Preparation of Fish Feed Ingredients: Reduction of Carotenoids in Corn Gluten Meal[†]

H. Park,[‡] R. A. Flores,[*,§] and L. A. Johnson[||]

Department of Agricultural and Biosystems Engineering and Department of Food Science and Human Nutrition, Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011, and Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506

Carotenoids in corn gluten meal (CGM) were eliminated by extraction with ethanol and butanol or by bleaching with soy flour as a lipoxygenase source. Corn gluten meal (50 g at 67% moisture basis) was extracted with 150 mL of ethanol or butanol followed by a second extraction with 100 mL of extractant. Of the carotenoids present in CGM, 87% and 94% were removed after the second extraction with ethanol and butanol, respectively. Total solid loss during extraction was greater with butanol (24%) than with ethanol (10%). Increasing the level of soy flour substituted from 5% to 15% did not improve the degree of bleaching but shortened the time to reach the maximum degree of bleaching at which 51% of carotenoids were bleached. Incorporating 5% soy flour, presoaking of soy flour, and aerating of the reaction medium reduced the reaction time to 30 min. The bleached and heat-dried product contained 62% less carotenoids compared with its unbleached counterpart.

Keywords: *Corn gluten meal; fish feed; carotenoids*

INTRODUCTION

Fish meal has been an essential ingredient in fish feed as a high-quality protein source. With the decline in worldwide supply and increased demand, the shortage of fish meal has intensified in recent past years, becoming a significant driving force to raise the cost of fish diets. Consequently, a search for an alternative protein source to partially or completely replace fish meal has become a focus for global aquaculture research.

Corn gluten meal (CGM), a major coproduct of corn wet milling, has received attention as an alternative source to fish meal along with other oilseed coproducts. Corn gluten meal is a high protein stream, and typical commercial CGM contains a minimum 60% of protein on a 10% moisture basis (Wright, 1987). Dewatered or wet CGM (WCGM) contains 35−40% solids and is further dried to obtain ~90% solids before being marketed. Corn gluten meal is used for animal feed, especially for poultry because of a low fiber content and a high carotenoid content which imparts a desirable yellow color to the skin. Since lysine and tryptophan are limiting amino acids in CGM, other ingredients complementing the amino acid profile of CGM are included in the feed formulation.

Corn gluten meal as a protein source in fish diets has been investigated for salmon, trout, sea-bass, catfish, and tilapia. High protein digestibility (over 90%) was reported with rainbow trout (Cho and Slinger, 1979), yearling channel catfish (Brown et al., 1985), and tilapia (Lorico-Querijero and Chiu, 1989). Wu et al. (1995) reported that the tilapia diets containing 16−18% CGM gave the higher weight gain, higher protein efficiency ratio, and better or equal feed conversion ratio values than an isoprotein commercial fish feed containing fish meal.

The yellow pigmentation of the flesh has long been recognized in certain white-fleshed fishes fed a diet containing CGM, reducing their market value (Lovell, 1984). Corn gluten meal is a rich source of carotenoids, the majority of which are xanthophyll, a term referring to a group of oxycarotenoids. Zeaxanthin and lutein are the predominant xanthophyll in CGM. Commercial CGM contains xanthophyll ranging from 224 to 550 mg/kg on a dry matter basis (Wright, 1987). Lee (1987) investigated the effect of the dietary concentration of carotenoids on the degree of yellowness in catfish flesh. In this study, catfish were fed diets supplemented with various levels of CGM, and the tissue concentrations of xanthophyll were found to be linearly related to the CGM level in the diet. The visual scores for flesh pigmentation correlated well with the pigment concentration in the tissues.

This pigmentation problem limits the utilization of CGM as a fish feed ingredient, yet no efforts have been made to circumvent this problem. The objective of this study was to establish a way to eliminate carotenoids in CGM on a laboratory scale. Solvent extraction has been used to produce depigmented zein from CGM. Hydrophobic solvents such as benzene, ether, and hexane have been widely used as extractants for carotenoids. Our preliminary study showed that WCGM was preferred to dried CGM as a starting material because of its greater dispersibility in the extraction medium and the expense of drying. Due to the high content of water in WCGM, and their low cost, the low aliphatic alcohols, ethanol and butanol, were chosen in this study. Cook et al. (1993) described a multiple extraction process to produce decolored zein using

[†] Journal Paper No. J-17144 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 3326 and supported by the Center for Crops Research and Information Center, the Iowa Agricultural and Home Economics Experiment Station, and the U.S. Department of Agriculture.

