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THE EFFECT OF PROCESSING ON PROTEIN VALUE OF CEREAL PROTEIN SUPPLEMENTS*

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The development of new food formulas, with high protein contents of good biological value, from vegetable meals has received much attention during the past five years. The increasing availability of pure amino acids, the development of improved processing techniques to prepare these foods, and the day-by-day findings in nutritional research suggest the necessity of a multidisciplinary approach to the problems of creating new food materials. This has, in turn, led the food technologists and nutritionists to work together.

There are numerous reports in the literature concerning the effects of heat on the nutritive value of various foodstuffs. Since the cereals and oilseeds will continue to play a large role in human feeding due to their content of utilizable protein, the effect of heat treatment on their nutritional values is of importance.

However, when it becomes necessary to process these products on an industrial scale, comparatively little is actually known concerning nutritive losses.

An example of the conflicting literature reports is shown in the following: Block *et al* report that the 3.4 Protein Efficiency Ratio (PER) of a raw cake mix was reduced drastically to 0.7 after heating by baking and toasting at 100 °C - 130°C for one hour. Yet after baking and air-drying on a radiator for 24 hours, the PER was 2.4. The mixture contained wheat flour, 51%; sucrose, 19.7%; egg white, 11.5%; lactoalbumin, 7.6%; oil, 5.2%; yeast, 3.0%; molasses 1.1%; and salt 0.9%. The inclusion of sugar is to be noted. Bressani *et al*[2] reported that 25 minutes of normal cooking of a cereal mixture, designated Mixture 9, had no effect on lysine, which remained essentially the same. They also state: "Nitrogen balance studies provided further evidence that protein quality of the mixture was not impaired by cooking." (The content of Mixture 9 was corn flour, 28%; sorghum flour, 28%; cottonseed flour, 38%; torula yeast, 3%; and dehydrated leaf meal, 3%.)

These findings suggest that the processing technique of all new products of this type must be carefully considered. In countries where there is an emergence from the small, home-cooking type of production to large, commercial operations this becomes very important from the nutritional aspect. The nutritional data used in calculations for new food products is based largely on published values for protein, vitamins, etc. which were determined on foods subjected to relatively mild cooking conditions. Cereal proteins and animal proteins under severe heat treatment and in the presence of carbohydrates

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(especially reducing sugars) can react in a Maillard-type reaction. The digestibility is lowered, as is the biological value of the heated protein, causing poor growth in animal feeding trials.[3,4]

On the industrial scale the technologist and the nutritionist must be aware of the effect of composition and processing variables if the nutritional quality of foods is to be improved or maintained. The amino groups of foods or those added may interact differently or may react at different rates. The carbohydrates of mixes which may give rise to reducing sugars or act as intermediates in the Maillard reaction are of particular importance during the industrial processes. Because of the great volume of cereals, the use of amino acids for supplementing the incomplete proteins of cereals in the diets of two billion people is foreseeable in the near future and makes this of increasing importance.

In our studies, which must be considered preliminary, mixtures of corn meal and soybean meal were enriched with amino acids and subjected to different processing conditions. We then tested the biological value of the proteins.

Procedure and Materials

The various mixtures and processes involved were prepared as described below. These mixtures were then used as a base for rations fed to rats in protein-efficiency feeding trials. The amino acid content of each mixture was adjusted either by formula manipulation or, where this was not possible, by the addition of amino acids to equal or slightly exceed the FAO amino acid pattern of 1965.

Mixture 1 (3 processing variations), consisting of:

41.00 kg of yellow dent corn (ground)
86.78 gm of L-Lysine
111.50 gm of L-Methionine
37.40 gm of L-Tryptophan
80.00 kg of water (distilled).

The amino acids were first dissolved in water in order to give a homogeneous distribution. The corn and water mixture was then heated to approximately 80°C in a steam-jacketed cooking kettle and held approximately 1/2 hour at a maximum temperature of 76.6°C with frequent stirring. No burn-on was encountered. The partially gelatinized mixture was then divided into 3 equal parts to be processed.

Mixture 2 (3 processing variations), consisting of:

23.30 kg of yellow dent corn (ground)
4.45 kg of soy meal
13.10 kg of corn starch
29.50 gm of L-Lysine
130.60 gm of L-Methionine
12.97 gm of L-Tryptophan
79.00 kg of water (distilled)

In both mixtures care was taken to maintain a 3:3:1 ratio of lysine-methionine-tryptophan.

