Available Lysine Losses during Thermal Processing of Unconventional Proteins with Glucose

Available lysine losses from cottonseed, yeast, bacterial, algal, casein, and purified bacterial protein sources were monitored following thermal processing. A model system consisting of sufficient protein source to yield 15% protein, 4% glucose, and microcrystalline cellulose was used. The model systems were prepared by mixing the protein, glucose, and cellulose with phosphate buffer to obtain a slurry with a pH of 7.0, freeze-drying, and then rehydrating in a desiccator over saturated cupric chloride to a water activity of 0.68. The model systems were heated for 2 min at 130 °C. Available lysine losses, as measured by Carpenter's fluorodinitrobenzene method, were dependent upon the protein source. Casein, which was included as a reference protein, and cottonseed exhibited the greatest losses of available lysine after processing followed by algal, yeast, and bacterial protein. In contrast, purified bacterial protein lost a greater proportion of its available lysine than any of the other protein sources.

Soy protein and casein are commonly used as protein sources in formulated foods. In the future, other protein sources will undoubtedly be used to a greater extent as a replacer for these relatively expensive proteins. If one protein source is substituted for another in a processed food product, then knowledge of how the nutritional value of each protein is altered by the processing procedure is needed. Loss in nutritional value of a protein due to thermal processing can be measured by determining the available lysine loss (Lea and Hannan, 1950; Carpenter, 1973).

There is limited literature comparing the loss of available lysine between various protein sources during storage or processing. Schnickels et al. (1976) evaluated the loss of available lysine during storage of several pure proteins when included in model food systems (a_w = 0.68, temperature = 35 °C, glucose = 10%). They found available lysine destruction rates to vary with the type of protein used and reported available lysine half-lives (time until half the original amount remained) of 22 days for casein, 25 for whey, 7 for soy (TVP), 19 for fish concentrate, >60 for egg albumin, and 40 for wheat gluten.

Ko et al. (1975) reported on the loss in available lysine of four proteins when stored in the presence of oxidizing lipids. They found available lysine losses of 61% in casein, 54% in egg albumin, 32% in soybean protein, and 35% in wheat gluten when stored for 7 days at 50 °C (a_w = 0.8) under fluorescent light in a model system containing ethyl linoleate and glucose. Casein lost nearly twice as much available lysine as compared to soybean protein.

Ben-Gera and Zimmerman (1972) evaluated the loss of available lysine during the storage of several intact foodstuffs. Temperature (20, 30, or 40 °C), water activity (0.4 or 0.6) and environment (metal cans at 20 in. vacuum or cloth bags exposed to atmosphere) were controlled during 24 months of storage. They reported available lysine losses of 87.6% in nonfat dry milk, 35.7% in cottonseed meal, 33.3% in peanut meal, 30% in chick peas, 43.3% in wheat, 18.5% in rice, 41.9% in soybean, and 7.5% in soybean meal for the same storage conditions. Differences in loss of available lysine during storage of the various foodstuffs were attributed to quantity of reducing sugars present and occurrence of lipid oxidation.

The study was done to evaluate the loss of available lysine during high-temperature heating of "unconventional" proteins in model food systems at a water activity of 0.68.

MATERIALS AND METHODS

Model System. The proteins used in this study were casein (95% protein, Technical Grade, Coleman Bell, Inc.), algal (57% protein, species unknown, prepared at the University of Florida), bacterial (74% protein, *Pseudomonas methylotropha*, Imperial Chemical Industries, Ltd.), yeast (59% protein, Torula yeast, British Petroleum Proteins, Ltd.), cottonseed (68% protein, Plains Cooperative Oil Mill), soy protein (97% protein, Promine D, Central Soya). Proximate analysis of the soy isolate and cottonseed protein are shown in Table I. Endogenous levels of glucose in the protein sources were all less than 0.5% except for the cottonseed preparation (3.2%). Model systems were prepared by dry blending one of the proteins with glucose (4%) and microcrystalline cellulose (to make 100%). The cottonseed model system contained 4.6% glucose due to glucose in the cottonseed protein concentrate. The blended samples were mixed with a minimum amount of pH 7.0 phosphate buffer (0.1 M), frozen, and then freeze-dried. The dried samples were crushed using a mortar and pestle to a fine powder, and then approximately 10 g was placed into a desiccator over $CuCl_2$ (a_w = 0.68), a vacuum drawn, and stored for 4 days to allow equilibration.