[‡] Department of Agricultural and Biosystems Engineering, Iowa State University.

[§] Kansas State University, formerly with the Departments of Agricultural and Biosystems Engineering, and Food Science and Human Nutrition, Iowa State University (e-mail raf@wheat.ksu.edu).

[||] Center for Crops Utilization Research, Iowa State University.

S0021-8561(96)00972-7 CCC: $14.00 © 1997 American Chemical Society

ethanol. However, the residual carotenoid content at each state of extraction was not reported.

Lipoxygenase is widespread in the plant kingdom, and its action in catalyzing lipid oxidation and pigment bleaching was recognized in the 1930s. Loss of yellow lutein pigment caused by lipoxygenase activity in durum wheat was reported by Irvine and co-workers (1950). Soybeans are known to be a rich source of lipoxygenase, and soy flour is widely used as an aid to bleach wheat flour for bread making (Wolf, 1975; Frazier et al., 1973). Bleaching carotenoids in CGM using soy flour was investigated in this study.

MATERIALS AND METHODS

Materials. Wet corn gluten meal was supplied by Grain Processing Corp. (Muscatine, IA) and contained ~33% solids, of which ~78% was protein. The carotenoid content in CGM ranged from 515 to 530 mg/kg CGM on a dry matter basis. Two types of unidentified and variety 5252 Vinton bulk soybeans were dehulled and ground to pass through a 50-mesh U.S. standard sieve to prepare full-fat soy flour. The 5252 Vinton variety and the unidentified mixture that presumably contained different varieties of soybeans were obtained from a local seed company and a local elevator, respectively. Two commercial enzyme-active soy flours were donated by Cargill Co. (Cedar Rapids, IA) and Archer Daniels Midland (Olathe, KS). Soybean meal was purchased from Waterloo Mills Co. (Waterloo, IA) and ground to pass through a 50-mesh U.S. standard sieve. Linoleic acid with ~99% purity was purchased from Sigma Co. (St. Louis, MO).

Methods. *General Methods.* Moisture, nitrogen, crude free lipid, and ash contents were determined by AACC Methods 44-19, 46-13, 30-25, and 08-03, respectively (AACC, 1995). Protein was estimated by using the conversion factor of Nx6.25.

Determination of Carotenoids Content. Carotenoids in CGM were determined using AACC Method 14-50 (1995). Water-saturated butanol (40 mL) was added to 0.500−1.000 g of sample, and the content was allowed to stand for 3 h with occasional gentle shaking. After standing, the contents were shaken again and filtered using Whatman No. 1 paper. The carotenoid content of the extract was determined by using a spectrophotometer (model UV-160 Shimadzu Corp., Kyoto, Japan) at 435.8 nm and $E_{1\%} = 1.6632$ in water-saturated butanol (Johnston et al., 1980).

Extraction of Carotenoids in CGM with Ethanol or Butanol. Wet corn gluten meal (50 g at 67% mb) was extracted with 150 mL of ethanol or butanol by stirring on a magnetic stirrer for 15 min at room temperature (25 ± 1 °C) and then filtering the slurry through Whatman No. 1 filter paper. In one experiment, WCGM was stirred for 60 min, and a small portion of slurry (~2 mL) was collected periodically and centrifuged. The supernatant was syringe filtered and subjected to carotenoid determinations on a spectrophotometer at 438 nm. For the second extraction, the retenate was suspended in 100 mL of ethanol or butanol, stirred for 15 min, and refiltered. The third and fourth extractions were completed by repeating the steps of the second extraction. After completion of extraction, retenate was washed with distilled water (~1.5 L), lyophilized, ground to a fine flour, and subjected to carotenoids and proximate analysis. The filtrate was collected and subjected to solid content measurement. All the experiments were replicated.

