

STUDIES ON THE "BROWNING REACTION" OF MILK AND MILK PRODUCTS II. SOME FACTORS IN THE "BROWNING REACTION" OF HEATED SKIM MILK

著者	ADACHI Susumu
journal or publication title	Tohoku journal of agricultural research
volume	4
number	2
page range	167-181
year	1954-02-20
URL	http://hdl.handle.net/10097/29108

STUDIES ON THE "BROWNING REACTION" OF
MILK AND MILK PRODUCTS
II. SOME FACTORS IN THE "BROWNING
REACTION" OF HEATED SKIM MILK

By

Susumu ADACHI

*Department of Animal Husbandry, Faculty of Agriculture,
Tohoku University, Sendai, Japan*

(Received, January 5, 1954)

Milk and milk products, that is to say, condensed milk and milk powder are discolored to brown by prolonged heating, or by long time storage. This phenomenon generally is referred to as the "browning reaction" or "Maillard reaction", since in 1912 the French chemist Maillard had investigated the reaction between amino acid and reducing sugars¹⁾ and found the formation of dark brown colored substances, so-called "mellanoidin". After Maillard's investigation, the term "browning reaction" has involved the reaction of nitrogen compounds, namely, amino acids, peptides, and proteins with reducing sugars. This reaction is of interest and significance in the field of not only biological chemistry²⁾³⁾ but also food technology⁴⁾. Various basic and applied research works have been reported extensively and the literature reviewed in detail⁵⁾⁷⁾. As a result, in the field of food technology, it was found by several workers that the browning reaction appear to contribute to the color, flavor, taste and nutritive value of some foods, whether these incidental phenomena are desirable or deteriorative for foods.⁷⁾ Perhaps, it seems that this reaction participates in not only the discoloration but also the flavor and other properties of milk and milk products.⁸⁾⁹⁾

Although many investigations concerning the browning reaction of milk and milk products has been performed, the milk constituents taking part in this reaction has been investigated slightly in order to understand the mechanism of this reaction. Ramsey, Tracy, and Ruehe,¹⁰⁾ who worked on the use of corn sugar in sweetened condensed skim milk, said: "The brown discoloration of sweetened condensed skim milk and . . . is due to sugar protein condensation products instead of caramelization. Caramelization plays no role in the

discoloration of the dairy products." and then, discussed that milk proteins that is potential supply of free amino nitrogen reacts with aldol and keto sugars in dairy products. While investigating the factors responsible for the formation of color in lactose solution during the heating of milk, Webb¹¹⁾ investigated the development of color in aqueous solution of lactose and various nitrogen compounds. He found that the color development in lactose solution during heating at 120° C is increased with increasing concentration of hydroxyl ions, lactose, urea, amino acids, namely, asparagine, ammonium salts, phosphates, and oxygen. In order to explain the formation of color of autoclaved and evaporated milk, Kass and Palmer¹²⁾ studied the effect of inorganic and organic buffer contained in soluble milk salts on lactose and caseinate solution at 120°C. They pointed to the conclusion that the origin of this color may be accounted for on the basis of the caramelization of lactose by casein, and neglected the effect of the soluble phosphate and the other dissolved milk salts in this reaction. Hadson¹³⁾ suggested that the hexone bases, namely, arginine, histidine, and lysine of milk proteins are participated the browning reaction in evaporated milk, by his work on the amino acid losses during the prolonged storage of evaporated milk. Recently, it was shown that inorganic phosphates are not of direct importance in the heat induced browning reaction in milk, but casein and lactose are the principal reactants by Patton,¹⁴⁾ who investigated the factors involved in the browning reaction of skim milk in the report on browning of heated milk.

As described, above many recent works only dealt with the protein-sugar system reaction which is principally responsible for the browning reaction of heated milk, and not particularly with the other factors. In this report, in order to make the basis for succeeding research of the browning reaction of dairy products, I have dealt with the chemical factors of some milk components affecting the browning reaction of heated skim milk, and found that the urea-lactose system reaction is predominant in this reaction, except the last opinion that the protein-lactose system is important.

Methods and Materials

Materials

As the color formation was very sensitive to impurities, it was necessary to subject the organic compounds employed to rigorous purification. Lactose, which is one of the principal reactants in this reaction, was recrystallized from methyl alcohol solution in order to obtain the riboflavin-free lactose.

