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FOOD STORAGE STABILITY

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chapter six

Biochemical aspects: nutritional bioavailability

K. Ananth Narayan

Contents

Introduction....126
Overview of nutrient degradation....126
Driving forces affecting nutrient bioavailability....126
Distinction between nutrient content and bioavailability....127
Scope....127
Biological availability of proteins....127
Protein–carbohydrate interaction....127
Nature of the reaction....127
Amino acid models....129
Protein–carbohydrate models and food systems....130
Influence of temperature....134
Influence of water activity, A_w....135
Influence of pH....137
Influence of the type of carbohydrate....137
Influence of antioxidants and metal ions....138
Protein-oxidized lipid interaction....139
Nature of the reaction....139
Influence of the type of lipid or protein....141
Influence of temperature....141
Influence of pH....141
Influence of metal ions and antioxidants....141
Influence of water activity....142
Nutritional effects....142
Protein interactions....143
Protein–protein interactions....143
Protein modification by thermal treatments....143
Protein modification by alkali treatment....144
Protein–polyphenol interactions....145
Protein–phytic-acid interactions....146
Blocking undesirable side reactions....146

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Protein–carbohydrate interaction 146
Protein-oxidized lipid interaction 149
Protein interactions 150
Protein-polyphenol interactions 150
Intermediate moisture food interactions 150
Detection of protein quality losses and quantification 150
Fluorodinitrobenzene method 151
Trinitrobenzene sulfonic acid method 152
Dye-binding method 152
Quanidation 152
Furosine 152
Sodium borohydride reduction 152
Lysine decarboxylase 153
Microbiological methods 153
In vitro estimate of protein digestibility 153
In vitro determination of enzymatically available amino acids 155
Corrected chemical scores for essential amino acids 157
True digestibility of proteins by rat balance methods 159
Bioavailabilities by rat growth assay 160
Protein efficiency ratio 161
Net protein ratio 161
Biological value and net protein utilization 162
Conclusions 163
References 165

Introduction

Overview of nutrient degradation

It is recognized that micronutrients such as thiamin, ascorbic acid, pyridoxine, folacin, tocopherol, retinol, and carotene are to varying extents vulnerable to external and internal stresses, such as temperature, light, water activity, and pH. There is a need to assess and investigate the retention and biological availabilities of macro- and micronutrients as a result of interactions between nutrients and with other constituents. Among those interactions that need further investigation are between proteins and reducing sugars[1–5]; between autoxidized lipids and proteins[6–16]; between dehydroascorbic acid and amino compounds[17]; between lipids and carbohydrates, especially during extrusion processing of foods[18–22]; between minerals and oxalates, phytates, and fibers[23–25]; between cobalamin and ascorbic acid[26]; between riboflavin and ascorbic acid[27]; between niacin and peptides or carbohydrates[28]; between thiamin and carbohydrates[29]; between thiamin and municipal drinking water[30] or brewed tea[31]; among thiamin, nicotinamide, and cobalamin[32]; and between riboflavin and thiamin.[33]

Driving forces affecting nutrient bioavailability

It is almost axiomatic to state that temperature is the major driving force influencing nutrient retention and bioavailability in stored foods. Other prominent factors are water activity (A_w), pH, packaging, and visible and ultraviolet radiation. Another important factor is the presence, abundance, and chemical structure of the interacting species. For example, the maintenance of high protein quality in protein-rich foods is affected by even small concentrations of reducing sugars or oxidizing lipids.

Distinction between nutrient content and bioavailability

The distinction between nutrient content and nutrient bioavailability is in many instances (e.g., vitamins) not clearly evident. Nutrient content represents the sum total of the nutrient as measured by recognized assay procedures, which in many cases is a chemical method; nutritional bioavailability represents only that portion of the nutrient which is digested, absorbed, and metabolized. Because there is a need to minimize animal experimentation, reliance upon *in vitro* enzymatic procedures for nutrient bioavailability has increased. In such instances, nutritional bioavailability may be narrowly redefined as that portion which is available after enzymatic digestion for absorption and metabolism in the body.

Some examples illustrating the difference between nutrient content and nutritional availability are instructive. The iron content of spinach is high, but only a small portion (1.3%) is available for absorption due to chelation of iron by oxalic acid in spinach, even when the ingested dose is small (2 mg). Similarly, substantial levels of fat-soluble vitamins ingested with high levels of synthetic fats, such as sucrose polyesters, may be less available due to interference in absorption by these fats. Food proteins interact with oxidizing polyunsaturated fats (PUF) during storage and might be poorly digested by proteolytic enzymes. Thus, biological availability is compromised, whereas the total content of the proteins as determined by Kjeldahl nitrogen remains largely unaffected.

Scope

This review will be restricted to foods in the pH range of 5 to 7. Nutrient–vitamin and nutrient–mineral interactions and their influence upon the bioavailabilities of vitamins and minerals are not covered. The focus will be on those interactions that affect the protein quality of foods. In general, due to the lack of data, the retention of vitamins rather than their bioavailabilities have been reported in the literature. Baker reported on the poor biological availability of several vitamins in some foods: niacin (10%) in corn; biotin (40%) and pathothenic acid (60%) in wheat and barley; and vitamin B_2 (60%) in corn and soymeal.[34] The numerous interactions that influence the bioavailabilities of vitamins and minerals have been the subject of many reviews and research articles.[35–47] The metabolic interrelationships among vitamins, minerals, and hazardous elements have been the subject of an earlier conference.[39] Accordingly, the thrust will be on protein availability changes during storage of foods, with some discussion on processing effects.

This review will not address the metabolic consequences of either macro- or micronutrients, especially where they are in great excess. For example, it will not address the question of nutritional consequences of ingesting of excess lysine, leucine, protein, fat, or fiber in the diet.

Biological availability of proteins

The bioavailability of food proteins is compromised when they become less digestible by proteolytic enzymes as a result of crosslinking reactions between proteins and carbohydrates, proteins and oxidized lipids, and proteins and other components in food.

Protein–carbohydrate interaction

Nature of the reaction

By far the most damaging reactions are those between proteins (or free amino acids) and carbonyl compounds, particularly reducing sugars. The Maillard reaction[48–50] is a complex multistage reaction and has been the subject of several excellent reviews and investiga-

tions.[51–67] An extensive review of the early literature (1915–1950) on heat injury to dietary protein is also available,[68] and one must acknowledge that some of the current considerations of this subject were recognized, investigated, and properly interpreted over 80 years ago. For example, in 1915, McCollum and Davis understood the cause of the loss of nutritive efficiency in heated milk.[69] In 1925, Morgan and King reported retardation of growth in rats fed browned food products, such as toasted crackers, toasted whole wheat, toasted white bread, puffed wheat, puffed rice, or the crust of white bread,[70] which has been confirmed and extended in subsequent investigations.[71,72] Tsen et al. showed that microwave and steam-baked breads were superior in protein quality to conventionally baked bread.[71] Browned albumin following interaction with glucose was demonstrated to be a poor protein source.[72]

In the first phase of the Maillard reaction, the Schiff base formed between the free ϵ-amino groups in lysine of proteins and the carbonyl group of reducing sugars cyclize to the corresponding *N*-substituted glucosylamine and then undergoes an irreversible Amadori rearrangement to form ketose sugar derivatives (*N*-substituted 1-amino-1-deoxy, 2-ketose, commonly referred to as the Amadori compounds [ACs]). The ACs decompose in the subsequent stage of the reaction to give rise to a variety of scission products, carbonyl compounds, heterocyclic flavor compounds, as well as brown melanoidin pigments.[1,51] The ACs, as well as the carbonyl compounds formed upon their degradation, are recognized for their specific reducing properties and afford a simple means of monitoring the reaction. Some of these compounds are powerful lipid antioxidants and, hence, are extremely valuable in extending the shelf life of foods containing polyunsaturated fats.[73] The compounds produced[1, 74] during the second stage of the reaction are responsible for many of the pleasing aromas and flavors that are cherished so much in foods, such as bread, coffee, cocoa, roasted nuts, maple syrup, beer, and wine. They enhance the sensory acceptance of foods and indirectly improve shelf life. Unfortunately, these components also degrade and produce undesirable off-flavors.

From a nutritional standpoint, the compromise to protein quality occurs at the first stage of the Maillard reaction (MR), because the ACs are not digested by mammalian proteolytic enzymes and so they limit the biological availability of lysine (LYS) in foods. Data indicates considerable loss of LYS due to MR in model systems during relatively mild storage conditions.[4,75,76] The loss of LYS in an LYS-GLU-cellulose model (where GLU refers to glucose) at a low A_w of 0.2 as a function of time and temperature is shown in Figure 1. As much as 25% of LYS was lost in this model system in 6 days at 40°C. Further LYS loss is significantly less rapid since the reaction obviously does not follow zero-order kinetics. The brown color developed during the final stages is sometimes used erroneously as a measure of damage to protein quality. Confirming other literature data, the study clearly illustrated that as much as 70% of the LYS was lost in the LYS-GLU-cellulose model system *before* there was a significant increase in color (Figure 2) or in fluorescence (not shown).[4] However, furosine correlated well ($r = 0.98$) with LYS loss.

The reaction between LYS and GLU has generally been considered to be first-order. However, semilog plots of LYS concentration vs. time do not give straight lines (beyond 50–70% LYS loss).[4] It has been argued that the reaction more closely follows second-order kinetics than first-order kinetics.[77] After a period of time, LYS concentration levels off faster in a second-order reaction than in a first-order reaction.

From a practical standpoint, however, protein-rich foods containing reducing sugars and stored for long periods (such as in the case of military rations) may not lose their protein quality as rapidly as would be predicted by first-order kinetics. An alternative is to use curve-fitting approaches to predict shelf life over extended periods. Narayan and Andreotti fitted the data from LYS-GLU-cellulose and *N*-alpha-acetyllysine (NAL)-GLU-cellulose models to double exponential models.[4] These adequately describe LYS loss

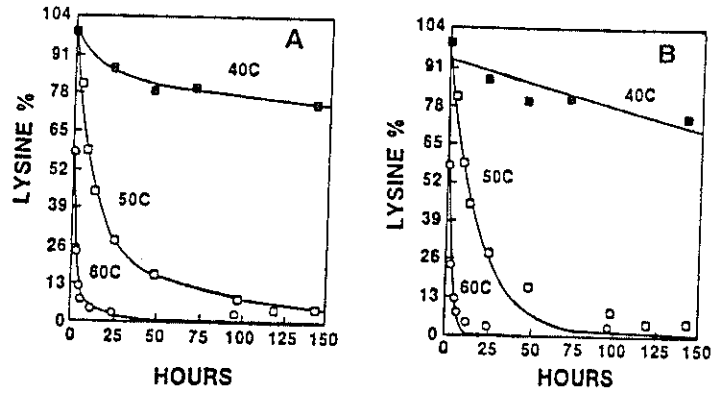


Figure 1 Lysine loss at various temperatures and low A_w in the range of 0.19 to 0.21 with compressed lysine-glucose models. (A) lysine data fit to the double exponential equation, $y = Ae^{Bx} + De^{Fx}$; and (B) lysine data fit to the first order exponential equation, $y = Ae^{Bx}$. (From Narayan, K.A. and Andreotti, R.E. 1989. J. Food Biochem. 13: 105-125. With permission).

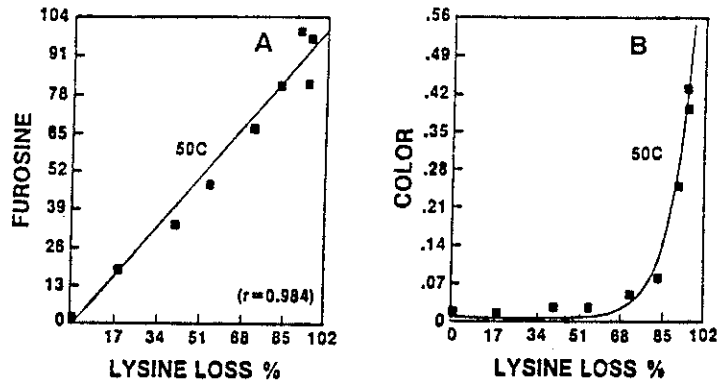


Figure 2 Correlation between the increase in (A) furosine ([10⁷] Data Module 930 peak area units/mg lysine) and (B) color absorbance at 410 nm/mg lysine with the decrease in lysine in the compressed lysine–glucose–cellulose model at 50°C. (From Narayan, K.A. and Andreotti, R.E. 1989. J. Food Biochem. 13: 105-125. With permission).

during storage. An excellent fit ($r > 0.99$) between observed and predicted values is seen for LYS-GLU-cellulose models,[4] as well as for NAL-GLU-cellulose models[77] at 40°C, 50°C, and 60°C. The equation used is

$$C = ae^{bt} + ce^{dt}$$

where C = concentration of lysine (mg/g model) expressed as percentage of initial concentration; t = time; and a, b, c, and d are derived constants.

Amino acid models

Amino acid-reducing sugar models are very useful for determining the effects on the Maillard reaction of temperature, A_w, pH, additives, and blocking agents. The early work of Hannan and Lea, who reacted NAL with GLU at 37°C and 20%, 40%, and 60% RH, showed that as much as 25% of NAL was lost in 3 days at an RH of 20%.[76] Eichner investigated the reactivity of LYS-GLU-cellulose models and observed significant effects

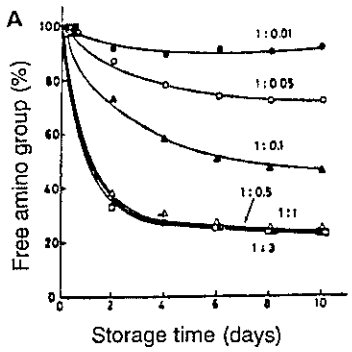


Figure 3 Maillard reaction between ovalbumin and aldohexoses at 50°C and 65% relative humidity. Decrease in free amino group of ovalbumin stored with various levels of glucose and galactose, respectively for 10 days. (From Kato, T., et al. 1986. J. Agric. Food Chem. 34: 351-355. With permission.)

of variables such as A_w, glycerol, and cysteine.[73] From an Arrhenius plot utilizing rate constants for Maillard browning at 80°C, 90°C, and 100°C for glucose–glycine and glucose–aspartame models, Stamp and Labuza have accurately predicted the number of days to reach an optical density (OD) of 0.1 at a low (45°C) temperature, estimating 62 vs. observing 60 days.[78] Amino acid models, such as dextrose and lysine as well as tryptophan and cysteine, have been used in an attempt to predict the shelf life of parenteral nutrition solutions for human use.[75]

Protein–carbohydrate models and food systems

Because of the ease with which optical absorption measurements can be made, Maillard browning rather than Maillard protein quality decrement has been widely investigated. Interesting observations were made by Kato and colleagues in their studies with ovalbumin (OVA)-aldohexose models at 50°C and RH of 65% incubated for 0–26 days.[79,80] The loss in free amino groups was essentially the same with all three sugars (GLU, mannose (MAN), and galactose (GAL)) used (Figure 3).[80] In all cases, the free amino groups decreased with increase in the ratio of sugar to protein. However, GAL produced maximum browning[80] and a high degree of protein insolubilization (Figure 4), which is of concern because this sugar may be present in stored dairy products. In studies comparing OVA-GLU (1:1 w/w) and OVA-lactose (LAC) (1:2 w/w) models (79), it was observed that there was virtually no browning with the LAC model (Figure 5A), but there was substantial loss (70–80%) in free amino groups in both models over a period of 26 days. Once again, this emphasizes that although stored dairy products may be virtually colorless, they could have suffered a significant loss in protein quality. However, such high losses in LYS with a protein–dissacharide model is contrary to normal experience and may be due to the high A_w (65% RH) used.

