Use of Algae *Dunaliella* as a Protein Supplement in Bread[1]

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ABSTRACT

Commercial preparations of the halotolerant and osmotolerant algae of the genus *Dunaliella* are unicellular green algae that contain large quantities of protein and intracellular glycerol, 3-5% β-carotene, and about 30% salt. The commercially available dried alga (alga 1), the alga from which the β-carotene had been removed (alga 2), and that from which β-carotene, glycerol, and salt had been removed (alga 3), together with their water-soluble and water-insoluble fractions, were evaluated for functional properties in breadmaking. Algae 1, 2, and 3 contained 24.5, 25.6, and 55.3% protein (N × 6.25, 14% mb), respectively. Gas production of the 90:10 wheat flour and algae blends was only 7.4 and 5.1 gasograph units for algae 1 and 2, respectively, because of high salt levels. Gas productions for blends with the salt-free, water insolubles of algae 1 and 2, however, were satisfactory and approached the calculated value of 49.4 gasograph units. The commercial conditions for the removal of β-carotene (alga 2) and glycerol and salt (alga 3) adversely affected the loaf-volume potentials of the water-insoluble fractions of algae 2 and 3. The contribution of the high-protein, water-insoluble fraction of alga 1 to loaf volume was essentially equal to that of the 10% of replaced wheat flour. The chlorophyll of the algae, although probably unobjectionable in very dark specialty breads, would be highly objectionable in light-colored breads.

Commercial cultivation of the unicellular, halotolerant green algae of the genus *Dunaliella* is of interest in Australia (Borowitzka and Borowitzka 1981), the United States (Waaland and Mumford 1981), and Israel (Ben-Amotz and Avron 1981), because large quantities of glycerol and β-carotene can be produced and the dry algal meal contains a relatively high protein content. The algal organisms contain no rigid polysaccharide cell wall, but instead are surrounded by a thin elastic membrane (Gibbs and Duffus 1976). According to Ben-Amotz and Avron (1980), species of *Dunaliella* possess outstanding adaptability and tolerance toward a very wide range of salinities, from sea water to saturated salt solutions. The capability of the cell to thrive in high salt concentrations depends on its unique ability to produce photosynthetically high concentrations of intracellular glycerol and thus maintain an osmotic balance with the extracellular salt concentration. Field-cultivated *Dunaliella* yielded up to 12 g/m^2 per day of glycerol. Certain species of *Dunaliella* can accumulate, in addition to glycerol, β-carotene in concentrations up to 8% on a dry-weight basis. Harvested *Dunaliella* can yield, therefore, three major valuable products: glycerol, β-carotene, and a remaining high-protein dry algal meal. A typical high yield obtained in open pond culture was 11 g of high-protein meal, 8 g of glycerol, and 400 mg of β-carotene/m^2 per day.

The purpose of this study was to examine the commercially available dried algae and the algal meal from which the β-carotene alone or together with glycerol and salt had been removed. The three commercial products and their water-soluble and water-insoluble fractions prepared in our laboratory were evaluated for their gross composition and related functional properties when used as a protein supplement in white pan bread.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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MATERIALS AND METHODS

Materials

CS-80, the control straight-grade flour, was milled from a composite of many hard winter wheat varieties harvested at many

locations throughout the Great Plains in 1980. CS-80 flour contained 12.9% protein (N × 5.7, 14% mb). It had excellent loaf volume potential and a medium-long mixing requirement of 3 3/4 min.

Three dried algae samples were from Koor Foods Ltd., Haifa, Israel. The algae were cultivated in salty ponds. Sample 1 contained β-carotene, glycerol, and about 30% salt; the β-carotene (3–5%) had been removed in sample 2, and both β-carotene and glycerol had been removed in sample 3, which contained less than 1% salt (manufacturer's data), because it was washed before drying. Ground samples 1 and 2 were greenish dark brown and greenish light brown, respectively, and sample 3 was dark green.

Analytical Methods

Protein and moisture contents were determined by AACC approved methods 46-11 and 44-15A, respectively (AACC 1976).

