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EXTRUDED CORN GRITS-QUINOA BLENDS: I. PROXIMATE COMPOSITION, NUTRITIONAL PROPERTIES AND SENSORY EVALUATION

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ABSTRACT

Quinoa, a high protein, high lysine grain, was blended with corn grits at levels of 10, 20, and 30%. These blends were extruded under various conditions. Proximate composition, nitrogen solubility and in-vitro *digestibility of the raw materials and products were determined. Sensory analysis was performed to judge the acceptability of the products. Quinoa addition produced unprocessed and extruded products which were higher in protein, fiber, ash and some amino acids than 100% corn grit products. The products containing quinoa had a greater nitrogen solubility and a somewhat lower* in-vitro *digestibility than the products containing only corn grits. Sensory evaluation of the extruded blends indicated that the products were acceptable.*

INTRODUCTION

Quinoa is a grain crop which originated from the high Andes of Ecuador, Peru, Bolivia and Chile. It is normally grown at altitudes of 2000 to 4000 meters above sea level, where it is not possible to grow maize (Carmen 1984). The grain is frost and drought resistant and is able to grow on poor soils. In Colorado, quinoa has been grown at elevations ranging from 2000 to 3500 meters (Johnson and Croissant 1985).

Average protein of the grain is generally above that of wheat, maize and rice (Wilson 1985). Quinoa also has a better amino acid profile, since it is higher in lysine than either wheat or corn (VanEtten *et al.* 1963). The protein quality of cooked quinoa has been rated better than that of casein on the basis of nitrogen efficiency for growth (NEG) and similar to casein on the basis of protein efficiency ratio (PER) (Mahoney *et al.* 1975). Though much has been reported on

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the protein and amino acid content and quality of the quinoa grain itself (VanEtten *et al.* 1963; Quiros-Perez and Elvehjem 1957; DeBruin 1964; Mahoney *et al.* 1975; White *et al.* 1955), no information is available on the protein functionality, such as nitrogen solubility or *in-vitro* digestibility of this grain.

Bitter saponins are located in the pericarp of the quinoa grain (Simmonds 1965). These compounds make the grain unpalatable, so they must be removed before consumption (Risi and Galwey 1984). This can be done by washing and/or friction. Saponin removal, on a household scale by washing or friction, is relatively easy. On an industrial scale it is quite difficult due to the large amount of energy required (Risi and Galwey 1984). Saponin removal by abrasive dehulling has recently been studied (Reichert *et al.* 1986). Abrasive dehulling produced quinoa which was low in saponin content. After saponin removal, quinoa may be ground into a flour that is then made into a coarse bread called 'kispina,' biscuits, porridge, and hot or fermented beverages (Simmonds 1965; Weber 1978). Quinoa flour has also been successfully used as a partial substitute for wheat flour in breads, cookies and pasta (Weber 1978). Some of these foods are available commercially in natural food stores. Finding other food applications for quinoa would make it more attractive for farmers in the United States to produce it.

One such application could include the production of a snack-type product through extrusion processing. This process has become increasingly important in food processing, because it precooks, shapes, texturizes and restructures food constituents. Some extruded foods, which are now commonly consumed, include croutons, breakfast cereals and snack foods.

Corn grits, which is relatively low in protein content, is frequently used to produce commercially available snack products. By extruding blends of corn grits and quinoa, a product with improved protein content and amino acid quality could possibly be developed.

There is little information available on the effects of extrusion on the nutritional and functional properties of quinoa. The purpose of this study was to extrude quinoa in combination with corn grits and to determine various nutritional and functional properties. Extrusion of 100% quinoa is not economically feasible because of the high cost of the grain.

MATERIALS AND METHODS

Sample Identification

Quinoa was obtained from the Colorado State University Agronomy Department. The cultivar used was Colorado D407, a Chilean landrace. The grain was grown during the 1987 growing season on experimental plots located in the San Luis Valley of Colorado. Degerminated corn grits were obtained from Illinois Cereal Mills, Paris, IL.

Proximate Composition of Materials

Proximate analysis was performed on unprocessed quinoa, corn grits and selected extruded samples, which contained varying amounts of quinoa and were extruded at various temperatures and screw speeds. All analyses were done in duplicate.

Standard AACC (1969) methods were used for determinations of moisture, ether extract, ash and crude nitrogen. Total dietary fiber was determined using the FiberZym kit prepared by Novo BioLabs (Wilton, CT).

Saponin Removal

To remove saponins, quinoa was placed in a sieve, rinsed with warm water and then covered with cold water and allowed to soak overnight at 4–6°C. After rinsing, the quinoa was checked for remaining saponins by placing 10g of the grain into a screw top sample tube with warm water. The tube was then shaken vigorously for 30 s (Risi and Galwey 1984). If foaming occurred, the grain was rinsed again in warm water and soaked overnight at 4–6°C. When no saponins remained (no foaming occurred), the grain was placed in thin layers in aluminum baking pans and immediately dried in conventional ovens (approximately 68°C). Drying to 12% moisture required about 6 h.

Extrusion

The following blends of whole kernels of quinoa and corn grits were made: 100% corn grits; 90% corn grits: 10% quinoa; 80% corn grits: 20% quinoa; 70% corn grits: 30% quinoa. Two 2500 g blends of each combination were prepared in a Hobart mixer. One blend was adjusted to 15% and the other to 25% total moisture by adding the appropriate amount of water during blending in a Hobart A-200 mixer using a paddle. The mixtures were allowed to equilibrate overnight at room temperature in air-tight plastic bags.

A Brabender Plasticorder Extruder model PL-V500 with a 19.05 mm barrel diameter, a 20:1 length to diameter ratio and eight 0.79 × 3.18 mm longitudinal grooves was used. All blends were extruded using both a 1:1 and then a 3:1 compression ratio screw. The barrel was equipped with two electrically heated, compressed air cooled collars which were controlled by thermostats. Each blend was extruded at two sets of barrel temperatures: 80° and 100°C at the feed section and 100° or 150°C at the compression section, respectively. The extruder was equipped with a variable speed drive, allowing all samples to be run at three screw speeds: 100, 150 and 200 rpm. A $\frac{1}{8}$ in. diameter die was used for all trials. All products were air-dried and stored in glass jars at refrigeration temperature.

Amino Acid Determinations

The following samples were analyzed for amino acid content: degerminated corn grits, quinoa ground in a micromill (The Lab Apparatus, Cleveland, Ohio), a 70% corn grits: 30% quinoa extruded blend and that same blend fried (190°C for 25 s).

The method used was described by Lorenz *et al.* (1983). Samples containing approximately 10–15 mg of protein were weighed into 16 × 150 mm glass culture tubes. Ten mL of 3N p-toluenesulfonic acid containing norleucine as an internal standard was added, and the tubes were capped with polypropylene caps. The tubes were placed in an enclosed boiling water bath for 31 h of hydrolysis. Each sample was cooled, transferred to a 25 mL volumetric flask, neutralized with NaOH, diluted with pH 2.2 buffer, and frozen until analyzed.

Each sample was thawed and ultrafiltered through a 0.2 nm cellulose acetate membrane immediately before application to the ion-exchange column. Samples (185 μL) were applied to the long and short columns of a Beckman 120C automatic amino acid analyzer and eluted according to the Beckman 2 h hydrolyzate procedure (Lorenz *et al.* 1983).

Recoveries of amino acids were corrected for mechanical losses with norleucine and calculated to 100% on a protein basis. The Beckman automatic analyzer reproductibility is ± 3.0%.

In-Vitro Digestibility. *In-vitro* digestibility was determined using the method described by Maga *et al.* (1973). Quinoa and extruded blends were ground to pass 200 mesh (Cyclone sample mill, Udy Corp.). Based on their protein content, samples were weighed to contain 2 mg of nitrogen per mL. Samples were then suspended in 40 mL of distilled water and pH was adjusted to pH 7. The samples were permitted to rehydrate for 1 h at 5°C. The rehydrated samples were incubated in a 37°C water bath. Three mL of lyophilized trypsin (twice-crystallized) at a concentration of 40 mg/mL were added. Changes in pH were measured at 1 min intervals.

Nitrogen Solubility. Samples (0.2 g) were weighed into centrifuge tubes and distilled water was added to a volume of 9 mL. The pH of all samples was adjusted in a range of 2 to 10 using 1N, 0.1N and 0.01N HCl and NaOH. Samples are placed in a 25°C water bath and shaken for 1 h. Volume was made to 10 mL and the samples were centrifuged at 6000 rpm for 15 min. Samples were then filtered through Whatman No. 2 filter paper. Nitrogen content of the supernatant was determined using the method described by Mitchell (1972).

Sensory Evaluation

Two samples with optimum expansion ratios were chosen for sensory evaluation. These samples were a 70% corn grit: 30% quinoa blend and an 80% corn grits: 20% quinoa blend, both with a preextrusion moisture content of 15%

and extruded with a 3:1 compression ratio and a barrel temperature of 100–150°C at the feed and compression section, respectively. The 80% corn grits: 20% quinoa blend was extruded at a screw speed of 150 rpm and the 70% corn grits: 30% quinoa blend at 100 rpm.

Two sensory panels were used for evaluation of the quinoa extrudates. One trained panel consisted of ten members (six females, four males), who knew the samples were made with quinoa and knew some of the benefits of the grain. The second panel, which was untrained, consisted of eleven uniformed members (five females, six males). Both panels consisted of college-aged members.

The panel with knowledge of the products also evaluated a 80:20 blend with cheese. The panel with no knowledge of the products also evaluated a fried and an unfried 80:20 blend in the chip shape. Chips were made by flattening the extrudate with a set of rollers as it emerged from the die of the extruder and cutting into 1 in. pieces. Chips were fried at 190°C for 25 s. The products were evaluated for appearance, texture and flavor using a seven point hedonic scale. A score of 1 indicated "like extremely" and a score of 7 "dislike extremely."

All products were coded with a number and the evaluation was conducted in a quiet and odor-free room. Water was provided for rinsing in-between samples.

Statistics

Analysis of variance was done using the SAS program.

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of corn grits, quinoa and selected extruded products is shown in Table 1. Quinoa was higher in ash, ether extract, crude protein and total dietary fiber content than degerminated corn grits. These compositional differences were reflected in extruded blends of corn grits and quinoa. Thus, increasing quinoa in the blend caused an increase in the ash, ether extract, crude protein and fiber content of the extruded products.

The composition of quinoa would vary with variety and growing conditions, as do other grain crops. The quinoa used in this study was higher in crude protein, crude fat, ash and fiber than common cereals, such as wheat, rice and corn. This agrees with previous literature reports (White *et al.* 1955; DeBruin 1964).

Amino Acid Composition

The amino acid compositions of quinoa, degerminated corn grits, a 30% quinoa blend and that same blend fried are shown in Table 2. Quinoa had a much higher

TABLE 1.
PROXIMATE COMPOSITION OF RAW MATERIALS AND SELECTED EXTRUDED AND FRIED PRODUCTS (% DRY BASIS)

Material[1]	Ash	Ether Extract	Nitrogen	Crude Protein	TDF[2]
Unprocessed Corn Grits	0.41	1.28	1.35	8.43	5.04
Unprocessed Quinoa	2.92	5.82	2.44	15.25	9.80
Extruded 100% Corn Grits	0.46	0.24	1.36	8.50	4.70
Extruded 90% CG/10% Q	0.84	0.49	1.43	8.94	n.d.
Extruded 80% CG/20% Q	0.95	0.80	1.59	9.94	n.d.
Extruded 70% CG/30% Q	1.23	1.10	1.66	10.38	6.73
Extruded 80% CG/20% Q (Fried)	n.d.	18.00	1.37	8.56	n.d.
Extruded 70% CG/30% Q (Fried)	n.d.	22.56	1.21	7.56	n.d.

Protein = % Nitrogen x 6.25
[1]CG = Corn Grits
Q = Quinoa
[2]TDF = Total Dietary Fiber
n.d. = Not Determined

Blends were extruded at 25% moisture, a 3:1 compression ratio screw and 100°C and 150°C at the feed and compression sections, respectively.

lysine content than did corn grits. The extruded blend, containing 70% corn grits and 30% quinoa, had 50% more lysine than the corn grits alone. Quinoa was higher in threonine, valine and isoleucine than the corn grits. Histidine was comparable in both raw materials. Quinoa was somewhat lower in methionine and phenylalanine than the corn grits. The corn grits were much higher in leucine than quinoa. Quinoa is deficient in cystine (Risi and Galwey 1984).

Usually, there is a loss of lysine with heat treatment. Under the extrusion conditions used for this particular blend, there was no such loss. The highest temperature recorded for the product was 146°C while in the extruder barrel. These conditions were not severe enough to cause a loss of lysine in the short time the material was in the extruder. Frying (190°C for 25 s) did cause a loss in lysine content, because the temperature at which the material was fried was higher than the temperature under which the material was extruded.

In-Vitro Digestibility

Results of *in-vitro* digestibility tests are shown in Fig. 1 and 2. The pH drop in *in-vitro* digestibility is used as a means of measuring digestive acceptability of food products (Maga *et al.* 1973). There was a greater pH drop in raw corn grits than in either extruded or raw quinoa (Fig. 1). This larger pH drop indicated

TABLE 2.
AMINO ACID COMPOSITION[1] OF RAW MATERIALS, EXTRUDED AND FRIED PRODUCTS

	Raw Material		Extruded[2]	
	Degerminated Corn Grits	Quinoa	30% Quinoa 70% Grits	30% Quinoa 70% Grits (Fried)
Aspartic Acid	5.82	9.06	7.67	7.42
Threonine	3.42	4.13	4.00	3.65
Serine	5.30	5.41	5.19	5.25
Glutamic Acid	18.63	16.43	17.92	17.75
Proline	8.12	5.28	7.62	7.42
Glycine	2.90	5.69	4.37	4.16
Alanine	7.42	5.22	6.58	6.73
Half Cystine	2.04	1.51	1.59	1.49
Valine	3.45	3.82	3.61	3.60
Methionine	2.46	1.86	1.93	1.99
Isoleucine	2.65	3.06	2.73	2.64
Leucine	13.18	7.40	10.51	10.55
Tyrosine	4.83	4.10	4.45	4.51
Phenylalanine	5.91	5.19	5.38	5.66
Histidine	5.53	5.40	4.87	6.67
Lysine	2.36	5.70	3.54	2.80
Ammonia	1.76	1.66	1.52	1.71
Arginine	4.23	9.08	6.51	5.99

[1]Grams of amino acids per 100g protein corrected to 100% recovery basis.
[2]Products containing quinoa and corn were extruded at 15% moisture, 100 rpm, 3:1 compression ratio. 100° and 150°C at the feed and compression sections, respectively.

greater *in-vitro* digestibility for the corn grits. Extrusion caused a decrease in *in-vitro* digestibility. As the amount of quinoa increased in the extruded blend, the overall pH drop decreased (Fig. 2), indicating a significant decrease in *in-vitro* digestibility with quinoa addition ($P < 0.02$).

Heat treatment has been shown to increase the digestibility of some proteins (Maga *et al.* 1973; Peri *et al.* 1983). Heating may improve protein digestibility by rendering the protein more susceptible to hydrolysis due to structural changes and/or it may destroy all or part of the activity of enzyme inhibitors which may be present (Maga *et al.* 1973). Heating may not always be beneficial to digestibility, however, as demonstrated by the cooking of sorghum where digestibility decreased (Hamaker *et al.* 1987). Similarly, extrusion of corn grits decreased the initial rate of hydrolysis (Fig. 1). This may be due to interactions between the proteins and other components in the product, such as lipids and carbohydrates which may render the protein less available for enzymatic hydrolysis.

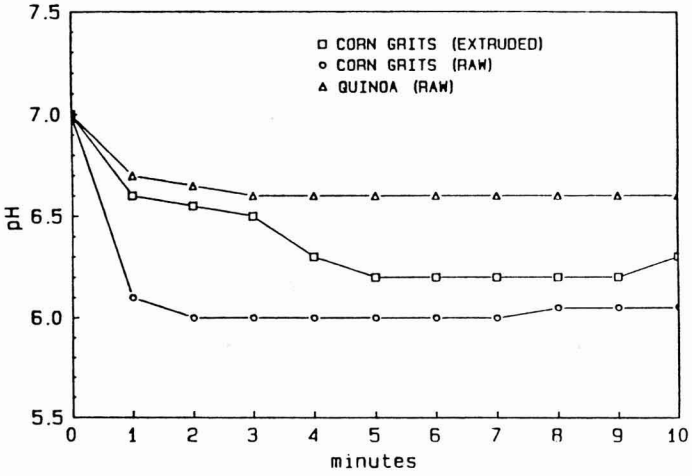

FIG. 1. *IN-VITRO* DIGESTIBILITY OF RAW MATERIALS AND 100% EXTRUDED CORN GRITS
(Extrusion Conditions for Corn Grits = 15% H_2O; Barrel Temperatures = 80° and 100°C, respectively, for Feed and Compression Sections, 3:1 Screw.)

Nitrogen Solubility

A nitrogen solubility profile, over a range of pH values, can be used as a guide to protein functionality, because it relates directly to many important

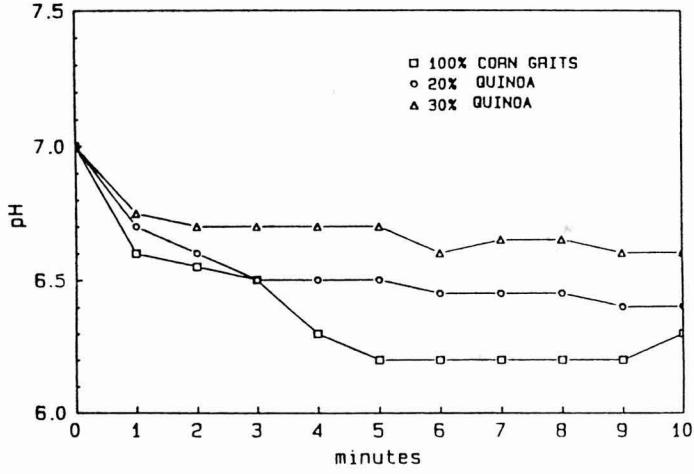

FIG. 2. *IN-VITRO* DIGESTIBILITY OF EXTRUDED PRODUCTS: EFFECT OF INCREASING QUINOA CONTENT
(Extrusion Conditions are the same as given for Fig. 1.)

properties, such as use in beverages, emulsification, foaming capacity and gelation (Kinsella 1976). Solubility data provide an indication of a protein's potential solubility in food systems and the degree of denaturation the protein may have undergone (Betschart 1974).

