Iron-Enriched Bread with Karkade (*Hibiscus sabdariffa*) and Wheat Flour

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ABSTRACT

Karkade (*Hibiscus sabdariffa*) was blended with wheat flour to make bread. When 0.5% Karkade was blended with wheat flour, maximum bread height and specific volume (cm^3/g) were obtained (pH 4.8–5.0); these properties gradually deteriorated with increased Karkade. The pH of the bread crumb decreased with increased Karkade, reaching pH 3.35 when blended with 10% Karkade. The pH of Karkade alone was 2.5, which was adjusted to ≈5.0 by the addition of alkali just before blending with wheat flour and making bread. Control of the Karkade pH resulted in bread height and specific volume recovering to the original optimal levels. In addition, the deep reddish purple color (513 nm) of the bread crumb changed to a brownish color crumb. The Fe content was 0.14 mg of Fe/gram of dry crumb, or 6.22 mg of Fe/60 g of fresh bread when 5% Karkade was blended with wheat flour.

Cereal Chem. 75(5):686-689

Karkade (Roselle or Jamaica-Sorrel) is cultivated in tropical and subtropical regions, and sometimes in warm regions of the United States. Its thickened calyxes and floral bracts are esteemed for the acid flavor they impart to jams, jellies, sauces, and beverages. Karkade (*Hibiscus sabdariffa* L.) is a native annual of the tropics, 1.2–2.0 m in height, with flowers 3–5 cm long that are pale yellow with a deeper yellow or purple center. Dried Karkade powder is made from the center of the deep-red portion. Karkade contains high levels of minerals such as Fe (188 mg/100 g), Mg (442 mg/100 g), Ca (1.28%), and Se (0.09 ppm).

The daily Fe intake among Japanese is 8–10 mg, which is insufficient. Men need 10 mg and women need 12 mg of Fe daily (Hallberg et al 1966, Tchai 1987, Itoh et al 1988,). We were interested in blending Karkade with wheat flour to make healthful, mineral-enriched bread. Bread produced with added Karkade flour would help meet the nutritional requirements for Fe in the Japanese diet. Karkade contains organic acids, so it is possible that when Fe reacts with acids, such as phytic acid, it forms a nutritionally unavailable complex. However, in yeast-leavened white wheat bread, phytic acid is effectively hydrolyzed by indigenous phytase (Harland and Harland 1980).

Acids can affect breadmaking properties when Karkade is blended in wheat flour. Seguchi et al (1997a) reported that gaseous acetic acid improved baking properties such as bread height and specific volume. However, those properties gradually worsened when the acetic acid treatment exceeded an optimal level. Seguchi et al (1997a,b) observed that the water-binding capacity (WBC) and viscosity of the flour-water suspension were increased by the addition of acetic acid, and those changes were related to improvements in breadmaking. We explored effects of adding Karkade flour, with and without pH modification, on breadmaking. To increase the dietary Fe intake of Japanese, we designed an Fe-enriched bread by blending pH controlled Karkade with wheat flour.

MATERIALS AND METHODS

Karkade Powder

Karkade was farm-cultivated (Yahiro Sangyo Co., Ltd., Japan) and the calyx and floral bracts (Fig. 1) were collected. Powder was prepared using a vacuum dehydrator with balance chamber dry system (Yahiro Sangyo). The protein (N × 5.7), lipid, carbohydrate, and ash content of Karkade powder was 13.1, 0.6, 55.9, and 8.4%, respectively; moisture content was 8.4%. Carbohydrate was measured by the phenol-sulfuric acid method (Dubois et al 1956). Lipid and ash were determined according to Approved Methods 30-26 and 08-01, respectively (AACC 1995). Moisture was determined by the method of Tsutsumi and Nagahara (1961).

Wheat Flour

Wheat flours used in these experiments were commercial brands Red Knight (Nitto Flour Milling Co., Ltd., Japan), and Ookan, Takara, and Rikishi (Fuji Flour Milling Co., Ltd., Japan) made from hard red spring wheat flour. The protein content of the wheat flours was 14.1, 14.2, 16.3, and 13.8%; ash content was 0.37, 0.36, 0.55, and 0.33%, respectively. Moisture content was 14.0% mb.

