fresh and frozen corn are preferred. But is fresh corn aroma preferred over frozen because of the high concentration of H_2S in frozen corn? Obviously, more research needs to be done in this area to answer this and other questions.

ACKNOWLEDGMENTS

The authors wish to thank Mildred Modery and Thelma Chase for their help in the processing, maturity evaluation, and sensory panel testing of the corn samples. Gratitude is also expressed to Larry Douglass of the University of Maryland's Dairy Science Department for his advice and counsel on statistical matters.

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Received for review March 13, 1974. Accepted May 15, 1974. Scientific Article No. A1976. Contribution No. 4913 of the Agricultural Experiment Station, Department of Horticulture.

Nutritional Quality of Processed Milk Containing Carrageenan

Rudolph M. Tomarelli,* Walter D. Tucker, Jr., Linda M. Bauman, Savini Savini, and John R. Weaber

Nutritional studies were conducted on rats fed diets containing carrageenan that had been mixed into skim milk at a concentration equal to that of the protein and heat sterilized under conditions routinely used in the manufacture of canned liquid milk. When fed in a simulated milk powder diet, the processed carrageenan, at a dietary level of 4%, had no influence (compared to cerelose or cellulose) on growth rate, diet energy efficiency, absorption of protein, fat, or calcium, blood coagulability, utilization of protein for growth (PER), or the utilization of iron (anemic rats). Gross and microscopic examination of the cecum and colon, after 6 months feeding, revealed none of the abnormalities associated with the feeding of degraded (hydrolyzed) carrageenan to susceptible species. These results support the conclusion that food grade carrageenan, at its present or anticipated use level in food, does not constitute a hazard.

Carrageenan is the generic name for a group of sulfated polygalactans of high molecular weight present in a number of red seaweeds. The principle commercial source of carrageenan is the alga *Chondrus crispus*, also known as "Irish moss" or "carragheen moss," in reference to the Irish coastal town of Carragheen, where for centuries it was collected for use in the preparation of the milk pudding, blanc mange.

The reactivity of carrageenan with milk protein to form a stable gel has found wide application in the dairy and related industries. Typical applications include the prevention of separation and sedimentation in aseptically canned and sterilized milk products, the suspension of cocoa particles in chocolate drinks, the prevention of whey separation and ice crystal formation in ice cream, and the stabilization of whipped products.

The carrageenan products of predominant use in the food industry are mixtures of κ- and λ-carrageenan, with the proportions selected determined by the characteristics desired, κ-carrageenan for gel formation and λ for increased viscosity.

Chemically κ-carrageenan consists of alternating units of sulfated *d*-galactose and 3,6-anhydro-*d*-galactose in approximate equimolar amounts (O'Neil, 1955); λ-carrageenan consists almost entirely of sulfated *d*-galactose (Smith *et al.*, 1955). The molecular weight of κ-carrageenan is between 1.8 and 3.2×10^5 and that of λ-carrageenan is between 4 and 7×10^5 (Smith *et al.*, 1954).

The safety of carrageenan for use in food products has been recognized in the United States by its inclusion in the first GRAS list (FDA, 1959), and subsequently as a regulated additive (FDA, 1961). The joint FAO-WHO Expert Committee on Food Additives (World Health Organization, 1970) established an acceptable daily intake for man of 50 mg/kg, *i.e.* approximately 3.5 g per day for the average man.

Several years ago the safety of carrageenan as a food additive was questioned as a result of a series of studies in England (Marcus and Watt, 1969; Watt and Marcus, 1969, 1970; Watt *et al.*, 1970) in which ulcerations of the cecum and proximal colon were found in rabbits, guinea pigs, and rats drinking water containing a degraded carrageenan product. The product (Ebimar), which had been used in Europe for over 10 years in treatment of peptic ulcer, is produced by the degradation of ι-carrageenan, usually by acetolysis, to a molecular weight of less than 30,000 (Anderson and Soman, 1966). ι-Carrageenan, isolated from *Eucheuma spinosium*, differs from κ-carrageenan in that sulfate is present at the C_2 position of the 3,6-anhydrogalactose.