Preparation of artificial milk

Before the examination for the browning reaction of milk itself, the tests concerned with the reaction of artificial milk, which is a model of normal milk,

were performed. The basal soluble milk salts solution was prepared according to the procedure of Van Slyke and Bosworth,¹⁵⁾ and Kass and Palmer,¹²⁾ as shown in table 1. The mixture of milk salts was dissolved in distilled water and filled up to 500 cc. The hydrogen ion concentration of this solution showed the value of pH 6.70, which was measured by the potentiometric method using the quinhydrone electrode.

Table 1. The composition of milk salts used in preparation of artificial milk

Salts	Weight (mg.)	Percentage
Calcium phosphate monobasic	1.743	19.42
Calcium chloride	1.186	13.21
Magnesium phosphate dibasic	1.025	11.42
Sodium citrate	2.215	24.70
Potassium citrate	0.519	5.78
Potassium phosphate monobasic	2.285	25.47
Total	8.973	100.00

450 g. of lactose and 25 cc. of 8 per cent solution of calcium caseinate prepared by the modified Van Slyke and Hart method were dissolved in this basal salt solution and filled up to 100 cc. in measuring flask with the same solution. Casein which was prepared by the Van Slyke and Bosworth method¹⁶⁾ was dissolved in lime water, and neutralized to pH 6.9 with the addition of dilute hydrochloric acid solution. The calcium caseinate solution described above varied from the opalescent to a milk-like appearance. This caseinate, which is precipitated from the solution with the addition of ethyl alcohol and is dried over sulfuric acid in a vacuum desiccator, contained 1.42 per cent of CaO. The hydrogen ion concentration of artificial milk prepared by the above-described method was pH 6.68, and this milky solution contained 2.13 per cent of casein.

Color Measurement

It had been found that the brown pigment is associated with milk protein, and that the tryptic digestion¹⁷⁾ or alkaline extraction procedure¹⁹⁾ to liberate the color from the milk proteins and spectrophotometric measurement method of reflectance¹⁸⁾ in milk have been developed in color measurements of browning in dairy products. As the milk proteins were coagulated by the super-heated treatment in this experiment it has been adopted that the color of coagulated protein-free whey are measured photometrically. A Pulfurich's step photometer with No. 48 filter was used to obtain the extinction coefficient. After the heat treatment was performed, the heated samples were centrifuged. The supernatant liquid was used for the final color measurement. Although, by

this method, the contribution of milk proteins to this browning reaction could not be determined satisfactorily, the sequel of this monograph will discuss the significance of milk protein which is a reactant in the browning reaction system of dairy products.

All samples were sealed in 40 cc test tubes and were subjected to the suitable heat treatment at 120~121°C. in an oil bath. The pH of the reaction system was determined potentiometrically by means of Nishigaki's potentiometer using a quinhydrone electrode. The concentration of total reducing substances was determined by the method of Fujita-Iwatake,²¹⁾ the weight of ferrocyanide being checked by thiosulfate titration, and was shown with the N/100 thio-sulfate solution volume (cc.) per 1 cc. of the samples.

Results

1. Browning reaction of lactose-glycine system

Few data are available dealing with the factors concerned with the browning reaction of lactose solution. At the start, lactose-glycine system which is most simplest in amino-sugar reaction system was used to determine the basic factors in the browning reaction of lactose-nitrogen compound systems which is employed in this experiment. 0.3 M. solution of lactose and glycine was heated at 120°C. An increase of exposure to heat is accompanied not only by an increase in color but also by a corresponding lowering in the final pH of the systems. These relationships are shown in table 2.

Table 2. The effect of time of heating upon color and final pH of lactose-glycine system

Compounds	Time (min.)	Color	pH Measured	pH Depression
Lactose	0	—	5.6	0
Lactose-glycine	0	—	5.4	0.2
Lactose-glycine	20	0.19	5.4	0.2
Lactose-glycine	60	0.26	5.3	0.3
Lactose-glycine	120	0.34	5.2	0.4
Lactose	180	0.13	5.3	0.3
Lactose-glycine	180	1.14	4.8	0.6

The result obtained indicates that the production of color is not a direct function of time, but that the reaction proceed rapidly after the induction period passed. It seems to be suitable for comparing the color formation to heat the systems at 120°C. for 180 minutes in this experiment. The glycine solution added to 0.3M. of lactose solution, and the concentration of glycine in this mixture was brought to 0.6, 0.2, 0.04, and 0.02M.. The glycine-lactose system

solution was heated at 120 for 120°C minutes to study the effect of glycine concentration upon the color development. The data obtained are given in table 3.