It is generally accepted that browning is an extension of the first phase of the Maillard reaction[1] in which the ACs first break down to 3-deoxyhexasone, methyl dicarbonyl intermediates, hydroxymethyl furfuraldehyde, and reductones, and subsequently undergo a series of condensation–polymerization reactions to form melanoidin pigments. This work has demonstrated that if the blocked protein is separated from the unreacted sugars, further browning virtually ceases even for the OVA-GLU blocked protein (Figure 5B).[79] Addition of GLU or LAC to the two blocked protein models free of unreacted sugars showed that GLU, but not LAC, was essential for further browning (Figure 5C, 5D).

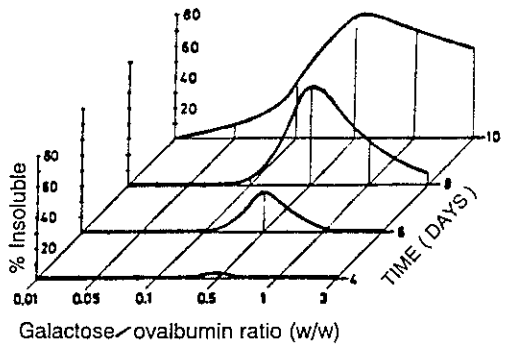
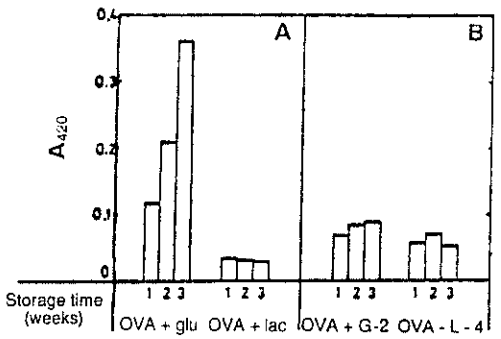


Figure 4 Insolubility of ovalbumin by storage with galactose for 4–10 days at 50°C and 65% RH. (From Kato, T., et al. 1986. J. Agric. Food Chem. 34: 351-355. With permission.)



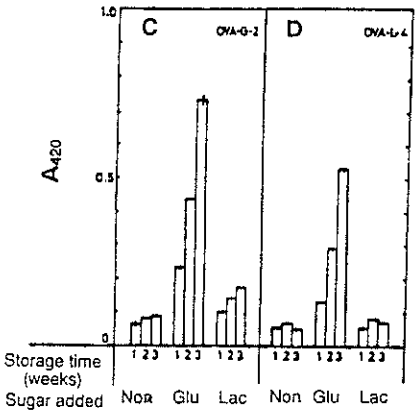


Figure 5 The effect of the type, presence, or absence of reducing sugar on the browning of ovalbumin (OVA). (A) OVA-glucose (GLU) and OVA-lactose (LAC) systems stored for 1–3 weeks at 50°C and 65% RH. (B) OVA-GLU and OVA-LAC systems freed from unreacted sugars after 2 and 4 days of reaction, respectively, and stored for 1–3 weeks. (C and D) OVA-GLU and OVA-LAC systems, freed of sugars, reincubated with or without free sugars (GLU or LAC) for another 3 weeks. "Non" is no sugar added. (From Kato, T., et al. 1988. J. Agric. Food Chem. 36: 806-809. With permission.)

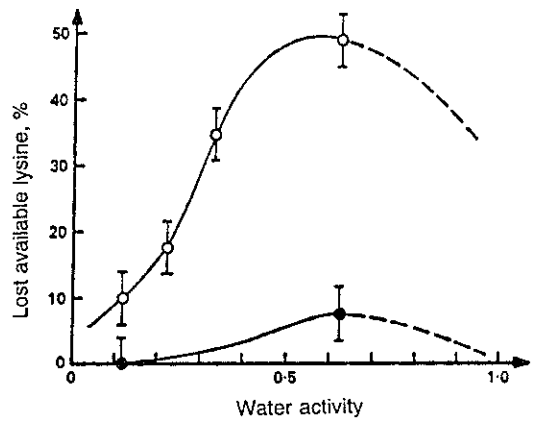


Figure 6 Loss of available lysine as a function of water activity at 1 month's storage. Solid circles, unhydrolyzed milk; open circles, lactose-hydrolyzed milk. The dashed lines are drawn to indicate the probable form of the curves. The bars indicate 95% confidence intervals. (From Burvall, A., et al. 1978. J. Diary Res. 45: 381-389. With permission.)

Hence, undesirable browning and off-flavors may be avoided if the reducing sugars remaining after processing can be encapsulated, neutralized, degraded, or bound.

Stability data at extreme temperatures (30–300°C) are available for various casein-reducing sugar models and have provided limited data on available lysine, protein efficiency ratios (PERs), and protein digestibilities.

There is considerable concern with regard to the stability of dairy products because of their relatively high levels of lactose. In ordinary milk powder, browning is remarkably low during storage.[81] Even at 25°C and an A_w of 0.62, only 25% of the lysine was lost in 9 months. However, with the lactose-hydrolyzed milk (Figure 6), 48% of the lysine was lost in 1 month of storage at 25°C. A loss of 28% and 35% in total lysine and methionine, respectively, has been reported for skim milk powder having $A_w = 0.57$ and stored at 40°C for 1 month.[82] In addition, the pepsin, trypsin, and chymotrypsin digestibilities were reduced. The effect of several processing treatments on lysine availability in milk[2] indicated extensive loss during roller drying (Table 1). The loss, especially in enzymatically available lysine loss, increased with decrease in the speed of the roller. Similar losses during roller drying of cheese whey have been reported.[83] Post-processing, the subsequent storage losses of this product, whether roller dried or spray dried, were similar.

The influence of temperature, A_w, and time have been studied with a diafiltered whey protein (2.1% lactose)–lactose system (LYS:LAC, 1:1).[84] At 121°C for 5000 s, irrespective of the A_w of 0.3, 0.5, or 0.7, the losses in enzymatically available lysine exceeded 75% (Figure 7). Even at 100°C and 500 s of heating time, the losses were excessive (40–50%). At an A_w of 0.97, the losses were modest (5–23%).

Besides changes in lysine, color, and digestibility, Hsu and Fennema have investigated changes in functionality (e.g., solubility, foaming properties, and emulsification ability) in a dry whey protein concentrate (52% protein, 36% lactose) during storage for 6 months at temperatures ranging from –40 to 40°C with A_w from 0.15 to 0.41.[85] The functional properties were relatively stable, but browning increased with storage time. Toughening of the texture of meats during storage is attributed, in part, to Maillard reaction. The differences in the two foods may be related to the proportions of fibrous and globular proteins.

Apart from A_w, the importance of the crystalline or amorphous (hygroscopic) nature of the reactants involved in Maillard reactions has been underscored by several authors.[86–88] At low A_w, the crystalline form has a low capacity for water absorption, while the amor-

Table 1 Influence of Various Treatments on Lysine Values of Standard Milk (g/16 g N)

Samples	Total Lysine TLV	Furosine FUR	Availability by Enzymic Method ALV_{enz}
	Non-treated milks		
Fresh milk	8.22	0	8.37
Freeze-dried	8.22	0	8.08
Spray-dried	8.32	0	8.22
Standard milk (SM)	8.13	0	8.20
	Sterilization of SM		
115°C, 10 min	7.97	0.30	7.77
115°C, 20 min	7.96	0.31	7.47
115°C, 30 min	7.80	0.34	6.51
115°C, 40 min	7.70	0.35	5.97
	Roller-drying of SM		
6 atu, 11 rpm	5.88	1.24	4.57
6 atu, 5.5 rpm	5.34	1.52	3.04
6 atu, 2 rpm	4.51	1.98	2.15

Note: Methods used: acid hydrolysis, furosine, and enzymatic digestion.

From Finot, P.A., et al. 1981. In: Progress in Food and Nutrition Science. Maillard Reactions in Food (C. Eriksson, ed.), Pergammon Press, Elmsford, NY, pp 345–355. With permission.

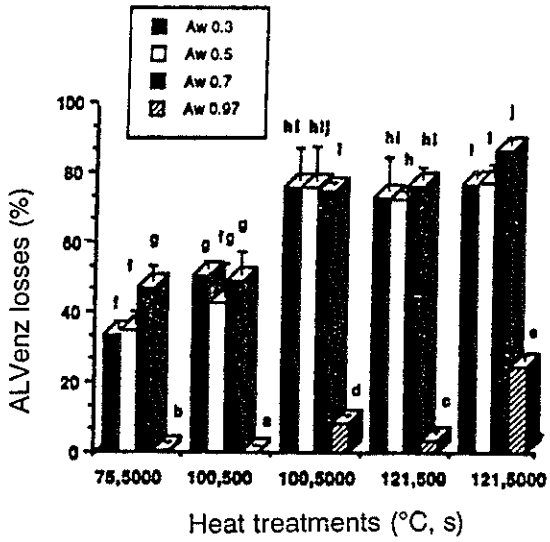


Figure 7 The effect of time, temperature, and A_w on lysine in a diafiltered whey-protein–lactose system. Results are expressed as enzymatically available lysine losses (%). Each bar is the average of three determinations (± SD). Means bearing a common letter are not different according to the LSD test (95% confidence level). (From Desrosiers, T., et al. 1989. J. Agric. Food Chem. 37: 1385-1391. With permission.)

phous form has high capacity. Nonhygroscopic whey powders have been reported to result in less browning and lower losses in lysine than hygroscopic whey powders with the same A_w due to release of water in the hygroscopic powders as the lactose crystallizes.[88] However, the differences in the two sets of data are significant only at A_w of 0.44 and 0.65 at 45°C,[88]

but not at the lower temperatures (25°C and 35°C). There is some ambiguity in this area, since Huss concluded that there is negligible damage to available lysine in dried skim milk powder when lactose is present in the amorphous form.[87]

Maillard browning of hygroscopic whey powders equilibrated to several A_w is greater in sealed than in open pouches, partly because of the water released during crystallization of amorphous lactose is trapped in sealed pouches resulting in higher A_w than in open pouches.[89] Hence, this factor must be taken into account in the packaging of hygroscopic foods. The role of glass transition in food models has been examined by a number of investigators.[90,91]

The storage stability tests on intermediate moisture food (IMF) models have indicated that significant losses in lysine occur before there is objectionable browning and that a humectant such as glycerol lowers the A_w to the range where the maximum rate for Maillard reaction occurs.[92]

Kramer has assembled a series of tables based on data from the early literature to estimate the maximum storage temperatures for limiting percentage of losses of several nutrients stored for various times.[93] Table 2 is a guide for maximum warehouse temperatures that can be tolerated in order to preserve protein quality of nonfat milk powder. Unfortunately, storage is never at one fixed temperature; since there is always a certain amount of cycling of the temperature during daytime and nighttime; moreover, packaging and other storage conditions can have an effect. Since the prediction of storage life is more complex in these situations, mathematical models making use of data obtained at constant temperatures have been developed and applied to specific foods.[94–96] Nielson et al. have determined the comparative losses of tryptophan (TRY), methionine (MET), and lysine (LYS) in several model systems.[97] During early and advanced stages of the Maillard reaction in whole milk powder stored at 50°C and 60°C for 9 and 4 weeks, respectively, MET and TRY were unchanged, but LYS losses were extensive (21% and 80%, respectively).

Influence of temperature

The reaction between proteins or amino acids and reducing sugars is greatly influenced by temperature, as judged by the Q_{10} values reported in the literature,[4,98] which impact protein bioavailability. This reaction proceeds significantly during normal storage and sometimes occurs even during freezer storage, which is a cause for concern.[99] Additional data are needed during low temperature storage to ensure the nutritional quality of susceptible products during long-term storage.

The loss of 25% of LYS and in a LYS–GLU–cellulose (CEL) model with an A_w of 0.2 stored for 6 days at 40°C was referred to earlier (Figure 1).[4] In subsequent studies with a NAL–GLU (1:1 mol/mol)–cellulose model, 70% of the NAL was lost in about 7 months at 40°C.[77] The estimated activation energy, E_a, of 36 kcal/mol for the NAL–GLU–cellulose model is lower than the value of 57 kcal/mol obtained for the LYS–GLU–cellulose model, which presumably reflects the blocking of the alpha-amino group in lysine by acylation. One can speculate that by acylating the free ϵ-amino group it might be possible to reduce the temperature sensitivity of the Maillard reaction. Lysine loss curves (Figure 1) during storage of LYS–GLU–cellulose models[4] suggest that a relatively simple way to retard protein quality loss is by reducing storage temperature. However, at freezer temperatures, the water in the food freezes out thereby resulting in concentrated reactants being in a medium with a more favorable A_w in which to react.[99]

The effect of time, temperature, and A_w on a low LAC whey protein (2–4% LAC)–LAC (1:1 LYS:LAC mol/mol) model[84] is shown in Figure 7. Based on the effect of heating samples with common A_w for 5,000 s at 75°C and 100°C, the enzymatically available LYS is almost three times as much at 75°C than at 100°C. Similarly, the available LYS is more

Table 2 Guide for Maximum Storage Temperature (°F) for Limiting Percentage of Loss of Protein in Dehydrated Products under Several Storage Conditions

	Months in storage			
	6	12	18	24
Wheat flour				
<10% loss				
in sealed jars	50	38	32	28
in bags	38	26	20	17
<20% loss				
in sealed jars	100+	100+	100	100
in bags	100+	100	70	44
Nonfat milk solids				
<10% loss				
in bags, 60°RH	30	−8	—	—
in bags, 40°RH	52	+6	—	—
in vacuum cans	100	58	38	+5
<25% loss				
in bags, 60°RH	76	30	0	−8
in bags, 40°RH	96	46	20	+8
in vacuum cans	100+	95	68	52
<50% loss				
in bags, 60°RH	80	62	46	38
in bags, 40°RH	100+	92	86	45
in vacuum cans	100+	100+	100	95

[a] Total protein nitrogen.

[b] Protein efficiency ratio.

From Kramer, A. 1974. Food Technol. 28: 50–60.

than twice as much at 100°C than at 121°C after 500 s of heating. In aspartame–glucose and glycine–glucose models with an A_w of 0.80,[78] the rate constants, the time to reach 0.1 absorption units, E_a, and Q_{10} were all estimated. The rate constant in the aspartame–GLU system at 100°C was sixfold greater than at 80°C; in the glycine–GLU system, this ratio was only 3.5, indicating that aspartame added to foods may degrade more rapidly.

The early work of Ben-Gara and Zimmerman provides a lot of information on the stability of several foods (nonfat dry milk, peanut meal, soybean, wheat, rice, etc.) at 20°C, 30°C, and 40°C and RH of 40% and 60%.[100] The decrease in chemically available lysine content of nonfat dry milk after being in 6 months storage at 40% RH and 20–40°C ranged from 18–66%, respectively; at 60% RH, it ranged from 27–84% for the two temperatures. The maximum losses during a 24 month storage at these temperatures were 88% for nonfat dry milk, 34% for peanut meal, 42% for soybeans, and 18% for rice.