The 10-g bread-making method included mixing to minimum mobility (optimum) at 100 rpm, optimum water, and 50 ppm of ascorbic acid. Additional formula ingredients were 10 g of flour (14% mb), 150 mg of salt, 300 mg of shortening, and 530 ± 20 mg of compressed yeast. Since the bread-making formula did not contain sucrose, 60 instead of 25 mg of barley malt (52 DU/g, 20° C) was added to maintain adequate gas production. Compressed yeast was a 50:50 blend of weekly shipments from Anheuser-Busch, Inc., and Standard Brands, Inc. Straight doughs were fermented 52 min to first punch, 77 (52 + 25) min to second punch, and 90 (77 + 13) min to pan. Controls were proofed 37 min (the time required to proof them to 4.1 cm) at 30° C. The wheat flour and algae blends were proofed 46 min (the time required to proof the water-insoluble fractions of algae 1 and 2 to 4.1 cm). Baking time was 13 min at 215° C. Loaf volumes were determined by dwarf rapeseed displacement about 2 hr after removal from the oven. Loaf volumes that differed by 3.0 cc were statistically significant at $P = 0.05$. Additional related details are given by Shogren and Finney (1984), Magoffin et al (1977), Finney et al (1976), and Finney (1984).

Doughs were prepared in a 10-g mixer. The mixer differed from that of the mixograph described by Finney and Shogren (1972) in that it was nonrecording, contained two instead of three pins in the bowl, and was operated at 100 instead of 88 rpm.

Gas production was determined on the gasograph (Rubenthaler et al 1980) except that doughs (10 g of flour), instead of slurries, were prepared the same as for breadmaking (Finney et al 1982). Gas production in 135 min (sugar formula) and 140 min (no-added sugar) was expressed as gasograph units (GU). Gas production values (average of duplicates) that differed by 1 GU were statistically significant at $P = 0.05$.

Mixograms (10 g of flour) were made as described by Finney and Shogren (1972).

Fractionation of the Algae

Each of the algal samples was fractionated into water-soluble and water-insoluble fractions. Ten grams of each algal material was suspended in 100 ml of water, stirred for 1 hr, and then centrifuged for 15 min at 2,000 rpm. After decanting the supernatant, the centrifugate was resuspended in 75 ml of water, stirred for 1 hr, and centrifuged as above. The supernatant was again decanted and the centrifugate extracted a third time. The combined supernatants were freeze-shelled and lyophilized. The centrifugate was resuspended in 75 ml of water, freeze-shelled, and lyophilized. After lyophilization, each centrifugate was powdery, and the water-soluble fractions of algae 1 and 2 were hygroscopic, sticky, and had a greasy appearance and consistency.

RESULTS AND DISCUSSION

Protein and Ash Contents and Fraction Yields

Protein content of alga 1 was 24.5% (Table I). Removal of β-carotene from alga 1 increased protein content to 25.6% (alga 2). When β-carotene, glycerol, and salt were removed (alga 3), protein content was more than double (55.3%) those of algae 1 and 2. Protein content of alga 3 (55.3%) compares well with the value of 52% reported by Gibbs and Duffus (1976). It should be noted, however, that about six percentage points of the crude protein in *Dunaliella* is nonprotein nitrogen (NPN). The high growth rates in unicellular organisms are generally associated with high values of NPN, because of their relatively high nucleic acid content.

Average ash content of algae 1 and 2 (47.9%) was 25.5 percentage points higher than that of alga 3 (22.4%), because algae 1 and 2 contained the sea-water salts acquired osmotically during their growth and development (Table I). Thus, 25.5% is an estimation of their average sea-water-salt content in addition to that directly associated with the organic composition of the plant organism. The ash contents of the water-soluble fractions of algae 1 and 2 remained high, because the sea-water-salt and glycerol contents of algae 1 and 2 were concentrated by a factor of about 2.25. Most of the protein, organic ash, and other organic components were in the water-insoluble fraction. Of course, the ash contents of the water-insoluble fractions of algae 1, 2, and 3 were relatively low, because those fractions were essentially free of sea-water salt.

The water-soluble fraction of alga 1 was large (60.1%) and its protein content was low (7.5%) because it contained all of the glycerol and salt and relatively little water-soluble protein (Table I). About 82% of the proteins were insoluble in water.

Similar results were obtained for the fractions of alga 2 from which the carotene had been removed industrially. Thus, the decrease in yield of the water-insoluble fraction from 39.9 to 33.4% and the increase in protein content from 50.5 to 60.6% were largely attributable to the removal of carotene.

