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CONTENTS

ANTIOXIDATIVE MAILLARD REACTION PRODUCTS III. APPLICATION IN COOKIES

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ABSTRACT

The effect of Maillard reaction products (MRP) on the storage stability of two variations of a basic type of cookie (containing lard and vegetable fat, respectively) was studied. The following additions were made to the dough of the cookies: (A) histidine and glucose, (B) MRP from histidine and glucose, and (C) BHA/BHT[1] . Cookies without additions were baked as well and used as a control. All cookies were stored at 30° C. During the storage period samples were withdrawn for sensory evaluation and for gas chromatographic analysis of volatile compounds.

In the cookies containing lard, which were the least stable of the two types of basic cookie, the development of rancid flavor as well as the formation of n-hexanal was effectively retarded by the addition of histidine and glucose. Similar results were indicated in the experiment with cookies containing vegetable fat, although the effect was less pronounced owing to the higher stability of these cookies. The antioxidative effect is claimed to arise from MRP formed during the baking.

INTRODUCTION

The storage stability of dry foods, such as cookies, is mostly limited by lipid oxidation. Hence, phenolic antioxidants can be used to extend the storage time of this type of product. In products containing vegetable fat, the stability is also partly enhanced by the tocopherols naturally occurring in the fat. In products based on animal fat, like lard, containing very small amounts of tocopherols, however, the need for antioxidants is more pronounced. In fact, oxidation is a main limitation to using lard as a baking fat.

In model systems, Maillard reaction products (MRP) from amino acids and sugars were shown to possess antioxidative properties (Lingnert and

[1] BHA = Butylated Hydroxy Anisole
BHT = Butylated Hydroxy Toluene

Eriksson 1979). In particular, the basic amino acids, like histidine or lysine, were found to form potent antioxidants when reacted with reducing sugars like glucose, fructose or xylose.

The purpose of this investigation, therefore, was to study whether antioxidative MRP are able to inhibit lipid oxidation in food. Cookies were chosen as the test food, since MRP are naturally formed during the baking process, for which reason possible color or aroma contribution from the added MRP probably could be accepted. Furthermore, observations on increased fat stability caused by browning from added glucose in cookies have been reported previously (Griffith and Johnson 1957).

In the present investigation, it was studied how the storage stability of cookies is affected by the addition of histidine and glucose, as well as of preformed MRP from histidine and glucose, to the dough of the cookies.

MATERIALS AND METHODS

Synthesis of MRP from Histidine and Glucose

MRP were obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate (Fluka, Switzerland) and 50 mmol D-glucose (Fisher Scientific Comp., USA) in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h. The final volume of the reaction mixture was 35 ml.

Baking of the Cookies

Two variations of a basic type of cookie were baked, one containing lard as the fat component and the other one containing a shortening of vegetable origin. The following recipe was used:

	G	
Wheat flour	600	
Potato flour	20	
Fat	165	
Sucrose	201	
Sodium chloride	7.5	
Baking powder	6.5	1000 g
Water	150	1150 g

The ingredients were mixed and the dough kneaded for 7 min. The dough was then rolled to a thickness of 2 mm and round cookies, 40 mm in diameter, were cut out. The cookies were baked at 220°C. Those

containing vegetable fat were baked for 12 min. Those containing lard were baked for 14 min.

Besides these cookies without additions, both types of basic cookie were baked with the following additions:

Histidine and Glucose. 1.0 g L-histidine monohydrochloride monohydrate was added to 1000 g of dough, as calculated without added water, while 10 g of the sucrose in the recipe was replaced by the same amount of D-glucose. The histidine and the glucose were dissolved in the water to be added to the dough.

MRP from Histidine and Glucose. 2.5 ml of the Maillard reaction mixture (corresponding to 1.0 g dry matter) was added to 1000 g of dough, as calculated without added water. The MRP were mixed with the water to be added to the dough.

BHA/BHT. The commercial antioxidant Tenox 4 (Eastman Kodak Co., USA), consisting of 20% BHA, 20% BHT and 60% corn oil was used. Forty mg Tenox 4 was added to 1000 g of dough, as calculated without added water, which corresponds to 0.1 g BHA + BHT per 1000 g fat, which is the highest concentration allowed in Sweden. The antioxidant was dissolved in about half of the (melted) fat used, before incorporation into the dough.

The cookies were stored at 30° C in heat-sealed, laminated aluminum pouches, each containing 15 cookies. At regular intervals, samples were withdrawn for sensory evaluation and gas chromotographic analysis of volatile compounds.

Sensory Evaluation

A five-member, experienced panel at a cookie factory was used for the sensory evaluation. The evaluations were performed in individual testing booths. At each storage time investigated, one cookie of each of the eight kinds was presented simultaneously to each member of the panel. The order of presentation between the coded samples was varied from one storage time to another, but was the same for all judges. The evaluations were not replicated.

The panel was asked to judge (A) the "total impression of flavor" of the cookies, by indicating a value 0-4, where 0 = not accepted, 1 = not fully accepted, 2 = accepted, 3 = well accepted, and 4 = excellent, and (B) the "intensity of rancid flavor", using a scale 0-4, where 0 = no rancid flavor and 4 = very high intensity of rancid flavor. The definition of "rancid flavor" of the cookies was made by the judges themselves, on the basis of their previous experiences of sensory evaluation of cookies.

Mean values were calculated from the five judges. Statistical analysis of variance was applied to the data obtained and least significant difference values at the 1% level were calculated according to the method of

Scheffé (1953).

Gas Chromotographic Analysis of Volatile Compounds

Volatile compounds formed in the cookies were analyzed gas chromatographically by a headspace technique previously described by von Sydow *et al.* (1970). Three cookies from the same pouch were crushed in a mortar. To 8 g of the crushed cookies was added 30 ml distilled water, which had been filtered through activated charcoal, and the mixture was homogenized for 3 min with an Omnimixer (Sorvall, USA). The homogenate was transferred to a 250 ml glass flask, previously flushed with helium. An additional portion of 10 ml distilled water was used to rinse the mixer flask and the funnel. The flask was rotated in an inclined position for 30 min at 25° C in order to achieve equilibrium of volatiles between the gaseous and the liquid phase. A 175 ml sample of the headspace gas was conveyed to the cold trap of the pre-column concentration accessory of the gas chromatograph. The condensed material was injected onto the gas chromatographic column by heating the trap with a silicone oil bath maintained at 140° C.

The gas chromatography was performed in a Perkin-Elmer 900 instrument provided with a flame ionization detector (FID) and the pre-column concentration accessory. The open tubular column used consisted of a 0.76 mm ID × 181 m stainless steel tube coated with SF 96/Igepal CO 880 (95/5%). The oven temperature was programmed 20-140° C at 2° C/min after an initial isothermal period of 3 min. The injector temperature was 110° C and that of the detector 170° C. The carrier gas flow (nitrogen) was 12 ml/min. The FID-signal was fed into an Infotronic CRS-101A electronic integrator provided with digital print-out equipment.

Determination of Free Histidine

The amount of unreacted L-histidine in the histidine-glucose reaction mixture and in the cookies after baking was determined by decarboxylating the histidine with L-histidine decarboxylase (SIGMA, USA) and measuring the amount of carbon dioxide formed manometrically, using a Warburg apparatus (B. Braun, GFR). To the major compartment of the Warburg vessel was transferred 2.5 ml of 0.1 M sodium acetate buffer, pH 5.0, containing 20 μl of the histidine-glucose reaction mixture or a lyophilized water extract of the cookies. A standard solution of 2×10^{-3} M L-histidine in 0.1 M sodium acetate buffer was 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 approx. 0.7 U of histidine decarboxylase.

Twenty grams of the cookies was first ground in a mortar and passed through a sieve (DIN 6), then extracted with 4 × 40 ml distilled water, and centrifuged. The combined extracts were lyophilized. The lyophilized extracts from cookies with added MRP were solubilized and diluted to 20 ml with 0.1 M sodium acetate buffer, whereas extracts from cookies with added histidine and glucose were diluted to 50 ml. Of these solutions 2.5 ml portions were used for the determination of the histidine content.

Determination of the Peroxide Value

Forty grams of cookies, ground in a mortar and passed through a sieve (DIN 6), was extracted with 100 ml of acetic acid-chloroform (3:2) on a magnetic stirrer for 30 min. The suspension was filtered and two 30 ml aliquots were used for the determination of the perioxide value according to the official method AOAC (1975).

As separate experiments showed that 30 ml extract contained 2.1 g fat, this value was used when calculating the peroxide values.

RESULTS

Cookies Containing Lard

Figure 1 shows the development of rancid flavor during storage in the cookies containing lard. It should be pointed out that the sensory data are regarded here as "absolute values", that can be compared from one test occasion to another. This is, however, a doubtful procedure for taste data, and for other behavioral data, which are subject to inconsistencies caused by variations due to time, scale usage, ranges of samples, etc. However, a cautious comparison of values over time is considered warranted.

A rapid increase of the intensity of rancid flavor occurred in the cookies with no additions after 4 months, and in the cookies with addition of MRP from histidine-glucose after 6 months. A somewhat slower increase was observed in the cookies with added BHA/BHT after 6 months. The cookies with added histidine and glucose, however, were considered to have low intensity of rancid flavor during the first 14 months, after which time a rapid increase in the intensity of rancid flavor occurred. Samples of the three kinds first mentioned were not analyzed by sensory evaluation, when stored for a longer period than 14 months.

After being stored for 6 months, the cookies with added histidine and glucose, as well as the cookies with added BHA/BHT, were significantly

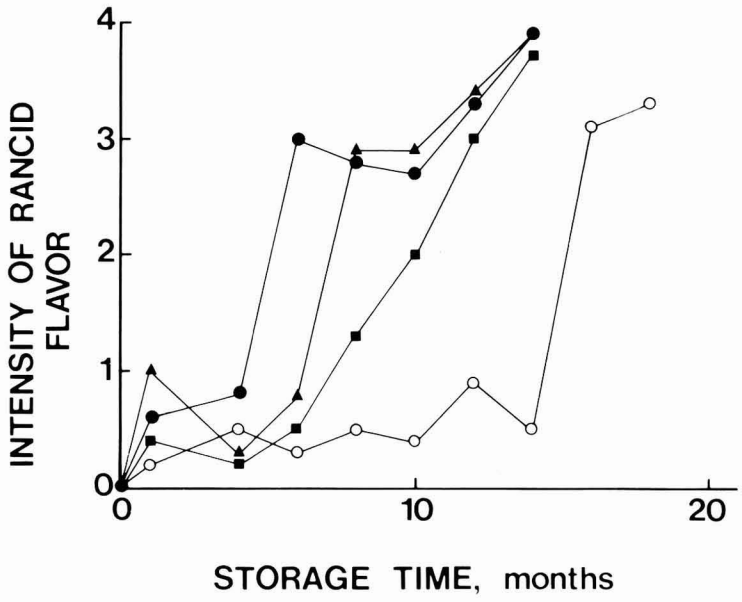

FIG. 1. DEVELOPMENT OF RANCID FLAVOR IN COOKIES CONTAINING LARD

Each point is based on 5 values (5 judges; 1 replicate)

Symbol	Addition to the dough
●——●	no addition
○——○	0.1% L-histidine monohydrochloride monohydrate + 1.0% D-glucose
▲——▲	0.1% Maillard reaction mixture (calculated as dry matter), obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h.
■——■	0.0016% BHA + BHT.

different from the control without additions, with respect to the intensity of rancid flavor. After 8 months the cookies with added histidine and glucose were significantly different from the control and from the cookies with added MRP. After 10 months, significant differences were found only between the cookies with added histidine and glucose and the cookies with added MRP, while after storage for 12 and 14 months the former was significantly different from all the three other cookies.

The results from the evaluation of the total impression of flavor of the cookies containing lard are shown in Fig. 2. As can be seen, these curves are approximately an inversion of the curves in Fig. 1, showing the increase in "rancidity". The cookies without additions were, how-

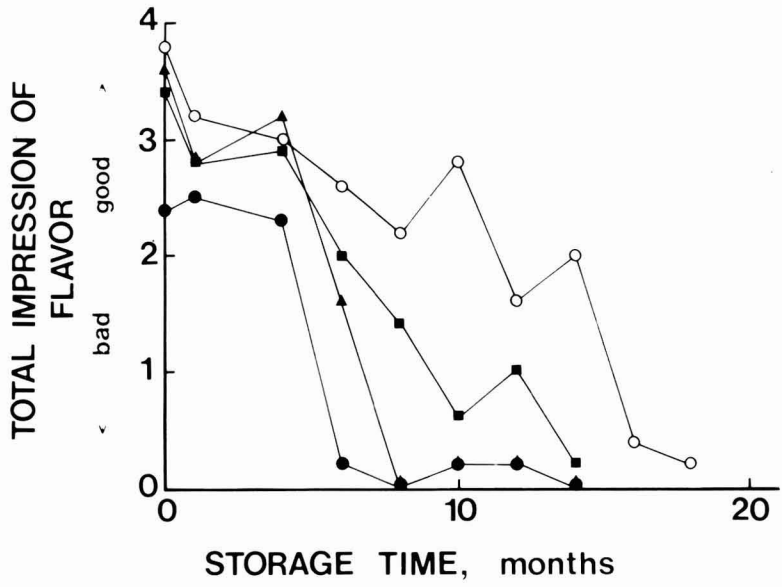

FIG. 2. TOTAL IMPRESSION OF FLAVOR OF COOKIES CONTAINING LARD

Each point is based on 5 values (5 judges; 1 replicate)

Symbol	Addition to the dough
●——●	no addition
○——○	0.1% L-histidine monohydrochloride monohydrate + 1.0% D-glucose
▲——▲	0.1% Maillard reaction mixture (calculated as dry matter), obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h.
■——■	0.0016% BHA + BHT.

ever, judged to be inferior to the others already at the start of the experiment. This observation is difficult to explain, but is probably due to other reactions than lipid oxidation, since the lower values in the "total impression of flavor" were not accompanied by the corresponding higher intensities of rancid flavor. It can, however, not be ruled out that lipid oxidation is responsible. Owing to the complex nature of the flavor derived from lipid oxidation, these early stages of deterioration are perhaps not perceived as "rancid" flavor.