Table 3. The effect of the concentration of glycine upon color development in lactose-glycine system heated at 120°C. for 120 minutes.

Concentration (M)	Color		pH		
	Measured	Difference	Before	After	Difference
—	0.355	—	5.6	5.6	0.1
0.02	0.376	0.021	5.5	5.5	0.1
0.04	0.397	0.043	5.4	5.3	0.1
0.20	0.410	0.055	5.4	5.2	0.2
0.60	0.422	0.067	4.6	4.4	0.2

It is indicated that the color formation in this system is increased by the addition of glycine and is not due to the catalytic action of glycine but due to the interaction between lactose and glycine. The theory that the color formation results from the interaction between lactose and the amino group of glycine is evidently demonstrated by the following experiment that some of the glycine derivatives, that is to say, acethylglycine, glycinethylester, instead of glycine in this system were added in lactose solution before heating to determine their effect upon color development. The results obtained are given in table 4. Acethylglycine and glycinethylester were synthesized by means of the Herbst and Shemin,²¹⁾ and Curtius²²⁾ method respectively. pH of the mixture was adjusted to 6.6 by the addition of NaOH or HCl solution.

Table 4. The effect of some glycine derivatives upon browning reaction in 0.3 M lactose solution heated at 120°C. for 120 minutes.

Substances	Structural formula	Concentration (M)	Color	pH	
				Before	After
—	—	—	0.22	6.6	6.4
Glycine	NH ₂ CH ₂ COOH	0.6	0.60	6.6	6.2
Acethylglycine	CH ₃ CONHCH ₂ COOH	0.6	0.29	6.6	6.0
Glycinethylester hydrochloride	NH ₂ CH ₂ COOCH ₂ CH ₃ -HCl	0.6	0.35	6.6	2.6

The pH depression of glycinethylester hydrochloride-lactose system may be due to the liberation of hydrochloric acid accompanied by the degradation of glycinethylester hydrochloride, and contributes less color formation of glycinester-lactose system comparing with the glycine system. The differences in color development of these compounds originates the cover of the amino group or carboxyl group in these compounds. It appears that the color

development of the glycine-lactose system is based upon the interaction between lactose and the amino group of glycine.

The color development is also affected by the hydrogen ion concentration to decrease the tendency toward color formation. The addition of acid has little effect on the appearance of the brown color, but the addition of alkalis has a profound effect on the development of color. The hydrogen ion concentration of 0.3M. solution of the lactose-glycine system was adjusted to suitable pH by the addition of N/10 H₂SO₄ or N/10 NaOH solution before heating at 120°C. for 120 minutes. These prescribed relationships are shown in table 5.

Table 5. The effect of pH upon the color development, final pH, and degradation of lactose in 0.3 M lactose-glycine solution heated at 120°C. for 120 minutes.

Initial pH	Lactose			Lactose-glycine		
	Color	Final pH	Reducing value	Color	Final pH	Reducing value
6.4	—	6.3	0.06*	0.41	6.0	0.05*
5.2	0.04	5.2	0.07	0.31	4.8	0.06
4.4	0.04	3.8	0.09	0.25	4.2	0.07
3.0	0.07	2.4	0.11	0.14	2.0	0.07
2.2		1.4	0.14	0.13	1.6	0.06

*... The values are difference of reducing value between before and after heating.

It is indicated that the hydrogen ion concentration of the system is one of the main factors in color formation in this solution. The fact that the reducing value is increased by decreasing the hydrogen ion concentration may be due to the hydrolysis of lactose. The reducing value of lactose-glycine solution is less than the lactose solution. The difference of these reducing values are explained the reaction by which the reducing group of lactose are reacted with the amino group of glycine and are formed the mellanoidin.

2. Interaction of milk components

In order to determine the effect of milk components on color development, the mixture of two components among the entire milk components which are contained in milk over 1 mg per cent were heated at 135°C. for 120 minutes. The concentration of milk components which are mixed with the equivalent volume of the others is shown in table 6.

The results of combination of the two components which were mixed are shown by representative data, upon the color development, initial pH and final pH, given in table 6, 7, and 8 respectively. The color formation is shown qualitatively by symbols using —, +.

