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Effect of Browning on Chemical Properties of Egg Albumin†

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Egg albumin was stored at 37°C, under 68% relative humidity in the presence of excess glucose for 40 days to study the effect of browning on chemical properties of the protein. Binding of glucose to egg albumin occurred mostly in the early stage of browning whereas brown color started developing after 10 days. Amino acid analyses showed large lose in lysine, arginine, serine, histidine and tryptophan. Dye binding methods demonstrated some relationships to the nutritional value of browned egg albumin, however they were not accurate enough to follow the change in the nutritional value of egg albumin during storage. Relatively close correlation between the amount of glucose bound and the loss of nutritional value was observed.

The significance of the browning reaction in the preservation of foods has been reviewed by many workers.[1~5] The consequences of the reaction is of particular importance from a nutritional standpoint. There are numerous reports that browning of milk and its products during manufacturing process and storage causes a decreased digestibility and destruction of amino acids, resulting in a lower nutritional value of the protein.[6~8]

It has been quite difficult to unravel the chemistry of the overall reaction in the complex mixture encountered in most every foodstuff and the solution is not at hand despite the studies which have been done since the early years of this century. Therefore, studies of the browning reaction have been usually carried out in model systems to simplify the reactions. Even this approach, however, has not yielded all of the answers, since even in simple systems possible reactions are numerous.

Little information is available on the nutritional consequence of the browning reaction in systems other than milk. For this reason the effect of the browning reaction on the chemical properties of egg albumin and its relationship to the nutritional quality were studied.

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MATERIALS AND METHODS

Preparation of browned egg albumin. Egg albumin powder (Nutritional Biochemical Co.) and D-glucose powder (Fisher Scientific Comp.) were mixed mechanically in the ratio of 3 parts egg albumin to 2 parts D-glucose. Distilled water was added to give a moisture content of approximately 15%. The mixture was stored at 37°C in sealed glass chambers for 10 (10 D), 20 (20 D), 30 (30 D) and 40 (40 D) days. Relative humidity (R.H.) inside of the chambers was kept constant at 68% using 40% sulfuric acid solution. The browned mixture was then ground and kept in the freezer (−20°C) until used.

Determination of the amount of glucose bound to egg albumin. The browned mixture was dissolved in distilled water and filtered through Whatman No. 1 filter paper. Free glucose in the filtrate was determined by the Glucostat reagent, according to the directions of the manufacture, Worthington Biochemical Corp., Freehold, N. J. The glucose bound to the protein was calculated by the difference in free glucose at the beginning and after different times of storage at 37°C and 68% R. H.

Determination of color and fluorescence development. Optical density of the filtrate prepared for the glucose determination was measured at 420 nm by a Beckman spectrometer, type DB-G against the filtrate of the untreated egg albumin sample (O.D.). Fluorescence of the filtrate was measured with an Aminco fluorocolorimeter (American Instrument Co., Inc.) calibrated with quinine sulfate after it was diluted 200-fold.

Amino acid composition of egg albumin. Egg al-

bumin was hydrolyzed at 110°C for 20 hr in 6 N hydrochloric acid. For the tryptophan determination, 5 N sodium hydroxide was used as the hydrolytic agent at 110°C. Total amount of each amino acid was determined by ion-exchange chromatography (Technicon autoanalyzer). Enzymatic hydrolysis with pepsin and pancreatin was also performed as described by Akeson and Stahmann.[9]

Reactions between dyes and browned egg albumin. The method established by Frölich[10] was used to study the reaction between cresol red and browned egg albumin. Orange G was used to determine the number of basic groups in protein molecule at pH 2.2 as described by Moran *et al.*[11] The number of acidic groups in browned egg albumin was determined by using safranin 0 at pH 11.5.[12]

RESULTS AND DISCUSSION

The amount of glucose bound to egg albumin during storage at 37°C, 68% R. H. is shown in Table I. The results are expressed as % bound