As the value 2 was defined as the limit for acceptance, Fig. 2 shows that the cookies without additions and the cookies with added MRP were judged unacceptable after 6 months. The cookies with added

BHA/BHT were judged unacceptable after 8 months and the cookies with added histidine and glucose after 12 months.

The concentrations of n-hexanal in the cookies containing lard, obtained from the results of the gas chromatographic analysis, are presented in Fig. 3. n-Hexanal, which is known to be derived from lipid oxidation, gave one of the largest peaks in the chromatograms. It should be observed, that a logarithmic scale is used for the concentration of n-hexanal in Fig. 3. A sudden, drastic increase in the formation of n-hexanal occurred in the cookies without additions and in the cookies with added MRP after 8 months of storage, in the cookies containing BHA/BHT after 12-16 months, and in the cookies with added histidine and glucose after 18 months. This increase of the concentration of n-hexanal hence occurred later than the increase of the intensity of rancid flavor, as measured by sensory evaluation. There is, however, good agreement between the results with respect to the order between the different kinds of cookies.

The peroxide value was determined only occasionally. The results are shown in Table 1. The peroxide value went through a maximum in the cookies without addition and in the cookies with added MRP. This is a common course, since the hydroperoxides, being intermediate products in the lipid oxidation sequence, are subject to further reactions. The results from the peroxide value determinations support the results obtained with the other methods.

Cookies Containing Vegetable Fat

The cookies containing vegetable fat were found to be considerably more stable than those containing lard. Table 2 shows the intensity of rancid flavor, the concentration of n-hexanal, and the peroxide value of the cookies containing vegetable fat after various times of storage. A marked increase of the intensity of rancid flavor was not observed in the cookies without additions and in the cookies with added MRP until after 26 months. After this period of time these cookies were significantly different from the two others, which were still judged to give rather low intensities of rancid flavor. No significant differences were obtained before.

A small increase of the concentration of n-hexanal was observed in the beginning of the storage period. The concentration of n-hexanal was then rather constant until about 20 months of storage, after which time an increased formation of n-hexanal was observed in some of the cookies. The cookies without additions and the cookies with added MRP contained high concentrations of n-hexanal after 30 months. In the cookies with added BHA/BHT the increase was not so pronounced, and the

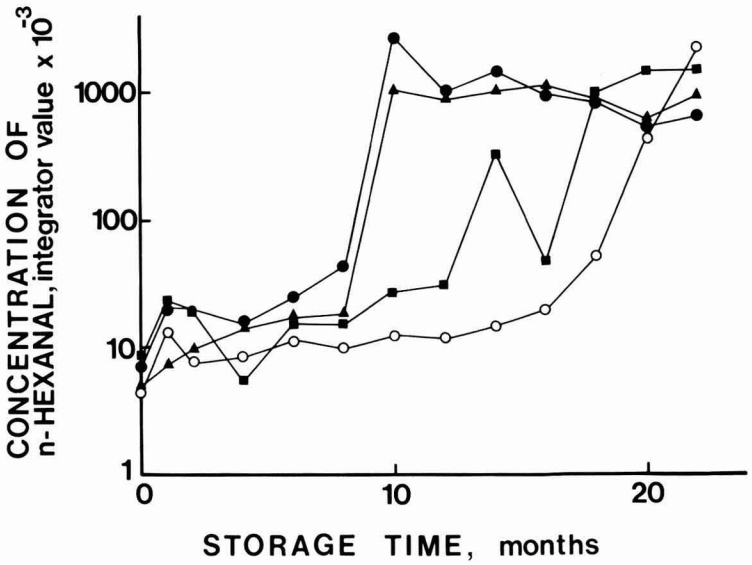

FIG. 3. FORMATION OF n-HEXANAL IN COOKIES CONTAINING LARD

Symbol	Addition to the dough
●——●	no addition
○——○	0.1% L-histidine monohydrochloride monohydrate + 1.0% D-glucose
▲——▲	0.1% Maillard reaction mixture (calculated as dry matter), obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h.
■——■	0.0016% BHA + BHT.

cookies with added histidine and glucose still had a relatively low concentration of n-hexanal.

The increase in the peroxide value was also considerably smaller in the cookies with added histidine and glucose than in the other three cookies. After 31 months of storage the highest peroxide value was obtained with the cookies containing BHA/BHT.

Altogether, these results with the cookies containing vegetable fat indicate good agreement with the results obtained with the cookies containing lard, with regard to the effect of the investigated additions. Storage for a longer period of time would, however, be necessary with the cookies containing vegetable fat to verify the observed tendencies.

Table 1. Peroxide values (meq/kg fat) of cookies containing lard

Addition	Storage Time (months)			
	0	8	13	22
No addition	0	18	352	47
Histidine + glucose [a)]	0	0	6	96
MRP [b)]	0	16	203	61
BHA/BHT [c)]	0	5	22	271

a) 0.1% L-histidine monohydrochloride monohydrate and 1.0% D-glucose were added to the dough
b) 0.1% Maillard reaction mixture (calculated as dry matter) was added to the dough. The Maillard reaction mixture was obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml potassium phosphate buffer, pH 7.0, for 5h
c) 0.0016% BHA + BHT was added to the dough

Free Histidine in the Cookies

The amounts of free histidine remaining in the Maillard reaction mixture and in the cookies were determined in order to assess the extent of histidine reactions during the baking. As is shown in Table 3, about 90% of the histidine reacted when histidine and glucose were refluxed for 5 h in order to obtain MRP. After the baking, about 95% of the initial amount of histidine had reacted in the cookies with added MRP, while about 70% had reacted in the cookies with added histidine and glucose. However, since about three times as much histidine was added to the cookies in the latter case, these cookies contained twice as much "reacted histidine" as those with added MRP.

DISCUSSION

A number of reports on the antioxidative effect of MRP, as evaluated in model systems, have been published (*e.g.* Kirigaya *et al.* 1968; Eichner 1975; Lingnert and Eriksson 1979). Reports on applications of the antioxidative effect of MRP in foods are, however, rare. Griffith and Johnson (1957) found that cookies with 30% sucrose (calculated on dough weight including water) became rancid in a shorter time than cookies prepared with 27.5% sucrose and 2.5% glucose. This effect was ascribed to reductones formed by the Maillard reaction during the baking.

Tomita (1972) reported the stability of "fried rice cake chips" to be improved by the addition of tryptophan and glucose. Since the processing included heating to 120° C for 30 min and deep fat frying at 180° C

Table 2. Intensity of rancid flavor, concentration of n-hexanal and peroxide value of cookies containing vegetable fat after various times of storage

Addition	Intensity of Rancid Flavor			Concentration of n-hexanal (Integrator Value $\times 10^{-3}$)			Peroxide Value (meq/kg fat)		
	0	22	26 Months	0	22	30 Months	0	22	31 Months
No addition	0.1	1.7	3.6	7	90	680	0	40	469
Histidine + glucose [a)]	0	1.2	0.7	3	27	39	0	17	46
MRP [b)]	0.1	1.1	3.4	6	50	770	0	54	375
BHA/BHT[c)]	0	0.7	1.1	6	53	200	0	42	609

a) 0.1% L-histidine monohydrochloride monohydrate and 1.0% D-glucose were added to the dough
b) 0.1% Maillard reaction mixture (calculated as dry matter) was added to the dough. The Maillard reaction mixture was obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h
c) 0.0016% BHA + BHT was added to the dough

Table 3. Amounts of free histidine added to the doughs and remaining in the cookies after the baking

Fat Used in the Baking	Addition	Amount of Free Histidine (mg/1000 g of Cookies)			Amount of Reacted Histidine in Baked Cookies (mg/1000 g of Cookies)
		Initially	After Refluxing	After Baking	
Lard	Histidine + glucose	740 [a]	—	250	490
	MRP [b]	280	30	10	270
Vegetable fat	Histidine + glucose	740 [a]	—	230	510
	MRP [b]	280	30	20	260

a) 1.0 gL-histidine monohydrochloride monohydrate was added to 1000 g dough (as calculated without added water)
b) The MRP were obtained by refluxing 25 mmol L-histidine monohydrochloride monohydrate and 50 mmol D-glucose in 25 ml 0.1 M potassium phosphate buffer, pH 7.0, for 5 h. The final volume of the reaction mixture was 35 ml, 2.5 ml of which was added to 1000 g dough (as calculated without added water)

for 3 min, MRP formed were supposed to be responsible for the antioxidative effect.

In both these cases, the reactants were added to the food product and the MRP were formed during the normal heat processes. In the present investigation, preformed MRP were added to the dough, as well as the free amino acid and the reducing sugar. The latter addition was, however, the only effective one. A question is, thus, whether the added histidine per se or reaction products formed during the baking were responsible for the antioxidative effect in the cookies. In the latter case the antioxidative reaction products could theoretically be formed by reaction between histidine and glucose or by reaction of either histidine or glucose with other reactants in the cookie dough. Histidine could possibly be effective as a carbonyl scavanger by reacting with, for instance, volatile, odorous aldehydes and ketones derived from the lipid oxidation (Yong and Karel 1978), which might partly explain the ability of added histidine to retard the development of rancid flavor. Antioxidative effect of histidine alone has been observed previously (Marcuse and Fredriksson 1969; Tjhio and Karel 1969). However, when measuring in model systems (Lingnert *et al.* 1979) we have not noticed any antioxidative effect of histidine at such low concentrations at which MRP from histidine-glucose are found highly antioxidative (unpublished observations).

Since only 30% of the added histidine could be recovered as free histidine in the cookies after the baking, a reasonable assumption is that MRP from histidine contributed to the antioxidative effect. To what extent the added histidine reacted with either the added glucose or with other carbonyl compounds present in the dough is still a question. Thus, no final proof can be presented by now that a histidine-glucose reaction product is responsible for the antioxidative effect in stored cookies. The results of Griffith and Johnson (1957) who added only glucose to the dough indicate that products obtained by reaction of glucose with other reactants than the added histidine also can contribute to the antioxidative effect. However, their results support the assumption that MRP formed during the baking were the active antioxidants.

If mainly histidine-glucose reaction products were responsible for the antioxidative effect in the cookies, it should be asked why preformed MRP did not exert any antioxidative activity. It may be that the concentration of *antioxidative* MRP was in fact lower when adding preformed MRP than when adding free histidine. As was shown in Table 3, the cookies with added histidine and glucose contained more "reacted histidine" than did the cookies with added MRP. It is also possible that the reaction conditions during the baking, for example the low water activity, are favorable for the formation of *antioxidative* MRP.

To be able to use the addition of histidine and glucose to retard lipid oxidation in cookies and similar products in general, further knowledge of the influence of changes in the cookie recipe or in the baking process on the formation of antioxidative products is needed. Possibly can the antioxidants formed be extracted from the product after the baking, for measurement of their antioxidative effect in model systems. If so, it could be checked whether the additions are still effective, when modifications of the products or the process have been made.

The highest legal concentration of BHA/BHT influenced the storage stability very little. Part of the antioxidants were possibly lost during the baking. It is, however, difficult to compare the effect of BHA/BHT with the effect of the other additions in the present investigation. About fifty times more histidine than BHA + BHT was, for example, added, as calculated on the basis of weight. The amount of antioxidative products formed during the baking is, however, unknown.

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ANTIOXIDATIVE MAILLARD REACTION PRODUCTS IV. APPLICATION IN SAUSAGE

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ABSTRACT

The effect of Maillard reaction products (MRP) on the oxidative stability of sausage during frozen storage was studied. Sausage of the frankfurter type was made from batter containing (A) no addition, (B) 0.08% *MRP from histidine and glucose, (C)* 0.16% *MRP from histidine and glucose, (D)* 0.08% *histidine +* 0.32% *glucose, (E)* 0.16% *histidine +* 0.32% *glucose, and (F)* 0.16% *MRP from an enzymic hemoglobin hydrolysate and glucose. The sausages were wrapped in aluminum foil and stored at* $-20^{\circ}C$. *At regular intervals during storage, samples were withdrawn for sensory evaluation and for gas chromatographic analysis of volatile compounds formed, as well as for the determination of the peroxide value and the content of free fatty acids. The development of rancid flavor, as determined by sensory evaluation, was found to be effectively retarded in the three types of sausage containing MRP. No effect was obtained by the addition of free histidine and free glucose. Neither the concentration of volatiles, nor the peroxide value was found able to reflect the sensory changes during storage. Some differences in the concentrations of n-hexanal between the samples were observed, which were consistent with the results from the sensory evaluation.*

INTRODUCTION

Maillard reaction products (MRP) were previously found to be capable of retarding the development of rancidity in foods such as cookies (Griffith and Johnson 1957; Lingnert 1980) and "fried rice cake chips" (Tomita 1972). In those three investigations the antioxidative effect was obtained by adding sugars or sugars and amino acids to the unprocessed products, and antioxidative MRP were formed during the normal heat treatment. Addition of preformed MRP (from histidine and glucose) to cookie dough failed to retard the lipid oxidation of cookies (Lingnert 1980), possibly because of the concentration of antioxidative MRP used being too low.