The implication of the Marcus and Watt studies, that degraded carrageenan was a hazardous therapeutic agent, was contested (Bonifils, 1970; Maillet *et al.*, 1970; Sharratt *et al.*, 1971), and further investigations on the biological properties of native and degraded carrageenan have been published (Beattie *et al.*, 1970; Dewar and Maddy,

Wyeth Labs, Inc., Research Division, Radnow, Pennsylvania 19087.

Table I. Composition of Basal Diets

Ingredient	A	B	C
Skim milk powder[a]	559	282	
Casein			240
Cellulose	40	40	50
Cerelose	109	536	568
Salt mix[b]		40	40
Fe, Cu, Mn[c]	10		
Vitamin mix[d]	1	1	1
Choline chloride	1	1	1
Fat blend	280	100	100

[a] Low-heat spray dried nonfat milk solids. [b] Bernhart and Tomarelli (1966). [c] Cerelose concentrate (g/10 g): $FeSO_4 \cdot 7H_2O$, 1.25; $CuSO_4 \cdot 5H_2O$, 0.2; and $MnSO_4 \cdot H_2O$, 1.54. [d] Vitamin content per gram: (in milligrams) thiamine·HCl, 6.5; riboflavine, 12.5; pyridoxine·HCl, 6; niacin, 75; calcium pantothenate, 40; 2-methylnaphthoquinone, 0.5; biotin, 0.5; folic acid, 2.0; ascorbic acid, 50; *p*-aminobenzoic acid, 100; inositol, 100; *dl*-α-tocopherol, 120; and vitamin B_{12}, 25 μg; vitamin A palmitate, 6000 IU; and vitamin D_2, 3000 IU.

1970; Marcus and Watt, 1971; Watt and Marcus, 1971; Abraham *et al.*, 1972; Benitz *et al.*, 1973; Grasso *et al.*, 1973; Fabian *et al.*, 1973; Poulsen, 1973).

Recently the Food and Drug Administration, in a review of the above and studies of their own, have concluded that the native carrageenan of food use, at its present and anticipated level of use, did not constitute a hazard, and to ensure against the introduction of degraded carrageenan into the food supply have amended the present regulation by stipulating that the carrageenan have an average molecular weight exceeding 100,000 as indicated by a specified test (FDA, 1972).

In the early feeding studies on the nutritional properties of carrageenan, no adverse effects were noted in mice or rats until dietary concentration exceeded 10%; between 10 and 25% growth retardation and diarrhea were found, effects attributable to the high intake of undigestable bulk (Nilson and Schaller, 1941; Nilson and Wagner, 1959; Hawkins and Yaphe, 1965). In these studies, the carrageenan product fed had not been subjected to the processing attending the manufacture of a food product. It was the purpose of the present investigation (1) to study the biological properties of carrageenan that had been exposed to the heat treatment of canned milk sterilization and (2) to determine if the presence of carrageenan in the sterilized milk had any influence on the nutritional quality of the milk.

MATERIALS AND METHODS

Processed Carrageenan. Calcium carrageenan (Sea Kem, type II, Marine Colloids, Co.) was added to skim milk in the proportion of 1 part of carrageenan to 1 part of protein (N × 6.38). Preliminary studies had shown that this preparation would remain fluid during sterilization; on cooling a soft gel would form, but reheating restored the fluid state. The well-blended mix was pasteurized, sealed in 1-qt cans, and sterilized at 265°F for 3.68 min. This preparation was used in all experiments except those in which the nutritive value of protein was under study. For protein study the ratio of carrageenan to protein in the processed preparation was set at 0.4 so that diets could be prepared that contained 10% protein and 4% carrageenan. The processed liquid products were dried either by lyophilization or spray drying.