Influence of water activity (A_w)

Protein–carbohydrate interactions are profoundly influenced by the A_w of the system. One way to greatly impede this reaction is to make use of dry foods (such as freeze-dried foods) with as low an A_w as possible (i.e., less than 0.2). On the basis of the early studies, such as those of Loncin et al.,[101] it is generally accepted that Maillard browning or lysine loss vs. A_w conforms approximately to a bell-shaped curve with an A_w for maximum damage being in the range of 0.6–0.7 (Figure 8). There is a considerable shift downward to lower A_w for maximum damage when glycerol is included in the reaction medium. Without any additives at low A_w, it is likely that the mobility of the reactants is severely

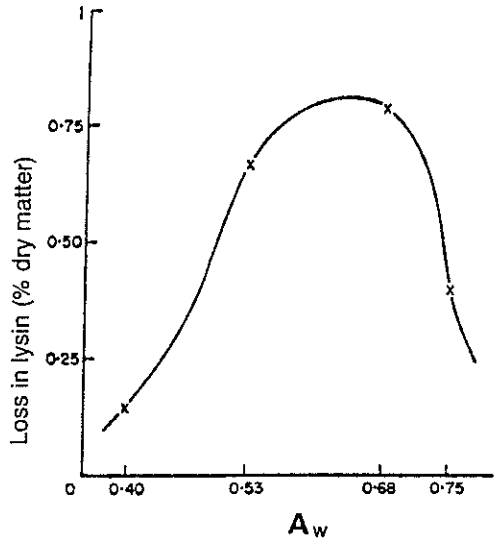


Figure 8 The loss in free (available) lysine of milk powder kept at 40°C for 10 days as a function of A_w. (From Loncin, M., et al. 1968. J. Food Technol. 3: 131-142. With permission.)

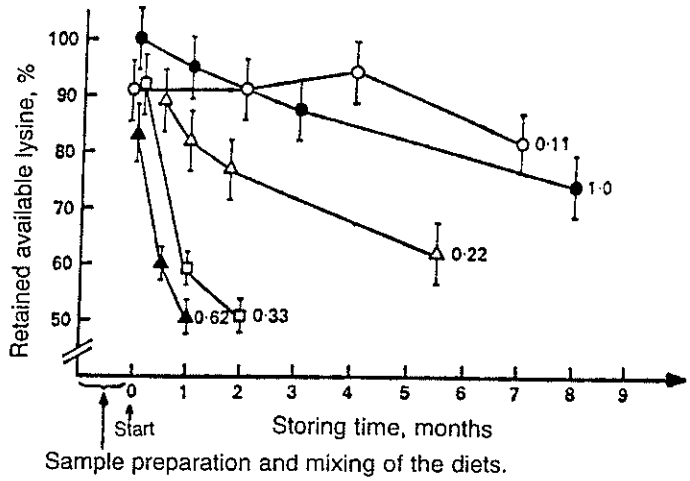


Figure 9 Storage of lactose-hydrolyzed milk. Retention of available lysine is shown as a function of storage time at 25°C at different A_w (0.11, 1.0, 0.22, 0.33, and 0.62 from top to bottom). The bars indicate 95% confidence intervals. (From Burvall, A., et al. 1978. J. Dairy Res. 45: 381-389. With permission.)

restricted due to low diffusivity, which is responsible for low reactivity. At the other end, at high A_w the reaction rate decreases due to dilution of the reactants.

The effect of several different A_w on lysine availability in lactose-digested milk, LHM, is shown in Figure 9. This product is stable at an A_w of 0.11. It is highly unstable at A_w in the range of 0.33 and 0.62. The rate falls off at an A_w of 0.22. At $A_w = 1.0$, there is a limited, but measurable, loss. Ordinary milk powder is quite stable at $A_w = 0.3$ and A_w approaching 1.0. The greater activity of hygroscopic whey powder as compared with nonhygroscopic whey powder has been discussed above.

The advantages of intermediate moisture foods (IMF) are well recognized: (1) they can be consumed readily without heating (important for many field feeding situations);

(2) they need no refrigeration; (3) they can be conveniently packed in flexible pouches; (4) they can be consumed any time after opening the package, since even open packages are microbiologically safe; and (5) they retain nutrients better than canned products due to limited heat treatment. IMFs are rendered microbiologically safe by reducing their A_w to 0.85. However, the amount of glycerol necessary to reduce the A_w to 0.85 is high (43%). Furthermore, the investigations of Narayan and colleagues on the nutritional effects of glycerol showed that rats fed high levels of glycerol in a low polyunsaturated fat diet produced fatty livers and increased levels of serum lipids, chylomicrons, and high-density lipoproteins.[102–104]

A strategy involving a combination of pH and other adjustments (O_2 tension, antimicrobial agents, and/or mild heat treatments) can be used to control microbial growth in the A_w range of 0.900–0.950.[105,106] Similarly, Fox et al. have also suggested that a combination of mild processing methods and controlled pH and A_w may be used to enhance nutrient quality and acceptance of IMF through minimization of adverse effects caused by humectants and increased processing temperatures.[107] With regard to Maillard browning, the lag time was high at all A_w, so it was not a problem. In order to overcome some of the objections to high levels of humectants (flavor, texture) in present IMFs, it may be possible to reduce the humectants and still stabilize IMF having high A_w foods by reducing pH, by treating mildly with heat, and by adding antimicrobial agents. The combined use of multiple factors such as A_w, temperature, pH, preservatives, redox potential, as well as modified atmosphere packaging, edible coatings, Maillard reaction products, bacteriocin, ultrahigh pressure, etc., result in a series of hurdles which simultaneously disturb the homeostatic mechanisms of microorganisms collectively and synergistically. This results in a safe, nutritious, and highly acceptable product that could not have been possible through the individual action of any one of these hurdles acting separately.[108–110]

Influence of pH

As might be expected, the rate of Maillard reaction is low at low pH and increases progressively as the pH is increased. The effect of pH on amino–carbonyl interaction has been investigated by Wolfrom et al., who showed that in an alanine–glucose system Maillard browning is most intense at pH 7, followed by pH 5 and then by pH 3.[111] Ashoor and Zent have shown the maximum browning occurred at a pH of about 10 for 21 amino acids tested with glucose, fructose, and lactose at 121°C for 10 min.[112]

The effect of pH was studied in solid NAL–GLU–CEL models by Narayan, Cross, and Bibeau (unpublished results). The reactants were dissolved in large volumes of deionized water or various buffers with pHs of 9.0, 7.0, and 4.0, frozen, and then concentrated upon freeze drying. The rates of reaction in samples that were originally at the higher pHs (ca. 9.0 and 4.0) were considerably greater at all times after 4 h than the rates in samples made from pH 7.0 in the powder systems with moisture content 1.7% and an A_w of 0.2 and heated at 60°C. The sample made with deionized water (Milli Q) reacted more rapidly than the samples prepared at pH 7.0 or pH 4.0. It is intriguing that there is a significant effect of pH and buffer salts on reactivity in such dry systems.

Influence of the type of carbohydrate

While only reducing sugars interact with proteins via the Maillard reaction, browning during the storage of model systems containing sucrose and amino compounds has been reported.[113] Under the slightly acidic conditions (pH 5–6) in most foods, the hydrolysis of sucrose could proceed at a slow rate and produce the reactants. If the food contains an acid-like citric acid, the hydrolysis is fairly rapid. In the presence of enzymes, as in the case of lactase in cultured milk or yogurt, the hydrolysis to glucose and galactose is rapid.

Reducing disaccharides and reducing oligosaccharides are less reactive than monosaccharides. The pentoses are, in general, more reactive than the hexoses and the aldoses are more reactive than the ketoses.

Hashiba has listed the order of reactivity with glycine of the several sugars at 120°C: ribose, glycuronic acid, xylose, arabinose, galacturonic acid, galactose, mannose, glucose, and lactose.[114] The large difference in Maillard browning between OVA–GLU and OVA–LAC models (Figure 5) has already been mentioned. Similarly, Burvall et al.[81] and Finot et al.[2] have shown that the reduced lactose milk is far more reactive than ordinary milk powder. As would be expected, the sugar/protein ratio is important; in one study,[115] the rate constant for lysine loss increased with increase in this ratio from 0.5 to 5.0. What is generally not recognized is that in the reaction between free LYS and GLU, as many as four GLU moieties may react with a single free LYS giving rise to tetrafructosyllysine. In principle, this process could lead to a tightly crosslinked polymer through the union of the carbonyl groups in this compound with additional lysine compounds. The bulkiness of the compound formed together with the propensity of ACs formed to degrade easily to carbonyl and amino compounds could hinder such unrestricted polymeric growth. In proteins, the bound LYS can react with two GLU moieties and the two AC carbonyl groups can react with two other LYS moieties, subject to any constraints of steric hindrance.

Influence of antioxidants and metal ions

Experiments have been conducted that appeared to suggest that antioxidants (AOs) are effective in inhibiting the Maillard reaction.[116,117] The data showed that several AOs (ascorbic acid, tocopherol, propyl gallate, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA)) were effective in minimizing the loss in lysine and the formation of furosine after acid hydrolysis in a casein–GLU–AO model incubated at 37°C and 60% RH for 0 to 60 days.[116] However, certain anomalies are apparent. The browning appears to have proceeded at the same rate for 30 days in all systems (with or without AOs), which would be unlikely if the early stages of the Maillard reaction had been arrested. Based on the furosine yield, which correlates linearly with lysine loss (Figure 2), the predicted LYS loss for samples without AOs at the end of 60 days should have been in the range of 35–50%, rather than the 10–18% reported.[116]

It is known that ACs, and especially the carbonyl compounds formed from them, are powerful lipid antioxidants.[1,73,118] Yet it has been observed that Maillard reaction products (MRP) from glutamic acid and either glucose or fructose[119] showed no antioxidant properties. The high stability of fructose–glutamic acid is known. Protein hydrolsates are poor antioxidants, but after reaction with glucose their antioxidative activity improved greatly. Strong antioxidants were obtained from MRP of dipeptides and glucose; the MRP obtained by reacting L-histidylglycine with xylose proved to be a very effective lipid antioxidant.[120] The potential use of such specific products in actual foods containing PUF to inhibit their oxidation remains to be tested.

There is indication that copper, iron, and cobalt salts may enhance amino–carbonyl interactions.[121] Bohart and Carson have reported that even small amounts of manganese (0.4 ppm) inhibits the rate of browning in air.[122] Other metal ions inhibit browning including tin.[121–123] Rendleman and Inglett have extensively investigated the influence of Cu^{2+} in the Maillard reaction and have concluded that cupric ions enhance the rate of Maillard browning in several model systems.[123] The differential effects of metal ions (Na^{+}, Cu^{2+}, Fe^{3+}, and Fe^{2+}) on this reaction have also been reported by others.[124,125] Melanoidin pigment exhibited a strong affinity for Ca^{2+} and Cu^{2+} ions.[125] Whether this affinity has any significance in the biological availability of calcium and copper remains to be determined.

Protein-oxidized lipid interaction

Nature of the reaction

Polyunsaturated lipids can readily autoxidize during processing or storage in the presence of air, especially when antioxidants are depleted, and can also interact with food proteins to form tightly bound complexes. Following the pioneering investigations by Tappel[6] on the binding of oxidized fatty acids and esters to form stable complexes with proteins, Narayan and colleagues reported on studies relating to the nature of these complexes and the factors influencing their formation.[7,126,127]

These reactions and interactions are of interest in food science as well as in other disciplines. The complex reactions that occur during the oxidation of fats in the presence of proteins are considered to be responsible for the yellowing of bacon and rusting of fish during storage.[128] Physiologically, oxidized fats have been implicated in the formation of brown pigments in the adipose tissue of vitamin E deficient rats as well as ceroid pigments in choline deficient rats.[128,129] Hartroft has reported on the presence of ceroid pigments in the aortas of men exhibiting atheromatous changes; he also observed the formation of ceroid-like pigments upon reacting red cells with cod liver oil in air.[130,131]

Narayan and colleagues investigated the chemical and physical properties of complexes between a food protein (egg albumin) and either oxidized linoleic acid, thermally oxidized (TOCO), or thermally polymerized (TPCO) corn oil.[126,127] With a highly polymerized oxidized corn oil and after 48 h at 60°C, as much as 41% of the lipid was bound and could not be extracted with several solvents by the Soxhlet procedure. With trilaurin, oleic acid, unoxidized linoleic acid, and unoxidized corn oil subjected to a similar treatment, little or no lipid was found bound to the protein. On the basis of further reaction with lauroylchloride, it was concluded that the reactive amino, hydroxy, or sulfhydryl groups in the protein were not involved in the interaction with oxidized lipids. Based on reactions of three aldehydes having differing chain lengths (C2, C9, and C12), it was suggested that such binding is not due to a simple aldehyde–amine interaction. Malonaldehyde, a dialdehyde, was not used, since it is not formed from linoleic acid oxidation. Secondary forces, primarily hydrogen bonding and noncovalent bonding, were deduced to be responsible for binding the oxidized lipid to the aggregated protein.

Many other investigations of the reactions between oxidized lipids and proteins have since been conducted,[8–10,132–139] and it is instructive to consider them briefly because they may have a bearing upon storage stability of foods and could suggest possible strategies for minimizing this interaction. Desai and Tappel[8] proposed a free radical mechanism where the lipid peroxy and lipid alkoxy radicals would interact with the protein and result in lipid–protein copolymers.[8] Nonradical mechanisms involving oxidized lipid scisson products (mainly malonaldehyde) and protein–amino groups have also been proposed.[132] A free radical mechanism has been proposed, in which lipid free radicals lead to the formation of protein free radicals, resulting in protein polymerization with the occlusion or association of the oxidized lipid in some type of lipid–protein complex that does not involve covalent bonds between the two compounds.[9] Roubal later suggested that the denatured protein polymer could effectively trap and stabilize lipid radicals.[134] Gamage et al. separated radical and nonradical products of linoleate oxidation and reacted them with ribonuclease.[137] They reported that the radical product fraction was responsible for protein polymerization, whereas the nonradical product fraction resulted in fluorescence of the reaction product.

Of interest in this connection is an excellent but not often cited review of free radicals in low moisture systems[10] that provides general and specific information on (1) free radical formation in food as a result of ionizing radiation, photolysis, mechanical energy, freezing, and dehydration; and (2) free radical reactions in foods and their effect upon nutrients.

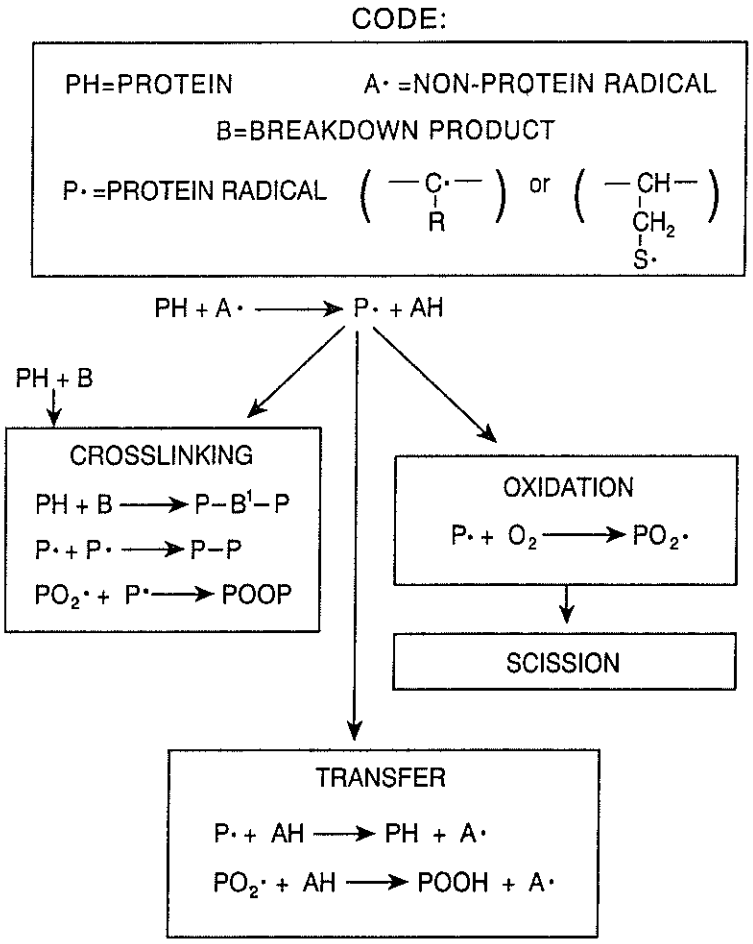


Figure 10 Schematic representation of reactions of proteins with peroxidizing lipids. Redrawn with permission from (139), Copyright, 1975, American Chemical Society, Washington, D.C.