The nitrogen solubility of quinoa, corn grits and extruded blends are shown in Fig. 3. The protein in quinoa was much more soluble than that of corn grits. With increasing quinoa content, nitrogen solubility increased. A shift in the isoelectric point of the material toward the isoelectric point of quinoa's protein, which is about pH 4.0, was also observed. Heat treatment reportedly has a deleterious effect on solubility (Kinsella 1976; Peri *et al.* 1983).

Frying two of the extruded blends exposed them to more severe conditions (180°C for 25 s) than did the extrusion. Frying under these conditions had a deleterious effect on the nitrogen solubility of the two extruded blends (Fig. 4). Again, it was shown that blends with more quinoa had higher solubilities. This was true both before and after frying.

Sensory Evaluation

Results of the sensory evaluation of selected extruded products are presented in Table 3. Most of the quinoa products were judged to be in the "neither liked nor disliked" category when evaluated for flavor, texture and appearance. This would make the products moderately acceptable. A slightly darker color and bitter aftertaste of these products, compared to a commercial corn curl, were stated as drawbacks in their acceptability.

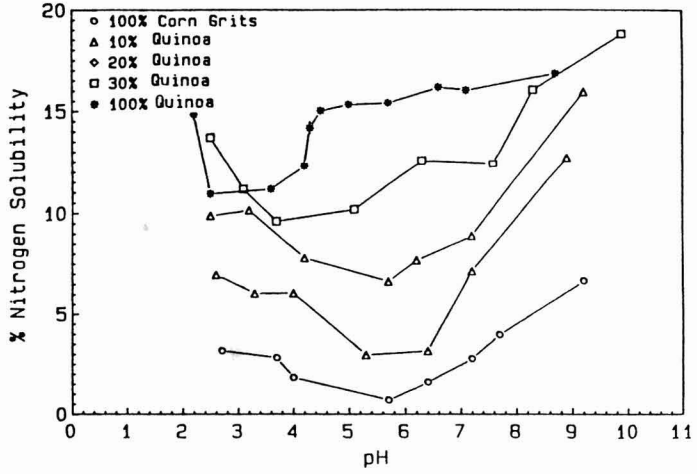

FIG. 3. NITROGEN SOLUBILITY OF UNPROCESSED CORN AND QUINOA AND OF EXTRUDED PRODUCTS: EFFECT OF INCREASING QUINOA CONTENT
(Extrusion Conditions are the same as given for Fig. 1.)

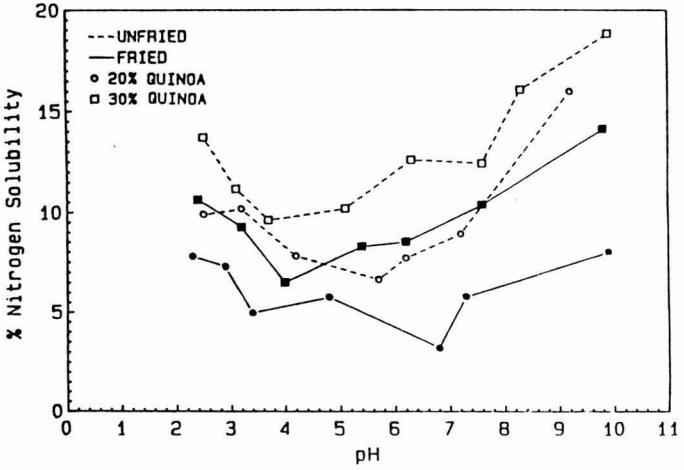

FIG. 4. NITROGEN SOLUBILITY OF EXTRUDED PRODUCTS: EFFECT OF FRYING
(Extrusion Conditions are the same as given for Fig. 1; Extruded Products were fried in Vegetable Oil for 25 s at 190°C.)

Knowledge of quinoa and the extrusion process had no significant effect on the acceptability of the product. There was no significant difference between the product containing 20% quinoa and the product containing 30% quinoa. However, overall, most products containing 20% quinoa received slightly higher

TABLE 3.
SENSORY EVALUATION OF SELECTED EXTRUDED PRODUCTS[1]

Panel[2]	% Quinoa in Blend	Shape	Flavor	Texture	Appearance
K	20	Curl	4.6^{a}	3.1^{ab}	4.0^{a}
U	20	Curl	4.9^{a}	4.3^{a}	4.2^{a}
K	30	Chip	4.6^{a}	3.9^{ab}	4.3^{a}
U	30	Chip	5.3^{b}	4.2^{a}	4.3^{a}
K	20	Cheese Curl	4.6^{a}	3.0^{b}	3.8^{a}
U	20	Chip	4.4^{a}	3.6^{ab}	3.8^{a}
U	20	Fried Chip	4.1^{a}	2.9^{b}	3.2^{a}

[1]1 = like extremely, 7 = dislike extremely
[2]K = knowledge of quinoa (average of 10 panelists)
U = no knowledge of quinoa (average of 11 panelists)

Values in the same column with the same superscript are not significantly different. Extrusion conditions: 15% moisture; 3:1 screw; 100° and 150°C at feed and compression section, respectively.

scores for texture and appearance than did the products containing higher amounts of quinoa. A slightly bitter aftertaste was reported for these products. Adding cheese, frying and changing the shape of the product from a curl to a chip had no significant effect on product scores. Characteristics which were liked by some panelists included crunchy texture, unique shape and flavor, which was described as nutty or wheaty.

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EXTRUDED CORN GRITS—QUINOA BLENDS: II. PHYSICAL CHARACTERISTICS OF EXTRUDED PRODUCTS

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ABSTRACT

Quinoa was blended with corn grits at levels of 10%, 20% and 30% and extruded at 15% and 25% moisture respectively, using screws with a 1:1 or 3:1 compression ratio. Extrusion temperatures were 80° and 100°C at the feed section and 100° or 150°C at the compression section, respectively. RPM values were 100, 150 and 200.

Quinoa addition produced a darker, less yellow product than corn grits alone. Density, expansion ratio and shear strength were lower for products containing greater levels of quinoa. The most favorable products were produced at a 15% initial moisture content and a 3:1 compression ratio. Products extruded under these conditions had the greater expansion, lower density and lower shear strength than products extruded at 25% moisture or at a 1:1 compression ratio.

SEM showed larger, more uniform cells in the 100% corn grits product indicating a more even expansion. A product containing 30% quinoa had a rougher texture, many broken cell walls and less evenly distributed air cells.

INTRODUCTION

Today, most of the world's food comes from a mere twenty or so species of plants. This means the vast majority of the world's edible plants have yet to be developed to their potential (Vietmeyer 1986).

The lesser-known food crops have not been rejected because of any inherent inferiority. Many have been overlooked merely because they are native to the tropics, a region generally neglected because the world's research resources are concentrated in the temperate zones. Others are neglected because they are scorned as 'poor people's plants.'

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A remarkable collection of poor people's crops is to be found in the highlands of South America, among them the grain called quinoa (Chenopodium quinoa). An annual, broad-leaved herb, quinoa's white or pink seeds occur in large sorghum-like clusters. The seeds, containing 12–19% protein, are one of the richest sources of protein among grain crops. Moreover, their protein possesses an attractive amino acid balance for human nutrition because of its high levels of lysine and methionine (Risi and Galwey 1984).

Of equal importance is the hardiness of this plant. Quinoa thrives with low rainfall; high altitudes, thin, cold air; hot sun; subfreezing temperature; and even poor, sandy, alkaline soil. It is this ability to thrive where few other food crops can that has allowed quinoa to remain the staple food of descendants of the Inca and makes it a viable crop for farmers in the Rockies of North America and other mountainous, cool and semi-arid regions of the world (Theurer Wood 1985).

However, quinoa has one major shortcoming: the seed pericarp contains toxic saponins which are extremely bitter and form a soapy solution in water (Reichert *et al.* 1986). For food use, the saponins must be removed by laborious hand scrubbing in water. Some agriculturists maintain that a saponin-free strain of quinoa should be developed but the bitter-tasting saponins probably also prevent insect and bird predation (Risi and Galwey 1984).

Currently, only one numbered variety, D407, is available for experimental production in Colorado. It has early maturity, a semi-dwarf growth habit, yellow compact heads and medium-small kernels. In 2 years of trials, it has produced consistent yields of 1200 lb/acre in the San Luis Valley of Colorado (Johnson and Croissant 1985).

It was the purpose of this work to develop new food applications of quinoa. The grain was extruded in blends with corn grits. The proximate composition, nutritive properties and sensory evaluations of these extruded blends have been described (Coulter and Lorenz 1991). In this paper we discuss the physical characteristics of the extruded blends.

MATERIALS AND METHODS

Sample Identification

Quinoa was obtained from the Colorado State University Agronomy Department. The cultivar used was Colorado D407, a Chilean landrace. The grain was grown during the 1987 growing season in experimental plots located in the San Luis Valley of Colorado.

Degerminated corn grits were obtained from Illinois Cereal Mills, Paris, Illinois.

Proximate Composition

After removal of saponins, as described previously (Coulter and Lorenz 1991), the proximate composition (moisture, ash, protein, fat and total dietary fiber) of quinoa and that of corn grits and selected extruded samples was determined as reported in a previous paper (Coulter and Lorenz 1991).

Extrusion

One hundred percent corn grits and the following blends: 90:10, 80:20, and 70:30 corn grits to quinoa, were extruded. Extrusion batch size was 2500 g of dry blend adjusted to 15 or 25% moisture by appropriate moisture addition, blending in a Hobart mixer and equilibration overnight at room temperature in air tight plastic bags. A Brabender Plasticorder Extruder model PL-V500 with a 19.05 mm barrel diameter, a 20:1 length to diameter ratio and eight 0.79 × 3.18 mm longitudinal grooves was used. Extruder screws having 1:1 or 3:1 compression ratio were used on all samples. The barrel was equipped with two thermostatically controlled, electrically heated, compressed air cooled collars. Barrel temperatures were 80° and 100° or 100° and 150°C, at the feed and compression sections, respectively.

The extruder variable speed drive was set at 100, 150 and 200 rpm, respectively. A $\frac{1}{8}$ in. diameter die was used for all trials. Torque was read from the torque indicator. Dough temperatures at two sections of the extruder barrel were recorded using temperature probes. All products were air dried after extrusion.

MEASUREMENTS AND EVALUATION OF EXTRUDED SAMPLES

Extrusion Rate

Extruded samples were collected for 15 to 90 s, depending on product throughput. Weights were divided by the time of collection and multiplied by 60 to determine extrusion rate per minute.

Expansion Ratio

Expansion ratio was determined as the ratio of extrudate diameter to die diameter. Three randomly selected extrudates, from each experiment, were used for the measurement.

Density. Density was measured as the volume of rapeseed displaced per unit weight of sample using a rapeseed displacement meter.

Color. The color of ground samples (micromill, The Lab Apparatus Co., Cleveland, Ohio) was measured using a Hunter lab meter D25D (Hunter As-

sociates Laboratory, Inc., Fairfax, Virginia, U.S.A.) with standard values of L = 78.2, a = −2.4, b = 21.9.

Color changes in the Hunter scale were recorded and the color difference, ΔE, was calculated as: $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$ (Clydesdale 1976)

where ΔL = L (standard) − L (sample)
Δa = a (standard) − a (sample)
Δb = b (standard) − b (sample)

Shear Strength. Shear strength was determined on five replicates of each sample, using an Instron Universal testing machine. Peak Newton force was calculated by multiplying the peak height by the factor corresponding to the setting used on each sample. Shear strength (N/mm^2) was then calculated by dividing Peak Newton force by the average cross-sectional area of the extrudate.

Scanning Electron Microscopy (SEM). Corn grits, 80:20, and 70:30 blend extrudates were attached to circular specimen stubs with silver paste, covered with 100 A each of carbon and gold overlayer using a Technics Hummer sputter coater, and photographed under various magnifications using a Phillips SEM.

Statistics

Data were statistically analyzed using the GLM procedure on the Statistical Analysis System (SAS). The significance of compression ratio, screw speed, barrel temperature, moisture content and quinoa content was determined on the following product characteristics: Expansion ratio, shear strength, density and color L and ΔE values.

RESULTS AND DISCUSSION

Physical property data, including extrusion rate, density and expansion ratio are presented in Tables 1 and 2. Because of the large amount of data collected, only those of extrudates manufactured with a 3:1 screw are reported. Statistical data are shown in Table 3.

Extrusion Rate

Product extrusion rate increased with increasing quinoa content due to quinoa's fat content, which is higher than that found in degerminated corn grits. Fat has been found to be a determining factor (de Muelenaere and Buzzard 1969). Fat increases the rate because of an adverse effect on expansion and density. Products containing quinoa and extruded at 25% moisture were found to have higher extrusion rates than did the same materials with only 15% moisture. Higher

TABLE 1.
EXTRUSION RATE, DENSITY AND EXPANSION RATIOS OF EXTRUDATES[1]

Product Composition	Moisture in Blend (%)	Screw Speed (RPM)	Extrusion Rate (g/min)	Density (g/cm^3)	Expansion Ratio
100% CG	15	100	112.4	0.12	2.41
90% CG - 10% Q	15	100	91.4	0.10	3.36
80% CG - 20% Q	15	100	113.2	0.11	2.73
70% CG - 30% Q	15	100	111.4	0.16	2.52
100% CG	15	150	143.0	0.09	3.78
90% CG - 10% Q	15	150	115.6	0.10	3.67
80% CG - 20% Q	15	150	126.4	0.10	3.04
70% CG - 30% Q	15	150	165.2	0.12	2.41
100% CG	15	200	157.4	0.07	3.99
90% CG - 10% Q	15	200	154.4	0.08	3.46
80% CG - 20% Q	15	200	159.0	0.09	3.36
70% CG - 30% Q	15	200	185.4	0.13	2.83
100% CG	25	100	106.5	0.46	1.57
90% CG - 10% Q	25	100	122.2	0.36	1.47
80% CG - 20% Q	25	100	135.2	0.37	1.47
70% CG - 30% Q	25	100	148.1	0.43	1.26
100% CG	25	150	133.4	0.53	1.89
90% CG - 10% Q	25	150	161.7	0.36	1.47
80% CG - 20% Q	25	150	196.4	0.36	1.47
70% CG - 30% Q	25	150	233.0	0.38	1.57
100% CG	25	200	145.9	0.38	2.31
90% CG - 10% Q	25	200	187.5	0.37	1.99
80% CG - 20% Q	25	200	249.6	0.37	1.89
70% CG - 30% Q	25	200	306.3	0.53	1.47

[1] Compression Ratio = 3:1; barrel temperature = 80–100°C (feed and compression sections, respectively) CG = Corn grits; Q = Quinoa

moisture may cause increased lubrication in the extruder barrel, thus contributing to extrusion rate in a manner similar to fat.

A higher compression ratio resulted in decreased extrusion rate. Similar results were seen in an earlier study dealing with the extrusion of corn grits (Harmann and Harper 1973). As would be expected, increasing screw speed increased the rate of all quinoa containing products.

Extrusion rate of products containing quinoa increased at higher barrel temperatures. In other studies, rice and corn extrudates were found to have increased rates at higher extrusion temperatures while potato flour and corn grit extrusion rates seemed to be independent of extrusion temperatures (Kim and Maga 1987; Harmann and Harper 1973). A possible explanation for the increase in rate seen with the quinoa added products at higher barrel temperatures is that at the higher temperatures used in this study, the viscosity of the material in the extruder would be lower, allowing for faster flow through the extruder and thus a higher extrusion rate.

TABLE 2.
EXTRUSION RATE, DENSITY AND EXPANSION RATIOS OF EXTRUDATES[1]

Product Composition	Moisture in Blend (%)	Screw Speed (RPM)	Extrusion Rate (g /min)	Density (g /cm^3)	Expansion Ratio
100% CG	15	100	110.2	0.08	3.36
90% CG - 10% Q	15	100	119.0	0.06	3.15
80% CG - 20% Q	15	100	112.8	0.09	2.73
70% CG - 30% Q	15	100	140.4	0.14	2.62
100% CG	15	150	197.6	0.10	3.04
90% CG - 10% Q	15	150	149.0	0.09	3.36
80% CG - 20% Q	15	150	172.8	0.08	3.15
70% CG - 30% Q	15	150	181.2	0.12	2.52
100% CG	15	200	175.2	0.08	3.99
90% CG - 10 Q	15	200	174.4	0.08	3.67
80% CG - 20 Q	15	200	206.8	0.15	3.36
70% CG - 30 Q	15	200	210.0	0.08	2.83
100% CG	25	100	100.0	0.23	2.62
90% CG - 10% Q	25	100	126.8	0.27	1.78
80% CG - 20% Q	25	100	130.0	0.30	1.68
70% CG - 30% Q	25	100	166.0	0.26	2.10
100%	25	150	120.4	0.16	2.10
90% CG - 10% Q	25	150	173.8	0.22	1.78
80% CG - 20% Q	25	150	208.8	0.31	1.89
70% CG - 30% Q	25	150	232.8	0.17	1.78
100% CG	25	200	115.6	0.23	2.62
90% CG - 10% Q	25	200	216.2	0.26	2.10
80% CG - 20% Q	25	200	276.0	0.33	1.89
70% CG - 30% Q	25	200	307.2	0.35	1.89

[1]Compression Ratio = 3:1; barrel temperature = 100–150°C (feed and compression sections, respectively) CG = Corn grits; Q = Quinoa

Product Density

Quinoa addition had a significant effect ($p > 0.002$) on product density (Tables 1 and 2). In general, adding quinoa to corn grits resulted in a slightly more dense extruded product with the 3:1 compression, possibly due to a higher protein content. Generally, higher protein increases the firmness of the plasticized extrudate, preventing expansion due to flash evaporation of moisture on exit from the die.

Moisture content had a more significant effect ($p > 0.0001$) than quinoa addition on the density of the extruded products. Density of extruded products was higher in those which initially had higher moisture contents. Similar results have been reported for the extrusion of corn gluten meal/soy protein blends, and defatted corn grits (Bhattacharya *et al.* 1986; Harmann and Harper 1973). This increase in density is related to a decrease in expansion. Products with higher moisture contents did not expand as well as those with lower moisture contents, resulting in more dense products. The screw speeds used in this study had no effect on the density of extruded corn grit and quinoa blends.