Animals and Diets. Sprague-Dawley rats (Madison, Wis.) were used in all experiments. The ages and sex of the rats used in the various experiments are listed in the following sections. The rats were housed individually in screen-bottomed cages with free access to food and water.

The experimental diets were either of a skim milk base or formulated from purified ingredients; the objective of the particular experiment determined the choice of the basal diet. The composition of the three diets is shown in Table I. Basal diet A, a milk-base diet of adequate protein content, approximately 20%, consisted of skim milk powder to which cerelose was added to reduce the lactose content to a level more readily tolerated by the rat. A fat blend of oleo, safflower oleic, soybean, and coconut oils was added at the 28% level to equal the caloric density of whole milk powder; this diet was fortified with vitamins and with those minerals lacking in milk, namely Fe, Cu, and Mn. In the study of iron utilization, the ferrous sulfate addition to this diet was omitted.

Basal diet B, used in the study of protein utilization, also contained skim milk powder but at a level to supply 10% protein. This diet contained a complete mineral mixture.

Basal diet C, formulated from purified ingredients, was used in experiments determining the absorption of unprocessed carrageenan, *i.e.*, carrageenan not subjected to heat sterilization in a skim milk solution. The carrageenan concentration of the experimental diets was 40 g/kg.

Analytical Methods. Calcium and iron were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1971), nitrogen by the Kjeldahl method (AOAC, 1970), hemoglobin by the cyanmethemoglobin method (Hoffman, 1970, p 154), blood coagulation factors by the determination of prothrombin time (Hoffman, 1970, p 191) and partial thromboplastin time (Hoffman, 1970, p 193), and food and fecal fat by the method of van de Kamer *et al.* (1949).

Carrageenan was determined by the procedure of Yaphe and Arsenault (1965), which is based on the colorimetric reaction between acid resorcinol and 3,6-anhydrogalactose, a monosaccharide constituent of carrageenan. The method was modified for fecal analysis by the introduction of a correction "blank" for nonspecific color formation; the optical density of a sample treated with resorcinol-free reagent was subtracted from that obtained with the complete color reagent. With this modification, feces from rats fed milk-base diets with cerelose or cellulose substituted for the carrageenan yielded zero values. It was essential that the carrageenan used for the calibration curve be from the same lot as that used in production of the experimental samples since color reactivity varied with the different batches.

EXPERIMENTAL PROCEDURES AND RESULTS

Long-Term Feeding Studies. Two experiments of 6 months duration were conducted to determine the effect of feeding relatively large amounts of processed carrageenan for prolonged periods. In the first experiment two dietary groups were maintained: (1) a control group fed the basal diet A of Table I, and (2) a carrageenan group fed the basal diet in which equivalent amounts of the skim milk powder and cellulose were replaced by the processed milk-carrageenan product. In the second experiment a third dietary group was included, a group fed the basal diet in which cerelose was substituted for the cellulose. This group served as a control to assess the influence of the relatively large amounts of indigestible carbohydrate presented by the carrageenan or cellulose.

The dietary groups of the first experiment consisted of 25 males and 25 females; in the second there were 12 males and 12 females per group. In the second experiment half the rats of each dietary and sex group were killed at the fourth month.

The rats were weighed twice weekly. At intervals 6–8 rats from the groups were placed in metabolism cages for 5–7 days for the study of the absorption of nutrients. At autopsy the organs were examined for gross lesions with

Table II. Effect of Carrageenan on Diet Utilization for Growth[a]

Dietary variable	Food,[b] g/day	Wt gain, g/day	Food efficiency, g/100 kcal	Water consumption, ml/day
Cerelose	14.2 ± 0.4[c]	5.2 ± 0.3	7.24 ± 0.26	23.4 ± 1.5
Cellulose	14.6 ± 0.5	5.4 ± 0.3	7.45 ± 0.34	27.3 ± 1.0*[d]
Carrageenan	14.9 ± 0.8	5.3 ± 0.3	7.17 ± 0.48	30.2 ± 1.1**

[a] Experiment 2, basal diet A, 7-day study during the fifth week, 12 males/group. [b] Cerelose diet, 5.08 kcal/g; carrageenan and cellulose diets, 4.92 kcal/g. [c] Standard error. [d] Statistically significant difference from cerelose group; one asterisk, $P = 0.05$; two asterisks, $P = 0.01$.

