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Effect of Protein Substitution on Nonenzymatic Browning in an Intermediate Moisture Food System

Raymond A. Schnickels, Henry C. Warmbier, and Theodore P. Labuza*

The effect of various proteins on the loss of lysine and rate of nonenzymatic browning was studied in an intermediate moisture food system containing glucose as 10% of the solids. The system was prepared to a water activity range of 0.6–0.8 and the samples were held at 35 °C to accelerate the reaction. It was found that proteins which showed long induction times before color development also had lower browning rates. The proteins also showed over 50% loss of available lysine soon after initial color development occurred. Free lysine added to the formulation caused very rapid browning. No pattern of browning rate and lysine loss existed with the total lysine availability.

Nonenzymatic browning reactions occur very widely during processing and storage of food materials. The colors produced range from pale yellow to very dark brown, depending on the type of food and/or the extent of the reaction. Reviews of the reaction have been made by Hodge (1953), Bender (1970), Carpenter and Booth (1973), Ellis (1959), and Reynolds (1963, 1965).

During food processing and storage, nonenzymatic browning can take place when reducing sugars and proteins react in the presence of H_2O to form brown pigments. This results in the production of off-flavors (Markova et al., 1972) and loss of solubility and protein nutritional value (Rao and Rao, 1972; Lea, 1958). This loss of protein nutritional value is due to the fact that lysine, an essential amino acid, is a primary reactant, although other amino acids are reactive. Nonenzymatic browning is especially important in intermediate moisture foods because the amount of water present results in a much greater reaction rate than in dry food systems (Lea and Hannan, 1949, 1950; Labuza, 1971). The rate usually has a maximum in the intermediate moisture food (water activity 0.6–0.85). Because of the high reaction rate in this a_w region, processors have a problem in the use of reducing sugars such as dextrose or corn syrup solids for food formulations.

Many intermediate moisture foods have been developed recently, such as complete breakfast replacements and high nutrition dietary bars. The intermediate moisture range was chosen for these products because of the good palatability while still maintaining the stability of the food toward microorganisms (Labuza, 1971). Unfortunately, even though microbial stability can be achieved, chemical degradation cannot. For example, loss of protein nutritional value as discussed previously, rancidity, and vitamin losses all can occur unless the proper additives or formulations are used (Chou et al., 1973; Lee and Labuza, 1975). It is quite evident that more information is needed on the stability of these foods.

The purpose of this study was to examine the effect of protein substitution in an intermediate moisture food model system on the rate of nonenzymatic browning. The supply and/or the cost of the various proteins are such that food companies may find it necessary to substitute their usual protein source with a different protein source. The problem lies in that the new protein may alter the stability of the finished product, specifically with respect to the rate of nonenzymatic browning during storage.

There are three important factors related to nonenzymatic browning when considering using a protein for an intermediate moisture food formulation. The first consideration is the induction time prior to visual detection of an increase in brown color. Second is the overall change in color due to the pigment production during the expected shelf-life of the product. The last consideration is the

Burger King Corporation, Minneapolis, Minnesota 55455 (R.A.S.), the American Can Company, Neenah, Wisconsin 54956 (H.C.W.), and the Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108 (T.P.L.).

Table I. Composition of Model System

Ingredient	%
K-sorbate	0.3
Glucose	10
Glycerol	20
Protein	30
Apiezon B oil	20
Microcrystalline cellulose	20
Water	As per Table II

amount of available lysine lost.

MATERIALS AND METHODS

Model System. The proteins that were examined in this study were casein (Technical grade, Coleman Bell, Inc.), egg albumin (Technical grade, Coleman Bell, Inc.), whey protein (prepared at the University of Minnesota, spray dried, delactosed), fish protein concentrate (Bureau of Commercial Fisheries), wheat gluten, and spin textured soy protein (General Mills, Inc.). The wheat gluten also was studied with 10% lysine addition (weight/weight of protein). These proteins were substituted on a strict weight basis; thus the initial levels of available lysine (Table II) and inherent reducing sugars would be different. However, this was done as it might be used commercially. The amount of residual reducing sugars in the protein should make no difference in the rate of browning since the amount of glucose added exceeds the lysine by a 3:1 molar ratio as shown by Lea and Hannan (1950). Above this ratio the browning rate is at a maximum. The composition of the dry model system is shown in Table I.

The moisture content was determined on the "dry" mixed ingredients for each system by a GLC technique as described by Tjhio et al. (1969). Deionized water was then added to each system to achieve approximately 20% moisture in the final system. The final water activities were determined by the vapor pressure manometric technique (Labuza et al., 1976). The results are presented in Table II.

