

The Early Stages of Nonenzymatic Browning

Introduction

The nonenzymatic browning or MAILLARD reaction (1) is very important in many fields, ranging from the processing of food to cataract formation and diabetes. Many of its facets have been studied in the last 70 years but there are still many unanswered questions. Much of the work reported in a recent symposium (2) concerned the MAILLARD reaction of proteins and carbohydrates, particularly where the amino group is supplied by the α -amino group of lysine, and only a few papers dealt with simple amino acids and peptides. While protein may be very important in MAILLARD browning reactions in many foods, foods of plant origin especially, such as potatoes (3) and tomatoes (4), contain high concentrations of free amino acids and possibly peptides. In potatoes, 40%–50% of the nitrogen-containing compounds are free amino acids (3, 5, 6). Also parenteral nutrition solutions (7) contain high concentrations of free amino acids and free sugars which may cause browning. In most cases, the development of brown color has been used to monitor the reaction. But browning does not occur in the earliest reaction stage (8, 9). The first step is the simple addition of the amino compound and the carbonyl group of the reducing sugar (8, 10). This can be detected by the ninhydrin reaction (11) and may be separated on the amino acid analyzer (4, 7, 12), but the results from natural mixtures are very complicated. This paper is a continuation of an earlier study (12) of these first-stage MAILLARD reactions using simplified model systems, filter paper disks heated in deep fat, with emphasis on the amino acids and sugars found in potatoes. Twenty-two amino acids were tested with glucose and some of them with other sugars. Most were 1:1 concentrations but several were with varying ratios, and in one case, two amino acids competed for the same sugars. Most of the reactions were at 103°C but a few at room and at refrigerator temperatures were calculated. A new method of correlation of the reaction involved is outlined. Possibly the most important combinations in potatoes are asparagine, glutamine, and *gamma*-amino butyric acid with glucose.

Experimental

Standard solutions (10^{−3} M per disk of 0.1338 M in water) of amino acids and sugars were added separately to 9 mm disks of filter paper, drying after each addition. The disks were stored in the refrigerator or at room temperature or heated in deep fat at 103°C for varying lengths of time. The latter were rinsed in carbon tetrachloride to remove the fat. The disks were placed directly on the ion-exchange column of the amino acid analyzer, to minimize changes before and during measurements, and washed in with rinses of buffer in the usual manner. The calibration constant of the parent amino acid was used for the new peaks. This follows the observation of INGLES and REYNOLDS (11), which apparently was based on crystalline compounds obtained by them. HEYNS *et al.* (13) and EICHNER and CINER-DORUK (4) have used different color constants for the parent amino acids and the intermediates, apparently based on amorphous compounds, which gave single peaks on chromatography. Sugars and other compounds could have been present which would not react with ninhydrin. The detailed procedure has been described by TALLEY and PORTER (12).

The data obtained were correlated as follows: the time of heating in seconds of each sample was converted to the \log_{10} time (s) basis. These values were plotted against the corresponding residual amino acid content or the intermediate equivalent values in micromoles. Usually straight line sections are readily apparent (Fig. 1). The equations of the straight line sections were calculated, including the correlation coefficients. Lines were drawn from the equations as a check on the calculations (Fig. 2).

Results and Discussion

Fig. 1 is a plot of the data obtained from the glycine and glucose sets using \log of the time instead of straight time as was done for threonine and glucose in Fig. 2 of the previous paper (12). The curve of glycine and glucose in Fig. 1 is quite typical of most of the samples run. The plot of residual micromoles of glycine (Y) vs. \log_{10} of the time of heating in