As pH of the mixture is not similar with the others, the results obtained are too different to be discussed. It is seen, however, that the color formation owing to the reaction between these substances is not remarkable except for the reaction of lactose with the other substances, and that the color development is increased by the more shifting of pH of the mixture, though there is no regularity between pH

Table 6. milk constituents

Organic substances*	Per cent
Calcium caseinate	4.56
Lactose	9.00
Creatine	0.004
Creatinine	0.002
Ascorbic acid	0.008
Urea	0.04
Uric acid	0.004
Cystine	0.002
Citric acid	0.80
Milk salts**	Per cent
Calcium phosphate monobasic	0.36
Potassium phosphate monobasic	0.46
Potassium citrate	0.10
Magnesium phosphate dibasic	0.20
Sodium citrate	0.44
Calcium chloride	0.24

* The values are taken and estimated from Davis (24)

** The values are taken and estimated from Van Slyke (15)

Table 7. The interaction of milk components for the color formation

Components	Concentration	Components														
		Ca-caseinate	Lactose	Citric acid	Urea	Uric acid	Creatine	Creatinine	Cystine	Ascorbic acid	CaHPO ₄	MgH ₄ P ₂ O ₈	K ₂ HPO ₄	Na ₃ -citrate	K ₃ -citrate	CaCl ₂
Ca-caseinate	1.50	#														
Lactose	4.50	##	+													
Citric acid	0.40	#	#	+												
Urea	0.012	-	##	-	-											
Uric acid	0.002	-	+	-	-	-										
Creatine	0.002	#	+	-	-	-	-									
Creatinine	0.001	+	##	-	-	-	-	-								
Cystine	0.001	-	#	-	-	-	-	-	-							
Ascorbic acid	0.004	-	##	-	+	-	+	-	-	#						
CaHPO ₄	0.180	-	+	-	-	-	-	-	-	-	-					
MgH ₄ P ₂ O ₈	0.100	#	+	-	-	-	-	-	-	-	-	-				
K ₂ HPO ₄	0.230	#	##	-	-	-	-	-	-	#	-	-	-			
Na ₃ -citrate	0.220	-	##	-	+	-	-	-	-	-	-	-	-	-		
K ₃ -citrate	0.052	-	#	-	-	-	-	-	-	#	-	-	-	-	-	
CaCl ₂	0.120	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

shifting and color development. Ascorbic acid, calcium caseinate, and lactose take part in color formation, but the main reactants for color development of these components react markedly in color formation with the other substances as follows: calcium caseinate, ascorbic acid, creatinine, urea, potassium

Table 8. pH of the milk component mixtures before heating.

Components	Components									
	Ca-caseinate	Lactose	Citric acid	Urea	Uric acid	Creatine	Creatinine	Cystine	Ascorbic acid	CaHPO ₄
Ca-caseinate	6.4									
Lactose	6.6	4.6								
Citric acid	4.6	2.6	2.4							
Urea	6.2	5.6	2.4	4.0						
Uric acid	6.6	5.6	2.6	4.4	5.6					
Creatine	6.4	5.4	2.4	4.6	4.4	4.2				
Creatinine	6.4	4.8	2.6	4.4	4.6	4.4	4.4			
Cystine	6.0	5.4	2.6	4.8	5.0	5.6	5.4	5.6		
Ascorbic acid	6.0	5.6	2.6	5.2	5.4	5.2	5.6	5.4	5.0	
CaHPO ₄	6.8	6.2	2.6	5.8	5.0	5.6	5.2	5.6	5.4	6.0
MgH ₄ P ₂ O ₈	5.8	2.8	2.4	2.8	2.8	2.8	2.6	4.4	4.2	2.8
K ₂ HPO ₄	6.6	7.0	2.8	6.4	6.6	4.6	6.8	7.0	7.0	7.0
Na ₃ -citrate	6.4	6.4	3.6	6.4	6.6	5.6	5.8	5.8	5.8	5.8
K ₃ -citrate	5.8	5.6	2.6	5.8	5.8	4.8	5.6	5.8	5.2	5.8
CaCl ₂	6.0	5.6	2.4	4.8	4.8	3.8	4.4	5.2	5.0	5.6

Table 9. pH of the milk component mixture after heating.