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Sato *et al.* (1973) found that the rapid development of oxidized flavor in refrigerated cooked meats, so-called warmed-over flavor (WOF), was inhibited by water extracts obtained from retorted beef. MRP formed during heating were presumed to be responsible for the antioxidative effect, since retorted solutions of sugars and amino acids were demonstrated to inhibit the development of WOF as well. Recently, Einerson and Reineccius (1977) reported similar results from experiments with turkey meat.

The purpose of the present investigation was to study whether preformed MRP were able to inhibit the development of rancidity in sausage during frozen storage. Since MRP from enzymic protein hydrolysates and sugars previously were shown to possess antioxidative properties in model systems (Lingnert and Eriksson 1980b), MRP from an enzymic hemoglobin hydrolysate and glucose as well as MRP from histidine and glucose were tested. In order to investigate whether antioxidative MRP could be formed even during the limited heat treatment included in the production of the sausage, additions of free histidine and free glucose to the sausage batter were made as well. Sausage with no additions was used as a control. Sensory evaluation and chemical analyses were performed during storage.

MATERIALS AND METHODS

Synthesis of MRP

MRP from histidine and glucose were obtained by refluxing 81 g of L-histidine monohydrochloride monohydrate (Fluka, Switzerland), which corresponds to 60 g of histidine, and 60 g of D-glucose (Fisher Scientific Co., USA) in 240 ml of 0.1 M potassium phosphate buffer, pH 7.0, for 5 h. The final volume of the reaction mixture was 335 ml.

Furthermore, MRP were obtained by refluxing 40 g of hemoglobin hydrolysate and 40 g of D-glucose in 160 ml of 0.1 M potassium phosphate buffer, pH 7.0, for 5 h. The final volume of the reaction mixture was in this case 210 ml. The hemoglobin hydrolysate was obtained by enzymic hydrolysis as described by Stachowicz *et al.* (1977). The bovine erythrocytes were hemolyzed by dilution with water and the dissolved protein was denatured by alkali treatment at pH 11 for 1 h. After adjustment of pH to 9 the material was filtered through cheese cloth and glass wool. The filtrate was further diluted with water to a protein content of about 7%. The protein was then hydrolyzed at 50°C by the enzyme alcalase® (NOVO, Denmark) and ultrafiltrated through a PM 10 membrane (Romicon Inc., USA).

Production of Sausage

A frankfurter type sausage was used. The sausage batter was produced in batches of 12 kg according to the following recipe:

	g
Beef (*M. biceps femoris*)	5570
Tallow	1750
Derinded backfat	1170
Blood plasma (frozen)	1200
Potato flour	450
NaCl containing 0.6% $NaNO_2$	240
Water	1620
Spices	12
Ascorbic acid	2.4

In order to get as uniform raw materials as possible, all the beef was minced, mixed in a big container, and frozen in portions corresponding to the recipe. The tallow and the backfat were cut into cubes and were likewise mixed and frozen in portions. The beef, tallow, and backfat portions were stored at −30° C until use.

The sausage batter was made in a bowl chopper (Rohwer Kolbe, GDR) according to the following procedure: (1) Mixing of beef and potato flour for 20 s. (2) Addition of salt. Mixing for 10 s. (3) Addition of blood plasma. Mixing for 20 s. (4) Addition of spices, ascorbic acid and, where applicable MRP or histidine + glucose together with half of the water. Mixing for 20 s. (5) Addition of the remaining water. Mixing for 20 s. (6) Addition of tallow and backfat. Mixing for 30 s. (7) Emulsification of the batter at a high rate for 130 s, resulting in a final temperature of 13-16° C.

The batter was filled in casings, 50 mm in diameter and 600 mm long, and the sausages were kept in a 0° C water bath until the day's production was finished. The sausages were then kept first in a 30° C water bath for 30 min and were then moved to a 75° C water bath and kept there for 45 min. The sausages were cooled in ice water for 30 min and were then allowed to hang in a room kept at 0° C for 1 day. They were thereafter wrapped separately in aluminum foil and stored at −20° C.

Sausages with the following additions (as calculated for 12 kg of sausage batter) were produced, three batches of each:

A. No addition
B. 28.0 ml of Maillard reaction mixture from histidine and glucose, corresponding to 10.0 g of histidine + glucose (0.08%).

C. 56.0 ml of Maillard reaction mixture from histidine and glucose, corresponding to 20.0 g of histidine + glucose (0.16%).
D. 13.5 g of L-histidine monohydrochloride monohydrate, corresponding to 10.0 g of histidine (0.08%) + 40.0 g of D-glucose (0.32%).
E. 27.0 g of L-histidine monohydrochloride monohydrate, corresponding to 20.0 g of histidine (0.16%) + 40.0 g of D-glucose (0.32%).
F. 52.5 ml of Maillard reaction mixture from hemoglobin hydrolysate and glucose, corresponding to 20.0 g (0.16%).

Sensory Evaluation

At each session, three samples of sausage were presented to a panel of nine to eleven judges. For each sample the judges were asked to judge (1) the "intensity of rancid flavor", using a scale of 0-9, where 0 = no rancid flavor and 9 = very high intensity of rancid flavor, and (2) the "total impression of flavor", using a scale of 0-9, where 0 = very bad and 9 = very good.

The samples within each of the groups I or II below were always compared in the same session, except for the two longest storage times (28 and 36 weeks). Only the three samples with MRP were then evaluated, since the other ones had been deleted due to too intensive rancidity.

I. A. Sausage without addition.
B. Sausage with added MRP from histidine and glucose (0.08%).
D. Sausage with added histidine (0.08%) and glucose (0.32%).

II. C. Sausage with added MRP from histidine and glucose (0.16%).
E. Sausage with added histidine (0.16%) and glucose (0.32%).
F. Sausage with added MRP from hemoglobin hydrolysate and glucose (0.16%).

The three samples within each session were presented coded and in a random order, which was varied for different judges. A total of three replicates of each evaluation was made for each storage time.

The frozen samples were thawed overnight at +4° C, cut into 15 mm thick slices which were fried in fresh margarine at 180° C, 2.5 min on each side. The fried slices were placed one by one in stainless steel petri dishes with covers, which were placed on hot plates kept at 70-75° C in the testing booths. The evaluations were performed as soon as possible after the frying.

The eleven-member panel consisted of employees at the Institute. At least nine of them participated in each session. They were experienced in sensory evaluation of various food products, including evaluations of

rancidity. Before the present investigation was started, the panel was introduced to the flavors likely to occur in the product by evaluating different kinds of sausages that had been stored for different periods of time.

For each storage time and for each attribute, mean values of the 27 to 33 evaluations were calculated. The significance of the differences among the three mean values within each group of samples (I and II above) was determined by two-way analysis of variance (variables: samples and judges). Finally, least significant difference values at the 5% level were calculated according to the method of Scheffé (1953).

Gas Chromatographic Analysis of Volatile Compounds

Volatile compounds formed in the sausages were analyzed by gas chromatography using a headspace technique previously described by von Sydow *et al.* (1970). To 50 g of sausage was added 50 ml of distilled water, which had been filtered through activated charcoal, and the mixture was homogenized for 3 min with an Omnimixer (Sorvall, USA). The homogenate was transferred to a one-liter glass flask, previously flushed with helium. The flask was once again flushed with helium when containing the homogenate. The flask was rotated in an inclined position for 30 min at 25° C in order to achieve equilibrium of volatiles between the gaseous and the liquid phase. A 700-ml sample of the headspace gas was conveyed to the cold trap of the pre-column concentration accessory of the gas chromatograph. The condensed material was injected onto the gas chromatographic column by heating the trap with a silicone oil bath maintained at 140° C.

The gas chromatography was performed in a Perkin-Elmer 900 instrument provided with a flame ionization detector (FID) and the pre-column concentration accessory. The open tubular column used consisted of a 0.76 mm ID × 181 m stainless steel tube coated with SF 96/Igepal CO 880 (95/5%). The oven temperature was programmed 20-140° C at 2° C/min after an initial isothermal period of 3 min. The injector temperature was 110° C and that of the detector 170° C. The carrier gas flow (nitrogen) was 12 ml/min. The FID-signal was fed into a Perkin-Elmer PEP-1 data processor.

Determination of the Peroxide Value and of the Content of Free Fatty Acids

The determination of the peroxide value and of the content of free fatty acids was performed on chloroform extracts of the fat, as described by Pearson (1970). One hundred grams of sausage, cut into cubes, was homogenized with 60 ml of chloroform in an Omnimixer (Sorvall, USA)

for 2 min. The chloroform was decanted, and the material was extracted another four times, each with 40 ml of chloroform. The five extracts were combined, filtered, and distributed as follows: (1) 15 ml was transferred to each of two weighed petri dishes for determination of the total fat content, (2) 20 ml was transferred to each of two 250-ml flasks for determination of the peroxide value, and (3) 25 ml was transferred to each of two 250-ml flasks for determination of the content of free fatty acids. The peroxide value was determined according to Wheeler's method (Williams 1950), starting with the addition of 30 ml of acetic acid to the chloroform extract. The determination of the content of free fatty acids was performed according to the method outlined by Pearson (1970).

RESULTS

The results from the sensory evaluation of the "intensity of rancid flavor" are shown in Fig. 1. Mean values of the judgments are given. As can be seen, the samples containing added MRP (samples B, C, and F) were at each test occasion considered to have lower intensity of "rancid flavor" than the three samples without MRP (A, D, and E). The latter seemed to develop rancid flavor more rapidly and were even judged to be more rancid already at the start of the experiment.

Caution must, however, be used, when comparing intensity scores for sensory attributes, *e.g.* "rancidity," obtained on different test occasions (even if the panel has exactly the same composition on all occasions). The reason for this is that behavioral data, such as taste data, are subject to inconsistencies caused by variations due to time, scale range, range of samples, etc. It should, thus, be kept in mind that the samples in this investigation were always compared within two groups (consisting of the samples A, B, D and the samples C, E, F respectively), which were always evaluated separately. This has to be considered when conclusions are drawn from results such as those presented in Fig. 1.

The statistical analysis was, therefore, at each storage time applied only to the data within each of the two groups. Within the group consisting of the samples A, B, and D, the intensity of rancid flavor was significantly lower in sample B than in sample A for the storage times of 4, 6, 12, and 16 weeks. For the storage times of 4, 12, and 16 weeks, significant differences were also found between the samples B and D. At the start of the storage period (0 weeks), sample D was significantly different from both sample A and sample B.

Within the group consisting of the samples C, E, and F, the intensity of rancid flavor was significantly lower in sample C than in sample E for

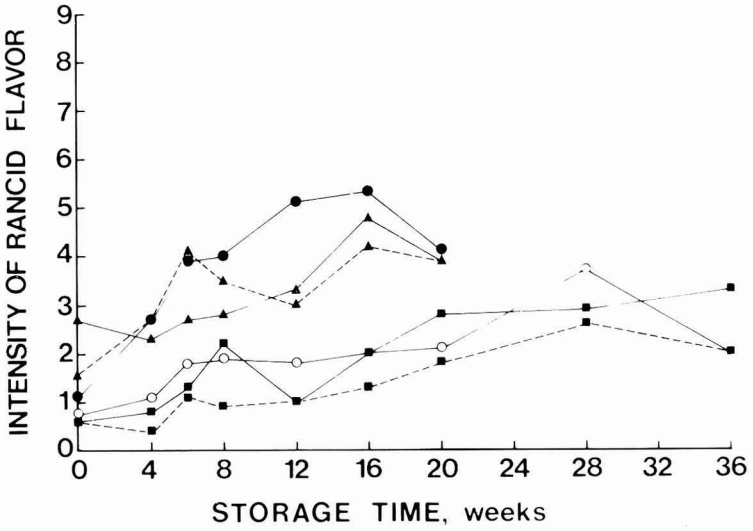

FIG. 1. DEVELOPMENT OF RANCID FLAVOR IN SAUSAGE DURING STORAGE AT −20°C

The values are based on 27—33 judgments (9—11 judges; 3 replicates).

Symbol	Sample	Additions to the sausage batter
●——●	A	No addition
■——■	B	0.08% MRP from histidine and glucose
■- - - -■	C	0.16% MRP from histidine and glucose
▲——▲	D	0.08% histidine + 0.32% glucose
▲- - - -▲	E	0.16% histidine + 0.32% glucose
○——○	F	0.16% MRP from hemoglobin hydrolysate and glucose

all the storage times investigated, except at 0 weeks. For storage times of 4 and 6 weeks, sample F, as well, gave significantly lower intensity of rancid flavor than sample E.

Figure 2 shows the results from the evaluations of the total impression of flavor of the samples. The curves appear approximately to be inversions of the corresponding curves in Fig. 1. As compared with the sausages without addition, some new types of flavors were caused by the addition of MRP, which were described by the judges in terms like "burnt" and "sweet". From the results in Fig. 2, however, these new flavors do not seem to impair the overall impression of flavor to any great extent. It should be mentioned that the addition of MRP also had some effect on the color of the sausages, causing somewhat darker brown color, as compared with the more reddish color in sausages without addition.