A schematic representation of protein-oxidized lipid interaction as envisaged by Karel et al.[139] is shown in Figure 10. Crosslinking due to interaction of proteins with lipid radicals or lipid breakdown products (dialdehyde), as well as to protein–protein interaction initiated by lipid free radicals, is indicated here. Protein oxidation and scission are also known to occur.

The interaction between thermally oxidized corn oil and food proteins under normal or physiological conditions is important because of the possible formation of such oxidative polymers of vegetable oil in some commercial deep fryers.[140] Rats fed used corn oil from a commercial potato chip fryer were shown to have their growth depressed in comparison with those fed fresh oil.[140,141] The growth depression was greatly overcome by increasing the protein content of the diet from 10% to 20%,[140] which may suggest that the effect is due partly to loss of protein as a consequence of the oxidized lipid–protein interaction and associated diminished protein availability. In addition, there appears to be a synergistic effect of heated fats in carcinogenesis, based on studies with rats fed an extremely low level (0.005%) of *N*-2-fluorenylacetamide (FAA), a hepatocarcinogen.[142] Thermally oxidized lipids and acetaminophen have been implicated in accelerating the induction of liver cirrhosis in rats given 3% ethanol.[143] Oxidized lipids have been identified in human atherosclerotic plaques along with substantial quantities of α-tocopherol and

ascorbate.[144] Dietary oxidized lipid has been found in rat very low density lipoproteins as well as in human chylomicrons.[145–146] The role of oxidized low density lipoproteins in atherogenesis has been the subject of a recent symposium.[12–15] It has been suggested that oxidized lipids present in the American diet may be an important risk factor for atherosclerosis.[16]

Influence of the type of lipid or protein

In studying the reactions of peroxidizing lipids with proteins, linoleic acid, methyl linoleate, linolenic acid, methyl linolenate, methyl arachidonate or ethyl esters of menhaden oil (73% docosahexaenoate and 23% eicosapentaenoate), or oxidized/polymerized corn oil have been generally used. Trilaurin, lauric acid, oleic acid, linoleic acid, or corn oil were ineffective in binding to egg albumin after heating for 2 h at 60°C in the presence of air.[126] Linoleic acid reacted only to the extent of 8% after 24 h, while thermally oxidized corn oil bound to egg albumin to the extent of 4.4% in 2 h.

In the presence of a large amount of water, only egg albumin and lactalbumin were able to form insoluble complexes with oxidized lipids,[126] while amino acids, peptones, gelatin, and sodium caseinate did not yield any similar complexes. However, a number of investigators have reported the complexing of oxidized lipids with gelatin, insulin, lysozyme, cytochrome, and ribonuclease under different experimental conditions.[8,9,134,137,139] Further, Tappel has shown copolymer formation with sodium caseinate, casein hydrolysate, and glycine.[6] These data indicate that food proteins can interact with lipids peroxidizing under both processing and storage conditions and may adversely affect protein quality. In addition, oxidized fat present in foods may interact with digestive enzymes and interfere with the digestion and absorption of foods. At low $A_w (< 0.05)$, protein free radical mobility is restricted, but at high A_w approaching 1.0, their mobility is increased, which it is suggested enhances protein polymerization, decreases solubility, and reduces enzymatic activity.[138]

Influence of temperature

The influence of temperature has not been explored in detail, although it is important in connection with storage of foods containing proteins and fats, especially PUF. The early data of Narayan and Kummerow with egg albumin and thermally oxidized corn oil showed that 60°C was optimum; however, only two other temperatures (30°C and 90°C) were investigated.[126] The rate of the reaction was greater at 30°C than at 90°C.

Influence of pH

Under the conditions that were standardized by Narayan and Kummerow for the reaction between egg albumin and oxidized corn oil,[126] a pH of 7.0 was optimum for maximizing the binding of oxidized lipid (4.3%) to this protein. A pH of 5.0 was found to maximize the formation of polymerized protein-oxidized lipid aggregates, but the amount of lipid bound was only 2.6%. Several investigators have routinely used a pH of 7.0 in their experiments on oxidized lipid–protein interactions.[8,9,136]

Influence of metal ions and antioxidants

The formation of complexes of the type described here between lipids and proteins takes place only when the lipid becomes oxidized.[6,7,126] For example, both unoxidized linoleic acid and unoxidized corn oil reacted very little with egg albumin.[126] The addition of a catalyst such as hematin greatly accelerated the reaction.[6,9] The presence of antioxidants such as BHA and BHT delayed the onset of the characteristic fluorescence by as much as 7 days.[135] Thus, it is possible to retard this reaction by use of metal chelating compounds,

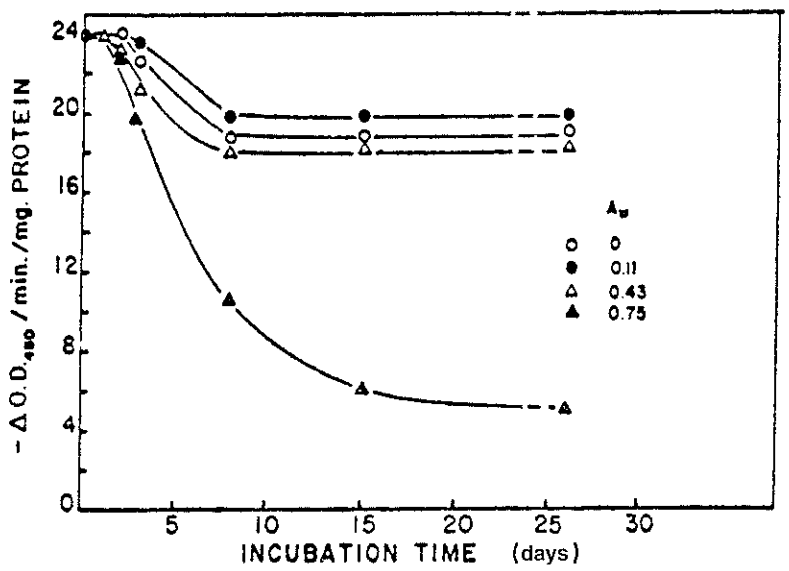


Figure 11 The influence of water activity upon oxidized lipid–protein interactions: Loss of enzymatic activity of lysozyme as a result of reaction with peroxidizing methyl linoleate. Methyl linoleate (400 mg) was emulsified with 2 g of lysozyme in 50 ml of water, freeze dried, equilibriated to various A_w and incubated at 37°C for 0–30 days. (From Kanner, J. and Karel, M. 1976. J. Agric. Food Chem. 24: 468-472. With permission.)

and by including antioxidants or oxygen scavengers, depending upon the type of food. However, it should be noted that α-tocopherol is not an effective antioxidant, as is propylgallate or BHA, in these systems.[6]

Influence of water activity

It has been shown by Kanner and Karel that the greatest loss in enzyme activity as a consequence of the interaction between oxidized linoleate and lyzozyme was at an A_w of 0.75, approaching 80% in 26 days (Figure 11). This loss parallels the changes seen (76%) in protein insolubilization. At A_w of 0, 0.11, and 0.43, the corresponding losses were between 16% and 33%. Nielson et al. investigated the effect on amino acid availabilities of two A_w in a whey-protein-oxidizing methyl linolenate model; they reported that considerably greater damage to lysine quality occurred at an A_w of 0.84 than of 0.33.[147] Major damage to ovalbumin by oxidized lipids[126] was seen at high water content rather than at low water content.

Nutritional effects

From the nutritional standpoint, the extensive destruction of amino acids due to this type of reaction is of concern. Data on amino acid yield and composition upon 6N HCl hydrolysis of oxidized lipid–protein complexes in several experiments[8,135,136] show that there is extensive damage in almost all amino acids (17–54%)[8] and in specific amino acids (22–40%); such damage is consistent with the mechanism of free-radical-induced protein polymerization. Matoba et al. reacted casein or egg albumin with methyl linoleate and observed amino acid losses in the range of 9–41% in methionine (MET), LYS, and histidine (HIS) based upon acid hydrolysis.[136] In the casein-oxidized linoleate model stored at 80% RH for 10 days at a high temperature of 50°C, they observed even greater losses, based upon enzymatic hydrolysis, in LYS, HIS, and MET (41–94%).

Early data by Johnson and Narayan (unpublished results, 1957) indicated that the growth of weanling rats was not reduced when the oxidized corn oil–egg albumin complexes (either 7% or 32% lipid) were given to them at a level corresponding to 30% of protein. However, when the protein complex level was reduced to 10% and the amount fed was restricted to 5 g/rat/day, there was a substantial loss in weight of rats fed the complex in contrast to the gain in weight in control rats.

Extensive investigations have been conducted by Nielson and colleagues on the effects of A_w, temperature, O_2 limitation, and storage time on amino acid losses in an oxidizing methyl linolenate–whey protein model system.[97,147,148] The destruction of LYS and TRY increased with increase in A_w, storage time, and temperature, as indicated by chemical analysis, and approached losses of 71% and 31%, respectively, under extreme conditions. Methionine loss was very extensive (up to 100%) due to oxidation to methionine sulfoxide (see below). The losses in histidine approached 51%. Other amino acids were unaffected.

By the rat balance methods,[147] it was observed that at low A_w the bioavailabilities of LYS and TRY were essentially unaffected. At high temperature (55°C) and high A_w (0.84), there were extensive losses in LYS (88%) and TRY (71%). These losses are higher than indicated by chemical methods. When the chemical assay values were adjusted for lower digestibility (47–97%), the new values were similar to those obtained by rat assay. Methionine sulfoxide is as highly available as methionine in the rat and, therefore, there appears to be no problem with the bioavailability of methionine. The possible importance of cysteine loss, indicated by a 28% decrease in bioavailability in an oxidizing methyl linolenate–whey protein model limited to 1 mol O_2 uptake per mole lipid, and its role in oxidized lipid–protein interaction, remain to be explored. Karel et al. have also demonstrated the formation of sulfur radicals in cysteine, cystine, and in proteins containing free sulfhydryl groups that were exposed to oxidized methyl linoleate.[139] The conclusion drawn by Nielson et al. was that at low A_w and with limited access to O_2 (e.g., freeze dried foods), the losses in LYS, TRY are of minor significance in human foods.[147]

Protein interactions

Protein–protein interactions

The protein–protein interactions are important because they affect chemical, physical, and biochemical characteristics of foods. Such interactions are involved in gelling phenomenon[149]; they may adversely affect digestibility[150–152]; and they may influence spun characteristics[153] of food fibers. The desirable type of food fiber for use as meat analogs can be conveniently obtained by suitable selection of temperature, pH, and ionic strength, rather than by amino acid modification through either genetic or other means.

Protein modification by thermal treatments

Thermal treatments of proteins may cause considerable diminution of protein and amino acid digestibilities. Several reactions are known to occur[154]: oxidation of methionine to methionine sulfoxide[148]; formation of lactone ring through reaction of serine or threonine hydroxy groups with carboxyl groups[1]; isopeptide formation due to the reaction of the amino group of lysine with the carboxyl group of aspartic and glutamic acids[155]; and unfolding of proteins and attendant changes in conformational structure or, under extreme cases, gel formation due to ionic and hydrophobic interaction.[156] Additionally, it has been suggested that new three-dimensional crosslinks may be formed and could sterically hinder enzymatic activity. Accordingly, the availabilities[154] of several amino acids (aspartic acid, glutamic acid, threonine, serine, methionine, tyrosine, and lysine) were found to be reduced as a result of heating diafiltered low lactose (2.4%) whey protein to 121°C at $A_w = 0.97$ for 5000 s (Figure 12). The damage[157] was far greater at an A_w of 0.97 than at 0.7,

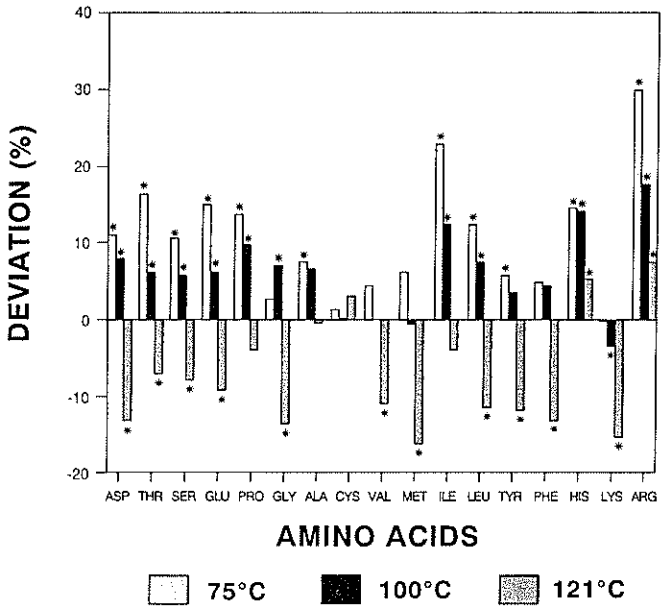


Figure 12 Effect of heat treatment for 5000 s on *in vitro* digestibility of totally delactosed whey protein (with 2.4% residual lactose). Results are expressed as percentage of deviation in digestibility from control (unheated) sample. For each amino acid (AA) shown with a three letter code, there are three bars shown in sequence and represent heating temperatures of 75°C, 100°C, and 121°C, respectively. Percentage of deviation in AA digestibility equals 100 × [(digestibility of heated sample − digestibility of control)/(digestibility of control)]. *Significantly different ($p < .05$) from the unheated control sample. Redrawn with permission from (154), Copyright, 1987, Institute of Food Technologists, Chicago, IL.

0.5, and 0.3 for most amino acids (Figure 13). Hakansson et al. have demonstrated that the biological value and available lysine of whole grain wheat were reduced (15–35% and 14–60%, respectively) during thermal processing, such as autoclaving or popping.[158] Substantial losses in thiamin (63–95%) and in folacin (16–71%) under these conditions were also reported. Severe popping decreased sulfur amino acids (30%), tryptophan (27%), and threonine (15%). Extrusion processing of white flour even under severe conditions had no effect upon either the biological value of the proteins or upon available lysine. Drum-dried products unexpectedly exhibited an increase in biological value.

Protein modification by alkali treatment

An amino acid containing two primary amino groups, two carboxyl groups, and one secondary amino group (lysinoalanine, LAL) has been found in acid hydrolysates of proteins severely damaged by treatment with alkali.[159,160] Such damaged proteins are less digestible and have reduced net protein utilization values.