Barrel temperature also exhibited a significant effect on product density ($p > 0.004$). Products extruded at higher temperatures were less dense than those extruded at lower temperatures. This is in agreement with other studies, which saw similar decreases in density with extruded corn grits and extruded soybean products (Harmann and Harper 1973; Cumming *et al.* 1972). The decrease in density with increased temperature could result from: (a) decreased viscosity from temperature increases, allowing the dough to expand more readily and (b) increased vapor pressure as temperature increases causing increased flashing and puffing upon exit from the die (Harmann and Harper 1973).

Like moisture, compression ratio had significant effects ($p > 0.0001$) on the density of the extruded products. Materials extruded at 1:1 compression ratios were more dense than those extruded at 3:1 compression ratios. Materials extruded with the 3:1 compression ratio reached higher dough temperatures in the barrel of the extruder. Therefore, the reasons for differences observed in density between compression ratios are the same as those noted above for differences due to barrel temperature. A statistical summary of the factors affecting product density is presented in Table 3.

Expansion Ratio

Quinoa addition had a very significant ($p > 0.001$) effect on the expansion of the extruded products (Tables 1 and 2). Samples containing greater percentages of quinoa did not expand as much as those containing no quinoa. This effect of quinoa addition was more apparent at higher compression ratios. Quinoa may have caused a decrease in expansion in products because it has a higher protein and lipid content and a lower amylose content than the corn grits. All three of these factors have been linked to decreased expansion. The addition of whey protein concentrate caused a decrease in the expansion of rice and corn flour products (Kim and Maga 1987). Addition of up to 11% gluten caused a decrease in expansion of wheat starch (Faubion and Hoseney 1982b). Because protein content of the quinoa added to the corn grits was high (14.75%), it may have contributed to a reduction in expansion. The addition of 1% free flour lipids to wheat starch caused a decrease in expansion, while defatted flours gave products with increased expansion (Faubion and Hoseney 1982b). The addition of quinoa to corn grits increased the lipids content of the material. This increase in lipid content may then be partially responsible for the observed decrease in expansion with quinoa addition. Fat added to extruded products tends to weaken the resulting dough and reduces the strength of the product and increases plasticity as it leaves the extruder (Harper 1981b). This may then result in decreased expansion.

Initial moisture contents of the extruded material also had a significant ($p > 0.0001$) effect on the expansion of the products. Products extruded at 15% moisture content were more expanded than products extruded at 25% moisture. Similar results have been reported by a number of researchers (Harmann and

TABLE 3.
STATISTICAL EVALUATION OF PRODUCT CHARACTERISTICS

Source	Degrees Freedom	Density		Expansion Ratio		Shear Strength		Color L Value	
		F Value	P-Value	F Value	P-Value	F Value	P-Value	F Value	P-Value
Compression Ratio	1	117.06	0.0001	64.53	0.0001	275.34	0.0001	5.30	0.0230
Screw Speed	2	0.38	0.6822	1.51	0.2253	1.04	0.3576	0.05	0.9509
Barrel Temperature	1	8.63	0.0038	2.18	0.1422	0.02	0.8863	1.05	0.3079
Quinoa Content	2	5.01	0.0025	5.71	0.0011	9.11	0.0001	153.38	0.0001
Moisture Content	1	117.95	0.0001	45.84	0.0001	100.11	0.0001	230.96	0.0001

Harper 1973; Mercier and Feillet 1975; Faubion and Hoseney 1982a and 1982b; Gomez and Aguilera 1984; Owasu-Ansah *et al.* 1984; Paton and Spratt 1984). At higher moisture contents a simultaneous decrease in viscosity reduces the temperature attained in the die and increases the rate of contraction after the product leaves the die, resulting in reduced expansion (Park 1976).

Like quinoa and moisture content, compression ratio had a statistically significant effect ($p > 0.0001$) on expansion ratio. Samples extruded with the 3:1 compression ratio were more expanded than those extruded at the 1:1 compression ratio. The extent of puffing or expansion depends on the pressure differential between the die and the atmosphere (Bhattacharya *et al.* 1986). As with lower moisture materials, higher temperatures and greater pressures are produced with the 3:1 compression ratio than with the 1:1 compression ratio. Higher temperatures and higher pressure differentials between the die and atmosphere then result in a more expanded product (Park 1976; Bhattacharya *et al.* 1986).

Screw speed and barrel temperature did not exhibit statistically significant effects on expansion of the extruded quinoa/corn grit blends. A statistical summary of the variables affecting expansion ratio is presented in Table 3.

Shear Strength

Quinoa addition had a significant effect on the shear strength of the extruded products ($p > 0.001$). Shear strength decreased with quinoa addition. This effect was greater at 15% moisture. The protein and lipid content of the corn grit/quinoa blends was higher than that of the corn grits alone. This may have contributed to the decrease in shear stress observed. The textural strength of extruded wheat flour was increased with the removal of lipids (Faubion and Hoseney 1982b). In the same study, lipid addition to wheat flour and starch caused a decrease in the textural strength of the extruded products. Since quinoa addition also added lipid to the material, this may have contributed to the observed decrease in shear strength. Gluten has been shown to decrease textural strength (Faubion and Hoseney 1982b).

Moisture exhibited a very significant effect ($p > 0.0001$) on the shear strength of the extruded products. The shear strength of the extruded corn grits/quinoa blends was higher at higher initial moisture contents when extruded at the 3:1 compression ratio (Fig. 1). These products are, therefore, harder than lower moisture level products. Other investigations have reported similar effects of moisture (Mercier and Feillet 1975; Bhattacharya *et al.* 1986). The extruded corn grits/quinoa products with higher initial moisture contents were more dense than those at lower moisture levels. Products with higher density, due to high moisture contents, then require more work to shear the product, therefore shear strength is increased (Bhattacharya *et al.* 1986).

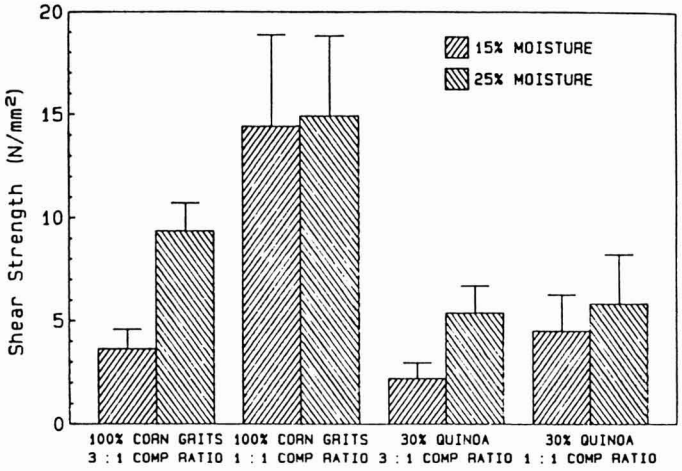

FIG. 1. EFFECT OF MOISTURE CONTENT ON SHEAR STRENGTH OF EXTRUDED PRODUCTS
(Products were extruded at 100–150°C, Feed and Compression Sections, respectively; RPM = 150.)

Compression ratio also exhibited a significant ($p > 0.0001$) effect on the shear strength of the products. The lower compression ratio (1:1) used also produced products which were harder than those produced with the larger compression ratio (3:1). This increase in hardness was exhibited as an increase in shear strength. Products which were extruded at the 1:1 compression ratio are subjected to lower temperatures and less pressure than products extruded at the 3:1 compression ratio. Lower temperatures produce more dense products.

As shown in Table 3, screw speed and barrel temperature did not have a statistically significant effect on the shear strength of extruded quinoa/corn grits blends.

Shear strength has been shown to be interrelated to other extrudate product characteristics. Shear strength was found to be lowest in products with minimum density and maximum expansion (Phillips and Falcone 1988). The shear strength of extruded corn grits and blends of corn grits and quinoa was found to be related to both the density and the expansion of the products. A negative relationship was found between shear strength and expansion. As shown in Fig. 2, shear strength decreases as expansion increases. The correlation coefficient for this relationship was determined to be -0.8480.

A positive relationship was found between shear strength and density (Fig. 3). As density increased, shear strength increased. The correlation coefficient for this relationship was 0.8781.

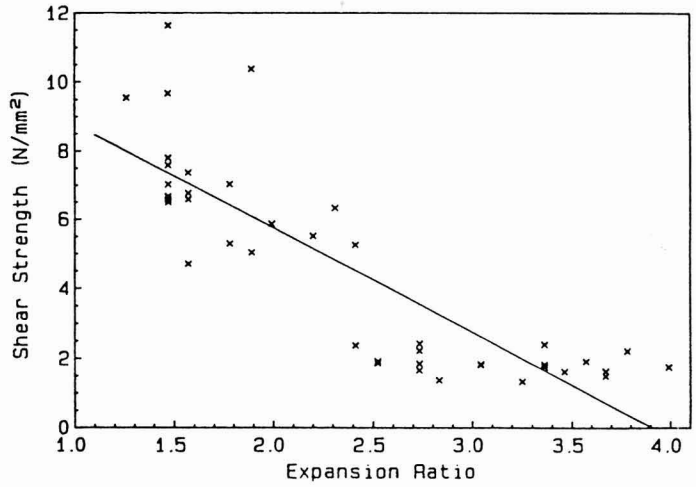

FIG. 2. RELATIONSHIP BETWEEN SHEAR STRENGTH AND EXPANSION RATIO OF EXTRUDED PRODUCTS

Color

Color data of some extruded products are given in Table 4.

Color was not significantly affected by the two barrel temperatures or by the three rpms used. Quinoa addition significantly ($p > 0.0001$) affected both Hunter L and $\triangle E$ values. Since quinoa is darker than corn grits, it would be expected

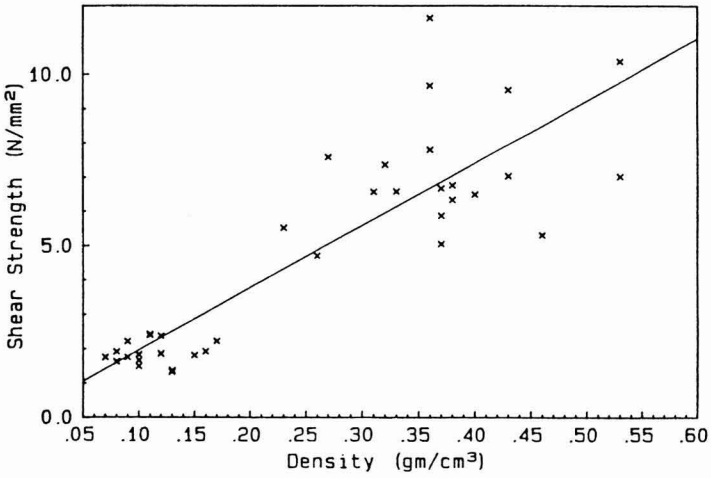

FIG. 3. RELATIONSHIP BETWEEN SHEAR STRENGTH AND DENSITY OF EXTRUDED PRODUCTS

TABLE 4.
COLOR DATA OF EXTRUDED PRODUCTS[1]

Blend Composition	Moisture of Blend (%)	Compression Ratio	Screw Speed (RPM)	L	a	b	ΔE
100% CG	15	1:1	100	71.6	1.3	31.4	12.14
90% CG - 10% Q	15	1:1	100	66.0	1.5	26.3	13.54
80% CG - 20% Q	15	1:1	100	62.0	1.3	22.9	16.65
70% CG - 30% Q	15	1:1	100	57.9	1.4	21.0	20.67
100% CG	15	1:1	150	72.5	1.5	31.2	11.58
90% CG - 10% Q	15	1:1	150	65.6	1.6	25.8	13.78
80% CG - 20% Q	15	1:1	150	62.3	1.2	23.1	16.35
70% CG - 30% Q	15	1:1	150	58.4	1.3	21.2	20.15
100% CG	15	1:1	200	72.6	1.4	31.2	11.46
90% CG - 10% Q	15	1:1	200	64.6	1.8	25.4	14.66
80% CG - 20% Q	15	1:1	200	59.9	1.5	23.0	18.74
70% CG - 30% Q	15	1:1	200	57.6	1.5	21.2	20.98
100% CG	25	3:1	100	68.4	-0.5	32.7	14.71
90% CG - 10% Q	25	3:1	100	57.6	1.5	24.5	21.13
80% CG - 20% Q	25	3:1	100	52.0	2.0	20.5	26.60
70% CG - 30% Q	25	3:1	100	50.1	2.0	19.1	28.58
100% CG	25	3:1	150	69.0	-1.6	31.4	13.25
90% CG - 10% Q	25	3:1	150	58.0	1.8	24.6	20.81
80% CG - 20% Q	25	3:1	150	52.9	1.9	20.5	25.70
70% CG - 30% Q	25	3:1	150	50.9	2.0	18.4	27.87
100% CG	25	3:1	200	67.7	-1.5	31.1	13.99
90% CG - 10% Q	25	3:1	200	58.5	1.7	24.8	20.33
80% CG - 20% Q	25	3:1	200	53.6	1.8	20.6	24.99
70% CG - 30% Q	25	3:1	200	49.8	2.0	18.0	29.00

[1] Extruded at 100-150°C (feed and compression sections, respectively)
CG = Corn Grits; Q = Quinoa
Standard values: L = 78.2, a = -2.4, b = 21.9

that material with quinoa added would be darker and thus would have lower L values.

Moisture content and dough temperature were more important factors than barrel temperature and screw speed in determining color properties of the extrudates. Those materials with lower moisture contents reached higher dough temperatures inside the barrel of the extruder, because these materials produced stiffer doughs in the extruder than the higher moisture materials. Stiffer doughs caused more friction in the extruder barrel, resulting in higher dough temperatures. Hunter L and ΔE values were significantly ($p > 0.0001$) affected by moisture content. Blends which had higher initial moisture contents produced darker products as is illustrated in Fig. 4. Since the lower moisture products had more expansion and were less dense than the higher moisture products, they appeared lighter in color.

Quinoa is less yellow than corn grits, therefore color b values decreased with quinoa addition. Slightly more positive a values were seen in higher moisture products (Fig. 5). These differences decreased with increased quinoa content.

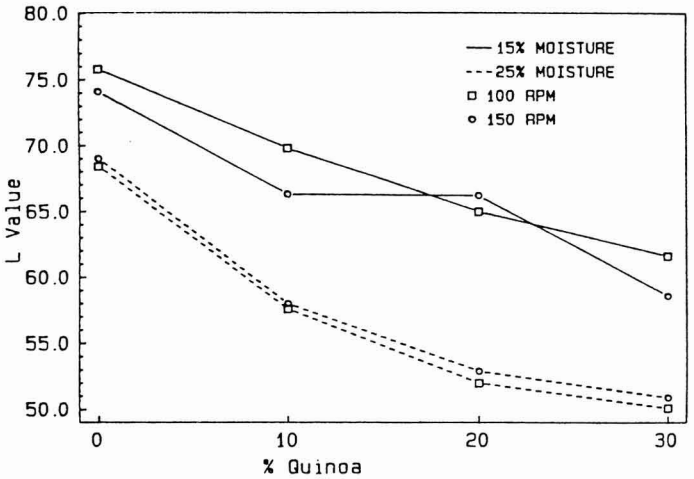

FIG. 4. COLOR L VALUES OF EXTRUDED PRODUCTS: EFFECT OF MOISTURE CONTENT
(Products were extruded at 100–150°C, Feed and Compression Section, respectively; Compression Ratio = 3:1.)

Very little difference was apparent in the 20 and 30% quinoa product. The opposite occurred with color b values (Fig. 5). Products with lower initial moisture contents had slightly higher b values than those with higher initial moisture contents.

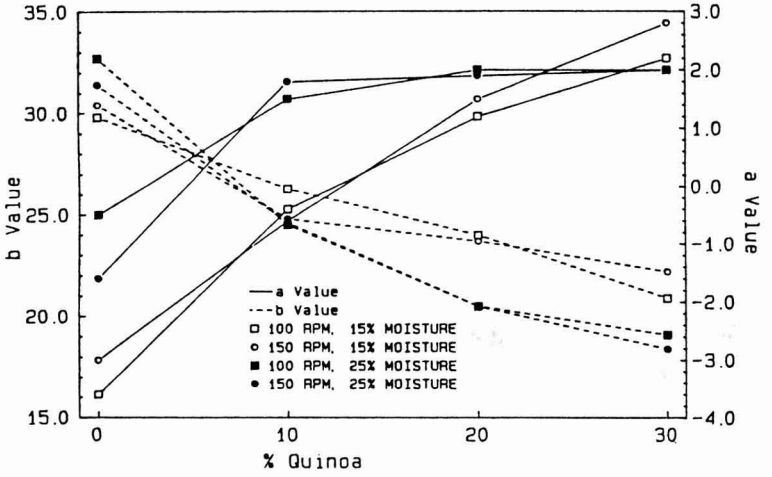

FIG. 5. COLOR A AND B VALUES OF EXTRUDED PRODUCTS: EFFECT OF MOISTURE CONTENT
(Products were extruded at 100–150°C, Feed and Compression Section, respectively; Compression Ratio = 3:1.)

Compression ratio exhibited a significant effect on both color L ($p > 0.02$) and $\triangle E$ ($p > 0.01$) of the extruded products. Color b values were not affected by the two compression ratios used in this study.

The color a value of products extruded with a 1:1 compression ratio changed very little with quinoa addition, indicating very little pigment destruction or color formation. Greater pigment destruction occurs with higher compression ratios. With this compression ratio (3:1), higher temperatures are obtained within the barrel of the extruder, causing greater thermal destruction of the pigments. Table 3 shows a statistical summary of factors affecting Hunter L and $\triangle E$ values.