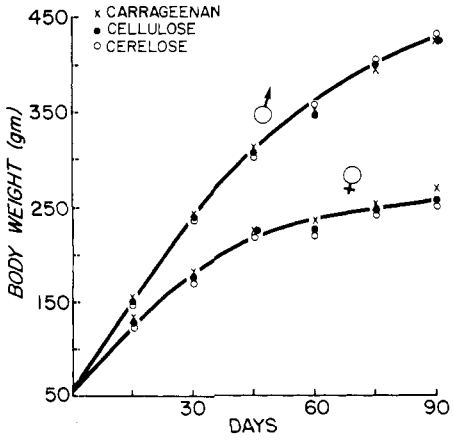

Figure 1. Average weight gain of rats fed simulated milk powder diets containing 4% of either carrageenan, cellulose, or cerelose.

particular attention directed to the lower gastrointestinal tract. To facilitate the detection of intestinal lesions, just before being killed, the animals were injected intravenously in the tail with a 4% aqueous solution of Direct Sky Blue (Wyeth). The gastrointestinal tract was removed, opened, the contents removed by gently swabbing, and examined under magnification for mucosal damage. Mucosal injury would be indicated by the presence of dark blue areas against a pale blue background, produced by the diffusion of the protein-bound dye through the damaged mucosa. This technique, essentially that of Brodie *et al.* (1970), will permit the detection of lesions as small as 0.3 mm in diameter. For histological examination, the entire cecum and colon were placed in 10% neutral buffered formalin. Three tissue blocks of the two intestinal segments, representing proximal, mid-length, and distal portions, were processed by routine histologic procedures, and stained with hematoxylin-eosin (Luna, 1968).

Throughout the 6-month feeding studies the animals of all the groups of both experiments appeared to be in normal health. There were no significant differences in the average body weights of the various dietary groups at any time. The uniformity of the growth response of the groups is illustrated in Figure 1. During the first month the males gained 6.1 g/day which is in the maximal range for this strain of rats.

The utilization of dietary energy for growth, *i.e.*, g of gain/100 kcal, was not influenced by the heat-processed carrageenan; typical data are presented in Table II. The rats fed the carrageenan diet drank more water than those of the cerelose control group. This was also true for the cellulose group but to a lesser degree (Table II). The hydrophilic nature of the carrageenan was also evidenced by the excretion of moist, bulky, lighter colored feces.

Gross examination at autopsy revealed no abnormalities. There were no differences of statistical significance in the weights of organs of the rats of various groups. The organs weighed were: liver, kidneys, adrenals, testes, and ovaries.

Table III. Excretion of Carrageenan[a]

% excretion	
Untreated[b]	Processed in milk[c]
105.7[d]	98.8
92.0	99.1
106.2	93.9
100.1	89.2
90.2	107.9
98.6	92.7
	101.2
	103.2
98.8 ± 2.7%[e]	98.3 ± 2.2%

[a] 5-day feeding study; see text for details. [b] 5% of semisynthetic basal diet C. [c] 4% of skim milk basal diet A. [d] Values for individual animals. [e] Standard error.

Histological examination of the multiple sections taken from the cecum and colon revealed normal tissue morphology. No evidence of typhlitis or colitis was found, and there were no erosions or ulcerations of the mucosal surfaces.

Absorption of Carrageenan. Absorption studies were conducted on unprocessed samples and samples of carrageenan that had been heat sterilized in milk solution.

