Matoba, T., Nagayasu, C., Hayashi, R., Hata, T., *Agric. Biol. Chem.* **33**, 1662 (1969).
Minamiura, N., Matsumara, Y., Fukumoto, J., Yamamoto, T., *Agric. Biol. Chem.* **36**, 588 (1972).
Noguchi, M., Arai, S., Yamashita, M., Kato, H., Fujimaki, M., *J. Agric. Food Chem.* **23**, 49 (1975).
Shroeder, W. A., *Methods Enzymol.* **25**, 203 (1972).

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Effect of Maillard Reaction Products on Disaccharidase Activities in the Rat

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Effect of nonenzymatically browned products on the activities of mucosal disaccharidase in the small intestine of young rats both in vivo and in vitro was studied using browned products prepared from a natural food system (apricot) and model systems (glucose, egg albumin, and glucose–tryptophan). Disaccharidase activities of rats fed a diet containing browned products were found to be significantly reduced. Using a model system (glucose–tryptophan), the in vitro study on the mode of inhibitory effect of browning products on maltase activity revealed that the fructose–L-tryptophan (Amadori compound) fraction showed a competitive inhibitory effect (K_i = 3.5 mol or 1.28 mg), whereas the fraction free from fructose–L-tryptophan exhibited a noncompetitive inhibitory effect (K_i = 0.42 mg). The fructose–L-tryptophan free fraction contained mostly colored and fluorescent compounds. This fraction appeared to increase in quantity with an increase in reaction time and temperature.

Mucosal carbohydrases in the small intestine play a fundamental role in mediating digestion and absorption of carbohydrates, being responsible for the hydrolysis of poorly absorbable carbohydrate into easily absorbable monosaccharides. Most studies of dietary effects on carbohydrases have been focused on their activity in response to dietary carbohydrate. The feeding of maltose to rats caused a specific increase in maltase activity and similarly the feeding of sucrose increased sucrase activity (Deren et al., 1967; Reddy et al., 1968). However, contradictory results were observed when similar attempts were made with a lactose diet. Some reports showed increased lactase levels in rats after feeding a high lactose diet (Bolin et al., 1969; Bolin et al., 1971; Goldstein et al., 1971; Jones et al., 1972), while others showed no adaptive increase in the lactase activity upon increasing dietary lactose (Cuatrecasas et al., 1965; Bayless and Huang, 1969; Keusch et al., 1969; Leichter, 1973). Furthermore, an adverse effect of antibiotics on carbohydrase activities was observed and the decreased intestinal carbohydrase activities resulting from antibiotic treatment caused intestinal malabsorption (Paes et al., 1967; Cain et al., 1968).

In our previous study, it was observed that diets containing Maillard reaction products formed from a reaction mixture of reducing sugar and protein (or amino acid) caused diarrhea as well as depressed growth in rats (Lee et al., 1977). The extent of diarrhea varied with the nature of Maillard products and became pronounced with the increased intensity of browning. It was speculated that such diarrhea could be developed as a result of reduced activity of mucosal enzymes due to the ingestion of browned products in such a way that the poorly hydrolyzed products cause increased retention in the lumen contents. The present work was undertaken to verify the effect of the Maillard reaction products on the activities of mucosal disaccharidase and to reveal the underlying mechanisms by which such an effect would occur.

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Table I. Composition of Control and Browned Apricot Diet

Ingredients	% control	% test
Casein	10	13[a]
Apricot powder	74	71
Corn oil[b]	10	10
Salt mixture[c]	5	5
Vitamin mixture[d]	1	1
Choline chloride[e]	0.01	0.01
Total	100.01	100.01

[a] 3% casein was added to the test diet at the expense of carbohydrate in order to supplement protein in browned apricot which is assumed to be nutritionally unavailable (Sgarbieri et al., 1973). [b] Mazola Pure Corn Oil (Best Foods, Division of CPC, International Inc.). [c] Jones and Foster (1942), Salt Mixture (Nutritional Biochemicals Corp.). [d] Vitamin mixture (Nutritional Biochemicals Corp.). [e] Additional requirement, in addition to the quantity in the vitamin mixture, which has been recommended for rats by the National Academy of Science (1972).