Scanning Electron Microscopy

Two extruded samples are shown in Figs. 6 and 7. Figure 6 shows a product containing 100% corn grits, while Fig. 7 shows a product containing 30% quinoa and 70% corn grits. Both products were extruded with initial moisture contents of 15%. The product containing only corn grits had larger, more evenly round

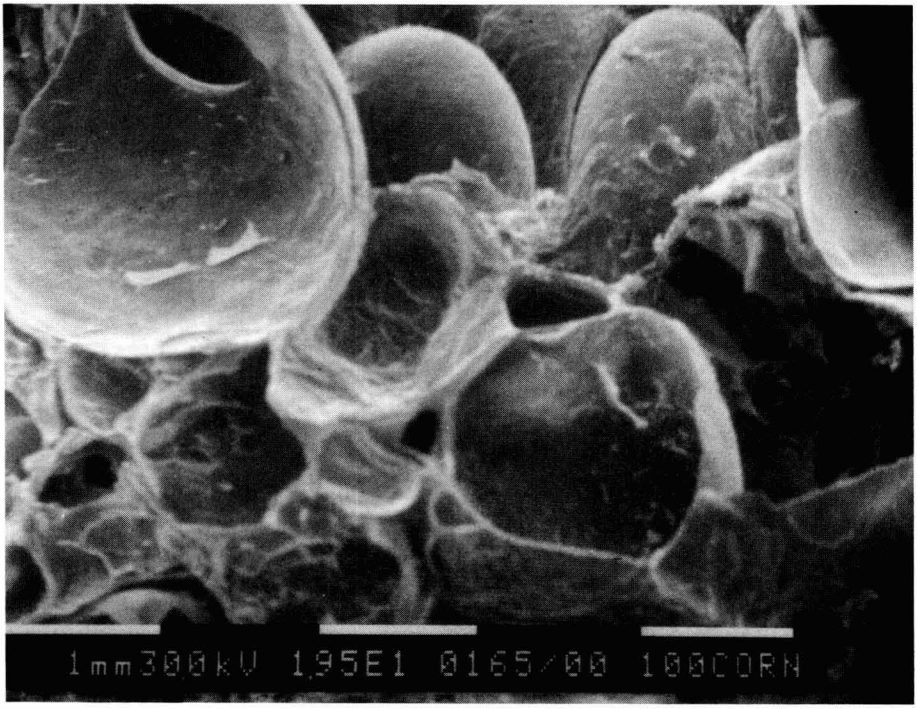

FIG. 6. SEM OF EXTRUDED PRODUCT CONTAINING 100% CORN GRITS
(Product was extruded at 100–150°C, Feed and Compression Section, respectively; 15% Moisture, 3:1 Compression Ratio; 100 RPM.)

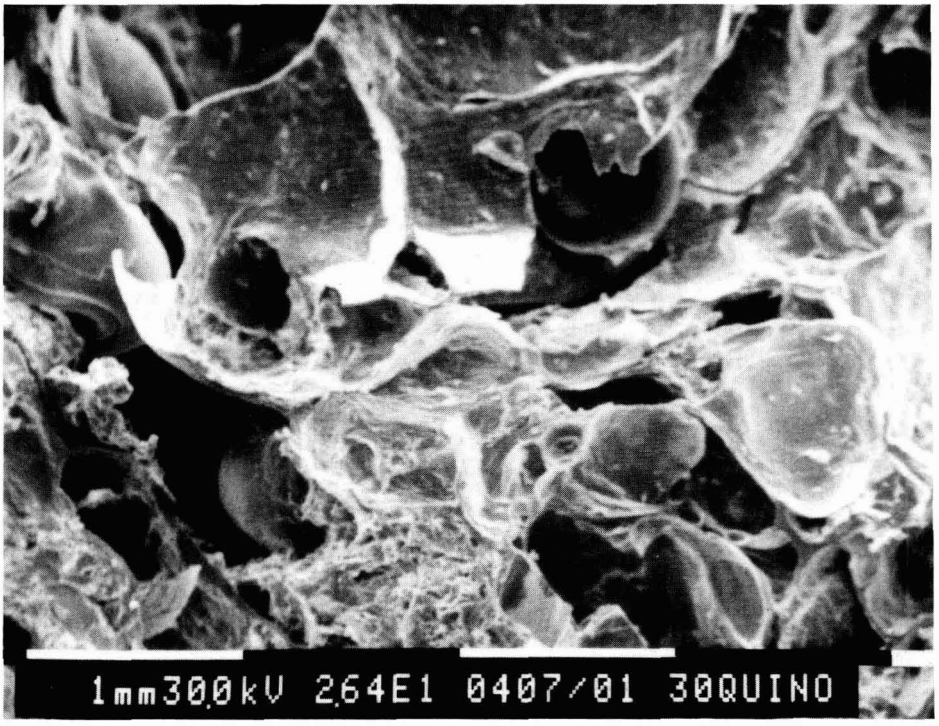

FIG. 7. SEM OF EXTRUDED PRODUCT CONTAINING
30% QUINOA AND 70% CORN GRITS
(Product was extruded at 100–150°C, Feed and Compression Section, respectively; 15% Moisture; 3:1 Compression Ratio; 100 RPM.)

cells than did the product containing quinoa, which had smaller, more compact cells. The larger more uniform cells of the 100% corn grit product indicated a more even expansion of that product. The product containing the quinoa had a rougher texture, many broken cell walls, less evenly distributed air cells and more open spaces than the 100% corn grit product. This helps to explain the lower shear strength observed in the products containing quinoa.

CONCLUSION

Extruded blends of corn grits and quinoa were darker and less yellow than extruded corn grits alone. Products extruded at 15% initial moisture and a 3:1 compression ratio had greater expansion, lower density and lower shear strength than products extruded at 25% moisture or at a 1:1 compression ratio. Quinoa addition produced products which were higher in protein, fiber, ash and some amino acids than 100% corn grits products. These products had a greater nitrogen

solubility but a somewhat lower *in-vitro* digestibility than products containing only corn grits. Sensory evaluation of the extruded corn grits—quinoa blends indicated that the products were moderately acceptable.

ACKNOWLEDGMENT

We thank Dr. D. Johnson, Department of Agronomy, Colorado State University for supplying the sample of quinoa.

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THE EFFECT OF ETHYLENE DIAMINE TETRACETIC ACID ON PRESERVING THE COLOR OF AN AVOCADO PUREE

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ABSTRACT

In this work, the effect of ethylene diamine tetracetic acid (EDTA) is evaluated as a potential additive to preserve the green color of avocado purees. Four batches of avocado purees were made, adding different concentrations of EDTA. Evaluation of color, chlorophylls, pheophytins as well as microbiological and sensory analysis were carried out during six months of storage. Results show that the addition of EDTA to the avocado purees in concentrations from 300 to 350 ppm, preserve better the green color and disminished pheophytin content as compared with purees without EDTA and 250 ppm of same, kept under refrigeration at 2–4°C.

INTRODUCTION

Attempts to preserve avocado purees, have been carried out at low temperatures, adding preservatives to prevent browning, rancidity and microbiological

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spoilage. Taste has been acceptable after three months of storage under refrigeration (2–4°C), as the results of sensory evaluation (Ortiz 1983). Nevertheless, preservation of original green color has not been very successful. Chlorophylls responsible for green color, are susceptible to change to pheophytins where magnesium is replaced with hydrogen ions and turn to a brownish color. On the other hand, chelating agents such as EDTA (Ethylene Diamine Tetracetic Acid), can be used to control the degradation reactions catalyzed by trace metals and has been used for this purpose (Gupta and Gangulli 1980; Song and Cunningham 1985). The present work deals with the effect of EDTA on the color and chlorophyll and pheophytin contents, as well as sensorial evaluation of taste, in four lots of avocado purees, stored under refrigeration (2–4°C), during six months.

MATERIALS AND METHODS

Avocados Hass were obtained from Uruapan, state of Michoacan, and transported to Mexico City. The experiments were carried out, as soon as the avocados reached a lipid content of 17% ± .3 and showed good sensorial characteristics, such as a uniform texture and an even black color of the peel.

Preparation of Samples

Considering the results of a previous work (Ortiz 1983), four lots of avocado puree were prepared according to the method presented in Fig. 1. The composition of each lot is shown in Table 1. Finally, the avocado purees were packed under vacuum in cellopolyfoil bags. Analysis were carried out over a period of 6 months as follows: weekly during the first two months and then monthly until the end of the experiment.

Peroxides

Peroxide index was performed following the method described in the AOAC (1980).

pH

It was determined in a pH meter Corning model 7.

Chlorophylls and Pheophytins

Chlorophylls and pheophytins were determined spectrophotometrically according to Vernon (1960). Chlorophylls (a and b) present in the purees extracts were calculated employing Eq. 9. Pheophytins (a and b) present in purees were equal to the difference between total pheophytins obtained after conversion with

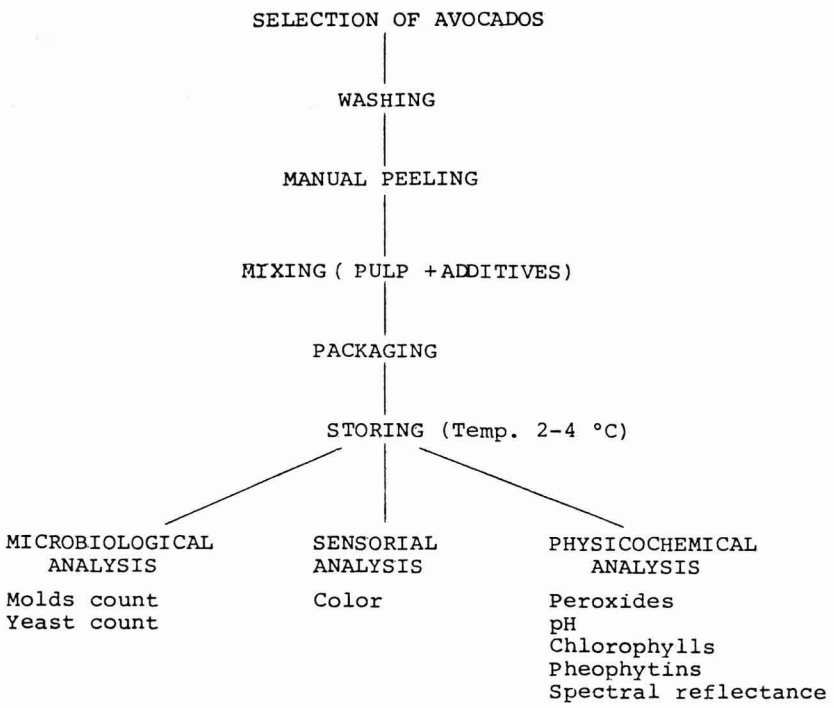

FIG. 1. PROCEDURE FOR THE ELABORATION AND STORAGE OF AVOCADO PUREE

oxalic acid (Eq. 12) and the chlorophylls. Reference was made to the quantity of puree employed to obtain the extract, to express results in ppm.

Color

Relative spectral reflectance was determined in an Agtron Spectrophotometer utilizing two monochromatic lights: red and green. Color was also evaluated using a Sensorial Ranking Test (IFT 1981), performed under daylight as follows: coded samples on white plates were distributed among 13 panelists to judge for their preference as compared one with the other three samples based on the green color. They were asked to assess the color according to a scale ranking from 1 to 4.

Statistical Analysis

Least squares analysis of variance of the data with equal numbers was used to obtain means for sensorial evaluation, spectral reflectance, pH, peroxides,

TABLE 1.
ADDITIVES CONCENTRATION IN THE FOUR LOTS OF AVOCADO PUREE.

Lot No.	Sodium Metabisulfite (ppm)	Sodium Bisulfite (ppm)	Ascorbic Acid (g/100g*)	Fat Antioxidant BHT + BHA (g/100g*)	Sodium Benzoate (g/100g*)	EDTA (Na^+ Salt) (ppm)
I	150	350	0.7	0.05	0.15	0
II	150	350	0.7	0.05	0.15	250
III	150	350	0.7	0.05	0.15	300
IV	150	350	0.7	0.05	0.15	350

*100 g of avocado purée

chlorophylls and pheophytins contents. Differences between means were tested using the Tukey test (Bruning and Kintz 1977).

Microbiological Analysis

Mold and yeast counts were determined, using the recommended methods for the microbiological examination of foods by Sharf (1966).

RESULTS AND DISCUSSION

Color

Change of color evaluated by reading the reflectance of avocado paste, using green light can be observed in Fig. 2. Lot I blank, show a statistically significative difference with respect to lots III and IV, at a level of 0.05, during the first 60 days of storage. After that period of time, differences between the blank and these two lots became even more significant (level of 0.01).

A similar behavior was obtained using a red light, as shown in Fig. 3, which indicated that a level of 300 and 350 ppm of EDTA has an effect on preventing deterioration of the original color of avocado puree.

Color was also evaluated based on the preference of the panelists towards a green color. Figure 4 shows that an average value assigned for sensory preference

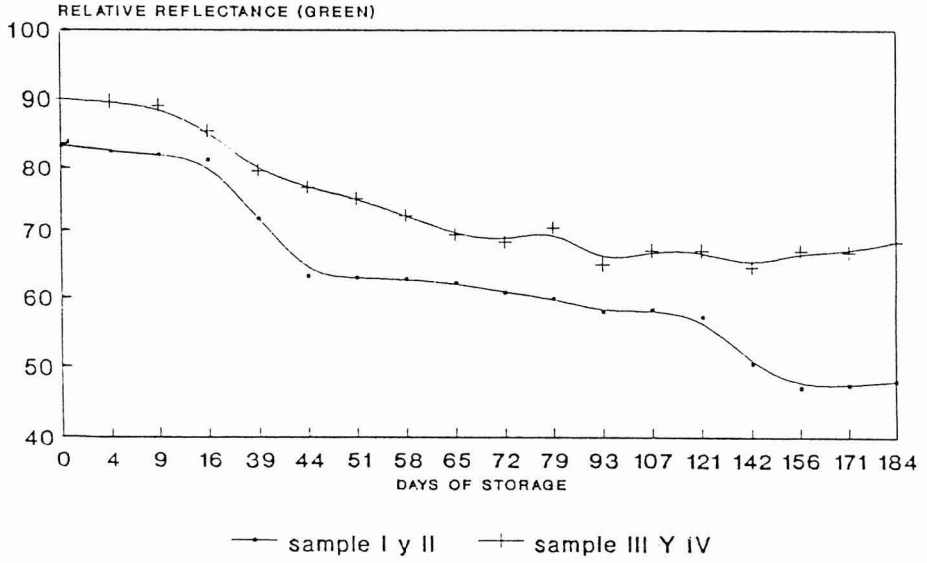

FIG. 2. GREEN LIGHT REFLECTED BY AVOCADO PUREES

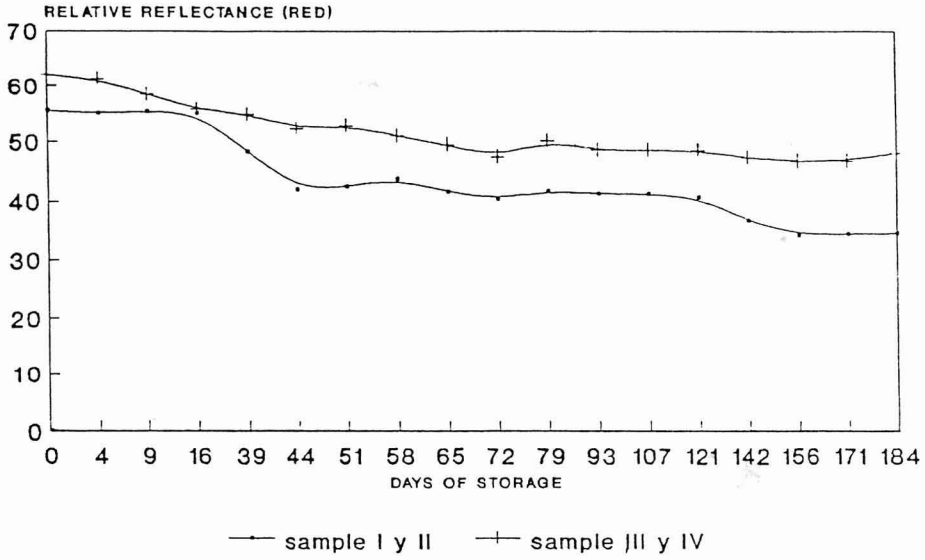

FIG. 3. RED LIGHT REFLECTED BY AVOCADO PUREES

scores is markedly higher for lots III and IV than for lots I and II, mainly after sixty days of storage. Color of lots I and II was observed to turn yellowish green; however, lots III and IV retained more of their characteristic green color.

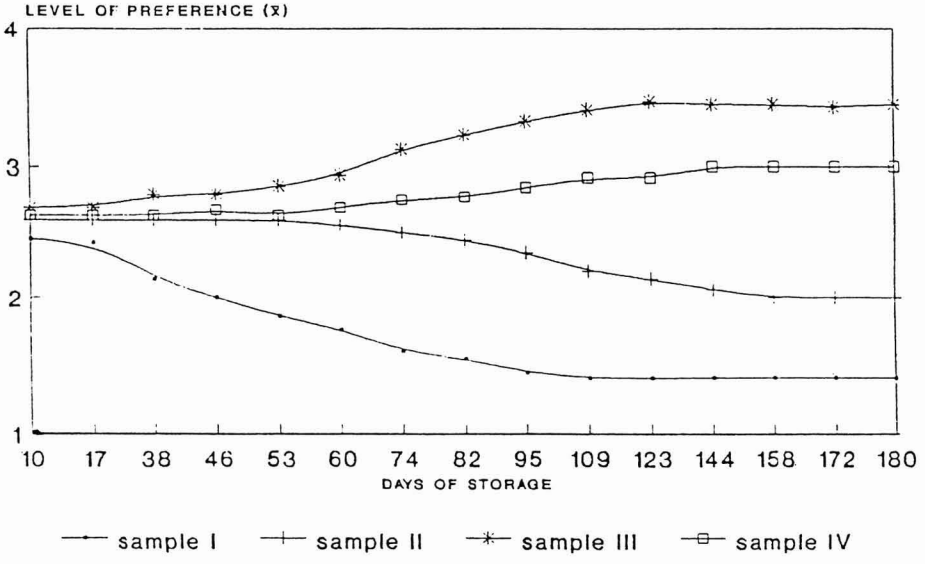

FIG. 4. LEVEL OF PREFERENCE OF COLOR OF SAMPLE

Chlorophylls and Pheophytins Contents

Evaluation of chlorophylls (Fig. 5), show that lots III and IV of avocado puree, containing 300 and 350 ppm of EDTA, maintained practically a value of 31 ppm in their chlorophylls content, during 180 days of storage. There is a moderate difference (level of 0.10) between means of lot I (blank), and lots III and IV. At the end of the experiment, lot I blank presented a loss of 3 ppm of its original chlorophylls, that is 9% of the original content. Pheophytins concentration increased in lots I and II during storage whereas in lots III and IV, pheophytins remained essentially constant (2 ppm); this can be seen in Fig. 6. There is a significant difference, at a level of 0.01, when the blank and lots III and IV are compared.

