In the first experiment, commercial baker's yeast + *K. alvarezii* (1%) was used as control (C1), wild yeast + *K. alvarezii* (1%) as K1, wild yeast + Wakame (1%) as W1, and wild yeast + Aosa (1%) as A1. In the second experiment, fresh garlic was added for the purpose of suppressing the odor of the *K. alvarezii* seaweed, Wild yeast + *K. alvarezii* (0.5%) as K0.5, wild yeast + *K. alvarezii* (1.5%) as K1.5, wild yeast + *K. alvarezii* (0.5%) + garlic (1 g) as K0.5G, and wild yeast + *K. alvarezii* (1.5%) + garlic (1 g) as K1.5G. As a result of the bread making, the ratio of the addition of *K. alvarezii* was determined, and four kinds of bread were made to compare the influence of Aosa and Wakame on the physical characteristics of bread. The ingredients for bread making are shown in Table 2. The ingredients and seaweed were put into a Siroca home bakery SHB-112 (Siroca, Inc.), and were baked automatically. The first fermentation time was 40 min and the second fermentation time was 50 min. After baking, the weight of these breads was measured. The height of these bread was measured in order to check the volume rise of bread.

2.4 Sensory Evaluation

In order to clarify whether the bread containing *K. alvarezii* is acceptable to a Japanese population, the sensory evaluation was conducted using the bread made with Japanese seaweed. The sensory evaluation was performed using a method based on the appearance, scent, sweetness, softness (texture), and

Table 1 Seaweed and garlic used for bread making.

	Seaweed	Fresh garlic
C1*	*Kappaphycus alvarezii* (1%)	
K1	*K. alvarezii* (1%)	
W1	Wakame (1%)	
A1	Aosa (1%)	
K0.5	*K. alvarezii* (0.5%)	
K1.5	*K. alvarezii* (1.5%)	
K0.5G	*K. alvarezii* (0.5%)	1 g
K1.5G	*K. alvarezii* (1.5%)	1 g

* Commercial baker's yeast.

Table 2 Ingredients for bread-making.

	Ratio (%)	Amount of use (g)
Hard flour	100	320
Butter	8	25
Sucrose	4.7	15
Honey	3.2	10
Salt	1.8	6
Skim milk	2.5	8
Yeast (dry yeast)	2.8 (1)	9 (3.2)
Water	68	220

overall preference for four kinds of confectionery breads. The panel included 30 female students of the Department of Human Nutrition at Seitoku University. The analysis method was verified by Spearman's rank correlation coefficient. For the analysis of the results, Friedman's test was conducted. Differences in values between groups were tested by Scheffe's multiple-range test. A p-value of less than 0.05 was considered statistically significant.

2.5 Analysis of Organic Acid Composition

The bread was lyophilized and powdered by a grinder. A 1 g sample was extracted with 10 mL of 70% ethanol at 50 °C for 30 min and centrifuged at 1,500 *g* for 10 min, at 4 °C. The residue was further extracted two more times, and all the extracts were combined and evaporated using rotary vacuum evaporation. The concentrates were diluted to 3 mmol/L of the perchloric solution and filtered through 0.45 µm filters (Millipore, Molford, MA, USA).

An organic acid in the four breads was analyzed by HPLC (Hitachi UV L-7405). The samples were applied to a GL-C610H-S column (300 mm × 7.8 mm; eluent, 3 mmol/L of perchloric solution, reaction liquid, bromothymol blue solution, flow rate, 0.5 mL/min, oven temperature, 60 °C, injection volume, 20 µL, Hitachi Chemical Co, Ltd, Japan), and visible (VIS) absorbance was measured at 440 nm [12].

3. Results and Discussion

3.1 Characterization of Seaweeds

The nutritional composition of *K. alvarezii* was compared with that of Japanese seaweeds (Table 3). Although the calcium level in *K. alvarezii* was lower than that in Japanese seaweeds, the carbohydrate, fiber and ash contents in *K. alvarezii* were higher. *K. alvarezii* is a red alga with particularly high carbohydrate content. Functional foods such as red seaweeds could serve as potential therapeutic options for metabolic syndrome, a constellation of risk factors for type 2 diabetes and cardiovascular disease [13, 14]. There are some evidences that carrageenan of *Kappaphycus* has antioxidant and blood lipid-lowering properties [15, 16]. Wanyonyi *et al.* [3] reported that the major biochemical components of dried *Kappaphycus* are carrageenan, a soluble fiber at 34.6%, and a low overall energy content for whole seaweed. It was assumed that the *K. alvarezii* from Sri Lanka contained a large quantity of carrageenan. Quantification of carrageenan in the *K. alvarezii* used in this study is necessary.