Breadmaking

Breadmaking was performed according to Seguchi et al (1997a), mixing wheat flour (100%), compressed yeast (2.9%), sugar (5.0%), salt (1.0%), and water as determined from the baking absorption estimated from farinograph absorption at 500 BU. The ingredients were put into a computer-controlled National automatic breadmaker (SD-BT 6, Matsushita Electric Ind. Co., Ltd., Japan) until the first proof step after 2 hr, 20 min. Total time of the process was 2 hr, 20 min, which included 15 min for the first mixing, a 50-min rest, 5 min for the second mixing, and a 70-min fermentation. Wheat flour was replaced with Karkade flour. The dough was then divided into 120-g pieces, then rounded, molded, and placed in a baking pan. In this experiment, Approved Method 10-10A (AACC 1995) was slightly modified, with the dough being divided into 120-g pieces. The dough was subjected to a second proof for 22 min at 38°C, and baking was performed at a constant temperature of 210°C (oven model DN-63 Yamato Science Co., Ltd., Japan) for 30 min. After baking, the bread was removed from the pan and left for 1 hr at constant temperature (26°C) and humidity (43%). The bread height (mm), weight (g), and volume (cm^3) were measured. Bread crumb was evaluated visually.

Methods

The pH of Karkade, Karkade-wheat blended flour, and bread crumb were determined by Approved Method 02-52 (AACC 1995). Organic acids, such as oxalic, malic, succinic, acetic, and fumaric acid were determined by HPLC.

Oxalic acid was determined by suspending a 1-g sample in 50 mL of 5% perchloric acid, shaking for 10 min, filling to 100 mL with 5% perchloric acid, and centrifuging at 1,700 × *g* for 20 min. The supernatant solution (10 µL) was subjected to HPLC at 220 nm. A liquid chromatograph (LC-10 AD, Shimadzu) was connected to an 8- × 500-mm column (Shodex Ionpak C-811) and equilibrated with 0.5% phosphate solution at 40°C. Flow speed was 0.7 mL/min.

Malic, fumaric, and acetic acid were determined by suspending 1 g of sample in 5 mL of 5% perchloric acid and 30 mL of water, shaking for 10 min, filling to 50 mL with water, and centrifuging. The supernatant solution (10 µL) was subjected to HPLC at 445 nm. A liquid chromatograph (SPD-6AV, Shimadzu) was connected to an 8- × 500-mm column (Shodex Ionpak C-811) and equilibrated

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Publication no. C-1998-0806-01R.

with 3 m*M* perchloric acid at 40°C. The reaction solution was 15 m*M* di-sodium hydrogen phosphate containing 0.2 m*M* bromothymol blue (BTB). Flow speed was 1.0 mL/min.

Succinic acid was determined by suspending 1 g of sample in 5 mL of 5% perchloric acid and 30 mL of water, shaking for 10 min, filling to 50 mL with water, and centrifuging. The supernatant solution (10 μL) was subjected to HPLC at 445 nm. A liquid chromatograph (SPD-6AV, Shimadzu) was connected to an 8- × 500-mm column (Shodex Ionpak C-811) and equilibrated with 3 m*M* perchloric acid at 60°C. The reaction solution was 15 m*M* di-sodium hydrogen phosphate containing 0.2 m*M* BTB. Flow speed was 1.0 mL/min.

Fe content in Karkade and bread crumb was determined by Approved Method 40-41A (AACC 1995).

Farinograph measurements were made on 300 g of Karkade-wheat blended flour (Approved Method 54-21). The baking absorption was determined from the farinograph absorption at 500 BU.

Karkade aqueous suspension (2%) was subjected to thin-layer chromatography on a silica gel plate. Karkade color was ascended in a solvent of water-saturated phenol solution. Karkade-wheat blend bread crumb was suspended in water, shaken, and centrifuged at 1,700 × *g* for 20 min. The absorbance of the supernatant was measured at 330–700 nm in a UV-visible recording spectrophotometer (UV-250 Shimadzu).