This mixture was prepared in the same manner as mixture 1. The processing for both mixtures was carried out as follows:

Process I: Dehydration

The cooked mixture, while still hot, was forced through a die to form a ribbon, which was distributed on a screened tray for drying in a tunnel dehydrator. The process was carried out at 54.4°C for 3 hours, with an uncontrolled dewpoint and no recycle. It was dried to a moisture of 4%, ground to a meal fineness, and stabilized to 6% moisture.

Process II: Freeze-drying

The cooked sample was frozen in a still chamber at -20°C, with the sample distributed on special trays previous to freeze- drying. This process took approximately 30 hours per batch (1 mix per batch). The resulting cake was then ground.

Process III: Canning

The cooked sample, still hot, was canned in number 202 x 308 cans. Then the cans were processed for 130 minutes at 115.5°C in a still retort. This time was calculated for a non-acid food heated by conduction.

Protein Efficiency Testing

Weanling rates (Sprague-Dawley strain) were used as the test animal to determine the PER of each of the 6 variations prepared. A modification of the A.O.A.C. PER method was employed in the determinations. The formula fed was supplemented with vitamins, (1%); corn oil, (3%); minerals, (4%); and water added to bring the total moisture to 8%. The protein content of all samples was adjusted to 10%. The amino acid analysis of each variation was measured by the Moore and Stein technique using a Technicon Autoanalyzer. Total nitrogen of the samples was determined by macro-Kjeldahl method. Tryptophan determinations were made by microbiological assay using *Lactobacillus plantarum*.

Discussion

As shown in Table I, mix 1, the PER's for the air-dried and freeze-dried samples were very similar (1.92 and 1.99); however, the canned sample had a greatly decreased PER of 0.81. In mix 2, the air-dried sample was lower than the freeze- dried sample, the latter being higher than the casein control. As in the first group, the canned sample showed a lower PER of 1.30.

These data parallel the amino acid data shown in Table II. In mixes 1 and 2 the content of amino acids of the two different canned samples was lower in all cases than that of the dehydrated or freeze-dried samples. The drop would account for a reducrion of growth, but not, it is felt, to the extent experienced. It was suspected that a possible interaction of amino acids and carbohydrates may have occurred.

The lower PER found in both canned samples is of significance as it is widely accepted that the normal canning process results in minimal nutritive loss.[5] However, Halevy and Guggenheim[6] refute such a view, stating, "...by autoclaving wheat gluten with glucose, 50% of the lysine was destroyed and the amount of lysine released by pancreatin was likewise reduced by one-half."

In this trial, working with corn and soy, it is also very possible that the canned samples present the same phenomenon of destruction of lysine through the browning reaction.

Recent studies by Boctor and Harper[7] show that heating egg white in an autoclave at 121°C for 2 hours did not reduce its content of available lysine, but when it was heated for 12.5 hours the loss of available lysine was about 13%. Quoting from their work, "When samples containing different amounts of glucose were similarly heated for 12 hours, the sample with 2% of glucose not only showed the value for lysine low, but also the values for methionine, histidine and arginine." These findings agree with our results. We are confronted with the fact that under certain conditions amino acids are made unavailable by heat, pH and carbohydrates present. The important point is that in this study these changes have been produced under normal commercial canning procedure conditions. In the case of the canned samples, the reaction between protein and hydrolyzed cereal starch possibly involves a condensation of the carbonyl group of the hydrolysate with the free amino groups of the amino acids to produce an indigestible linkage. This seems a valid reason for the markedly low PER observed. This thinking is reinforced in that the air-dried samples and the freeze-dried samples, where only mild heat treatment was applied, show a PER value close to that of the control.

In order to confirm the findings of the trail just under discussion, a second trial was conducted. In this follow-up investigation the original conditions of canning, i.e., 115.5°C for 130 minutes, were employed (using 202 x 308 cans as before). However, in this trial the influence of type of carbohydrate, interaction of free amino acids and level of free moisture were considered. Four of the five variations were designed to encourage the Maillard reaction as influenced by either sucrose or cornstarch. One of the four groups was further modified by the addition of 10% of dextrin. The unheated control group received the same amino acid mixture as the others. Natural protein used in all cases was vitamin-free ANRC casein. All groups were formulated so as to contain 10% protein [9% from casein, 1% from free amino acids (mixed according to the NRC amino acid requirements for the rat)]. Fat, fiber, mineral and vitamins were supplied at the levels recommended by the A.O.A.C. for the standard PER test. Moisture of all groups except the control was approximately 35%. Table 3 summarizes diets, treatments, and PER's resulting from a four-week feeding trial.