¹ Deceased, May 21, 1985

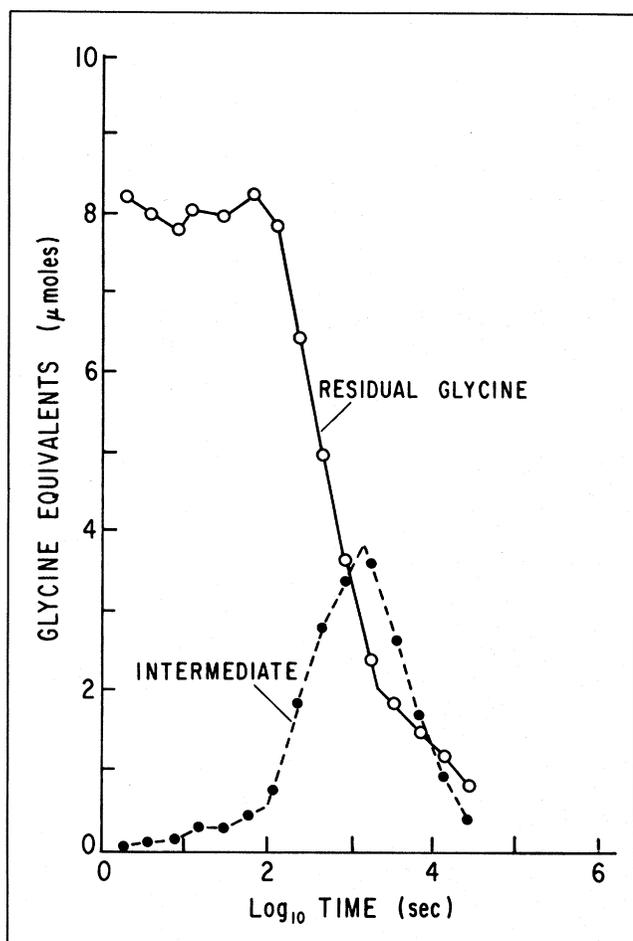


Fig. 1 Changes in concentration of glycine and of the major intermediate with \log_{10} time during heating with glucose at 103°C

Similar data (for threonine) were used in Fig. 2 of TALLEY and PORTER (12)

Open circles connected by solid lines represent micromoles of residual glycine. Solid circles connected by dashed lines represent micromoles of the intermediate.

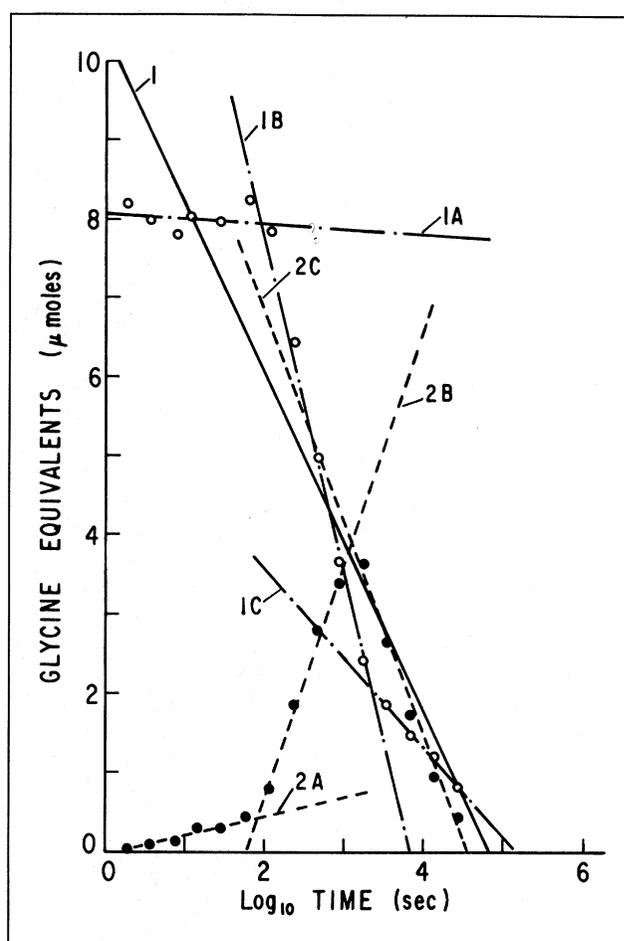


Fig. 2 Changes in concentration of glycine and of the major intermediate with \log_{10} time during heating with glucose at 103°C

Here the lines of the calculated equations have been drawn in

1 refers to the residual amino acid (the line calculated from all the points (-) and does not have a letter) (This line corresponds to Eqn. (1) GLY 275 in Tab. 1.); 1A is the first straight section (---) (7 points); 1B is the second section (-.-) (6 points); and 1C is the third section (---) (4 points). 2 refers to the formation and loss of the intermediate (----) (the letters have the same significance as for 1). (2B corresponds to Slope (2B) GLY 90 in Tab. 2.)