MATERIALS AND METHODS

In Vivo Study. *Preparation of Diets.* Sundried apricots (Royals variety, received no chemical treatment and were purchased from the Prune and Apricot Growers Association, Calif.) were rehydrated in a growth chamber (Sherer Gillett, Marshall, Mich.) in order to bring the moisture content to 12% which facilitated maximum browning according to our previous study (Lee, 1974). The rehydrated apricots were then stored at 45 °C and 70% relative humidity (RH) for 3 months. The apricots browned as such were dehydrated by hot-air blast until

Table II. Composition of Extract Diet

Ingredients	% control	Extract % water	Extract % ether
Casein	15	15	15
Corn starch	71	51	66
Extract	0	20[a]	5[a]
Corn oil	5	5	5
Salt mixture	5	5	5
Fiber[b]	3	3	3
Vitamin mixture	1	1	1
Choline chloride	0.01	0.01	0.01
Total	100.01	100.01	100.01

[a] The percentage of extracts in the diet is given on the dry basis. [b] Alphacel (cellulose, Nutritional Biochemicals Corp.).

moisture content reached that of the control apricot (8%). The apricot diet was formulated by substituting apricot for the carbohydrate portion of the basal ration. The composition of the control and browned apricot diet is given in Table I.

For the preparation of extract diet, browned apricots were extracted with absolute ethanol (USP reagent quality, U.S. Industrial Chemical Co.) using a large continuous extractor (Corning Glass Works, Corning, N.Y.). After removal of solvent, the ethanol extract was separated into water-soluble and ether (diethyl ether)-soluble fractions. The water-soluble fraction was extracted repeatedly with ether until most of the ether-soluble substances were removed from the water phase. The water-soluble fraction was then freeze-dried after removal of residual solvent at 50 °C by a rotary evaporator. The ether remaining in the ether-soluble fraction was removed at 40 °C in the same manner. The solvent used for extraction was recovered by condensation. The temperature of the condenser was maintained below 0 °C by a bath cooler (PBC-2, Neslab Instruments, Inc.), and the recovered solvent was recycled for the next extraction in order to minimize the loss of low-boiling compounds during evaporation. Extract prepared from either the water-soluble or the ether-soluble fraction was incorporated into the basal ration at the expense of carbohydrate. The composition of the extract diet is given in Table II.

Synthetic apricot was prepared following the amino acid (Table III) and sugar composition (Ehearst and Mason, 1967) of apricot in a proportion of 3 parts of amino acids and 61.7 parts of sugars (consisting of 35.4 parts sucrose, 18.2 parts glucose, and 8.1 parts fructose) by weight. The formulated synthetic apricot mixture was dissolved in methanol and refluxed for 48 h to allow a sufficient browning. Following the removal of methanol, the concentrate was reconstituted with water and freeze-dried. The freeze-dried browned synthetic apricot was then incorporated into the basal ration at the expense of carbohydrate. For the control diet, the formulated synthetic apricot was substituted for the carbohydrate portion without any treatment.

The preparation of browned egg albumin and the supplementation of amino acids were done according to the procedure reported previously (Sgarbieri et al., 1973). The browned egg albumin diet was formulated as the apricot diet except that corn starch was substituted for the apricot. The formulations of all the diets conformed to the nutrient requirements for rats as recommended by the National Academy of Science (1972).

Animals and Feeding. Four male Sprague-Dawley weanling rats (Charles River Breeding Lab., CD Strain, Wilmington, Mass.), weighing 80 g as an average, were used. The animals were caged individually and fed for 2 months. For the test of the supplemented browned egg albumin diet, four male rats aged 2–3 months were used for each group and fed for 1 month. All the feeding tests were done by pair-feeding.