TABLE I
PER of Mixture I and mixture 2
(according to the method of Campbell[9]).

Treatments	PER Mixture I corn meal and amino acids	PER Mixture II corn and soy meal plus amino acids
Raw Mix	1.24 ± 0.2	2.20 ± 0.4
Air-dried	1.92 ± 0.4	1.88 ± 0.4
Freeze-dried	1.99 ± 0.4	2.95 ± 1.0
Canned	0.81 ± 0.1	1.30 ± 0.2

(casein control, PER 2.50)

TABLE II
ESSENTIAL AMINO ACID CONTENT OF THE MIXTURES AND TREATMENTS

	Mixture 1 Corn meal & Amino Acids			Mixture 2 Corn meal & Soy meal & Amino Acids		
Amino Acid	Air-Dried	Freeze-Dried	Canned	Air-Dried	Freeze-Dried	Canned
	gm amino acid/100 gm sample					
Threonine	.295	.319	.273	.381	.385	.348
Valine	.422	.456	.355	.576	.520	.434
Methionine	.381	.404	.377	.407	.417	.295
Isoleucine	.289	.311	.275	.447	.391	.346
Leucine	.946	1.011	.943	.999	.901	.846
Phenylalanine	.448	.475	.423	.584	.543	.465
Lysine	.464	.452	.380	.560	.498	.383
Histidine	.261	.291	.277	.315	.299	.256
Arginine	.387	.436	.379	.676	.628	.500
Tryptophan*	.033	.034	.027	.069	.071	.034

*Microbiological assay.

TABLE III
SUMMATIONS OF DIETS AND TREATMENTS FOR TRIAL No. 2

DIET #	COMPOSITION	TREATMENT	PER
Control	Sucrose, Amino Acids, Casein	unheated	3.50
2	Sucrose, Amino Acids, Casein	heated	1.00
3	Cornstarch, Amino Acids, Casein	heated	2.97
4	Cornstarch, Amino Acids, Casein	heated unheated	2.97
5	Sucrose, Dextrin, Amino Acids, Casein	heated	3.39

TABLE 4
SELECTIVE ESSENTIAL AMINO ACIDS OF CONTROL RATION AND #2 RATION
(gm amino acids/100 gm sample)

Amino Acids	Control ration	#2 ration
Methionine	0.393	0.393
Lysine	0.633	0.477
Histidine	0.324	0.268
Arginine	0.331	0.211

It should be noted that the processing temperatures employed were calculated on the basis of heat penetration (by conduction) to achieve a safe level of internal can heat. Temperatures necessary will vary with material, but, as used in this instance, are not unusual.

The pigmentation and bitter odor of the browning reaction were evident in both #2 and #5 groups (Table 3). Diet #2 was the most obviously affected both in physical appearance and biological value. Diet #5 was of lighter color, although darker than Diets #3 and #4, but its protein value was unimpaired. This protein-sparing activity, protective action or even growth-promoting property of dextrin has been noted by Harper and Elvejehm[8] as well as mentioned in NRC publication 990.

The amino acid analyses of the #2 ration shown in Table 4 revealed, as in the first trial, a substantially lowered lysine level (30%) as well as similar decreases in arginine (36%) and histidine (20%). These findings are amply verified in the literature.[1,4,6]

At the end of the four-week test period, 4 rats of group #2 were fed for an additional two weeks on their usual formula supplemented with 0.5% L-lysine and showed immediate improvement in physical appearance with weekly weight gains averaging 25 grams each, compared to 7 grams/week previously. Two other rats of the same group were switched to the control ration and averaged a gain of 40 grams/week. Again this appears to indicate that lysine, while the major factor, is not the only amino acid rendered unavailable through carbohydrate action and/or processing procedures.

Conclusions

The results obtained lead us to the following conclusions:

1. With the increasing use of high carbohydrate cereals, care must be exercised when choosing processing techniques to avoid hydrolysis of the starch, thus keeping reactive carbonyl groups to a minimum.
2. The addition of sucrose or dextrose to a product destined for processing under conditions favorable to developing the Maillard reaction may create a nutritional problem if the product is to be a major source of nutrients for any segment of a population.
3. Definite work in the field of amino acid activity and interaction under the variety of conditions peculiar to food processing should be of high priority. This is of special importance because of the potential use of amino acid mixtures.

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