Adult males, placed in metabolism cages, were fed a basal diet for 7 days, followed by 5 days of feeding the carrageenan diet, and then 2 more days on the basal diet. Preliminary study had shown that carrageenan excretion was completed 48 hr after the last feeding. The feces for each 7-day period for each rat were combined, dried, pulverized, and analyzed for carrageenan. In the study of the absorption of unprocessed carrageenan, a basal diet of purified ingredients (diet C, Table I) was used. It had been anticipated that with a basal diet containing skim milk, undigested lactose in the feces might complicate the analysis for carrageenan. However, with the analytical procedures employed, no "apparent carrageenan" was found in the feces of rats fed the skim milk basal diet (diet A), and this more appropriate diet was used for the study of the absorption of the carrageenan processed in skim milk.

The results of the absorption studies, presented in Table III, show that carrageenan, untreated or heat sterilized in milk, is quantitatively excreted by the rat. The 98% average excretion values are, statistically, insignificantly different from 100% excretion ("*t*" test).

Protein Utilization. The influence of carrageenan on protein utilization was studied by the determination of the protein efficiency ratio of milk preparations containing carrageenan. In experiments 5 and 7 (Table IV) the effect of native carrageenan (no heat treatment in milk) was measured by feeding diets in which carrageenan powder was mixed into the skim milk powder diet (basal B, Table I) at the expense of the cellulose. In experiment 6, the processed carrageenan preparation consisted of carrageenan in liquid skim milk that was canned and heat sterilized under commercial conditions. In experiment 7,

Table IV. Influence of Carrageenan on the Nutritive Value of Milk Protein

Expt	Milk and carrageenan treatment[a]	Protein efficiency ratio, g of gain/g of protein
5	Skim milk powder	3.07 ± 0.07[b] (8)[c]
	Skim milk powder and carrageenan powder	3.08 ± 0.11
6	Canned liquid skim milk, commercial sterilization	3.03 ± 0.09 (8)
	Canned liquid skim milk with carrageenan, commercial sterilization	3.15 ± 0.07
7	Autoclaved liquid skim[d]	2.50 ± 0.08 (6)
	Autoclaved liquid skim with carrageenan[d]	2.72 ± 0.08
	Skim milk powder	2.94 ± 0.01
	Skim milk powder and carrageenan powder	2.90 ± 0.08

[a] Basal diet B containing either 4% carrageenan or 4% cellulose; see text. [b] Standard error. [c] Number of weanling male rats per group in each experiment. [d] 45 min at 15 psi.

Table V. Effect of Processed Carrageenan on Iron Utilization

	Hemoglobin, g/100 ml				
	Low Fe diet at weeks			10 mg of Fe/kg of diet at weeks	
Group[a]	1	3	4	2	4
Cellulose	9.97	6.45	6.61 ± 0.37[c,d]	8.87	10.89 ± 0.39
Carrageenan[b]	9.97	6.49	6.07 ± 0.23	8.24	10.65 ± 0.42

[a] Ten rats per group; initial average body weight, 75 g; basal diet A without iron. [b] Processed product, 4% carrageenan in the diet. [c] Standard error. [d] None of the differences between the carrageenan and cellulose groups were of statistical significance.

excessive heat treatment was applied by autoclaving carrageenan (or cellulose) in liquid skim milk for 45 min at 15 psi.

All diets contained 10% milk protein; the carrageenan or cellulose concentration was 4%.

The protein efficiency ratios of Table IV indicate that the native carrageenan, carrageenan in skim milk sterilized under manufacturing conditions, or autoclaved carrageenan in skim milk had no effect on the utilization of protein for growth.

Iron Utilization. The possibility that carrageenan might interfere with the absorption of iron was investigated by determining the effect of dietary carrageenan on the depletion and regeneration of hemoglobin in rats fed a low-iron diet.