The water-soluble fraction of alga 3 was small (16.3%) and its protein content relatively high (39.8%), because the β-carotene, glycerol, and salt had been removed previously (industrially). About 88% of the total protein was in the large (83.7%) water-insoluble fraction.

Gas Production

Sugar formula. Gas production of CS-80 control flour, with 6% added sugar in the formula, was 59.2 GU (Table II). When 1 g of each alga replaced 1 g of CS-80 flour, gas productions of flour and

TABLE I
Protein Contents of Three Samples of Algae *Dunaliella* and Yields and Protein Contents of Their Water-Soluble and Water-Insoluble Fractions[a]

Commercial Algae				Fractionated Algae					
Sample				Water-Soluble			Water-Insoluble		
No.	Description	Protein[b] (%)	Ash (%)	Yield (%)	Protein[b] (%)	Ash (%)	Yield (%)	Protein[b] (%)	Ash (%)
1	Nonextracted	24.5	45.4	60.1	7.5	59.7	39.9	50.5	19.9
2	No β-carotene	25.6	50.4	66.6	8.8	61.9	33.4	60.6	25.0
3	No β-carotene, glycerol, or salt	55.3	22.4	16.3	39.8	...	83.7	59.4	21.5

[a] Data are on a 14% mb.
[b] Protein expressed as N × 6.25.

algae 1 and 2 blends were only 8.1 and 4.0 GU, respectively. However, gas production of flour and alga 3 blend was higher than expected (57.7 GU). A 10% reduction in gas production might be expected, other factors being equal, because only 9 instead of 10 g of wheat flour was used. Good gas production of alga 3 was attributable mainly to the absence of salt. To promote rapid cell growth of *Dunaliella*, the sea water was enriched with $NH_4H_2PO_4$. Ammonium ions would be utilized by yeast as a ready source of nitrogen. It is likely that sample 3 contained only trace amounts of ammonium phosphate, because of exhaustive washing.

No-added-sugar formula. Gas production of CS-80 flour, with no added sugar in the formula, was 54.9 GU (Table II). When 1 g of each reconstituted alga replaced 1 g of CS-80 flour, gas productions of flour and algae 1 and 2 blends were only 7.4 and 5.1 GU, respectively, and were in good agreement with the corresponding values when the formula included sugar. There may be an inhibitor of amylase activity in the water-insoluble fraction of alga 3, which could account for the relatively low value (35.8 GU) for 1 g of reconstituted alga 3 compared to that (57.7 GU) for the sugar formula that is not dependent on amylase activity.

Gas productions of the blends of flour and water solubles from 1 g of algae 1 and 2 were 7.5 and 5.0 GU, respectively, and were essentially equal to the corresponding values of the reconstituted algae 1 and 2. However, the blend of flour and water solubles from 1 g of alga 3 had a gas production of 52.0 GU, which was higher than would be expected for 9 g of CS-80 flour.

When the water insolubles from 1 g of each alga replaced 1 g of CS-80 flour, gas productions (41.1–47.1) were less than that (54.9 GU) of the control flour. Values for blends of alga 1 (44.4 GU) and especially for alga 2 (47.1 GU) approached what would be expected for 9 instead of 10 g of CS-80 flour.

The gas-production data were the basis in breadmaking for proofing the wheat flour and algae blends to 46 min (the time required to proof the water-insoluble fractions of algae 1 and 2 to 4.1 cm) instead of 37 min (the time required to proof the control flour to 4.1 cm).

TABLE II
Gas Production Data for Blends of Wheat Flour with Each of Three Samples of Algae *Dunaliella* and with Their Water-Soluble and Water-Insoluble Fractions[a]

Composition of Blends	Gas Production (GU)
Sugar formula	
CS-80 Control (10 g)	59.2
9 g CS-80 plus:	
1 g alga no. 1	8.1
1 g alga no. 2	4.0
1 g alga no. 3	57.7
No added sugar	
CS-80 Control (10 g)	54.9
9 g CS-80 plus:	
Water solubles from 1 g of alga 1	7.5
Water insolubles from 1 g of alga 1	44.4
1 g of reconstituted alga 1	7.4
Water solubles from 1 g of alga 2	5.0
Water insolubles from 1 g of alga 2	47.1
1 g of reconstituted alga 2	5.1
Water solubles from 1 g of alga 3	52.0
Water insolubles from 1 g of alga 3	41.1
1 g of reconstituted alga 3	35.8

[a] Data are on a 14% mb.

