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METHIONINE SUPPLEMENTATION OF SOY MILK TO CORRECT CYSTINE LOSS RESULTING FROM AN ALKALINE SOAKING PROCEDURE

INTRODUCTION

THE STUDY of the effects of alkaline pH on protein quality have for the most part been limited to corn protein. Bressani et al. (1958) have reported the effects of lime (CaO) and heat during the production of tortillas on the nutritive value of zein. Of the essential amino acids they found decreases in leucine, cystine, histidine and lysine. DeGroot and Slump (1969) studied the effects of severe alkaline treatment on isolated soy protein and found significant changes in the amino acid levels and the net protein utilization as well as the formation of the amino acid derivative lysinoalanine. More severe treatment caused a decrease in digestibility.

In an earlier paper Badenhop and Hackler (1970) reported some of the effects of soaking soybeans in a sodium hydroxide solution as a pretreatment step in the preparation of soy milk. The results of that study indicated that an alkaline treatment with 0.05N NaOH produced a soy milk with a pH of 7.4 which had a significantly better flavor. A further increase in pH resulted in a poorer flavor, however. Other effects of alkaline pretreatment included an increase in available niacin, reduced trypsin inhibitor activity, reduced cystine content and decreased nutritive value as indicated by protein efficiency ratio measurements. The present investigation was undertaken to better define the effects of an alkaline soaking treatment on the nutritional value of the protein.

EXPERIMENTAL

FOUR SOY milk samples were prepared using the procedure outlined in the previous report (Badenhop and Hackler, 1970). Soaking solutions of 0.05N, 0.075N and 0.100N NaOH were prepared and the soybeans soaked for 2 hr at 50°C. A sample was also prepared using distilled water as a soaking medium. The fluid milk samples were divided into two lots one of which was filled into #10 cans and heat processed for 10 min at 121°C. All samples were then freeze-dried for chemical analyses and incorporation into dry test diets.

The dried samples were analyzed for protein (N × 6.25) by a slight modification of the micro-Kjeldahl procedure as outlined in AOAC (1940). Samples were prepared for amino acid analysis by acid hydrolysis of the protein using 6N HCl at 110°C for 22 hr. The hydrolysates were filtered, dried and taken up in pH 2.2 sodium citrate buffer. Amino acid composition of the samples was determined by ion-exchange column chromatography using a Technicon Amino Acid Autoanalyzer equipped with four columns containing type C-2 resin. Cystine was analyzed by conversion to cysteic acid using the method of Moore (1963) followed by chromatography. Tryptophan was determined using the method of Horn and Jones (1945).

Protein efficiency ratios (PER) were determined in growth studies using male weanling rats of the Holtzman strain. The soy milk supplements were used as the sole protein source in the test diets at a level of 10% crude protein. After the dietary ingredients were thoroughly mixed they were divided into two lots, one of which was fed as is while the other was supplemented with 0.35% of L-methionine. The diets were fed to groups of rats ad libitum for 28 days. The PER values obtained on the experimental diets were adjusted based on a control diet containing 10% casein. Other details of the experimental methods used in the rat growth studies have been reported previously (Hackler et al., 1963).

Trypsin inhibitor measurements were obtained using the procedure of Learmonth (1952) as modified by Van Buren et al. (1964). This method compares the retardation of gelatin liquefaction by the enzyme trypsin in the presence of various dilutions of the sample. Results are expressed as the percent of the activity present in the non heat-treated soybeans.

RESULTS & DISCUSSION

A PREVIOUS REPORT of the effects of alkali-soak treatment by Badenhop and Hackler (1970) indicated that the only amino acid which was significantly affected was cystine. The present study was concerned with establishing more clearly whether the availability of any of the essential amino acids is affected by the alkaline treatment.

Amino acid analyses

The results of essential amino acid analyses (Table 1) confirm that the amino acid most affected by alkaline conditions is cystine. Cystine was the only amino acid observed to decrease with increasing pH in both the raw and heat-processed soy milk samples. (The terms raw or unprocessed milk is used interchangeably to refer to soy milk not processed at 121°C.) The raw soy milk prepared at pH 8.97 contained 15% less cystine than the pH 6.50 soy milk. In the heat-processed samples the decrease was 25% at pH 8.97. A decrease was also noted in tryptophan (14%).