TABLE I. BINDING OF GLUCOSE TO EGG ALBUMIN DURING STORAGE

Storage time (days)	Bound glucose (%)	Binding moles glucose[a] per mole protein
0	0	0
10	18.5	41.5
20	19.6	42.5
30	25.5	55.7
40	26.2	57.7

[a] Calculated on the basis of 46,000 M.W. for egg albumin and 162 M.W. for the sugar moiety.

glucose and also as moles of glucose per mole of egg albumin (46,000 molecular weight). Binding of glucose to egg albumin occurred mostly in the first 10 days of storage. Color and fluorescence development is shown in Fig. 1. It is interesting to note that the rate of color development did not correlate with the amount of glucose bound to egg albumin. A marked induction period for the color development was observed at the first stage of the browning reaction. On the other hand, the development of fluorescence was almost linear with the storage time, up to 20 days.

The results of the amino acid analysis of egg albumin samples hydrolyzed by 6 N hydrochloric acid are presented in Table II. Table III shows the amino acid composition of pepsin and pancreatin digests. The largest loss was observed in lysine and arginine residues; serine, histidine and tryptophan residues were next (Table II). It is of considerable interest that glutamic acid, 1/2 cystine, isoleucine and leucine showed fairly large losses (between 12 and 17%) after 40 day storage although these amino acids, especially isoleucine and leucine, are known to be nonrective in the browning reaction.[13~15] Acid hydrolysis resulted in a 57% degradation of lysine after 40 days of storage, while only 13% of lysine was recovered by enzymatic digestion (Table III). Acid hydrolysis breaks the linkages between amino acids and reducing sugars which are not attacked with digestive enzymes, thus the smaller losses would be found for some amino acids such as lysine, threonine, and methionine in the acid hydrolyzate. According to Lea and Hannan,[16] a 24 hr acid hydrolysis recovered all of the combined methionine and two-thirds of the combined lysine, but none of the arginine and histidine.

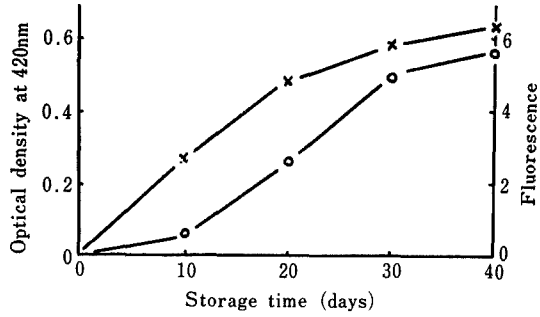

FIG. 1. Development of Color and Fluorescence with Storage Time.
○—○, Optical density at 420 nm; ×—×, fluorescence.

Table IV illustrates the biological value and the absorptive property of browned egg albumin for dyes. Frölich[10] observed that properly heated soybean oil meal absorbed or reacted with significantly more phenolphthalein than unheated samples; over-heated samples, more than the properly heated ones. However other dyestuffs such as methyl red,

TABLE II. AMINO ACID COMPOSITION (g/16 gN) OF BROWNED EGG ALBUMIN HYDROLYZED BY 6 N HYDROCHLORIC ACID

Amino acids	0 D	10 D	20 D	30 D	40 D	% loss in 40 D protein
Aspartic acid	10.12	10.15	10.42	9.58	10.58	—
Threonine	3.50	3.39	4.22	3.45	3.53	—
Serine	7.26	6.40	6.57	6.88	5.72	21.2
Glutamic acid	11.01	9.78	10.24	10.01	9.44	14.3
Proline	2.39	2.82	2.50	2.41	2.40	—
Glycine	3.99	3.42	3.65	3.33	3.88	2.8
Alanine	7.52	5.51	6.50	5.84	6.82	9.3
Valine	6.53	6.24	6.77	6.37	6.49	—
1/2 Cystine	3.44	2.46	2.62	2.71	2.86	16.9
Methionine	3.34	3.47	3.55	3.28	3.07	9.1
Isoleucine	5.97	5.71	5.36	5.37	5.25	12.1
Leucine	8.97	9.13	8.97	8.27	7.46	16.8
Tyrosine	3.33	2.97	3.02	3.04	3.04	9.7
Phenylalanine	5.26	5.92	5.82	5.52	4.93	6.3
Lysine	7.68	5.92	4.19	4.04	3.29	57.2
Histidine	2.76	2.53	2.36	2.39	2.12	23.2
Arginine	3.68	3.26	3.01	2.22	1.78	51.6
Tryptophan[a]	1.61	1.47	1.34	1.24	1.26	21.7

[a] Tryptophan was determined after alkaline hydrolysis.