Table I. Analysis of Cottonseed Protein and Soy Protein Isolate

	Soy isolate[a] percent	Cottonseed percent
Moisture	4.7	5.4
Protein (N × 6.25)	91.8	62.9
Protein (moist free basis)	96.5	68.3
Crude fat	<0.1	1.0
Ash	3.4	7.3
Carbohydrate	<0.1	23.4[b]

[a] From Technical Literature (Promine D). [b] 3.2% glucose, 20.2% other carbohydrates.

Purification of Bacterial Protein. A portion of the bacterial source was boiled in 1 N HCl (w/v, 1:2) for 20 min. After boiling, the sample was adjusted to pH 7 with a NaOH solution and then centrifuged at 6000 rpm using a GSA Head for 0.5 h. The supernatant fluid was discarded and the precipitate soaked in 10 M urea for 48 h. The urea soluble constituents were dialyzed against distilled water to remove the urea, frozen, and freeze-dried. The freeze-dried preparation was incorporated into the model system and processed.

Thermal Processing. The equilibrated samples (5 g) were sealed in retortable foil pouches (50 M-35F-300C79, R2 retort shock, Continental Group Inc.) and heated in miniature retorts at 130 °C for 2 min. Each pouch contained no more than 0.07 g of sample/cm^2 of pouch surface area. The heating and cooling lags which occurred in these

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Table II. Retention of Available Lysine during the Thermal Processing of Various Protein Sources

Protein source	Available lysine (fraction remaining)[a]
Algal	0.65 ± 0.10
Bacterial	0.75 ± 0.03
Casein	0.46 ± 0.06
Cottonseed	0.48 ± 0.12
Yeast	0.82 ± 0.02
Soy	0.60 ± 0.09
Purified bacterial protein[b]	0.17 ± 0.20

[a] Mean and standard deviation of three determinations. [b] See text for purification procedure.

small samples were offset by increasing the processing time by 6 s. This adjustment was based on the numerical corrections determined by a method similar to the one described by Gondo et al. (1972).

Available Lysine. Available lysine was determined using the fluorodinitrobenzene (FDNB) procedure of Carpenter (1960) as modified by Booth (1971).

RESULTS AND DISCUSSION

Available lysine losses during heating of model systems containing selected proteins are shown in Table II. The loss of available lysine in the soy isolates, compared to casein, appears to be deviate from the data of Schnickels et al. (1976). They reported lysine half-lives (time until half the original amount remained) of 22 days for casein and only 7 days for spun texture soy protein (TVP). Our results appear to indicate that casein loses available lysine quicker than the soy isolate, the opposite of Schnickels et al. (1976). However, different types of soy protein were utilized, and this prevents a comparison of data. Also, after 35–45% lysine loss, available lysine loss in our protein isolate does not follow first-order reaction kinetics. A no-loss phase is entered before 50% loss is achieved (Jokinen et al., 1976; Thompson et al., 1976; Wolf et al., 1977). The samples utilized in our experiments were processed well into the no-loss phase. Therefore, casein, which obeys first-order reaction kinetics, continued to lose available lysine while the soy isolate stopped losing available lysine. Schnickels et al. (1976) results indicated that TVP soy protein does not have a no-loss phase. Therefore, one would expect continued available lysine loss in their system. Thus, the no-loss phase exhibited by our system accounts for the apparent inconsistency in available lysine loss. As can be seen in the table, the single-cell proteins lost substantially less available lysine during heating than did the processed or isolated proteins. One might expect the protein in the single-cell products to be protected by the organism's cellular organization. For example, cellular proteins may be physically separated from cellular reducing sugars. Also, since the cellular protein is encased in a cell wall, one would not expect a reaction of protein with free reducing sugars added to the model system. The cottonseed, casein, and soy products have all been subjected to some degree of processing and isolation techniques. It is generally assumed that denaturation due to chemical or thermal treatment of a protein opens the protein structure and makes it more subject to enzymatic and chemical reaction. Therefore, the processed proteins would be expected to have their natural structural protection removed and configuration changed so they are subject to greater chemical reaction.

It is known that disruption of the cell wall renders single cell protein more digestible and, therefore, of greater nutritional value (Cunningham et al., 1975). Since it appears logical that disruption of cellular structure would also make the cell protein more subject to chemical reaction, we treated a sample of the *Pseudomonas methylotropha* so as to disrupt the cell structure and increase protein purity. The effect of this treatment on available lysine losses is shown in Table II. Thermal processing of the disrupted cells resulted in a fourfold greater loss of available lysine than processing of the intact cells. It is obvious that the cellular structure and, perhaps, protein configuration, provided substantial protection against chemical reaction of the lysine.

A practical consideration should be noted from this study. Isolation and purification of proteins derived from single-celled organisms may result in increased chemical reactivity. This increased reactivity could be very significant when considering losses in protein nutritional value.

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