Open circles represent micromoles of residual glycine. Solid circles represent micromoles of the intermediate.

seconds (X) (open circles) indicates three straight line sections, although the entire 15 points calculated as a straight line is:

$$Y = -2.13X + 10.31 \quad \text{Eqn. (1)}$$

with a correlation coefficient of -0.94 (Line 1 in Fig. 2). The first 7 points (1A in Fig. 2) gave an equation:

$$Y = -0.06X + 8.06; \text{corr} = -0.21 \quad \text{Eqn. (1A)}$$

(The low correlation coefficient is partly associated with the short line segment.) The second section 1B has 6 points:

$$Y = -4.17X + 16.09; \text{corr} = -0.99 \quad \text{Eqn. (1B)}$$

The third section, 1C, of 4 points is:

$$Y = -1.12X + 5.82; \text{corr} = -0.99 \quad \text{Eqn. (1C)}$$

The intermediate, 2, has three sections as follows:

$$2A \text{ GLY } 90 \text{ } Y = 0.27X - 0.07; \text{corr} = 0.96 \text{ (6 points)}$$

$$2B \text{ GLY } 90 \text{ } Y = 2.99X - 5.35; \text{corr} = 0.99 \text{ (4 points)}$$

$$2C \text{ GLY } 90 \text{ } Y = -2.68X + 12.20; \text{corr} = -0.99 \text{ (5 points)}$$

(2A, 2B, and 2C represent the 1st, 2nd, and 3rd straight line sections of the group of equations for the GLYcine + GLUcose intermediate, which was eluted at 90 ml (Fig. 2).

Some of the results obtained for the residual amino acids are listed in Tab. 1 and for the intermediates found are listed in Tab. 2.

In general, the results for all the sugar-amino acid combinations tested are similar. In a few cases, a "D" section was found for the intermediate, where the rate slows down at the long heating times. The "A" section is missing in a number of cases but the "B" and "C" sections are present for all the intermediates. In a number of cases, more than one intermediate was found, as reported in the previous paper (12). Sets of equations for these intermediates were calculated where data was available. Possibly all the small peaks would give similar results if sufficient data were available. These may be due to isomeric forms similar to those reported by

Tab. 1 Slopes of equations for residual amino acids, log₁₀ time (second) (X) vs. μmoles amino acid (Y), fried at 103°C, ratio amino acid to sugar is 1:1, except where noted. (Line 1 in Fig. 2, all points)

With glucose										
AA ¹	GLY	SER	MSG	GLN	ARG	THR	PHE	ILE	GLU	TRP
EV ²	275 A	175A	242A	178A	630B	169A	543A	448A	245A	390B
Slope ³	-2.13	-1.97	-1.87	-1.83	-1.80	-1.71	-1.67	-1.58	-1.56	-1.54
AA	LYS	BAL	VAL	PRO	ALA	ASN	TYR	HIS	GAM	LEU
EV	258B	700A	358A	220A	297A	178A	530A	338B	182B	470A
Slope	-1.49	-1.45	-1.28	-1.27	-1.26	-1.16	-1.15	-1.10	-0.91	-0.86
AA	ASP	MET	NH ₃		MSG	MSG	GLU	GLU		
EV	154A	442A	233B		a242	b240	a245	b245		
Slope	-0.72	-0.66	-0.64		-1.74	-1.87	-1.56	-1.46		
	ASparagiNe		MSG		GAM			ILE	ILE/2	
Sugar ⁴	ASC	NAS	GLU	2GLU	GLU	4GLU	8GLU	16GLU	GLU	GLU
Slope	-0.74	-0.40	-1.74	-2.00	-0.91	-2.01	-1.88	-1.97	-1.58	-0.76
	ASparagiNe			LEUcine			GAM			
Sugar	GLU	FRU	ASC	NAS	GLU	FRU	SUC	GLU	FRU	MAN
Slope	-1.16	-1.62	-0.74	-0.40	-0.86	-0.87	-0.79	-0.91	-1.60	-1.55
	GAM		HISidine		LYSine					
Sugar	SUC	FRU6	GLU	FRU	SUC	GLU	FRU	SUC	0.0	
Slope	-0.68	-0.93	-1.10	-1.75	-1.12	-1.49	-1.94	-0.20	-0.06	
	MSG + VAL + 2GLU									
	(Slopes of line sections as indicated in text and in Fig. 2.)									
225 (MSG)	1,	-2.29;	1A,	-1.27;	1B	-4.22;	1C	-0.82		
358 (VAL)	1,	-1.43;	1A,	-1.36;	1B	-3.11;	1C	-0.29;	1D,	-1.60

¹ AA = Amino Acid, in addition to the usual symbols, MSG = MonoSodiumGlutamate; GLN = GLutaminNe; TRP = TRyPtophan; BAL = β-ALanine; ASN = ASparagiNe; GAM = gamma-aminobutyric acid; NH₃ = ammonium sulfate; ILE/2 refers to half of the normal volume of solution applied.