LAL and lanthionine (LAN) occur as a result of the β-elimination reaction in proteins containing specific seryl or cystinyl residues and the subsequent interaction of the intermediate dehydroalanine formed with the ϵ-NH_2 group of lysine residues. LAL has been shown to cause histological lesions in rat kidneys.[161] While the initial focus was upon alkali damage to proteins, later work demonstrated that alkali treatment was not a necessary prerequisite, but that heat treatment could also result in limited formation of LAL.[159] The ubiquitous presence of LAL in home-cooked and commercially processed foods is now recognized.[159]

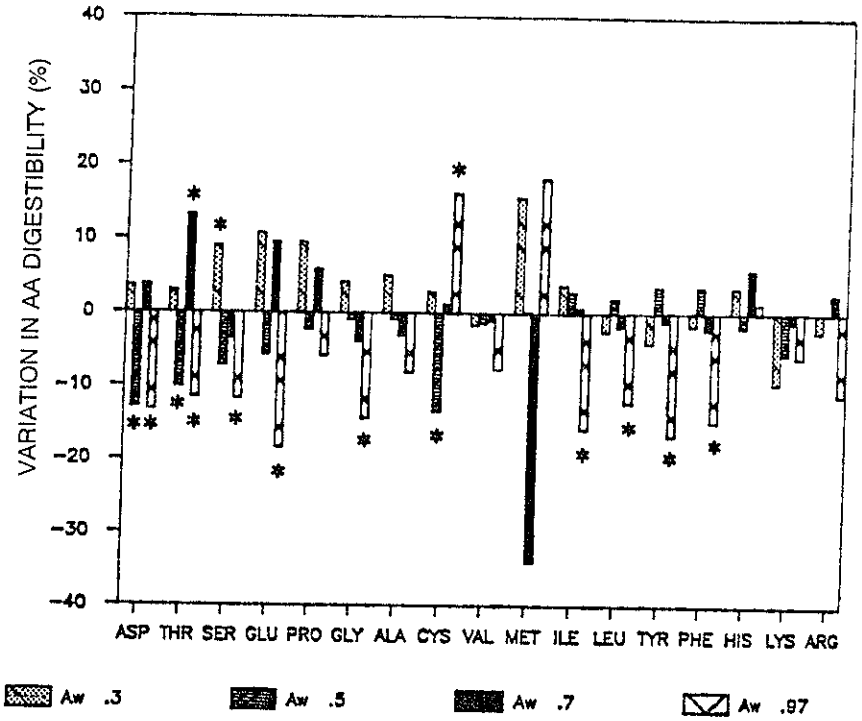


Figure 13 Effect of severe heat treatment at 121°C at four A_w for 5000 s on the *in vitro* amino acid (AA) digestibility of totally delactosed whey protein (2.4% residual lactose). Results are shown as variation in AA digestibility expressed as percentage of deviation from unheated control values. Variation in AA digestibility (%) = 100 × [(digestibility of heated sample − digestibility of control)/(digestibility of control)]. Shown are four bars for each amino acid, represented as a three-letter code for each of the four A_w, 0.3, 0.5, 0.7, and 0.97, in sequence. (From Desrosiers, T., et al. 1987. J. Dairy Sci. 70: 2476-2485. With permission.)

Finley and Friedman have cautioned that the dehydroalanine could also react with other amino acid residues (containing histidine, arginine, etc.) to form histinoalanine (HAL) and arginoalanine (AAL).[162] However, their biological effects are not established. Finley et al. have shown that LAL formation can be suppressed by the inclusion of mercaptoamino acids.[163]

Racemization must also be considered. Due to the antimetabolic effects of some D-amino acids and due to unnatural peptides being formed that are resistant to hydrolysis (e.g. D-L, D-D, and L-D peptides), there could be nutritional consequences. Racemization is generally brought about by treatment with alkali, for example, by heating proteins at 80°C for 1 h in 0.2 *N* NaOH.[164] Even without alkali, partial racemization (15–40%) can occur upon heating for prolonged periods. In plasma albumin and in fat-extracted chicken muscle heated at 121°C for 27 h, aspartic acid, cysteine, and serine were partially racemized (e.g., 32%, 15%, and 40%, respectively, in the case of albumin).

Protein–polyphenol interactions

Phenolic compounds fall broadly into four chemical groups (benzoic acids, cinnamic acids, flavonoids, and terpenoids) and are widely distributed in plants foods.[165] The action of polyphenol oxidase in enzymatic browning and resulting loss in protein quality[166] are known. *o*-Diphenols, such as caffeic acid, can be oxidized at alkaline pH to *o*-quinone or

o-semiquinone radicals. These quinones can react with the free amino groups and sulfhydryl groups under appropriate conditions. Hurrel et al. reacted casein–caffeic acid mixtures under several conditions (nonenzymatically at pH 10 or enzymatically at pH 7) and determined their nutritional properties.[167] They observed that this reaction reduced the protein quality of casein and has an effect similar to the Maillard reaction. Lysine and tryptophan were drastically reduced (50%). Histidine and tyrosine were also degraded to a lesser extent (14–22%). The determination of chemically available FDNB–lysine agreed fairly well with rat assay for lysine availability. The enzymatic reaction has a pH optimum of 7.0. For the nonenzymatic reaction, a pH of 10 or above is essential. The data obtained also established that the nonenzymatic reaction is more rapid and caused greater loss in lysine than the enzymatic reaction. Additionally, in the range of temperature of 20–60°C, maximum loss in available lysine (24–56%) was encountered at 60°C for the nonenzymatic reaction, while the loss in available lysine (13–21%) due to the enzymatic reaction was unaffected by temperature.

It is also evident that, in order to minimize losses in lysine quality and, more generally, in the protein quality of a plant food, alkaline treatment should be eliminated or, at the very least, the pH should be kept below 8.0. To suppress damage brought about by polyphenol oxidases in plant food being processed or stored, the pH should be kept below 6.0. Clearly, keeping the pH below 6.0 minmizes both types of degradation.

Protein–phytic-acid interaction

Phytic acid (meso-inositol hexaphosphoric acid) occurs extensively in cereals, nuts, and legumes. In bran products, the levels could be as high as 5%.[168] Based on its structure, it would be expected to interact with positively charged compounds, such as metal ions and proteins, in foods. At low pH, proteins have a maximum positive charge and readily bind the negatively charged phytic acid. As the pH is raised toward its isoelectric point, this binding becomes weaker because of two factors: (1) the net charge on the protein approaches zero[165]; and (2) proteins tend to become insoluble near this point. At neutral or basic pH, another kind of association occurs between phytic acid and proteins. Under these conditions, cations such as Ca^{2+}, Mg^{2+}, or Zn^{2+} can bind both the negatively charged proteins and phytate ions.[165] These complexes are important because of their effects on the bioavailability of proteins and of such physiologically critical elements. Stunted growth among the population in parts of Iran has been attributed to the zinc deficiency caused by excess ingestion of phytate in cereal diets. Salterlee and Abdul-Kadir have shown that the protein quality of high protein bran flour, but not soy isolate, is affected by their phytate content.[169]

Blocking undesirable side reactions

Protein–carbohydrate interaction

It is difficult to effectively block the first phase of the MR, although sulfites have long been well known to retard browning in foods.[170] Other suggestions that have been advanced to limit Maillard-induced LYS loss have included acetylation of free amino groups in proteins, use of aromatic aldehydes, manipulating A_w and pH, and adding specific amino acids or protein hydrolysates to *redirect* the reaction.[1, 170–171]

It has been shown that Schiff bases of aromatic aldehydes, such as benzaldehyde and salicylaldehyde, are as fully available as lysine in the rat.[172] This opens the possibility of utilizing these or other aromatic aldehydes, such as pyridoxal, to minimize reaction with aliphatic amines. Schiff bases of aromatic aldehydes are stable and will not undergo the Amadori rearrangement. However, even if this approach were to be successful, the

stoichiometry of the reaction makes it impractical. It would also be objectionable from biological and sensory considerations to use an excess of aromatic aldehydes in the food.

Acetylation of the free amino groups in proteins is a way of preserving lysine[173] by blocking the amino–carbonyl interaction. However, this reaction may not be simple to accomplish in foods and is likely to adversely affect the functionality of the foods.

Freeze drying of foods to low A_w is yet another approach to minimize Maillard-induced lysine losses. However, it is recognized that serious losses in lysine can be encountered even in low A_w systems.[4] Shifts in pH are difficult to accomplish because normal foods have pHs in the range of 5–6, and a decrease in pH could lead to unacceptable tartness.

A unique suggestion to block the Maillard reaction through the use of antioxidants was made by Yen and Lai.[116] The rationale for this approach is based upon the proposed mechanism[117] in which free radicals are formed prior to the Amadori rearrangement, so it involves trapping these radicals with antioxidants (AO) before they start to propogate. Using a casein–glucose–antioxidant model at 37°C and a RH of 60% for 60 days, the observed losses in lysine were considerably decreased when comparing the model with and without AO (13% vs. 60%, respectively). However, it is clear from Figure 14 that there is a lag period before the lysine starts to fall precipitously. The lysine was estimated by the trinitrobenzene sulfonic acid method, which is generally recognized to underestimate lysine loss. An alternative explanation of these findings is that the small amounts of residual lipids in the casein autoxidized, formed lipid-free radicals during incubation, and contributed to the loss in lysine. This would account both for the lag period (which is uncommon for browning reactions) as well as for the effectiveness of the AO. Because of residual lipid, one must use hot-alcohol extracted, vitamin-free casein[174,175] to create an essential fatty acid (EFA) deficiency or a vitamin deficiency in animals. Further, the minor amount of residual linoleic acid in starch is sufficient to delay the onset of EFA deficiency in rats.[175] Whether or not the small amount of lipids in the casein is sufficient to sustain the propogation of lipid-free radicals needs to be determined. Despite these concerns about experimental procedures, the suggestion to use AO as a Maillard blocking agent is novel and should be further explored. A recent paper by Djilas and Milic[176] has described the inhibition of Maillard browning and the inhibition of the formation of free pyrazine cation radical by naturally occurring phenolic compounds. It would be worthwhile to use simple models such as the actyllysine–glucose models, under the relatively mild conditions used by Narayan and Cross,[77] and establish by HPLC procedures whether or not lysine losses can be eliminated through the inclusion of natural antioxidants, since it would have a profound impact upon the storage stability of foods.

The use of cysteine to reduce Maillard browning was demonstrated (Figure 15) in lysine–glucose–cysteine–Avicel model systems.[73] The mole ratio of cysteine to lysine was 1.0, which is excessive for food applications. Pham and Cheftel have observed reduced Maillard induced lysine loss and browning by adding cysteine, aspartic acid, glutamic acid, sodium chloride, ammonium chloride, or urea.[177] The blocking action of several of these compounds is impressive, but they were used in large amounts and several are unsatisfactory for human consumption. Cysteine and the dicarboxylic amino acids form stable Amadori compounds[178], which are less susceptible to browning reactions.[112]

Glutamic acid and aspartic acids were shown to inhibit the rate of browning in lysine-reducing sugar models at a pH of 8.0.[179] However, when these experiments were repeated using controlled pH conditions, it was found that the earlier observations of inhibition were due to a large decrease in pH caused by adding these dicarboxylic acids to phosphate buffers (Narayan, K.A., Splaine, M., Neidhart, A., and Jason, K., unpublished results).

The ability of sodium sulfite to prevent enzymatic and nonenzymatic browning in foods is well recognized. However, it has not been convincingly demonstrated whether

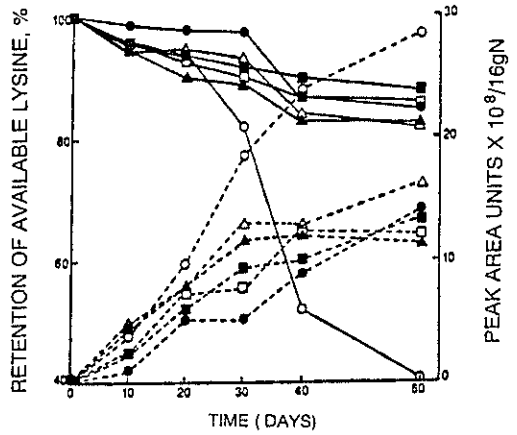


Figure 14 Effects of antioxidants on the retention of available lysine (top solid lines) and the formation of furosine in a casein–glucose (1.5:1, wt/wt) system at 37°C and 60% relative humidity. The antioxidants were added at 20–260 ppm to a paste of casein–glucose, homogenized, quick-frozen, and stored in a dessicator containing 40% sulfuric acid for 0–60 days, ○ control, ● ascorbic acid (260 ppm), □ - tocopherol (100 ppm), ■ propylgallate (20 ppm), △ BHT (200 ppm), ▲ BHA (200 ppm). (From Yen, G.-C. and Lai, Y.-H. 1987. J. Food Sci. 52: 1115-1116. With permission.)

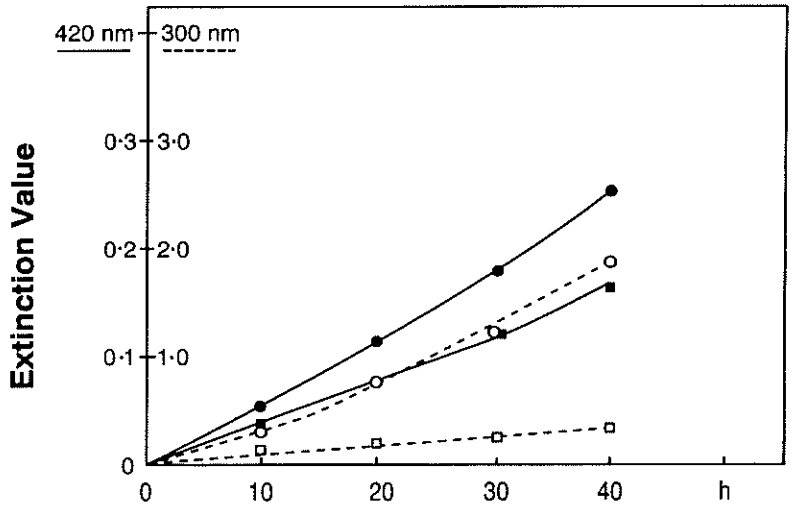


Figure 15 The effect of cysteine on browning in a freeze-dried glucose–lysine–cysteine model system (2:1:1 mol, 14 g Avicell per gram glucose) compared with a corresponding cysteine-free model at an A_w of 0.4. Data at 300 nm (UV-absorbing, browning intermediate) and at 420 nm (visible brown pigments) are shown. Circles, no cysteine added: ●, 420 nm and ○, 300 nm. Squares, cysteine added: ■, 420 nm and □, 300 nm. The predominant effect is on the intermediate compound. (From Eichner, K. 1975. In: Water Relations of Food (R.B. Duckworth, ed.), Academic Press, New York. With permission.)

sulfite also prevents the formation of the Amadori compound in which lysine is irreversibly bound or if it inhibits only the latter stages of the Maillard reaction. Early studies of Friedman and Kline suggested that sodium bisulfite drastically reduces Maillard browning in protein hydrolysate–glucose systems, but has only a minor effect upon the preservation of amino acids, which were determined by microbiological methods.[180]

Investigations of Friedman and Molnar-Perl have shown that far less than stoichiometric levels (0.02 to 0.2 *M*) of inhibitors were required for optimum inhibition of browning in the reaction between amino acids and glucose.[181–183] For high protein foods, either 0.1 *M* sodium bisulfite or 0.25 *M* *N*-acetylcysteine was sufficient to suppress browning. It was implied, but not stated, that these and other sulfur compounds (L-cysteine and reduced glutathione) may further help preserve amino acid and protein quality. Earlier work of Friedman and Kline,[180] based upon microbiological assay alone, as well as that of Farmar et al.[184] and Cerami[57] would appear to suggest that sodium bisulfite intervened after the first stage of the MR where lysine is already compromised. Hirsch and Feather have recently demonstrated the usefulness of aminoguanidine as an effective inhibitor of the Maillard reaction.[60] Unfortunately, aminoguanidine does not prevent the formation of Amadori compounds but interferes with the subsequent crosslinking reactions. Therefore, from the standpoint of nutrition and bioavailability, this compound is ineffective.