Storage of Samples. The samples were sealed in 202 × 214 cans, with the ends sealed with an epoxy resin to prevent water loss. The cans were stored at 35 °C for 60 days and sampled periodically for nonenzymatic browning, pigment production, and loss of available lysine. Duplicate samples were taken at each time used.

Browning Determination. The Maillard browning reaction was monitored by measuring NEB (melanoidin) pigment production by a trypsin digest, aqueous extraction procedure of Choi et al. (1949) as modified by Labuza (1971). The available lysine content was determined by the FDNB method of Booth (1971).

RESULTS AND DISCUSSION

The results of pigment production as a function of time are shown in Figure 1. The rate of browning is calculated

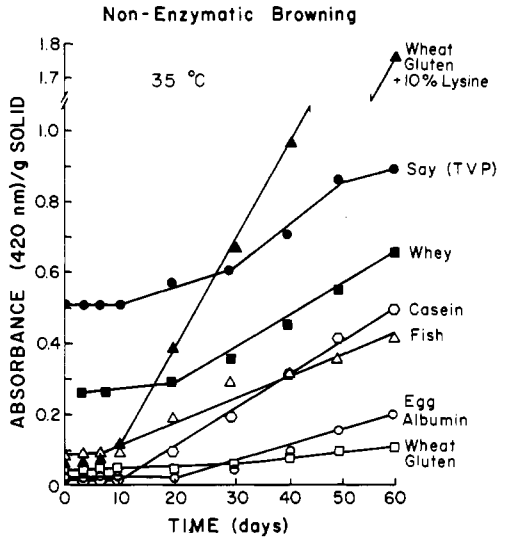

Figure 1. Extent of nonenzymatic browning in an intermediate moisture model food system with different proteins added at 35 °C.

by the slope of the line after measurable browning started occurring. The induction time was determined as the time up to where the amount of browning starts to increase by a zero-order reaction. These are presented in Table II. As seen, the wheat gluten has the largest induction time and the slowest rate, while the wheat gluten with lysine fortification has the shortest induction time and the greatest browning rate. Figure 1 also shows the different initial brown pigment which is due to preprocessing. This obviously has some effect on the build up of reacting intermediates. As noted in Table II, there is some influence between the induction time and browning rate, with a lower rate if the induction time is longer, except in the case of the fish protein concentrate system. Also, egg albumin reacts about as fast as the other proteins even though there is a low available lysine in the initial sample. This low value may have been due to initial overprocessing as generally one considers egg to be a high quality protein.

Table II also shows the time to reach a 50% loss of available lysine in the model systems. As seen, the destruction of lysine occurs very rapidly. In the case of the fortified gluten and the soy, this occurred prior to any increase in brown pigment over the initial value. For the other proteins over 50% loss occurred within about twice the induction time except for egg albumin. The fastest lysine loss occurs in the fortified product as would be expected.

For the other proteins a different pattern exists for lysine loss than found for nonenzymatic browning. Soy has the most rapid lysine loss as compared to casein which has the highest rate of browning. There is no pattern, however,

Table II. Results of Browning and Lysine Loss during Storage at 35 °C

Proteins	Total available Lys[a] content, mg/100 g of solids	Moisture content, g of H_2O/100 g of solids	a_w	Induction time, days	Browning rate, OD/day × 10^3	Time for 50% lysine loss, days
Wheat gluten + 10% free lysine	3300	20.72	0.72	7	35.0	<1
Casein	1416	18.34	0.70	13	10.2	22
Whey	857	18.53	0.78	20	8.5	25
Soy (TVP)	762	20.00	0.73	20	7.5	7
Fish concentrate	1283	18.25	0.68	8	5.9	19
Egg albumin	360	19.76	0.63	20	4.9	>60
Wheat gluten	303	19.05	0.66	30	0.9	40

[a] By FDNB procedure.

for browning and lysine loss even based on initial lysine content. Obviously this variability is probably due to the reaction of other amino acids such as arginine, glutamine, asparagine, histidine, and tyrosine, as well as to structural availability of the amino acids.

This simple study shows that protein substitution in formulated IMF products is a complicated situation with respect to chemical and nutritional stability. The processor might substitute with a protein product of lower lysine content but this does not ensure either increased stability to browning or retention of the lysine content. In addition, this work verifies that supplementation with free lysine in an IMF product which contains reducing sugars cannot be done. Lastly, this work points out that some accelerated test for browning should be used in product development to determine applicability of a protein in an IMF product such as done by Mizrahi et al. (1970).