² EV = Effluent Volume, A refers to the acid-neutral column, and B refers to the basic column; a refers to replicate run a, and b to replicate run b.

³ Slope is the X coefficient of the calculated equation.

⁴ Sugar is the sugar of the reaction; ASC = ASCorbic acid; NAS = sodium ascorbate; GLUcose; 2GLU = 2 equivalents of GLUcose to one of amino acid. 4GLU, 8GLU, and 16GLU are 4, 8, and 16 equivalents of GLUcose per equivalent of GAM in this case; FRUctose; SUCrose, MANnose; FRU6 = FRUctose-6-phosphate; 0.0 means no sugar added, lysine only.

HEYNS *et al.* (13). (They used more specialized elution protocols and possibly larger analytical samples.) Some long term storages were made at room temperature (around 20°C) and at refrigerator temperatures (3–4°C) and apparently the same type of equations are obtainable (the unit of time was days). Insufficient data were obtained, especially in the early stages, to be sure. A few cases, where the data were more complete, were calculated. Residual amino acid concentrations did decrease with time and intermediates did form and disappear and the paper disks did brown after sufficient time in a similar manner to that of the heated samples. Only selected data, not including the “A”, “B”, and “C” sections, are listed in Fig. 1 and the “A” and “D” sections, in Fig. 2. In the entire lot of equations calculated, the correlation coefficients were 0.90 or larger for 71% of the residual equations (including the equations not broken down into sections) and for 90% of the intermediates (sections only).

We found that plotting micromoles of amino acid or of intermediate equivalent vs. log₁₀ time gave straight line sections. To our knowledge, this has not been reported directly in the literature. Recently, LINFIED *et al.* (14) obtained similar results in enzyme catalyzed reactions, however, their straight lines are without breaks. At first glance, WESTPHAL and CIESLIK (15) have related brown color to time to give a similar curve but closer examination shows that they plotted log absorbance at 430 μm against time which gave a curve resembling the one we obtained using residual amino acid vs. log time. When the data of Fig. 1 were plotted with log₁₀ residual concentration of the amino acid vs. time the plot showed considerable curvature in the early stages although it did approach a straight line without a break after about 1 h of heating. A plot of ln(C₀-C_t), logarithm of concentration of amino acid reacted, vs. time gave an almost vertical line for the first 2 min curving into an almost horizontal line after

about 2 h. HEYNS *et al.* (13) have plotted concentration of the intermediate against doubling reaction time periods. Most of our time periods between individual points are very nearly double the preceding time period and when the points were plotted in the HEYNS' fashion, they gave similar curves, with much lower deviations from straight lines. The log

time plot seems to give slightly smaller deviations than the doubling of times with the same data. Probably the greater differences in the two sets of results are due to the fact that our samples were on air dry paper disks instead of in solution and reached the temperature of the heated fat instantaneously. The same was true on removal since the sample disks

Tab. 2 Slopes of equations for intermediates, log₁₀ time (second) (X) vs. μmoles intermediate (Y), fried at 103°C, ratio amino acid to sugar is 1:1, except where noted. (Lines 2B and 2C in Fig. 2.)