Mixograms

When the control flour contained 1.25 or 2.5% of sodium chloride, the widths, heights, and points of minimum mobility (peaks) of the mixograms increased in proportion to the concentration (Fig. 1). The two salt levels corresponded approximately to the salt in 5% (0.5 g) and 10% (1 g), respectively, of alga 1 (as received) and alga 2 (less β-carotene). The increases in width, height, and mix time of the mixograms for blends with 5 and 10% of alga 1 were essentially identical to and those with alga 2 were somewhat greater than those for 1.25% and 2.5% of sodium chloride, respectively. The mixogram data indicate that alga 1 contained 25–30% salt and alga 2 at least 30%. Mixograms of the alga 3 (less β-carotene and glycerol) blends were similar to the control flour with no sodium chloride, because alga 3 also contained less than 1% salt.

Breadmaking—Algae Samples and Their Fractions

Alga 1—Loaf volume. The blend of CS-80 wheat flour and alga 1 had a loaf volume of only 33 cc that approached the volume expected (about 26 cc) if the gluten protein, its gas retention, or yeast activity was zero (Table III, Fig. 2, loaf 1).

The blend containing the water solubles of 1 g of alga 1 (loaf 2) also had a volume of only 30.8 cc, because it contained essentially all of the salt and glycerol in 1 g of alga 1.

The blend containing the water-insoluble fraction of alga 1 (loaf 3), however, had a loaf volume of 83.8 cc for a total weight of only 9.399 g (flour plus alga) instead of 10 g for the control (84.8 cc). A

TABLE III
Bread-making Data for Blends of Wheat Flour with Each of Three Samples of Algae *Dunaliella* and with Their Water-Soluble and Water-Insoluble Fractions[a]

Composition of Blend	Loaf No.[b]	Flour Plus Alga (g)	Loaf Volume (cc)	Crumb Color[c]	Bake	
					Absorption (%)	Mix Time (min)
CS-80 Flour (10 g)	Control	10.00	84.8	C-W	61.4	3¾
9 g CS-80 plus:						
1 g of alga 1	1	10.00	33.0	G-B	58.8	7
Water solubles from 1 g	2	9.601	30.8	C-T	52.8	7¼
Water insolubles from 1 g	3	9.399	83.8	G-B	59.6	3⅝
1 g of alga 2 (no β-carotene)	4	10.00	33.3	Gish-T	58.5	7¼
Water solubles from 1 g	5	9.666	30.5	C-T	53.8	7¾
Water insolubles from 1 g	6	9.334	74.0	Gish-T	60.6	3⅝
1 g of alga 3 (no β-carotene, glycerol, or salt)	7	10.00	62.0	G	58.9	3⅝
Water solubles from 1 g	8	9.163	80.3	C-W	56.9	4
Water insolubles from 1 g	9	9.837	62.0	G	61.2	3⅞

[a] Data are on a 14% mb, no added sucrose in formula.

[b] Loaf 1 is for alga 1 that contained β-carotene, glycerol, and salt; loaves 2 and 3 are for the W-S and W-IS fractions, respectively, of alga 1. Loaf 4 is for alga 2 that contained glycerol and salt but no β-carotene; loaves 5 and 6 are for the W-S and W-IS fractions, respectively, of alga 2. Loaf 7 is for alga 3 that contained no β-carotene, or glycerol, or salt; loaves 8 and 9 are for the W-S and W-IS fractions, respectively, of alga 3. The bread-making formula contained no added sucrose.

[c] C-W = creamy-white; G-B = green-brown; C-T = creamy-tan; Gish-T = greenish-tan; G = green.

volume of 79.7 cc (9.399 g × 84.8 cc)/10 would be expected, assuming that 0.399 g of the insolubles of alga 1 contributed a volume equal to that of 0.399 g of the control flour.