The amino acid, threonine, appears to increase slightly in soy milk prepared from beans soaked in NaOH (Table 1). Peptide bonds containing the amino groups of threonine have been found to be quite labile (Hill, 1965). For this reason one would expect to obtain complete release of threonine during the 22 hr hydrolysis procedure employed in the

Table 1—Essential amino acid composition of soy milk as influenced by an alkaline soaking procedure and heat processing for 10 min at 121°C

	10 min at 121°C				No heat treatment			
pH	6.50	7.21	7.81	8.97	6.50	7.21	7.81	8.97
Amino acid	(g/16g of nitrogen)							
Histidine	2.37	2.40	2.48	2.37	2.36	2.37	2.47	2.40
Lysine	6.69	6.75	6.65	6.70	6.79	6.90	6.84	6.78
Phenylalanine	5.08	5.02	4.98	4.92	5.01	5.05	5.00	5.02
Tyrosine	3.79	3.73	3.86	3.71	3.86	3.85	4.06	3.92
Tryptophan	1.95	1.92	1.89	1.70	1.79	1.81	1.91	1.84
Methionine	1.25	1.25	1.23	1.20	1.26	1.29	1.21	1.25
Cystine	1.50	1.51	1.39	1.13	1.70	1.63	1.64	1.45
Threonine	3.89	3.83	4.06	4.08	3.95	4.01	4.35	4.44
Leucine	7.85	7.93	7.91	7.90	7.88	7.91	7.92	7.99
Isoleucine	4.65	4.69	4.75	4.75	4.71	4.77	4.77	4.77
Valine	4.73	4.63	4.74	4.85	4.80	4.82	4.98	4.93

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preparation of the samples for analysis. However, threonine destruction during acid hydrolysis to the extent of 5–10% has also been reported (Rees, 1946). The soy milk data appear to suggest that the presence of a small quantity of NaOH has a protective effect on threonine. However, when gelatin and an amino acid standard were treated with alkali to confirm or deny the possible protective effect of alkali on threonine, no protective effect was observed (Table 2). Thus the observed increase in Table 1 must be an anomaly.

Results of the trypsin inhibitor measurements (Table 3) show that for the processed samples a heat process of 10 min at 121°C in #10 cans is sufficient to destroy at least 96% of the original trypsin inhibitor activity. In the raw milk samples the effect of pH on trypsin inhibitor is seen in that the activity dropped with each increase in pH. The activity present in the pH 8.97 sample was approximately one-half of that in the pH 6.50 sample.

Animal feeding study

Table 4 shows the relationship of the adjusted PER values from the rat feeding experiment to the pH of the fluid soy milk, as well as other growth data. The samples which were not supplemented with methionine confirm the results obtained in the earlier study (Badenhop and Hackler, 1970) in that the PER of the heat-processed, unsupplemented samples decrease with increasing pH. This decrease in nutritive value is in agreement with the results of amino acid measurements which have already been discussed. These two methods of nutritional evaluation indicate quite clearly that the additive effects of high pH and temperature quickly reduce the nutritive value of soy protein due mainly to cystine destruction. The raw soy milk samples were observed to improve in nutritive value with increasing pH which can be directly attributed to the increased lability of trypsin inhibitor at elevated pH (Table 3).

The effect of methionine supplementation is quite apparent from an examination of Table 4. The adjusted PER values obtained from the heat-processed samples were quite consistent except for the pH 8.97 sample. Increases in adjusted PER values of 28, 32, and 27% (10 min at 121°C vs. 10 min at 121°C + 0.35% methionine) were obtained for the pH 6.50, 7.21 and 7.81 samples, respectively. For the pH 8.97 sample, however, the increase in adjusted PER was 92%. This dramatic increase at pH 8.97 indicates that high temperature and pH may cause both a destruction and decrease in availability of sulfur amino acids. The raw milk samples were significantly improved by methionine supplementation at each pH. The percentage increase was unaffected by pH. The observed increases in adjusted PER due to addition of methionine to the non heat-treated samples were 54, 53, 49 and 52% for the pH 6.50, 7.21, 7.81 and 8.97 soy milks, respectively. The fact that the relative improvement was independent of pH is particularly interesting in view of the observation that the trypsin inhibitor activity is inversely related to pH (Table 3). This would indicate that the level of residual trypsin inhibitor activity has no affect on the relative improvement due to methionine supplementation.

The effect of trypsin inhibitor on cystine and methionine metabolism has been studied by Barnes and Kwong (1965). It appears from their work that an increase in the conversion of methionine to cystine is brought about by trypsin inhibitor. Therefore the apparent effect of methionine supplementation on the PER values observed for the raw sam-

Table 2–Effect of various pretreatments on threonine determined by acid hydrolysis

Gelatin	Threonine g/16g N
No pretreatment	1.66
pH ≃ 7	1.68
pH ≃ 9	1.64
Amino acid standard	**μM**
No pretreatment	0.72[a]
pH ≃ 9	0.72

[a]The sample hydrolyzed (amino acid standard) contained 0.8 μM of threonine; therefore, 90% of the threonine was recovered.