With glucose										
AA ¹	GLY	SER	SER	MSG	MSG	GLN	ARG	THR	PHE	ILE
EV ²	100A	83A	87A	75A	80A	77A	255B	83A	300A	149A
TP ³	2B	2B	3B	2B	3B	2B	2B	2B	2B	2B
Slope ⁴	1.38	1.59	0.59	2.39	0.12	1.67	1.94	2.22	1.72	1.07
TP	2C	2C	3C	2C	3C	2C	2C	2C	2C	2C
Slope	-1.73	-1.56	-0.72	-2.46	-0.19	-2.60	-1.32	-2.07	-2.18	-1.67
AA	GLU	TRP	TRP	LYS	LYS	LYS	BAL	VAL	VAL	PRO
EV	75A	80B	130B	61B	94B	129B	410A	93A	100A	87A
TP	2B	2B	3B	2B	3B	4B	2B	2B	3B	2B
Slope	0.67	0.07	0.72	1.23	1.08	1.11	1.20	0.90	1.45	2.03
TP	2C	2C	3C	2C	3C	4C	2C	2C	3C	2C
Slope	-1.64	-0.05	-0.47	-0.80	-0.79	-0.63	-1.71	-0.68	-1.16	-1.89
AA	ALA	ASN	ASN	TYR	HIS	GAM	GAM	GAM	LEU	ASP
EV	95A	62A	70A	285A	147B	47B	90B	104B	170A	64A
TP	2B	2B	3B	2B	2B	2B	3B	4B	2B	2B
Slope	1.92	0.04	1.86	1.35	1.09	0.09	0.22	0.22	1.26	1.05
TP	2C	2C	3C	2C	2C	2C	3C	4C	2C	2C
Slope	-0.81	-0.07	-1.60	-0.64	-1.26	-0.19	-2.03	-0.19	-1.07	-1.06
AA	MET	NH ₃	NH ₃		MSG	MSG	MSG	GLU		
EV	136A	75B	130B		a73	b75	b80	75		
TP	2B	2B	3B		2B	2B	3B	2B		
Slope	1.95	0.47	2.82		2.21	2.39	0.12	0.67		
TP	2C	2C	3C		2C	2C	3C	2C		
Slope	-1.59	-0.35	-0.62		-2.42	-2.46	-0.19	-1.64		
ASparagiNe										
Sugar ⁵	ASC	ASC	NAS	GLU	GLU	GLU	2GLU	2GLU	ILE + GLU	ILE/2 + GLU
EV	42A	50A	42A	a73	b75	b80	74	81	149	151
TP	2A	3A	2A	2B	2B	3B	2B	3B	2B	2B
Slope	0.05	0.02	tr	2.21	2.39	0.12	2.84	0.16	1.06	1.23
TP				2C	2C	3C	2C	3C	2C	2C
Slope				-2.42	-2.46	-0.18	-2.67	-0.12	-1.67	-1.65
GAM										
Sugar	GLU	GLU	GLU	4GLU	4GLU	8GLU	8GLU	16GLU	16GLU	
EV	47	90	104	47	91	48	91	47	89	
TP	2B	3B	4B	2B	3B	2B	3B	2B	3B	
Slope	0.09	0.22	0.22	0.20	2.14	0.29	2.44	0.35	2.17	
TP	2C	3C	2C	2C	3C	2C	3C	2C	3C	
Slope	-0.19	-2.03	-0.19	-0.27	-4.45	-0.33	-4.98	-0.54	4.73	
ASparagiNe										
Sugar	GLU	GLU	FRU	FRU	ASC	ASC	NAS	GLU	FRU	SUC
EV	62	70	61	70	42	50	50	170	169	169
TP	2B	3B	2B	3B	2A	3A	2A	2B	2A	2A
Slope	0.04	1.86	0.22	0.82	0.05	0.02	tr	1.26	tr	tr
TP	2C	3C	2C	3C				2C		
Slope	-0.07	-1.60	-0.57	-0.90				-1.07		
LEUcine										

Tab. 2 Slopes of equations for intermediates, log₁₀ time (second) (X) vs. μmoles intermediate (Y), fried at 103°C, ratio amino acid to sugar is 1:1, except where noted. (Lines 2B and 2C in Fig. 2.) (continued)

GAM									
Sugar	GLU	GLU	GLU	FRU	FRU	MAN	MAN	MAN	SUC
EV	47B	90B	104B	67	86,90 ⁶	47	86	97	91
TP	2B	3B	4B	2B	3B	2B	3B	4B	2A
Slope	0.09	0.22	0.22	0.33	0.88	0.20	1.60	0.19	0.01
TP	2C	3C	4C	2C	3C	2C	3C	4C	
Slope	-0.19	-2.03	-0.19	-0.32	-0.89	-0.18	-2.43	-0.24	

GAM (continued)					HIS			
Sugar	FRU6	FRU6	FRU6	FRU6	GLU	FRU	FRU	SUC
EV	25, 25, 29B	50A	65A	70A	147	107	142	140
TP	2B	2A	3B	4B	2B	2B	3B	2B
Slope	0.55	0.006	0.35	0.09	1.09	0.18	0.43	0.63
TP	2C		3C	4C	2C	2C	3C	2C
Slope	-.51		-0.39	-0.05	-1.26	-0.08	-0.24	-0.06

LYSine								
Sugar	GLU	GLU	GLU	FRU	FRU	SUC	SUC	0.0
EV	61	94	129	94	129	99	130	nothing
TP	2B	3B	4B	2B	3B	2B	3B	
Slope	1.23	1.08	1.11	0.48	0.27	0.06	0.08	
TP	2C	3C	4C	2C	3C			
Slope	-0.80	-0.79	-0.63	-0.73	-0.17			

MSG + VAL + 2GLU			
AA	GLU	VAL	VAL
EV	74	88	98
TP	2B	2B	3B
Slope	2.23	1.64	1.21
TP	2C	2C	3C
Slope	-2.69	-1.82	-1.29

¹ See footnote 1, Tab. 1.