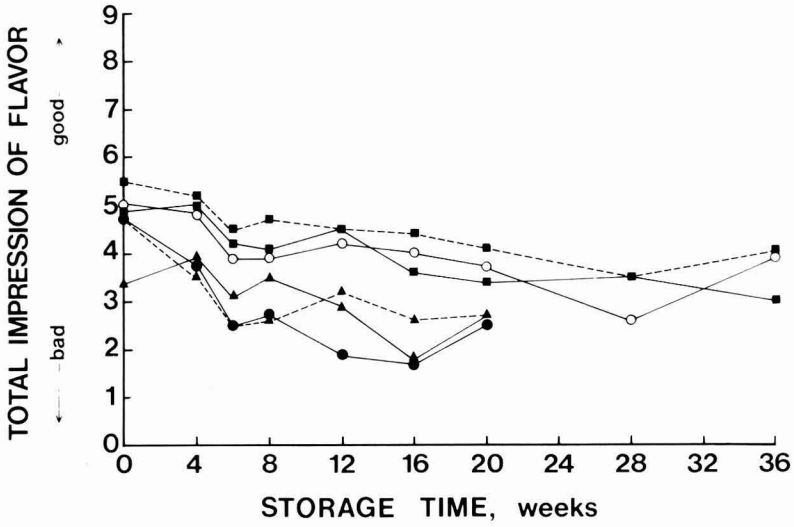

FIG. 2. TOTAL IMPRESSION OF FLAVOR OF SAUSAGE DURING STORAGE AT −20°C

The values are based on 27—33 judgments (9—11 judges; 3 replicates).

Symbol	Sample	Additions to the sausage batter
●——●	A	No addition
■——■	B	0.08% MRP from histidine and glucose
■- - - -■	C	0.16% MRP from histidine and glucose
▲——▲	D	0.08% histidine + 0.32% glucose
▲- - - -▲	E	0.16% histidine + 0.32% glucose
○——○	F	0.16% MRP from hemoglobin hydrolysate and glucose

The concentrations of volatiles, as analyzed by the gas chromatographic method, were found not to change very much during storage. The results are exemplified in Fig. 3, showing the concentration of n-hexanal, one of the largest peaks in the chromatograms. Analyses were made of samples stored up to 66 weeks. In spite of this long storage period, sample A was the only one, for which an increasing tendency in the concentration of n-hexanal possibly could be noticed. Considerable variations were, however, observed between different sausages of the same sample, or even between different parts of the same sausage. Based on six replicates, the concentration of n-hexanal in sample E after storage for 6 weeks, for example, gave a mean value of 2.7 with a standard deviation of 0.8. Differences between the six samples can, however, be observed in Fig. 3. The samples with added MRP (B, C and E) are on a lower concentration level than at least samples A and D during the whole storage period, which is consistent with the results from the sensory evaluations.

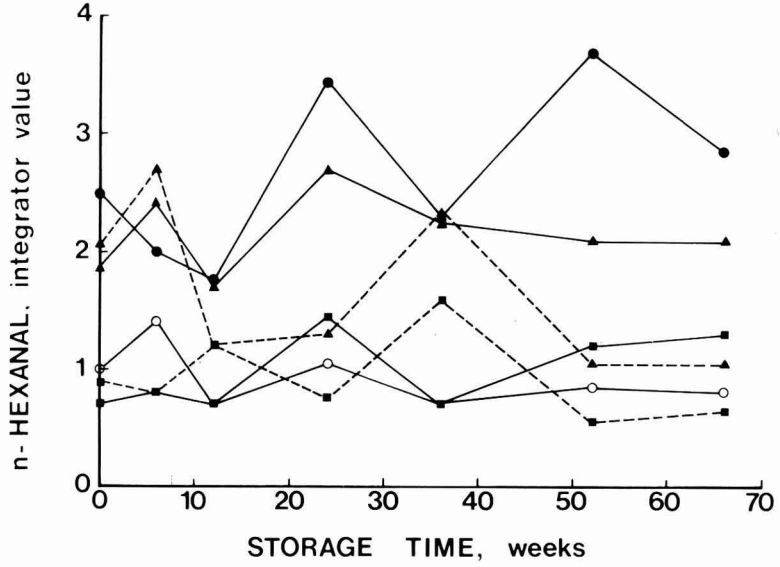

FIG. 3. FORMATION OF n-HEXANAL IN SAUSAGES DURING STORAGE AT −20°C

Symbol	Sample	Additions to the sausage batter
●——●	A	No addition
■——■	B	0.08% MRP from histidine and glucose
■- - -■	C	0.16% MRP from histidine and glucose
▲——▲	D	0.08% histidine + 0.32% glucose
▲- - -▲	E	0.16% histidine + 0.32% glucose
○——○	F	0.16% MRP from hemoglobin hydrolysate and glucose

No increase in the peroxide value could be established during the storage period. Nor could any clear differences between the six samples be observed. Great variation within one and the same sample was, however, found even here.

It can thus be concluded that neither the peroxide value, nor the concentration of the volatiles recorded in our procedure is a very sensitive measure of rancidity for this product. None of them reflected the sensory changes during storage.

The content of free fatty acids was found to vary less than the peroxide value. No obvious changes over storage time or differences between the six samples could be noticed.

DISCUSSION

Although the antioxidative effect of the MRP during storage of sau-

sage was difficult to prove by chemical measurements, this effect was clearly observed by the sensory evaluations. With the exception of experiments showing MRP to inhibit the formation of WOF in ground beef (Sato *et al.* 1973) and in turkey meat (Einerson and Reineccius 1977), inhibition of lipid oxidation in foods by added preformed MRP has, as far as we know, not been reported previously. In our previous experiment with cookies, for example, only MRP formed during baking, from amino acid and sugar added to the dough, retarded the oxidation (Lingnert 1980). In the present investigation the heat treatment included in the sausage production was obviously insufficient for the formation of antioxidative MRP. This was not surprising, since no extensive Maillard reaction is to be expected during 45 min at such low temperature as 75° C. The conditions during cookie baking are probably more favorable in this respect, particularly owing to the low water activity.

An interesting question is why preformed MRP were ineffective in cookies at the 0.1% level, while effective in sausage at approximately the same concentration. It should, however, be noted that the MRP used in the two experiments were not identical. A higher molar ratio of histidine to glucose was used when synthesizing MRP for the sausage experiment. This has been found to be favorable for the formation of antioxidative products (Lingnert and Eriksson 1980a). Another possible explanation of the difference in effectiveness of preformed MRP between the two products may be differences in the lipid oxidation mechanism. It might, for example, be possible that the dominating heme catalysis in the sausage was effectively inhibited by the MRP.

There were also obvious differences between the two experiments with respect to the applicability of the chemical methods of measurement. In the cookie experiment the formation of volatiles as well as the peroxide value were very useful to demonstrate the antioxidative effect. In the sausage experiment neither of them reflected the sensory changes. This points at the difficulties to measure lipid oxidation. Owing to its complex nature and its dependence of so many factors no single method of measurement works in all food systems. For instance, since heme compounds catalyze not only the oxygenation step in the lipid oxidation sequence, but also the further breakdown of lipid hydroperoxides, the peroxide value may be a rather insensitive measure of lipid oxidation in a heme catalyzed system. Similarly, the formation of volatiles may be directed in different ways in different reaction systems. The volatiles responsible for the rancid flavor in the sausage were possibly not recorded with our procedure. The changes could also be too small to be effectively observed, due to the considerable variations between the analyses.

To enable establishment of possible, small changes (in volatile compounds, for instance) over time in the sausage experiment, it had been necessary to make a considerably greater number of replicates of the analyses. Since it is very difficult to uniform inhomogeneous raw materials such as beef, tallow, and backfat in large amounts, it is likely that differences in lipid composition or in initial degree of oxidation between different sausages or even between different parts of the same sausage will occur, leading to differences in the lipid oxidation during storage. These variations could be observed from the sensory evaluations as well but were suppressed because of the large number of analyses (9—11 judges, judging three replicates of each sample from at least two sausages).

As was shown in Fig. 1, all samples containing MRP were judged less rancid already at the beginning of the experiment. This is possibly due to that some lipid oxidation occurred during the production of the sausages with no added antioxidants, while lipid oxidation in the other sausages was inhibited by the added MRP. If that is the case, the present results point at the importance of applying lipid oxidation protection as early as possible in the manufacture of a product. In this case, the early onset of lipid oxidation may be caused by the same mechanism as the usual formation of WOF, since the process included cooking in 75° C water for 45 min, followed by keeping at 0° C for one day. According to a recent review on WOF (Pearson *et al.* 1977), experiments have shown that the development of rancidity occurs most rapidly in meat heated at 70° C for 1 h, while the extent of rancidity decreased if the cooking temperature was raised above 80° C.

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DRIED FISH PATTIES: STORAGE STABILITY AND ECONOMIC CONSIDERATIONS[1]

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ABSTRACT

Storage study was conducted on dried fish patties from the mixture of several comminuted fish species (rockfish, Pacific cod, ling cod and Pacific herring) with structured vegetable protein, modified tapioca starch, sodium chloride and/or sodium sorbate and antioxidants. Moisture, pH, total volatile nitrogen (TVN), thiobarbituric test (TBA), total pour plate counts, and yeast and mold counts were conducted monthly during four months storage period at 25° C. Slight variations in the values of these tests indicated the stability of this product. Proximate analysis, amino acid profile and protein efficiency ratio (PER) in the dried patties indicated the nutritional quality of the product. A brief economic study indicated good feasibility of the dried patties in the market.

INTRODUCTION

During recent years, considerable effort has been expended in increasing the use of resources from the sea for human consumption. Programs emphasizing total utilization of sea resources, new sources of protein, utilization of unconventional fish species, and total recovery and utilization of fish flesh have received considerable attention (Kreuzer 1965; Martin 1974; Pigott 1976).

Countries such as Venezuela have a wide variety of fish species of high economic potential that have not yet been exploited, including Bagres (*Arius* spp.), Cazones (*Mustelus* spp.) and Perla (*Lepophidium* spp.) among others. In general, these fish have been considered of low economic value because the cost of harvesting and preparing them for human food is higher than the consumer is able or is willing to pay.

[1] This paper was presented at the Fifth International Congress of Food Science and Technology, Kyoto, Japan, September 17—22, 1978.

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Even the high proportion of low value fish caught incidental to the shrimp fishery is considered of no value by the fishermen, and these fish are discarded. In some areas of the country there are nutritional problems in the population such as infant malnutrition, low growth rates in children, anemia (particularly in pregnant women), and others due to deficiency in total food intake and particularly in high quality protein products (Osio *et al.* 1976). In areas where low income families have limited transportation and refrigeration facilities, dried, salted fish and meat are popular products that give a practical solution to problems of storage of perishable food of animal origin. However, progressive increases in the price of this type of product reduces the possibility of maintaining a satisfactory protein intake in the daily diet.

A satisfactory dried product has been developed utilizing mixed species. Minced fish flesh is combined with soy fiber, starch and salt (Bello and Pigott 1979). The process is simple, requiring uncomplicated technology and unsophisticated equipment that can be operated by people at medium educational level (Fig. 1).

Advantages of the process include (a) maximum utilization of the fish flesh, where the total recovery ranges from 40 to 64% (Table 1); (b) efficient use of raw materials, allowing mixed species utilization; (c) socio-economic advantages for fishermen and the fishing industry; and (d) nutritious high-protein products.

The purpose of this study was to gain some information about the nutritional quality of this product and its stability during storage at room temperature. Also, the operating economics were considered to determine the feasibility of adding this product to existing, traditional fish plants in Venezuela and other Latin American and Caribbean countries.

MATERIALS AND METHODS

Fish: Rockfish (*Sebastes sp.*), Pacific cod (*Gadus macrocephalus*), ling cod (*Ophidon elongatus*), and Pacific herring (*Cuplea harengus pallus*).

These fish were obtained fresh from fishermen. The iced fish were transported to the laboratory and processed immediately. The viscera and head were removed manually, followed by washing in tap water, and deboning in a Yanagiya separator, Model Y-200. The minced fish flesh was packed in plastic bags, each containing 10 kg, frozen, and stored at −34° C. Rockfish, Pacific cod and ling cod were stored at −34° C for one month and Pacific herring was stored frozen for four months prior to use.

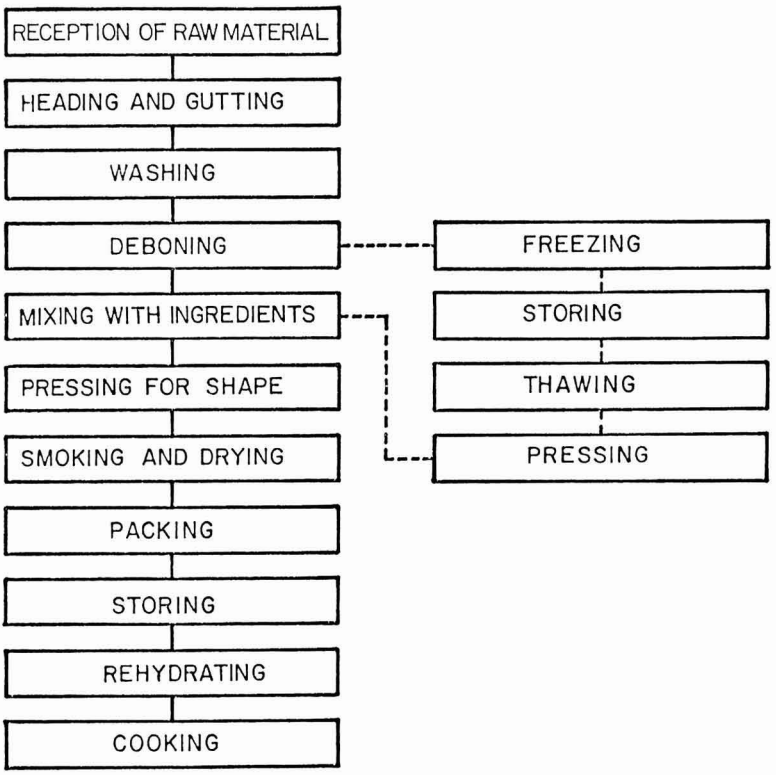

FIG. 1. FLOW DIAGRAM FOR THE PROCESSING OF DRIED FISH PATTIES

Twenty-five percent of each species of minced fish flesh was mixed together. The ingredients were added to this fish flesh mixture in the following proportions:

Fish Flesh	89.5%
Structured Soy Protein	5.0%
Modified Tapioca Starch	5.0%
Sodium Chloride	0.5%

Sample A consisted of a mixture of the fish flesh and ingredients plus potassium sorbate as an antimicrobial agent (0.05% in the dried product) and an antioxidant mixture (Tenox-26 from Eastman Kodak) calculated to be 0.015% of the fat content in the product. Sample B consisted of the mixture of fish flesh and ingredients plus potassium sorbate (0.05% in the dried product) and Sample C consisted of the same fish flesh and ingredients mixture plus the antioxidant mixture (0.015% of the fat content in the final product). Potassium sorbate was mixed with sodium

Table 1. Recovery of the deboned fish flesh from different species of fish

Fish Species	Raw Material (%)	Waste 1[a] (%)	Headed & Gutted Fish (%)	Waste 2[b] (%)	Total Waste 1 & 2 (%)	Recovered Flesh (%)
Herring	100	20.68	79.32	15.50	36.18	63.82
Rockfish	100	38.60	61.40	24.30	62.90	37.10
Lingcod	100	42.70	57.30	15.80	58.50	41.50
Pacific cod	100	38.50	61.50	21.00	59.50	40.50
Average	100	35.12	64.88	19.15	54.27	45.73

a = head and guts
b = skin and bones

chloride and the antioxidant was used as a spray.