It is well known that chlorophylls and carotenoids are among the main pigments in avocados. Literature reports 25 ppm of chlorophyll in the avocado Hass (Hulme 1971). A chlorophyll content of 31 ppm was found in this work. Chlorophylls impart a brilliant green color to avocado flesh, which is lost when degradation of the pigment occurs. Chlorophylls degradation is known to involve one or more of the following reactions: (1) a replacement of magnesium of chlorophylls by hydrogen to form pheophytins, (2) hydrolysis catalyzed by chlorophyllase and (3) oxidation reactions to give uncolored products. On the other hand, in the present work, values of pH show a reduction in all samples from 6 to 5.1, during the first two months of storage (Fig. 7); this is due to an increase of

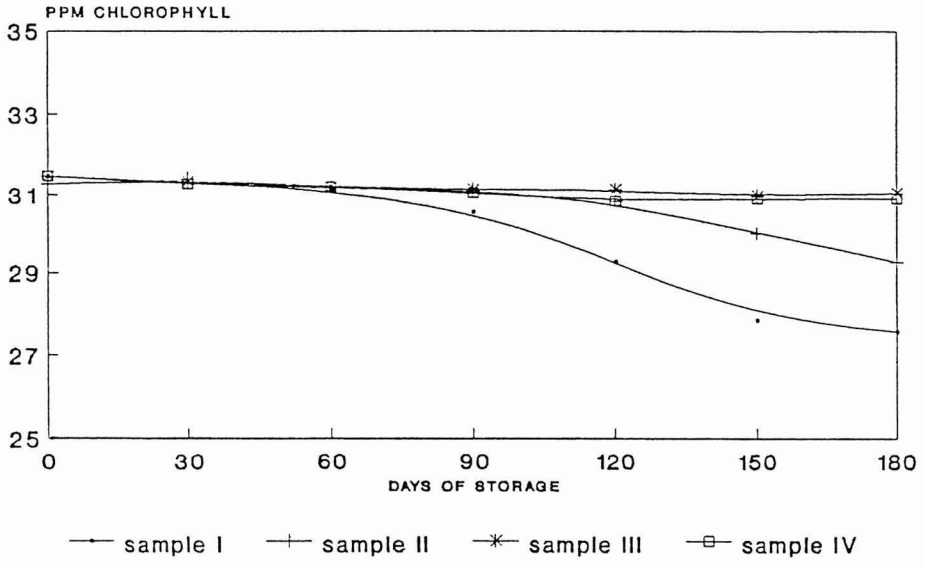

FIG. 5. CHLOROPHYLL IN AVOCADO PUREES

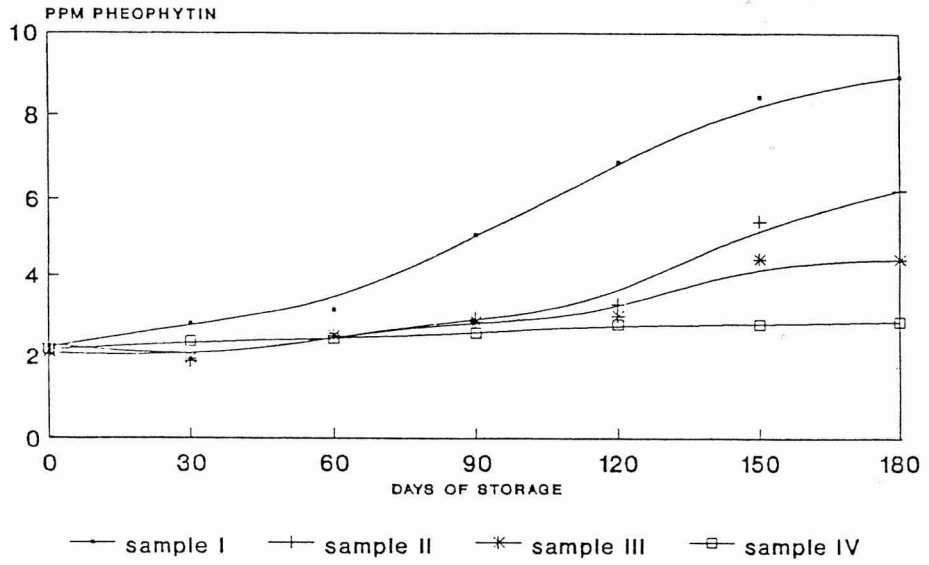

FIG. 6. PHEOPHYTIN IN AVOCADO PUREES

hydrogen that might replace the magnesium of chlorophylls and cause a change in color. This statement is supported given the results presented in Fig. 2 and 3 in which a decrease in spectral reflectance in all samples, during the first two months of storage can be observed. Conversion of chlorophylls to pheophytins began to be significant after pH diminished to a value of 5.1.

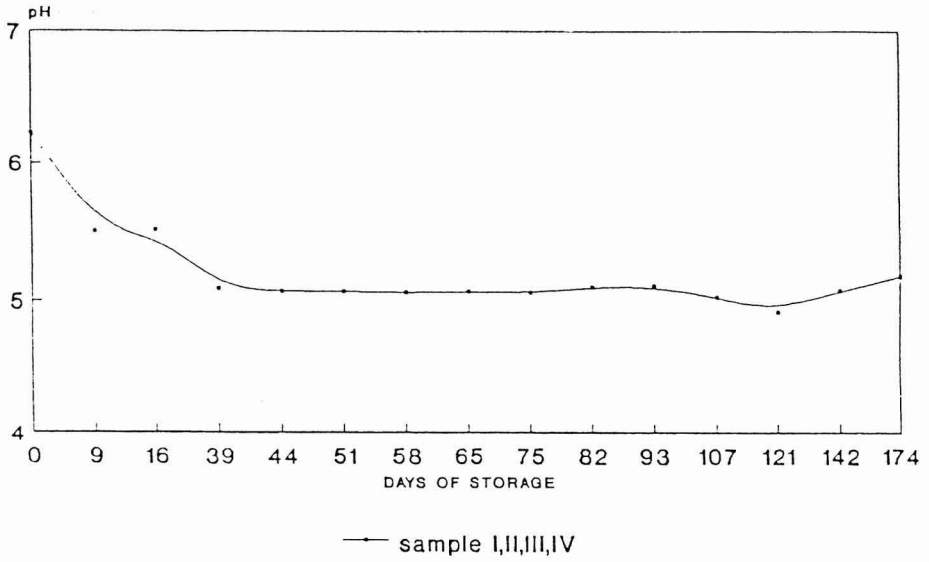

FIG. 7. CHANGE IN pH DURING STORAGE

Buckle and Edwards (1970), reported that changes in pH is a key factor affecting color at High Temperature-Short Time processed-green pea puree.

Peroxides

Variations in Peroxide index during time of storage, were very similar for the four lots, due to the fact that they contained the same antioxidant concentration. The graphs of Fig. 8, show two peaks in the peroxide index: one appears approximately at 40 days of storage and the other at 120 days. This can be explained considering peroxides are intermediate products in the oxidation of lipids, and that antioxidants used were not effective enough to prevent oxidation of fatty acids. Gomez and Bates (1970), reported a logarithmic relationship between the peroxide index and storage time in air, of freeze dried avocado puree. In this work no correlation between peroxides and contents of chlorophylls and pheophytins was found. Nevertheless we observed that when peroxides show maximum values at 40 and 120 days of storage there is a decrease in green reflectance of samples with no EDTA (Fig. 2 and 8). This suggests that oxidation reactions and peroxides could also have an effect on reflectance, apparently affecting other substances than chlorophylls.

Microbiological Analysis

Molds were practically absent. The initial content in puree of the four lots were between 0 to 20 colonies/g. After sixty days of storage, this figure remained unchanged.

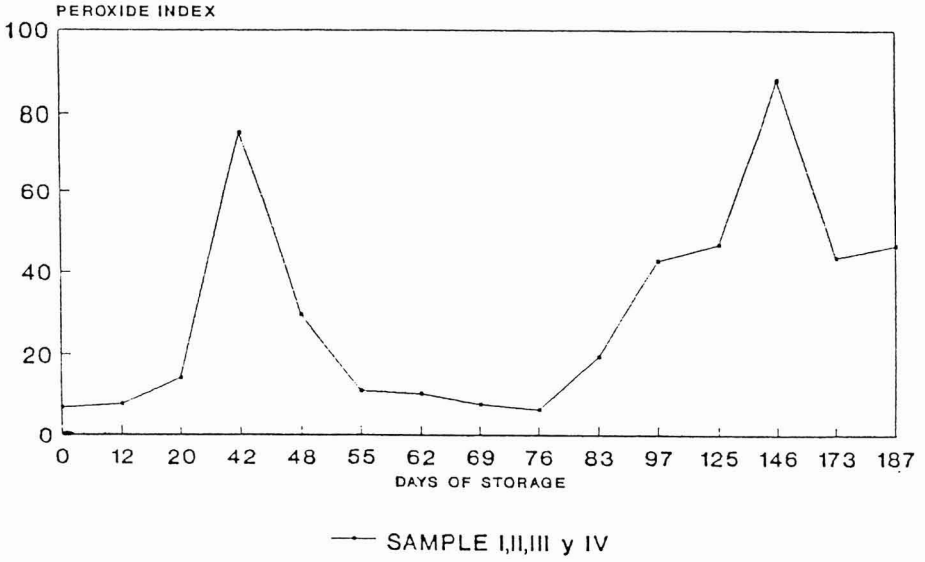

FIG. 8. PEROXIDE INDEX OF THE AVOCADO PUREES

The initial yeast count in the four lots varied from 280 to 360 colonies/g. After forty days of storage, yeasts were not detected. As it was expected, EDTA concentration had no influence on the growing of microorganisms and the presence of benzoate and sulfites as well as storage at low temperature decreased the yeast count to a value of zero after 40 days.

Considering the results of this work, it can be concluded that EDTA mixed with avocado puree in a concentration of 300 and 350 ppm, helps to prevent change of the original green color, compared with a blank with no EDTA. Chlorophyll content is practically constant in lots containing 300 and 350 ppm and the concentration of pheophytins in avocado purees did not increase at those concentrations.

EDTA has been used to control degradative reactions catalyzed by trace metals in foods (Gupta and Gangulli 1980). The stabilization of the original color in salad dressings, prevention of discoloration in retorted whole egg (Song and Cunningham 1985), and maintaining the stability of the buffalo casein micelles, are examples of this. In the present work, EDTA prevents change of color in avocado puree during storage, probably forming complexes with trace metals, that catalyze different reactions such as replacement of magnesium in chlorophylls. This helps to stabilize chlorophylls and prevents the formation of pheophytins.

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TRAY-DRYING OF CAROTENOPROTEINS RECOVERED FROM LOBSTER WASTE

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ABSTRACT

Drying characteristics of carotenoproteins were evaluated in an air-drier using air temperature (45, 55 and 65°C) and relative humidity (5 and 15%) as main factors. The study indicated that the carotenoprotein slurry exhibited a short constant rate period drying followed by classical diffusion drying. Higher temperatures achieved faster drying rates, but adversely affected its nutritional composition and/or quality. The proximate composition of carotenoprotein air dried at 45°C and relative humidity of 5% or 15% were comparable to that obtained by freeze drying.

INTRODUCTION

Enhancing the value of food processing waste for use as food and/or feed supplement has intrigued food technologists for many years. Crustacean processing waste is a rich source of protein, pigment and flavor compounds which may be recovered for use as feed supplement for farmed salmon, trout or lobster. Various methods have been devised to use crustacean wastes as fish feed. One method is to directly incorporate fresh or frozen offal in the diet (Saito and Regier 1971). Although pigmentation of fish flesh is achieved by this approach, the procedure has several disadvantages (eg., variable pigment levels, the tendency of the raw offal to deteriorate rapidly, bulkiness and high transportation cost, high chitin and ash content). Crustacean offal has also been processed into meals to reduce bulkiness, transportation costs and instability of raw offal, however the product is not well suited for incorporation into fish diets because

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of its low pigment content as well as high calcium and chitin levels (Lambertsen and Braekkhan 1971). Extraction of carotenoid pigment with organic solvent or soy oil reduces both the ash and chitin levels and achieves a good recovery of pigment, but the product thus obtained is devoid of protein, thereby decreasing the stability of the carotenoid pigments to oxidation (Spinelli *et al.* 1974). Recent studies in our laboratory have shown that both pigment and protein can be recovered from shrimp waste as a stable complex by extraction techniques which employ chelating agents like EDTA and with aid of trypsin (Simpson and Haard 1985). This method overcomes several disadvantages associated with traditional extraction procedures and recovers a product enriched in protein and carotenoid pigments, as well as depleted of anti-nutrients such as chitin and ash.

The objectives of this study were to determine the suitability of the trypsin-aided extraction process for recovery of carotenoprotein from lobster waste and to evaluate the quality of the product obtained by simple drying techniques such as tray drying, in relation to the high quality product obtainable from the more expensive freeze-drying process.

MATERIALS AND METHODS

Raw Material

Cooked lobster waste comprising of head, carapace and soft tissue were obtained from Westmorland Fisheries Ltd and stored at −80°C. The frozen lobster waste was thawed at 4°C overnight, rinsed in tap water and dried in a fumehood. The dried material (dried lobster waste) was then chopped into small pieces using a mincer (Reliance Electric Co.) and stored at −80°C prior to extraction of carotenoprotein.

Extraction of Carotenoprotein

Carotenoprotein was recovered from dried lobster waste using a modified trypsin aided process (Simpson and Haard 1985), schematically shown in Fig. 1. The ground sample (1.5 kg) was mixed with three volumes of 0.5 M trisodium EDTA (pH 7.7) and homogenized with a Hobart mixer. About 1.5 g trypsin was added to the homogenate to facilitate extraction of carotenoprotein at 4°C for 18 h with continuous stirring. The digested slurry was passed through a screen of 120 mesh and adjusted to pH 7.5 with 2N HCl and made to 45% saturation with solid ammonium sulfate. Carotenoprotein was recovered after centrifugation at 5000 × g and 4°C for 35 min. The pellet thus obtained was dissolved in two volumes of 5mM phosphate buffer (pH 7.0, 4°C) and dialyzed for 18 h against three changes of 8 L of the same buffer at 4°C. The dialyzed carotenoprotein was centrifuged once more to remove the major portion of water and stored at −20°C prior to freeze or tray-dehydration.

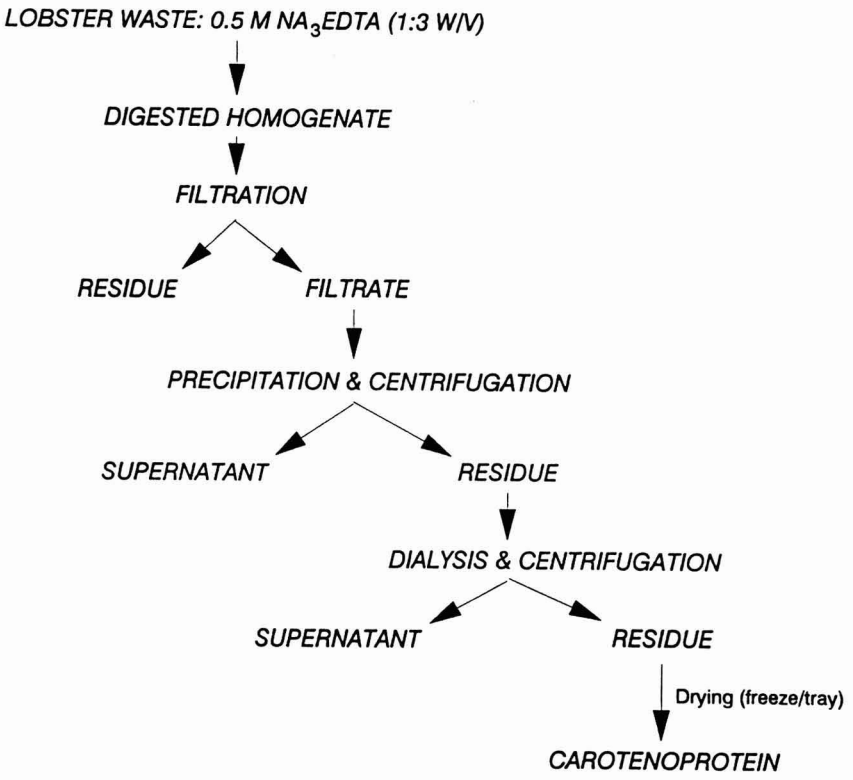

FIG. 1. FLOW-CHART FOR RECOVERY OF CAROTENOPROTEIN FROM LOBSTER WASTE

Freeze-Dried Control

The recovered carotenoprotein from the crustacean waste was freeze-dried to obtain a high quality product to use as control for comparing the quality of tray-dried product.

Design of the Tray Drier

A cross-flow tray dehydration technique employing air at a controlled temperature and relative humidity was used. The tray drier was a modified form of a simple home drier (Equi-Flow Food Dehydrator, Marysville, WA) as shown schematically in Fig. 2. The drier required an initial start-up time of about two hours to achieve stable conditions following which it was possible to control the setpoint temperature to $\pm$ 1°C. The relative humidity of the drying air was controlled by saturating the incoming air at an appropriate temperature. For this purpose, the incoming air was sprayed with water at a temperature predetermined

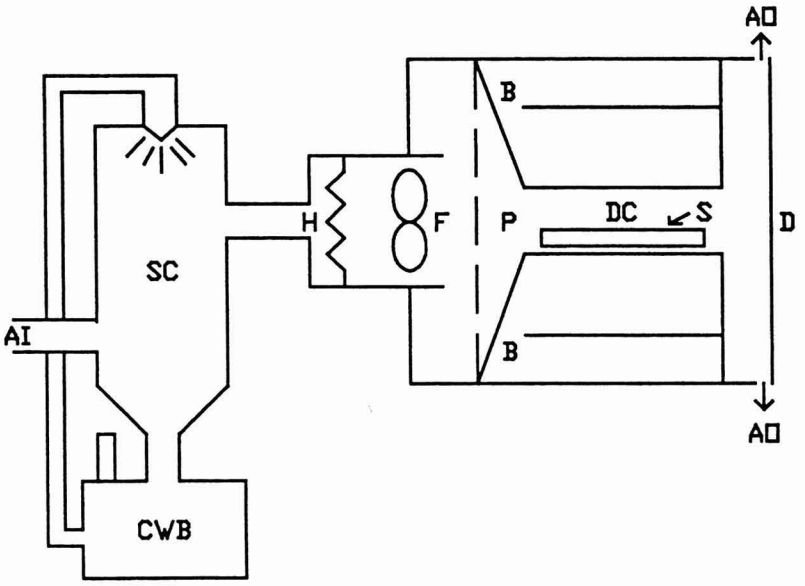

FIG. 2. DIAGRAM OF THE TRAY DRIER

AI	Air inlet	SC	Spray chamber
CWB	Circulating water bath	H	Heater
F	Fan	P	Perforated plate
B	Blocking plenum	DC	Drying chamber
AO	Air Outlet	D	Door
S	Sample tray		

using a psychometric chart which upon heating to the drying temperature, would give the desired relative humidity. For example, in order to maintain a condition of 65°C and 15% relative humidity, the incoming air was sprayed with water at 28°C to saturation. A circulating water bath equipped with a temperature controller was used to control water temperature as well as to spray it in the designated chamber. Drying was carried out only on one tray and the air was directed toward this tray by using a blocking plenum as shown in the figure. The air velocity over the sample tray was measured using an air velocity meter (Thermo-System Inc.). The measured average air velocity was 0.2 m/s.