Because of the need for substances that are highly effective for inhibiting Maillard-induced lysine and protein quality loss in stored foods, it is important to further explore these approaches.

Protein-oxidized lipid interaction

Although it is well-recognized that lipid oxidation is favored at very low A_w, it is interesting to note that the greatest damage to proteins by oxidized lipids has been shown to occur at $A_w = 0.75$.[139] This observation has been explained as due to the greater protein free radical mobility at $A_w = 0.75$, even though the concentrations of lipid and protein free radicals are higher at low A_w. On the basis of these and other data on optimum water content for this interaction,[126] it would seem that protein-rich canned foods and frozen entrees may be susceptible to protein insolubilization caused by lipid oxidation, which would adversely impact protein quality, food texture, and overall food acceptance. A low A_w wherever feasible would help to minimize these interactions, but favor lipid oxidation.

Since O_2 is essential for lipid oxidation, the use of antioxidants, vacuum packaging, and O_2 scavengers would be most helpful in retarding these reactions. As indicated above, tocopherol is not as effective as propyl gallate or BHA in inhibiting these reactions. Using chelators to bind metal ion catalysts in the food or in packaging systems would help to reduce the rate of lipid oxidation as well as its interaction with proteins. Their use must be balanced against the possibility of reducing the bioavailability of minerals.

Narayan and Kummerow demonstrated that thermally oxidized corn oil and autoxidized linoleic acid, but not thermally polymerized corn oil, could be tightly bound by alumina.[7] Roubal and Tappel[9] and Roubal[135] have concluded that oxidized lipids are unlikely to act as crosslinking agents, as initially suggested by Desai and Tappel,[8] and that oxidized lipids bound to insoluble protein polymers were in some way occluded or associated noncovalently in such complexes. This conclusion is consistent with the early observations of Narayan and colleagues concerning the nature of interaction between egg albumin and oxidized lipids.[7,126,127] The idea[9] that denatured polymeric proteins could provide a matrix to trap and stabilize lipid free radicals is important and may provide a means to block undesirable reactions compromising other proteins. The observation of Roubal,[185] which has been referred to by Karel,[10] that powder glass, quartz wool, and amino acids were ineffective for such trapping, whereas polysaccharides were partially effective as traps, is worthy of further study as a way to block protein polymerization as well as to inhibit other undesirable reactions.

Protein interactions

Racemization of amino acids, as well as the formation of abnormal amino acids, such as LAL, LAN, HAL, AAL, etc., can be greatly minimized by reducing the temperature and alkalinity of the food medium during processing. Finley et al. have shown that several mercaptoamino acids (such as cysteine and reduced glutathione) added at a 1% level can substantially reduce (13-fold) the formation of LAL during alkali treatment of soy proteins.[186] This area deserves further exploration and exploitation in all aspects (chemical, nutritional, and sensory). To minimize intramolecular reactions in food proteins, such as those between lysine and glutamic acid or between threonine and aspartic acid, the inclusion of small quantities of mixtures of carefully fractionated short peptides from partial protein hydrolysates should be considered.

Protein–polyphenol interactions

Protein–polyphenol interactions can be substantially reduced by keeping the pH of the foods being processed at 6 or less. This approach would counteract the damage caused by enzymatic (polyphenol oxidases) as well as by nonenzymatic reactions.

Intermediate moisture food interactions

For intermediate moisture foods (IMF), the suggestion has been made to increase A_w and decrease pH so that less of the humectant is required for the same degree of protection against microbiological degradation. As a means of boosting the morale of the troops in the front lines and as a way of enhancing the acceptance of carbohydrates in MRE and T-rations, Berkowitz and colleagues have developed an MRE pouch bread that is highly acceptable even after 3 years of storage at 27°C.[187,188] The successful outcome of this product was possible through the use of a combination of hurdle techniques and through the inclusion of an innovative antistaling compound (sucrose polyester). Pouch bread samples stored at 38°C and 49°C for 3 months and beyond were digested by α-amylase to a lesser extent than those stored at –18°C, which coincided with corresponding decreases in sensory acceptance scores (Narayan et al.[189]). From the discussion above, it is clear that reducing processing time, reducing temperature, and lowering pH of the food would significantly contribute to enhancing nutrient bioavailabilities.

Detection of protein quality losses and quantification

The principles upon which the detection and assessment of protein quality losses are based include (1) chemical estimation of reactive groups, especially the ε-amino group of lysine; (2) growth of microorganisms in the presence of test proteins or protein hydrolysates; (3) *in vitro* estimation of protein digestibility using protease mixtures; (4) *in vitro* determination of amino acids after enzymatic digestion; (5) chemical scores for essential amino acids corrected for digestibility; (6) true digestibility of protein and amino acids by rat balance methods; (7) biological availability of amino acids by rat growth; (8) protein efficiency ratio (PER) by determining protein contribution for rat growth and net protein ratio (NPR) by determining protein contribution for rat growth as well as for rat maintenance; (9) biological value (BV) by determining the proportion of the absorbed nitrogen retained for maintenance and/or growth; and (10) net protein utilization (NPU) by determining the proportion of the ingested nitrogen retained for maintenance and/or growth. Brief discussions of these methods are given below. A much-needed reference on the

Table 3 Comparison of Several Methods for Estimating Available Lysine in Eight Animal and Nine Vegetable Food Proteins

Food	Ratio A/E	Ratio B/E	Ratio C/E	Ratio D/E
A. Animal products:				
Casein	1.03	1.01	1.13	1.03
Tuna fish	1.03	0.98	1.03	1.26
Beef salami	0.93	0.84	0.97	1.80
Milk, nonfat	0.91	0.90	1.03	0.85
Beef stew	1.10	1.03	1.21	1.69
Chicken frankfurter	0.98	0.67	1.11	0.96
Milk, nonfat, heated	1.57	1.46	1.94	0.73
Breakfast sausage	1.12	1.07	1.32	1.08
B. Samples containing vegetable material:				
Pea protein concentrate	1.12	1.06	1.40	1.47
Pinto beans	1.52	1.45	1.62	1.44
Soy protein, isolated	1.11	1.05	0.99	1.05
Chick peas	1.09	1.05	1.37	1.20
Macaroni & cheese	1.09	1.09	1.31	1.49
Rolled oats	1.04	1.02	1.35	1.06
Peanut butter	1.00	0.94	0.96	—
Whole wheat cereal	1.04	1.01	1.25	0.90
Rice-wheat gluten cereal	1.50	1.31	2.30	0.15

Note: Values shown are ratios to rat assay values determined as part of a USDA collaborative study. A: total lysine by acid hydrolysis; B: fluorodinitrobenzene lysine; C: dye-binding lysine; D: *o*-phthalaldehyde lysine; E: rat assay values.

From Carpenter, K.J., et al. 1989. Plant Food Human Nutr. 39: 129-135. With permission.

bioavailability of nutrients for animals has recently appeared[40]; specific additional literature pertaining to amino acid and protein bioavailability include References 190–192.

Fluorodinitrobenzene method

This reagent was originally used by Sanger[193] and Sanger and Tuppy[194] in their classical experiment on the determination of free amino acid groups of insulin. Its application to foods to estimate the chemical availability of lysine was suggested by Carpenter[195] and is generally considered the method of choice. However, it is not without problems: (1) the fluorodintrobenzene (FDNB) interacts to some extent with the ACs, thereby overestimating available lysine; and (2) as much as 30% of dinitrophenyllysine is lost during acid hydrolysis of starchy foods.[3]

Carpenter et al. have shown (Table 3) that for 12 out of 16 foods the FDNB difference method provided estimates of lysine availability comparable to those obtained by rat assay.[196] In the case of pinto beans and heated nonfat milk, the chemically available lysine values were grossly overestimated by 45%. With chicken frankfurters, there was a 33% *underestimate* of the value obtained by rat assay. Thus, in foods in which the Maillard reaction occurs, the AOAC FDNB method[197] is likely to provide a more favorable view of lysine quality.

o-Phthalaldehye (OPA) is frequently used to estimate available lysine residues in proteins as well as to derivatize amino acids for fractionation by high-performance liquid chromatography. The data[196] shown in Table 3 illustrates that the OPA method *overestimates*

LYS availability in 7 out of 16 foods. In addition, LYS availability in the rice/wheat gluten cereal was 85% underestimated by this method.

Trinitrobenzene sulfonic acid method

Kakade and Liener have developed a simple method for estimating free amino groups in proteins, and they developed a means of distinguishing ϵ-amino groups in lysine from the terminal alpha-amino groups.[198] Unfortunately, the Amadori carbonyls react almost totally (85%) with this reagent.[1] Consequently, the data obtained might overestimate available lysine and should be validated by other methods.

Dye-binding method

Acid Orange 12 is an acidic dye used by the milk industry to estimate the protein content of milk, because it determines the basic groups (LYS, HIS, and ARG) in proteins. It has been made specific for lysine by measuring the reactivity before and after propionylation.[3] Of the three basic amino acids, only LYS is propionylated and upon acylation does not interact with the dye. Some of the data in the literature indicates that this method also overestimates available lysine by as much as 40%.[1,199]

Studies by Carpenter et al. showed that this dye-binding method overestimated lysine availability in heated nonfat milk by as much as 94% compared with rat assay (Table 3).[196] Additionally, this method[196] resulted in unexplained high values for beef stew (21%), sausage (32%), and several vegetable products (pea proteins, pinto beans, chick peas, macaroni and cheese, rolled oats, whole wheat cereal, and rice/wheat gluten cereal (25–130% increase, Table 3)). These results, therefore, call into question the general applicability of the dye method for lysine availability in foods.

Quanidation

The reactivity of the Amadori products led to the search of a lysine-specific reagent that would yield a product that would be stable during hydrolysis.[1] *o*-Methylisourea reacts with lysine to form homoarginine, which is detected by ion-exchange or gas chromatography. However, this reaction is not quantitative and requires further investigation.

Furosine

Maillard-reacted proteins, upon 6N HCl hydrolysis, release about 50% of the bound lysine together with two new compounds, furosine and pyridosine[2] to the extent of about 20% and 10%, respectively. Furosine formed correlated directly with the amount of Amadori compound formed[2] and with the loss in lysine (Figure 2) in model systems.[4] This method would clearly not be applicable to proteins altered by oxidized lipids, heat, alkali, or polyphenols.

Sodium borohydride reduction

A method developed by Hurrell and Carpenter stabilizes the Amadori product against subsequent acid hydrolysis.[3,200] This stabilization is accomplished using sodium borohydride, which efficiently hydrogenates the keto group in the reducing sugar bound to the lysine in protein. Acid hydrolysis neither releases bound lysine nor produces furosine. Therefore, the free lysine liberated after hydrogenation and acid hydrolysis corresponds to the available lysine. One drawback of this procedure is that the Schiff bases are also hydrogenated, which could lead to a reduced estimate of lysine availability.

Lysine decarboxylase

A specific enzyme, lysine decarboxylase, has been explored for determining lysine availability.[201] Its use has been limited, because of the need to acid hydrolyze Maillard-damaged protein products, which will release a considerable amount of lysine from deoxyketosyllysine derivatives.

Microbiological methods

A number of microbiological methods are available for measuring either protein quality or amino acid bioavailabilities in foods.[202,203] Several organisms have been used with varying degrees of success: *Leuconostoc mesenteriodes, Streptomyces faecalis, Streptococcus zymogenes,* and *Tetrahymena pyriformis. S. zymogenes* is generally favored, because it needs only a short incubation time in comparison with *T. pyriformis.* However, this microorganism does not have an absolute requirement for lysine, threonine, and phenylalanine.

Methods to make use of protazoa *T. pyriformis* or *Tetrahymena* WH_{14} are attractive for several reasons: (1) their essential amino acid requirements are similar to those of humans, and (2) whole proteins can, in principle, be utilized by these microorganisms. In general, these methods have met with mixed success.[204,205] Anderson et al. have concluded that *Tetrahymena* is unsuitable for "early" Maillard products, because it can utilize Amadori compounds as a source of lysine.[205] Shepherd et al. have used a novel marker for estimating cell growth; they have extracted tetrahymenol from cell cultures and quantified it by gas liquid chromatography.[206] This procedure appears to have improved the reproducibility.

The use of *Escherichia coli* mutants to assess amino acid bioavailabilities was investigated by Hitchins et al.[207] While a reasonable correlation has been obtained between biological and chemical assays, it is important to recognize that deoxyketosyllysine formed in Maillard-reaction-altered foods may be a source of lysine for the mutants.

In vitro estimate of protein digestibility

There have been many attempts to correlate enzymatic digestibility of proteins with true digestibility as determined by animal assay procedures.[208–222] Satterlee et al.[208] and Hsu et al.[211] developed a simple *in vitro* method to estimate protein digestibility based upon a pH drop during enzymatic digestion. Later, Pederson and Eggum[212] and McDonough et al.[213] modified this procedure to function at constant pH. The enzymes used were a mixture of trypsin, chymotrysin, and intestinal peptidase. The volume, B, of 0.1 N NaOH required in the titration to maintain a pH of 7.98 for exactly 10 min was used to compute[212] the true digestibility (TD) using the equation

$$\mathrm{TD} = 76.14 + 47.77B$$

This regression was derived by relating B to the TD obtained from rat balance studies. To correct for certain anamolies, McDonough et al.[213] have used a 30 min pretreatment with 0.1 N NaOH and a short enzymatic digestion (5 min).

While excellent correlation has been observed for 57 protein sources (vegetable proteins and vegetable–animal protein mixtures), it must be noted that there is a very large intercept in this equation, implying that the digestibility cannot fall below 76%. Data (Table 4) of Bodwell et al. indicate that heated nonfat dry milk is well digested[214]; values as high as 98% (range 84 –98%) digestibility have been reported using this *in vitro* method. However, this finding needs confirmation using other Maillard-reaction-altered proteins and food models, because extensive crosslinking is known to occur. Even *in vivo*

Table 4 Comparison of the Results of Protein Quality Determinations on Seven Foods

	Digestibility of N, %									Ess. Am. Ac. Index			
	Rat Values		*In vitro*			Relative PER		Relative NPR		Relative NPU	Uncorrected	Corr. for N Dig. %	Corr. for Am. Acid Dig. %
Reference Numbers	[1]	[2]	[1]	[6]	[10]	[3]	[5]	[3]	[5]	[1]	[3]	[3]	[3]
Casein	97	99	96	92	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	100	100	100
Tuna	93	97	92	100	99	96	104	98	106	101	100	100	100
NFDM	93	97	95	89	100	98	109	99	102	103	100	100	100
NFDM (heated)	90	90	92	84	98	59	58	67	79	95	89	80	78
Canned pinto beans	73	79	90	82	80	21	3	46	44	64	80	63	42
Chick peas	88	89	100	90	87	66	56	80	72	76	74	66	61
Pea protein concentrate	94	92	93	92	99	49	41	80	64	79	74	68	67

Note: Studies carried out in different laboratories as part of a USDA collaborative study. Reference numbers indicate the numbers for the laboratories. Values expressed as a percentage of the corresponding vlaue obtained for casein.

From Bodwell, C.E., et al. 1989. Plant Food Human Nutr. 39: 3-12. With permission.

digestibility values can be in error due to the metabolism of undigested protein residues by colon bacteria.

For most native proteins, the initial phase of the digestion proceeds rapidly. However, protein–sugar–protein crosslinked cores are likely to be poorly digested, so their presence might not be observed from titration data based on the first 10 min of reaction.