Table III. Amino Acid Composition of Apricot

Amino acid	mg/100 g of sample	%
Aspartic acid	150	10.52
Threonine and serine	144	10.12
Asparagine	830	58.28
Glutamic acid	22.4	1.57
Proline	7.4	0.54
Glycine	3.8	0.27
Alanine	49.7	3.48
Valine	10.8	0.75
Cystine	Tr	
Methionine	Tr	
Isoleucine	3.92	0.28
Leucine	3.65	0.26
Phenylalanine	Tr	
γ-Aminobutyric acid	19.9	1.39
Ammonia	178.7	12.54
Lysine	Tr	
Total		100.00

[a] Dried sample was hydrolyzed with 6 N HCl at 110 °C for 24 h and amino acids were analyzed using autoanalyzer (Technicon Auto-Analyzer, Technicon Instrument Co., Tarrytown, N.Y.).

Preparation of Mucosal Homogenates and Disaccharidase Assay. The preparation of the intestinal homogenates was carried out as described by Madge (1970). The homogenates were centrifuged at 16 400*g* at 4 °C for 20 min. The supernatants were stored at −25 °C and assayed in 1–3 weeks. The assay of disaccharidase activity was performed according to Dahlqvist (1964) using 0.1 M sodium maleate buffer with a pH of 6.2 (Siddons and Coates, 1972). The glucose liberated was assayed by a Tris–glucose oxidase reagent which was prepared from the Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.) following the procedure described by Dahlqvist (1964). Protein was measured by the method of Lowry et al. (1951).

In Vitro Study. *Preparation of Browning Products.* The preparation of the browning products was initiated by dissolving equimolar amounts of D-glucose and L-tryptophan in methanol to 30 mM. Refluxing was continued for approximately 50 h until the glucose level of the reaction mixture no longer decreased. The concentrate obtained after rotary evaporation at 40 °C was applied to a cellulose column (Whatman CM_{11}, fibrous powder, 12 × 600 mm) prewashed with water-saturated 1-butanol. The water-saturated 1-butanol was also used as an eluent. Each fraction was tested by thin-layer chromatography using silica gel plates (Type Q1, Arthur H. Thomas Co., Philadelphia, Pa.) with a solvent system consisting of 1-butanol, acetic acid, and water (4:1:1, v/v). The chromatogram was assayed by UV light and compared with that of fructose–L-tryptophan. Fructose–L-tryptophan was prepared following the procedure of Heyns and Noack (1964), and its purity was ensured by chromatographic examination. On the other hand, the fructose–L-tryptophan free fraction containing mostly colored and fluorescent compounds was reconstituted with water and freeze-dried after complete removal of 1-butanol with a rotary evaporator.

Study of Enzyme Kinetics. Two series of reaction mixtures with different substrate concentrations were prepared using maltose as a substrate. The procedure described by Dahlqvist (1964) was used for the enzyme assay. Different amounts of browning products were added

Table IV. Disaccharidase Activities in the Intestinal Mucosa of Rats Fed Control, Brown, and Supplemented Brown Egg Albumin Diets

Dietary group	Body weight, g	Activity, units/g		
		Lactase	Sucrase	Maltase
Control	338 ± 3.6	10.6 ± 1.02	35.7 ± 3.32	221 ± 16.3
Supplemented brown	300 ± 12.3[a²]	8.1 ± 1.31[a¹]	30.8 ± 0.78[a¹]	205 ± 11.3
Unsupplemented brown	278 ± 10.7[a³,b¹]	6.0 ± 1.20[a²]	24.6 ± 1.24[a²,b²]	172 ± 13.7[a²,b²]

[a] Significantly different from the control ($a^1 = P < 0.05$, $a^2 = P < 0.01$, $a^3 = P < 0.001$). [b] Significantly different from the supplemented brown diet ($b^1 = P < 0.05$, $b^2 = P < 0.01$).