² See footnote 2, Tab. 1.

³ Under Type of Eqns: [2], [3], and [4] refer to the number of the intermediate peak as it came off the column, 2 is first, etc. A corresponds to the initial or lag phase, B the increasing main reaction, + slope, C the decreasing main reaction, - slope, and D the final phase when the reactant concentrations are quite low.

⁴ See footnote 3, Tab. 1.

⁵ See footnote 4, Tab. 1.

⁶ Unresolved peaks, calculated as one.

were rinsed immediately with CCl₄ to remove the fat. The more important difference however, may be that the HEYNS' samples were concentrated under reduced pressure to obtain analytical samples while ours did not require additional preparation. (They were placed directly on the ion-exchange resin column and extraction carried out by the buffer rinses and eluting buffers right on the column and separation from other reactants began immediately.)

In most cases, the data from our work, when plotted as concentration against log₁₀ time gave straight line sections with sharp breaks, which are readily apparent. Errors in the determinations or calculations are readily detected if the least square lines are plotted with the points. The picture is surprisingly consistent, especially considering that all the plotting was done and the equations calculated after the experimental work was completed and the lab equipment was no longer available.

The equations labeled "A" ("1A" and "2A" in Fig. 2) evidently correspond to a lag phase, which is nonexistent in some cases, especially with *gamma*-amino butyric acid (GAM), but was up to 300-400 sec in other cases and was 66 min in one case (ASN & ASC). The "1B" sections of the residual amino acid equations correspond to a period of fairly rapid intermediate formation and subsequent disappearance of starting material. The "2B" and "2C" sections of the intermediate equations correspond roughly to this same time period with "2B" covering a fairly rapid rise in intermediate concentration followed by "2C", showing an about equally rapid fall in concentration in many cases but not in all. The "1C" sections of the residual amino acid equations correspond to a slower rate of disappearance of amino acid and apparently overlap in part the "2C" section of the intermediate equation. The concentrations of the sugar and amino acid involved are becoming very low at this

point. The "2D" section of the intermediate equation corresponds to a much slower rate of disappearance of the intermediate. It was obvious in only a few cases, it might be more obvious if the heating periods were extended. The "3A, 3B, 3C, and 3D" sections were calculated from different effluent peaks and correspond to different intermediates. GAM (gamma-aminobutyric acid) and LYS with sugar showed a third intermediate peak in a few cases. These equations were calculated as a "4" series.

EICHNER and coworkers (16–18) have suggested the use of the intermediates for monitoring damage due to certain drying regimes. They reported a lag in the formation of brown color and off-flavors but not in the formation of the intermediates. In the plots in our earlier paper (12), the lag did not show up either. This lag is shown in the double time plots of HEYNS *et al.* (13) but is not as definite as in our work. We also found that the paper disks were colorless initially and that the brown color appeared only after heating or after longer storage at room or refrigerator temperatures. The brown color began to appear on the discs at about the 2B stage of the reaction. As pointed out previously (12), the paper of the disks was not involved appreciably in the reactions. (Lysine heated at 103°C on paper without sugar addition showed only a slight loss (about 4–5% in 8 h).

Many authors have used the formation of brown color to follow the MAILLARD reaction. A number of these indicate color formation from the beginning and some have plots showing brown color present at zero time. They have not detected the initial phases discussed in this paper. For example: "It is remarkable, that there is no induction period in the formation of Amadori compounds in contrast to browning shown in Fig. 5" (4). LABUZA and SALTMARCH (19) show in their Fig. 1, brown pigment formation before their zero time. As pointed out above, our model system gives much less chance for continuing reactions to occur during isolation.

Most of our measurements were carried out at 103°C since this gave an optimum rate for measuring and in the frying of potato chips and especially french fries, the temperature of the piece is very close to that of boiling water, held down by evaporation of the water in the piece. Only at the surface and during the last portion of the chip-frying is the piece approaching the temperature of the oil.