Components	Components									
	Ca-caseinate	Lactose	Citric acid	Urea	Uric acid	Creatine	Creatinine	Cystine	Ascorbic acid	CaHPO ₄
Ca-caseinate	6.4									
Lactose	5.2	4.8								
Citric acid	4.4	2.6	2.6							
Urea	6.8	5.8	5.8	5.6						
Uric acid	6.4	4.4	2.6	6.2	5.8					
Creatine	6.6	4.6	3.2	5.8	5.6	5.6				
Creatinine	6.2	4.6	2.6	5.8	7.2	5.8	5.0			
Cystine	6.4	4.8	2.6	5.6	8.6	6.4	6.2	7.0		
Ascorbic acid	6.4	4.4	2.6	5.6	5.8	5.4	4.4	4.8	4.4	
CaHPO ₄	5.8	5.0	2.6	6.4	6.6	5.2	6.4	6.4	4.6	—
MgH ₄ P ₂ O ₈	5.0	2.6	2.4	4.4	6.4	2.8	3.8	6.0	2.6	6.2
K ₂ HPO ₄	6.6	4.6	3.0	7.0	7.8	7.2	7.0	6.0	6.4	7.8
Na ₃ -citrate	7.0	4.6	3.6	9.6	9.6	9.6	5.0	6.2	6.6	6.2
K ₃ -citrate	7.0	4.8	2.6	8.2	9.0	6.6	6.4	5.8	6.2	7.8
CaCl ₂	6.6	5.2	2.4	5.8	5.8	5.0	4.0	5.4	5.6	5.6

phosphate monobasic, and sodium citrate. Above all, the reaction of lactose-urea system is most remarkable.

As it was thought advisable to investigate the artificial milk for applying the above described results with the browning reaction of heated milk, the artificial

milk, whose preparative method was described in the part of experimental method, was used in the following experiments. The artificial milk solution was prepared by mixing the basal artificial milk as described with creatinine, ascorbic acid, and urea, and the concentrations of these systems in this solution were 0.002, 0.004, and 0.012 per cent respectively. To determine the effect of the components in this artificial milk on the color formation the milk in which one component in these substances was absent, was heated to 120°C. for 120 minutes. The supernatant liquid which was obtained by centrifugation was used in color measurement. The color development in these systems is shown in table 10.

Table 10. The browning reaction in artificial milk heated at 120°C for 120 minutes.

Lack compounds	Color	Initial pH	Final pH	Reducing value
Lactose	0.22	6.2	5.6	—
Calcium caseinate	0.60	6.4	5.4	17
Ascorbic acid	0.59	6.2	5.4	20
Creatine	0.65	6.2	5.4	24
Urea	0.52	6.2	5.4	19
Mineral salts	0.53	7.0	5.8	23
Control	0.65	6.2	5.4	20

The results obtained show that lactose is a main carrier of the browning reaction, and that urea and mineral salts are co-carriers. Though the effect of the calcium caseinate on the color formation are, unexpectedly, less than that of the substances above described, it may be due to the fact that the browning substances absorbed the calcium caseinate when coagulated by heat treatment. Accordingly, these experiments described above leave doubt for the effect of caseinate on the browning reaction. However, It may be concluded that the urea-lactose system reaction is predominant in the browning reaction of the heated artificial milk from the above experiments, and it is very interesting.

3. Factors in the browning reaction of heated skim milk

It was concluded that the urea-lactose, ascorbic acid-lactose, sodium citrate-lactose, and potassium phosphate-lactose systems, except the amino-lactose system in milk proteins, take part in the browning reaction of heated milk. Therefore skim milk that is separated from milk fat by a centrifugal cream separator and contains 0.01 per cent of fat was heated at 120°C. for 120 minutes to determine the factors in the browning reaction in it by the addition test using urea, ascorbic acid, potassium phosphate monobasic, and sodium citrate as the adding agents. These added agents were added to the skim milk before heating within the normal variation range presented in milk. After the heat treatment the milk proteins were coagulated at the bottom of the test tube. As shown

in the section on method, the color of supernatant clear liquid was measured. The experimental evidence obtained in heated skim milk is shown in table 11.

Table 11. The effect of added substances upon the browning reaction in skim milk heated at 120°C for 120 minutes.