Drying Procedure

Prior to the start of each drying run, a 100 g sample of the recovered carotenoprotein slurry was removed from the freezer and left overnight at 4°C to thaw. For each run the equipment was stabilized for 2 h at the specified conditions. The slurry was spread uniformly on a nonstick metal tray (25 × 17 × 1.5 cm) and placed in the drying chamber. The sample tray was weighed every

30 min (with an additional weighing after the first 15 min) and quickly returned to the drier to monitor the progress of drying. Drying was continued until the weight of sample remained relatively constant.

Experimental Conditions

The experimental conditions consisted of three different temperatures (45, 55 and 65°C) and two levels of relative humidity (5 and 15%). Three replicate runs were carried out for each test condition.

Analyses

The air temperature was measured with a digital thermometer (HI 8053, Hanna Instruments Inc., Woonsocket, RI) and relative humidity was measured with a digital hygrometer (HI 8564, Hanna Instrument Inc., Woonsocket, RI).

Initial moisture content was determined by drying in a vacuum oven at 70°C for 24 h. The equilibrium moisture contents (Me) were obtained after drying for 24 h under respective drying conditions.

Moisture content, total nitrogen, crude fat and ash were determined using standard AOAC procedures (AOAC, 1980). Crude protein was determined from Kjeldahl analysis for total nitrogen and multiplying results obtained by a factor of 6.25 to convert crude protein-N to crude protein. Ash was determined by burning samples at 600°C, while carbohydrate was estimated by difference.

Chitin was determined by the method of Spinelli *et al.* (1974) and total astaxanthin was determined by the method of Saito and Regier (1971) as modified by Simpson and Haard (1985).

Drying curves were prepared by plotting the residual moisture ratio $[(M - Me)/(Mi - Me)]$ versus drying time (t), where Mi and Me were the initial and equilibrium moisture contents (dry basis) and M was the moisture content at time t. The constant rate drying parameters were obtained from the early drying period data which showed a linear relationship between the moisture ratio and drying time (usually the first 30 min). The critical moisture content (Mc) which represented the end point of the constant rate drying period (tc) and the beginning of the falling rate drying was obtained from the above curve. The falling rate period drying parameters were obtained from a plot of $\ln [(M - Me)/(Mc - Me)]$ versus falling rate drying time.

RESULTS AND DISCUSSION

Dried Lobster Waste versus Freeze-Dried Carotenoprotein

Proximate compositions of dried lobster waste and freeze-dried carotenoprotein are summarized in Table 1. The freeze-dried product was enriched in protein by

TABLE 1.

PROXIMATE COMPOSITION OF DRIED LOBSTER WASTE AND FREEZE-DRIED CAROTENOPROTEIN EXTRACT

Component	Dried Lobster Wastes (% wb)	Carotenoprotein Extract (% wb)
Crude protein	34.5±0.88	60.1±0.58
Protein*	30.3±0.03	57.5±0.25
Crude fat	2.2±0.17	14.0±2.15
Ash	45.8±0.31	16.8±0.03
Moisture	7.9±0.40	5.6±0.03
Carbohydrate	13.8±0.91	6.1±2.46
Chitin	28.1±0.61	5.9±0.03

* after correction for non-protein nitrogen

about 90%, in crude fat by 536% as compared with the dried lobster waste and also had low ash and chitin levels.

Effect of Drying Conditions On Moisture Removal

The average moisture content of carotenoprotein prior to drying was 82% (wet basis). The drying conditions employed are detailed in Table 2. Typical constant rate and falling rate period drying curves at the three temperatures and two relative humidity levels are shown in Fig. 3 and 4. Within the range of different parameters studied, temperature was the major factor influencing the rate of moisture removal during both constant and falling rate period drying. Higher temperatures produced steeper curves (faster removal of moisture) than lower temperatures. The drying rate was also faster at the lower 5% relative humidity as compared with 15%.

The rate of drying remained constant during the first half-hour but decreased steadily thereafter. During the constant rate period, drying curves could be described by the linear model:

$$(M_t - M_e)/(M_i - M_e) = A - B.t; t < tc$$

TABLE 2.

DRYING CONDITIONS AND DRYING RATE PARAMETERS

Run	Temp.	RH[a]	Mc[b]	Regression details			
				Constant rate[c]		Falling rate[d]	
	(°C)	(%)	(%wb)	B(h^{-1})	R^2	D(h^{-1})	R^2
1	45	5	77.3	0.563	1.00	1.819	0.99
2	45	5	77.4	0.558	1.00	1.541	0.98
3	45	5	77.0	0.566	1.00	1.482	0.97
4	55	5	75.3	0.705	1.00	3.882	0.99
5	55	5	74.5	0.738	1.00	2.760	1.00
6	55	5	75.0	0.734	1.00	3.157	1.00
7	65	5	78.3	0.899	1.00	5.107	1.00
8	65	5	78.3	0.896	1.00	3.630	1.00
9	65	5	78.1	0.872	0.98	3.419	1.00
10	45	15	59.5	0.492	1.00	1.810	0.97
11	45	15	56.8	0.507	1.00	1.488	0.98
12	45	15	57.1	0.504	1.00	1.340	0.91
13	55	15	80.5	0.636	0.99	2.451	0.90
14	55	15	79.9	0.632	1.00	2.046	0.97
15	55	15	80.0	0.659	1.00	2.056	0.99
16	65	15	79.4	0.740	1.00	2.473	1.00
17	65	15	80.0	0.731	1.00	3.188	0.99
18	65	15	79.5	0.730	1.00	2.955	1.00

[a] = Relative Humidity ; [b] = Critical Moisture Content

[c] = (M-Me)/(Mi-Me) = A - Bt; $t < t_c$ [d] (M-Me)/(Mc-Me) = C e^{-Dt_f}

A = C = 1.0; t_c = constant rate drying period; t_f = falling rate drying time.

where the intercept coefficient A was essentially the initial moisture ratio, 1.0 and the slope coefficient B was constant rate period drying rate. During the falling rate period, the drying curves could be described by the conventional diffusion-drying equation:

$$(M_t - M_e)/(M_c - M_e) = C\, e^{-D.t_f}$$

where C, the intercept coefficient representing the critical moisture ratio, 1.0 with t_f expressed in falling rate drying time, and D, the slope coefficient representing the falling rate period drying rate, were parameters dependent on the drying conditions. These regression parameters, the R^2 and the critical moisture content below which the product undergoes a falling rate drying, are given in

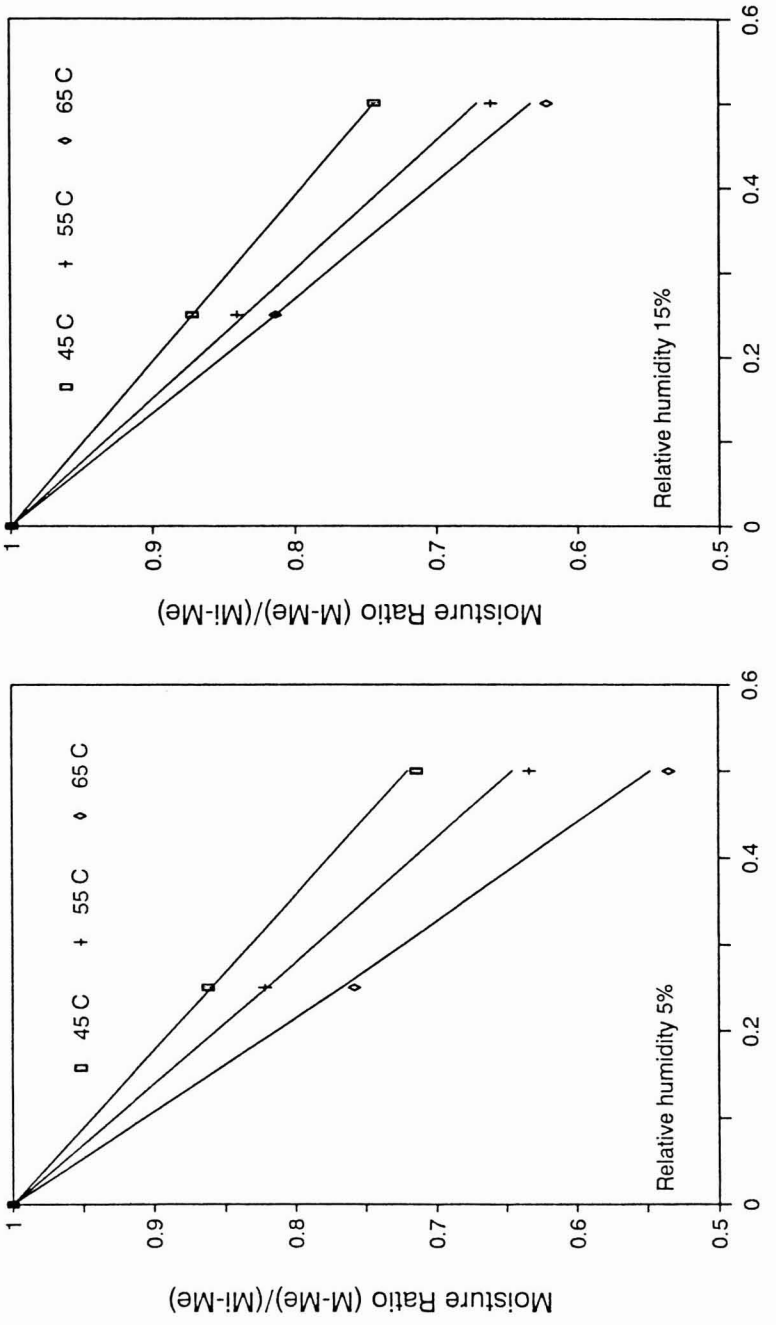

FIG. 3. CONSTANT RATE DRYING CURVES AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES

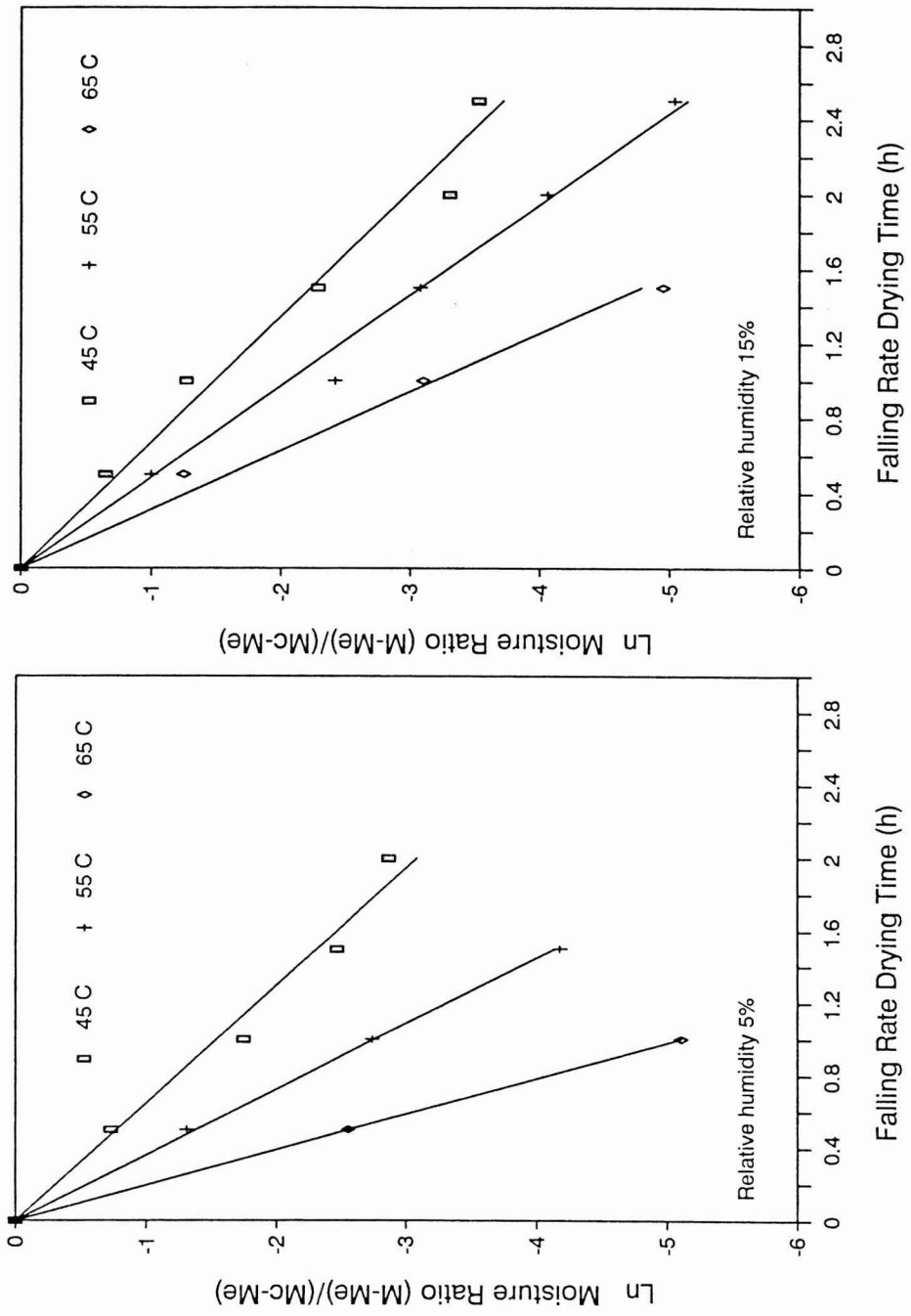

FIG. 4. FALLING RATE DRYING CURVES AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES

Table 2. The relatively high R^2 values found confirmed the diffusion controlled drying during the falling rate period. The pattern of drying coefficients (rates) in both constant and falling rate period drying were consistent with higher temperatures and lower relatives humidities favoring faster drying (Table 2; Fig. 3 & 4).

Composition and Carotenoids in Dried Samples

Proximate composition of tray-dried carotenoprotein is presented in Table 3. The protein and carbohydrate content (wet basis) of the carotenoprotein prepared by tray drying at various temperatures were about 60–80% and 520–570% higher than those in the dried lobster waste. The product prepared at 45°C was more comparable in quality with the freeze-dried product than samples prepared at the higher temperature.

TABLE 3.

PROXIMATE COMPOSITION OF CAROTENOPROTEINS TRAY-DRIED* UNDER DIFFERENT CONDITIONS

Component	Relative humidity (%)	Drying Temperature (°C) 65	55	45
Protein**	5	49.3±0.2	49.7±0.2	48.1±1.2
	15	48.3±0.4	49.8±0.6	48.1±0.8
Crude fat	5	15.1±0.8	14.0±0.9	14.9±0.5
	15	12.7±0.7	14.3±0.7	14.9±0.5
Ash	5	19.9±0.3	13.2±0.6	12.8±0.9
	15	14.0±0.2	12.8±0.8	13.2±0.2
Moisture	5	9.5±1.4	10.9±0.7	18.9±2.0
	15	11.4±0.4	13.4±0.5	15.0±0.5
Carbohydrate	5	12.2±1.6	12.2±1.8	5.3±3.1
	15	13.6±0.6	5.9±2.13	8.8±0.2

* Drying times: 65°C - 5% RH, 2 h; 15% RH, 2.5 h
55°C - 5% RH, 2.5 h; 15% RH, 3 h
45°C - 5% RH, 3 h; 15% RH, 3.5 h

** after correction for non-protein nitrogen

The quantity of astaxanthin, the principal carotenoid color compound, of lobster wastes, freeze and tray-dried carotenoproteins are shown in Table 4. The level of the carotenoid pigment associated with freeze-dried carotenoprotein prepared by trypsin-aided process (freeze-dried sample) was approximately 3 times that of the dried lobster waste. A similar level of the carotenoid pigment was found with tray-dried product obtained at 45°C while the samples dried at 65°C and 55°C had relatively lower levels of carotenoid pigments. Thus high drying temperature had a detrimental effect on total astaxanthin content of carotenoprotein. However, even the lower pigment levels associated with products dried at 65°C or 55°C were still higher than the levels associated with the raw material.

These drying rate versus quality (carotenoid color and nutrients) analyses indicate that although higher temperatures promote faster drying rates, they are not the best choice for obtaining high quality carotenoprotein. In fact the opposite was observed; the product dried at the lower temperature of 45°C retained better color and quality than the product dried at higher temperatures.

CONCLUSIONS

The results of the present study demonstrated that carotenoprotein extracted from lobster wastes by a trypsin aided process could be converted into a stable,

TABLE 4.

TOTAL ASTAXANTHIN CONTENT OF DRIED LOBSTER WASTE, FREEZE DRIED (CONTROL) AND TRAY-DRIED CAROTENOPROTEINS

Treatment	Total Astaxanthin (μg/g)
Dried Lobster Waste	97±1
Freeze-dried Product	343±3
Tray-dried Product (45°C, 5% RH)	316±11
Tray-dried Product (45°C, 15% RH)	295±27
Tray-dried Product (55°C, 5% RH)	235±20
Tray-dried Product (55°C, 15% RH)	98±20
Tray-dried Product (65°C, 5% RH)	219±26
Tray-dried Product (65°C, 15% RH)	115±19

high quality from by tray drying. Based on this study tray drying may be used as a more economical method of dehydrating carotenoprotein from crustacean wastes for use as a feed ingredient for cultured fish instead of the relatively more expensive process of freeze-drying.