Bleaching of Carotenoids in CGM with Soy Flour. The bleaching of carotenoids in CGM was carried out at room temperature (25 ± 1 °C) by using soy flour as a source of lipoxygenase. The total amount of CGM plus soy flour for each set of experiments was kept constant at 20 g on a dry matter basis. Wet corn gluten meal was suspended in distilled water (75 mL), and the pH of the slurry was adjusted by adding dropwise NH_4OH, when necessary. Then soy flour was added to the slurry at 5, 10, and 15% substitution levels for CGM (w/w, db), and the contents were stirred for 30-min intervals up to 120 min on a magnetic stirrer. In one experiment, soy flour was presoaked in 15 mL of distilled water for 60 min. Then the hydrated soy flour and the excess water were added to the slurry, and the contents were stirred for 60 min with or without aeration. Immediately after completion of the reaction time, the pH of the reaction medium was readjusted below 2.5 by adding concentrated HCl to stop enzyme activity (Surrey, 1964). The slurry was centrifuged, and the supernatant was discarded. The resulting sediment was lyophilized or heat-dried at 130 °C for 1 h, ground, and subjected to carotenoid content determination. Along the experiments, CGM treated with heat-treated (enzyme-inactive) soy flour served as a control. The enzyme-inactive soy flour was prepared by heating soy flour in an oven at 130 °C for 1 h. The percentage of carotenoids bleached was calculated on the basis of the carotenoids present in the control. All experiments were replicated twice.

Determination of Lipoxygenase Activity in Soy Flour and Soybean Meal. Lipoxygenase activity in soy flour and soybean meal was determined by a technique described by Surrey (1964) as modified by Lao (1971). Soy flour or soybean meal (0.500 g at 10% mb) was weighed and extracted with sodium phosphate buffer (pH 7.2, 0.05 M) by stirring for 30 min at room temperature (25 ± 1 °C). The slurry was centrifuged at 2000*g* for 15 min. The supernatant was syringe-filtered (pore size 0.22 μm) and used as the enzyme source.

The substrate was prepared as follows: Tween 20, 0.5 mL, was dissolved in 10 mL of borate buffer of pH 9.0, and 0.5 mL of linoleic acid was added dropwise. The contents were thoroughly mixed to disperse the linoleic acid into a fine emulsion. Then 1.3 mL of 1 N NaOH was added, and the mixture was once again agitated until a clear transparent solution was obtained. To this solution was added 90 mL of borate buffer, and the final volume made up to 200 mL with water. Finally, the solution was adjusted to pH 7 with concentrated HCl.

The reaction was carried out at room temperature (25 ± 1 °C) in a mixture of enzyme (1 mL) and substrate (9 mL), with air continuously passed through the reaction mixture. Periodically (2, 4, and 6 min) 0.5-mL samples were transferred into each of several centrifuge tubes containing 2 mL of absolute ethanol. To each tube was later added 7.5 mL of 60% ethanol to make a total volume of 10 mL. The tubes were centrifuged, if necessary. Absorbance of the clear alcoholic solutions were read at 234 nm against a blank on a spectrophotometer. The blank solution was prepared using the same volumes of substrate and enzyme as those used in the test samples. Enzyme was added to 2 mL of absolute alcohol. After mixing, the tube was allowed to stand for a few minutes. Sixty percent alcohol was then added, followed by substrate.

A unit of lipoxygenase was defined as the activity that produced an optical density of 1 at 234 nm in 1 min in a total volume of 10 mL of 60% ethanol solution. To represent the rates of linoleate oxidation per min, slopes of the least-squares lines were calculated from the linear portions of each activity curve.

Statistical Analysis. Statistical analyses were performed by using the Statistical Analysis System (SAS, 1990). Means were compared by the least significant difference (LSD) test at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Extracting Carotenoids with Ethanol and Butanol. Carotenoids in CGM were rapidly extracted into ethanol, and there was no further increase in absorbance after 5 min (Figure 1). In contrast, butanol extracts gradually increased in absorbance up to 1 h (Figure 1). This can be explained by the greater dispersibility of CGM observed in ethanol than in butanol. The limited dispersibility of CGM in butanol might restrict the surface area of CGM exposed to the extraction medium, extending the extraction time. This can be improved by vigorous agitation of the CGM suspension to separate and disperse the particles.

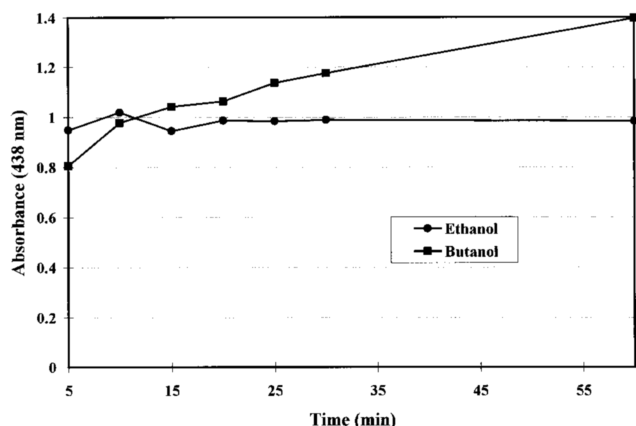

Figure 1. Extraction patterns of carotenoids in CGM. CGM (50 g at 67% mb) was extracted with 150 mL of ethanol or butanol at room temperature, a small portion of the slurry was taken periodically and centrifuged, and the absorbance of the supernatant was read on a spectrophotometer. LSD was 5.6% at the $\alpha = 0.05$ level.