All the samples were shaped into patties (90 mm in diameter and 12.7 mm in thickness), dried at 71—82° C for 10—12 h in a small cabinet drier to reduce the moisture content to 5—8%, packed in plastic bags and stored at 25° C for four months. Fifteen minutes in tap water was required for rehydration before cooking.

Determinations of pH, moisture, total volatile nitrogen (TVN), thiobarbituric acid test (TBA), total plate count, and yeast and mold counts were conducted monthly during the storage time. Amino acid analysis and proximate analysis were performed on the dry patties that had been stored for two months. The protein efficiency ratio (PER) was determined on the final product.

Analytical Methods

Moisture by weighing before and after drying the sample at 100—102° C for 16—18 h (AOAC 1975); ash by weighing the sample after carbonizing in a furnace at C.A. 525° C (AOAC 1975); protein (nitrogen × 6.25) by micro-Kjeldahl method (AOAC 1975); crude fat by extraction with anhydrous ethyl ether, using goldfish equipment (AOAC 1975); pH (hydrogen-ion concentration) by mixing the sample with water 1:3 (AOAC 1975); and by using "Corning" pH meter; oxidation of fats by thiobarbituric acid test (TBA), by distillation method of determination of malonaldehyde, Tarladgis *et al.* (1960), as modified by Castell and Boyce (1966); total volatile nitrogen (TVN) by microdiffusion method of Conway (1958); a_W by using Dow Point Hygrometer, Model 91 HF (Yellow Springs Instrument Company); amino acid analysis by chromatography procedure (Spackman *et al.*, 1958; Spackman 1969); tryptophan analysis by a modified Fischl procedure (Inglis and Leaner 1964; Sodek *et al.* 1975).

Microbiological Methods

Total pour plate count by using plate count agar (Difco) according to Gilliland *et al.* (1976), and yeasts and molds, using malt agar (Difco) according to Koburger (1976).

Biological Assay

Protein efficiency ratio (PER) (AOAC 1975). The rats used for this study were Sprague-Dawley, all male, 23-28 days old, with average weight of 51 ± 5 g, purchased from Simonsen Laboratories (Gilroy, California).

Table 2. Results of the physiochemical determinations in the dried fish patties

Determination	
Moisture (%)	5.5
Ash (%)	4.6
Protein (nitrogen × 6.25) (%)	55.5
Crude fat (%)	19.0
Carbohydrates (%)	15.4
TBA (mg/1000 g)	3.1
TVN (mg/100 g)	33.4
pH	6.2
a_w	0.3

RESULTS AND DISCUSSION

Table 2 shows the proximate analysis of the dried fish patties, indicating the protein, fat, carbohydrate and ash content in the product. The amino acid profile (Table 3) shows good retention of essential amino acids. In addition, the biological evaluation of the protein quality (PER) gave a value of 3.18 for the dried patties, while its reference casein value was 2.75.

During the four-month storage period, there was essentially no change in pH (Table 4). Variations in the moisture content from 3.7% to 8.3% can be attributed to the drying process itself, due to the high load in the small cabinet drying used in this experiment. For instance, moisture values of 3.5 and 8.5% were recorded in the dry patties situated closer to and farther from the heater, respectively. Reduction in the load of the dryer and rotation of the patties is recommended to prevent such variations in the moisture content.

Small variations in the TVN values were detected during the storage time. The minimal variations (30-40 mg/100 g) certainly indicate the stability of the product. The slight variation in the TBA values (Table 4) might be attributable to the variations in the fat content of the dried samples, which depended directly on the proportion of already rancid frozen herring used in this product. The contribution to the rancidity by the other fish species used would be negligible because rancidity in lean fish such as Pacific cod, even with long frozen storage, is very small (MacLean and Castell 1964; Castell *et al.* 1966). Lipid oxidation is minimal at the a_w value of the product (Table 2). However, some of the oxidation reactions which were started before or during the drying process

Table 3. Amino acid analysis in the dried fish patties and the reference casein

Amino Acid	Dried Fish Patties (%)	Reference Casein (%)[1]
Hydroxylysine	0	—
γ-Aminobutyric acid	0	—
Ornithine	0.12	—
Ethanolamine	0.08	—
1-Methylhistidine	0.11	—
Lysine	8.89	7.3
Histidine	2.35	2.7
3-Methylhistidine	0	—
Tryptophan	2.07	1.1
Arginine	6.42	3.6
Taurine	0.58	—
Aspartic acid	10.59	6.3
Hydroxyproline	0	—
Threonine	5.01	4.3
Serine	4.06	5.6
Glutamic acid	14.96	20.0
Proline	3.96	10.1
Glycine	4.38	2.4
Alanine	5.99	2.6
Valine	5.49	6.4
Cystine	0.80	0.3
Methionine	3.38	2.5
Isoleucine	5.00	5.4
Leucine	8.49	8.2
Tyrosine	2.90	5.6
Phenylalanine	4.34	4.4
β-Alanine	0	—

[1] Reported by Sheffield Chemical (Norwich, New York)

Table 4. Results of the physiochemical determinations in the dried fish patties with the following treatments: (A) Fish sample with antioxidant and antimicrobial agent; (B) Fish sample with antimicrobial agent; (C) Fish sample with antioxidant. During storage

Determination	Sample	Storage Time (Months)				
		0	1	2	3	4
Moisture (%)	A	4.35	5.33	4.83	5.28	8.3
	B	7.66	5.72	7.80	5.69	4.92
	C	3.74	7.14	5.79	4.11	5.25
pH	A	6.2	6.2	6.2	6.2	6.2
	B	6.2	6.1	6.2	6.2	6.2
	C	6.2	6.1	6.1	6.2	6.1
TBA mg MA/ 1000 g	A	3.32	3.70	2.97	4.18	3.68
	B	2.77	4.21	4.61	3.17	3.27
	C	3.19	5.29	3.67	4.25	4.66
TVN mg/100 g	A	28.71	34.16	37.87	33.60	32.11
	B	34.27	36.19	42.87	32.11	38.67
	C	37.16	40.22	30.21	41.41	37.14

cannot be stopped even with antioxidants. If rancidity is expected to be reduced in the dried product the antioxidant must be added immediately after mincing the flesh and before the freezing or mixing process. Furthermore, it is important to remember that a slightly rancid flavor in the dried, salted product is accepted by people in Venezuela and other Caribbean countries. Therefore, rancidity should be controlled according to the area where the fish will be sold, the species of fish available, the proportion of fat fish used and the acceptability to people in the specific area.

Total counts were around 10^2-10^3 cfu/g during the storage time (Table 5). Differences in the total counts which were dependent on the treatments were not observed. A few variations were observed, and these might be attributed to the samples which had small differences in moisture content. Low counts of yeasts and molds were recorded, as shown in Table 5, and no differences due to storage time or treatments were detected. The effect of potassium sorbate was negligible, and it would not be considered necessary to maintain control on the molds and the general microbial quality of the product during the storage time under the experimental conditions. The heat treatment and the a_w are enough to control microorganisms in the product.

Variations in values for moisture, TVN, TBA and microbial counts during zero and storage time could be attributed partially to the mixing process and partially to the drying process, which creates products with different moisture contents in addition to variations due to storage. The mixing of additives and ingredients was done manually for a short time in order to avoid more disruption of the fish flesh and prevent the formation of a compact dough which increases the drying process, alters the rehydration capacity of the patties and affects their sensory characteristics. The manual mixing procedure does reduce the uniformity of the patties. However, a satisfactory mixture and product were obtained using a mechanical mixer for the final developed product (Bello and Pigott 1979).

Figure 2 shows the fish patty process incorporated with regular fish plant operation. A brief economic study based on operating in Venezuela indicates that the product has a great deal of feasibility in the market (Table 6). First the production and operating costs are relatively low; second, the cost of building equipment and facilities and annual fixed costs are low if the fish patty processing plant is annexed to an existing facility; finally, even with a low selling price, the anticipated net profit is high.

Table 5. Results of the microbiological determination in dried fish patties with the following treatments: (A) Fish sample with antimicrobial agent and antioxidant; (B) Fish sample with antimicrobial agent; (C) Fish sample with antioxidant; during storage

Test	*Sample*	Storage Time (Months)				
		0	1	2	3	4
Total Plate Count Aerobic (Col/g)	A	710	250	<64	350	990
	B	680	<170	420	1300	<100
	C	240	650	<95	600	<60
Total Plate Count Anaerobic (Col/g)	A	120	0	0	200	<100
	B	250	0	<28	350	0
	C	0	<100	0	<50	0
Molds and Yeasts (Col/g)	A	0	<30	<11	0	<10
	B	<30	0	<34	<100	0
	C	0	<53	<18	0	0

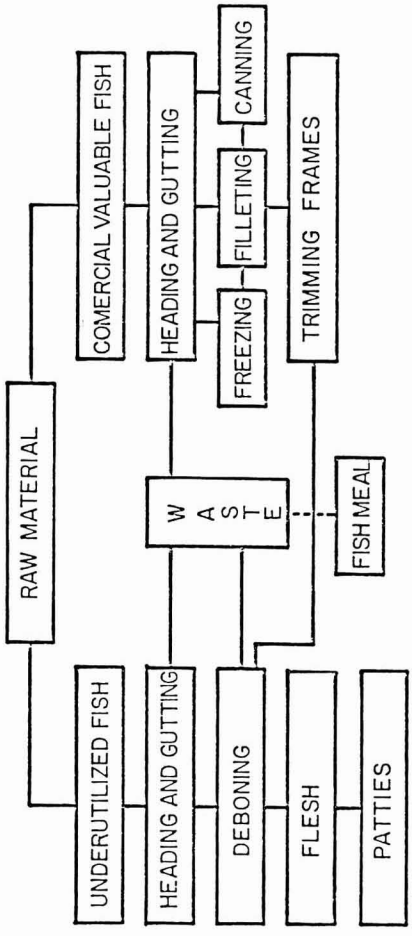

FIG. 2. DIAGRAM OF FISH PLANT OPERATION WITH A PROPOSED DRIED FISH PATTIES PROCESSING LINE

Table 6. Economic figures related to the production of dried fish patties in Venezuela

Raw Fish/Day:	1,000 kg (recovery 45%)		
Patties/Day:	5,000		
Daily Operating Costs			
A. Raw Materials:			
Fish		$ 330.00	
Soy Fiber		26.50	
Starch		17.93	
Salt (NaCl)		0.38	
Sodium Sorbate		0.61	
Antioxidants		2.63	
Plastic Bags		36.40	
Total		$ 414.45	
B. Daily Processing Costs:			
(a) Estimated labor cost: 10 men × 8 h/day × $3/h		$ 240.00	
(b) Power, water, wood miscellaneous		45.55	
		$ 285.55	
Processing cost/unit		$ 0.057	
Total Daily Operating Costs		$ 700.00	
Daily operating cost/unit (patty)		0.14	
Estimated Value of Plant Equipment and Facilities			
A. Equipment (Tray, press, heat sealer, smokehouse, deboner, mixer, misc.)			$ 21, 820.00
B. Remodeling and annexing of building and facilities			5, 000.00
Total			$ 26, 820.00
Annual Fixed Costs			
A. Salaries and Fees		$ 14, 600.00	
B. Insurance		5, 000.00	
C. Depreciation		2, 432.00	
D. Operating contingency reserve, improvements and repair, taxes, miscellaneous		4, 000.00	
Total annual fixed costs		$ 26, 032.00	
Selling price/unit		$ 0.25	
Total daily sales		1, 250.00	
Daily operating income		550.00	
Annual income (210 working days)		115, 500.00	
Total Annual Income		$ 89, 468.00	

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PROTEIN DEGRADATION IN HERBAGE EXTRACTS DURING MEMBRANE FILTRATION PROCESS: THE EFFECT OF REDUCING AGENTS ADDITION

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ABSTRACT

The degree of protein degradation in herbage extracts concentrated by the membrane filtration technique was measured as an effect of proteolytic enzyme activity which resulted in a substantial reduction of protein recovery in protein extraction procedure. Changes in enzymatic activity during the membrane filtration process were measured by the photo-chemiluminescence technique, while amino nitrogen in the permeate was determined colorimetrically using the ninhydrin method and protein in the concentrate was measured by the Orange G method. Protein concentration at 0° C reduced protein degradation without enzyme deactivation simultaneously decreasing the filtration action. Supplementation of herbage extracts with the reducing agents (ascorbic acid or sodium sulphite) prevented protein losses in extracts processed at 28° C due to the inhibition of enzymatic activity with a simultaneous 60-70% increase in the filtration rate as compared to process conducted at 0° C.