ACKNOWLEDGMENTS

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SELECTED CHARACTERISTICS OF EXTRUDED BLENDS OF MILK PROTEIN RAFFINATE OR NONFAT DRY MILK WITH CORN FLOUR[1]

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ABSTRACT

Various levels of milk protein raffinates (MPR) containing different concentrations of lactose and nonfat dry milk powders (NFDM) were co-extruded with corn flour. The extrusion experiments were performed on a twin-screw extruder at 100°C, 125°C and 150°C (last section temperature). Differential scanning calorimetry (DSC) results indicated that the starch in extrudates was completely gelatinized. Lower processing temperatures resulted in decreased browning, while the level of lactose in the raffinates did not appear to cause a significant difference in product browning. Extrudate expansion ratio decreased at levels higher than 5% NFDM or MPR. Also, as the MPR content increased, to a certain level, the breaking strength of extrudates increased and beyond that the trend was reversed. The level and type of milk protein incorporated and extrusion temperature also affected the water holding capacity, nitrogen solubility index and sorption characteristics of extruded samples. In general, milk proteins appeared to improve the textural properties of the extrudates when used at levels below 5%.

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INTRODUCTION

Snacks, breakfast cereals, pregelatinized flours and starches, pet foods and animal feeds are examples of food and feed products produced by thermal extrusion (Harper 1981; Linko *et al.* 1981). Normally, extruded snack foods do not possess superior nutritional qualities. However, some efforts have been made to fortify corn or wheat flours with milk, casein, soy or meat proteins to produce extruded products that could be used in both developed and developing countries as nutritious snacks (Maga and Lorenz 1978; van de Voort *et al.* 1984; Kim *et al.* 1989a,b). The addition of some proteins to starch based systems such as breakfast cereals and snack products has been found to improve the textural characteristics of the extruded products when incorporated in small amounts. In fact, proteins may reinforce the cell walls of the extrudates, thus improving the level of crispiness of the product (Chang 1986; Kelly 1990; Martinez-Serna and Villota; 1991).

Milk proteins are isolated by several processes including isoelectric precipitation, ultrafiltration, gel permeation, cryoprecipitation (Lonergan 1983); by separation of milk proteins from other milk constituents using membraneless osmosis (Antonov *et al.* 1982) or by selective extraction of lactose (Leviton and Leighton 1938; Leviton 1949). The latter technique is particularly suited for separation of the constituents in nonfat dry milk (NFDM) based on their relative solubility in specific solvents. Hoff *et al.* (1987) improved the procedure for producing milk protein raffinate from NFDM using ethanol-water mixtures.

The overall objective of this study was to evaluate the performance of milk proteins in the presence or absence of lactose in a starch-based system. Thus, various characteristics of coextrudates of corn flour and milk proteins were monitored. The milk proteins tested were NFDM and protein raffinates generated by ethanol extraction of lactose from NFDM.

MATERIALS AND METHODS

Corn Flour (CF)

The CF used in extrusion experiments was supplied by J. R. Short Milling Co., Kankakee, IL. The flour was of fine snack meal grade with a typical particle size distribution of $>$ 99% passing through US #20 (841 micron) sieve and at least 99% retained on US #40 (420 micron) sieve.

Milk Protein Samples

Nonfat dry milk was produced at a low temperature heat-treated and was obtained from Dairy Farm Product's, a division of Milk Marketing, Inc., Goshen, IN. The whey protein nitrogen content was 6.7 mg/g. Milk protein raffinate

(MPR) was produced by ethanol extraction of lactose from NFDM on a pilot plant scale (Hoff *et al.* 1987). Basically, the procedure for producing MPR consisted of stirring weighed amounts of NFDM into 95% ethanol, followed by addition of a calculated amount of water corresponding to a solvent-to-solid ratio of 15:1 (w/v) and a solvent composition of 72.9% ethanol (w/w) at 25°C. These extraction conditions were found to give optimal separation of protein and lactose on laboratory scale experiments (Hoff *et al.* 1987). The total suspension was rapidly separated in a stainless steel basket centrifuge (Western States Co., Hamilton, OH). The raffinate was washed with the extracting solvent mixture (5 times weight of NFDM) and allowed to dry at room temperature followed by oven drying at 50°C overnight. Finally, the dried raffinate samples were ground to a size of approximately 250 microns in a hammer mill.

Extrusion Equipment

A Werner Pfleiderer ZSK-30 twin-screw extruder (Werner Pfleiderer Corp., Ramsey, NJ) with high shear co-rotating intermeshing screws at a constant speed of 450 rpm was used. The raw material (blends of corn flour and milk protein) was introduced into the feed zone by means of a K-Tron Model T35 twin-screw volumetric feeder (K-Tron Corp., Glassboro, NJ) as a single premix stream, with flow rates regulated by a K-Tron Series 6300 digital speed controller. Water was added into the feed zone with a Masterflex pump (Cole-Palmer Instrument Co., Chicago, IL) to adjust the moisture content of the material undergoing extrusion. Other details of the equipment used were given by Hawkes and Villota (1987).

The extruder barrel contained independently controlled heating and cooling sections. Each section was electrically heated and cooled by water. The barrel temperature was regulated by a temperature controller (Barber-Coleman Co., Lovespark, IL) at five different points along the length of the extruder. The barrel temperatures were set at 35°C (first section), 50°C (second section), 75°C (third section), 90, 110 or 125°C (fourth section), and 100, 125 or 150°C (fifth section). The die consisted of two circular openings 3.0 mm in diameter.

Extrusion Tests

Snack-food type products were produced by extrusion of CF, either alone with MPR or with NFDM. Raffinate 1-A and 1-B were incorporated at 5, 10, and 20% (w/w) levels, and rafinates 2, 3, and 4 were incorporated only at the 5% level (Table 1). Extrusion tests were carried out for each composition.

Chemical Analysis of Raffinate

Lactose in the raffinate was estimated by measuring total carbohydrates according to the method of Dubois *et al.* (1956). Protein content was measured

TABLE 1
CHEMICAL ANALYSIS OF RAFFINATE AND NFDM SAMPLES USED IN EXTRUSION TESTS[a]

Sample	Moisture (%)	Ash (%)	Lactose (%)	Protein (%)	Total (%)
Raffinates					
1 - A	8.09 ±0.05	10.96 ±0.03	1.35 ±0.05	81.14 ±0.72	101.54
1 - B	8.64 ±0.02	11.04 ±0.03	0.95 ±0.03	81.43 ±0.33	102
2	6.55 ±0.06	11.11 ±0.02	3.53 ±0.01	80.33 ±0.76	101.52
3	6.69 ±0.09	10.27 ±0.00	7.16 ±0.08	75.42 ±0.85	99.54
4	5.95 ±0.06	8.89 ±0.01	20.93 ±0.53	65.68 ±0.68	101.45
NFDM	3.63 ±0.02	7.73 ±0.00	54.71 ±0.84	37.18	103.25

[a]Moisture, ash, lactose, and protein values are expressed as the mean of at least triplicate determinations ± standard deviation.

by the micro-Kjeldahl method, using a factor of 6.25 to convert % N to % protein. The moisture content of samples was determined by both the Karl Fisher method and by drying overnight (18 h) in a vacuum oven at 70°C, 686 mm Hg vacuum. Ash content was determined using a muffle furnace at 550° overnight (18 h).

Residual ethanol content of raffinates was determined by an enzyme assay kit (Ethanol UV-method, No. 176290, Boehringer Mannheim Corp., Indianapolis, IN) which utilized alcohol dehydrogenase and aldehyde dehydrogenase. Samples for the enzymatic determination of ethanol were prepared by treatment with a Carrez solution. Two moles of NADH are generated per mole of ethanol present and the absorbance of NADH is measured at 340 nm.

Chemical Analysis of Extrudate

All the samples were ground in water-cooled mills. Methods for the determination of moisture, protein, ash and residual ethanol content were the same as those used for raffinate samples. The carbohydrate content (% w/w) of the extruded samples was determined by differences between 100 and the sum of protein (%), ash (%), fat (%) and moisture (%).

Extent of Starch Gelatinization in the Extrudate

The starch gelatinization endotherm was measured with a DuPont Thermal Analyzer (Model 1090, DuPont Co., Wilmington, DE). The sample was prepared by finely grinding the extrudate and mixing it with 30% water. Tests were carried out by placing 20 mg of the sample in an aluminum pan which was then hermetically sealed.

The energy required for gelatinization was obtained by measuring the area under the endotherm with a planimeter. The extent of gelatinization was calculated from Eq. (1):

$$Eg = [1 - (D H_s - D H_c] \times 100$$

Color of Extrudates

The color of ground extrudates was measured by two methods: (1) colorimetric using a Hunter D25L colorimeter (Hunter Associates Lab., Inc., Reston, VA), and (2) spectrophotometric using a Spectronic 20 (Milton Roy Co., Rochester, NY) at 450nm, measuring the color of a trichloroacetic acid filtrate of the trypsin hydrolyzed mixture (Choi *et al.* 1949).

Sorption Characteristics of Extrudates

Extrudate strands were first crushed by a mortar and pestle and then ground in a mill. The initial moisture content of samples was measured by drying the samples overnight in a vacuum oven at 70°C and 686 mm Hg vacuum.

A small amount of each sample was placed in preweighed aluminum dishes and weighed accurately. The sample dishes were then placed in air-tight desiccators containing saturated salt slurries to provide a constant relative humidity environment. After three weeks, the samples were taken from the desiccators and weighed. The final moisture content (g H_2O/g solid) of the samples was calculated from their initial moisture content and the weight change after three weeks exposure to a known relative humidity environment.

Breaking Strength of Extrudates

Textural characteristics of extruded strands, dried in a vacuum oven at 70°C for 1 h, were measured using an Instron Universal Texture Analyzer. Representative samples were placed on a U-shaped angle iron and broken by a 9.5 mm diameter rod, using a 10 cm/min cross-head speed.

Expansion Ratio

The average diameter of extrudates was calculated from ten measurements taken with a Vernier caliper. Expansion ratio of extruded samples was calculated by dividing the average diameter of extrudates by the diameter of the die orifice.

Nitrogen Solubility Index (NSI)

NSI of extrudates was determined in triplicate by standard methods (Method 46-23, AACC 1983).

Water Holding Capacity (WHC)

WHC was measured on triplicate samples by a modified procedure of Quinn and Paton (1979).

Water Solubility Index (WSI)

WSI was determined by the method of Anderson *et al.* (1969).

RESULTS AND DISCUSSION

Proximate Analysis

A chemical analysis of raffinate samples used for extrusion is given in Table 1. Samples of raffinate for extrusion runs were grouped based on their lactose content: (1-A) 0–2.5%; (2) 2.6–4%; (3) 4.1–10%; and (4) 10.1–27.5% lactose. An additional raffinate sample with a lactose content of 0.95% (Table 1, sample 1-B) was also prepared for extrusion.

The results of chemical analysis of extruded samples from the first test are shown in Table 2. None of the samples showed any detectable amount of ethanol even though the MPR had up to 4.5% ethanol (data not shown). The residual moisture content of samples varied from approximately 9% to 12% (wb). The protein content of extrudates increased with increasing proportions of either NFDM or MPR, with a greater increase for MPR due to its higher protein content.

Extrudate Characteristics

For all the conditions studied, differential scanning calorimetry results indicated that complete starch gelatinization occurred even at the lowest extrusion temperature of 100°C in the fifth section (data not shown). In a study of wheat flour extrusion, Chiang and Johnson (1977) had also found that starch was completely gelatinized above an extrusion temperature of 110°C in a single-screw extruder. Thus, it is expected that differences in the characteristics of the

TABLE 2.
CHEMICAL ANALYSIS OF EXTRUDATES[a]

Sample Composition	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate[b] (%)
CF	11.55 ±0.83	0.59 ±0.03	7.72 ±0.34	0.29 ±0.11	79.85
CF + 5% NDM	8.99 ±0.10	0.96 ±0.042	9.53 ±0.27	0.24 ±0.022	80.28
CF + 10% NDM	11.19 ±0.97	1.28 ±0.041	11.16 ±0.42	0.34 ±0.06	76.03
CF + 20% NDM	11.08 ±0.58	2.05 ±0.05	14.31 ±0.24	0.33 ±0.12	72.23
CF + 5% Raffinate 1-A	10.69 ±0.03	1.12 ±0.02	11.58 ±0.46	0.30 ±0.08	76.31
CF + 10% Raffinate 1-A	11.96 ±1.89	1.62 ±0.055	15.17 ±0.29	0.43 ±0.14	70.82
CF + 20% Raffinate 1-A	9.39 ±0.07	2.68 ±0.06	22.90 ±0.12	0.39 ±0.02	64.64

[a]Average of triplicate determination (% wb) ± standard deviation.
[b]Calculated by difference.

extrudates were primarily due to composition and process conditions and not to the extent of starch gelatinization. It is, however, possible that certain levels of dextrinization may have occurred, depending upon the processing conditions. This more complex phenomenon was not monitored due to the extensive analytical procedures required for its evaluation. Nevertheless, in an extrusion study of corn, wheat and rice flours, Mercier and Feillet (1975) found that the starch was not degraded into maltodextrins even at temperature of 200°C. Observed variations in the solubility index may be a result of protein and/or starch fragmentation.

The addition of milk protein affected color (Hunter L-a-b scale) of the extrudates. As higher levels of milk protein were added to the feed, the level of browning increased (i.e., lower L value, higher a value, and lower b value) (data not shown). It was expected that upon incorporation of higher levels of NFDM or MPR, higher levels of lactose and protein were introduced into the system which might have accounted for some increase in browning. Similar results on browning of extrudates from blends of sugar and corn meal have been reported by Hsieh *et al.* (1990). Temperature also clearly influenced color development; browning increased with an increase in the temperature. Differences in levels of browning did not appear to be significant for systems containing MPR compared to NFDM as the protein source.

When the browning data for extrudates from either MPR- or NFDM-fortified CF were compared with those of either casein- or sodium caseinate-fortified CF, it became evident that browning was affected by the protein, lactose and CF levels of the feed (Kelly 1990). It was also evident that milk proteins were more reactive than corn proteins. It is expected that amino acids such as lysine present in the protein fraction, reducing sugars such as lactose in the milk-protein containing systems and reducing sugars from corn starch hydrolysis might have participated in the browning reaction. Cheftel *et al.* (1981) demonstrated that lysine participated in non-enzymatic browning of extrusion cooked biscuits.

Browning results as measured by formation of nonenzymatic reaction product and monitored spectrophotometrically (Fig. 1), exhibited similar trends as those measured on the powders using the Hunter Colorimeter. Information collected using the trypsin method to extract the browning compounds is more reliable since browning compounds are released from the protein component. It should be emphasized that these results corroborate that raffinates that contained relatively low lactose levels resulted in extrudates with significant browning development (NFDM vs MPR at 125°C). This indicates that other reducing sugars

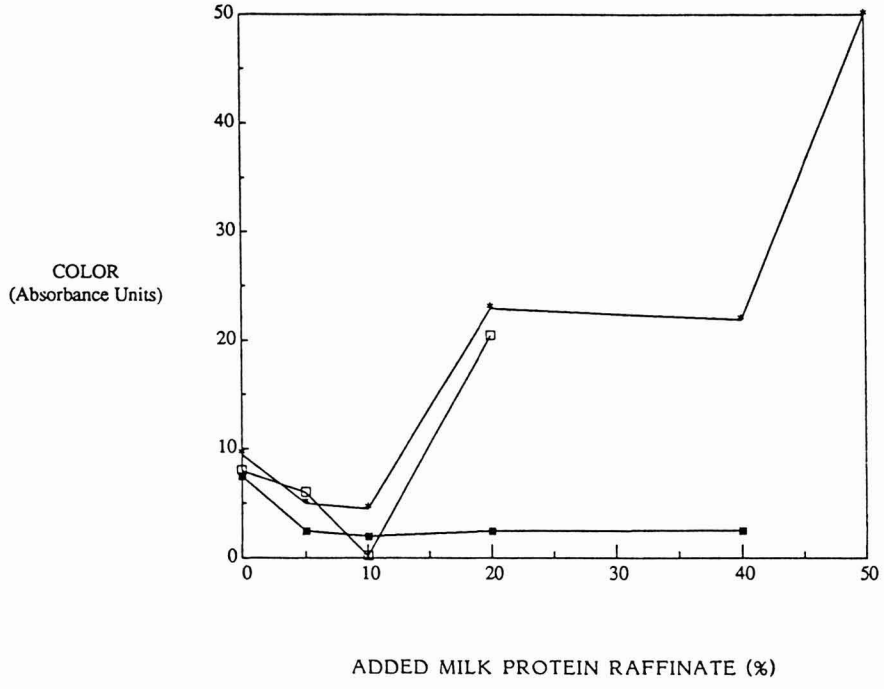

FIG. 1. NONENZYMATIC BROWNING COLOR FORMATION (SPECTROPHOTOMETRIC MEASUREMENT) IN EXTRUDATES AS A FUNCTION OF % ADDED MILK PROTEIN
Nonfat Dry Milk at 125°C (*), Nonfat Dry Milk at 100°C (■),
Milk Protein Raffinate at 125°C (□).

present in the corn meal or generated through starch hydrolysis, in addition to lactose present in the raffinate, contributed to the formation of browning. Variability in the lactose content of the raffinates would partially explain the fluctuation of the data in raffinate-containing extrudates.

Expansion ratio decreased ($P < 0.05$) at levels higher than 5% NFDM or MPR incorporation (Fig. 2). It was expected that enhanced protein-protein interaction at levels higher than 5% would result in lower expansion. A decrease in expansion can be also explained by the fact that the primary structuring agent is the starch present in the corn flour, and thus, as the starch fraction was reduced, expansion was also reduced. At high temperatures such as 150°C (fifth section temperature), a decrease in expansion may be associated with high rates of protein denaturation.

