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CONTENTS

PREDICTION OF ASCORBIC ACID RETENTION DURING DRYING II. SIMULATION OF RETENTION IN A MODEL SYSTEM

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ABSTRACT

The second part of this study to predict ascorbic acid retention during air-drying deals with the kinetics of degradation of ascorbic acid as a function of moisture content, temperature and time, and with the simulation of ascorbic acid retention. Ascorbic acid degradation was found to follow a first order reaction and to be highly moisture- and temperature-dependent. A reasonable prediction of ascorbic acid retention was obtained when local moisture rather than average moisture content was taken into consideration. This type of approach may be applicable to other nutrients or organoleptic qualities providing that the kinetics of the loss of these qualities can be established.

INTRODUCTION

The prediction of nutrient retention during processing has been an area of great interest in the past several years. This topic was treated by Labuza (1973) and Lund (1973).

Preliminary work in the prediction of ascorbic acid retention during storage was carried out by authors such as Karel and Nickerson (1964), Vojnovich and Pfeifer (1970), Lee and Labuza (1975) and Kirk *et al.* (1977). These authors showed dependency of ascorbic acid degradation as a function of water activity or moisture content, temperature and oxygen content in food systems. Wanninger (1972) postulated a mathematical model for the degradation of ascorbic acid. Excellent agreement was observed between the experimental data reported by Vojnovich and Pfeifer (1970) and the values predicted by the mathematical model developed by Wanninger.

Computer-aided prediction of ascorbic acid retention in systems affected by environmental changes of temperature, water activity and oxygen has been successfully achieved by several authors. Mizrahi and Karel (1977) predicted the loss of ascorbic acid in tomato juice powder

in water-vapor permeable containers by using accelerated tests which
involved subjecting the product to high rates of moisture uptake at
elevated temperatures. Riemer and Karel (1978) working with tomato
juice powder successfully predicted ascorbic acid retention during storage
by combining mathematical models describing ascorbic acid retention as
a function of time, temperature and water activity and mathematical
models describing the transport of water to the food.

Saguy *et al.* (1979) predicted ascorbic acid retention in grapefruit
juice using kinetic models in commercial thermal and concentration
processes.

In the preceding paper we reported on determination of moisture and
temperature profiles during drying of a model material containing ascor-
bic acid (AA). This paper deals with (1) determination of the kinetics of
ascorbic acid degradation as a function of moisture content and tempera-
ture (2) development of a mathematical model for ascorbic acid degrada-
tion as a function of moisture, temperature, and time (3) calculation of
ascorbic acid retention as a function of time and drying conditions by
combining the mathematical models for kinetics of degradation and
drying behavior and (4) comparisons of predicted values and values
obtained in drying experiments.

MATERIALS AND METHODS

Sample

For all experiments the model system used contained 300 mg of
ascorbic acid per 100 grams of sample. The remaining components of
the system were as follows:

Water	71.70%
Avicel	17.00%
C.M.C.	11.00%

The pH was adjusted with NaOH to 4.8 (in solution).

For the collection of kinetic information the material was thoroughly
blended and shaped in the form of small flakes (0.4 cm diameter and
0.2 cm thickness). The samples were shaped into this form and size in
order to obtain nearly instantaneous and uniform heating to the required
temperature during the collection of kinetic data. Relative concentrations
of Avicel, CMC, and water were selected after tests were performed to
develop a material with a minimum of shrinkage upon heat treatment.

For the drying experiments the cellulose model system was shaped in
the form of slabs (3.4 × 3.5 × 0.60 cm). This slab-shaped material was

previously tested to determine drying conditions suitable for the obtaining of drying kinetic information. The methodology followed for the determination of the drying behavior of the system was given by Villota and Karel (1980).

Sample Preparation

In order to adjust the samples to the desired moisture content, dehumidification of the samples was carried out in desiccators containing different saturated salt solutions. Dehumidification was carried out at 5°C in order to minimize the degradation of ascorbic acid.

Heat Treatment

The samples were placed in airtight containers with a capacity of 25 ml. Heat treatment was carried out in water baths at four different temperatures: 50, 60, 70, and 80°C. Duplicate samples were removed from the water baths at different time intervals. These time intervals were predetermined in preliminary experiments. Instantaneous cooling of the samples after heat treatment was obtained by freezing the samples in liquid nitrogen. No loss of ascorbic acid was observed upon freezing the samples.

Assay

Ascorbic acid was determined by high speed liquid chromatography on a strong anion exchange column (Zipax sax) according to the methodology described by Villota (1979). This method was found to be free of interferences by breakdown products.

Data Processing

Experimental data were fitted by using linear (BMD2R, Dixon, 1975) and nonlinear regression (BMDP3R). Computer programming was carried out on an IBM 370 system at the M.I.T. Information Processing Center.

Results and Discussion

A. Mass and Heat Transfer Analysis to Determine Moisture and Temperature Profiles as a Function of Drying. Mathematical models describing drying behavior of the model system were developed on the basis of data obtained in drying experiments in which average moisture content and temperatures were measured. The models which were based on applicability of Fick's law and on three-dimensional diffusion were used to calculate moisture and temperature distributions with time and distance.

The validity of these calculated distributions was confirmed by experimental determination of moisture content in subsections of the model which was being dehydrated. An attempt was made to correct effects of shrinkage during drying by using an average slab thickness.

Mass and heat transfer analysis during the drying process was carried out according to the methodology described by Villota and Karel (1980). A summary of the mathematical models required for the prediction of nutrient retention is given below.

For average moisture content:

$$\frac{\overline{w} - w_e}{w_o - w_e} = \left[\frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} e^{\left(-\frac{(2n+1)^2}{a^2} \cdot \frac{\pi^2}{4} \right) D^t} \right] \cdot$$

$$\left[\frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} e^{\left(-\frac{(2n+1)^2}{b^2} \cdot \frac{\pi^2}{4} \right) D^t} \right] \cdot$$

$$\left[\frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} e^{\left(-\frac{(2n+1)^2}{c^2} \cdot \frac{\pi^2}{4} \right) D^t} \right] \tag{1}$$

where $D^t = \int_o^t D(t)dt$ and $D(t) = D_o e^{-E/RT(t)}$ $\therefore$ $D^t = \int_o^t D_o e^{-E/RT\ (t)} dt$

In this equation
$$D_o = 82.500$$
$$E/R = 5649 \qquad \text{if } T_s < 60°C$$

$$D_o = 0.0150$$
$$E/R = 2781 \qquad \text{if } T_s \geq 60°C$$

and,

$$\ln T(t) = (0.000823\ T_{DB} - 0.2640)\ \ln (t \times 10^{-5})$$

$$+ (0.004738\ T_{DB} + 4.24142) \tag{2}$$

for $t \neq 0$

For local moisture content, the moisture distribution inside an infinite slab at any given time, t, was found to be given by:

$$\bar{w}(x) = w_e + \frac{4}{\pi}(w_o - w_e) \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} \cos\left[\frac{(2n+1)}{2a}\pi x\right] e^{\left(\frac{-\pi^2(2n+1)^2}{4a^2}\right)Dt} \tag{3}$$

where $Dt = \int_0^t D(t)dt$. Since tridimensional diffusion was considered an expression to describe the moisture distribution for the rectangular parallelepiped was obtained by considering three infinite slabs perpendicular to each other.

B. Determination of the Kinetics of AA Degradation as a Function of Moisture Content and Temperature. The approach taken to obtain kinetic information was the classical isothermal kinetics approach. The retention of the given nutrient was measured as a function of time at constant temperature, keeping the other independent variables constant. The reaction constant was determined from the equation

$$-\frac{dC}{dt} = KC^n \tag{4}$$

in which n, the order of the reaction, was found to be 1 for our system.

Since the degradation of ascorbic acid was studied as a function of temperature, the applicability of the Arrhenius equation was tested.

Arrhenius equation: $K = K_o e^{-Ea/RT}$ $\tag{5}$

where
- C = concentration of the nutrient at any time
- t = time
- n = order of the reaction
- K = reaction constant
- Ea = activation energy
- R = molar gas constant
- T = absolute temperature
- K_o = frequency of molecular collision

The kinetic information obtained for reduced and total ascorbic acid is presented in Fig. 1, 2, 3 and 4. The rates of degradation of total and reduced ascorbic acid increased with temperature in accordance with the Arrhenius equation.
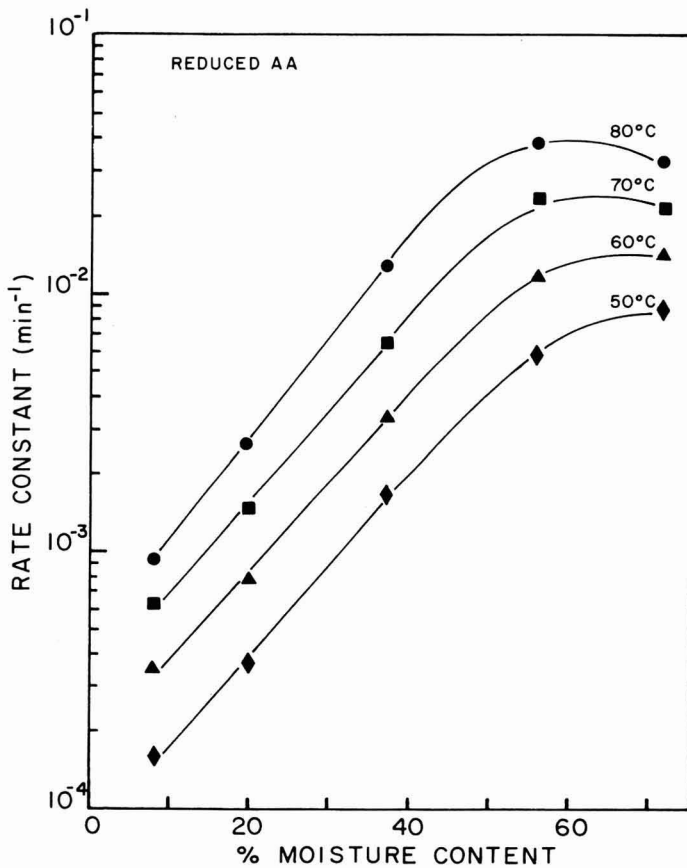
FIG. 1. REDUCED ASCORBIC ACID DEGRADATION AS
A FUNCTION OF MOISTURE CONTENT AND
TEMPERATURE

With respect to moisture content, a maximum rate of degradation of
AA occurs at around 60% moisture (Fig. 1). This maximum appears to
be more noticeable as temperature increases. A definite maximum in
the rate of degradation of total AA occurs at around 60% moisture
(Fig. 2). Restriction in the mobility of the reactant species below this
moisture level and dilution effects above it caused a slowing down of
the reaction.

Activation energies and correlation coefficients are given in Table 1.
Figures 3 and 4 show the effect of moisture content on the energy of
activation and the collision factor.

C. Mathematical Models for Ascorbic Acid Retention as a Function
of Moisture Content and Temperature. Although there is virtually a
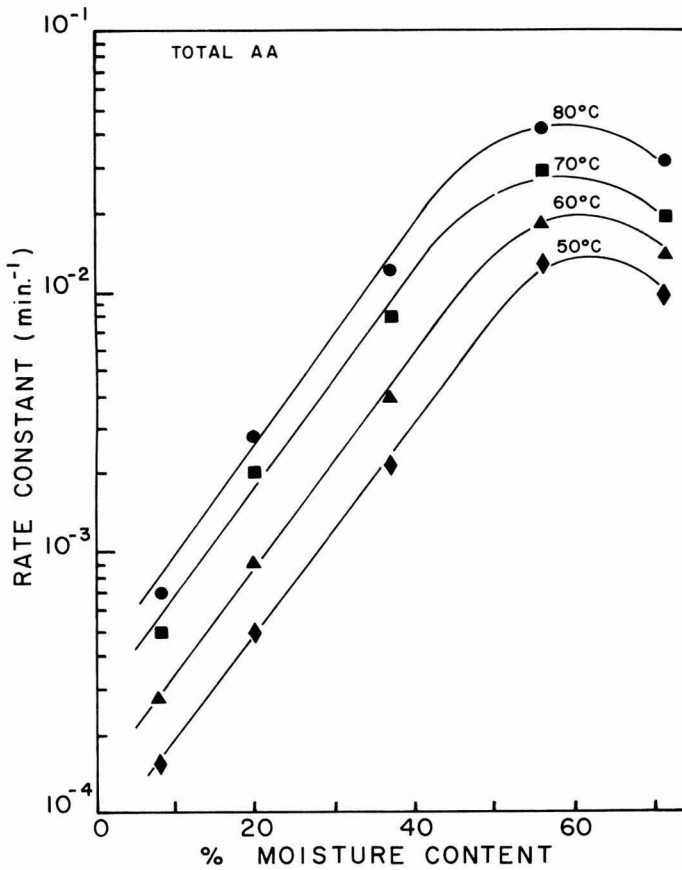
FIG. 2. TOTAL ASCORBIC ACID DEGRADATION AS A
FUNCTION OF MOISTURE CONTENT AND
TEMPERATURE

limitless choice of forms that can be used for fitting equations to experimental data, mathematical relationships between the rate of ascorbic acid degradation and parameters such as moisture content and temperature were developed using the following considerations.

1) A model as simple as possible.
2) Low error means square between the experimental values and those predicted by the model (S^2).
3) No discontinuities in the practical range of the independent variables.
4) The deviations of the predicted values from the experimental values should be uniform over the whole range of conditions under investigation.
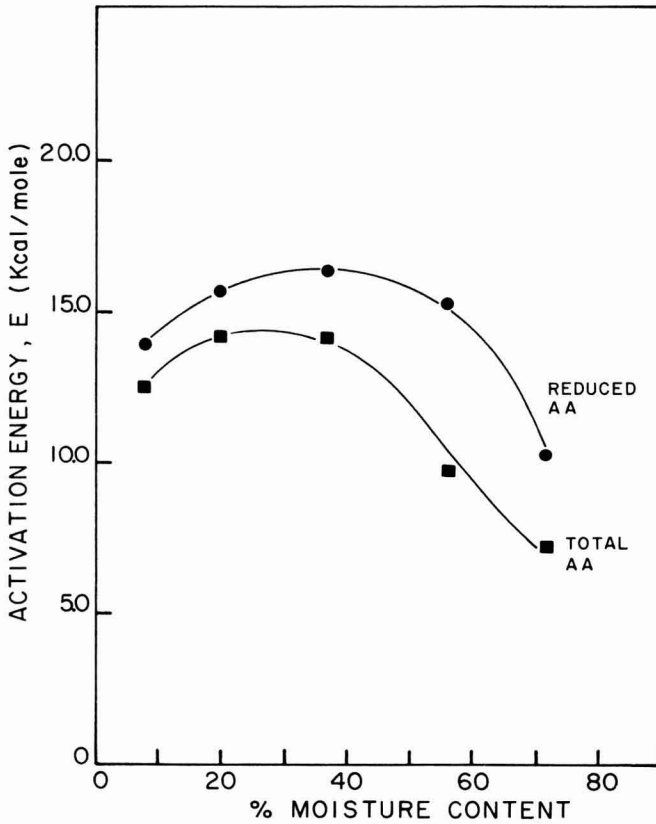
FIG. 3. ACTIVATION ENERGIES FOR REDUCED AND
TOTAL ASCORBIC ACID AS A FUNCTION OF MOIS-
TURE CONTENT

Models of One Independent Variable

The dependent variable (K) was first studied as a function of only one of the independent variables at a time. In this way the relationship existing between the dependent variable and each one of the independent variables was observed and decided what kind of mathematical expression could accurately describe this behavior. These relatively simple models were chosen based on theoretical considerations and on the shape of the different plots.

K vs m. A third degree polynomial equation with a general form,

$$\ln K = a_o + a_1 m + a_2 m^3 \tag{6}$$
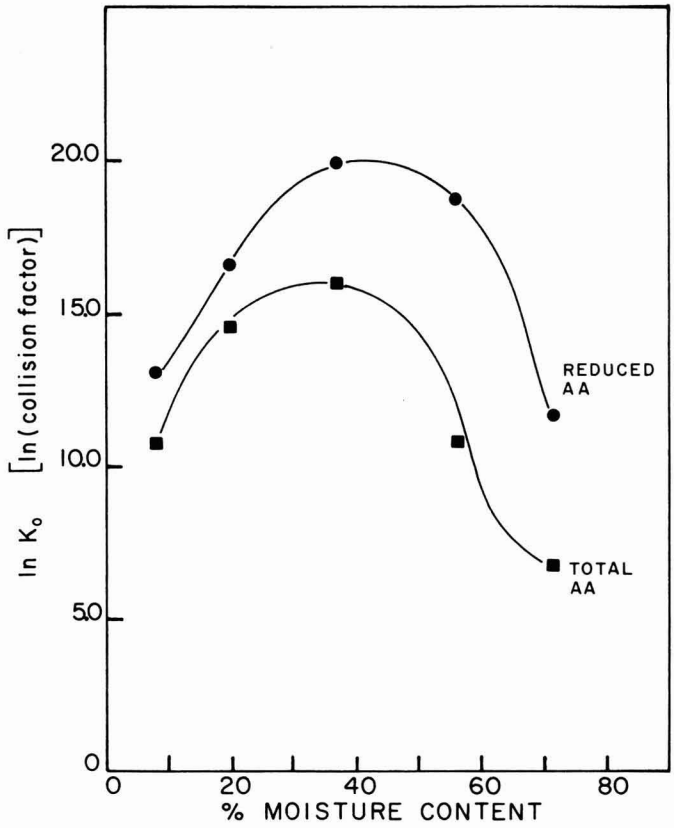
FIG. 4. COLLISION FACTOR (K_o) FOR REDUCED AND
TOTAL ASCORBIC ACID AS A FUNCTION OF MOISTURE
CONTENT

Table 1. Activation energies

	% Moisture Content (WB)	Activation Energy (kcal/mole)	Correlation Coefficient
Reduced AA	71.5	10.48	0.99
	56.0	15.26	0.99
	37.0	16.31	0.99
	19.8	15.68	0.98
	8.0	13.92	0.99
Total AA	71.5	6.75	0.90
	56.0	9.71	0.99
	37.0	14.17	0.99
	19.8	14.19	0.99
	8.0	12.48	0.99

described well the behavior of the system. In this equation the moisture content (m) is given in g H_2O/g sample. Correlations in which "m" was given as g H_2O/g solids were found to be less satisfactory. In Table 2 the actual values for the coefficients a_o, a_1 and a_2, as well as standard error of estimate and multiple correlation coefficient, are given for the equation obtained.