Added compounds	Addition weight (mg/100cc.)	Color	Initial pH	Final pH	Reducing value
Urea	5	1.85	6.6	5.6	10
Ascorbic acid	2	1.15	6.0	5.6	13
Sodium citrate	10	1.18	6.6	5.4	9
Potassium phosphate monobasic	10	1.20	6.6	5.4	17
Lactose	500	1.13	6.6	5.6	11

As the milk proteins had not been added in skim milk before heating, the effect of milk proteins on the color development in skim milk has not been observed and could not be compared with the other factors in color formation. Table 10 shows, however, that urea is one of the main factors affecting the browning reaction. The other factors described above are as given in the following series with regard to the degree of color development: potassium phosphate, sodium citrate, ascorbic acid, and lactose. In comparison with the contents of these compounds, though the order of color formation is like the above, the compounds which have influence upon the color formation with minor quantities are sodium citrate, potassium phosphate, especially urea and ascorbic acid. In spite of the addition of large quantities, lactose has little effect upon color development. This relationship was confirmed by the following experiment in which lactose was reacted with these substances, as shown in Table 12. These substances were added into 100cc. of 4.5 per cent lactose solution. The mixed systems were heated at 120°C. for 120 minutes. Since it has been found that furfural derivatives are formed in the course of browning reaction as intermediate products, acetic acid-anilin reaction tests²⁴⁾³⁵⁾ were performed qualitatively for furfural derivatives formed in this reaction mixture.

The order of color formation at the level of 50 mg per cent of added compounds is as follows:

urea > ascorbic acid > potassium phosphate > sodium citrate

The color was substantially increased by addition of urea in small quantities, and was slightly increased by potassium phosphate and sodium citrate. The fact that the acetic acid—anilin reaction test was markedly positive in the ascorbic acid—lactose system indicates that furfural derivatives were produced from ascorbic acid during heat treatment. The browning reaction

Table 12. The effect of urea, potassium phosphate monobasic, sodium citrate, and ascorbic acid on the browning reaction in lactose solution heated at 120°C for 120 minutes.

Compounds	Addition weight	Initial pH	Color	Reducing value	Furan test
Urea	(mg)				
	50	5.6	1.75	16	##
	15	5.6	0.84	10	+
	6	5.6	0.28	5	±
	3	5.6	0.21	5	±
K ₂ HPO ₄	230	5.0	0.60	31	##
	100	5.0	0.37	15	##
	50	5.2	0.14	15	+
	10	5.6	0.05	5	±
	5	5.6	0.04	4	±
Na ₃ -citrate	230	5.2	0.51	24	##
	100	5.2	0.34	20	##
	50	5.2	0.16	14	+
	10	5.4	0.05	4	-
	5	5.6	0.03	4	-
Ascorbic acid	100	4.0	0.35	—	##
	10	5.4	0.35	—	##
	2	5.6	0.34	—	##
Control	—	5.8	0.00	4	+

of the heated skim milk is affected by pH similarly to the lactose-glycine system, as shown in table 13. The pH of the skim milk was adjusted by the addition of dilute lactic acid solution within the range from 6.6 to 6.0 before heating at 120°C. for 120 minutes.

Table 13. The effect of pH on the browning reaction of heated skim milk.

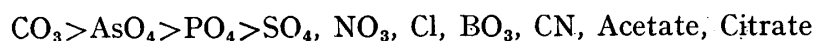
Sample number	Initial pH	Final pH	Color
1	6.6	5.6	1.11
2	6.5	5.6	0.97
3	6.4	5.4	0.93
4	6.3	5.4	0.84
5	6.0	5.4	0.86

Table 13 shows that the color development of skim milk containing no lactic acid is the highest degree and the velocity of browning reaction is decreased by the increasing acidity and decreasing pH of the skim milk. Therefore it appears that the color formation of sour milk is less than of normal fresh milk. The problems of the browning reaction of sour milk will be reported in a later paper.

Discussion

As the high temperature treatment applied to the manufacture of evaporated milk is a sterilization process in which the concentrated milk is heated at a temperature of $116^{\circ}\sim 120^{\circ}\text{C}$. for twelve to twenty minutes, the heat treatment in which the reaction systems were heated at 120°C . for 2 or 3 hours may be too long to compare directly with the browning reaction of evaporated milk. The difference in color development, however, owing to chemical factors in skim milk can be decided easily by long time high temperature heat treatment. The milk proteins in these systems are coagulated and contracted at the bottom of the reaction apparatus by super-heating, and the supernatant liquid which is used in color determination is separated from it. Ramsey, Tracy, and Ruehe have observed that the color substances formed from sugar amino acid condensation are really soluble in whey, but are absorbed by the casein. The work of Kass and Palmer has shown that the brown pigments developed in heated milk are adsorbed physically on the milk proteins. Therefore, it is necessary to extract the pigment from it for photolorimetric determination of color in heated milk, and some methods have been employed by Doob et al and Choi et al. In this experiment, however, as the supernatant liquid separated from coagulated milk protein curd was used in color measurement, it seems probable that the color determination may be uncertain, because the pigment was adsorbed in the coagulated proteins. Even if the pigment was liberated from the proteins by long heat treatment, it seems to be impossible to compare accurately the effect of milk proteins on the browning reaction with the effect of other components. The comparison of these effects will be decided in a supplementary report. Nevertheless, it seems that the tendency for the effect of milk components on the browning reaction of heated milk was confirmed substantially. Hitherto, the urea-lactose reaction, which may occupy an important place in the browning reaction of heated milk, has not been studied. The report by Webb of color development in lactose solution, suggests that urea is a reactant of the color production in lactose solution, but does not conclude that the urea-lactose system reaction indicated by the author is one of the main reactive systems in this reaction of heated milk. Though the urea-lactose system reaction is remarkably similar to the protein-lactose system, reported by some workers, the ratio of the two systems participated by the browning reaction remains a pending question. However, it may be seen that the browning reaction of the urea-lactose system in heated milk takes a conspicuous part in the browning of heated milk. As the urea-sugar system is the simplest of the amino-sugar systems, the reaction of this system may be interesting from the standpoint of the organic chemistry of amino-sugar reaction.