Table 3–Trypsin inhibitor activity of soy milk samples

pH	Trypsin inhibitor activity (%)[b]
6.50[a]	2.5
7.21[a]	4.4
7.81[a]	2.3
8.97[a]	2.2
6.50	50.0
7.21	42.2
7.81	36.7
8.97	26.7

[a]These samples were heat processed for 10 min at 121°C.

[b]Activity is expressed as the percent of that present in untreated raw beans.

Table 4–The effect of an alkaline soaking procedure and supplemental methionine on protein utilization by weanling rats[a]

pH	CP (%)	ADG (g)	ADF (g)	PER	Adjusted PER[b]
			No heat treatment		
6.50	10.00	1.51	9.35	1.62	1.44(.04)[a]
7.21	9.56	1.57	9.23	1.77	1.58(.08)[ab]
7.81	10.25	2.18	11.00	1.92	1.72(.09)[b]
8.97	10.38	2.10	10.26	1.96	1.75(.06)[b]
			No heat treatment + 0.35% L-methionine		
6.50	9.88	2.58	10.40	2.48	2.22(.07)[a]
7.21	9.81	3.13	11.79	2.69	2.41(.09)[ab]
7.81	9.81	3.55	12.59	2.86	2.56(.07)[b]
8.97	10.00	3.99	13.42	2.97	2.66(.08)[b]
			Processed 10 min at 121°C		
6.50	10.13	2.96	11.65	2.50	2.23(.06)[a]
7.21	10.13	2.64	11.30	2.31	2.06(.04)[a]
7.81	10.00	2.78	11.97	2.31	2.07(.03)[a]
8.97	10.13	1.45	8.53	1.65	1.47(.07)[b]
			Processed 10 min at 121°C + 0.35% L-methionine		
6.50	10.13	4.66	14.32	3.20	2.86(.04)[a]
7.21	10.25	4.13	13.20	3.04	2.72(.05)[a]
7.81	10.00	3.73	12.62	2.93	2.62(.06)[a]
8.97	9.81	4.17	13.45	3.16	2.82(.04)[a]

[a]Values within parentheses are the standard errors. CP = crude protein; ADG = average daily gain; ADF = average daily feed; and PER = protein efficiency ratio. Casein control diet: CP = 9.88; ADG = 3.53; ADF = 12.74; PER = 2.80. All adjusted PER values were corrected to the casein control diet = 2.50.

[b]Adjusted PER values within each grouping of four containing the same superscript are not statistically different at the 1% probability level as determined by Duncan's Multiple Range Test (Duncan, 1955).

ples of this study is probably the result of an interaction between the cystine content, total sulfur amino acid requirement and the trypsin inhibitor activity of the samples. Increasing the pH resulted in both a lower cystine content and lower trypsin inhibitor activity. Under these conditions an increase in PER would occur as a result of the lower trypsin inhibitor activity but since cystine is also limiting, the extent of the increase in PER will be diminished. While methionine supplementation should correct a cystine deficiency, it should be noted that the level of supplementation (0.35%) combined with the methionine and cystine already present results in a level of 0.65% or less for methionine plus cystine. This is more than the required level as found by Rama Rao et al. (1959) but only equal to or slightly less than the egg value used by Mitchell (1954) to calculate the essential amino acid index. If the metabolic conversion of methionine to cystine is affected by trypsin inhibitor activity as Barnes and Kwong (1965) have suggested, then in the lower pH samples, where the trypsin inhibitor activity is higher, more cystine would become available. The increased cystine level would tend to overcome part of the depressive effect of trypsin inhibitor and result in a compromise PER value. At higher pH the depressive effect of trypsin inhibitor is less, but the amount of available cystine is also less due both to cystine destruction at high pH and the decrease in conversion of methionine in the absence of trypsin inhibitor. Again a compromise PER would result.

In conclusion, it would appear that methionine supplementation is an effective means of improving the nutritive value of alkali-treated, heat-processed soy milk. Methionine supplementation also improved the nutritive value of raw soy milk. In no case, however, did supplementation of the raw milk make it equal in nutritive value to the supplemented, heat-processed soy milk. This indicates that methionine supplementation alone is not sufficient and that in order to obtain the most nutritious product both heat processing to destroy trypsin inhibitor and supplementation to correct cystine deficiency are required.

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