Table 2. Rate constant dependency on moisture content for different temperatures

					Correlation Coefficient	Std. Error
		a_o	a_1	a_2		
m: wet basis	80°C	−7.963	11.156	− 9.293	0.998	0.123
		−8.234	12.104	−10.502	0.998	0.139
	70°C	−8.299	10.000	− 7.036	0.996	0.198
		−8.567	12.048	−10.768	0.997	0.180
	60°C	−8.812	9.336	− 5.606	0.997	0.175
		−9.189	11.675	− 9.046	0.995	0.241
	50°C	−9.557	9.170	− 4.652	0.998	0.127
		−9.842	11.865	− 8.657	0.992	0.326

Header note: [Upper values are for AA / Lower values are for total AA]

K vs T. As previously stated, the Arrhenius equation can be applied to all the conditions studied (See Table 1).

Models of Two Independent Variables

The approach taken to develop these models was the one that considers that a functional relationship can be generated by fitting appropriate functions to the partial responses (K vs m and K vs T) and then multiplying together these functions to yield an approximate description of the overall response. For this purpose the BMD02R program was used. A series of transgeneration cards including all the terms of the product of models of one independent variable were used. Since we were unable to obtain an accurate description of the overall response, additional transgeneration cards representing simple forms of the independent variables were included.

The following relationships were established. For reduced ascorbic acid:

$$\ln K = a_1 m + a_2/T^3 + a_3 m^3 + a_4 m^2/T + a_5 m/T^2 + a_6 m^3/T^3 + a_7 \quad (7)$$

and for total ascorbic acid:

$$\ln K = a_1 m + a_2 m^2/T^3 + a_3/T^3 + a_4 m^3/T^2 + a_5 m/T^3 + a_6 \quad (8)$$

(m: g H_2O/g sample)

In these two equations the terms are arranged in order of importance to the fitting. The actual values of the coefficients and the degree of importance of each term are presented in Table 3.

Table 3. Models of two independent variables

	Reduced Ascorbic Acid			Total Ascorbic Acid		
	Coeff.	R	S	Coeff.	R	S
a_1	17.936	0.884	0.801	7.878	0.893	0.893
a_2	2.245×10^8	0.976	0.384	7.512×10^8	0.956	0.531
a_3	-33.33	0.996	0.159	2.503×10^8	0.979	0.377
a_4	5920.67	0.998	0.096	2.934×10^6	0.996	0.151
a_5	1.585×10^6	0.999	0.081	-9.878×10^7	0.997	0.153
a_6	4.711×10^8	1.000	0.044	-1.907		
a_7	-2.339					

As far as nonlinear models are concerned, it was concluded that no further improvement of the mathematical models could be achieved by using this approach. Since it is desired that the number of constants in an equation be as low as possible, the use of nonlinear models would be justified if a decrease in the number of constants could be obtained. If we observe Fig. 3, it can be concluded that the dependency of the activation energy on moisture content cannot be described by a simple function. The same reasoning applies to the collision factor (Fig. 4), therefore showing no advantage in the use of nonlinear models from this point of view. Nonlinear models, however, have the advantage that they might be more meaningful from the viewpoint of theoretical considerations of kinetics. Nonlinear models in our case would be based on the applicability of the Arrhenius equation which we have previously determined.

$$\ln K = \ln K_o - \frac{Ea}{RT} \qquad (9)$$

where K_o and Ea are a function of moisture content.

Another disadvantage present in mathematical models based on the Arrhenius equation is a decrease in sensitivity. The mathematical models established using linear models are expected to be more accurate since they were obtained based on a larger number of observations. The linear models would be more subject to cumulative sources of error.

D. Mathematical Model for Kinetics of Degradation and Dehydration Behavior (Moisture and Temperature Profiles) Combined to Calculate Retention as a Function of Time and Drying Conditions. A computer program was written in order to carry out the prediction of reduced and total ascorbic acid during drying. The mathematical models to describe temperature of the sample, average moisture, and local moisture as a function of time, as well as the kinetic information as a function of moisture content and temperature, and the equation for first order reaction kinetics were combined to predict retention (Fig. 5).
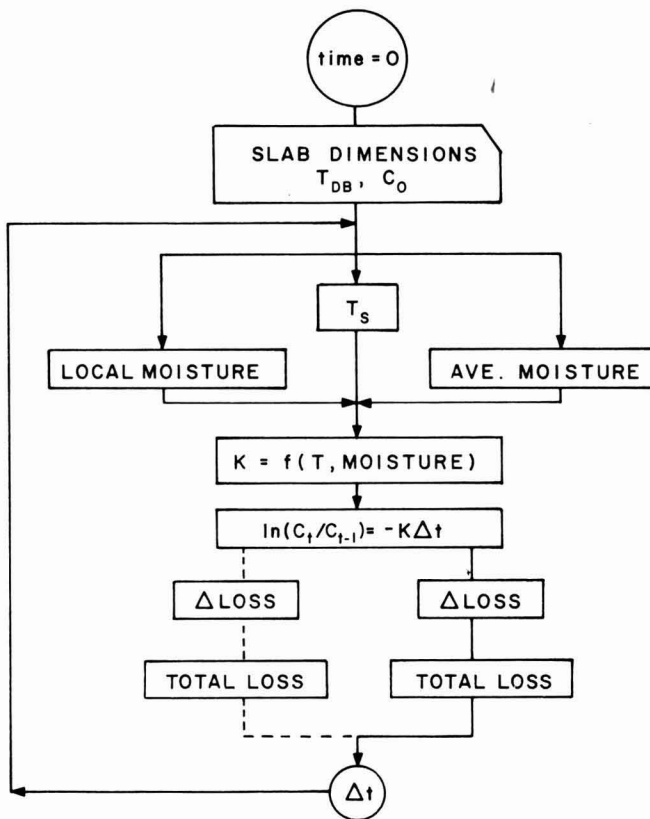
FIG. 5. GENERAL FLOW DIAGRAM FOR THE PREDIC-
TION OF ASCORBIC ACID RETENTION

Time intervals of five minutes were considered for our calculations. Every five minutes along the drying cycle a new rate constant for the degradation of total and reduced AA was calculated according to the

time-temperature-moisture profile of the sample. In the case of local moisture content, the slab was divided into eight identical sections. Each one of these sections was then divided into 125 subsections for which total and reduced AA were determined. These fractions were then combined to estimate the total loss at a given time.

Our results show that the higher the temperature of drying (T_{DB}), the lower the retention of AA (total and reduced) by the end of the drying cycle, although differences were more appreciable for reduced AA (Fig. 6, 7). It was also observed that as drying proceeds the differences in retention as a function of time become more noticeable. Retention of reduced ascorbic acid and total ascorbic acid as predicted by our mathematical models was found to be higher when local content was used for our calculations. The differences between predicted values of retention by using local and average moisture increase with temperature and become more marked as drying proceeds.
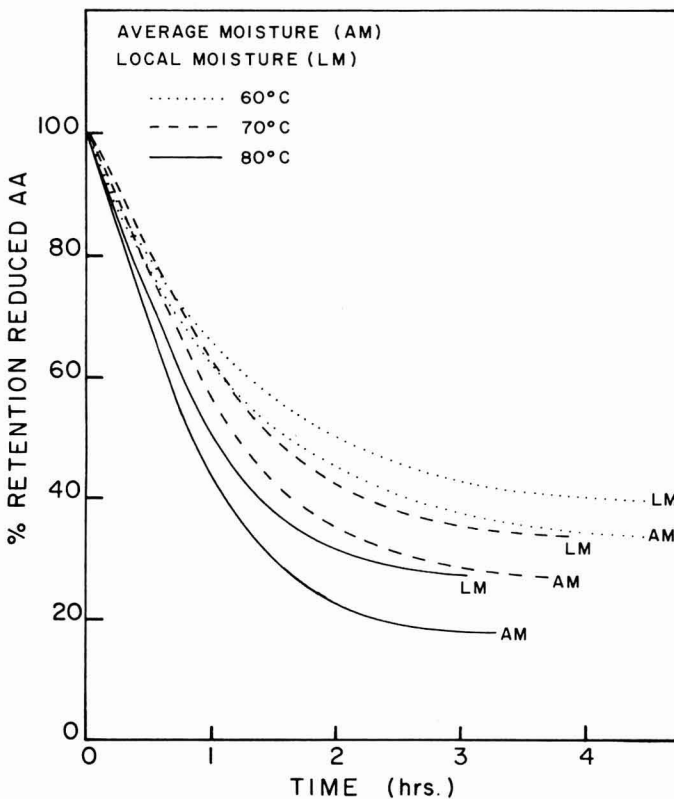
FIG. 6. REDUCED ASCORBIC ACID DEGRADATION AS
A FUNCTION OF DRYING TIME CONSIDERING LOCAL
AND AVERAGE MOISTURE CONTENT FOR THREE
DIFFERENT DRY-BULB TEMPERATURES

Information obtained by using mathematical methods.
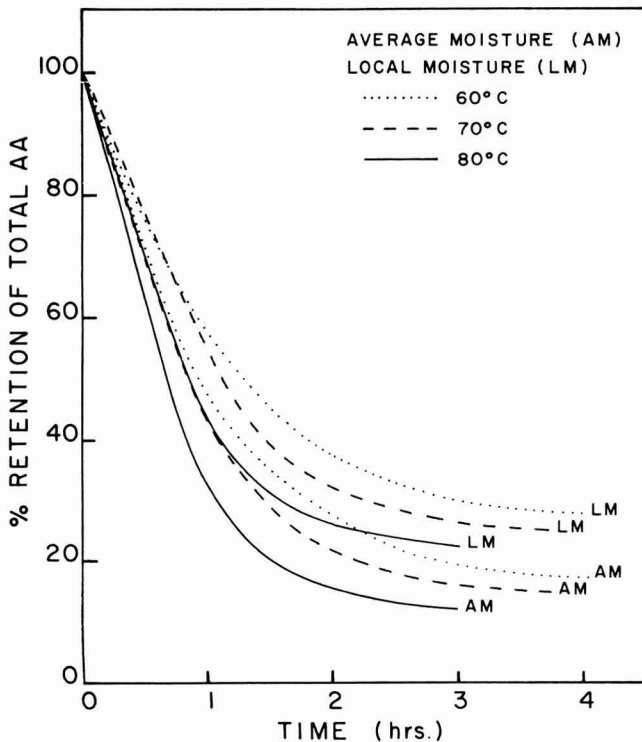
FIG. 7. TOTAL ASCORBIC ACID DEGRADATION AS A
FUNCTION OF DRYING TIME CONSIDERING LOCAL
AND AVERAGE MOISTURE CONTENT FOR THREE
DIFFERENT DRY-BULB TEMPERATURES

Information obtained by using mathematical methods.

It was observed that the effect of moisture content was predominant
over the effect of temperature at the beginning of the drying cycle. For
instance, retention at 60°C was lower than retention at 70°C approx-
imately during the first 30 min of drying. Beyond this drying time, the
effect of temperature is the predominant one.

E. Predictions of Ascorbic Acid Retention Tested in Drying Experi-
ments. Our results show that basically the trend of degradation of total
and reduced ascorbic acid with regard to moisture content and tempera-
ture can be predicted by our mathematical models. Our results from the
drying experiments suggest clearly that the behavior of the system in
regard to ascorbic acid degradation can be better described by our math-
ematical models when local moisture rather than average moisture is
considered. A better fit was observed for total ascorbic acid (Fig. 8, 9).
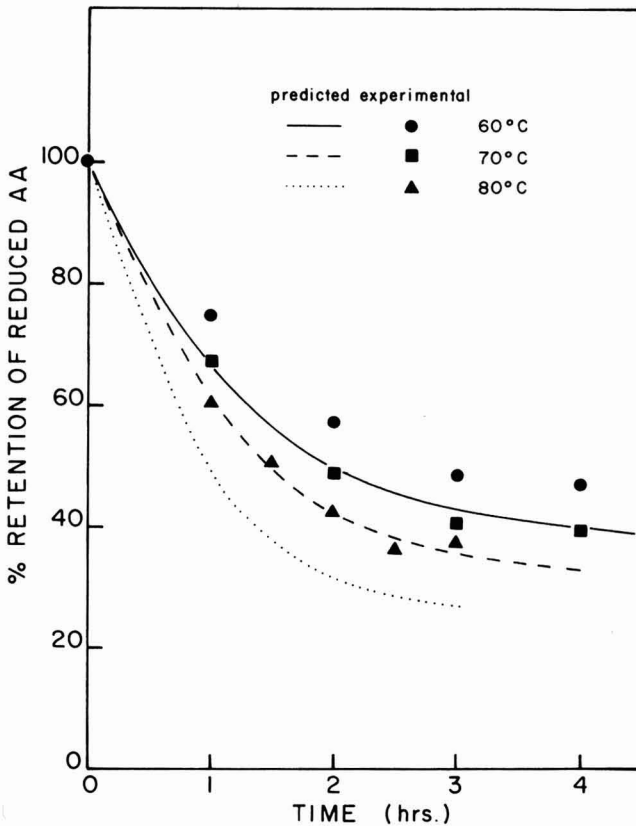
FIG. 8. PREDICTED VERSUS EXPERIMENTAL VALUES
FOR REDUCED ASCORBIC ACID DEGRADATION AS
A FUNCTION OF DRYING TIME AND DRY-BULB
TEMPERATURE

Several factors may contribute to the discrepancy between experimental data and predicted values.

1) Mathematical models for temperature, moisture, and kinetics involved some error since no perfect, but acceptable, fit was obtained. Cumulative errors would certainly lead to differences between experimental and predicted values.

2) Stresses on the structure of the slab as water is removed during drying caused shrinkage and cracking. Although cracking was practically eliminated by adjusting the drying conditions and the geometry of the sample, some samples, especially the ones exposed to higher T_{DB}, still showed a tendency to some minor cracking on the edges and/or the presence of pinholes toward the center. The presence of these defects
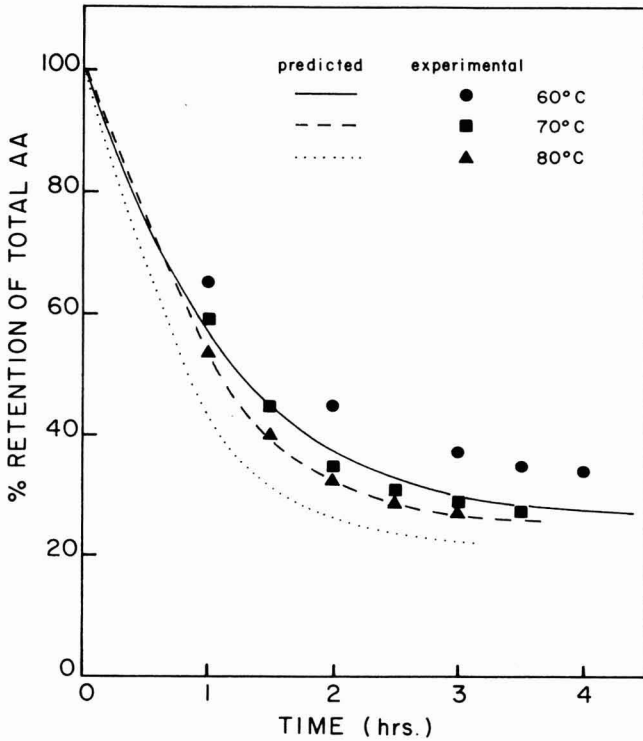
FIG. 9. PREDICTED VERSUS EXPERIMENTAL VALUES
FOR TOTAL ASCORBIC ACID DEGRADATION AS A
FUNCTION OF DRYING TIME AND DRY-BULB
TEMPERATURE

would create a model subject to some error since this type of sample
would not show ideal behavior, and as found experimentally, these
samples showed higher retention. It is clear that our model system is
very moisture dependent, and the presence of minor cracks and pinholes
would favor higher drying rates having as a consequence a faster change
from high moisture content (high rates of AA degradation) to lower
moisture content (low rates of AA degradation) than normally predicted
by our mathematical models. It should be mentioned that most major
changes in the structure of the slab occurred during the first hour of
drying which is precisely the most sensitive part as far as AA degradation
is concerned. In fact, lack of fit was higher for higher dry-bulb
temperatures.