Weanling male rats were fed basal diet A, without the ferrous sulfate ingredient, for 1 week. They were then divided into two groups of equal average body weight and hemoglobin level. The experimental group received the processed carrageenan incorporated into the basal diet at the expense of the cellulose. After 4 weeks on the deficient diets the control and carrageenan diets were then fortified with a suboptimal amount of ferrous sulfate, 10 mg of iron/kg diet. Hemoglobin values, presented in Table V, show that the feeding of the heat-processed carrageenan in the iron-deficient diet did not increase the severity of the anemia, nor did it impede the regeneration of hemoglobin when iron supplementation was instituted.

Absorption of Nutrients. The results of studies on the possible influence of carrageenan on the absorption of nutrients from a milk diet are summarized in Table VI.

The absorption of fat, calcium, and total solids was determined in the rats during the third month of the long-term feeding study. As shown in Table VI no differences of statistical significance were found between the groups fed the processed carrageenan or cellulose.

The excretion of fecal solids was examined because the voluminous quantity of feces from the rats fed the carrageenan diets suggested a possible loss of dietary energy. It was evident, however, that the bulky nature of the feces resulted from the retention of water by the carrageenan in the feces.

Calcium absorption was of interest since the carrageenan product added to the milk was in the form of the calcium salt. It was realized that the intestinal absorption of calcium is regulated by the animal's need, and with a more than adequate amount of calcium in the basal diet only a drastic interference with calcium absorption would affect the absorption values. The lower per cent excretion of calcium by rats fed the carrageenan diet, while not of statistical significance, reflects the greater excess of calcium in the carrageenan diet. Absolute absorptions during the 7-day metabolic study, for the cellulose and carrageenan diets respectively, were: 124 mg *vs.* 141 mg for the males and 56 mg *vs.* 53 mg for the females.

The direct relationship of intestinal absorption to requirement is well demonstrated in the marked difference in per cent calcium absorption between the males and females. The males were gaining weight at the ratio of 12 g/week, while the females, approaching the mature body weight plateau, were only gaining 5 g/week.

The effect of carrageenan on the absorption of protein was studied in young male rats fed the low-protein, basal diet B. The results of the 7-day metabolic studies (experiments 5, 6, and 7 of Table VI) show that carrageenan, unprocessed, commercially sterilized in skim milk, or excessively heated in skim milk, had no effect on nitrogen absorption.

Blood Coagulation. Carrageenan, in common with other sulfated polysaccharides, possesses anticoagulant activity (Hawkins and Leonard, 1963). Carrageenan, or degraded carrageenan, intravenously injected into rabbits has been shown to prolong the clotting time of blood (Anderson and Duncan, 1965). In "*in vitro*" tests with human blood, a dual response has been observed; low concentrations of carrageenan had procoagulant activity while higher concentrations produced an anticoagulant effect (Schwartz and Kellermeyer, 1969).

In the present investigation, coagulation tests conducted on blood samples taken at various times during the long-term feeding studies, experiments 1 and 2, or from rats fed the low-protein diet, experiment 6, revealed no differences between the carrageenan-fed rats and the cellulose controls. Typical data were: prothrombin times of 11.5 ± 0.1 and 11.7 ± 0.1 sec for the cellulose control and carrageenan groups, respectively; and partial thromboplastin times of 27.2 ± 0.6 and 26.9 ± 1.0 sec for the cellulose and carrageenan groups, respectively (average ±