In vitro determination of enzymatically available amino acids

Several enzymatic assay procedures are available; they vary in the technique, the enzyme used, the selection of end point, and the type of data obtained. The early method of Mauron et al.[215] and Mottu and Mauron[216] utilized digestion, first with pepsin, and then with pancreatin, followed by ion-exchange chromatography to determine available lysine and other amino acids in foods. It has been claimed that the data obtained by this procedure was in good agreement with rat bioassay methods.

Savoie and Gauthier automated the digestion procedure of Mauron et al. using a digestion cell with two concentric cylindrical chambers the inner to digest the sample and the outer to continuously sweep the amino acids and peptides as they were transported through a tubular dialysis membrane (1000 molecular weight cutoff) into a flowing buffer.[217] Pepsin digestion for 30 min, followed by 1–24 h treatment with pancreatin was used. Savoie et al. have provided data on the percentage of amino acids (Table 5) released following digestion by pepsin, pancreatin, and 6N HCl (24 h at 110°C).[218] The data indicate that lysine, arginine, tyrosine, and phenylalanine were released rapidly, whereas cystine, proline, aspartic and glutamic acids, serine, and threonine were released slowly. During the 6-h digestion with pancreatin, only 31–51% of the protein–nitrogen was digested, leaving an average of 60% of the sample protein undigested.

Investigations of this type using an innovative continuous digestion cell are critical for mimicking gastrointestinal digestion and minimizing animal experimentation. Before accepting this technique, it is necessary to consider an inconsistency. Although rat balance studies (Table 6) have shown that most of these foods are well digested (79–100%), the data from the collaborative study[214] demonstrated considerable differences in rates of digestion of the various amino acids during the 6-h period. It is possible that the digestion is limited and that acid hydrolysis compromises the validity of the enzymatic process.

Rayner and Fox have developed a digestion procedure using a high level (5% of the substrate) of protease (pronase) from *Streptomyces griseus* and incubating at 40°C for 48 h.[219] In untreated muscle, 84% of the lysine was released upon pronase treatment. Experience with beef stew has shown that aspartic and glutamic acids, leucine, and isoleucine are poorly released by this procedure.

To overcome the problem of incomplete digestion of food proteins with pronase, a three-stage digestion procedure was used in which pronase treatment was followed by carboxypeptidase and aminopeptidase.[220] Lysine was found to be completely released by pronase alone. Carboxypeptidase vastly enhanced the release of aspartic and glutamic acids, while aminopeptidase increased the release of isoleucine and leucine (Figure 16). This procedure was successfully applied to beef stew thermally processed in flexible pouches and in No. $2\frac{1}{2}$ cylindrical cans and freeze dehydrated. The data (Figure 17) demonstrated that lysine and methionine were better preserved in beef stew processed in flexible pouches compared with those processed in cans. The preconcentration step that preceded the freeze dehydration was also shown not to cause significant damage to lysine and methionine. Storage at 100°F for 12 months preserved (Figure 18) the enzymatic availability of lysine and methionine in these beef stew products.

A very different and interesting approach is that of Porter et al.[221] and Culver and Swaisgord[221,222] who have used two separate reactors—one containing pepsin immobilized

Table 5 Percentage of Digestibility after Treatment with Pepsin and Pancreatin in a Digestion Cell followed by 6N HCl (24 h at 110°C)

Protein Sources	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys
ANRC casein	35[a]	36	35	35	30	52	50	36	50	35	59	72	68	45	66	57	50
NFD milk	34	35	32	29	29	57	47	41	52	44	60	80	64	50	70	88	42
NFD milk (heated)	32	36	30	30	33	55	48	39	45	39	59	82	45	41	61	73	28
Macaroni and cheese	32	34	31	32	24	46	52	43	51	45	57	76	56	47	72	83	20
Tuna	29	34	34	27	30	40	39	42	44	44	52	60	56	77	62	57	30
Chicken franfurters	33	41	36	33	28	36	46	54	55	55	60	80	70	56	67	70	30
Salami	39	49	47	33	31	40	49	51	57	48	60	98	66	67	70	73	23
Breakfast sausage	22	25	21	19	15	19	23	32	39	35	40	53	44	38	57	46	16
Beef stew	26	31	26	29	25	34	39	42	42	38	43	66	51	46	55	65	20
Pinto beans	26	27	28	28	30	33	35	34	47	27	37	63	47	34	51	72	31
Kidney beans	29	33	29	27	47	40	45	45	61	44	48	70	50	367	51	61	36
Lentils	28	34	31	26	28	38	42	38	70	38	45	92	68	31	50	49	17
Pea concentrate	34	37	38	31	29	39	50	54	42	52	54	70	61	45	51	62	16
Chick peas	26	30	31	28	27	38	42	41	37	36	44	72	56	41	52	60	20
Soy isolate	27	34	37	23	21	40	51	55	59	50	64	65	60	46	76	65	17
Peanut butter	34	44	39	23	35	39	52	60	53	57	61	77	69	52	70	73	32
Instant whole wheat	29	34	28	25	19	30	38	40	40	46	45	61	41	39	57	55	22
Rolled oats	21	27	24	25	21	29	33	31	43	33	39	60	41	32	47	63	10
Rice-wheat gluten cereal	27	32	28	22	17	39	39	40	35	44	45	59	39	40	61	65	13

[a] Mean of triplicate analyses.

From Savoie, L., et al. 1989. Plant Food Human Nutr. 39: 93-107. With permission.

Table 6 *In Vivo* Digestibility of Protein and Specific Amino Acids in Various Food Products by the Rat Balance Method

Mixture	Protein	Lys	Met	Cys	Thr	Trp
Casein	99	100	99	100	100	100
Skim milk	95	96	92	94	95	98
Beef (roast)	100	100	100	100	100	100
Beef salami	99	99	99	100	100	100
Sausage	94	94	91	95	92	93
Egg white solids	98	97	98	97	96	97
Tuna fish	97	97	95	96	98	97
Chicken Franks	96	97	97	100	95	96
Pea flour	88	92	77	84	87	82
Pea, Century (autoclaved)	83	85	62	85	78	72
Pinto bean (canned)	79	78	45	56	72	70
Lentil (autoclaved)	85	86	59	75	76	63
Fababean (autoclaved)	86	85	59	75	76	63
Soybean	90	87	82	82	84	89
Soybean protein isolate	98	98	94	94	96	98
Rapeseed protein concentrate	95	91	92	93	91	93
Peanut	96	90	85	89	89	94
Peanut meal	91	88	89	89	87	—
Peanut butter	98	96	94	100	97	99
Sunflower meal	90	87	92	91	90	—
Wheat	93	83	94	97	91	96
Rolled Oats	94	90	92	98	90	97
Rice-wheat-gluten	93	85	81	95	88	92
Wheat flour-casein	95	91	91	89	90	90
Macaroni-cheese	95	95	93	98	92	98
Potatoes-beef	86	89	83	89	83	86
Rice-soybean	90	89	77	82	84	87
Corn-pea	83	85	84	86	82	80
Corn-soybean	93	93	87	94	93	98

From FAO/WHO. Report of the Joint FAO/WHO Expert Consultation on Protein Quality Evaluations. 1990. Food and Agriculture Organization of the United Nations, Rome and World Health Organization, Geneva, pp 1–66. With permission.

on glass beads and the other containing trypsin, chymotrypsin, and intestinal peptidase covalently attached to glass beads. This method has the potential of being very efficient, except for insoluble food proteins or foods where the interaction, if any, is bound to be slow.

Corrected chemical scores for essential amino acids

In minimally processed foods where the change due to Maillard reaction or due to oxidized lipids is relatively small or assumed to be small, the amount of amino acids in terms of milligrams per gram protein released by chemical hydrolysis (6N HCl, performic acid plus 6N HCl, and/or barium hydroxide) is used to estimate individual essential amino acid scores.[223] Acid hydrolysis with 6N HCl under N_2 at 110°C for 20 h is the standard procedure for determining most amino acids in proteins, except for sulfur amino acids and tryptophan.[224] For branched-chain amino acids (valine and isoleucine), a 96 h hydrolysis in 6N HCl is recommended. The sulfur amino acids (methionine and cysteine) in the intact protein are oxidized to methionine sulfone and cysteic acid residues, respectively, with cold performic acid (16 h at 0°C), hydrolyzed with 6N HCl (18 h at 110°C), and

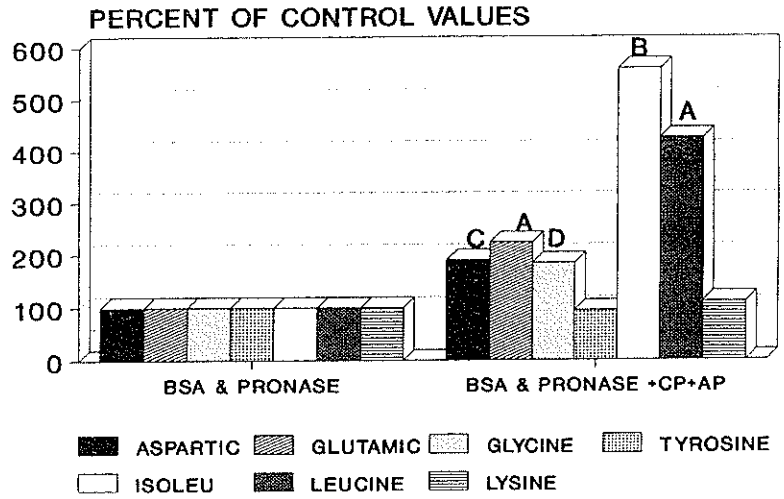


Figure 16 The influence of carboxypeptidase (CP) and aminopeptidase (AP) upon the digestion of proteins such as bovine serum albumin (BSA) after initial treatment with pronase. Bars with letters are statistically significant from corresponding controls. A: ($p < .001$); B: ($p < .005$); C: ($p < .025$); D: ($p < .05$).

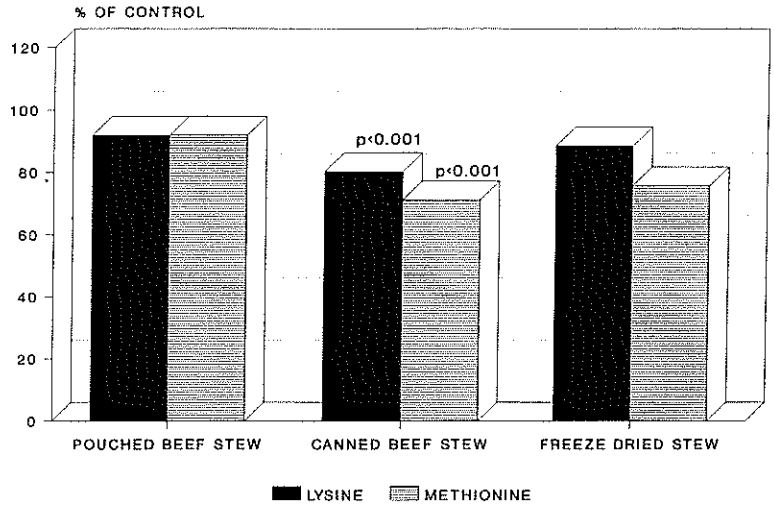


Figure 17 The influence of various processing treatments (thermal processing in flexible pouches; thermal processing in cylindrical cans (No. $2\frac{1}{2}$); preconcentration-freeze dehydration) of beef stew on the enzymatic availability of lysine and methionine. Bars marked $p < 0.001$ are statistically significant from corresponding controls. All other values are not statistically different from controls (i.e., preprocessed samples).

determined by HPLC.[224] Trytophan is determined after alkaline hydrolysis with lithium hydroxide, sodium hydroxide plus starch, or with barium hydroxide.[224]

The amino acid scores are derived from the ratios of the amino acid values for the test protein to a FAO/WHO reference pattern for amino acids.[223,225] The rules for assigning amino acid scores are as follows: (1) all ratios above 100 are considered as 100%; and (2) the lowest ratio represents the first limiting amino acid and is defined as the amino acid score for the test protein. Because all the protein may not be digested by mammalian

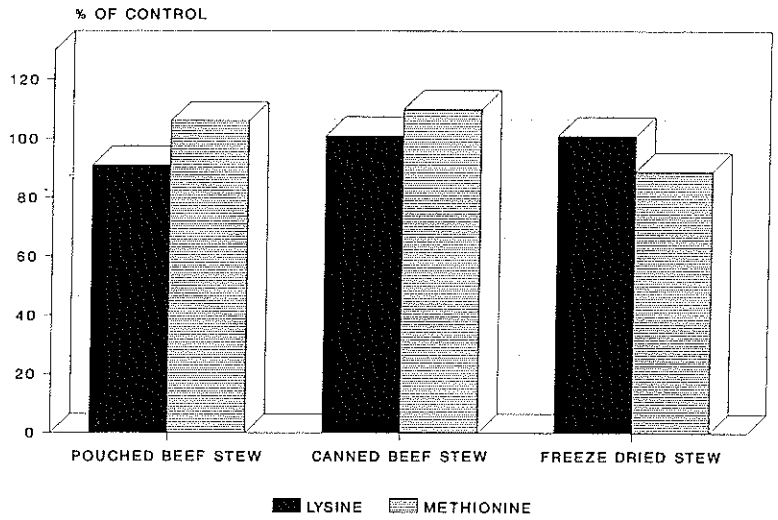


Figure 18 The effect of long-term storage (12 months at 100°F) on the biological availability of lysine and methionine in processed beef stew (see legend to previous figure). Statistical tests showed that none of those values were significantly ($p < .05$) altered from corresponding zero time controls.

proteolytic enzymes, a correction needs to be applied for true digestibility, obtained either by the rat balance method or by the *in vitro* method discussed above. The corrected amino acid scores give a reasonably good indication of the protein quality of foods and are in fair agreement with the NPR values (Table 4).

Carpenter et al. estimated available LYS by three chemical procedures in 17 test foods.[196] They observed that total LYS (determined by 6N HCl hydrolysis) was a reasonably good indicator of LYS availability, except for heated nonfat milk (Table 3) where by comparison with rat assay the LYS was overestimated (46–94%) by three methods: acid hydrolysis, FDNB, and dye binding. It is, therefore, necessary to interpret cautiously LYS data obtained using these for assessing damage to food proteins .

It is recognized that plant proteins are not complete proteins because they are low in one or more essential amino acids. For example, legumes and corn are deficient in tryptophan; cereals and millets are low in lysine and threonine; while legumes are deficient in sulfur amino acids (swaminathan[226]). Most nuts and oilseeds are low in methionine and lysine. Hence, the poor amino acid scores (42, 61, and 67) seen for pinto beans, chick peas, and pea protein is understandable (Table 4). On the other hand, legumes and soybean proteins are rich in lysine and threonine, while sesame and sunflower seeds have a high content of tryptophan and sulfur amino acids.

By mixing a low-quality vegetable protein source with a high-quality food protein (whether animal or vegetable) or with specific amino acids, the protein quality of the mixture can be increased substantially,[223,227] as seen in Table 7. The corrected amino acid score of wheat flour of 0.41 was increased to 0.67–0.91 by the addition of an equal part of soy protein, pea flour, beef, or casein. The corrected score of wheat gluten of 0.25 was greatly enhanced to 0.77 by adding beef. Similarly, beef increased the amino acid scores of peanut meal and sunflower protein isolates by 46–127% (Sarwar and McDonough[228]).