3) A factor that perhaps is of primary importance is shrinkage. For the
development of mathematical models to describe the m-T-t profiles, an

average of the initial and final dimensions was used. This was considered to be the best approach since most of the shrinkage occurs during the first hour of the drying cycle. Furthermore, when the initial or the final dimensions of the slab were used for the development of mathematical models, the disagreement between experimental and predicted values was more noticeable. Shrinkage will, therefore, affect our computations to predict ascorbic acid retention.

4) Another factor of importance as a possible source of error is temperature of the sample. Some error in measuring temperature of the sample is expected due to damage to the uniform structure of the slab caused by puncturing this with thermocouples. As a result of puncturing the cellulose material, recorded temperature of the sample is expected to be higher than the actual temperature of the sample if no damage had been inflicted to the material. Therefore, predicted ascorbic acid losses are expected to be higher than actual losses. Temperature of the sample may also be affected by heat conduction through the thermocouple which will result in higher recorded sample temperature than actual T_s. A variation of $1°C$ can originate discrepancies of ~1.5% in ascorbic acid retention.

SUMMARY AND CONCLUSIONS

A cellulose model system, in which ascorbic acid was incorporated, was used to develop a methodology for the prediction of nutrient retention during air drying of food materials.

Total and reduced ascorbic acid were measured using a modified method of assay utilizing HPLC which was found to be free of interferences by breakdown products, in contrast to the indophenol method which was found unsuitable.

The kinetics of ascorbic acid degradation were conducted by the classical isothermal method. Since moisture content was one of the variables, tests at each temperature were conducted at several constant moisture contents. A constant pH value of 4.8 (in solution) was used throughout the study. The kinetics of ascorbic acid degradation were found to be independent of initial concentration.

Degradation of total and reduced ascorbic acid was found to be a first-order reaction. Rates of degradation of total and reduced ascorbic acid increased with temperature in accordance with the Arrhenius equation. A maximum rate of degradation was observed to occur at approximately 60% moisture. The activation energy was found to be dependent on moisture content.

Mathematical models describing kinetics of ascorbic acid degradation as a function of moisture content and temperature and mathematical models describing drying behavior were combined to predict retention of ascorbic acid during drying. It was observed that a better agreement between predicted and experimental values is obtained when losses are calculated using local rather than average moisture content. Higher losses of ascorbic acid are calculated by using average moisture content. Our results show that the higher the temperature of drying (T_{DB}) the lower the retention of ascorbic acid by the end of the drying cycle. It was observed that the effect of moisture content seems to be the predominant effect at the beginning of the process of drying. As drying proceeds, the effect of temperature is the predominant one.

In general, a reasonable agreement between theoretical and experimental values was observed for both reduced and total ascorbic acid. Shrinkage, pinholes and mild cracking combined with lack of accuracy in determining temperature of the sample as a function of time are considered to be the most important sources of error. Lack of precise fit in mathematical models developed may also account for some of the discrepancies observed.

This study shows that predictive models can be developed which can form the basis for control of drying conditions in order to maximize nutrient retention. The same approach can also be used for prediction of retention of organoleptic qualities, provided the kinetics of the loss of these qualities can be established. The development of such predictive models for food products and optimization of processing conditions are potentially of great economic value.

NOMENCLATURE

a, b, c	half thickness of a slab, cm
$a_o, a_1, a_2 \ldots$	constants
D	diffusion coefficient, cm^2/s
D_o	collision factor
E	activation energy (moisture diffusion) cal/mole
Ea	activation energy (ascorbic acid degradation), cal/mole
K	reaction constant, min^{-1}
K_o	frequency of molecular collision
m	moisture content, g H_2O/g sample
n	order of the reaction
R	molar gas constant, 1.987 cal/$^{\circ}$K-mole
T	absolute temperature, $^{\circ}$K

T_s temperature of the sample, °K
T_{WB} wet-bulb temperature, °K
T_{DB} dry-bulb temperature, °K
t   time, s
w_o initial moisture content, g H_2O/g solids
$\underline{w}_e$ final equilibrium moisture content, idem
$\underline{w}$ average moisture content at a given time, idem
w(x)   local moisture content at a given time, idem
x   distance away from the piece surface, into the piece

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ANTIOXIDATIVE MAILLARD REACTION PRODUCTS.
I. PRODUCTS FROM SUGARS AND FREE AMINO ACIDS

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ABSTRACT

Maillard reaction products (MRP) obtained by reaction of glucose, fructose or xylose with arginine, cysteine, glutamic acid, histidine, lysine or valine were studied with regard to their antioxidative properties. Model systems consisting of emulgated linoleic acid were used to evaluate the antioxidative effect of the MRP. The antioxidative effect was found to be strongly dependent on the choice of reactants, especially the amino acid. While MRP from glutamic acid with glucose or fructose showed no antioxidative properties, potent antioxidative products were obtained from, for example, histidine or lysine with any of the three sugars, or from arginine with xylose only.

The reaction between histidine and glucose was studied with regard to the influence of pH and of the molar ratio of amino acid to sugar on the antioxidative effect of the MRP formed. Neutral or slightly basic initial pH was found to favor the production of antioxidative products. Furthermore, the antioxidative effect was found to be more favored by a high histidine concentration than by a high glucose concentration.

INTRODUCTION

The complex reaction between carbonyl compounds, such as reducing sugars, and amino compounds, such as amino acids, which is known as the Maillard reaction, is very common and important in food processing. This reaction is, for example, responsible for the formation of brown color in frying crusts or during bread baking and of various kinds of aroma compounds. The reaction may, however, also cause nutritional losses by making essential amino acids, especially lysine, biologically unavailable.

Observations of antioxidative effect of Maillard reaction products (MRP) have also been made. This was first reported by Griffith and Johnson (1957), who demonstrated that the addition of 5% glucose to

sugar cookies produced a marked browning in the cookies and resulted in a greater stability to oxidative rancidity. They also showed that MRP from glycine-glucose exhibited antioxidative properties. Later on, reaction products from various amino compounds and sugars were studied with regard to antioxidative properties (Kirigaya *et al.* 1968, 1969; Yamaguchi and Fujimaki 1974). Attempts to isolate the antioxidative products through fractionation by dialysis, gel filtration, or ion exchange chromatography have also been made (Yamaguchi and Fujimaki 1970; Kirigaya *et al.* 1971). It has, however, not yet been possible to identify the antioxidative active components. Eichner (1975) reported that MRP formed in dehydrated model systems inhibited fat oxidation in these systems.

In the present investigation, various combinations of amino acids and sugars were reacted and the antioxidative effect of the reaction products was evaluated by adding them to model systems consisting of emulgated linoleic acid. The influence of the various amino acids and sugars, as well as of reaction conditions on the formation of antioxidative products was studied.

MATERIALS AND METHODS

Synthesis of MRP

MRP were obtained by refluxing 5 mmol amino acid and 10 mmol sugar in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h, where not otherwise stated. Potassium hydroxide or hydrochloric acid was used to obtain the desired initial pH. The following amino acids were used: L-arginine monohydrochloride, L-histidine monohydrochloride monohydrate (Fluka, Switzerland), L-cysteine, L-glutamic acid, L-lysine monohydrochloride and L-valine (Merck, GFR). The sugars used were: D-glucose (Fisher Scientific Comp., USA), D-fructose and D-xylose (Merck, GFR). At least two replicates were made of each synthesis. The reacted mixtures of cysteine-fructose, lysine-glucose and of the combinations of valine contained precipitations. Only the soluble fraction of the reaction mixtures was used in all measurements.

Measurement of Antioxidative Effect

The antioxidative effect of the reaction mixtures was evaluated by the polarographic and gas chromatographic methods, described previously (Lingnert *et al.* 1979). When using the polarographic method, 20 μl of the Maillard reaction mixture was added to 10 ml of the 10 mM linoleic acid emulsion, pH 6.5. Hemin was used to catalyze the oxidation.

When using the gas chromatographic method, 250 μl of the Maillard reaction mixture was added to 400 ml of the 3 mM linoleic acid emulsion, pH 6.8. The headspace samplings were standardized to one occasion before the addition of catalyst, heat activated horseradish peroxidase, followed by samplings after 1 h, 3.5 h, 24 h, and 26 h. On each occasion 600 ml of headspace gas was sampled. At the beginning and at the end of the incubation period, 0.2 ml aliquots of the emulsion were withdrawn for spectrophotometric measurement of the absorption at 234 nm. The aliquots were immediately diluted to 5.2 ml in a mixture of equal volumes of 0.2 M potassium borate, pH 9.0, and 95% ethanol.

Determination of Remaining Glucose and Fructose

The amounts of unreacted glucose and fructose in the reaction mixtures were determined spectrophotometrically by enzymatic methods, using hexokinase, glucose-6-phosphate dehydrogenase and phosphoglucose isomerase (Bergmeyer 1970). The determinations were made with Boehringer Mannheim's test-combination (Boehringer Mannheim, GFR; Cat. No. 139106).

Determination of Remaining Amino Acid

The amount of unreacted arginine or lysine remaining in the reaction mixtures after the reaction was analyzed by decarboxylating the amino acids with L-arginine decarboxylase and L-lysine decarboxylase respectively (SIGMA, USA) and measuring the amount of carbon dioxide formed manometrically, using a Warburg equipment (B. Braun, GFR). 25 μl of Maillard reaction mixture was solubilized in 6 ml of 0.1 M sodium acetate buffer, pH 5.0. 2.5 ml was transferred to the major compartment of the Warburg vessel. Standard solutions of 2×10^{-3} M arginine or lysine in 0.1 M sodium acetate buffer were run in parallel. The decarboxylation was performed at 37°C and was started by adding, from the side arm of the Warburg vessel, 0.5 ml of acetate buffer containing approximately 1.1 U of arginine decarboxylase or approximately 0.8 U of lysine decarboxylase.

RESULTS

Influence of Reaction Time
on the Antioxidative Effect of MRP

The reaction between histidine and glucose (starting with 100 ml reaction mixture) was allowed to proceed for 200 h, during which time

5 ml samples were withdrawn at intervals for analysis of antioxidative effect, color and pH. Antioxidative effect was measured by the polarographic method and absorption at 450 nm was used as a measure of the color of the samples. Figure 1 shows the development of color and antioxidative effect as a function of reaction time. The antioxidative effect, as well as the color intensity, went through a maximum after about 20 h of reaction. This is, however, at least partly explained by the fact that precipitation of polymerized brown material started at that time and the measurements were made on the soluble part of the reaction mixture.
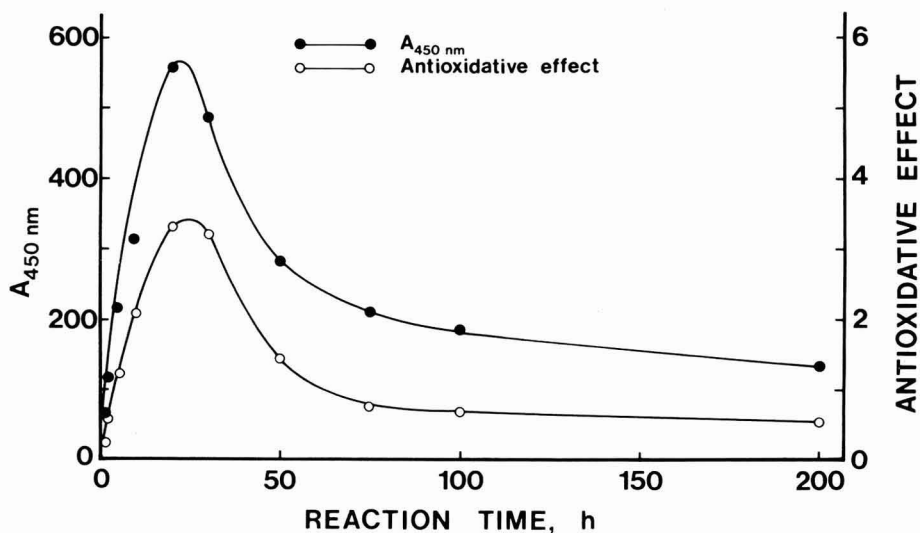
FIG. 1. DEVELOPMENT OF COLOR $(A_{450 \text{ nm}})$ AND ANTIOXIDATIVE EFFECT AS A FUNCTION OF TIME IN A HISTIDINE-GLUCOSE REACTION MIXTURE

0.1 mol histidine and 0.2 mol glucose were refluxed in 100 ml 0.1 M potassium phosphate buffer, pH 7.0. At intervals 5 ml of the reaction mixture was withdrawn for spectrophotometric measurement of color (450 nm) and polarographic measurement of antioxidative effect.

It is well known that the Maillard reaction involves a decrease in pH. This is illustrated in Fig. 2 for the reaction between histidine and glucose, which shows a considerable pH drop during the first hours of reaction.
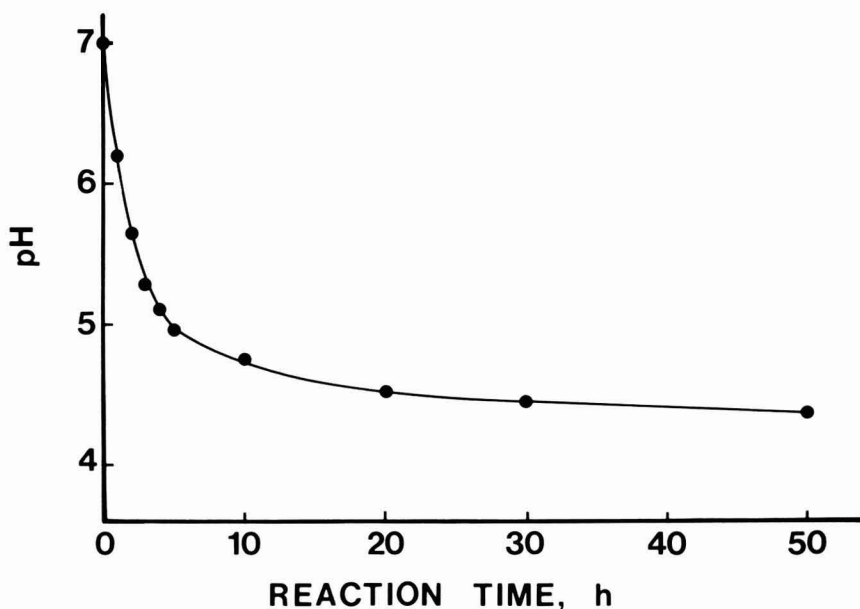
FIG. 2. pH OF A HISTIDINE-GLUCOSE REACTION MIXTURE AS A
FUNCTION OF TIME

0.1 mol histidine and 0.2 mol glucose were refluxed in 100 ml 0.1 M potassium
phosphate buffer, pH 7.0. At intervals 5 ml of the reaction mixture was with-
drawn for measurement of pH.

Different Amino Acid-Sugar Combinations

The antioxidative effect of MRP obtained by reaction between the
six different amino acids and the three sugars for 5 h, as measured by
the polarographic method, is shown in Table 1. The table also shows the
final pH and the color (A_{450nm}) of the reaction mixtures, as well as the
amounts of arginine, lysine, glucose and fructose remaining in the reac-
tion mixture.

As can be seen, the arginine-xylose and lysine-xylose reaction mix-
tures were by far the most antioxidative. As to the rest of the combina-
tions, the antioxidative effect was more influenced by the choice of
amino acid than by the choice of sugar. While reactions between glutamic
acid and glucose or fructose gave products with no detectable antioxida-
tive effect, products with considerable antioxidative effect were obtained
especially from lysine, histidine and arginine.

Except for the case of cysteine, fructose formed either lesser amounts
of or less efficient antioxidative products than the other two sugars. As
can be seen in Table 1, reactions with fructose also involved less color

Table 1. Final pH, color ($A_{450\,nm}$), remaining amino acid and sugar and antioxidative effect of MRP from different amino acid-sugar combinations[a]

| Reactants | | Final pH | A_{450} nm | Remaining Amino Acid[b] (%) | Remaining Glucose[c] (%) | Remaining Fructose[c] (%) | Antioxidative Effect[d] |
Amino Acid	Sugar						
Arginine	Glucose	3.7	128	31	29	1	0.7
Arginine	Fructose	4.3	65	59	2	60	0.4
Arginine	Xylose	3.7	182	28			5.6
Cysteine	Glucose	3.6	20		40	0	0.2
Cysteine	Fructose	4.5	3		1	68	0.3
Cysteine	Xylose	3.2	52				0.2
Glutamic acid	Glucose	4.9	115		32	4	0.0
Glutamic acid	Fructose	5.2	71		3	51	0.0
Glutamic acid	Xylose	4.7	228				0.3
Histidine	Glucose	4.8	324		10	3	0.9
Histidine	Fructose	5.6	169		2	37	0.7
Histidine	Xylose	4.7	355				0.9
Lysine	Glucose	3.1	336	33	23	0	1.3
Lysine	Fructose	3.5	141	67	1	62	0.8
Lysine	Xylose	3.1	389	58			3.9
Valine	Glucose	3.9	192		32	1	0.5
Valine	Fructose	4.4	81		1	68	0.3
Valine	Xylose	3.8	105				0.4

[a]5 mmol amino acid and 10 mmol sugar were refluxed in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h

[b]The amount of arginine or lysine in the final reaction mixtures was determined by manometric technique, using the enzymes arginine decarboxylase and lysine decarboxylase respectively. The remaining amount is expressed as % of the initial amount of amino acid

[c]The amounts of glucose and fructose in the final reaction mixtures were determined spectrophotometrically by enzymatic methods. The amounts are expressed as % of the initial amount of sugar

[d]The antioxidative effect was measured by the polarographic method

formation and a higher final pH. Furthermore, less arginine and lysine had reacted with fructose than with glucose or xylose. Irrespectively of the amino acid used, more glucose than fructose reacted as well. Altogether these facts indicate that an explanation of the less antioxidative reaction mixtures from fructose can be that fructose is less reactive than the other two sugars.