Alga 2—Loaf volume. The blends of CS-80 flour with alga 2 and its fractions had loaf volumes that were essentially equal to the corresponding values for alga 1 (Table III, Fig. 2), except for the water-insoluble fraction (74.0 cc, loaf 6). A volume of 79.2 cc (9.334 g × 84.8 cc)/10 would be expected, assuming that 0.334 g of the insolubles of alga 2 contributed a volume equal to that of 0.334 g of the control flour. Commercial conditions during the removal of β-carotene appear to have significantly reduced the loaf volume potential (gas production) of the water-insoluble fraction of alga 2.

Alga 3—Loaf volume. The blend of CS-80 flour and alga 3 had a loaf volume (62.0 cc) that was materially less than expected when the β-carotene and especially the glycerol and salt had been removed (Table III, Fig. 2, loaf 7). The relatively low volume for the blend of flour and alga 3 was attributable to the loaf volume of 62.0 cc for the water-insoluble fraction (loaf 9). The commercial conditions for the removal of glycerol and salt appear to have greatly reduced the volume potential (gas production) of the water-insoluble fraction of alga 3.

The blend containing the water solubles of alga 3 (loaf 8), however, had a volume of 80.3 cc for only 9.163 g instead of 10 g for the control flour and alga 3 blend. A volume of 77.7 cc (9.163 g × 84.8 cc)/10 would be expected, assuming that 0.163 g of the solubles of alga 3 contributed a volume equal to that of an equal weight of the CS-80 flour. The water-soluble fraction from alga 3 was highly functional (directly or indirectly by interaction) and contributed almost three times as much to loaf volume as a comparable weight of CS-80 wheat flour.

Bake absorption. Average bake absorption of the blend of CS-80 flour and each of the three algae samples was about 2.7 percentage points lower than that of the CS-80 control (Table III).

Average absorption for the blends containing the water solubles of algae 1 and 2 was 5.9 percentage points lower than expected for 9 g of CS-80 plus the weight of a water-soluble fraction, assuming that the absorption of each water-soluble fraction would be the same as that of an equal weight of CS-80 flour. The bake absorption of 9 g of CS-80 flour plus 0.163 g of the water solubles of alga 3 was somewhat higher (56.9%) than expected (56.3%).

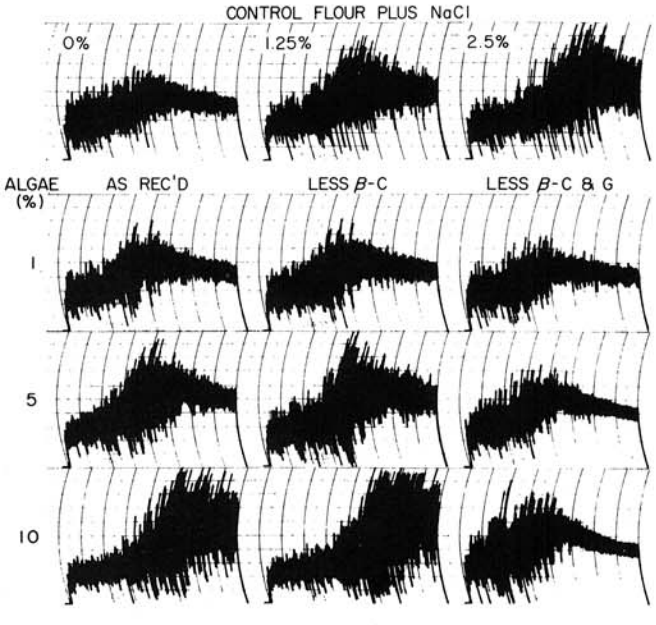

Fig. 1. Mixograms for 99:1, 95:5, and 90:10 blends of wheat flour with each of three commercial samples of algae *Dunaliella*. Alga 1 (as received) contained β-carotene (β-C), glycerol (G), and about 30% salt. Alga 2 (less β-C) contained G and about 30% salt but no β-C. Alga 3 (less β-C and G) contained no β-C, or G, or salt. Concentrations of 1.25 and 2.5% of sodium chloride (top) correspond approximately to the salt in 5 and 10%, respectively, of algae 1 and 2.

Average bake absorption of the blends of CS-80 flour and each of the water-insoluble fractions was 2.0 percentage points more than expected when considering the weights of the water-insoluble fractions.

Bake mix time. For algal samples 1 and 2 and their fractions that contained salt, bake mix time was 7–7 3/4 min (Table III). Bake mix time was only 3 5/8–4 min for blends with alga 3, its fractions, and the water-insoluble fractions of algae 1 and 2, because none contained salt.