TABLE III. AMINO ACID COMPOSITION (g/16gN) OF BROWNED EGG ALBUMIN DIGESTED SUCCESSIVELY WITH PEPSIN AND PANCREATIN

Amino acids	0 D	10 D	20 D	30 D	40 D
Aspartic acid	0.26	0.20	0.14	0.08	0.02
Threonine	0.21	0.15	0.07	trace	trace
Serine	0.04	trace	trace	trace	trace
Glutamic acid	0.36	0.20	0.05	trace	trace
Proline	—	—	—	—	—
Glycine	0.12	0.12	0.09	trace	trace
Alanine	0.27	0.16	0.13	0.09	0.09
Valine	0.42	0.36	0.35	0.19	0.28
1/2 Cystine	—	—	—	—	—
Methionine	1.51	1.42	1.21	1.25	1.10
Isoleucine	0.63	0.60	0.55	0.52	0.45
Leucine	4.23	4.01	3.46	3.50	3.42
Tyrosine	1.96	2.02	1.73	1.74	1.82
Phenylalanine	3.01	2.90	2.79	2.77	2.48
Lysine	1.34	0.41	0.27	0.21	0.18
Histidine	0.76	0.64	0.58	0.53	0.48
Arginine	1.55	1.20	0.88	0.76	0.72
Tryptophan	0.26	—	—	—	—

methylene blue and fuchsin were absorbed to about the same extent regardless of the heat treatment of the meals. Cresol red, a phthalein containing dye, appeared to react more with egg albumin with the increased length of its storage in the presence of glucose.

Orange G, an acid azo dye, combines stoichiometrically with basic protein groups at pH 2.2. The amount of orange G absorbed per gram of protein decreased significantly with the time of storage (Table IV) indicating

TABLE IV. ABSORPTIVE PROPERTY OF BROWNED EGG ALBUMIN FOR DYES AND ITS BIOLOGICAL VALUE

Dyes	0 D	10 D	20 D	30 D	40 D
Cresol red[a]	4.8	6.1	6.8	7.8	8.1
Orange G[a]	151.3	118.2	101.1	83.2	57.2
Safranin O[b]	280.2	297.5	316.6	320.3	328.6
Biological[c] value (%)	90.1	44.5	34.3	29.1	23.7

[a] Amount of dye absorbed/g protein/hr.
[b] Amount of dye absorbed/g protein/24 hr.
[c] Adult male Sprague Dawley rats, weighing 320~380 g, were divided into groups of six, each group having the same weight. 10% protein diets were used. After rats had been on their diets for four days during adaptation, feces and urine were collected for the next three days (see Reference 20).

that egg albumin lost its basic protein groups during storage. This is reasonable because the

basic amino acids such as lysine, histidine and arginine react with a reducing sugar in the first stage of the browning reaction. However, Ashworth[17] found that the absorption of acid orange 10 (acid azo dye) by milk proteins was not affected during the production of commercial sterilized evaporated milk even though this process is known to cause a decrease in available lysine. The basic dye, safranin 0, reacts with the carboxyl, phenolic and thiol groups of proteins at pH 11.5. Slight increase in the amount of safranin 0 absorbed indicates that number of acidic groups in egg albumin-glucose mixture increased during storage. Lea and Hannan[16] obtained the similar results and explained that it was probably due to the formation of a few acidic groups in the carbohydrate residues attached to the proteins.