During the Maillard reaction some isomerization between glucose and fructose was obtained, as is shown in Table 1, giving small amounts of fructose when starting with glucose and vice versa.

The amounts of remaining unreacted arginine and lysine were analyzed in order to assess whether the differences in antioxidative effect obtained in products from one amino acid with different sugars were due to varying degrees of reaction. As shown in Table 1, great differences in the amount of unreacted amino acid were observed. The antioxidative effect was, however, correlated neither to the amount of reacted amino acid nor to the amount of reacted sugar, when comparing the products from glucose with different amino acids or the products from fructose with different amino acids.

The antioxidative effect of most of the reaction products was also tested by the gas chromatographic method. Table 2 shows the concentration of n-hexanal, n-pentanal and n-hept-*trans*-2-enal, as well as the increase of the diene absorption at 234 nm, after oxidation for one day in experiments carried out with no addition or with the addition of 250 μl of the different amino acid-sugar reaction mixtures to 400 ml linoleic acid.

With this method as well the strongest antioxidative effect was obtained with reaction products from the basic amino acids. Especially products from histidine, irrespective of the choice of sugar, and products from arginine and xylose inhibited the formation of volatile compounds. The products from lysine were somewhat less effective. The choice of sugar still seems to be of minor importance, except in combinations with arginine, which, in combination with glucose and fructose, formed MRP with very weak antioxidative effect, while the reaction mixture of xylose and arginine contained potent antioxidants.

Influence of Initial pH on the
Antioxidative Effect of MRP

As was shown in Fig. 2, the pH decreases considerably during the amino acid-sugar reaction. In order to study the influence of pH on the antioxidative effect of MRP, the reaction between histidine and glucose was performed in water starting at pH 3.0, 5.0, 7.0, 9.0, and 11.0, respectively. The reaction was allowed to proceed for 5 h, after which

Table 2. The influence of MRP from various amino acid-sugar combinations on the formation of volatile compounds and conjugated diene compounds from linoleic acid after oxidation for 25 h[a]

Added MRP[b]	n-Pentanal (Integrator Value)	n-Hexanal (ppb)	n-Hept-*trans*-2-enal (Integrator Value)	$\Delta A_{234 nm}$[c]
No addition	46	1512	101	1.2
Arginine — Glucose	44	1303	102	1.2
Arginine — Fructose	39	1142	96	1.1
Arginine — Xylose	4	39	5	0.1
Cysteine — Fructose	76	1349	151	1.4
Cysteine — Xylose	28	848	78	1.2
Glutamic acid — Glucose	58	2452	117	0.9
Histidine — Glucose	2	29	6	0.2
Histidine — Fructose	2	25	4	0.1
Histidine — Xylose	2	21	3	0.1
Lysine — Glucose	7	85	13	0.3
Lysine — Fructose	16	149	22	0.5
Lysine — Xylose	10	72	13	0.2
Valine — Glucose	27	706	78	1.1
Valine — Fructose	31	1172	94	1.1
Valine — Xylose	38	1060	84	1.1

[a]The oxidation was catalyzed by heat activated horseradish peroxidase. The figures given are mean values of samplings after 24 h and 26 h
[b]The MRP were obtained by refluxing 5 mmol amino acid and 10 mmol sugar in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h. 250 μl were added to 400 ml 3 mM linoleic acid emulsion, pH 6.8
[c]Increase in absorption at 234 nm during 25 h oxidation

time pH, color ($A_{450 nm}$), antioxidative effect and remaining amounts of glucose and fructose in the reaction mixture were measured. The results are shown in Table 3.

The strongest antioxidative effect and the highest color intensity was obtained at intermediate pH. The formation of antioxidative MRP was especially favored by neutral or slightly basic initial pH, while the pigment formation was somewhat more favored by slightly acidic initial pH. The amount of unreacted glucose was found to decrease as the initial pH was increased. The isomerization of glucose to fructose was, however, also favored by high pH, whereby the sum of unreacted glucose and fructose in the reaction mixture was greater after starting at pH 11 than after starting at pH 9.

Table 3. The influence of initial pH on final pH, color ($A_{450\ nm}$), remaining sugar and antioxidative effect of MRP from histidine-glucose[a]

Initial pH	Final pH	$A_{450\ nm}$	Remaining Glucose[b] (%)	Remaining Fructose[b] (%)	Antioxidative Effect[c]
3.0	2.8	174	51	0	1.7
5.0	3.0	285	42	0	1.9
7.0	5.0	279	15	3	2.0
9.0	5.5	248	11	4	2.0
11.0	7.3	122	10	9	1.2

[a]5 mmol histidine and 10 mmol glucose were refluxed in 5 ml water for 5 h. Potassium hydroxide or hydrochloric acid was used to obtain the desired initial pH
[b]The amounts of glucose and fructose in the final reaction mixtures were determined spectrophotometrically by enzymatic methods. The amounts are expressed as % of the initial amount of glucose
[c]The antioxidative effect was measured by the polarographic method

Influence of the Molar Ratio of Histidine to Glucose on the Antioxidative Effect

The influence of the molar ratio of amino acid to sugar on the antioxidative effect of the products was investigated for various combinations of histidine and glucose, which were reacted for 5 h, starting at pH 7.0. The antioxidative effect of the reaction mixtures obtained was measured by the polarographic method. The final pH, absorption at 450 nm and content of unreacted glucose and fructose were measured in the reaction mixtures.

The results are shown in Table 4. Neither histidine nor glucose alone formed any antioxidative products. The antioxidative effect of products from histidine and glucose increased on increasing the concentrations of the reactants. The antioxidative effect was, however, influenced to a greater extent by changes in the histidine concentration than by changes in the glucose concentration. When the histidine concentration was kept constant, an increase of the amount of glucose from 2.5 to 10 mmol increased the antioxidative effect, as well as the color and the remaining amount of unreacted glucose, while the final pH decreased. When the glucose concentration was kept constant, however, an increase of the amount of histidine from 2.5 to 10 mmol increased the antioxidative effect even more. In the latter case unreacted glucose decreased and the final pH increased. The color was, however, not found to be influenced by changes in the histidine concentration.

Table 4. The influence of histidine and glucose concentration on final pH, color ($A_{450\ nm}$), remaining sugar and antioxidative effect of MRP from histidine-glucose

Reaction Mixture[a]		Final pH	$A_{450\ nm}$	Remaining Glucose[b] (%)	Remaining Fructose[b] (%)	Antioxidative Effect[c]
Histidine (mmol)	Glucose (mmol)					
0.0	5.0	5.9	8	50	19	0.0
5.0	0.0	7.0	0			0.0
2.5	5.0	5.2	158	18	3	1.0
5.0	2.5	6.4	106	0	2	1.1
5.0	5.0	5.8	165	3	3	1.6
5.0	10.0	4.8	324	10	3	1.9
10.0	5.0	6.5	161	0	0	2.4

[a]The amounts of histidine and glucose indicated were refluxed in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h
[b]The amounts of glucose and fructose in the final reaction mixtures were determined spectrophotometrically by enzymatic methods. The amounts are expressed as % of the initial amount of glucose
[c]The antioxidative effect was measured by the polarographic method

DISCUSSION

The antioxidative effect of MRP from amino acids and sugars was found to be strongly dependent on the choice of reactants. This was particularly true for the amino acid partner, while the choice of sugar, with few exceptions, was of minor importance. Some differences between the results obtained by the polarographic and the gas chromatographic measuring method were, however, observed, as is evident when comparing Table 1 and Table 2. Since the two methods are based on measurements at entirely different stages in the lipid oxidation sequence, these different results may possibly be explained by differences in the antioxidative mechanisms of various MRP.

Irrespective of the measuring method used, the strongest antioxidative MRP were, however, obtained from the basic amino acids. This is in accordance with the results of Kirigaya *et al.* (1969) who investigated MRP from various amino acids reacted with glucose and from various sugars reacted with glycine. Measuring the influence of added MRP on the peroxide value during oxidation of 40% ethanol solutions of fatty acids, they found strong antioxidative effect in products from the basic amino acids, especially histidine, with glucose. Of the sugars used, xylose was found to be the most effective one for forming antioxidative products with glycine. It is, however, not evident, that the most effective amino acid when reacted with one sugar is the most effective when reacted with other sugars. This was illustrated in the present investigation, in the case of arginine, for example.

The formation of antioxidative compounds was not correlated to the formation of pigments, neither when comparing different amino acid-sugar combinations nor when comparing one and the same combination at different reaction conditions, for example histidine-glucose in varying molar ratios. While the pigment formation in the latter case was almost entirely dependent on the glucose concentration, the antioxidative effect was to the greatest extent dependent on the histidine concentration. Since the formation of different reaction products is influenced in different ways by the reaction conditions, only part of the complex mixture of reaction products seems to possess antioxidative properties. Isolation and identification of these products and evaluation of their antioxidative mechanism still remain to be done.

The knowledge of how amino acids and sugars differ in their ability to form antioxidative products, and how the reaction conditions influence the antioxidant production, is of the greatest importance for making the best use of these types of antioxidants to inhibit lipid oxidation in foods, whether this is done by the addition of preformed antioxidative MRP or by optimizing the formation of antioxidative products from added or naturally present reactants during normal food processes.

ACKNOWLEDGMENTS

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ANTIOXIDATIVE MAILLARD REACTION PRODUCTS. II. PRODUCTS FROM SUGARS AND PEPTIDES OR PROTEIN HYDROLYSATES

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ABSTRACT

Maillard reaction products (MRP) obtained by the reaction of reducing sugar with dipeptides, or with protein hydrolysates formed by enzymic hydrolysis of some proteinous food by-products, were studied with regard to antioxidative properties. Model systems consisting of emulgated linoleic acid were used to evaluate the antioxidative effect. Dipeptides of histidine and glycine were found to form antioxidative MRP by reaction with xylose, the antioxidative effect being dependent on the amino acid sequence in the dipeptides. Stronger antioxidative effect was obtained when reacting histidylglycine with xylose than when reacting a mixture of histidine and glycine with xylose.

Although some antioxidative effect was achieved by some of the protein hydrolysates themselves, the effect was considerably increased by reacting them with glucose. The MRP from the protein hydrolysates and glucose were, however, found to be less antioxidative than histidine-glucose or arginine-xylose reaction products.

INTRODUCTION

The complex reaction between amino and carbonyl compounds, known as the Maillard reaction, is very common in food processing. By this reaction products with antioxidative properties may be formed, as has been demonstrated previously when reacting amino acids and reducing sugars in well-defined model systems (Kirigaya *et al.* 1968, 1969; Yamaguchi and Fujimaki 1974; Lingnert and Eriksson 1980). The antioxidative effect of the Maillard reaction products (MRP) was found to be dependent on which amino acid and which sugar take part in the reaction, as well as on the reaction conditions, such as pH and the molar ratio of amino acid and sugar (Lingnert and Eriksson 1980).

The antioxidative effect of soybean protein hydrolysates, as well as of

some dipeptides has previously been reported by Yamaguchi *et al.*
(1975a, b). Bishov and Henick (1975) reported that protein hydrolysates,
such as hydrolyzed vegetable protein and autolyzed yeast protein,
possess antioxidative properties. These hydrolysates also showed syner-
gism with BHA (Butylated Hydroxy Anisole) and other phenolic anti-
oxidants. However, no reports on the antioxidative effect of reaction
products of protein hydrolysates and sugars have, as far as we know,
been published.

In the present investigation model systems consisting of emulgated
linoleic acid were used to evaluate the antioxidative effect of reaction
products from reducing sugar with dipeptides of histidine and glycine,
whereby the influence of the sequence of the two amino acids was
studied.

Furthermore, reaction products from reducing sugar with some protein
hydrolysates were investigated with regard to the antioxidative effect.

MATERIALS AND METHODS

Synthesis of MRP

MRP from dipeptides were obtained by refluxing 1 mmol of
L-histidylglycine or glycyl-L-histidine hydrochloride (Sigma, USA) and
2 mmol D-xylose (Merck, GFR) in 5 ml 0.1 M potassium phosphate
buffer, pH 7.0, for 5 h. The products obtained in this way were com-
pared with MRP formed when refluxing 2 mmol L-histidine monohydro-
chloride monohydrate (Fluka, Switzerland) or 2 mmol glycine (Merck,
GFR) or a mixture of 1 mmol L-histidine monohydrochloride mono-
hydrate and 1 mmol glycine together with 2 mmol D-xylose in 5 ml
0.1 M potassium phosphate buffer, pH 7.0, for 5 h.

MRP were also obtained by refluxing 1 g of protein hydrolysate (from
malt sprouts, brewer's grains or bovine erythrocytes, mainly consisting
of hemoglobin) and 10 mmol of D-glucose (Fisher Scientific Comp.,
USA) in 5 ml buffer, as above, for 5 h. Comparisons were in this case
made with MRP produced in the same way from 5 mmol L-histidine
monohydrochloride monohydrate and 10 mmol D-glucose or 5 mmol
L-arginine monohydrochloride (Fluka, Switzerland) and 10 mmol
D-xylose in 5 ml buffer.

The hydrolysate of malt sprouts, as well as the hydrolysates of
brewer's grains, were kindly provided by Dr. Y. Mälkki, VTT, Otaniemi,
Finland. The hydrolysate of malt sprouts was obtained by autolysis,
while the hydrolysates of brewer's grains were obtained by treatment
for about 25 h with peptidases from *Pseudomonas fluorescens*

Mc 864/VTTE 8.7 (Mälkki *et al.* 1977) or with proteases from *Bacillus subtilis.* Mälkki *et al.* (1973) have previously described these types of hydrolysates.

The hydrolysate of hemoglobin was obtained by enzymic hydrolysis, as described by Stachowicz *et al.* (1977). The red cell fraction of ox-blood obtained after plasma separation was hemolyzed by dilution with water and denatured by adjusting pH to 11 with 5 M NaOH and retaining this pH for 1 h. pH was then adjusted to 9 with 1 M HCl and the sample was centrifuged. The supernatant was further diluted with water to a protein content of about 4%. The protein was then hydrolyzed at 50°C by the enzyme alcalase® (NOVO, Denmark) in a membrane reactor consisting of an ultrafiltration device of hollow fibre type (Romicon Inc., USA). The ultrafiltration membranes XM 50 or PM 10 (with nominal cut-off values of 50,000 and 10,000, respectively) were used. When using the XM 50 membrane, the hydrolysate obtained was found to contain 0.9% hemin, while the hydrolysate obtained by using the PM 10 membrane was found to contain 0.007% hemin as calculated on dry matter.

Measurement of Antioxidative Effect

The antioxidative effect of the MRP was evaluated both by a polarographic and a gas chromatographic method, as previously described (Lingnert *et al.* 1979). When using the polarographic method, 20 μl of the Maillard reaction mixture was added to 10 ml of the 10 mM linoleic acid emulsion, pH 6.5. 0.4 ml of 10 μM hemin solution was used to catalyze the oxidation.

In the gas chromatographic method, 0.25–1.0 ml of the Maillard reaction mixture was added to 400 ml of the 3 mM linoleic acid emulsion, pH 6.8. The headspace samplings were standardized to one occasion before the addition of the catalyst, heat activated horseradish peroxidase, followed by samplings after 1 h, 3.5 h, and 26 h. On each occasion 600 ml of headspace gas was sampled. At the beginning and at the end of the incubation period, 0.2 ml aliquots of the emulsion were withdrawn for spectrophotometric measurement of the absorption at 234 nm. The aliquots were immediately diluted to 5.2 ml in a mixture of equal volumes of 0.2 M potassium borate, pH 9.0, and 95% ethanol.

RESULTS

Reactions Between Sugar and Dipeptides

The antioxidative effects of the reaction products from xylose and the two dipeptides of glycine and L-histidine, glycylhistidine and

histidylglycine, as well as of the reaction products from xylose and the free amino acids were compared. The gas chromatographic method was used when evaluating the antioxidative effect. Table 1 shows the concentration of n-pentanal, n-hexanal, and n-hept-*trans*-2-enal in the linoleic acid emulsion, as well as the increase of the absorption at 234 nm, after 25 h of oxidation. The figures given are mean values of the samplings after 24 h and 26 h.

The table shows that the reaction products from glycine and xylose gave no antioxidative effect at the concentration tested, while histidine reacted with xylose gave considerable antioxidative effect. Products with intermediate antioxidative effect were obtained when reacting xylose with a mixture of the two amino acids. The comparison between MRP from xylose and the two dipeptides shows that the antioxidative effect was influenced by the sequence of the amino acids in the peptide. The peptide with histidine as the N-terminal amino acid formed equally effective antioxidants as those formed by histidine alone in the reaction with xylose, while MRP from xylose and glycylhistidine, having glycine as the N-terminal amino acid, gave antioxidative effect comparable with that of reaction products from xylose and a mixture of glycine and histidine.