Statistical Analysis

Statistical analysis was conducted using an analysis of variance (ANOVA). The significant calculated mean values were compared using Duncan's multiple range test at $\alpha = 0.05$ level of significance. All experiments at least duplicated.

Fig. 1. Photograph of Karkade (*Hibiscus sabdariffa*) (top) and a view of calyxes and floral bracts (bottom).

RESULTS AND DISCUSSION

Acids

Karkade is known to contain acids. Seguchi et al (1997a,b) reported that acids such as acetic acid are related to breadmaking properties. Therefore, it is possible that acids in Karkade may be detrimental to Karkade-wheat blend breadmaking properties. The 5% Karkade solution had a pH value of 2.5. To determine the amount of acids in Karkade, acids were extracted from Karkade powder into water and analyzed by HPLC. Citric acid, tartaric acid, malic acid, succinic acid, lactic acid, fumaric acid, acetic acid, and oxalic acid were used as standards. The results (Table I) show that the main organic acid in Karkade was oxalic acid (1.15%), followed by malic acid (0.29%), succinic acid (0.08%), fumaric acid (0.06%), and acetic acid (0.06%). Citric acid, tartaric acid, and lactic acid were absent in Karkade.

Farinograph Patterns

Karkade was blended with Red Knight wheat flour at levels of 1 and 5% and subjected to farinograph testing. Figure 2 shows the effects of Karkade on the farinograph pattern of wheat flour. The

TABLE I
Amount (%) of Organic Acid in Karkade[a]

Malic acid	0.29
Succinic acid	0.08
Fumaric acid	0.06
Acetic acid	0.06
Oxalic acid	1.15

[a] Mean values of two experiments.

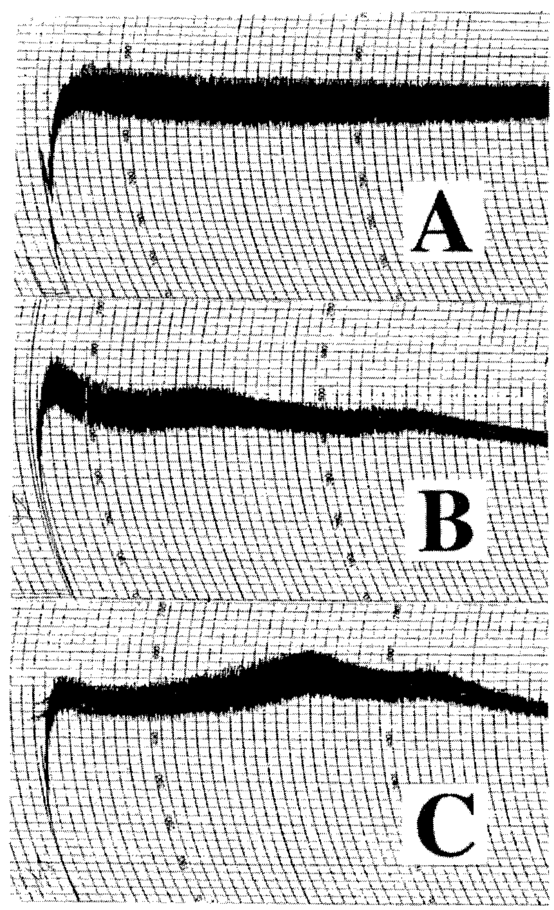

Fig. 2. Farinograms of flour from wheat (**A**), 1% Karkade-wheat blend (**B**), and 5% Karkade-wheat blend (**C**).

stable farinograph pattern of wheat flour alone showed some peaks appearing in the pattern and some interesting mixing behavior in modifying or extending the peak mixing time. However, those peaks disappeared when the pH of the Karkade-wheat blended flour was controlled at pH 5.0 (Fig. 3). These results indicate that the acids in Karkade had some effect on the mixing behavior of the wheat flour dough. Bennett and Ewart (1962) reported that the properties of wheat flour dough are sensitive to changes in pH. Acids react strongly with the proteins in the dough. Tanaka et al (1967) and Seguchi et al (1997a) reported that farinograph dough consistency was decreased by lowering the pH with acetic acid.