Table V. Disaccharidase Activities in the Intestinal Mucosa of Rats Fed Browned Apricot, Extract, and Synthetic Diets

Dietary group	Body weight, g	Activity, units/g		
		Lactase	Sucrase	Maltase
Control apricot	320 ± 39	7.45 ± 1.25	32.6 ± 7.08	219 ± 42.81
Browned apricot	280 ± 18[a]	5.21 ± 0.08[a]	16.9 ± 2.82[b]	142 ± 21.34[a]
Control extract	313 ± 13.9	5.19 ± 0.90	11.6 ± 3.88	126 ± 10.66
Water extract	275 ± 25.8[a]	3.15 ± 0.54[b]	7.35 ± 0.44[b]	87 ± 0.23[c]
Ether extract	321 ± 16.4	4.64 ± 0.04	16.79 ± 3.61	134 ± 13.04
Control synthetic	315 ± 28.5			171 ± 4.93
Browned synthetic	235 ± 29.3[b]			127 ± 9.07[c]

[a] Significantly different from the control group ($P < 0.05$). [b] Significantly different from the control group ($P < 0.01$). [c] Significantly different from the control group ($P < 0.001$).

to the series of reaction mixtures. A proper concentration of enzyme solution prepared from the mucosal homogenate was added to the reaction mixture at the final step. Mucosal homogenate was prepared from the intestine of the rats fed Purina Chow (Ralston Purina Co., St. Louis, Mo.). The determination of enzyme inhibitor constants followed the method reported by Dixon (1953).

RESULTS AND DISCUSSIONS

All three intestinal disaccharidase activities were significantly diminished in rats after feeding an unsupplemented browned egg albumin diet for 1 month, and, similarly, the supplemented browned diet reduced the lactase and sucrase activities (Table IV). The table also shows that the sucrase and maltase activities were significantly lower in the unsupplemented brown group than in the supplemented brown group. This indicates that the quantity of available protein in the diet has a significant effect on disaccharidase activities, although it was reported that neither a protein-free nor a protein-deficient diet, based on a standard formula containing no browned products, caused diminished intestinal disaccharidase activites in rats (Solimano et al., 1967; Prosper et al., 1968; Troglia et al., 1970). It seems that the condition to be developed upon feeding a diet containing browned products could be worsened with reduced available protein in the diet. The rats fed a browned apricot diet for 2 months also showed a significant reduction in all their disaccharidase activites. Disaccharidase activites were reduced significantly after feeding a diet containing water-extract fraction, whereas the diet containing ether-extract fraction did not alter disaccharidase activites (Table V). This indicates that the water-soluble fraction of browned apricot is responsible for the reduction in disaccharidase activites. Similar effects were also observed in the rats fed a browned synthetic apricot diet (Table V). The rats fed browned egg albumin, browned apricot, and water-extract diets no longer had diarrhea after 1 month of feeding even with reduced disaccharidase activities. However, the rats fed a synthetic apricot diet showed a persistent diarrhea throughout the entire feeding period with significantly diminished disaccharidase activities. It was also observed that diarrhea still occurred in rats when

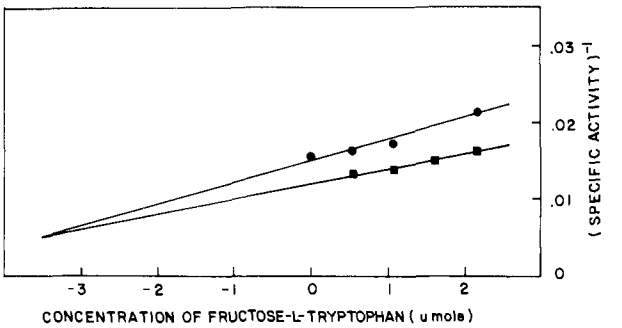

Figure 1. Effect of fructose–L-tryptophan on maltase activity in the intestinal mucosa; concentrations of substrate used were 0.056 M maltose (●) and 0.084 M maltose (■). The mean from duplicate run was used in each point.

the carbohydrate in the supplemented browned egg albumin diet was replaced with corresponding monosaccharides. These results and observations indicate that the reduced mucosal enzyme activities of the small intestine may not be the sole cause of diarrhea and that the susceptibility to diarrhea seems to depend upon the severity of the browning. A discrepancy between the disappearance of diarrhea during the latter part of the feeding period and reduced disaccharidase activities may suggest that the observed activities may tolerate the level of dietary disaccharide ingested.