The effect of phosphate and citrate on the browning reaction has been studied by a number of investigators. Especially, Webb, and Kass and Palmer indicated that phosphate is responsible for a catalytic influence on the browning reaction of heated milk in their model experiment using a lactose solution substituted for milk itself. But they presumed that the effect of phosphate is probably negligible in the reaction, since the concentration of the phosphate buffer is too low to change appreciably the color of the lactose solution. The effect of citrate on the browning reaction of glucose solution, is remarkable on the color development as the result of the reaction between the nitrogen free carboxylic acid and glucose.²⁵⁾ It is suggested that citrate may play an important role in the browning reaction. It is also probably found that the role of citrate and phosphate in the browning reaction of heated milk cannot be ignored, though their effect may not be remarkable in consequence of their low concentration in milk. Ashida and Sugio²⁶⁾ have stated that sugar oxidized in the presence of some salts, namely, the order of anions in these salts on the oxidizability of fructose solution is as follows:



It is found that the browning reaction between milk salts, that is to say, citrate, phosphate with lactose is different from the amino-sugar reaction in which amino acid is oxidized. In the former reaction lactose may be oxidized by phosphate and citrate as described above.

The fact that ascorbic acid is an important factor in the browning reaction has been observed in the deterioration of vegetable foods by some workers.²⁸⁾²⁹⁾ The color development of dried whole milk, manufactured by the Merrel Soul sprayer with much added L-ascorbic acid before spraying was indicated by the work of Nakanishi.²⁷⁾ Similarly, it is found that ascorbic acid in milk takes a partial part in the browning reaction of the heated milk, and the addition of ascorbic acid develops the color of the products. As ascorbic acid is changed to furfural by heating,³⁴⁾ furfural from which the brown pigment is easily produced³⁰⁾³¹⁾³²⁾³³⁾ may be formed from ascorbic acid in heated milk, and caused the color formation.

It has been shown by some reports that pH of the systems influences the velocity of the browning reaction of heated milk. pH of milk before heating, as shown in preliminary experiments using the glycine-lactose system, has a important role in the browning reaction, and the higher pH increases the velocity of the reaction. However, the reaction in milk is also affected by the addition of some neutralizers under the same pH conditions, the reaction is due to the kind of salts used as neutralizer, but to the pH. It is found that this relationship may be observed in the effect of milk salts on the reaction, as shown in the reports of Ashida and Sugio, and Lewis, Esseln, and Fellers.²⁵⁾

Summary

In order to determine the factors influencing the browning reaction of heated skim milk, the experiments using the following systems were performed: (1) glycine-lactose system reaction as a preliminary experiment to obtain the fundamental factors in the reaction, (2) artificial milk system which is synthesized from milk components presented over 1 mg per cent to determine the chemical factors in the reaction of this heated system as model system milk, for (3) skim milk which is used for an addition test of these components to decide the effect of these factors on the reaction of heated milk.

Most samples were heated at 120°C, for 120 minutes in sealed test tubes.

As a result, it was found that the factors affecting the browning reaction of heated skim milk, except the milk proteins, are as follows:

urea, ascorbic acid, potassium phosphate, sodium citrate and then these reactants are reacted with lactose in the browning. Above all, the urea-lactose system was the predominant reactant among these reactions. It is presumed that the courses of the browning reaction played by these reactants are different chemically and the amino-lactose reaction is not all in the browning reaction.