LITERATURE CITED

Bender, A. E., in "Evaluation of Novel Protein Products", Bender, A. E., Kihlberg, R., Lofqvist, B., and Munck, L., Ed., Pergamon Press, Elmsford, N.Y., 1970, p 115.
Booth, V. H., *J. Sci. Food Agric.* **22**(12), 658 (1971).
Carpenter, K. J., Booth, V. H., *Nutr. Abstr. Rev.* **43**(6), 423 (1973).
Choi, R. P., Koncus, A. F., O'Malley, C. M., Fairbanks, B. W., *J. Dairy Sci.* **32**, 580 (1949).
Chou, H. E., Acott, K., Labuza, T. P., *J. Food Sci.* **38**, 316 (1973).
Ellis, G. P., *Adv. Carbohyd. Chem.* **14**, 63 (1959).
Hodge, J. E., *J. Agric. Food Chem.* **1**(15), 928 (1953).
Labuza, T. P., Contract NAS 9-10658, National Aeronautics and Space Administration, Food and Nutrition Office, Houston, Tex., 1971.
Labuza, T. P., Acott, K., Tatini, S. R., Lee, R., McCall, W., Flink, J., *J. Food Sci.*, in press (1976).
Lea, C. H., "Fundamental Aspects of Dehydration and Storage of Dehydrated Foods", Society of Chemistry and Industry, London, 1958, p 178.
Lea, C. H., Hannan, R. S., *Biochim. Biophys. Acta* **3**, 313 (1949).
Lea, C. H., Hannan, R. S., *Biochim. Biophys. Acta* **4**, 518 (1950).
Lee, S. H., Labuza, T. P., *J. Food Sci.* **40**, 370 (1975).
Markova, J., Honischova, E., Hampl, J., *Sb. Vys. Sk. Chem.-Technol. Praze, Potraviny* (1972).
Mizrahi, S., Labuza, T. P., Karel, M., *J. Food Sci.* **35**, 804 (1970).
Rao, N. H., Rao, M. M., *J. Food Sci. Technol.* **9**(2), 66 (1972).
Reynolds, T. M., *Adv. Food Res.* **12**, 1 (1963).
Reynolds, T. M., *Adv. Food Res.* **14**, 168 (1965).
Tjhio, K., Labuza, T. P., Karel, M., *J. Assoc. Off. Anal. Chem.* **46**, 597 (1969).

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Preparation of Optically Active Proline. Optical Resolution of *N*-Acyl-DL-proline by Preferential Crystallization Procedure

Chikara Hongo, Masataka Shibazaki, Shigeki Yamada,* and Ichiro Chibata

To establish a practical method for the production of optically active proline, the optical resolution of DL-proline by a preferential crystallization procedure was investigated. DL-Proline was easily resolved by converting it to simple *N*-acyl derivatives, such as *N*-acetyl-, *N*-chloroacetyl-, *N*-*n*-butyryl-, and *N*-isobutyrylproline. The optically active acylproline obtained by this resolution method was hydrolyzed to the optically active proline without racemization. The undesired optically active acylproline was readily racemized into the racemic modification and could be reused for the resolution step.

Optically active proline is an important substance in the pharmaceutical and food industries. L-Proline, especially, as well as the essential amino acids, has been proven to be necessary in parenteral nutrition (Dolif and Juergens, 1971) and is widely used as a component of amino acid infusion.

L-Proline has been produced by hydrolysis of natural protein or by fermentation methods. To find a more economical method for the production of optically active proline, we have investigated the optical resolution of synthesized DL-proline. DL-Proline can be synthesized by several chemical methods, for instance, by the method of Albertson and Fillman (1949).

With respect to the optical resolution of DL-proline, chemical and enzymatic procedures have been reported (Greenstein and Winitz, 1961; Kovács et al., 1957; Vogler and Lanz, 1966). These conventional methods seem to be laborious and unsatisfactory for commercial production. On the other hand, the preferential crystallization procedure is considered to be one of the most useful methods for industrial application, since it enables the desired optically active isomer to crystallize preferentially from a supersaturated solution of the racemic modification. So far as proline is concerned, no report has appeared on optical resolution by this simple procedure. Therefore, the optical resolution of DL-proline by the preferential crystallization procedure has been investigated. The advantages of this simple resolution method and the screening methods for resolvable derivatives were described in our previous reports (Yamada et al., 1973a,b; 1975a,b).

DL-Proline itself had no properties suitable for this resolution method. It was reported by Hamer and Greenstein (1951) that the melting points of L isomers of *N*-acetylproline and *N*-chloroacetylproline are much higher than those of the corresponding racemic modifications. This satisfies one of the conditions under which they exist as a racemic mixture (conglomerate). Since the most desirable situation for the preferential crystallization procedure is that the racemic modification crystallizes as a racemic mixture, they might be expected to be resolved by this simple method. Therefore, *N*-acetyl-, *N*-chloro-

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Company, Ltd., 16-89, Kashima-3-chome, Yodogawa-ku, Osaka, Japan.