Crumb color of bread. Most of the green and brown of the green-brown crumb color of alga 1 was removed when the β-carotene was commercially extracted (alga 2, Table III). Yet when β-carotene, glycerol, and salt were removed (alga 3), the crumb was green.

Most of the brown of the greenish dark brown of ground alga 1 was removed when β-carotene was extracted. Thus, ground alga 2 was greenish light brown or tan. When glycerol and salt were removed in addition to β-carotene (alga 3), the light brown or tan of ground alga 2 was removed and the green color concentrated to the dark green of ground alga 3.

CONCLUSIONS

If algae *Dunaliella* are to be considered as a protein supplement in fermented dough products, it is imperative that the salt be removed. Algae 1 and 2 were useless for bread production, because of their salt content. Their water-insoluble fractions, however, were functional.

The commercial conditions for the removal of β-carotene (alga 2) and glycerol and salt (alga 3) materially reduced the loaf volume potentials of the water-insoluble fractions of algae 2 and 3. After we

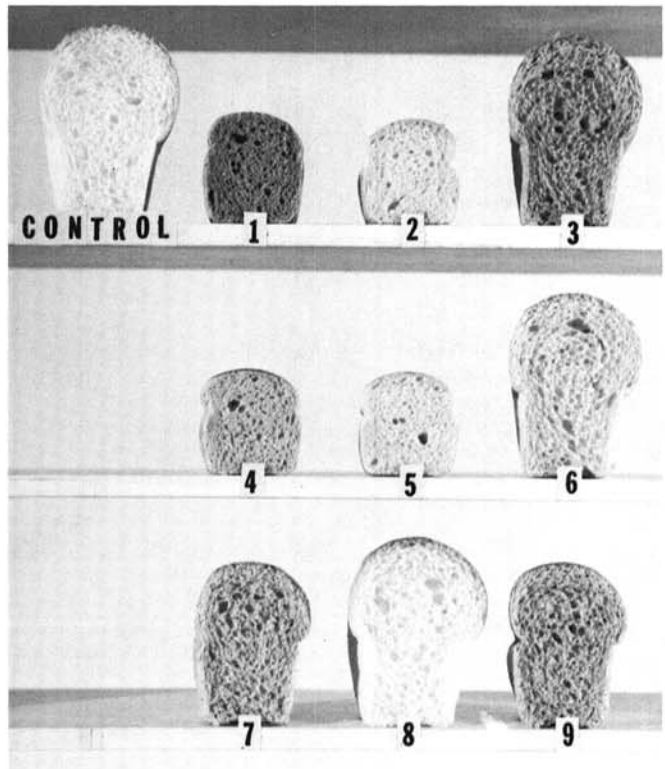

Fig. 2. Cut loaves of bread for blends of wheat flour (9 g) with each of three commercial samples of algae *dunaliella* (1 g) and with their water-soluble (W-S) and water-insoluble (W-IS) fractions from 1 g of each alga. Loaf 1 is for alga 1 that contained β-carotene, glycerol, and salt; loaves 2 and 3 are for the W-S and W-IS fractions, respectively of alga 1. Loaf 4 is for alga 2 that contained glycerol and salt but no β-carotene; loaves 5 and 6 are for the W-S and W-IS fractions, respectively, of alga 2. Loaf 7 is for alga 3 that contained no β-carotene, or glycerol, or salt; loaves 8 and 9 are for the W-S and W-IS fractions, respectively, of alga 3. The bread-making formula contained no-added sucrose.

extracted the water solubles (glycerol, salt, water soluble protein) of alga 1, the contribution of its high-protein, water-insoluble fraction to loaf volume was essentially equal to that of the 1 g (10%) of replaced wheat flour. The water-insoluble fraction of alga 1 has a high loaf-volume potential but also a very high ash content, which limits its use. Furthermore, the high nucleic acid content, usually associated with unicellular organisms, must be reduced before algae can be used as food.

The chlorophyll probably would be unobjectionable in very dark specialty bread. In light-colored breads, the chlorophyll would discolor the crumb and be highly objectionable. Some experience in color removal has been gained from numerous studies on leaf protein concentrates. Admittedly, meaningful removal of the green color is a most challenging task.

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