Protein concentrates recovered from the herbage extracts processed with an ascorbic acid (1% w/v) or a sodium sulphite (2% w/v) addition showed higher methionine contents as compared to LPC produced by membrane filtration at 28° C without reducing agents added. Reducing agents also have a positive effect on the nutritive quality of LPC as measured by the in vitro *protein digestibility and availability of the most essential amino acids; methionine, lysine and tryptophan.*

INTRODUCTION

The available data suggest that protein extraction from green vegetation — an ample source of protein — has a potential for the commercial production of protein in leaf protein concentrates (LPC) form (Ostrowski 1978a, 1979a, b). However, LPC's recoverable from green herbage represent a mixture of different types of proteins (Ostrowski 1979c) and other

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plant constituents, thus giving protein concentrates the distinctive dark green color, bitter taste and strong grassy smell. The unfractionated product is therefore not acceptable for direct human consumption (Pirie 1971; Ostrowski 1979b).

It has been shown previously (Ostrowski *et al.* 1979) that to recover purified LPC suitable for direct human nutrition in general practice, the most convenient is to separate chloroplastic, feed-grade proteins from the crude plant extracts by the use of simultaneous acid (ph 6) and heat (55° C) precipitation and to isolate the remaining cytoplasmic — food-grade protein fraction by the membrane filtration technique. Proteins recovered by membrane filtration after drying are still water-soluble (Knuckles *et al.* 1975) as opposed to those proteins recovered by heat or acid precipitation which are then made water-insoluble due to denaturation.

In the introductory trials (Ostrowski 1975, 1976b) however, it has been shown that protein extraction efficiency, as measured by the amount of protein recovered from herbage extracts, rapidly decreased with the time of extract storage prior to its processing.

Singh (1962) and De Fremery *et al.* (1972) also found significant losses in protein recoverable from different plant species due to extract storage at high temperature which was ascribed to the activity of the proteolytic enzymes in the herbage extracts (Brady 1961; Singh 1962; De Fremery *et al.* 1972; Ching Geh 1970). Therefore, it is reasonable to suppose that during the membrane processing, when working in a batch system, the amount of recoverable protein may be gradually decreasing due to the activity of the proteolytic enzymes present in extracts separated from macerated plant tissues.

In the present study, an attempt has been made to prevent loss of protein during membrane filtration by storing plant extract during processing at 0° C or at temperature of 28° C (which results in a fast filtration action) with a simultaneous addition of the reducing agents to prevent protein degradation by the inhibition of proteolytic enzymes activity. Biochemical evaluation of the recovered protein concentrates was also conducted in order to investigate the extent to which reducing agents added as preservatives to membrane processed extracts may affect the nutritional quality of the final product — LPC.

MATERIALS AND METHODS

Herbage Extracts

The protein extraction procedure, equipment and herbage, used as a

source of herbage extracts in this experiment were described earlier (Ostrowski 1979d).

Immediately after the mechanical herbage processing, the herbage extract was filtered through the stainless steel strainer (100 μ) and then through No. 54 filter paper on a Buchner Funnel. The extract was then allocated in a glass container with a mechanical glass stirrer. Membrane filtration was carried out at 28° C on a 10 liter volume of herbage extract, while another three, each of ten 1 volumes were stored and processed at either:

0° C,
28° C with ascorbic acid added at concentration 1% (w/v),
28° C with sodium sulphite added at concentration 2% (w/v).

Equipment and Automation

Experiment was conducted with five herbage extracts using the equipment shown on Fig. 1, composed of a membrane filtration circuit with a by-pass for concentrated extract. Enzyme activity, protein and dry matter contents were determined continuously in five minute circles and using a by-pass for permeate the determination of amino nitrogen was done in the continuous flow mode.

Sampling of concentrate and analytical operations as schematically outlined on Fig. 2, were controlled by the LKB 11300 Ultrograd gradient mixer through the three valves opening by-pass of concentrated extract, buffers and reagents for the period of time necessary to deliver appropriate quantities of samples to reaction chamber filled up with buffer and reagents. LKB 7000 UltroRac fraction collector was used for continuous collection of processed extract fractions mixed with Orange G solution in the constant ratio by the use of peristaltic pump and appropriate size of tubing. Peristaltic pump delivering buffer and reagents was controlled by programmable electronic clock with switch on-off action allowing refilling the reaction chamber with a new basic reactant. After every new development of a model oxydo-reduction reaction, an electronic clock switched off the buffer and reagent pump for some 2 min before extract administration, switching the pump on again with the completion of the reaction recording.

Membrane Filtration

The DDS Laboratory module (20.0-0.36-LAB) fitted with DDS-870 of a nominal 9×10^3 MW cut off membranes was used under the following conditions: inlet pressure — 170 KPA; outlet pressure — 140 KPA; temperature 28° C; circulation rate — 8.1 min^{-1}.

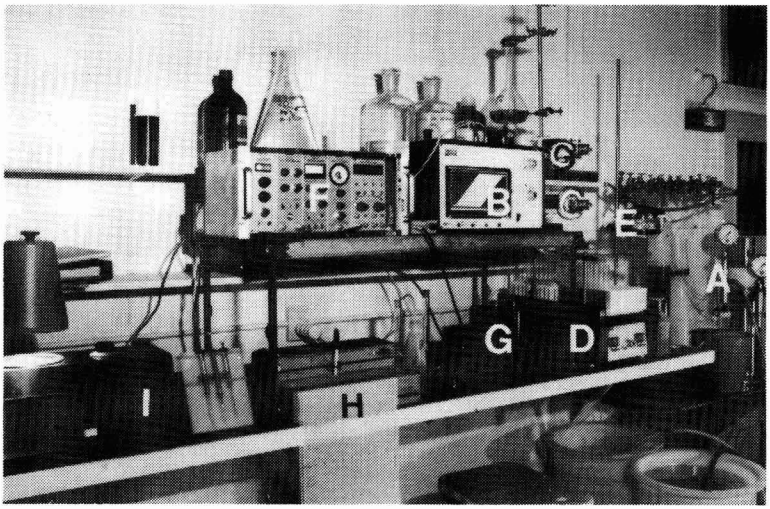

FIG. 1. THE EQUIPMENT ADAPTED FOR ANALYSIS OF OVERALL ENZYMATIC ACTIVITY IN MEMBRANE PROCESSED HERBAGE EXTRACTS BY THE USE OF BIOLUMINESCENCE TECHNIQUE

A, DDS laboratory membrane processing module (UF/RO);
B, LKB Ultrograd gradient mixer;
C_1 and C_2, LKB valves operated by the gradient mixer;
D, LKB UltroRac fraction collector;
E, On-line eluate concentrator;
F, Ortec equipment for bioluminescence measurement;
G, Philips scintillation counter and photomultiplier for bioluminescence detection;
H, Chart recorder for bioluminescence intensity recording;
I, Moisture balance used for rapid dry meter determination in herbage concentrate.

Membrane filtration circuit was operating each time until herbage extract was concentrated to approximately 10% DM at filtration rates as presented in Fig. 3. Further concentration of the unfractionated herbage extract was accompanied with the risk of irrecoverable membrane blockage. Operation conditions were similar to those described by Bungaard *et al.* (1972) and Ostrowski (1979d), and in principle comparable to those reported by Singh *et al.* (1974), Tragardh (1974) and Knuckles *et al.* (1975).

Photo-chemiluminescence (Bioluminescence)

Photo-chemiluminescence (bioluminescence) defined as the emission

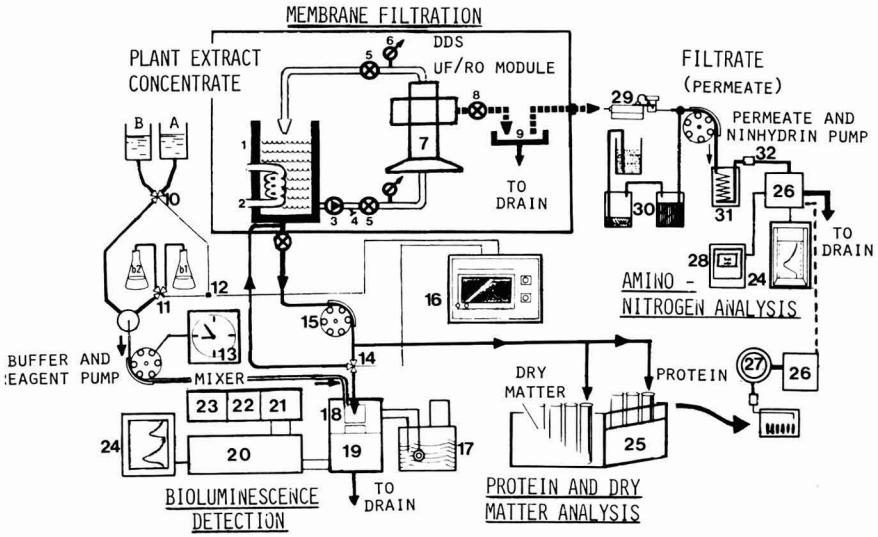

FIG. 2. SCHEMATIC DIAGRAM OF THE EQUIPMENT AND AUTOMATION USED IN ANALYTICALLY CONTROLLED MEMBRANE FILTRATION PROCESS

Analytical system composed of: DDS ultrafiltration circuit; circuit for continuous amino nitrogen determination in filtrate (permeate), system used for continuous protein and dry matter determination, circuit used for bioluminescence detection. 1. thermostated glass container for plant extract storage during concentration. 2. thermostat circulation; 3. pump; 4. flow rate adjustment; 5. valve; 6. pressure gauge; 7. DDS RO/UF laboratory membrane filtration module; 8. permeate valve, 9. plastic container for permeate collection; 10. buffer valve for Sorensen reagents A & B (in ratio 1:4); 11. reagent valve (0.4% hydrogen peroxide — b_1 and 0.1% pyrogellol-b_2 in ratio 1:4); 12. Parallel valves' connection; 13. electronic programmable switch on-off alarm clock; 14. sampling valve; 15. pump for concentrate sampling/by-pass of processed extract; 16. LKB gradient mixer; 17. thermostated (28°C) water bath; 18. flow cell with inlets for buffer and reagents mixture and sample injector; 19. lead house with a mounted in photomultiplier; 20. power stabilizer — high voltage power supply; 21. amplifier pulse high analyzer; 22. spectrum scanner; 23. log/lin rate meter; 24. multispeed chart recorded; 25. LKB UltroRac fraction collector; 26. 250 Gilford spectrophotometer; 27. Gilford automatic sampler; 28. date lister (printer); 29. On-line eluate concentrator; 30. Ninhydrine reservoir system; 31. reaction coil (100C) 32. Cooling unit.

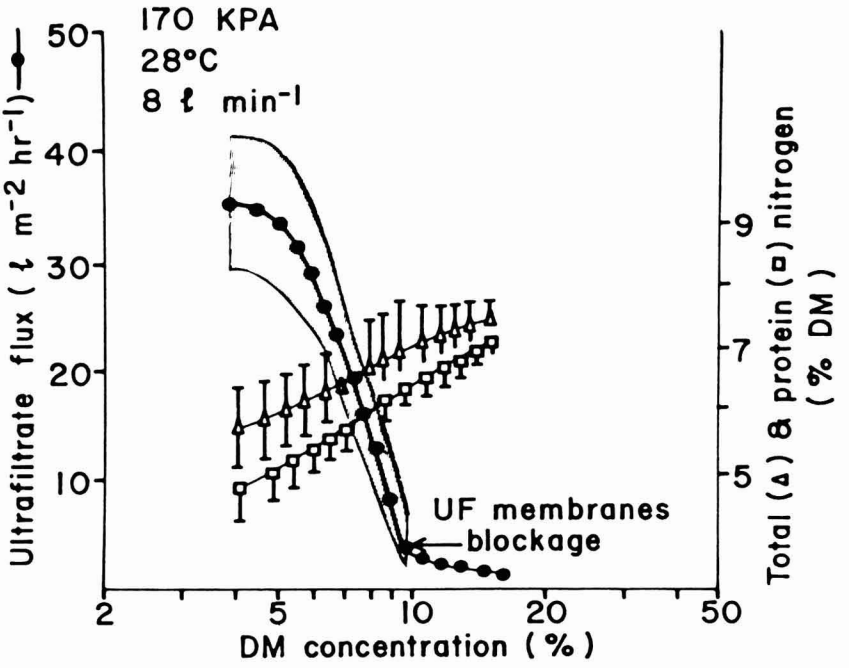

FIG. 3. VARIATION OF ULTRAFILTRATE FLUX WITH THE CONCENTRATION OF HERBAGE EXTRACT AND GRADIENT OF TOTAL AND PROTEIN NITROGEN CONCENTRATION (MEAN ± SE) AS OBTAINED BY THE USE OF DDS 870 MEMBRANE

of visible radiation in an enzyme catalyzed reaction, with the broad spectrum of quantum yields was measured by number of photons emitted by the standard volume of oxidased substrate herbage extract. Generally, chemiluminescence represents an efficient reaction with quantum yields from about 0.1 to merely 1.0 (Shimomura and Johnson 1970; Wampler *et al.* 1971) which at present may be directly measured with the photosensitive equipment. The enzymes present in the herbage extract provided the appropriate basic environment allowing for the appropriate transition levels (Cormier *et al.* 1973) as a result of enzymic catalysis of the oxidation of the substrate by enzyme(s) during which some quantum of energy was released creating a detectable, electronically excited state.