Table 1. Solids Loss during Extraction and the Chemical Composition of the Extracted CGM

extraction[a]	solids loss[b] (%)	protein[b] (%)	lipids[b] (%)	ash[b] (%)
control[c]		75.5	5.4	3.9
ethanol				
first extrctn	8.2	75.5	4.7	0.8
second extrctn	2.4	80.6	1.1	1
third extrctn	0	81	0.4	1.2
fourth extrctn	0	80.6	0.2	0.9
butanol				
first extrctn	23.6	80.7	1.2	1.3
second extrctn	0.4	78.9	0.3	1.4
LSD ($\alpha = 0.05$)	0.8	3.1	0.5	0.6

[a] CGM (50 g at 67% mb) was extracted with 150 mL of ethanol or butanol at room temperature for 15 min. The second, third, and fourth extraction stages were completed under the same conditions with the exception of 100 mL of extractant being used. [b] Values are expressed on a dry basis. [c] CGM without extraction.

Total solid loss after the second extraction was greater with butanol (24%) than with ethanol (11%), and the majority of loss occurred during the first extraction stage (Table 1). On the basis of solid loss and the chemical composition of CGM after extraction, the loss of individual components in CGM was estimated. The majority of solid losses during first extraction stemmed from the protein fraction of CGM: ~9% and 18% of the protein was lost with ethanol and butanol, respectively. It has long been known that zein, prolamine fraction of corn, is extractable with aqueous alcohol. Manley and Evans (1943) investigated the solubility characteristic of zein in various alcohol/water binary solvent systems. They found that zein was extractable in ethanol/water and butanol/water medium of which ratio ranged from 50/50 (w/w) to 85/15 (w/w) and from 70/30 (w/w) to 80/20 (w/w), respectively. Considering the moisture present in wet CGM, the titer of the first extraction medium is estimated to be ~80/20 (w/w), where coextraction of zein can occur. There was no significant protein loss after first extraction, which is attributable to the high titer of the extraction medium. With ethanol, 20% of the lipids were removed after the first extraction. An additional 62% were removed in the second extraction. In contrast, the majority of lipid (83%) loss occurred during the first extraction with butanol. Over 70% of the ash was removed after the first extraction with both ethanol and butanol. Despite the protein loss during extraction, the protein content in the depigmented CGM

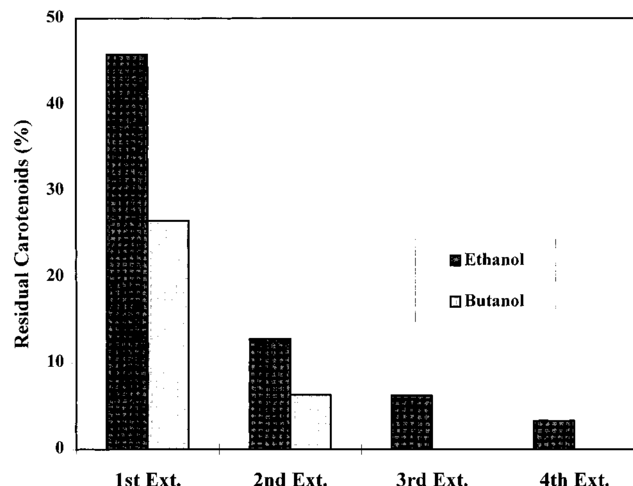

Figure 2. Residual carotenoids in the CGM extracted with ethanol and butanol. CGM (50 g at 67% mb) was extracted with 150 mL of ethanol or butanol at room temperature for 15 min. The second, third, and fourth extraction stages were completed under the same conditions with the exception of 100 mL of extractant being used.

remained the same or increased 3−5% corresponding to the loss of other components (Table 1).

With ethanol, 54% of the carotenoids present in CGM were removed after the first extraction stage and 97% after the fourth extraction stage (Figure 2). Butanol was more effective in removing pigments than ethanol; 94% of the carotenoids were removed after the second extraction (Figure 2). Butanol odor was still discernible in butanol-extracted CGM after water washing.