Reactions Between Sugar and Protein Hydrolysates

Two different hydrolysates of brewer's grains were investigated when refluxed alone for 5 h and when refluxed in the presence of glucose for 5 h. As a control the untreated hydrolysates were investigated. The antioxidative effect of the differently treated hydrolysates and the control, as measured by the polarographic method, are presented in Table 2.

As can be seen in the table, some improvement of the antioxidative effect of the protein hydrolysates alone was achieved by just heating them, possibly owing to presence of reducing sugars, since these types of protein hydrolysates have previously been shown to contain appreciable amounts of carbohydrates (Mälkki *et al.* 1973). Considerably higher antioxidative effect was, however, observed after reacting the protein hydrolysates with glucose. Furthermore, it can be seen that the hydrolysates, obtained from the same raw material by two different enzymes, differ in their ability to form antioxidative products when reacted with glucose.

In Table 3, MRP from glucose and some protein hydrolysates have been compared with those from histidine and glucose and from arginine and xylose, two of the most effective antioxidant producing amino acid-sugar combinations, according to a previous investigation (Lingnert and Eriksson 1980). None of the investigated MRP from protein hydrolysate

Table 1. The influence of added MRP on the formation of volatile compounds and conjugated diene compounds from linoleic acid emulsion after oxidation for 25 h[a]

MRP Precursor[b]	n-Pentanal (integrator value)	n-Hexanal (ppb)	n-Hept-trans-2-enal (integrator value)	ΔA_{234} nm [c]
No addition	46	1512	101	1.2
Glycine(2 mmol)	86	2656	133	1.2
L-Histidine(2 mmol)	11	193	25	0.5
Glycine(1 mmol) + L-Histidine(1 mmol)	16	305	34	0.7
Glycyl-L-histidine(1 mmol)	13	308	43	0.7
L-Histidylglycine(1 mmol)	10	185	26	0.5

[a]250 μl of MRP was added to 400 ml 3 mM linoleic acid emulsion. The oxidation was catalyzed by heat activated horseradish peroxidase. The figures given are mean values of samplings after 24 h and 26 h

[b]The amount of amino compound stated was reacted with 2 mm ol D-xylose in 5 ml 0.1 potassium phosphate buffer, pH7.0 by refluxing for 5 h

[c]Increase in absorption at 234 nm during 25 h oxidation

Table 2. The antioxidative effect of brewer's grains hydrolyzed by two different enzyme preparations and refluxed for 5 h in the absence and in the presence of glucose[a]

Hydrolyzing Enzyme Preparation Used	Treatment	Antioxidative Effect[b]
Peptidases from *Pseudomonas* *fluorescens*	No	0.4
	Refluxed	1.0
	Refluxed with glucose	10
Proteases from *Bacillus subtilis*	No	0.3
	Refluxed	0.5
	Refluxed with glucose	20

[a]1 g of the hydrolysate, either alone or together with 10 mmol glucose, was refluxed in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h
[b]The antioxidative effect was measured by the polarographic method

and sugar seemed to have any antioxidative effect when tested at the same concentration level as the products of the free amino acids and sugars. The amount of reaction products from protein hydrolysate and glucose added to the linoleic acid emulsion had to be increased at least four times in order to give an antioxidative effect comparable with that of the amino acid-sugar products at standard conditions.

Of the different protein hydrolysates, the malt autolysate was found to form the strongest antioxidative products when reacted with glucose, followed by the hydrolysate of hemoglobin ultrafiltrated through a PM 10 membrane and the hydrolysate of brewer's grains. The reaction mixture of glucose and the hemoglobin hydrolysate ultrafiltrated through a XM 50 membrane gave hardly any antioxidative effect, possibly as a consequence of its relatively high content of counteracting prooxidative hemin.

DISCUSSION

Previously, antioxidative MRP were shown to be formed by reaction between free amino acids and sugars, the antioxidative effect being primarily dependent on the choice of amino acid, but also on the choice of sugar (Lingnert and Eriksson 1980). The present investigation contains evidence that dipeptides also form antioxidative products when reacted with sugar. The antioxidative effect was in this case dependent not only on the types of amino acids in the peptide, but also on their

Table 3. The influence of MRP from sugars and amino acids or protein hydrolysates on the formation of volatile compounds and conjugated diene compounds from linoleic acid emulsion after oxidation for 25 h[a]

Added MRP[b]	Amount Added[c] (ml)	n-Pentanal (integrator value)	n-Hexanal (ppb)	n-Hept-trans--2-enal (integrator value)	$\Delta A_{234\,nm}$ [d]
No addition		46	1512	101	1.2
L-Histidine – Glucose	0.25	2	29	6	0.2
L-Arginine – Xylose	0.25	4	39	5	0.1
Hydrolyzed brewer's grains (proteases from *B. subtilis*) – Glucose	0.25	104	2612	125	1.2
	1.0	20	122	12	0.2
Autolyzed malt sprouts – Glucose	0.25	100	4580	147	0.8
	1.0	4	23	2	0.0
Ultrafiltrated (PM 10) hemoglobin hydrolysate – Glucose	1.0	9	54	6	0.2
Ultrafiltrated (XM 50) hemoglobin hydrolysate – Glucose	1.0	83	916	81	0.9

[a] The oxidation was catalyzed by heat activated horseradish peroxidase. The figures given are mean values of samplings after 24 h and 26 h
[b] The MRP were obtained by refluxing 10 mmol sugar together with 5 mmol amino acid or 1.0 g protein hydrolysate in 5 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h
[c] Amount of MRP added to 400 ml of the 3 mM linoleic acid emulsion
[d] Increase in absorption at 234 nm during 25 h oxidation

sequence. The two dipeptides of L-histidine and glycine were chosen in this study, since histidine was previously found to form potent antioxidants by reaction with xylose, while glycine-xylose reaction products were found to give no antioxidative effect at the concentration used in this study. As can be seen in Table 1, the reaction products from histidylglycin and xylose were nevertheless found to be as antioxidative as the reaction products from histidine alone and xylose, in spite of the fact that the latter combination contained twice as much histidine (equals the molar sum of histidine + glycine).

Compared with MRP from xylose and a mixture of histidine and glycine, histidylglycine-xylose reaction products were found to give stronger antioxidative effect. This can possibly be explained by the fact that histidine and glycine competed for the xylose in the first case, and products with no antioxidative effect were formed from glycine, while in the latter case, when the amino group of glycine was unavailable for reaction, histidine reacted with the xylose to a greater extent.

Yamaguchi et al. (1975a) found that, in the case of unreacted dipeptides as well, the antioxidative effect was dependent on the amino acid sequence. In their study the antioxidative effect of the dipeptides was in several cases found to be greater than the antioxidative effect of a mixture of the free amino acids which constituted the dipeptide.

If the antioxidative effect of MRP is to be applied to foods, it would be of great advantage from an economical point of view to be able to use protein hydrolysates rather than pure, free amino acids as the amino source in the reaction, since protein hydrolysates could be produced from several proteinous by-products from the food industries. Some protein hydrolysates obtained by enzymic hydrolysis of food by-products were therefore tested in this investigation in order to get an idea of their capacities for forming antioxidative MRP. The hydrolysates were used only to exemplify these possibilities and no attempts were made to characterize them regarding peptide chain lengths, amino acid composition, terminal amino acids etc.

Although some antioxidative effect was demonstrated for protein hydrolysates only, in accordance with previous reports (Bishov and Henick 1975; Yamaguchi et al. 1975b), the antioxidative effect was considerably increased by the reaction with sugar. The products were, however, inferior to MRP obtained from, for example, histidine and glucose or arginine and xylose. Small additions of some of them did even indicate prooxidative properties, causing increased formation of volatile lipid oxidation products during the oxidation of linoleic acid.

By the proper choice of, for example, proteolytic enzyme, hydrolysis conditions, and possibly separation methods, it should, however, be

possible to optimize the properties of the hydrolysate with regard to the content of peptides of suitable chain lengths and with suitable terminal amino acids that favor the formation of antioxidative products when reacted with sugars. In Table 2 it can, for example, be seen that hydrolyzing brewer's grains with two different enzymes gave hydrolysates with different abilities to form antioxidative products with glucose.

ACKNOWLEDGMENTS

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FACTORS AFFECTING THE MECHANISM OF FLUX DECLINE DURING ULTRAFILTRATION OF COTTAGE CHEESE WHEY

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ABSTRACT

The mechanism of fouling of ultrafiltration membranes during the processing of cheese whey was studied. Attempts were made to develop a mathematical model of the flux decline and to determine the contribution of individual proteins and the aqueous environment to fouling. In general, proteins dissolved in a salt-free system resulted in higher flux than in whey dialysate systems, implicating the salts as a major contributor to flux decline. Statistical analysis and evaluation of the parameters of the model indicated that fouling could be considered in two time periods. In the initial period of operation, α-lactalbumin appeared to cause greater flux decline and bovine serum albumin the least. After 10 min of operation, β-lactoglobulin was the major contributor to fouling. The native whey gave the most rapid rate of flux decline. These differences could be due to variations in protein structure or differences in the nature of the deposit on the membrane.

INTRODUCTION

The production of cheese whey, a by-product of the cheese manufacturing industry, increases annually and disposal of whey through sewage treatment plants is no longer a solution to the problem. Attempts have been made to treat whey using reverse osmosis (RO) and ultrafiltration (UF) to partially recover the solids for food and feed uses and to reduce BOD loads in treatment plants. The main problem associated with whey ultrafiltration is the decline in flux during operation due mainly to fouling of the ultrafiltration membrane, a phenomenon quite separate from flux-limiting effects due to concentration polarization. Lim *et al.* (1971) found that during concentration of cottage cheese whey by RO, the

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permeation rate decreased with time because of accumulation of a proteinaceous material on the membrane surface. Others (Lee and Merson 1975, 1976, Patel and Merson 1978, Fenton-May *et al.* 1971, Muller *et al.* 1973, Hayes *et al.* 1974) have observed the same phenomenon and many have tried chemical or physical treatments to the feed stream in order to prevent or reduce fouling. It appears that the microenvironment (i.e., pH, ionic strength and type of salts) as well as the nature and extent of membrane-protein interactions have a significant effect on the rate of fouling during ultrafiltration of cheese whey.

In this paper we present results of an investigation into the mechanism of fouling of ultrafiltration membranes during the processing of cottage cheese whey. The effects of salts, individual proteins and combinations of these on the rate of flux decline were determined and expressed in terms of an empirical mathematical model. Since the greatest rate of flux decline appears to occur immediately after the start-up of UF, this study was restricted to the initial 10 min of operation. The model UF system used was operated in the diafiltration mode and with adequate agitation to minimize effects due to concentration polarization.

MATERIALS AND METHODS

Cottage Cheese Whey

Cottage cheese whey was obtained from the Meadow Gold Plant in Champaign, Illinois. The whey was kept in a cooler at 4°C for not more than a week before use.

Simulated Skimmilk Ultrafiltrate

This was prepared according to the method of Jenness and Koops (1962) and used as a model solution simulating the aqueous salts system of cheese whey.

Whey Dialysate (WD) Protein System

This was prepared by the method of "ocean dialysis" (Murthy and Whitney 1956). 0.16% α-lactalbumin, 0.4% β-lactoglobulin (Sigma Chemical Company, St. Louis, Mo.) and 0.04% bovine serum albumin (Pentex Inc., Kankakee, IL.) were each dispersed in 300 ml deionized water with addition of 4.9% lactose to maintain volume. Each protein solution was then placed in a dialysis bag and individually immersed for 120 h in a vat containing 150—160 liters of whey at room temperature. 0.1% methyl p-hydroxybenzoate (paraban) was added to the whey

in order to prevent microbial growth. Agitation was provided with a submersible circulating pump placed in the vat. pH of WD protein solutions was 4.6.

Salt-free (SF) Protein System

Whey and individual proteins in the concentrations described above were dialysed against deionized water in the cooler for 120 h (when dialyzing against water no lactose or paraban were used). The dialysis bags, 150 ml each, were placed in a 4 liter Erlenmayer flask. The water was changed every 4—8 h during the 120 h of dialysis. Salt content was measured as Ca + Mg ions according to Ntailianas and Whitney (1964). A 100-fold decrease from the average original salt content was considered as a salt-free system. pH of SF protein systems was typically 5.2.

Ultrafiltration System

Figure 1 is a schematic of the model ultrafiltration system that was used. The ultrafiltration cell was the Amicon 202 diafiltration cell (Amicon Corporation, Lexington, Mass.) using 64 mm diameter flat sheet membranes. A polysulfone membrane was used (PTGC series, 10,000 molecular weight cut-off, Millipore Corporation, Mass.). Agitation was provided by a built-in magnetic stirrer adjusted to get a 3 cm deep vortex in the liquid in the cell to minimize concentration polarization effects. 100 ml sample of the solution under study was placed in the cell and the cell momentarily pressurized up to 40 psig before replacing fluid from the holding tank (whey dialysate without proteins in the case of whey and WD systems, and deionized water in the case of SF systems) was diafiltered through the cell. Permeate was collected in tared test tubes at 1 min intervals. Permeation volume was determined by weighing the filled tubes. Both cumulative volume and average flux per minute could be calculated. The system was free of metal parts. More detailed information on the mechanism of setting up the system and its operation is given by Merin (1979).

X-ray Analysis

This procedure was used to detect salts on the membrane surface. Pieces of the membrane were dehydrated in ethyl alcohol and air dried. They were then mounted on aluminum stubs which had previously been coated with a thick layer of dag 154 (Acheson Colloid Co., Port Huron, Much.) to achieve good conductivity of the specimen and to minimize interference by the aluminum while measuring x-ray counts. The membrane specimens were then coated with carbon in a Denton Vacuum
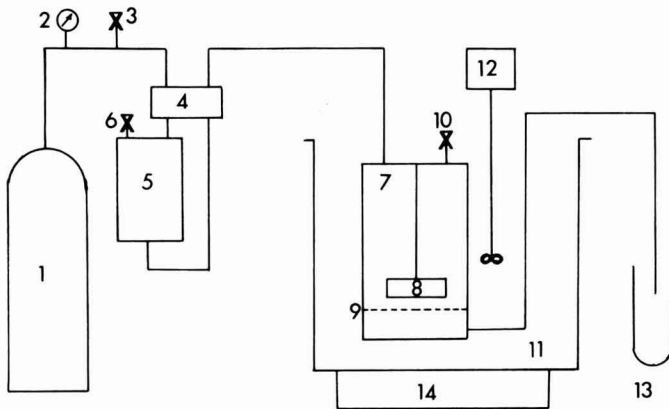
FIG. 1. SCHEMATIC LAYOUT OF THE ULTRAFILTRA-
TION SYSTEM

1. N_2 gas cylinder; 2. pressure gauge; 3. main N_2 valve;
4. three-way directory valve; 5. holding tank; 6. safety
(pressure release) valve; 7. UF cell; 8. magnetic stirrer;
9. membrane; 10. safety valve; 11. water bath; 12. heater/
stirrer; 13. collecting tube; 14. magnetic stirrer.

DU-502 evaporator to a brown color. Counts were measured using the
JEOL JSM U3 scanning electron microscope equipped with an Ortec
6200 Multi-Channel Analyser (Ortec, Inc., Oak Ridge, TN) at 9 kV
accelerating voltage in the backscattered mode. Counts were usually
taken either at 800 s or by setting 20,000 counts on the highest peak
(sulfur) as a reference standard. Since the Ortec 6200 collects the char-
acteristic emitted x-ray which is produced when an electron drops from
an upper energy level to fill in for an electron that has been knocked
out of its orbit by the electron beam, it was a convenient procedure
for the detection and identification of salts adsorbed on the membrane
surface.

Mathematical Model of Fouling

Each run was evaluated using the model shown below. The model
that best fit the data was one based on the assumption that the amount
of flux decline is a function of the cumulative volume processed similar
to the equation for instantaneous flux suggested by Matthews et al.
(1978). If J is the instantaneous flux at any time t, then

$$J = J_o V^{-b} \tag{1}$$

where V is the volume permeated and J_o is the initial flux at $t = 0$. Since $J = dV/dt$, Eq. (1) can be rewritten as

$$\ln dV/dt = A - b \ln V \tag{2}$$

where $A = \ln J_o$. When b is zero, it implies that no fouling occurs and the instantaneous flux at any time t should be equal to the initial flux J_o. Since experimental data is in terms of V and t, it is more convenient to use the integrated form of Eq. (2) for analysing the data:

$$V = [(b + 1) e^A]^{\frac{1}{b+1}} t^{\frac{1}{b+1}} \tag{3}$$

To allow for error in initial timing during the experiments, a $\triangle t$ term was introduced such that

$$V = k (t + \triangle t)^{\frac{1}{b+1}} \tag{4}$$

where

$$k = [(b + 1) e^A]^{\frac{1}{b+1}} \tag{5}$$

$$J_o = \frac{k^{b+1}}{b + 1} \tag{6}$$

k and J_o are constants for a particular protein system and is a function of operating parameters, physical properties of the feed and many unknown factors related to membrane performance such as pore size, shape and distribution, compaction effects and extent of gel formation, among others. The b value represents the slope of the plot of log of instantaneous flux vs log of cumulative volume (from Eq. 2) and hence it is an indicator of the rate of fouling during long-term operation. If b is zero, $J = J_o$ and it implies there is no fouling and the flux will remain steady.