3.2 Characterization of Seaweed Bread

In the first experiment, the degree of swelling in the four kinds of breads was nearly the same. However, the weight of the K1 was lower than that of the C1, W1 and A1. *K. alvarezii* has a peculiar seaweed smell, and thus is not popular among the Japanese population. Therefore, the bread was made with garlic added for a deodorization effect. The effect of different concentrations of *K. alvarezii* on bread is shown in Fig. 2. Figs. 2a and 2b show the breads made from K0.5, K1.5, K0.5G and K1.5G. Fig. 2c shows the breads made from W1 and K1. The heights of these

Table 3 Nutritional composition of seaweeds.

	Energy (kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Ash (g)	Potassium (mg)	Calcium (mg)	Phosphorus (mg)	Vitamin C (mg)
K. alvarezii[1]	121	8.6	3.6	1.5	53.7	48.1	32.6	2,384	181	12	20
Wakame[2]	134	7.2	16.7	1.2	47.4	31.7	27.5	1,800	960	320	20
Aosa[3]	130	16.9	22.1	0.6	41.7	29.1	18.7	3,200	490	160	25

[1] *K. alvarezii* (red algae); [2] *Undaria pinnatifida* (brown algae); [3] *Ulva linnaeus* (green algae).
[2, 3] Source: Standard Tables of Food Composition in Japan 2017.

breads were measured, and the results were as follows: (1) 12.5 cm and 12.3 cm; (2) 13.2 cm and 13.4 cm. The breads with garlic were higher than the breads not containing garlic. It was reported that fermentation was accelerated when yeast was cultured in a medium containing 0.17% to 0.35% garlic [17]. However, the growth of the yeast was inhibited when the amount of garlic added was more than 7%. It is important to provide fluffy bread in a fermentation process [18]. Garlic has several functional compounds such as flavonoids and antioxidants [19]. In this study, it was suggested that some ingredients of garlic increased the fermentation ability of yeast. It was planned to perform fermentation tests at various garlic concentrations to determine the optimum garlic concentration in bread-making.

3.3 Evaluation of Seaweed Bread

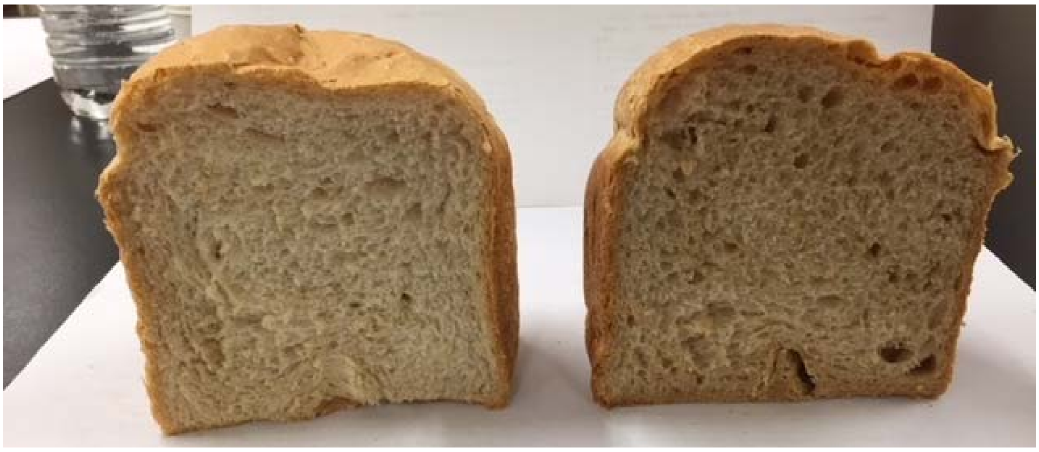
Table 4 shows the results of the sensory testing of the four types of bread. The bread most favored by students from the Department of Human Nutrition at Seitoku University was W1 because of its favorable flavor, preferable taste and overall quality. In regards to the fineness of the texture and the preferred texture, the most favored was K0.5G. The favorable flavor of W1 was significantly different from those of K0.5 and A1 ($p < 0.001$). There was a significant difference between A1 and W1 in the comprehensive evaluation ($p < 0.05$). Seaweeds are daily ingested such as miso soup and salad in Japan [20]. The Japanese students in this study had no experience of eating *K. alvarezii*. It was supposed that the breads made with Wakame were popular because the crushed Wakame grain was large and the original seaweed taste could be directly enjoyed.