Breadmaking Tests

The effects of Karkade on breadmaking properties were examined. The blending percentages of Karkade to Red Knight wheat flour were 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, and 10.0%. The results of the breadmaking are shown in Table II and Fig. 4. Bread height (mm) and specific volume (cm^3/g) were increased by increasing the percentage of Karkade up to 0.5%, after which the breadmaking properties gradually deteriorated. For bread crumb at 0.5 and 0.75% Karkade, the pH values were 5.02 and 4.83, respectively. Seguchi et al (1977a) reported that optimum bread height and specific volume were obtained by gaseous acetic acid treatment at 2.0 mL of acetic acid/kg of flour, with a bread crumb pH of 4.8. This is similar to the pH values we observed in 0.5–0.75% Karkade-wheat blend bread crumb. This indicates that the pH of Karkade-wheat blend bread crumb is related to breadmaking properties. The decrease in breadmaking properties by increased percentage of Karkade is related to the lower pH of the bread crumb. Thus, when breadmaking was performed with 1.0, 2.0, 3.0, and 5.0% Karkade, the pH of the bread dough was adjusted to ≈5.0 for obtaining the optimum breadmaking properties by the addition of alkali (5.0*N* NaOH) just before breadmaking. Table III and Fig. 5 show the effects of pH adjustment on breadmaking properties, with bread properties recovering to optimum levels. It was therefore concluded that even if large amounts of Karkade are blended with wheat flour, good breadmaking properties can be obtained by controlling the pH of the Karkade-wheat blend dough. But optimum results for pH modified or controlled blends occur at pH 5.3 (Table III), which suggests that the increase of the amount of Karkade in the blend would also positively affect the breadmaking properties with the pH control. The effects of Karkade on breadmaking properties with other wheat flours (Ookan, Takara, and Rikishi) were examined (Table IV) with similar results.

Color Analysis

Karkade alone showed a deep reddish purple color that was analyzed by thin-layer chromatography. The color was separated into

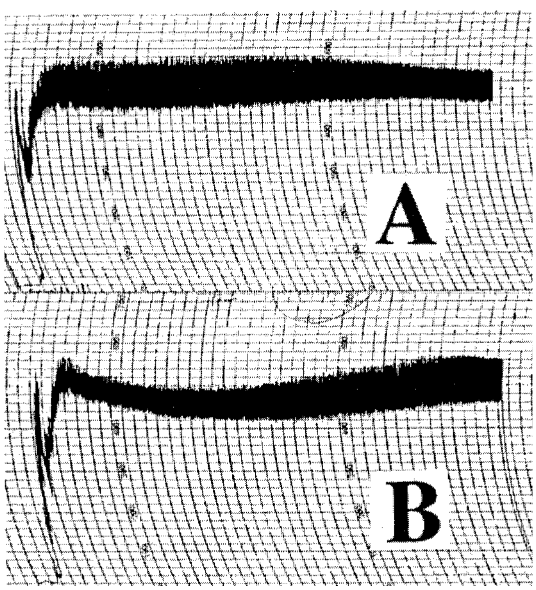

Fig. 3. Farinograms of flour from 1% Karkade-wheat blend (**A**) and 5% Karkade-wheat blend (**B**) controlled at pH 5.0.

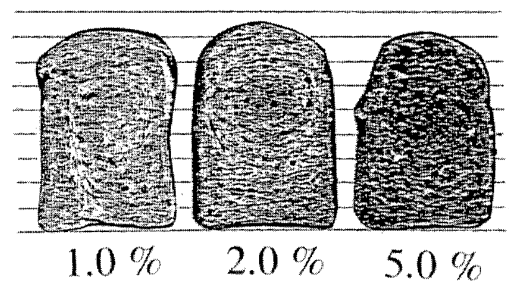

Fig. 5. Appearance of breads made from wheat flour blended with Karkade at various levels and pH controlled.