True digestibility of proteins by rat balance methods

This method is based upon feeding rats a diet containing 8% protein for a period of 28 days, measuring protein intake, fecal protein, and metabolic fecal protein, and then computing

Table 7 Amino Acid Scores for Some Protein Mixtures Illustrating the Enhancement in Their Quality

Mixture[b] (50:50 Protein Basis)	True Protein Digestibility %	Amino Acid Score	Protein Digestibility-Corrected Score
Wheat flour (WW)	90	0.46	0.41
WW + beef	93	0.91	0.85
WW + egg white	95	0.83	0.79
WW + casein	95	0.96	0.91
WW + rapeseed concentrate	93	0.72	0.67
WW + pea flour	92	0.89	0.82
WW + soy protein	92	0.78	0.72
Beef + rapeseed concentrate	95	1.12	1.00
Beef + rapeseed isolate	96	1.12	1.00
Beef + soybean concentrate	96	1.17	1.00
Beef + soybean isolate	98	1.07	1.00
Beef + peanut meal	95	0.80	0.76
Beef + pea concentrate	95	0.84	0.80
Beef + sunflower isolate	95	0.88	0.84
Beef + wheat gluten	95	0.81	0.77

From FAO/WHO. Report of the Joint FAO/WHO Expert Consultation on Protein Quality Evaluations. 1990. Food and Agriculture Organization of the United Nations, Rome and World Health Organization, Geneva, pp 1–66. With permission.

the true percentage of the protein that is retained by the rat. Metabolic fecal protein is determined in rats fed a nitrogen-free diet for 14 days. The amino acid digestibility values are similarly computed except that amino acid values, determined after chemical hydrolysis, are substituted for the protein values. Sarwar et al. demonstrated that for many foods the differences between protein and amino acid digestibilities were less than 5%.[229] However, for several vegetable products (beans and lentils), the true digestibility for methionine and cysteine were considerably lower (12–43%) than the digestibilities for crude protein. It is encouraging to note that the protein digestibility values were high for most products except for peas, pinto beans, and lentils. In general, the lysine digestibility values were similar to the protein digestibility values.

While the true lysine digestibilities of both unheated and heated nonfat milk were close to 92%,[230] the lysine availability in the heated nonfat milk had a significantly lower value of 66% compared to 108% for the unheated nonfat milk.[231] This difference between digestibility and availability may suggest that some microbial modifications of unabsorbed protein residues occur in the colon and lead to an overestimate by the rat balance method.[228,232]

Bioavailabilities by rat growth assay

Rat bioassay methods are available based upon dose-response curve for various amino acids, such as lysine, methionine, and tryptophan.[231-234] Growth curve responses are obtained using a basal diet that contains graded levels of the test amino acids, which at the same time provides 125% of the requirements of all other essential amino acids. Growth curves for rats fed experimental foods at two levels are then compared to the standard dose-response curves in order to estimate the bioavailability of the selected amino acid. As Table 8 shows, the lysine values were poor for pinto beans (73%) and for heated skim milk (66%). The latter value clearly demonstrated the loss in lysine due to the Maillard

Table 8 Relative Bioavailability of Lysine in Test Foods as Determined by Rat Growth

Source	Lysine (400 mg/100 g diet)[a]			Lysine (600 mg/100 g diet)[a]		
	Gain (g)	Intake (g)	Availability (%)	Gain (g)	Intake (g)	Availability (%)
Nonfat milk	38.9 ± 5.0[b]	0.545 ± 0.07	115 ± 19	65.3 ± 4.5	0.869 ± 0.06	108 ± 10
Nonfat milk-heated	32.5 ± 6.7	0.536 ± 0.08	61 ± 29	50.0 ± 4.5	0.852 ± 0.06	66 ± 7
Tuna	36.0 ± 3.8	0.539 ± 0.03	91 ± 20	63.8 ± 8.3	0.897 ± 0.07	96 ± 13
Chicken frankfurters	35.0 ± 7.9	0.480 ± 0.09	128 ± 30	53.1 ± 5.8	0.732 ± 0.08	104 ± 8
Beef salami	35.9 ± 7.4	0.523 ± 0.08	101 ± 17	65.4 ± 4.3	0.879 ± 0.05	105 ± 8
Breakfast sausage	30.9 ± 6.1	0.487 ± 0.09	83 ± 20	59.4 ± 9.3	0.878 ± 0.11	88 ± 8
Beef stew	41.6 ± 6.7	0.584 ± 0.08	111 ± 31	64.3 ± 7.7	0.927 ± 0.07	91 ± 9
Macaroni/cheese	36.5 ± 6.2	0.550 ± 0.06	88 ± 25	60.5 ± 8.7	0.873 ± 0.10	92 ± 6
Chick peas	40.9 ± 5.0	0.617 ± 0.06	84 ± 33	63.4 ± 5.9	0.939 ± 0.08	88 ± 8
Pinto beans	33.0 ± 4.0	0.567 ± 0.06	51 ± 28	54.6 ± 4.6	0.824 ± 0.06[c]	73 ± 11
Pea protein	37.5 ± 5.0	0.561 ± 0.06	90 ± 21	56.6 ± 7.2	0.835 ± 0.10	89 ± 4
Soy isolate	34.9 ± 7.1	0.548 ± 0.08	73 ± 27	57.7 ± 4.1	0.823 ± 0.06	90 ± 8
Peanut butter	28.4 ± 5.8	0.430 ± 0.08	102 ± 52	41.9 ± 6.6	0.585 ± 0.06	108 ± 8
Whole wheat cereal	34.1 ± 8.0	0.548 ± 0.08	67 ± 30	49.5 ± 5.5	0.705 ± 0.08	99 ± 10
Rice-wheat gluten cereal	31.9 ± 3.7	0.515 ± 0.03	72 ± 25	35.2 ± 5.1	0.571 ± 0.08[d]	70 ± 8
Rolled oats	41.5 ± 6.6	0.606 ± 0.07	95 ± 21	53.9 ± 4.8	0.750 ± 0.05[d]	101 ± 10

[a] Basal casein provided 300 mg lysine for all diets; thus, 25% and 50% of lysine were from test foods.

[b] All results are mean ($N = 8$) ± standard deviation.

[c] Low lysine content required feeding at 550 mg/100 g diet.

[d] Low lysine content required feeding at 500 mg/100 g diet.

From McDonough, F.E., et al. 1989. Plant Food Human Nutr. 39: 67-75. With permission.

reaction. Lysine availability was high (90–101%) in several plant proteins such as rolled oats, whole wheat, and soy isolate; it was low (70–73%) in rice/wheat gluten cereal and pinto beans (Table 8).

Protein efficiency ratio

A common means of assessing protein quality is to determine the protein efficiency ratio (PER), which is defined as follows:

$$\text{PER} = \frac{W_G}{P},$$

where W_G = weight gain of test group; P = protein consumed by test group. The rats are generally fed diets with 10% protein. The PER values are usually expressed as a ratio of the PER of test protein to the PER of casein, and which is normalized to a value of 2.5. Unfortunately, the PER value takes into account the contribution of the protein for growth, but not for maintenance. It does not provide a linear response for proteins of varying nutritional value. Furthermore, a test protein may have a PER of 0 (i.e., no growth in rats) but still be adequate for maintenance.[223,228]

Net protein ratio

A major modification of the PER method is the net protein ratio (NPR) procedure, which is defined as

$$\text{NPR} = \frac{W_G + W_L}{P},$$

where W_G = weight gain by test group; W_L = average weight lost by rats fed basal (nonprotein) diet; and P = protein consumed by test group.

This method is a significant improvement over the PER method, because allowance is made for both growth and maintenance, by introducing an additional group—a nonprotein group. The weight lost by this group is added positively to the weight gained by the test group. In order to scale the protein quality values from 1 to 100, the NPR values are conveniently expressed as a percent of the NPR of a reference protein (such as 10% casein or 8% casein + 0.1% methionine). These are referred to as relative net protein ration (RNPR) values. Similar scaling is not possible for PER because PER values are not proportional to protein qualities as mentioned.

Biological value and net protein utilization

Among the many methods used, the biological value (BV) using either animals or humans[223–236] is a highly reliable and exact indicator of protein quality. However, this method is time-consuming, laborious, and expensive. On the basis of a six-laboratory collaborative study to estimate protein quality variability in seven protein sources, Sarwar et al. have concluded that NPR and RNPR methods are the most suitable for rat growth assay and are both economical and reproducible.[237]

BV is defined as the proportion of absorbed nitrogen that is retained for maintenance and growth.

$$BV = \frac{B}{A} = \frac{I - (F - F_K) - (U - U_K)}{I - (F - F_K)},$$

where B = body nitrogen; A = absorbed nitrogen; I = nitrogen ingested; F = fecal nitrogen; F_K = endogenous fecal nitrogen; U = urinary nitrogen; and U_K = endogenous urinary nitrogen.

Net protein utilization (NPU) is defined as the proportion of ingested nitrogen that is retained for maintenance and growth. NPU is, therefore, the product of biological value and true digestibility. True digestibility, TD, is then defined as the proportion of food nitrogen that is absorbed:

$$TD = \frac{I - (F - F_K)}{I}.$$

From equations for BV and TD, we see that

$$NPU = (BV)(TD) = \frac{I - (F - F_K) - (U - U_K)}{I}.$$

NPU can also be experimentally obtained from carcass nitrogen.

Table 4 also compares the relative NPU and relative NPR values of seven foods. In several instances, the NPU values are higher than those for NPR. As explained by Bodwell

et al., this difference was due partly to the restriction in diet to 10 g/rat/day for the rats in the NPU assay and partly to the efficient recycling for maintenance of lysine, the limiting amino acid.[214] In any event, these kinds of data emphasize the difficulties in reaching conclusions from one protein quality detection method alone, be it the chemical or enzymatic, *in vitro* or *in vivo*, animal or human.

Conclusions

The vulnerability of micronutrients such as thiamin, ascorbic acid, pyridoxal, folacin, tocopherol, carotene, and retinol to stresses such as temperature, light, pH, and A_w is well recognized. In addition, the retention and biological availability of macro- and micronutrients may be severely compromised as a result of diverse nutrient–nutrient interactions. These interactions include those between proteins and reducing sugars; potentially deleterious interactions among autoxidizing lipids and proteins; between thiamin and carbohydrates, thiamin and tannins in brewed tea, among riboflavin or cobalamin and ascorbic acid; and between thiamin, nicotinamide, and cobalamin, to mention a few of the many reactions known.

The first stage of the Maillard reaction between reducing sugars and proteins has been known to render lysine unavailable for digestion and absorption in humans and animals due to the formation of Amadori compounds. Subsequent degradation of these in the second and third stages gives rise to a variety of highly desirable and undesirable flavor compounds and brown melanoidin pigments, as wells as to undesirable toughening of texture due to crosslinking reactions.

Studies with lysine–glucose model suggest that the Maillard reaction in dry foods could lead to substantial loss in lysine in a few months or years when it is not possible to block this reaction. High storage temperatures and "intermediate" moisture activities (0.5–0.7) favor the reaction, but even foods stored at normal or at low temperatures or low A_w are not immune from Maillard-induced losses. Amadori compounds in the presence of reducing sugars such as glucose, but not lactose, decompose to brown pigments.

Considerable attention has been focused upon lactose-hydrolyzed milk (LHM) and milk products for people with lactose intolerance. However, appropriate consideration should be given to the fact that LHM and LH dairy products are far more susceptible to Maillard-induced changes damage than is regular milk and regular dairy product due to the greater reactivity of monosaccharides compared with disaccharides. In this connection, the reactivity also differs, depending upon whether they are amorphous or crystalline; the former possibly leading to losses in hygroscopic foods higher than expected based upon A_w considerations. The use of humectants in intermediate moisture foods tends to shift downward the A_w for maximum rate of Maillard reaction, possibly due to increased mobility of the reactants in the presence of the humectant. To overcome the objections of high levels of humectants, such as poor flavor, tough texture, decreased acceptability, as well as deleterious nutritional consequences, a strategy involving a combination of pH, O_2 tension, antimicrobial agents, or mild heat treatments has been suggested and deserves further exploration.

It has been increasingly recognized that polyunsaturated lipids can readily autoxidize during processing and storage in the presence of air and then interact with food proteins to form tightly bound complexes under normal or physiological conditions (pH 7, 30°C, and high A_w), which could have a deleterous effect upon the biological availability of these proteins. In this respect, those interactions are similar to the Maillard reaction, but the nature of the associated reactions is far less understood. It is generally accepted that lipid

free radicals leads to protein free radicals, resulting in protein polymerization and consequent loss in biological availability of several amino acids. Although the binding between the lipids and the proteins appears to be noncovalent, it is strong and could be the result of secondary forces, occlusion, and/or adsorption. Copolymerization of lipid peroxy radicals and proteins is not as widely accepted as the nonradical mechanism involving condensation of malonaldehyde formed by lipid peroxidation and scission. For foods devoid of linoleic acid or higher polyunsaturated fatty acids, this nonradical mechanism is unlikely.[7,132]

From a practical standpoint, the consequences of the reaction between oxidizing lipids and proteins in foods is important, because it is considered to be responsible for a wide variety of problems: the yellowing of bacon; the rusting of fish upon storage; the formation of ceroid pigments in human aortas; and possibly for the depression of growth of rats fed used corn oil from a 1957 commercial potato chip fryer. In addition, large losses in the biological availability of important amino acids such as lysine, tyrptophan, and cysteine have been reported in proteins interacted with oxidized lipids. Extensive losses in almost all amino acids (17–54%) upon 6N HCl hydrolysis is consistent with the mechanism of free radical induced protein polymerization.

Besides racemization caused by alkali treatment of food proteins, which results in incompletely utilizable D-amino acids and in unnatural peptides, toxic compounds such as lysinoalanine are formed. *o*-Diphenols, such as caffeic acid, are capable of interacting with proteins either enzymatically through the action of polyphenol oxidase or nonenzymatically at pH 10, which reduces their nutritional quality and gives rise to brown pigments.

Numerous efforts have been made to block the Maillard reaction in food, but these have met with only limited success. Among the promising avenues are freeze drying to very low A_w, acetylation of the amino groups of food proteins, and where feasible the use of sulfite and sulfur compounds (cysteine or glutathione) and aminoguanidine. Most of the reports have provided data on the suppression of browning rather than on the retention of lysine quality; additional work in this area is highly desirable.

On the basis of a new mechanism involving the formation of free radicals prior to the Amadori rearrangement, the addition of antioxidants to a casein–glucose system has been investigated. The antioxidants appear to have been effective in minimizing lysine losses. However, this requires confirmation from other sources to eliminate other possibilities from clouding the picture such as oxidized lipid–protein interactions.

To inhibit oxidized lipid–protein interactions, the use of antioxidants, vacuum packaging, metal chelating agents, and O_2 scavengers have been recommended. Protein–polyphenol interactions can be greatly reduced by keeping the pH of foods at 6.0 or lower. This approach would minimize damage due to polyphenol oxidase as well as to nonenzymatic reactions.

Several methods are available to detect and assess protein quality changes in foods due to processing or storage. These include the following: (1) chemical estimate of reactive groups, especially the ε-amino groups of lysine; (2) microbiological estimation of the availability of proteins or protein hydrolysates; (3) *in vitro* or *in vivo* estimate of protein digestibility; (4) enzymatic determination of available amino acids; (5) determination of protein efficiency ratio or net protein ratio; (6) determination of biological availability of specific amino acids; (7) determination of biological value and net protein utilization. Most of these methodologies were used in an interlaboratory study initiated by USDA, Beltsville, MD, to assess protein quality and amino acid availability of several foods. These data have further emphasized the difficulties in reaching a definititve conclusion about protein quality based on a single method of assessment.

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