Table 3 shows the organic acid content of the breads. Citric acid, malic acid, succinic acid, lactic acid and acetic acid were detected in all of the breads. The highest total organic acid content was in K0.5G. Slight amounts of pyloglutamic acid were contained in K0.5G and K1.5G. Garlic was added to these breads during the bread-making process. The organic acid production of yeast results from the tricarboxylic acid cycle operating continuously, producing succinic acid and malic acid [21]. These organic acids are associated with the flavor and taste of bread made with yeast [9, 22].

In the sensory testing, although there were no significant differences between K0.5 and K0.5G, K0.5G was favored for the fineness of its texture and had the most preferred texture (Table 4). Additionally, the total organic acid level in the bread with *K. alvarezi* and garlic was the highest (Table 5). Some recipes call for broken rice to accelerate fermentation [23]. It is possible that some ingredients in the garlic accelerate the fermentation of yeast during bread-making as well as report of Mizuma [17].

K0.5 K1.5

(a)



K0.5G K1.5G

(b)

W1 A1

(c)

Fig. 2 Effect of different concentration of *K. alvarezii* on bread.

(a) Left side is *K. alvarezii* (0.5%), right side is *K. alvarezii* (1.5%); (b) left side is *K. alvarezii* (0.5%) + garlic (1 g), right side is *K. alvarezii* (1.5%) + garlic (1 g); (c) left side is Wakame (1%), right side is Aosa (1%).

Table 4 Sensory testing of the four types of bread.

Evaluation points	Bread	Average order	Friedman test X^2 value	p-value	Multiple comparison Scheffe
Favorable flavor	K0.5	2.33	16.777	0.001	** (K0.5G/A1 vs W1)
	K0.5G	2.93			
	A1	3.00			
	W1	1.74			
Preferable taste	K0.5	2.63	5.711	0.127	
	K0.5G	2.59			
	A1	2.78			
	W1	2.00			
Fineness of texture	K0.5	2.74	6.333	0.097	
	K0.5G	1.96			
	A1	2.63			
	W1	2.67			
Preferred texture	K0.5	2.93	6.244	0.100	
	K0.5G	2.19			
	A1	2.67			
	W1	2.22			
Overall	K0.5	2.56	8.200	0.042	* (A1 vs W1)
	K0.5G	2.52			
	A1	2.96			
	W1	1.96			

K0.5: *K. alvarezi* (0.5%); K0.5G: *K. alvarezi* (0.5%) + garlic (1 g); A1: Aosa (1%); W1: Wakame (1%). * $p < 0.05$, ** $p < 0.01$.

Table 5 Organic acid in the bread (mg/g).

	K0.5	K1.5	K0.5G	K1.5G	W1	A1	K1
Citric acid	1.11	1.02	1.17	1.23	0.85	1.04	1.19
Malic acid	0.11	0.15	0.04	0.17	0.39	0.18	0.45
Succinic acid	0.29	0.29	0.39	0.35	0.18	0.29	0.21
Lactic acid	0.48	0.34	0.61	0.39	0.05	0.38	0.09
Acetic acid	0.09	0.11	0.15	0.12	0.06	0.10	0.11
Pyroglutamic acid	-	-	0.04	0.03	-	0.01	-
Total	2.08	1.89	2.40	2.29	1.54	2.01	2.05

K0.5: *K. alvarezi* (0.5%), K1.5: *K. alvarezi* (1.5%); K0.5G: *K. alvarezi* (0.5%) + garlic (1 g); K1.5G: *K. alvarezi* (1.5%) + garlic (1 g); W1: Wakame (1%), A1: Aosa (1%), K1: *K. alvarezi* (1%).

4. Conclusions

This study demonstrated the strong potential to make fermented food using Sri Lankan seaweeds and Japanese wild yeasts. It was found that it contained much dietary fiber in *K. alvarezii*. It is possible to develop seaweed bread that is rich in dietary fiber and has excellent flavor by adding a suitable amount of garlic during the fermentation process.

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