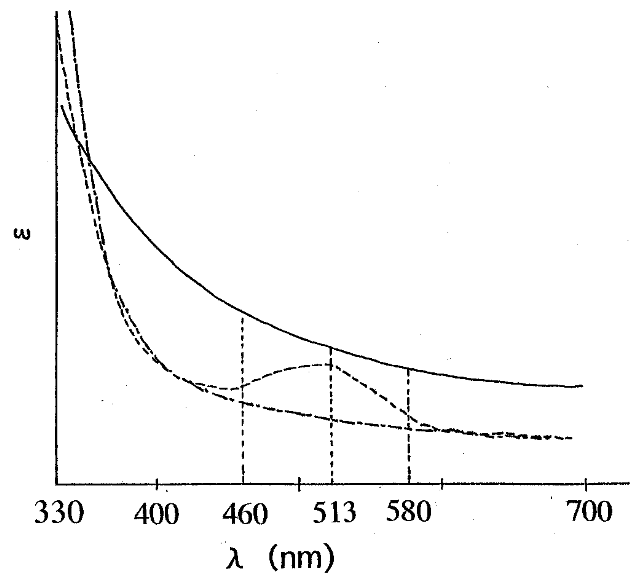

Fig. 6. Absorbance (330–700 nm) of water solubles in bread crumb baked with wheat flour (——), Karkade-wheat blend flour (- - - -), and pH controlled Karkade-wheat blend flour (– · – · –).

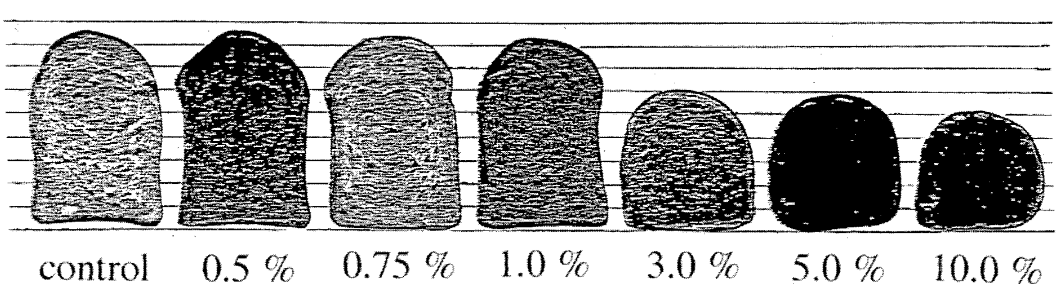

Fig. 4. Appearance of breads made with wheat flour blended with Karkade at various levels.

TABLE II
Effect of Karkade on Breadmaking Properties[a]

Karkade (%)	Bread Height (mm)	Specific Volume (cm^3/g)	Crumb pH
0.00	79.12(3.52)adm	3.68(0.18)a	5.42
0.25	82.63(1.64)bck	3.60(0.12)ab	5.18
0.50	85.64(2.30)ck	3.91(0.10)c	5.02
0.75	80.58(1.65)bdm	3.82(0.18)c	4.83
1.00	77.50(2.17)ade	3.35(0.22)d	4.65
2.00	59.43(2.09)f	2.26(0.06)e	4.36
3.00	58.26(4.93)fg	1.97(0.16)f	3.75
5.00	54.13(1.77)h	1.64(0.05)g	3.70
10.00	49.00(0.52)i	2.51(0.09)h	3.35

[a] Values represent means of four replicates with standard deviations in parentheses. Values followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE III
Effects of pH-Controlled Karkade on Breadmaking Properties[a]