Based on the slope of the overall equations for the residual amino acids, **Tab. 1**, glycine gave the fastest reaction, followed closely by serine, then monosodium glutamate, glutamine, arginine, threonine, phenylalanine, isoleucine, glutamic acid, tryptophan, lysine, *beta*-alanine, valine, proline, alanine, asparagine, tyrosine, histidine, *gamma*-aminobutyric acid (GAM), leucine, aspartic acid, methionine, and ammonium sulfate, all with glucose. This order is somewhat indefinite since a few duplicate runs made, showed minor changes in relative order. These relationships are indicated in **Tab. 1**, which lists some of the slopes of the all points equations.

Tab. 2 shows the effluent volumes of the intermediates and corresponding slopes from equations for sections from the intermediates. These show that in many cases the rates of formation and loss of the intermediates do not parallel the loss of the residual amino acids. The complete listing of all the equations calculated is not given here. Changes in the sugar and other conditions also changed the order, as will be shown below.

The pH of the dry mixtures on the paper disks probably has little meaning. However, judging by the slope of the equation for the disappearance of amino acid, glutamic acid with glucose may be compared with monosodium glutamate and glucose. Monosodium glutamate disappeared considerably faster, especially in the early stages, indication that a higher

pH increased the disappearance rate in this case. But the opposite was true with asparagine and ascorbic acid. Where the ascorbic acid was exactly neutralized with sodium hydroxide, the rate of disappearance was slower than with the unneutralized acid. (Ascorbic acid might not be thought of as a reducing sugar but some tautomeric forms probably exist with a potential carbonyl group. At any rate ascorbic acid does react with amino acids.)

When the concentration of glucose was doubled, the disappearance of monosodium glutamate increased to about 0.1 faster. Decreasing the isoleucine concentration to half of glucose decreased the rate to about half. With *gamma*-aminobutyric acid (GAM) and glucose, increasing glucose four times slightly more than doubled the loss of GAM. Increasing glucose to 16 times had little more effect.

Fructose caused a more rapid disappearance of asparagine than did glucose; with ascorbic acid and sodium ascorbate, it was slower. With leucine, the overall rates for glucose, fructose, and sucrose were about the same, but the rates increased considerably in the later stages with fructose and sucrose. With GAM, the overall rates for fructose and mannose were faster than for glucose with sucrose the slowest. The maximum fructose intermediate concentration with GAM was not as high as with glucose and the peaks appeared at slightly different places. Sucrose rate increased in the later stages; possibly due to hydrolysis or breakdown. Fructose-6-phosphate was similar to glucose. Sucrose, fructose, and glucose gave about the same rates with histidine, but fructose was higher. With lysine, glucose and fructose showed a similar rate but sucrose was much slower.

The combination of two or more amino acids with one sugar appeared to cause some interaction, as might be expected. Valine and monosodium glutamate (MSG) were applied to the same disks with glucose in a 1:1:2 ratio. MSG reacted faster at first and when most of it was gone, the rate of disappearance of valine increased. More complicated mixtures would be expected to be more difficult to follow. Probably ASparagiNe, GLutamiNe, and GAM are the most important reactors of the amino acids in potatoes. GAM, with no lag period, ranked third highest in concentration in a study of the free amino acids in potatoes (3). Asparagine and GLutamiNe were highest in concentration but have a longer lag period than most of the amino acids. Both the proline-sugar intermediates and residual proline gave higher absorption at the 440 μm wavelength than at the normal 570. High sugar concentration also may give early peaks with high 440 μm absorption.

A few attempts were made to isolate the sugar-intermediate using a scaled-up column (20) with pyridine-acetate volatile buffers (21). The sugar-amino acid intermediate was separated from the isoleucine very nicely but may have been contaminated with sugar. Removal of the buffer with a rotary evaporator left some acetic acid behind unless carefully done, and the intermediate tended to decompose. Freeze-drying was much more effective. But we did not obtain a crystalline product. ERBERSDOBLER *et al.* (22) have reported the isolation of lysine, methionine, and glycine products with glucose by ion exchange chromatography with pyridine-acetic acid buffers and subsequent freeze-drying of the eluate fractions but the report does not indicate whether the products were obtained crystalline. ROEPER *et al.* (23) and ALTENA *et al.* (24) report a number of crystalline Amadori-rearrangement products in which they used ion-exchange chromatography as one step in the purification. Possibly high performance liquid chromatography (HPLC) (25, 26) might be more effective, but even with HPLC, repeated chromatography apparently is necessary for complete purification.