Table VI. Effect of Processed Carrageenan on Nutrient Absorption

Expt and diet	Nutrient	% absorption[a]	
		Cellulose control	Carrageenan
Skim milk diet A, expt 1[e]	Total solids		
	6 ♂	88.6 ± 0.01	88.9 ± 0.1[b]
	6 ♀	88.4 ± 0.01	88.5 ± 0.1
	Fatty acids		
	6 ♂	94.9 ± 0.5	94.0 ± 0.7
	6 ♀	96.9 ± 0.3	96.9 ± 0.3
	Calcium		
	6 ♂	21.1 ± 2.9	17.2 ± 1.4
	6 ♀	12.9 ± 3.1	10.2 ± 2.7
Low protein skim milk diet B	Nitrogen		
Expt 5[f]	8 ♂	83.0 ± 1.0[c]	81.6 ± 0.6[c]
Expt 6[f]	8 ♂	83.2 ± 0.7[b]	83.7 ± 0.9[b]
Expt 7[f]	6 ♂	82.7 ± 0.2[d]	82.3 ± 0.5[d]
	6 ♂	84.6 ± 0.4[c]	84.6 ± 0.5[c]

[a] Apparent absorption, *i.e.*, food − fecal/food × 100%. [b] Commercially sterilized canned skim milk with cellulose or carrageenan. [c] Unprocessed; cellulose or carrageenan added to dry diet. [d] Excessive heat; autoclaved liquid skim milk with cellulose or carrageenan. [e] Seven-day study during the third month. [f] Seven-day study during the first month.

standard error of groups consisting of 5 males and 5 females after 6 months of feeding of diets of the basal A composition).

DISCUSSION

In the experiments reported here, carrageenan in skim milk, heat sterilized under the conditions employed in the manufacture of canned milk, produced no observable deleterious effects when fed to rats from the age of weaning to maturity. Gross and microscopic examination of the cecum and colon revealed none of the abnormalities reported with the feeding of degraded carrageenan to susceptible laboratory animals. The absorption of fat, protein, calcium, and iron from skim milk basal diets was not affected by the addition of heat-processed carrageenan to the diets, permitting the conclusions that the carrageenan did not react with the nutrients or their digestion products to form nonavailable complexes, or secondly, that the heat-treated carrageenan did not impair the absorptive capacity of the intestinal mucosa.

The utilization of protein, as measured by the biological assay of protein efficiency ratio, was not influenced by the presence of carrageenan in skim milk sterilized under routine commercial conditions or when the milk was subjected to excessive heat treatment. The results of the nitrogen aborption study confirm the earlier findings of Hawkins and Yaphe (1965), who found no effect on nitrogen absorption in the rat fed a semisynthetic diet until the dietary levels of a native carrageenan were in excess of 10% of the dry ration.

Carrageenan, and certain other sulfated polysaccharides, will inhibit the proteolytic action of pepsin (Houck *et al.*, 1960; Anderson and Harthill, 1967), a characteristic offering one rationale for the therapeutic activity of degraded carrageenan in the treatment of peptic ulcer. Vaughan *et al.* (1962), in a study of the effect of carrageenan on peptic digestion of a number of proteins, including that of a milk formula, have concluded that the levels used in foods do not interfere with normal digestion. In the present study in which a relatively large amount of carrageenan was fed in a milk diet, it would appear that if peptic digestion were inhibited it was of no physiological consequence, that tryptic digestion must have been adequate, since no interference with nitrogen absorption or protein utilization was observed.

In the carrageenan absorption studies reported here, native carrageenan, or carrageenan sterilized in skim milk, was, within experimental error, quantitively excreted. The results of the test with native, *i.e.*, unprocessed carrageenan, are in general agreement with similar studies in the rat conducted by Hawkins and Yaphe (1965) and Dewar and Maddy (1970), who found excretion values of 80–100%. The lower excretion values found by these investigators may be related to the particular carrageenan preparation fed, or perhaps to the analytical procedures employed.