The intensity of bioluminescence of herbage extracts was measured as an intensity of the photon emission of the bufferised system pyrogallol-hydrogen peroxide. Photo-chemiluminescence of the model reaction (0.1% pyrogallol and 0.1% hydrogen peroxide) in phosphate buffer (pH 7.0) was measured at temperature 28° C in continuous flow mode using Philips scintillation counter with built-in Pyrex flow cell and photomultiplier

tube within a sensitivity range between 290 and 600 nm. ORTEC equipment composed from high voltage power supply (Model 456), amplifier pulse height analyzer (Model 486), spectrum scanner (Model 484), Log/lin rate meter (Model 449), built on bin (Model 401A) was connected with Heothkit multispeed servochart recorder (Model IRI8M). The system was operated at 995 V and time constant 0.1 s at range of detection between 1 to 10×10^5 impulses. No optical filter was used to eliminate phosphoroscence which according to Fodor-Csanyi (1972) is less important in the system when bio- or chemiluminescence is measured of which intensity is much greater than phosphoroscence.

Photo-chemiluminescence intensity of the model reaction was measured in a container of bottom area identical to the scintillation counter surface (6.3 cm^2) during 5 min which allowed to reach stable reaction intensity level (approximately 1×10^2 impulses per second). Immediately after herbage extract was introduced to a model reaction photon emission intensity ($J \times 10^5$) was measured during the next 10 min with the display of the sums of photon emissions accumulated over each of the consecutive 60 s of the total reaction. Bioluminescence procedure in automatic mode was controlled by LKB gradient mixer and electric clock with switch on-off facilities.

Protein Determination in Plant Extracts using Orange-G (OG) Method

Protein nitrogen (PN) content in herbage extract was determined from dye-binding capacity using the Orange-G method (OG) according to Outen *et al.* (1966) at a 1:40 ratio of sample to dye volume (1 g/1). Protein was calculated as PN $\times$ 6.25.

The validity of the OG method was tested prior to the experiment using 30 samples of herbage extract obtained from 10 separate herbage processings. Herbage extract was sampled at the beginning, middle, and end of each extraction procedure for PN determination using both the OG method and the Kjeldahl (Kj) procedure with trichloroacetic acid (TCA) precipitation in a final concentration of 10% (w/v).

Based on the mean of the three determinations of PN in each sample by both OG and Kj methods, there was a direct relationship between the two procedures, the correlation between them being 0.94. The mean OG protein values were estimates of those determined by Kj procedure with a SE of $\pm$ 0.19.

The variation among repeated determinations was however greater for OG than for Kj. Analysis of the data for the herbage extracts obtained from the 10 herbage samples with triplicate determinations gave coefficients of variance (CV) of 4.6% for OG and 1.6% for Kj. Despite the higher CV, the OG method was chosen for PN determination in this

trial due to its convenience and rapidity of measurement.

Protein was determined by the continuous collection of concentrated herbage extract in the test tubes allocated from one side of the UltroRac fraction collector after previous proportional mixing with Orange-G solution. Proportional mixing was achieved by peristaltic pump and appropriate size of tubing. After the whole filtration process was completed, a set of test tubes was filtered through Whatman No. 44 filter paper and then Orange-G uptake by proteins was determined at 485 nm on GILFORD spectrophotometer (Model 250) with rapid sampling, programmer and data listing facilities (sample mode 360 samples per hour).

Dry Matter

Concentrated plant extract was continuously sampled during the membrane processing operation with fractions being collected parallel to samples of protein determination using the same peristaltic pump and opposite side of the LKB UltroRac fractions collector. Test tubes had been weighed earlier so that the collected concentrates' dry weight could be determined accurately in an air-drought oven at 105° C for 24 h.

To determine the end of the membrane filtration procedure 10 g of herbage concentrate was occasionally used for rapid (18 min) dry matter determination using an infra-red moisture tester.

Amino-Nitrogen Determination

Amino nitrogen was determined in filtrate (permeate) continuously sampled from the syphon and passed on through an AMICON "on line" eluate concentrator fitted with 90 mm DIAFLO UM2 membrane. Ultrafiltrate was then used for amino nitrogen determination after proportional mixing with ninhydrine solution. Mixed solution was passed through the coil immersed in 100° C bath and intensity of the ninhydrine reaction was determined at 570 nm on GILFORD 250 spectrophotometer fitted with flow through cuvette and data being recorded on a strip chart recorder and alternatively on a data printer in 10 s intervals.

Chemical and Nutritional Evaluation of Protein Concentrates

Herbage proteins concentrated by the membrane filtration in individual preservation treatments were freeze-dried before further chemical and biochemical tests. These tests were conducted according to the methodology published earlier (Ostrowski 1979c).

Methionine was determined by the Lorenzo-Andreu (1961) procedure and methionine availability by the method described by Pieniazek *et al.* (1975), total and available lysine as described by Ostrowski *et al.* (1970).

Tryptophan was measured by using the procedure described by Matheson (1974) and tryptophan availability was determined using *Tetrahymena pyryformis* according to Boyne *et al* (1975). Amino acids were determined using the analytical and sample preparation procedures recommended by Byers (1971a, b) for protein concentrates analysis.

RESULTS AND DISCUSSION

The Effect of Membrane Processing Conditions and Preservatives on Protein Recovery from the Herbage Extracts

Membrane processing at 28° C without any reducing agent added to the herbage extracts resulted in substantial losses of protein (Fig. 4). Processing at 0° C increased the recovery of protein. However, because of the slow rate of filtration such an alternative cannot be recommended as practical. Processing extracts from herbage at low temperatures (0° C) resulted in approximately 70% lower filtration rates as compared to 28° C.

A decrease in recoverable protein in the herbage extract processed at 28° C without any reducing agents, was accompanied by a parallel decrease in the photo-chemiluminescence measurements. Comparing the changes in the photon emission of herbage extracts stored and processed at both 0° C and 28° C, without any reducing agent added and with corresponding changes in protein contents, a highly significant correlation has been found between these two measurements ($r = 0.907$).

A decrease in bioluminescence results was also recorded in the extracts processed at 28° C with a reducing agent added. However, a similar decrease in protein content did not occur. This was due to the presence of reducing agents which have been found (Ostrowski-Meissner unpublished data) as inhibitors of the model oxydo-reduction reaction used in this study for the development of the photo-chemiluminescence phenomenon.

The decrease in protein content in processed herbage extract was associated with the gradual increase in amino nitrogen concentrations in the filtrate (permeate) obtained from membrane processing (Fig. 5). This indicates protein breakdown in the herbage extract with simultaneous liberation of free amino acids, peptides and polypeptides which passed semi-permeable membrane used in the filtration process. On the other hand, free amino acid nitrogen contents determined in the permeate obtained from the filtration of herbage extracts with the addition of reducing agents indicate that the reducing agents used in this study prevented protein losses. This may be ascribed to inhibition of proteolytic enzymes activity. When reducing agents: citric acid or sodium sulphate were added to the plant extracts immediately after juice expression from the plant

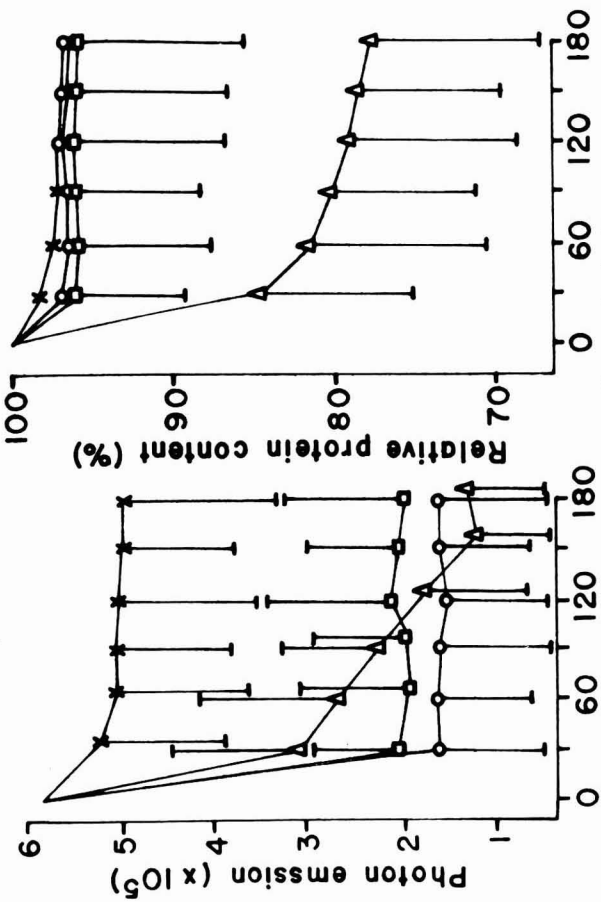

FIG. 4. CHANGES IN THE BIOLUMINESCENCE OF THE HERBAGE EXTRACTS AND THE DEGREE OF THE REDUCTION IN PRECIPITABLE PROTEIN STORED DURING 3 H MEMBRANE PROCESSING AT EITHER 0°C AND 28°C WITHOUT AND WITH REDUCING AGENTS ADDED TO THE EXTRACTS: ASCORBIC ACID (1%) AND SODIUM SULFITE (2%)

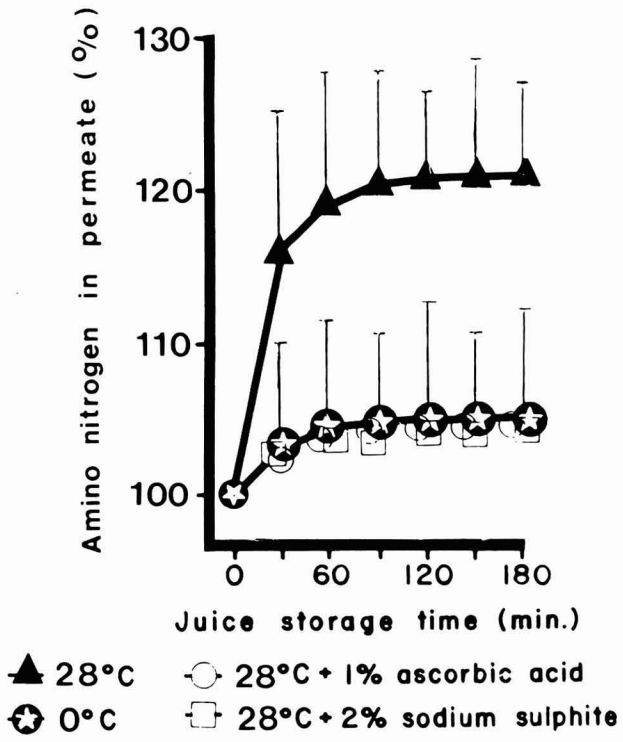

FIG. 5. CHANGES IN AMINO NITROGEN CONCENTRATION IN PERMEATE (FILTRATE) DURING HERBAGE EXTRACT PROCESSING AT 0°C AND 28°C WITHOUT OR WITH EITHER ASCORBIC ACID (1%) OR SODIUM SULPHITE (2%) SUPPLEMENTATION TO THE EXTRACT AS THE REDUCING AGENTS

cells, then despite the membrane processing at 28° C the recovery of protein was similar to that extract processed at 0° C (Table 1). The higher recovery of protein from herbage extracts stored and processed at low temperature as compared to 28° C, may be due to thermal inactivation of the proteolytic enzymes responsible for breakdown of proteins in the herbage extract, stored and processed at high temperatures.

Once the temperature rises, inactivated enzymes can still destroy proteins in the extract due to proteolytic activity which has been suppressed by the low (0° C) storage temperature. On the other hand, rising temperatures in the processed extract, containing a reducing agent as an inhibitor of proteolytic enzymes, does not effect proteins which can be recovered in a membrane processing operation without losses being observed in

Table 1. Protein yields, chemical and nutritional characteristics of LPC obtained from herbage extracts processed by membrane filtration conducted in various processing conditions[1]

Measurement	0°C	28°C	28°C with Citric Acid (1% w/v)	28°C with Sodium Sulfite (2% w/v)
LPC's Production Efficiency				
Protein yield (g per 1 l herbage extract)	61[a]	43[b]	62[a]	60[a]
Chemical Characteristic of LPC				
Protein nitrogen (% DM)	7.6[b]	8.8[a]	7.9[b]	7.5[b]
Total essential amino acids (g per 100 g recovered)	48[a]	52[a]	47[a]	48[a]
Biochemical Characteristic of LPC				
Protein digestibility *in vitro* (%)	88[a]	91[a]	90[a]	92[a]
Amino acid concentration (g/16gN) and (availability — %)				
— Lysine	5.9 (84) aA	5.6 (61) aB	5.7 (82) aA	5.8 (79) aA
— Methionine	1.8 (77) aA	1.1 (64) bB	1.9 (79) aA	1.9 (77) aA
— Tryptophan	2.0 (86) aA	2.0 (70) aB	1.7 (87) aA	1.8 (89) aA

[1] Each value represents the mean of the three individual LPC obtained in separate membrane filtration procedures

Values in the same line with unlike superscrips indicate significant differences at $P<0.05$ as determined by Duncan's multiple range test. Capital letters are related to figures in brackets, i.e. to significance in amino acid availability

juice processed at a temperature of 28° C without any reducing agent.