Statistical analysis of data was done using Eq. (4) according to Deming (1944). A curve was considered fitted when the sum of squares of deviations was not greater than the chi-square value at the 5% level for the given degrees of freedom. The calculated values were analysed using the analyses of variance in a factorial design according to Steel and Torrie (1960) using orthogonal comparisons. From these comparisons, mean k, J_o and b values were calculated ($\triangle t$ was not treated because it does not contribute to the shape of the curve, since it is a correction for timing which will result in a displacement of the curve on the x-axis).

RESULTS AND DISCUSSION

Water Flux

To compile data about the membrane operating characteristics, it was necessary to define an indicator of clean (i.e., unfouled) membrane performance. Water flux is a generally accepted indicator used by membrane manufacturers to measure this performance. However, even after cleaning the membrane as suggested by the manufacturer, water fluxes varied greatly during initial operation of the system, ranging from 4 to 40 ml/min. This is possibly due to the fact that pores in a new, unused membrane do not possess their final characteristics and performance; for example, pores may compact or reduce slightly in size upon the application of pressure, and may not revert back completely to the original size upon release of pressure. To obtain reproducible data and to allow comparison of different runs we found it necessary to prime or temper the membrane by a series of wetting, soaking and pressurizing steps in order to bring all membranes to a similar starting point (Merin 1979). Thus different pieces of the same membrane had the same initial water flux and this enabled the statistical comparison of data between different experimental variables. The importance of proper cleaning and pretreatment of membranes was also emphasized by Sirkar *et al.* (1979).

Figure 2 is a graph of the average water flux for the initial 10 min period. The vertical lines projecting at each point represent the standard deviation for six different trials (each with a fresh, primed, membrane). One of the problems when comparing these results with literature values is that many investigators do not mention water fluxes at all, and if the membranes used did not have the same starting point of water flux, it is possible that some of the fouling effects reported had nothing to do with fouling but are the results of an uneven initial performance. All the membranes used in this work were primed and had a steady water flux. The average water flux was 4.56 ml/min (91.2 liters/ m^2 /h).

Ultrafiltration of Salt Solutions

A decline in flux was observed while processing the simulated milk salt solution (Fig. 2). This was unexpected, although sometimes reported in the literature, since based on pore size measurements (Merin 1979), this PTGC ultrafiltration membrane should allow salts to permeate freely through and not reject them. X-ray analysis of the membrane surface revealed that various salts in cheese whey, among them chlorides
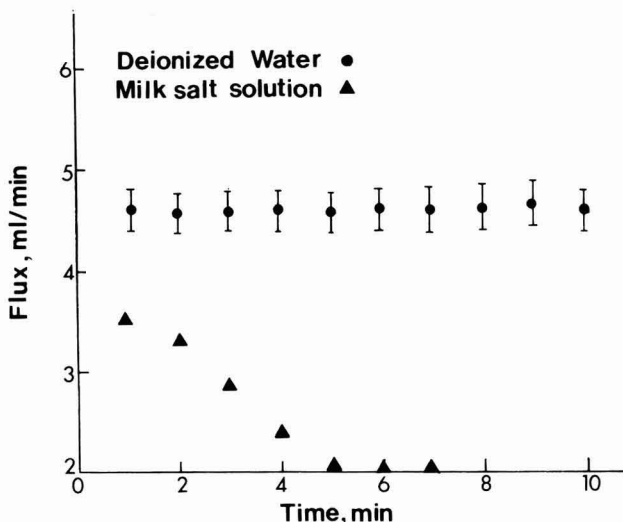
FIG. 2. FLUX FOR DEIONIZED WATER AND SIMU-
LATED MILK SALT SOLUTION THROUGH PTGC
ULTRAFILTRATION MEMBRANE

Membrane area = 32.17 cm^2. Temperature = 50°C.
Pressure = 40 psig. (Vertical bars indicate one standard
deviation of the mean.)

and phosphates of calcium, potassium and magnesium, were adsorbed
on the membrane surface or possibly even trapped in the pores of the
membrane. Indeed, even merely soaking the membrane briefly in cheese
whey caused a significant adsorption of salts (Table 1). Similar adsorp-
tion of salts by the membrane when immersed in simulated milk salt
and calcium chloride solutions were also observed (Merin 1979). In
Table 1, the sulfur (S) peak, corresponding to the sulfone group of the
membrane material, was the largest peak and it was set at 20,000
counts for each reading to serve as a reference standard.

Unfortunately, little is known about the precise chemical structure
and methods of manufacture of the membrane and it is difficult to
hypothesize the mechanism of membrane-salt interaction, except pos-
sibly as due to charge effects. Lee (1977) also noticed a 20—50% de-
cline in flux while processing salt solutions with three different types
of membranes, including polysulfone, and concluded that calcium and
phosphorus compete for binding sites on the membrane surface. Joseph-
son *et al.* (1975) found that 60% of the phosphorus of cheddar cheese
whey is retained by the ultrafiltration membrane. Mehta (1973) reported
that an increase in salt concentration (both calcium and phosphorus)
decreased the solute permeability and water flux.

Table 1. X-ray counts obtained from membrane after immersion in whey at different pH[1]

KeV[2]	Element	pH 3.0	4.6	5.5	7.0
1.23	Mg	2,382	2,566	4,654	4,560
2.01	P	3,378	3,544	6,426	6,366
2.30	S[3]	20,000	20,000	20,000	20,000
2.62	Cl	2,415	2,072	3,341	3,270
3.31	K	1,233	1,720	3,016	3,964
3.69	Ca	1,516	1,854	3,371	3,381

[1] pH of whey was adjusted with lactic acid or KOH
[2] KeV values listed for individual elements are the main energies detectable with the Ortec 6200 (Merin 1979)
[3] The maximum peak (sulfur) was set at 20,000 counts as a reference point

The x-ray data in Table 1 also indicates that higher pH increases the amount of adsorption or "binding" of salts by the membrane. (Part of the increase in potassium is due to the KOH used to adjust the pH.) This could be related to the solubility of the salts themselves, rather than due to any increase in charge effects per se; higher pH results in lower solubility of phosphates, which will cause them to precipitate out and deposit on the membrane. Lowering the pH, on the other hand, will cause less deposition on the membrane and presumably less resistance to flow of liquid through the pores. This could explain why Breslau *et al.* (1977) observed higher flux when the pH of cottage cheese whey was lowered to 1.5.

The presence of salts on the membrane surface could aid in the binding of protein to the membrane, by acting as a "salt bridge" between membrane and protein, thus resulting in faster fouling of the membrane. This implies that removal of salts should have a beneficial effect on fouling rates, since it will result in a slower build-up of the fouling layer. This hypothesis will be tested further.

Ultrafiltration of Protein Solutions

Individual proteins of the whey system were made up to the concentration as they exist in whey and ultrafiltered as described earlier. To separate the effect of salts and protein on fouling, two different aqueous systems were investigated with each protein: a salt-free (SF) and a whey dialysate (WD) environment. Figure 3 shows typical data of cumulative volume vs time obtained during ultrafiltration runs with each protein in the whey dialysate environment and with native whey. The lines are the fitted curves and the plotted points are experimental observations. Each line could be characterized by a set of k, b and △t values. Mean
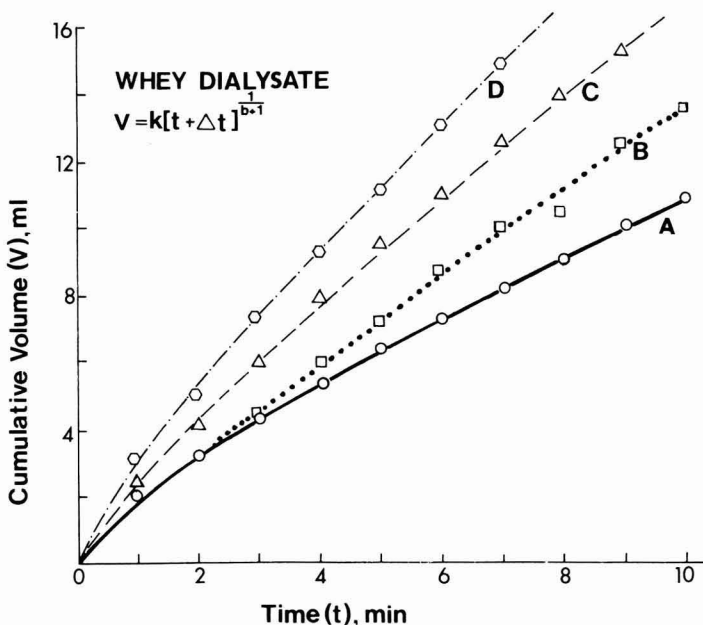
FIG. 3. PERMEATION OF WHEY AND INDIVIDUAL PRO-
TEINS IN THE WHEY DIALYSATE SYSTEM IN THE INITIAL
TEN MINUTES OF ULTRAFILTRATION

Same conditions as in Fig. 2. Lines are the fitted curves accord-
ing to Eq. 4. Points are the experimental data. (A = Whey,
B = α-lactalbumin, C = β-lactoglobulin, D = bovine serum albumin.)

values of the constants k, J_o and b for each of the eight protein systems
were obtained from the regression equation and are listed in Table 2.
From the constants and the differentiated form of Eq. (4), the instan-
taneous flux at any time could be obtained. This is shown in Fig. 4 for
the whey dialysate environment. A similar set of curves was obtained
for the salt-free system (Cheryan and Merin 1979).

With the experimental set-up used in this study, it is difficult to ob-
tain reproducible data in the first 30—60 s of ultrafiltration, due mainly
to errors in initial timing or in accounting for the hold-up in the appara-
tus. Nevertheless, similar two-stage flux decline as shown in Fig. 4 is
commonly observed for many macromolecular systems (Howell and
Velicangil 1979, Sirkar *et al.* 1979, Matthews *et al.* 1978).

Since J_o is essentially the intercept at t = 0 of the flux-time curve,
higher values of J_o imply higher flux at the start of the ultrafiltration
process. In practice, of course, the highest value of J_o should be 4.56
ml/min, equal to the water flux. In general, J_o values of the protein
systems are one-third to one-fourth that of the water flux, a phenomenon

Table 2. Computed values of constants[1] of mathematical model for each protein/salt system

Component	Environment	k	J_o	b
Water	—	—	4.56	0.000
Whey	— Native	1.56	1.37	0.297
	— SF	1.98	1.90	0.163
α-lactalbumin	— WD	1.80	1.72	0.123
	— SF	1.66	1.55	0.160
β-lactoglobulin	— WD	2.65	2.69	0.214
	— SF	3.08	3.16	0.136
Bovine serum albumin	— WD	2.76	2.78	0.147
	— SF	1.89	1.85	0.069

[1] Mean values obtained from regression equation (Merin 1979)
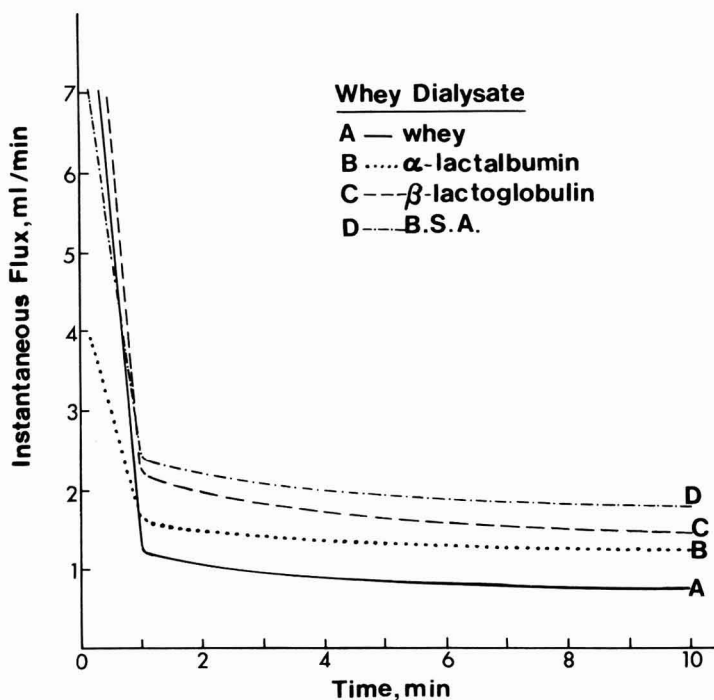SF = salt free system; WD = whey dialysate system

FIG. 4. RATE OF FLUX DECLINE AS A FUNCTION OF TIME WITH WHEY AND INDIVIDUAL WHEY PROTEINS IN THE WHEY DIALYSATE ENVIRONMENT

Instantaneous flux was calculated as the first derivative of Eq. 4 and using values of k and b from Table 2.

also observed by Howell and Velicangil (1979). In protein ultrafiltration, J_o is dependent on the operating parameters and physical properties of the feed and can be predicted fairly well using membrane permeability or mass transfer limited models (Cheryan 1977). In other words, J_o is an indication of the resistance to solvent transport by the membrane as well as the concentration polarization layer formed on the membrane by the proteins. Lower values of J_o indicate greater tendency for formation of the gel layer.

Among individual proteins, Table 2 indicates that α-lactalbumin has the greatest gel-forming tendency (lowest J_o values) while β-lactoglobulin has the least. One possible explanation is that since the α-lactalbumin molecule has the lowest molecular weight of the whey proteins investigated here, it may be small enough in size and shape to just "fit" in the pore opening and effectively block it by "crowding" the opening, analogous to the situation existing during ultrafiltration of sugar solutions (Baker *et al.* 1972). If the α-lactalbumin molecule sits firmly enough in the pore, it may not be removed from its place on the membrane surface by the usual shear forces due to stirring of feed. On the other hand, β-lactoglobulin normally exists as an octamer at the pH of the whey (Hayes *et al.* 1974, Whitney 1977) and this spherical molecule will simply "roll-off" from the membrane surface by high shear rates. This hypothesis is confirmed to a certain extent by Muller *et al.* (1973) and Hayes *et al.* (1974) who observed that changing the pH of the whey so that β-lactoglobulin will dissociate to its dimer form, having a lower molecular weight than the octamer form, results in lowered flux. It is apparently easier for normal shear forces to remove larger molecules than smaller ones.

Our finding that α-lactalbumin has the greatest effect on initial flux doesn't necessarily contradict other researchers (Lee and Merson 1975, 1976, Lee *et al.* 1975) who consider β-lactoglobulin to be the principal foulant. These researchers apparently only considered the long-term effects of fouling while we consider α-lactalbumin to be the cause of immediate loss of flux due to its apparently greater gel-forming tendencies, i.e., in the short-term period. A better indication of long term fouling effects is obtained from the b value, which is essentially the slope of log flux vs log volume, as shown in Eq. (2) and in Fig. 5. Among the individual proteins, β-lactoglobulin has the greater effect on long-term fouling (highest b value) while bovine serum albumin has the least (higher values of b imply greater fouling effects). Furthermore, if the experimental curve was extrapolated beyond the observed points, β-lactoglobulin would appear to eventually have a lower flux than α-lactalbumin, in essential agreement with the other workers mentioned earlier.
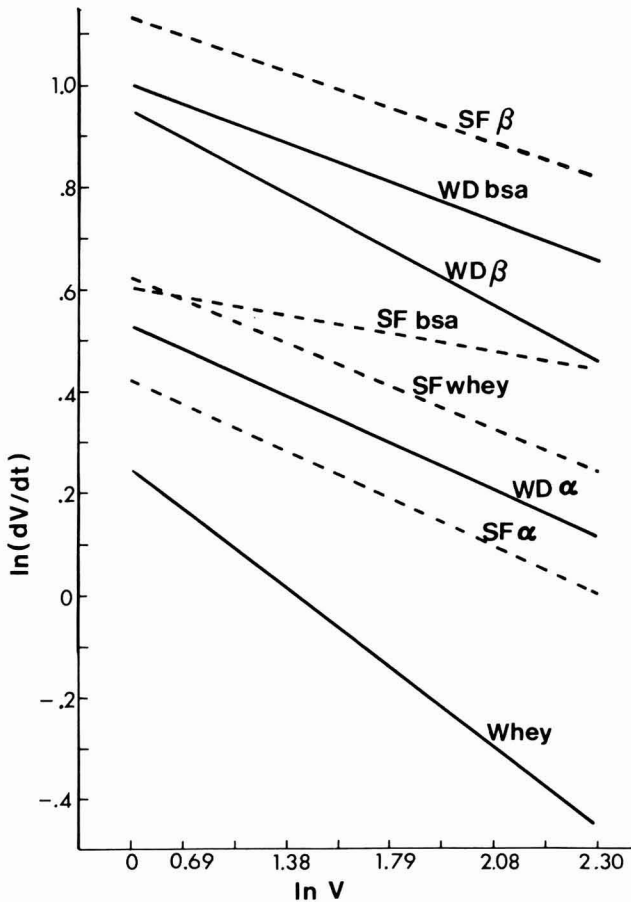
FIG. 5. RATE OF FLUX DECLINE AS A FUNCTION OF
CUMULATIVE VOLUME

Lines calculated using Eq. 2 and k and b vlaues from Table 2.
(WD = whey dialysate buffer system, SF = salt-free buffer
system. α = α-lactalbumin, β = β-lactoglobulin, bsa = bovine
serum albumin).

We interpret b values as indicative of true fouling effects, due perhaps
to specific membrane-solute interactions. The combined effect of the
individual proteins is represented by the native whey system, which of
course has the highest b value and lowest flux of the solutions studied.