The skim milk–carrageenan product fed in the present study was prepared by adding food grade carrageenan to skim milk at a concentration equal to that of the protein, the highest concentration that could be added with a reasonable assurance that the product would remain liquid and be completely subjected to heat treatment during the sterilization. When incorporated into the experimental diet of a composition simulating whole milk powder, the carrageenan concentration was 4%. On a liquid basis this diet was equivalent to that of whole milk containing 0.5% carrageenan. A canned liquid milk product containing this amount of carrageenan could not be marketed since the product would soon become a semisolid. The concentration of carrageenan in general use for the stabilization of milk falls in the range of 0.01–0.03%; infant milk formulas, particularly the concentrated formulas, usually contain less (Infant Formula Council, 1972). Thus, the concentration of carrageenan in the milk diets fed to the rats in studies reported here exceeded by 15- to 50-fold the concentration that would be found in a marketed product.

The feeding of this relatively large amount of carrageenan that had been heat sterilized in milk without evidence of any of the adverse effects reported with the feeding of degraded carrageenan offers reassurance that the carrageenan used in this country for the stabilization of milk and milk formulas is not being degraded during the manufacturing process.

The results of these studies support the conclusions of the FDA (1972) that the use of food grade carrageenan in any present or anticipated use level in foods does not constitute a hazard. In addition, in these studies no evidence was obtained that would indicate that carrageenan in commercially processed milk impairs the nutritional quality of the milk.

ACKNOWLEDGMENT

The authors are indebted to E. R. Eckhardt and D. B. Droscha for the production of the experimental milk samples and to R. Oswald and Nancy A. Powell for assistance in the histological studies.

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Received for review January 25, 1974. Accepted May 14, 1974.

Analysis of Lead in Evaporated Milk by Flameless Atomic Absorption Spectroscopy

Hugh L. Huffman, Jr., and Joseph A. Caruso*

A procedure for determining lead in canned evaporated milk is evaluated by flame atomic absorption and the flameless atomic absorption technique using the tantalum ribbon and carbon-cup atomization devices. A fairly rapid pre-ashing procedure to eliminate the mechanical loss of lead encountered during the dry-ash sequence of the flameless method is described. A least-squares fit of the data taken using the method of standard additions is used to determine the Pb in each sample. The results from the analyses of actual samples taken from supermarket shelves fall within the federal guideline of <0.5 ppm.

In order to establish a maximum allowable content of heavy metals in canned foods it is necessary to have a rapid and reliable means of analyzing for these metals. Evaporated milk is widely used in preparing baby formulas and in various recipes prepared by the housewife. It is known to contain some amount of lead as a contaminant, presumably arising from the canning process which is discussed by Shea (1973). Recent reports by the U. S. Food and Drug Administration (FDA) and Consumers Union (AP and UPI releases, 1973; *Consumer Reports*, 1973; Fiorino *et al.*, 1973) indicate lead levels ranging from 0.02 to 0.37 ppm with an average of 0.12 ppm and in the range of 0.56–0.84 ppm with an average of 0.70 ppm. The FDA guideline for lead content in evaporated milk is <0.5 ppm (AP release, 1973).

The purposes of this study are: (1) to evaluate the lead content in canned milk using the recently developed technique of flameless atomic absorption spectrometry (AAS) and to compare the lead content of samples taken randomly from supermarket shelves with the federal guideline, and (2) to compare the carbon-cup and tantalum ribbon flameless techniques with the more cumbersome and time-consuming flame technique.

Amos (1972), in a review of nonflame atomization in AAS, describes the various designs of filament and furnace atomizers presently in use and includes a detailed description of the carbon-cup atomizer used for this study. Kurz *et al.* (1973) describe the operation of the carbon rod atomizer and the determination of its optimum settings. Additional descriptions of the carbon rod atomizer are given by Brodie and Matousek (1971) and Matousek (1971). Manning (1973) describes a single-point calibration determination of lead in milk using a Perkin-Elmer graphite furnace without any sample pretreatment; however, neither data nor statistical analyses are given for real samples. Hwang *et al.* (1971, 1972), Donega and Burgess (1970), and Takeuchi *et al.* (1972), describe the use of the tantalum ribbon in flameless AAS. Absolute sensitivities and absolute detection limits for the flameless methods are in the picogram range with the tantalum ribbon

Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221.