The orange G-binding property of a food protein should correlate with the results of biological tests of protein quality since the biological value of a protein is limited by its basic amino acid content. The binding of orange 10 with fish meals[11,18] and meat meals[19] supports this statement. Absorbability of three different dyes used in this study showed some relationships to the nutritional value of browned egg albumin, but it appeared that the dye binding method was not accurate enough to assess the change in the nutritional value of protein during storage.

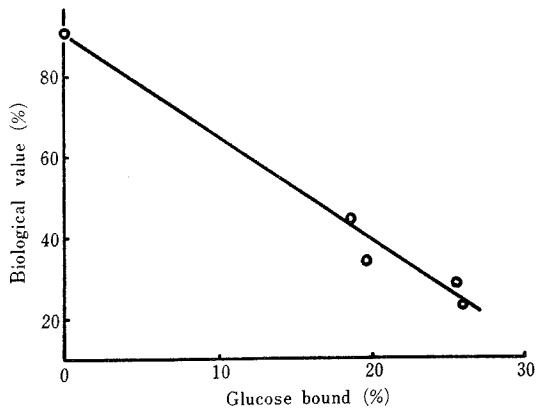

FIG. 2. Correlation between the Amount of Glucose Bound to Egg Albumin and the Biological Value.

It is important to note that the biological value of egg albumin decreased 50% within the first 10 days of storage at 37°C, 68% R. H.[20] Development of color did not follow the change of the biological value, however the amount of glucose bound to the protein corresponded very well with the change (Fig. 2). This correlation could be useful for assessing the nutritional value of browned egg albumin since, relative to the biological value, other *in vitro* methods such as protein score, chemical score, essential amino acid index, pepsin-pancreatin digest index and available lysine overestimated the nutritional value of the stored egg albumin.[20]

REFERENCES

1) J. E. Hodge, *J. Agr. Food Chem.*, **1**, 928 (1953).
2) H. D. Lightbody and H. L. Fevold, *Advances in Food Research*, **1**, 149 (1948).
3) E. R. Stadtman, *ibid.*, **1**, 325 (1948).
4) T. P. Labuza, *CRC Critical Reviews in Food Technol.*, **September**, 217 (1972).
5) S. T. Coulter, R. Jenness and W. F. Geddes, *Advances in Food Research*, **3**, 45 (1951).
6) J. Mauron, F. Mottu, E. Bujard and R. H. Egli, *Arch. Biochem. Biophys.*, **59**, 433 (1955).
7) S. Patton, *J. Dairy Sci.*, **38**, 457 (1955).
8) N. M. Rao and M. N. Rao, *J. Food Sci. & Technol. (Mysore)*, **9**, 66 (1972).
9) W. R. Akeson and M. A. Stahmann, *J, Nutrition*, **83**, 257 (1964).
10) A. Frölich, *Nature*, **174**, 879 (1954).
11) E. T. Moran, Jr., L. S. Jensen and L. J. McGinnis *J. Nutrition*, **79**, 239 (1963).
12) D. C. Udy, *Cereal Chem.*, **31**, 389 (1954).
13) A. R. Patton, E. G. Hill and E. M. Foreman, *Science*, **107**, 623 (1948).
14) V. C. Sgarbieri, J. Amaya, M. Tanaka and C. O. Chichester, *Arch. Latinoamericanos de Nutricion*, **23**, 363 (1973).
15) V. C. Sgarbieri, J. Amaya, M. Tanaka and C. O. Chichester, *J. Nutrition*, **103**, 1731 (1973).
16) C. H. Lea and R. S. Hannan, *Biochem. Biophys. Acta*, **5**, 433 (1950).
17) U. S. Ashworth, *J. Dairy Sci.*, **49**, 133 (1966).
18) A. W. Boyne, K. J. Carpenter and A. A. Woodham, *J. Sci. Food Agr.*, **12**, 832 (1961).
19) J. Bunyan and S. A. Price, *ibid.*, **11**, 25 (1960).
20) M. Tanaka, J. Amaya, T. C. Lee and C. O. Chichester, Abstracts of Papers, 4th International Congress of Food Sci. and Technol., Madrid, Spain, September, 1974, p. 31 (Session 7a).