Acknowledgment: I wish to express my hearty thanks to Profs. R. Sasaki and T. Nakanishi for their kind guidance and criticism throughout the course of this study. I have received valuable suggestions for the synthesis of magnesium phosphate dibasic from Prof. A. Fujiwara in this project.

References

- 1) Maillard, L. C. (1912). *Comptes. Rendus*, **154**, 66.
- 2) Pigman, W. W. (1951), *Science*, **114**, 554.
- 3) Lea, C. H. & Hannan, R. S. (1950). *Nature*, **165**, 483.
- 4) Lea, C. H. (1950). *Chem. & Indust.*, 155.
- 5) Barnes, H. M. & Kaufman, C. W. (1947). *Ind. & Engineering Chem.*, **39**, 1167.
- 6) Stadtman, E. R. (1948). *Advances in Food Research*, **1**, 325.
- 7) Kaufman, C. W. (1946). *Food Industries*, **18**, 12.
- 8) Henry, K. M., Kon, S. K., Lea, C. H., & White, J. C. D. (1948). *Jour. Dairy Research*, **15**, 292.
- 9) Hunziker, O. F. (1949). *Condensed Milk and Milk Powder* 7 ed., 14, 291, 495.
- 10) Ramsey, R. J., Tracy, P. H., & Ruehe, H. A. (1933). *Jour. Dairy Science*, **16**, 17.
- 11) Webb, B. H. (1935). *Jour. Dairy Science*, **18**, 81.
- 12) Kass, J. P. & Palmer, L. S. (1940). *Ind. & Engineering Chem.*, **32**, 1360.
- 13) Hadson, A. Z. (1952). *Food Research*, **17**, 467.
- 14) Patton, S. (1952). *Jour. Dairy Science*, **45**, 1053.
- 15) Van Slyke, L. L. & Bosworth, A. W. (1915). *J. Biol. Chem.*, **20**, 135.

- 16) Van Slyke, L. L. & Bosworth, A. W. (1913). *J. Biol. Chem.*, **15**, 203.
- 17) Choi, R. P., Koncus, A. F., O'Malley, C. M., & Fairbanks, B. W. (1949). *Jour. Dairy Science*, **32**, 580.
- 18) Nelson, V. (1948). *Jour. Dairy Science*, **3**, 409.
- 19) Doob, H., Willman, A., & Sharp, P. F. (1942). *Ind. & Engineering Chem.*, **34**, 1460.
- 20) Fujita, S., & Iwataka, D. (1932). *Biochem. Z.*, **242**, 43.
- 21) Herbst, R. M., & Shemin, D. (1948). *Organic Synthesis*, **1**, 11.
- 22) Curtius, T. (1883). *Berichte*, **16**, 754.
- 23) Davis, W. L. (1939). *The Chemistry of Milk* (Chapman & Hall) p. 155.
- 24) Stillings, R. S. & Browning, B. L. (1940). *Analy. Chem.*, **12**, 494.
- 25) Lewis, V. M., Esslen, W. B., & Fellers, C. R. (1949). *Ind. & Engineering Chem.*, **41**, 2591.
- 26) Ashida, J. & Sugio, Y. (1953). Lecture at Meeting of the Agr. Chem. Soc. Japan.
- 27) Nakanishi, T. (1952). Unpublished data.
- 28) Joslyn, M. A., March, G. L., & Morgan, A. F. (1934). *J. Bol. Chem.*, **105**, 17.
- 29) Morgan, A. F., Field, A., & Nichols, P. F. (1931). *Jour. Agr. Research*, **42**, 35.
- 30) Hass, V. A., Stadtman, E. R., Stadtman, F. H., & Mackinney, G. (1948). *J. Amer. Chem. Soc.*, **70**, 3756.
- 31) Singh, B., Dean, G. R., & Cantor, S. M. (1948). *J. Amer. Chem. Soc.*, **70**, 517.
- 32) Patton, S., & Josephson, P. V., (1949). *J. Dairy Science* **32**, 222.
- 33) Patton, S. (1950). *J. Dairy Science* **33**, 324.
- 34) Roe, J. H. (1936). *J. Biol. Chem.* **116**, 109.
- 35) Allport, N. L. (1947). *Colorimetric Analysis* (Chapman & Hall), p. 400.
- 36) Webb, B. H., & Holms, G. E. (1930). *J. Dairy Science* **13**, 25.