Karkade (%)	Bread Height (mm)	Specific Volume (cm^3/g)	Crumb pH
0.00	79.12(3.52)aj	3.68(0.18)a	5.42
0.50	85.64(2.30)bf	3.91(0.10)b	5.02
1.00	77.50(2.17)ac	3.35(0.22)cj	4.65
1.00[b]	90.95(4.40)d	4.16(0.14)d	5.28
2.00	59.43(2.09)e	2.26(0.06)e	4.36
2.00[b]	86.38(1.78)f	4.10(0.08)df	5.08
3.00	58.26(4.93)eg	1.97(0.16)g	3.75
3.00[b]	96.53(4.01)h	4.76(0.23)h	5.30
5.00	54.13(1.77)i	1.64(0.05)i	3.70
5.00[b]	82.33(2.60)f,j	3.46(0.09)j	5.40

[a] Values represent means of four replicates with standard deviations in parentheses. Values followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] pH controlled.

four components: red, deep red, purple, and deep purple. The color of Karkade-wheat blend bread crumb was determined by the absorbance at 330–700 nm. Figure 6 shows the broad absorbance of 5% Karkade-wheat blend bread crumb at 460–580 nm with an optimum absorbance at 513 nm. However, after pH adjustment to ≈5.0, 5% Karkade-wheat blend bread crumb showed a brownish color, and the absorbance at 460–580 nm disappeared.

Fe Content

Data of Fe content in Karkade-wheat blend bread are shown in Table V. The Fe content in Karkade powder was 1.88 mg/g of dried crumb. The Fe content in both the control bread crumb and the 0.5% Karkade-wheat blend bread crumb was 0.02 mg/g of dried crumb, and 1.0, 3.0, and 5.0% Karkade-wheat blend bread showed 0.05, 0.13, and 0.14 mg/g of dried crumb, respectively. When 5.0% Karkade is blended in white bread, one piece (60 g) of fresh bread contains 6.22 mg of Fe. Thus, two pieces of this bread would meet the daily Fe-intake requirements of the Japanese.

CONCLUSIONS

The edible-plant flower Karkade was blended with wheat flour and made into bread. With its high Fe content, Karkade would be a useful nutrient supplement. However, Karkade contains high levels of organic acids that were deleterious to breadmaking properties when higher percentages of Karkade were blended with wheat flour. But when the pH of the bread dough was adjusted by alkali to ≈5.0, the breadmaking properties showed a marked improvement, resulting in the production of a Karkade-wheat blend bread enriched with Fe.

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[Received January 14, 1998. Accepted May 2, 1998.]

Erratum

Cereal Chemistry, Vol. 75, No. 5, September-October 1998

Iron-Enriched Bread with Karkade (*Hibiscus sabdariffa*) and Wheat Flour

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On page 689, Tables IV and V should have been included with the article.

TABLE IV
Effects of pH-Controlled Karkade on Various Wheat Flours[a]

Wheat Flour	Karkade (%)	Bread Height (mm)	Specific Volume (cm3/g)	Crumb pH
Ookan	0.0	81.45(7.01)a	3.62(0.07)a	5.00
	2.0	58.63(0.56)b	2.43(0.02)b	4.00
	2.0[b]	92.65(2.89)c	4.07(0.09)c	4.92
Takara	0.0	85.98(1.28)a	4.26(0.03)a	5.67
	2.0	76.65(2.93)b	3.53(0.10)b	4.62
	2.0[b]	94.60(3.00)c	4.65(0.11)c	5.51
Rikishi	0.0	76.18(2.57)a	3.17(0.05)a	5.00
	2.0	55.90(3.11)b	2.00(0.05)b	3.95
	2.0[b]	82.68(1.65)c	3.66(0.04)c	5.00

[a] Values represent means of four replicates with standard deviations in parentheses. Values followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] pH controlled.

TABLE V
Fe Content in Karkade-Wheat Blend Bread Crumb[a]

Karkade Blend (%)	Dry Crumb (mg of Fe/g)	Fresh Bread (mg of Fe/60 g)
No addition	0.02	0.89
0.5	0.02	0.89
1.0	0.05	2.22
3.0	0.13	5.78
5.0	0.14	6.22

[a] Mean values of two experiments.