Bleaching of Carotenoids with Soy flour. Soybean lipoxygenase consists of two types of isozymes, which differ in optimum pH, substrate, and carotenoid bleaching activity. The type I lipoxygenases have an optimum pH of 9 and possess a low carotenoid bleaching activity, whereas the type II lipoxygenases have an optimum pH of 6.5 and have carotenoids bleaching activity (Grosch, 1976). The effect of the reaction medium pH on the degree of bleaching was examined at various pHs with a soy flour substitution level of 5%. At the end of 60 min of reaction, the pH was slightly shifted toward the acidic side by 0.2 to 0.3 units. At pH 6.5, 48% of the carotenoids were bleached, whereas at pH 9.0, 32% were bleached (Figure 3). There was no significant difference ($p < 0.05$) between pH 6.5 and pH 7, giving a rather wide window for the optimum pH range. Without any pH adjustment, the reaction medium gave a pH of 4.3 at which the bleaching was abruptly diminished (Figure 3). This result agreed with that of Surrey (1964) in which the activity of crude lipoxygenase from soybean meal declined sharply toward the acidic side.

At all substitution levels, carotenoids were bleached rather rapidly up to 60 min and then leveled off (Figure 4). The loss of carotenoid increased as the substitution level increased at the early stages of the reaction. However, the differences among the substitution levels gradually decreased after 30 min and eventually disappeared (Figure 4). This observation agreed with that of Matsuo et al. (1970) in which doubling the level of wheat lipoxygenase did not affect the extent of pigment destruction in semolina during spaghetti processing. They also found that, in the presence of added linoleic acid, increasing the level of lipoxygenase increased pigment loss, and oxygen by itself had little effect on pigment loss. This might explain why adding extra soy

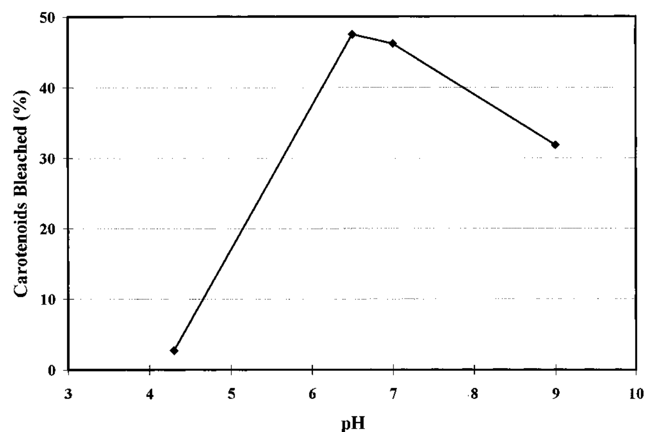

Figure 3. Effects of reaction pH on the degree of bleaching. Soy flour was substituted for CGM at the 5% level, and the reaction was carried out at room temperature for 60 min. Carotenoids bleached (%) was calculated on the basis of the control containing enzyme-inactive soy flour. Carotenoid content in the controls was not affected by pHs tested. LSD was 2.7% at the α = 0.05 level.

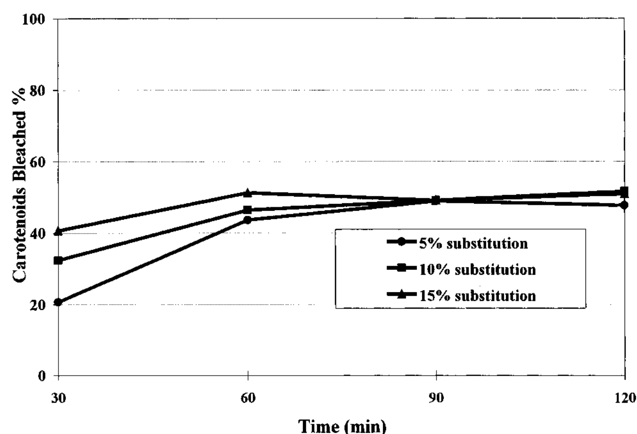

Figure 4. Effects of soy flour substitution levels and reaction time on the degree of bleaching. Reaction was carried out in the reaction medium of pH 7 at room temperature. Carotenoids bleached (%) was calculated on the basis of the control containing enzyme-inactive soy flour. LSD was 6.1% at the α = 0.05 level.

flour failed to enhance the destruction of carotenoids. Sumner and Sumner (1940) reported that the mechanism of carotenoid bleaching by lipoxygenase is a coupling process, coupled with oxidation of linoleic acid, especially free linoleic acid, in the presence of oxygen. Oxidation of carotenoids in CGM might be limited by the initial level of free linoleic acid present in CGM.