Conclusions

The rate of intermediate formation was found to vary with the amino acid and sugar involved. Plots of concentration vs. log time gave straight line correlations. Most of these showed a lag or slower reaction at the beginning. Browning was not visible in the initial stages. The general behavior of the reaction was the same at 5° as at 103°C though considerably slower at the lower temperature. The rate varied with amino acid and sugar involved and was affected by the relative concentrations. Most of the tests were with a single amino acid with a single sugar. Interaction appeared to occur when more than a single pair were involved. Probably the most important pairs involved in potatoes are asparagine, glutamine, and *gamma*-aminobutyric acid with glucose.

Acknowledgments

We wish to acknowledge the assistance and encouragement of *William L. Porter* in this work and to thank *John F. Sullivan* and *Mike Kozempel* for their discussion and encouragement.

References

- 1 MAILLARD, L. C., *Compt. Rend.*, *154*, 66 (1912)
- 2 ERICKSSON, C., editor, *Progress in Food and Nutrition Science*, Pergamon Press, Oxford, UK, 1981 5, No. 1-6, 501 pp.
- 3 TALLEY, E. A., FITZPATRICK, T. J. and PORTER, W. L., *Amer. Potato J.*, *47*, 231 (1970)
- 4 EICHNER, K. and CINER-DORUK, M., *Prog. Food Nutr. Sci.*, *5*, 115 (1981)
- 5 EPPENDORFER, W. H., EGGUM, B. O. and BILLE, S. W., *J. Sci. Food Agr.*, *30*, 361 (1979)
- 6 MARKAKIS, P., *Nutr. Clin. Nutr.*, *1*, Pt. 2, 471 (1975)
- 7 STEGINK, L. D., FREEMAN, J. B., DEN BESTEN, J. and FILER, L. J., JR., *Prog. Food Nutr. Sci.*, *5*, 265 (1981)
- 8 MAURON, J., *Prog. Food Nutr. Sci.*, *5*, 5 (1981)
- 9 FEENEY, R. E. and WHITAKER, J. R., *ACS Symp. Ser.*, *206*, 201 (1982)
- 10 HODGE, J. E., *J. Agr. Food Chem.*, *1*, 928 (1953)
- 11 INGLES, D. L. and REYNOLDS, T. M., *Aust. J. Chem.*, *11*, 575 (1958)
- 12 TALLEY, E. A. and PORTER, W. L., *J. Agr. Food Chem.*, *16*, 262 (1968)
- 13 HEYNS, K., MÜLLER, G. and PAULSEN, H., *Justis Liebigs Ann. Chem.*, *703*, 202 (1967)
- 14 LINFIELD, W. M., O'BRIEN, D. J., SEROTA, S. and BARAUSKAS, R. A., *J. Amer. Oil Chem. Soc.*, *61*, 1067 (1984)
- 15 WESTPHAL, G. and CIESLIK, E., *Nahrung*, *25*, 749 (1981)
- 16 CINER-DORUK, M. and EICHNER, K., *Z. Lebensm.-Unters. Forsch.*, *168*, 9 (1979)
- 17 EICHNER, K. and WOLF, W., *Chem.-Ing.-Tech.*, *54*, 270 (1982)
- 18 EICHNER, K. and WOLF, W., *ACS Symp. Ser.*, *215*, 317 (1983)
- 19 LABUZA, T. P. and SALTSMARCH, M., *J. Food Sci.*, *47*, 92 (1981)
- 20 TALLEY, E. A. and PORTER, W. L., *J. Chromatog.*, *3*, 434 (1960)
- 21 SCHROEDER, W. A., JONES, R. T., CORMICK, J. and McCALLA, K., *Anal. Chem.*, *34*, 1570 (1962)
- 22 ERBERSDOBLER, H. F., BRANDT, A., SCHARRER, E. and VON WANGENHEIM, B., *Prog. Food Nutr. Sci.*, *5*, 257 (1981)
- 23 ROEPER, H., ROEPER, S., HEYNS, K. and MEYER, B., *Carbohydr. Res.*, *116*, 183 (1983)
- 24 ALTENA, J. H., VAN DEN OUWELAND, G. A. M., TEUNIS, C. J. and TJAN, S. B., *Carbohydr. Res.*, *92*, 37 (1981)
- 25 TAKEOKA, G. R., COUGHLIN, J. R. and RUSSELL, G. F., *Liq. Chromatogr. Anal. Food Beverages (Proc. Symp. Anal. Foods Beverages)*, *1*, 179 (1979)
- 26 MOLL, N., GROSS, B., VINH, T. and MOLL, M., *J. Agric. Food Chem.*, *30*, 782 (1982)