The kinetic study with the browning products formed in the glucose–tryptophan systems revealed that the fructose–L-tryptophan fraction showed a competitive inhibitory effect (inhibitor consts, K_i = 3.5 mol, equivalent to 1.28 mg of fructose–L-tryptophan) (Figure 1), and that the fraction free from fructose–L-tryptophan exhibited a noncompetitive inhibition (K_i = 0.42 mg) (Figure 2). It shows that the inhibitory effect of fructose–L-tryptophan free fraction was markedly greater than that of fructose–L-tryptophan and cannot be counteracted by excessive substrate which would be able to keep the reaction site from the inhibitor. The fructose–L-tryptophan free fraction emerged early from the column during fractionation of browned products prepared from a glucose–tryptophan mixture. It contained mostly colored sub-

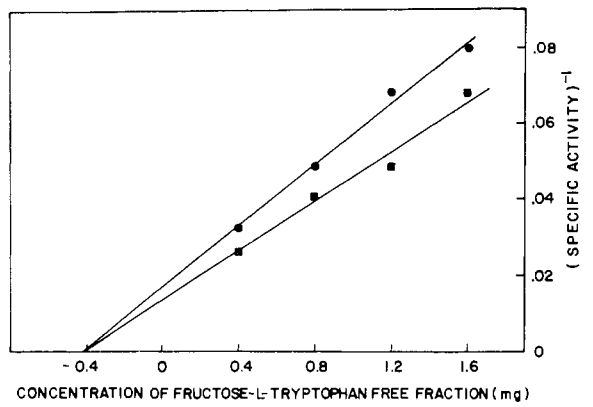

Figure 2. Effect of fructose–L-tryptophan free fraction on maltase activity in the intestinal mucosa; concentrations of substrate used were 0.056 M maltose (●) and 0.084 M maltose (■). The mean from duplicate run was used in each point.

stances as well as highly fluorescent compounds formed during browning. A study of the kinetics of browning of glucose–tryptophan system (Lee, 1974) showed that the intensity of color and fluorescence increased with reaction time and temperature. It seems, therefore, that the more browned product causes a greater effect on the digestive system in such a way that browned products reduced disaccharidase activities. It is suggested from in vitro observations that the instantaneous occurrence of diarrhea upon feeding diets containing browned products is at least a partial consequence of the direct inhibitory effect of browning products on the mucosal enzyme system of the small intestine. This observation was different from those reported by Paes et al. (1967) and Cain et al. (1968), who found that antibiotics failed to depress enzyme activity in the in vitro system and suggested that reduced enzyme activity in vivo caused by antibiotics resulted from decreased activity of the formed enzymes. The inhibitory effect of browning products on the digestive enzyme was also demonstrated by our group (Amaya, 1975). The Maillard dipeptide, fructosyl-glycyl-L-leucine, was not hydrolyzable by leucine-amino-peptidase in vitro, and the addition of 50% (mole ratio with respect to glycyl-L-leucine) of the Maillard peptide completely stopped the hydrolysis of the normal peptide by leucine-amino-peptidase. A stability study of fructose–L-tryptophan showed that fructose–L-tryptophan remained stable in the mucosal enzyme solution, indicating that none of the mucosal enzymes has an ability to hydrolyze this compound.

From both the in vivo and in vitro studies, it is suggested that diarrhea occurred as a result of an increase in lumen osmolarity; this was in turn caused by the accumulation of undigestable products formed during browning as well as unhydrolyzed substrates resulting from the reduced mucosal enzyme activities caused by the browning products. The lowered serum glucose level subsequent to the depletion of the liver glycogen of the rats fed diets containing browned products (Lee et al., 1977) is probably due to the decrease in available glucose as a result of reduced hydrolytic capacities of the disaccharidase activities as well as the degradation of carbohydrates during browning (Lee, 1974). Further investigations are necessary to determine whether the accumulated effect observed in the in vivo system is wholly the result of the direct inhibitory effect found in the in vitro system and to elucidate the mode of inhibitory action at a molecular level of browing products with special emphasis on the non-Amadori fraction containing mostly colored substances and fluorescent compounds.