Effect of Removal of Salts on Fouling Rates

It is interesting to compare the effects of a salt-free environment with
that of the environment of native whey, as represented by the WD sys-

tem. The b value for the native whey, β-lactoglobulin and bovine serum albumin is lower in the salt-free (SF) system, while the b value for α-lactalbumin is slightly higher in the SF system than the WD system. This indicates a possible change in the physical properties and fouling behavior in the presence of whey salts, although the behavior is not consistent enough between individual proteins to pinpoint a single major contributor to fouling.

Tables 3 and 4 show the analysis of variance for k and b, respectively. The k values are not significantly different between the SF and WD systems, which might indicate that salts have little effect on the gel forming ability of the proteins, or it might be a reflection of the wide range of k values between the individual proteins. However, the b values in Table 4 show that there is a significant difference between the two systems as well as significant differences between individual proteins. It appears that each protein has its unique fouling pattern during the early period of ultrafiltration considered in this study and that salts have a significant influence on long-term fouling rates. These results suggest that the complete or even partial removal of salts from whey should substantially improve flux. Hayes *et al.* (1974) and Delaney and Donnelly (1975) confirmed this when they pretreated whey by ion-exchange or electro-dialysis prior to ultrafiltration and obtained an improvement in flux. In this study also, removal of salts significantly reduced the rate of fouling of native whey (Table 2, Fig. 5).

Table 3. Analysis of variance for k

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F (cal)
System (S)	1	0.0372		0.504⁻
Protein (P)	3	5.8027	1.9342	26.173 **
Whey vs others	1	1.4090		19.066 **
α vs β + BSA	1	3.2490		43.964 **
β vs BSA	1	1.1446		15.488 **
$(\alpha$ vs $\beta)^a$	(1)	(4.3930)		(59.444)**
$(\alpha$ vs BSA)^a	(1)	(1.0528)		(14.246)**
S $\times$ P	3	1.9101	0.6367	8.616 **
S $\times$ whey vs other	1	0.3604		4.877 **
S $\times$ α vs β + BSA	1	0.0367		0.497⁻
S $\times$ β vs BSA	1	1.5129		20.472 **
(S $\times$ α vs $\beta)^a$	(1)	(0.2015)		(2.727)⁻
(S $\times$ α vs BSA)^a	(1)	(0.6100)		(8.255)**
Error	15	1.1085	0.0739	
Total	22	8.8586		

[a]Not orthogonal comparisons
⁻ = Not significant at less than 5% level; ** = Very significant
For meaning of symbols, see Fig. 5.

Table 4. Analysis of variance for b

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F (cal)
System (S)	1	0.0240		22.463 **
Protein (P)	3	0.0509	0.0169	15.875 **
Whey vs others	1	0.0353		33.031 **
α vs β + BSA	1	0.0021		1.974⁻
β vs BSA	1	0.0134		12.618 **
(α vs β)[a]	(1)	(0.0095)		(8.957)**
(α vs BSA)[a]	(1)	(0.0003)		(0.313)⁻
S $\times$ P	3	0.0250	0.0083	7.809 **
S $\times$ whey vs other	1	0.0098		9.231 **
S $\times$ α vs β + BSA	1	0.0135		12.666 **
S $\times$ β vs BSA	1	0.0016		1.530⁻
(S $\times$ α vs β)[a]	(1)	(0.0146)		(13.695)**
(S $\times$ α vs BSA)[a]	(1)	(0.0064)		(6.067)**
Error	15	0.0160	0.0010	
Total	22	0.1160		

[a]Not orthogonal comparisons
See Fig. 5 for meaning of symbols

In conclusion, salts appear to have profound influence on the rate of flux decline during ultrafiltration of cottage cheese whey. The exact role of salts is unclear, but it could cause changes in configuration or shape of individual protein molecules that would cause a greater build-up of the protein fouling layer, or it could form a "salt bridge" between protein and membrane, thus leading to more rapid fouling. Attempts were made to correlate these observed effects with the nature of the protein deposits on the membrane as examined under the electron microscope; these studies are reported elsewhere (Cheryan and Merin 1979).

Among the individual proteins, as seen in Fig. 5, α-lactalbumin has the greatest overall flux-depressing effect. This is especially surprising if one remembers that this component is not only one of the smallest of the whey proteins investigated, but is also present in a very low concentration (Merin 1979, Whitney 1977). The flux-depressing effect of α-lactalbumin would appear worse if considered on an equal percent protein basis. As mentioned earlier, it is difficult to compare the results of this work with others since experimental conditions such as ionic strength, salt composition and pH differed between this and other studies. In addition, our experience with initial water flux indicates it is necessary to prime the membrane before starting investigations of this nature, a step that other investigators have not particularly stressed in their publications.

Based on these results, it appears that it is very important to minimize interactions of the membrane with feed components such as salts and proteins. The easiest way is to deionize or remove salts from the feed prior to ultrafiltration. The cost-benefit ratio of such a treatment, however, may not be favorable and will have to be evaluated by individual processors.

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EFFECT OF BAKING AND TOASTING ON THE PROTEIN QUALITY AND LYSINE AVAILABILITY OF BREAD[1]

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ABSTRACT

The protein efficiency ratio (PER) and net protein ratio (NPR), the protein digestibility in vivo *(in rats) and* in vitro *(multienzyme digestion), and the lysine availability* in vivo *(in rats) and* in vitro *(fluorodinitrobenzene method) of unbaked bread mix, bread, crust, crumb, and bread toasted at two levels of darkening were determined. Compared to an adjusted PER of 0.75 for the unbaked bread ingredients, the PER of the entire loaf was 0.46, but that of the crumb was 0.91, while the crust showed a negative PER, −0.23. The corresponding NPR's were 1.51, 1.22, 1.62, and 0.69. Light toasting reduced the PER of the bread to 0.40 (NPR 1.11) and dark toasting to 0.16 (NPR 0.95). The protein digestibility decreased as the bread ingredients were exposed to greater amounts of heat (baking, light and dark toasting), but the crumb protein of untoasted bread was slightly more digestible* in vivo *than the protein of the raw bread ingredients. The total lysine content also decreased with increasing exposure to heat, the crumb lysine content being an exception. The available lysine content, as measured by either the biological or the chemical methods, suffered significant decrease as the heat exposure increased, and correlated very highly with PER (available lysine* in vivo *vs PER, r = 0.988; available lysine* in vitro *vs PER, r = 0.979).*

INTRODUCTION

An area of active current research is the bioavailability of nutrients in foods subjected to various processes. One nutrient on which consider-

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able interest has been focused is lysine, because it is an essential amino acid which limits the nutritional value of cereal proteins and readily reacts with other food constituents. Baking is thought to have a damaging effect on the protein quality of bread because of lysine destruction (Rosenberg and Rohdenburg 1951; Sure 1952; Sabiston and Kennedy 1957; Ericson *et al.* 1961; Jansen *et al.* 1964; Tsen *et al.* 1977). Toasting further aggravates the damage on the nutritive value of bread protien (Tsen and Reddy 1977).

In this study the heat effect of baking and toasting on the nutritive quality of bread protein was assessed in terms of rat growth, protein digestibility and lysine availability; an attempt was also made to differentiate the heat effect on the crust from that on the crumb of the bread.

MATERIALS AND METHODS

Unbleached bakery flour containing wheat flour, malted barley flour and potassium bromate was obtained from International Multifoods, Minneapolis, MN. The following solid ingredients (bread mix) were used: flour 100 parts, sugar 6 parts, yeast (granular) 3 parts and salt 1 part. The dough was developed with 65 parts of water in a Hobart mixer, shaped into loaves weighing approximately 1.7 kg and placed in $37 \times 10 \times 11$ cm pans. After a 30 min initial fermentation at $30°C$ the loaves were punched down and left to ferment for another 40 min at the same temperature. The baking was done in a preheated oven at $190°C$ until a gold-brown crust was formed (approx. 55 min).

Part of the bread was separated into crumb and crust with a knife and part of it was cut into 1.1 cm thick slices. The slices were toasted in a household toaster either for 1.5 min or 2.5 min. The air temperature in the center of the toast slot reached $270°C$ and $350°C$ during the 1.5 and 2.5 min toasting cycle, respectively. The 1.5 min toasting produced light toasted bread (LTB) and the 2.5 min toasting resulted in dark toasted bread (DTB). Additional bread was prepared from a mix that contained 4 parts of non-fat-dry-milk-solids per 100 parts of flour. This mix will be indicated as NFDM-mix and the bread made from it as NFDM-bread.

Bread, crust, crumb and toast were dried overnight in a forced-air cabinet at $35°C$. They were then ground to pass through a 0.5 mm mesh screen and kept refrigerated until used. Moisture, fat, ash and nitrogen were determined by AOAC (1975) procedures.

Color Measurement

The color of the ground samples was measured in a Hunter Color Difference Meter, D-25. The instrument was calibrated with standard tile No. 2814 (L 83.0; a_L −3.5; b_L 26.5). Four readings were taken and averaged for each sample after rotating the sample disk by 90°.

Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR)

PER was assessed by the AOAC (1975) procedure, modified to the extent that N X 5.75 was used to calculate bread protein content. Ten male, Sprague-Dawley, 21-day old rats were used per diet. One group of ten rats was fed a non-protein diet and the weight loss of that group was used in calculating NPR.

Apparent nitrogen digestibility was measured *in vivo* and *in vitro*. For the percent apparent N digestibility (% AND) *in vivo* the following formula was used:

$$\% \text{ AND} = \frac{\text{N in diet consumed} - \text{N in feces}}{\text{N in diet consumed}} \times 100$$

The amounts of consumed and fecal nitrogen were measured using the rats of the PER assay during the period from the 16th to the 26th day of the test. For estimating % AND in vitro the trypsin-chymotrypsin-peptidase technique of Hsu *et al.* (1977) was used. In this test the pH of the sample suspension and the pH of the multienzyme solution are adjusted to 8.0; the pH is measured again after mixing the two systems for 10 min. The drop of pH is converted to % AND by means of a formula. The buffer capacity of common foods is not expected to affect the results, but it is advisably measured.

Available Lysine by Rat Growth Assay

For this assay a reference curve was prepared relating weight gain of weanling rats to lysine consumed from a diet containing pure amino acids as the only source of nitrogen. The diet contained 18 amino acids; all except lysine were present in quantities equal to those suggested by the National Academy of Sciences (1972) plus an amount corresponding to 80% of that present in wheat flour (Kuiken and Lyman 1948); e.g. the amount of L-proline suggested by NAS is 0.44 g/100 g diet, 80% of the L-proline content of wheat flour is 0.20 g/100 g flour (Swaminathan 1967) and the total in the reference diet is 0.64 g/100 g diet. Since L-lysine was added to the basal diet at five different levels, that is 0.0, 0.1, 0.2, 0.3 and 0.4 g/100 g diet, it was necessary to prepare five refe-

rence diets. These diets also contained 5.5% corn oil, 5% salt mixture, 1% vitamin mixture, 1% nonnutritive fiber and sufficient quantities of corn starch and sucrose to complete the composition (to 100%). The salt and vitamin mixtures were the same with those used for the PER assay. The wheat products to be tested for lysine availability were incorporated into the basal NAS diet by replacing a portion of the carbohydrates of the diet; a sufficient quantity was added to make the diet contain 10% wheat product protein. A similar technique was successfully used by Calhoun et al. (1960). Six rats meeting the AOAC specifications for the PER test were assigned to each reference and test diet. According to Gupta et al. (1957) the lysine availability of the reference diets should be close to 100%.

Available Lysine by the FDNB Procedure

Carpenter's direct FDNB method as modified by Booth (1971) was used. A recovery factor was calculated by using synthetic ε, DNP-lysine as internal standard in quantities similar to those contained in the samples. The values for available lysine in the samples were corrected for only half the losses of the internal standard (Booth 1971).

Amino Acid Analysis

All amino acids, except methionine, cystine and tryptophan, were determined by the method of Moore et al. (1958), after hydrolysis of the proteins with 6N HCl. The S-containing amino acids were determined after performic acid oxidation according to the procedures of Shram et al. (1954) and Lewis (1966). Tryptophan was measured by the Spies Procedure W (1967).

RESULTS AND DISCUSSION

Proximate Analysis

Table 1 shows the moisture, protein, ash and fat contents of the dried and ground bread samples. Crumb represented 75% and crust 25% of the weight of the loaves.

Color

Table 2 contains the color measurements. The L-values reflect the darkening observed in the bread items as the heat treatment became more severe.

Table 1. Composition of dried and ground samples

	Moisture %	Protein, % (N × 5.75)	Ash %	Crude Fat, %
Bread mix[1]	11.5	13.1	1.3	0.6
Whole bread	4.7	13.3	1.3	0.5
Crumb	4.9	13 4	1.3	0.6
Crust	4.5	13.1	1.4	0.5
LTB	4.6	13.0	1.3	0.6
DTB	4.3	13.0	1.3	0.6
NFDM-mix[1]	10.1	13.6	1.1	0.6
NFDM-bread	4.8	13.5	1.3	0.7

[1] These two mixtures were not dried

Table 2. Color of the dried and ground samples. Hunter Color Difference Meter values

	Bread Mix	Whole Bread	Crumb	Crust	LTB	DTB	NFDM-Mix	NFDM-Bread
L	81.8	61.6	74.9	51.5	60.1	47.9	81.9	62.0
a	1.0	3.9	.75	6.0	4.2	4.1	.95	3.7
b	9.7	14.8	12.50	16.6	14.4	12.1	9.85	15.3

PER and NPR Values

Table 2 summarizes the results of the PER and NPR tests. The protein of the crust was damaged most severely. The PER of the crust was negative (-0.23), as the young animals not only did not grow, but lost weight (average loss 4.7 g), when crust was the only source of protein during the 28-day test period. Extensive was the damage suffered by the protein of DTB, the PER of which was only 21% that of the unbaked bread mix. The PER of LTB was 53% and that of the bread (baking only) 61% that of the unbaked mix. Light toasting caused a 13% decrease in the PER of the baked bread, while dark toasting caused a 65% decrease in the PER of the baked bread. Crumb had a PER 21% higher than that of the unbaked bread mix. Apparently, the mild heating of the crumb during baking caused a slight denaturation of the proteins and thus increased their utilization by the rat. Fortification of the bread mix with 4% NFDM solids resulted in a PER 56% higher than that of the plain bread mix; the PER of NFDM-bread was 63% over that of plain bread.

The NPR values ranked the proteins of the samples in the same order as the PER method. However, the NPR values were higher than the cor-

responding PER values. This is understandable as NPR takes into account the maintenance requirements of the rats in addition to the growth requirements.

Digestibility

Table 4 shows the apparent N digestibility of the samples tested *in vivo* and *in vitro*. The samples show a variation in their apparent N digestibilities *in vivo* which are, in general, parallel to the intensity of heat treatment. Both breads (plain bread and NFDM-bread) had apparent N digestibilities lower than the mix of ingredients from which they were made. Crust, LTB and DTB had lower values than the less heated whole bread, crumb and bread mix. The *in vivo* N digestibility of the crumb was slightly greater than that of the non-heated bread mix. The *in vitro* N digestibility values were in general agreement with their corresponding values *in vivo*. The buffer capacities of the protein samples did not differ much from each other and were not expected to have affected the measurement of the *in vitro* digestibilities.

Table 3. PER (adjusted to PER = 2.50 for casein) and NPR values of bread products

	Adjusted PER[1]	NPR[1]
Bread mix	0.75[c]	1.51[c]
Bread	0.46[b]	1.22[b]
Crumb	0.91[d]	1.62[d]
Crust	−0.23[a]	0.69[a]
LTB	0.40[b]	1.11[b]
DTB	0.16[a]	0.95[a]
NFDM-mix	1.17[e]	1.84[e]
NFDM-bread	0.75[c]	1.51[c]
Casein	2.50	3.23

[1] Values with different letter superscripts differ significantly ($P < 0.05$), according to Duncan's multiple range test

Amino Acid Analysis

The total amino acid composition of the samples is shown in Table 5. A comparison of the essential amino acid values of the samples with those of the 1973 FAO/WHO reference pattern of essential amino acids reveals that lysine is the first limiting amino acid in all samples with threonine being the second limiting amino acid. The total lysine content of the products decreased with increasing heat treatment, the values

Table 4. Apparent N digestibility (%) of experimental diets, in vivo and in vitro. Buffering capacity

	Bread Mix	Whole Bread	Crumb	Crust	LTB	DTB	NFDM-Mix	NFDM-Bread	Casein
In vivo	86.3	83.8	88.3	79.9	82.7	77.2	88.8	83.8	91.9
In vitro	86.1	81.5	83.4	79.5	81.8	81.6	86.4	82.9	88.6
Buffering capacity[1]	11.4	7.3	7.3	8.2	7.3	7.6	8.2	8.0	9.4

[1] Milliliters of 0.01 HCl required to decrease the pH of the sample from 8 to 6.45 in 10 min

Table 5. Total amino acid composition of samples (expressed as g residue/100 g protein)

	FAO/ WHO	Bread Mix	Whole Bread	Crumb	Crust	LTB	DTB	NFDM- Mix	NFDM- Bread
Lys	5.5	2.7	2.5	2.7	2.1	2.4	2.1	3.2	3.0
Thr	4.0	2.1	2.3	2.4	3.0	2.7	2.4	3.0	2.3
Cyst	3.5	2.0	1.9	1.9	1.8	1.9	1.9	2.0	2.0
Met		1.7	1.8	1.8	1.7	1.8	1.8	1.7	1.7
Val	5.0	3.9	4.1	3.9	4.4	4.1	4.1	4.4	4.4
Ile	4.0	3.6	3.7	3.4	3.9	3.8	3.7	3.5	4.0
Leu	7.0	6.2	7.2	7.6	7.7	7.6	6.4	6.4	8.5
Tyr	6.0	2.7	3.0	2.6	3.6	3.3	3.0	3.1	3.2
Phe		4.0	4.3	4.0	5.1	4.8	4.5	4.4	4.8
Trp	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
His	—	2.0	2.5	2.1	3.5	2.1	1.6	2.0	2.2
Arg	—	3.6	4.9	4.9	4.3	4.5	3.8	4.8	4.7
Asp	—	3.4	4.1	3.7	3.9	4.3	3.9	4.8	4.6
Ser	—	3.6	3.5	3.8	3.9	4.2	3.7	3.7	4.0
Glu	—	43.7	39.4	42.4	29.8	36.5	41.2	37.8	36.7
Pro	—	9.4	9.0	63	11.8	8.3	9.2	9.2	7.8
Gly	—	3.5	3.5	3.6	3.8	3.7	3.8	3.2	3.1
Ala	—	2.7	2.9	2.6	3.4	3.7	2.8	2.8	2.9

varying from 2.7 g/100 g protein for the bread mix, to 2.1 g/100 g protein for the crust. The NFDM fortification increased the lysine content of NFDM-mix to 3.2 g/100 g protein and that of NFDM-bread to 3.0 g/100 g protein.