The effect of enzyme activity on bleaching was investigated with various soy flours and soybean meal at the 5% substitution level. Enzyme activity of soy flour ranged from 4.56 to 9.96, yet there were only marginal differences (1−3%) on the degree of bleaching among the soy flours (Table 2). Again, this observation indicated that lipoxygenase activity, as long as it reached a certain level, was not a determining factor on bleaching. Soybean meal showed little enzyme activity and as a consequence little bleaching (Table 2). Soybean lipoxygenase is reported to be heat-liable (Rice et al., 1981), and heat treatment in the processing of soybean meal might have deactivated the lipoxygenases.

As substitution levels of soy flour decreased, longer reaction time was required to reach the maximum degree of bleaching (Figure 4). To increase the bleaching rate, two treatments, presoaking soy flour and aeration through the reaction medium, were tested at the 5% substitution level over 60 min of reaction time. Aeration alone significantly improved the degree of bleaching, whereas presoaking showed only marginal improvement. (Table 3). The combination of presoaking and aeration showed synergistic effects, bleaching 51% of carotenoids within 60 min (Table 3). After bleaching, the wet contents were dried in an oven at 130 °C for 1 h to reduce the moisture content to 8−10%. During the heat-drying, an additional 18% of the carotenoids were bleached, giving a total of 69% of the carotenoids destroyed. This bleached and heat-dried product contained 165 mg/kg (db) of carotenoids. Corn gluten meal taken from a corn wet milling stream contained 463 mg/kg (db) of carotenoids after being heat-dried under the same condition. Corrected for the 5% substitution level of soy flour, the final product contained 62% less carotenoids than its unbleached counterpart. In addition to providing a bleaching effect, soy flour improves the nutritive values of finished products. The amino acid pattern of soy flour complements that of CGM, soy flour protein being deficient in methionine and cystine but rich in lysine and tryptophan.

Table 2. Effects of Lipoxygenase Activity on the Degree of Bleaching[a]

sample	lipoxygenase activity[b] (units/mg of sample)	carotenoids bleached[c] (%)
soy flour		
unidentified	7.85	46.2
vinton	4.56	46.9
commercial 1	9.42	47.1
commercial 2	9.96	44.3
soybean meal	0.83	3.1
LSD (α = 0.05)		1.9

[a] Soy flour was substituted for CGM at the 5% level and the reaction was carried out in the reaction medium of pH 7 at room temperature for 60 min. [b] One unit of lipoxygenase was defined as that activity which produced an optical density of 1, at 234 nm in 1 min, in a total volume of 10 mL of 60% ethanol. The sample weight was at 10% mb. [c] Calculated on the basis of carotenoids content in a control containing enzyme-inactive soy flour.

Table 3. Effects of Presoaking Soy Flour and Aerating of the Reaction Medium on the Degree of Bleaching[a]

treatment	reaction time (min) 30	60
control[b]	32.1[c]	43.7
presoaking[d]	33.8	45.3
aeration	39.7	47.5
presoaking + aeration	46.2	51.5
LSD (α = 0.05)	3.0	3.0

[a] Soy flour was substituted for CGM at the 5% level, and the reaction was carried out in the reaction medium of pH 7 at room temperature. [b] Control received neither presoaking nor aeration. [c] All values were expressed in carotenoids bleached (%), based on the carotenoid content in a blank containing enzyme-inactive soy flour. [d] Soy flour was presoaked in water (15 mL) for 60 min, and the hydrated soy flour and the excess water were added to the slurry.

CONCLUSIONS

The solvent extraction was more effective in removing carotenoid in CGM compared to the bleaching method using soy flour. However, a substantial amount of solids was lost during the extraction process, to a greater extent with butanol than with ethanol. After the second extraction, 87% and 94% of carotenoid were removed with ethanol and butanol, respectively. The bleached and heat-dried CGM contained 62% less carotenoids

compared with its unbleached counterpart. Considering the processing costs and solid loss, the bleaching process might be more practical than the extraction process for use in fish feeds.

ABBREVIATIONS USED

CGM, corn gluten meal; WCGM, wet corn gluten meal; mb, moisture basis; db, dry basis; w/w, weight by weight.

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Received for review December 20, 1996. Accepted March 11, 1997.[⊗] We express our appreciation to Grain Processing Co. (Muscatine, IA) for providing corn gluten meal and the Midwest Feeds Consortium for the financial support.

JF960972J

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.