LITERATURE CITED

Amaya, J., "Biological Inactivation of Protein by the Maillard Reaction", Ph.D. Dissertation, University of Rhode Island, Kingston, R.I., 1975.
Bayless, T. M., Huang, S. S., *Am. J. Clin. Nutr.*, **22**, 250 (1969).
Bolin, T. D., Pirola, R. C., Davis, A. E., *Gastroenterology* **57**, 406 (1969).
Bolin, T. D., McKern, A., Davis, A. C., *Gastroenterology* **60**, 432 (1971).
Cain, G. D., Reiner, E. B., Patterson, M., *Arch. Intern. Med.* **122**, 311 (1968).
Cuatrecasas, P., Lockwood, D. H., Caldwell, J. R., *Lancet* **No. 7373**, 14 (1965).
Dahlqvist, A., *Anal. Biochem.* **7**, 18 (1964).
Deren, J. J., Broitman, S. A., Zamcheck, N., *J. Clin. Invest.* **46**, 186 (1967).
Dixon, M., *Biochem. J.* **55**, 170 (1953).
Ehearst, J. F., Mason, B. S., *J. Am. Diet Assoc.* **50**, 130 (1967).
Goldstein, R., Klein, T., Freier, S. F., Menczel, J., *Am. J. Clin. Nutr.* **24**, 1224 (1971).
Heyns, K., Noack, H., *Chem. Ber.* **97**, 415 (1964).
Jones, J. H., Foster, C., *J. Nutr.* **24**, 245 (1942).
Jones, D. P., Sosa, F. R., Skromak, E., *J. Lab. Clin. Med.* **79**, 19 (1972).
Keusch, G. T., Troncale, F. J., Thavaramara, B., Prinyanont, P., Anderson, P. R., Bhamarapravathi, N., *Am. J. Clin. Nutr.* **22**, 638 (1969).
Lee, C. M., "Physiological Consequences of Non-enzymatic Browning", Ph.D. Dissertation, University of Rhode Island, Kingston, R.I., 1974.
Lee, C. M., Chichester, C. O., Lee, T-C., "Physiological Consequences of Browned Food Products", *Proc. Int. Congr. Food Sci. Technol., 4th*, in press, (1977).
Leichter, J., *J. Nutr.* **10e**, 392 (1973).
Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Madge, D. S., *Comp. Biochem. Physiol.* **36**, 467 (1970).
National Academy of Science, "Nutrient Requirement of Laboratory Animals: Nutrient Requirement of Laboratory Rats", No. 10, Washington, D.C., 1972, pp 56–93.
Paes, I. C., Searl, P., Rubert, M., Faloon, W. W., *Gastroenterology* **53**, 49 (1967).
Prosper, J., Murray, R. L., Kern, F., Jr., *Gastroenterology* **55**, 223 (1968).
Reddy, B. S., Pleasants, J. R., Wostmann, B. S., *J. Nutr.* **95**, 413 (1968).
Sgarbieri, V. C., Amaya, J., Tanaka, M., Chichester, C. O., *J. Nutr.* **103**, 657 (1973).
Sgarbieri, V. C., Amaya, J., Tanaka, M., Chichester, C. O., *J. Nutr.* **103**, 1731 (1973).
Siddons, R. C., Coates, M. C., *Brit. J. Nutr.* **27**, 101 (1972).
Solimano, G., Burgess, E. A., Levin, B., *Brit. J. Nutr.* **21**, 55 (1967).
Troglia, O. M., Laughrey, E. G., Henley, K. S., *Gastroenterology* **58**, 669 (1970).

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