Available Lysine

The values for available lysine obtained by the rat growth assay and by the FDNB method are shown in Table 6 along with the total lysine values. The difference in total lysine between each sample and the non-heated (bread mix) sample represents destroyed lysine, i.e. that portion of the lysine that is not recovered after HCl hydrolysis. The difference between total lysine and available lysine by rat growth assay for each sample gives the inactive lysine. The sum of inactive and destroyed lysine constitutes the total unavailable (nutritionally lost) lysine.

Correlation Between Lysine Content and Nutritional Value of Bread Protein

Tables 3 and 6 suggest a multiple relationship between lysine content and PER or NPR values. Indeed, the correlation coefficients between these two measures of nutritional quality of protein and a) available lysine content by rat growth assay (ALB), b) available lysine content

Table 6. Total, destroyed, inactive and available lysine values of bread products (g/100 g protein)

	Bread Mix	Whole Bread	Crumb	Crust	LTB	DTB	NFDM-Mix	NFDM-Bread
Total lysine (HCl acid hydrol.)	2.7	2.5	2.7	2.1	2.4	2.1	3.3	3.0
Destroyed lysine	0.0	0.2	0.0	0.6	0.3	0.6	0.0[1]	0.3[1]
Available lysine (rat growth assay)	2.1	2.0	2.4	1.0	1.9	1.5	2.7	2.2
Inactive lysine	0.6	0.5	0.3	1.1	0.5	0.6	0.6	0.8
Available lysine (FDNB-method)	2.4	2.2	2.6	1.5	2.0	1.6	2.9	2.5

[1] Calculated on the basis of NFDM-mix lysine content

by the FDNB test (ALC), and c) total lysine content (TL) are large, as
Table 7 indicates. The same Table contains the linear regression equa-
tions corresponding to these correlation coefficients. It appears that the
process by which lysine loses nutritional value in baking and toasting
follows first order reaction kinetics (the unavailable lysine is propor-
tional to the total lysine). The FDNB test, which is simple and quick,
emerges as a reliable test for estimating PER.

Table 7. Correlation coefficients (r) and regression equa-
tions relating PER and NPR values to available lysine con-
tent determined biologically (ALB), available lysine content
determined chemically (ALC) and total lysine content (TL)

Link	r	Regression equation
PER—ALB	0.988	PER = 0.833 (ALB) − 1.109
NPR—ALB	0.973	NPR = 0.686 (ALB) − 0.057
PER—ALC	0.979	PER = 0.892 (ALC) − 1.427
NPR—ALC	0.845	NPR = 0.770 (ALC) − 0.397
PER—TL	0.918	PER = 0.979 (TL) − 1.998
NPR—TL	0.934	NPR = 0.853 (TL) − 0.912

The results of this work show that both conventional baking and
toasting decrease the nutritive value of wheat flour protein for the grow-
ing rat and this decrease is related to lysine availability. It must be point-
ed out, however, that the drop in protein value caused by baking is due
to changes occurring in the crust, and that the crumb contains more
available lysine and a higher PER than the unbaked bread mix. It is
ironic that the part of the bread which many people like best, the crust,
is the least nutritious, and that both the nutritional damage to the pro-
tein and the sensory appeal of the crust are chiefly due to the Maillard
reaction. It is nutritionally fortunate, on the other hand, that modern
commercial bread making has been successful in minimizing the pro-
portion of crust.

In a similar type of investigation, Tsen and coworkers (1977) showed
that steaming and microwave baking result in bread of higher nutritional
value than conventional baking and that toasting further reduces the
nutritive quality of bread. Our work focuses on the role available lysine
plays in both the improvement of protein quality in the crumb and the
lowering of said quality in the crust and the whole bread, as a result of
baking and toasting.

Regarding methodology, the FDNB test appears to consistently over-
estimate lysine availability in the products analyzed here. In the crust,

the FDNB estimate is considerably higher than that of the biological assay. This may be due to increased cross-linking between protein molecules at higher temperatures, resulting in the formation of undigestible peptides, which, however, contain FDNB reactive lysine units (Valle-Riesta and Barnes 1970).

ACKNOWLEDGMENT

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A NEW TECHNIQUE OF DERIVATIVE THERMAL MONITORING OF CALCIUM ORTHOPHOSPHATE — A FOOD PRESERVATIVE — IN A BINARY SYSTEM

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ABSTRACT

The preservative and nutritional qualities of calcium orthophosphate (TCP) has been previously reported. However, its protective use in processed foods and flour is mainly based on the purity of the salt. Most of the commercial salts screened were found to contain dicalcium orthophosphate as an impurity which is beneficial to insect breeding. A new quantitative method has been described for the estimation of TCP content in a binary phosphate system. The method is based on monitoring the solid phase decomposition of two salts under a controlled thermal environment. A unique technique of measuring the intermediary decomposition phase and estimating the adulterant in TCP is described and the thermal reactions are discussed.

INTRODUCTION

For the protection of wheat flour, semolina and other processed foods from insect infestation, calcium orthophosphate (TCP) is increasingly being used as a preservative (Majumder and Bano 1964). The salt while enhancing the nutritional quality of the food commodity causes pathological changes in the insect pests and inhibits their breeding (Bano and Majumder 1965). For effective insect control calcium orthophosphate of high purity is essential (Rao *et al.* 1971).

While screening different commercial samples of TCP, dicalcium orthophosphate (DCP) was found as a common contaminant of TCP. This binary salt promotes insect breeding (Bano and Majumder 1968) resulting in the reduction of nutritional quality and increase in uric acid content thereby, endangering human health. The proper monitoring of TCP for any impurity is imperative before using this salt as a protectant in processed foods. There have been no reliable analytical methods for

monitoring a binary mixture of calcium phosphate. This paper describes a new method which has been developed for the quantitative estimation of DCP-TCP mixtures using Derivative thermogravimetry (DTG).

Principle of the Method

A thermally sensitive compound in controlled dynamic thermal environment undergoes mass change (W). The first derivative of this change recorded against temperature (T) or time (t) depicts the characteristic reaction rate specific to the compound.

MATERIALS AND METHOD

Calcium orthophosphate $Ca_3(PO_4)_2$ obtained from Monsanto Chemical Company USA and Dicalcium orthophosphate $CaHPO_4 \cdot 2H_2O$ obtained from E-Merck Ag. DERMSTADT were used in the present study.

A binary model of the above salts were prepared by thoroughly mixing DCP with TCP at 1%, 10%, 25%, 50% and 75% levels in a Braun laboratory powder mixer. Further the samples were then ground in agate mortar and passed through −100 +120 Tyler standard sieves. The samples were placed in a 5 cm diameter petri dishes and equilibriated at 55% RH and 25°C for 48 h using a Gallenkamp humidity chamber.

One gram of the sample was taken in a Mullite sample holder of 100 mm X 10 mm dia and hooked up into a thermal analysis (TA) system (Venugopal et al. 1977). The sample was placed in an air environment. A linear program of 5°C/min ± 0.3°C for the Furnace was employed and the first derivative of the binary mass reactions (dw/dt) were recorded and decomposition peaks were characterized.

RESULTS AND DISCUSSION

The thermal reaction of TCP-DCP binary mixture (Fig. 1) indicated major decomposition phases between 70−116°C and at 320°C. They also exhibited many minor decomposition steps around 256°C and from 400−600°C. Many of these decomposition peaks were more due to the level of contaminants in the various binary mixtures. The major decomposition peak at 320°C showed a proportional relationship with the composition of the binary mixtures and also exhibited a characteristic weight loss pattern at 230°C (Fig. 2). A linear relationship between weight loss and salt composition was evident. The reaction derivatives were inversely proportional to TCP content (Fig. 2).
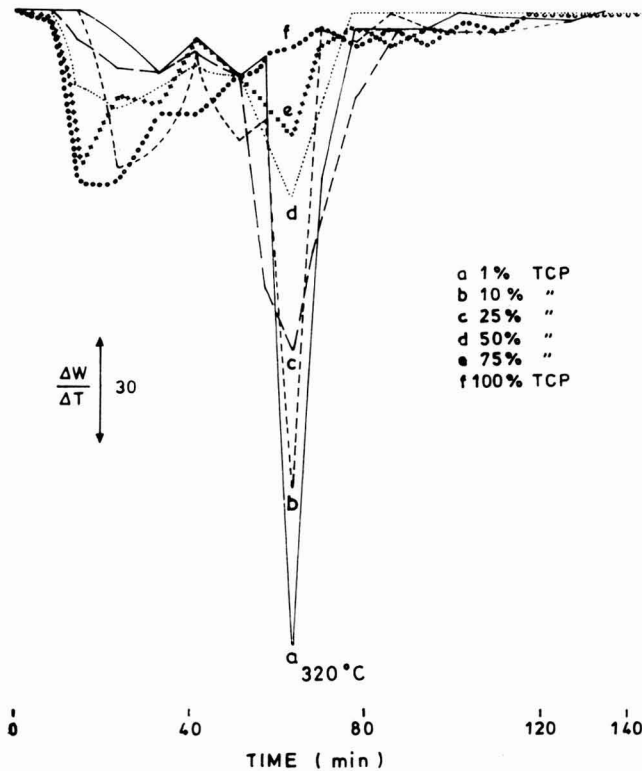
FIG. 1. DTG OF TCP-DCP BINARY MIXTURE

In the first decomposition stage (Fig. 1) a curious phenomena was observed. While pure TCP showed a major inflection, an addition of 1—25% of DCP (abc) significantly reduced the magnitude instead of the expected increasing trend. The peak observed around 70—116°C for TCP, was due to the normal loss of hydration. The presence of DCP, although in small amounts, enhanced the hydroxyl environment in the binary system. At the temperature of 164°C, DCP liberated one mole of water which was taken up by TCP. β-phase hydroxy apatite was formed due to solid state change (Boulle 1954; Thilo and Grunze 1957) which was stable till 210°C. With the increase in temperature DCP component of the binary system decomposed further, liberating second mole of water at 288°C forming a pyrophosphate (CPP) phase (Wallacy 1952). With the progressive loss of hydrated water in the binary system, the pyrophosphate phase increased proportionately attaining a maximum reaction rate at 320°C. Thus the energy level of CPP phase was markedly sensitive to the interaction of DCP. Furthermore, the pyrophosphate phase which originates normally around 430°C in
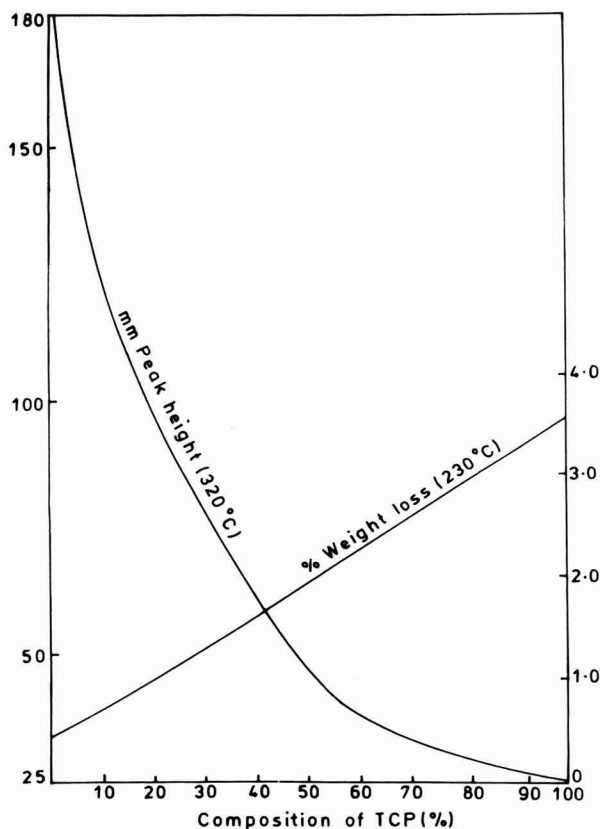
FIG. 2. RELATIONSHIP OF TCP CONCENTRATION
WITH PEAK HEIGHT AND MASS CHANGE

TCP (Venugopal *et al.* 1978) showed a significant lowering of the energy level by about 180°C with the addition of small concentration of DCP. Therefore the pyrophosphate phase in binary system appeared at a much lower temperature than pure TCP. The varying peaks observed at 320°C in the binary mixtures were due to the pyrophosphate content. Since the amount of CPP formation is linked with the available DCP the level of contamination in TCP can easily be determined (Fig. 2).

The above study has demonstrated a sensitive method of estimating the DCP adulterant in TCP. The method has application in flour milling, processed food industries and in bakery at dough premix stage. The analysis is fairly rapid and can also be extended for the detection of super phosphate quality.

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BOOK REVIEW

Tropical Foods: Nutrition and Chemistry, Volumes 1 (340 pp) and 2 (701 pp). Edited by George E. Inglett and George Charalambous. Academic Press. New York. 1979.

These two volumes are the Proceedings of an International Conference on Tropical Foods Chemistry and Nutrition held in 1979. Many different types of plant-derived foods are discussed including fruits, vegetables and cereals. Some of these fruits and vegetables are almost unknown in the United States. No more than a dozen pages are devoted to foods of animal origin such as meat, fish, eggs and milk. Some of the more exotic sources of "meat" include bats, reptiles, insects and larvae.

A general theme running through both volumes is the improvement of the general nutritional status of people in underdeveloped and developing regions of the Tropics through utilization of indigenous foods. Since foods of marine origin could provide a substantial portion of total protein needs, it seems unfortunate that they were given such short shrift. However, the plant-derived foods are covered quite broadly and selected ones are covered in considerable depth, e.g., papaya, citrus fruits, avocado, banana, prickly pear, mirlitons and eggplants, yams and pulses. The food processes described range from simple village industry processes for soybeans to the sophisticated temperature-accelerated short-time evaporation (TASTE) process for producing citrus concentrate for freezing. Food products covered range from very specific items such as sugar to broad classes of products such as foods from rice, wheat, maize, sorghum and pearl millet, soybeans, peanuts and bananas. A number of the authors discuss fermented foods such as tempeh, soy sauce and other lesser known products. In some cases, chemistry is covered in great detail, e.g., the papaya. The nutritional properties of these tropical foods is stressed in a number of papers.

The good news is that these two volumes provide a ready source of reference material on plant-derived tropical foods in general and on specific tropical foods of a more exotic nature. One would have to sift through a lot of foreign journals to unearth this much information.

However, there is also bad news. These volumes suffer from all of the problems and shortcomings of symposia proceedings put together in this fashion. The individual papers were reproduced directly from the typed manuscripts in no less than a half dozen different type styles. The material is not organized in any coherent pattern. Some papers are reviews. Others are strictly research reports and might be more appropriate for publication in scientific journals. Some papers are very general;

others are very specific. There is some overlap and duplication, as for example, between Standal's paper "Tropical Foods of the Pacific Region" and Sakai's paper "Aroid Root Crops: Acridity and Raphides." There is no consistency in the referencing: virtually all possible referencing systems have been used. The index for both volumes in in Vol. 2 which could cause some inconvenience.

There are many instances of typographical errors, misspelled words, etc. There are other errors which could have been prevented by judicious editing; for example, in the paper by Casier *et al.*, the authors concluded that higher pentosan content promoted shelf life and compressibility of bread made from starchy tropical crops, yet they gave no data to support this conclusion. In fact, they did not even describe methodology for measuring these parameters. With all due respect to the Belgian authors of this particular paper, their English is atrocious. Their paper appears to be Part III of a series. One can only guess as to where to find Parts I and II. Admittedly, the task of collecting and editing manuscripts for a publication such as this is not an easy one. In this particular case, however, it seems more appropriate that the term "edited by" be replaced by "assembled by."

W. BREENE

F
N
P
JOURNALS AND BOOKS
IN
FOOD SCIENCE AND NUTRITION

JOURNAL OF FOOD SERVICE SYSTEMS — G. E. Livingston and
   C. M. Chang

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GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. 57, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

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