Breaking strength of extrudates was altered by the addition of milk proteins. As the MPR content increased, to a certain level, the force required to break the extrudate increased ($P < 0.05$) and beyond that the trend was reversed (Fig. 3). These textural changes were affected by both the type of protein incorporated and the process temperature. The breaking strength of extrudates processed at

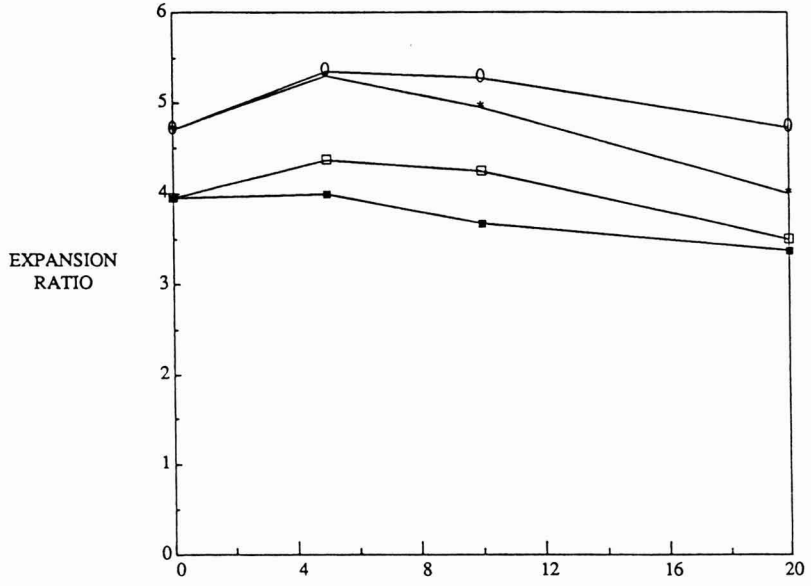

FIG. 2. EXPANSION RATIO OF EXTRUDATES AS A FUNCTION OF % ADDED MILK PROTEIN
Milk Protein Raffinate at 125°C (○), Milk Protein Raffinate at 150°C (□), Nonfat Dry Milk at 125°C (*), and Nonfat Dry Milk at 150°C (■).

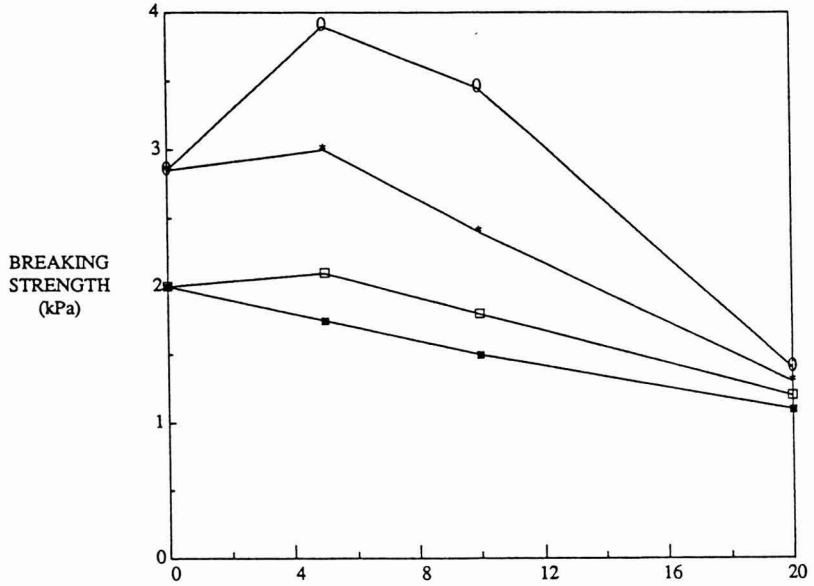

FIG. 3 BREAKING STRENGTH OF EXTRUDATES
AS FUNCTION OF % ADDED MILK PROTEIN
Milk Protein Raffinate at 125°C (○), Milk Protein Raffinate at 150°C (□),
Nonfat Dry Milk at 125°C (*), and Nonfat Dry Milk at 150°C (■).

125°C (fifth section temperature) was greater than that required for those obtained at 150°C (fifth section temperature). Higher temperatures are associated with the higher levels of protein denaturation and starch gelatinization. Higher levels of expansion are normally associated with an increase in the levels of gelatinization, thus resulting in a fragile structure (Fig. 4). Similar results have been reported by Chinnaswamy and Hanna (1988) and Hsieh *et al.* (1990). The protein fraction is expected to reinforce the cell walls, thus resulting in stronger and more expanded extrudates. However, interactions between the protein and the carbohydrate components, as determined by their reactivity and the rates and extent of protein denaturation, control the breaking strength of the extrudates. High rates of protein aggregation or denaturation will lower the reactivity and functionality of the protein. At both 125°C and 150°C, an increase in protein content decreased ($P < 0.05$) the breaking strength of the extrudate (Fig. 3), implying that the primary function of the protein fraction within the 20% level of incorporation, was to reinforce the cell walls of the extrudate. This would prevent collapse of the extrudate and the formation of a compact structure. Above this

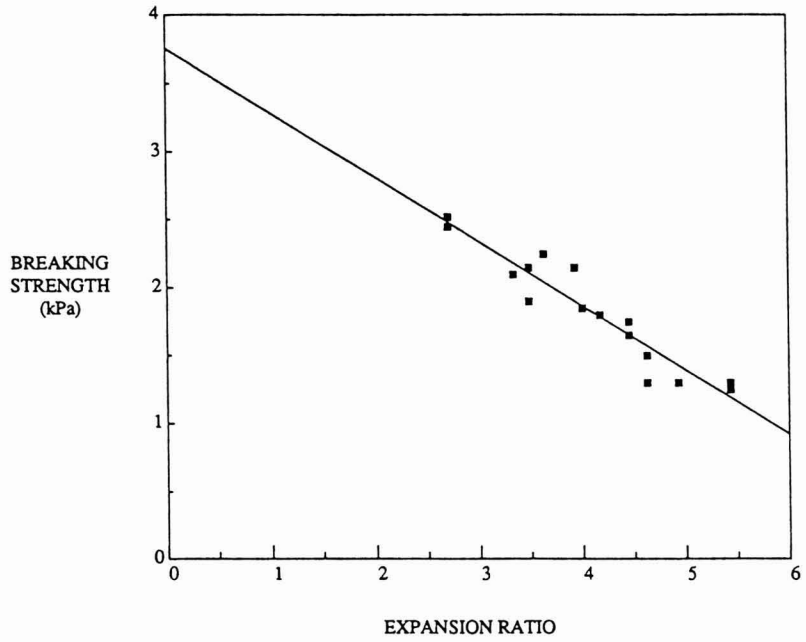

FIG. 4. BREAKING STRENGTH AS A FUNCTION OF EXPANSION RATIO FOR EXTRUDATES MADE FROM VARIOUS BLENDS OF MILK PROTEIN AND CORN FLOUR

level, the breaking strength increased (data not shown), due to more protein-protein interactions. The extent of starch hydrolysis was also expected to affect expansion and structural characteristics of extrudates.

Water holding capacity (WHC) of extrudates decreased with increasing concentration of NFDM in the blends (Fig. 5). These results are expected since the water holding capacity of gelatinized starch is higher than that of proteins. However, a higher WHC was observed with a higher processing temperature, which may be associated with the ability of the extruded sample to imbibe water. It is also possible that high temperatures favored lactose-protein interaction, thus minimizing lactose/starch water competition resulting in increased rates of starch gelatinization. Lower levels of protein/starch interaction would also be expected under these conditions due to rapid protein denaturation. The presence of lactose in the formulations appeared to have a positive effect on the ability of the extrudate to retain water (Fig. 6). The high solubility of lactose and its ability to bind water are possible explanations to the observed trends. The sorption isotherm results (Fig. 7) also support the WHC results which indicate that the presence of lactose in the formulations has a positive effect on the ability of the extrudate to bind water (comparison of isotherms for CF + NFDM vs CF + 20% Raffinate 1-A).

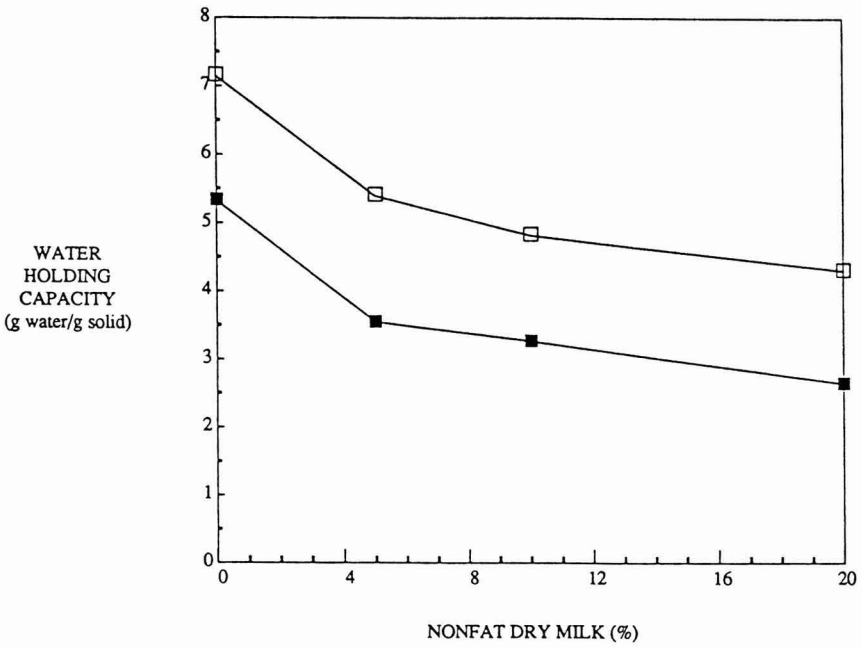

FIG. 5. WATER HOLDING CAPACITY OF EXTRUDATES AS A FUNCTION OF % ADDED NONFAT DRY MILK 125°C (■) and 150°C (□).

A regression analysis of soluble protein in the extrudate indicated that it was positively correlated to the protein, soluble protein, and CF content of the feed (Eq. 2).

$$PS = 1.5\,PC + 0.5\,SP + CF - 109 \qquad (R^2 = 0.92)$$

It is expected that protein-lactose interactions contributed to the decrease in soluble protein in the extruded products. The presence of CF led to higher levels of soluble protein. Similar results have been reported for rice starches. It is speculated that CF, besides providing some reducing sugars that will denature the protein, may also provide a stabilizing effect, and prevent protein denaturation.

The total solubility of the extrudate was significantly affected by the contents of total protein, soluble protein and CF. It was positively correlated with the soluble protein and CF levels, but it was negatively correlated with the protein level (Eq. 3).

$$SM = 0.1\,SP - 0.4\,PC + 0.3\,CF + 23 \qquad (R^2 = 0.98)$$

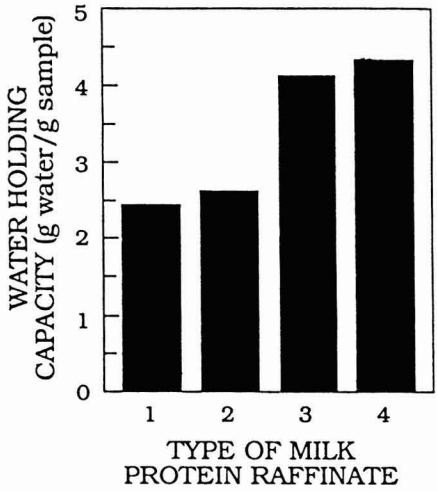

FIG. 6. WATER HOLDING CAPACITY OF EXTRUDATES FORTIFIED (5% LEVEL) WITH MILK PROTEIN RAFFINATE
1 = Average of 0.95 and 1.35% Lactose, 2 = 3.53% Lactose, 3 = 7.16% Lactose, 4 = 20.93% Lactose.

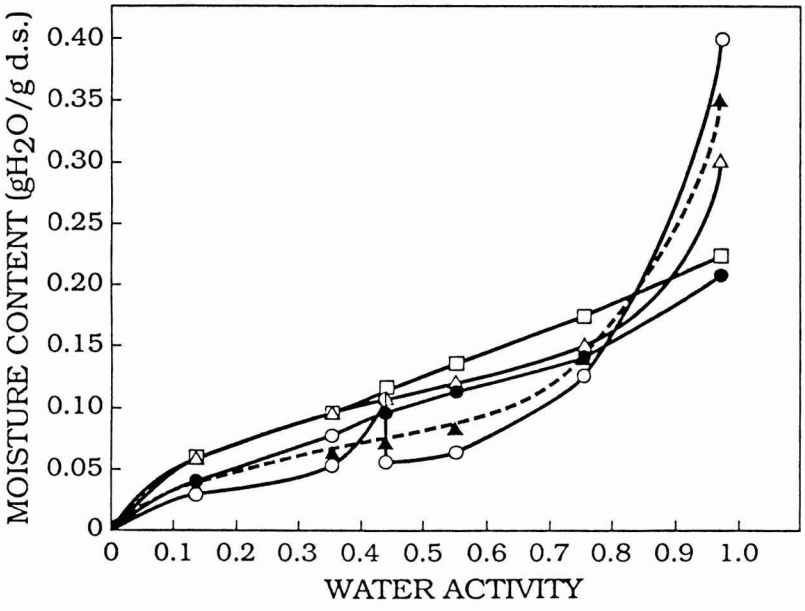

FIG. 7. SORPTION ISOTHERMS OF RAW MATERIAL AND EXTRUDED SAMPLES
Nonfat Dry Milk (○), Milk Protein Raffinate 1-A (▲), Corn Flour (△), Extrudate of Corn Flour and 20% Milk Protein Raffinate 1-A (●), and Extrudate of Corn Flour and 5–10% Nonfat Dry Milk (□).

This is most likely due to the fact that the protein as a whole was insoluble which would lower the solubility as compared to the other components present in the system. The increased solubility of CF after extrusion was due to gelatinization. The water solubility index was higher at a higher temperature in samples without NFDM or with low levels of NFDM incorporation (Fig. 8). Higher water solubility values were most likely due to higher levels of starch gelatinization associated with high temperatures. However, at higher levels of NFDM incorporation, the results were reversed. It is expected that higher temperatures increase the rates and extent of protein denaturation, and thus, decreased the solubility of the protein fraction. Starch-protein interactions will also influence protein solubility. An increase in solubility values was observed with 5% added NFDM. This result was somewhat expected since the concentration of lactose in the overall system was also increased. Due to its large concentration in NFDM, it is possible that a large amount of lactose remains unreacted through nonenzymatic browning related interactions.

A general decrease in solubility was observed as the concentration of lactose increased in the raffinate (Fig. 9). As the concentration of lactose reached levels higher than 7%, the solubility increased. It is expected that the decrease in

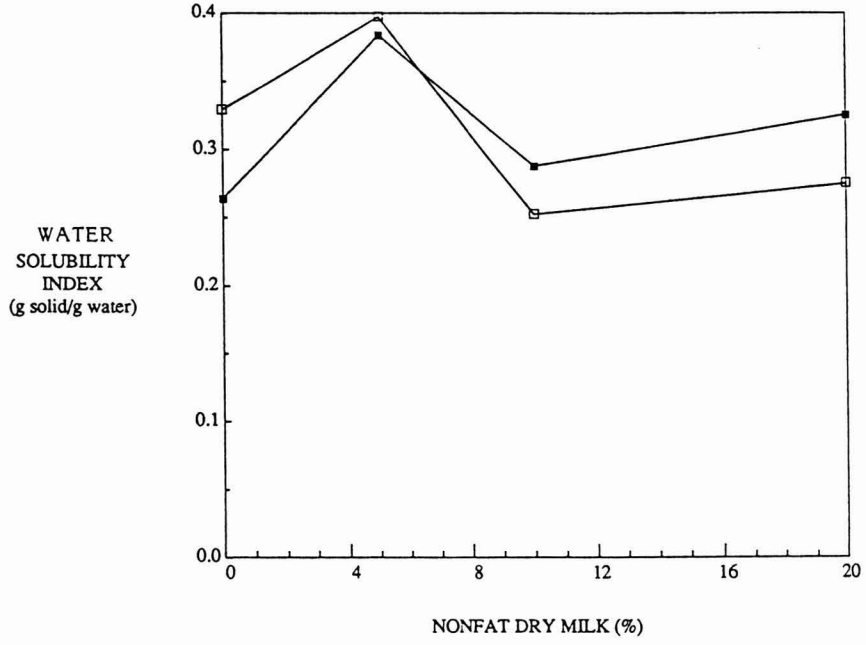

FIG. 8. SOLUBILITY INDEX OF EXTRUDATES AS A FUNCTION OF % ADDED NONFAT DRY MILK 125°C (■), and 150°C (□).

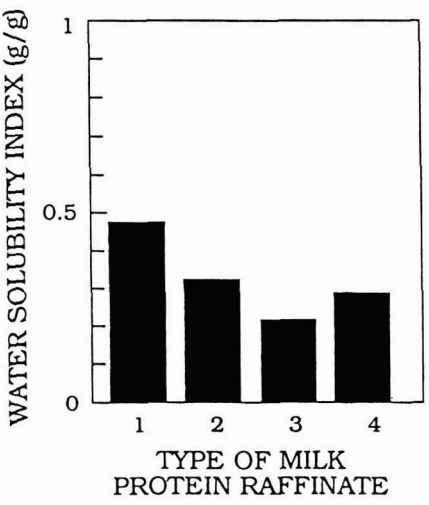

FIG. 9. SOLUBILITY INDEX OF EXTRUDATES FORTIFIED (5% LEVEL) WITH MILK PROTEIN RAFFINATE
1 = Average of 0.95 and 1.35% Lactose, 2 = 3.53% Lactose, 3 = 7.16% Lactose, 4 = 20.93% Lactose.

solubility was due to an increase in lactose interactions. Beyond a certain concentration level, it is expected that lactose would be responsible for an increase in solubility since excess lactose would be present. In another study on extrusion of CF, Chinnaswamy *et al.* (1989) showed that the change in solubility was associated with molecular interactions between starch and protein molecules.

CONCLUSIONS

(1) Lower processing temperatures resulted in decreased browning, even at high levels of protein incorporation. Based on the lactose content of samples, CF and MPR blends with low lactose content were expected to provide a better product than CF and NDM blend. Although some improvement in color was observed, it was evident that other reducing sugars generated through starch hydrolysis and low levels of lactose still remaining in the MPR resulted in significant browning in extruded products.

(2) Lactose content showed a significant effect on the overall characteristics of the extrudate in terms of (a) starch gelatinization due to water competition, (b) expansion and breaking strength and (c) WHC, sorption phenomenon and solubility of the extruded systems.

(3) Incorporation of MPR, even at the 5% level, resulted in significant changes in the physical characteristics of the extrudates. Very high levels of protein

addition resulted in products where protein-protein rather than carbohydrate-protein interactions controlled the physico-chemical characteristics of the extrudates.

NOMENCLATURE

CF = Corn flour in feed (%)
Eg = Extent of starch gelatinization (%)
DH_c = Enthalpy of the control (J/g)
DH_s = Enthalpy of the sample (J/g)
PC = Protein content in feed (%)
PS = Protein solubility of extrudate (%)
SM = Total soluble matter (g solid/100g water)
SP = Soluble protein in feed (%)

ACKNOWLEDGMENTS

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