According to Singh (1962) who studied the effect of juice storage after extraction from several plants in hot climate conditions on protein recovery in protein extraction procedure, the decrease of up to 40% in the yield of protein may be observed due to juice storage in elevated temperatures without preservatives added as compared to freshly extracted juice. Noteworthy — up to 50% reduction in protein recovery from alfalfa juice stored at 50° C was also reported by De Fremery *et al.* (1972). The reduction in protein recoverable in precipitable form from stored juice was ascribed to the activity of proteolytic endogenous enzymes which in herbage juice caused the degradation of precipitable protein particles into soluble peptides and amino acids which are not recoverable in precipitation procedure (Singh 1962; Ching Geh 1970; De Fremery *et al.* 1972). At lower temperatures these enzymes according to Pirie (1971) are not active enough to cause a notable reduction in protein yield. This was demonstrated also in the present study in reference to herbage extracts processed at 0° C, where the decrease in recoverable protein yield after 3 h was minimal.

None of the workers however, dealing with enzymatic protein degradation (Singh 1962; De Fremery *et al.* 1972) did indicate or ascribe the protein degradation to any particular proteolytic enzyme. Based on the general concept of overall enzymatic catalysis in the herbage extracts which as was shown in this study may be measured by the photo-chemiluminescence phenomenon developed in the model oxydo-reduction reaction (Fig. 6) and looking simultaneously at the protein degradation as indicated by protein loss in the herbage extracts (see Fig. 4) as well as at the free amino nitrogen appearances in permeates during the membrane filtration process, one can ascribe enzyme activity mostly to proteolytic enzymes. It appears from the results presented so far that active proteolytic enzymes may be responsible for lowering the recovery of protein during the membrane filtration process.

The close agreement between the degree of protein degradation and consequential loss of protein yield obtained with the time of juice storage and the bioluminescence values expressed as a maximum photon emission, showed that this method may be a useful procedure in enzymatic protein degradation studies. The bioluminescence method was also shown in the earlier studies as a simple bio-physical technique suitable for measuring the changes in enzymes activity in stored plant material (Ostrowski 1971a, b; 1972b). While in general the technique looks a promising one for application in such studies it will be necessary to improve the method which would be selective for proteolytic enzyme(s) exclusively while other enzymes would not be interfering in the selec-

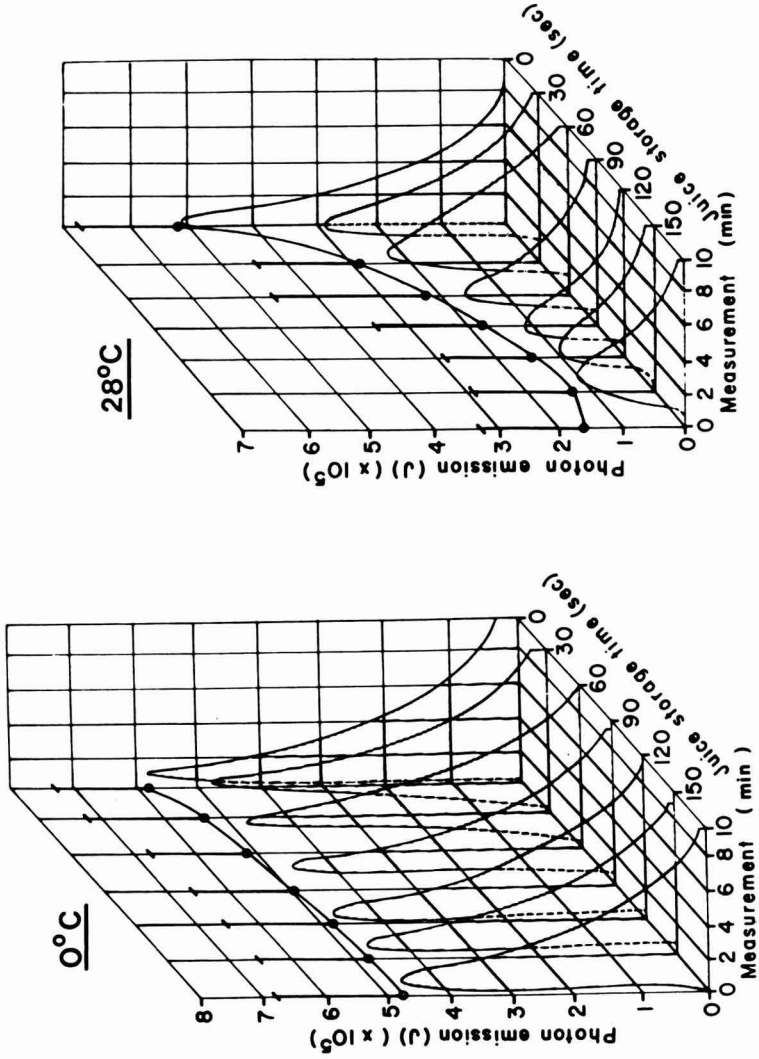

FIG. 6. BIOLUMINESCENCE CHARACTERISTIC OF THE HERBAGE EXTRACTS BEING STORED DURING PROCESSING AT EITHER 0°C OR 28°C WITHOUT REDUCING AGENT ADDED

tive model reaction. Photo-chemiluminescence (bioluminescence) as a method to detect the superweak luminescence using commercially available instrumentation (photomultiplier electronic pulse recorded) has been already described (Duquesney *et al.* 1970) as of great value in photochemistry, photobiology and photophysics and also in analysis of several biological materials (Ostrowski 1972a, b; 1978c; Ostrowski *et al.* 1972).

Supplementation of the herbage juice with the reducing agents — particularly with sodium sulphite in concentration 2% (w/v) — notably reduced enzyme activity while in juice cooled down before being stored the enzymes still remained with their high activity as recorded at the time of expression from the plant material. The reduction in enzyme activity due to reducing agents supplementation suggests that such chemicals be used for juice preservation when storage and/or processing of the herbage extracts at ambient temperatures are invisaged.

The Effect of Membrane Processing Conditions and Preservatives on Nutritive Quality of the Recovered Protein Concentrates

The higher yields of protein from herbage extract with the membrane filtration conducted at 0° C or at 28° C with the reducing agents added, as compared to the membrane processing at 28° C without reducing agents (see Table 1) was associated with the lowering in protein nitrogen concentration in the final product — LPC. LPC recovered during the processing at 28° C without reducing agents characterised by a slightly higher total essential amino acid concentration with a simultaneous substantial decrease in methionine content as opposed to LPC's recovered at low temperature and at 28° C but with preservatives added. There were however, no differences in the concentrations of lysine and tryptophan neither in the *in vitro* digestibility of protein recovered by the membrane filtration procedure conducted at various conditions; digestibility figures being within the range of values reported by Byers (1971a), Subba Rau *et al.* (1969, 1972), and Hartmann *et al.* (1967) to be satisfactory for such a type of product, but lower than those reported by Akeson and Stahmann (1965) and Saunders *et al.* (1973).

LPC produced by the membrane filtration at 28° C without preservatives added was also inferior to LPC's recovered in the other three conditions in terms of methionine, lysine and tryptophan availability. The contents of the three essential amino acids in LPC's, whatever the membrane filtration conditions, were in the range of concentrations reported by Byers (1971a, b), except for the lower methionine values in the LPC recovered from the processing at 28° C without preservatives added. The amino acid compositions of protein concentrates and lysine concentration in particular, has been previously found to be dependent on the technique

used for protein separation from plant juice (Bhatty and Finlayson 1973; Girault 1973; Ostrowski 1979c). In this paper methionine appeared to be the amino acid most sensitive to membrane processing conditions. However, all LPC's recovered in this study by the membrane filtration technique despite the processing conditions, showed methionine deficiency as compared to amino acid composition of the 1965 FAO/WHO protein standard. Since the latest FAO/WHO (1973) protein and amino acid standards have been criticized as being inadequate for health mean (Gorza *et al.* 1977), the earlier (FAO/WHO 1965) standard amino acid profile, different to the later (FAO/WHO 1973) criticized publications, was used as a reference in this study. Despite the total lysine and tryptophan "sufficiency" detected in all membrane processed LPC's, due to observed lowering in the availability of these amino acids, the LPC which was ultrafiltered at 28° C without reducing agents added showed biological deficiency of lysine and tryptophan. This is in addition to a further decrease in biologically accessible methionine.

Lowering in lysine availability can be explained by the processing during which plant extract is exposed to both elevated temperature and physical forces (Lund 1973). In such conditions ε-amino groups of lysine being especially reactive, can crosslink with reducing sugars present in heated herbage liquor (Maillard reaction), or in the absence of sugars may form alternative crosslinks with other amino acids in protein particles (protein-protein linkages) (Hurrel and Carpenter 1977; Ostrowski 1978b). With the membrane filtration at 28° C, in the presence of reducing sugars detected in the herbage extract (Ostrowski 1979c; Free and Satterlee 1975) there are more opportunities for lysine to be damaged — via the Maillard reaction — than in ultra-filtration process at either low (0° C) or at 28° C but with reducing agents added to the extract which appeared to have a protective action on lysine during herbage extract processing. Since only lysine molecules with free ε-amino groups may be utilized by single-stomached organisms for their metabolism and growth (Carpenter 1973), LPC's which contained less "available" lysine represent protein of lower nutritive value as opposed to LPC's containing a similar total lysine but of a slightly higher availability.

In this study tryptophan — biologically available to monogastric organisms — (i.e. total tryptophan contents corrected for its availability) has been shown as a limiting amino acid too. This due to a lowering in its availability.

Wallace (1973) who analyzed losses in the nutritional quality of proteins due to their processing indicated that the nutritive value of processed vegetable proteins tends to be limited more by the sulphur amino acids than by lysine and so damage to sulphur amino acids (or methionine)

is generally more significant in nutritional practice than damage to lysine. The results obtained in this study would indicate that this is so. However, due to the substantial loss in availability, the essential amino acids: lysine and tryptophan — in addition to methionine — also become deficient in biological terms as judged by the FAO/WHO (1965) amino acid standard.

ACKNOWLEDGMENTS

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SENSORY THRESHOLD OF STYRENE AND THE MONOMER MIGRATION FROM POLYSTYRENE FOOD PACKAGES

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INTRODUCTION

Migration of low-molecular weight compounds, including residual monomers, from polymeric packaging materials into packaged foods and drugs has been the object of growing interest and concern in recent years, primarily due to the discovery that some of these compounds involve health hazards, up to and including carcinogenicity. The latter was the case with vinyl-chloride monomer (VCM), and as a result considerable attention was given to polyvinyl-chloride (PVC) and assorted additives (plasticizers, stabilizers, lubricants, etc.) used in it, Figge (1972), Daniels and Proctor (1975), Downes and Gilbert (1975), Gilbert (1976), Chudy and Crosby (1976) and Morano *et al.* (1977).

As regards styrene, its acute toxicity has been thoroughly investigated by Gerarde (1960) and it was found by Withey (1976) to irritate human eyes, throat and skin. In rats, coma and death supervened within 1 h of exposure to a level of 5000 ppm. Milvy and Garro (1976) and Loprieno *et al.* (1976) found that styrene oxide is formed in a metabolic process, and while no evidence has been produced that styrene itself is mutagenic, its oxide induced forward mutations in yeast-and *Salmonella* strains. On this basis it was concluded that styrene may be activated to a potentially carcinogenic compound. Van Duuren (1969) actually found that styrene epoxide induces tumors on animal skin. There is no evidence to-date, however, that styrene is, or may become, a carcinogen for humans. Apart from the above, styrene has a very strong and repellent smell and its migration from the packaging material into the contents imparts a highly disagreeable off-flavor. Thus, even if styrene is eventually proved safe below a certain concentration level, foods contaminated by it may be rejected by consumers due to off-tastes.

The present work was carried out with the aim of establishing the residual styrene in polystyrene food packages as well as of determining

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the sensoric threshold of this monomer in foods and its migration into food simulants.

EXPERIMENTAL

Residual Monomer Determination

The residual styrene content in two types of food-grade polystyrene containers obtained from a food-processing plant was tested: (1) cups for yogurt and sour cream, (2) cups for 9% fat, soft cream cheese. Two methods of analysis were compared: (1) The Food and Drug Administration method (1977), according to which a sample of the polystyrene is dissolved in methylene chloride and a measured amount of the solution is injected into a gas chromatograph; (2) an alternative method, in which a 10g polystyrene sample is extracted with 200 cc acetone in a Soxhlet apparatus, and a predetermined volume of the solution is injected into a GC.

Migration Study

The cups (wall thickness approximately 0.3 mm) were cut into pieces, about 0.5 cm × 1.0 cm in size. Weighed samples were placed in vials of 60 cc nominal size (exact volume predetermined), filled with soybean oil or water as food simulants. The vials were sealed with a rubber stopper and an aluminum crimp and stored at ambient temperature (~23° C). After a predetermined period, a 5 cc sample of the oil or water was withdrawn from the vial and injected into another stoppered vial, which was kept in an oven for half an hour (at 125° C for the oil, 110° C for the water). Finally, 5 cc of the headspace were withdrawn and immediately injected into the gas chromatograph for styrene determination.

Gas-chromatographic Procedure

A Varian 1700 gas chromatograph, equipped with a dual flame ionization detector, and a 6 ft. × 1/4 in. stainless steel column filled with chromosorb W/AW, DMCS with a 15% SE 30 coating was used. Flow rates were: nitrogen (carrier gas) — 35 cc/min; hydrogen — 60 cc/min; air — 400 cc/min. Temperatures: injection port — 165° C, column — 122° C, detector — 175° C.

Two calibration curves were constructed by injecting known amounts of a styrene in acetone solution and plotting the area under the peak against the weight of injected styrene. The lower styrene concentrations necessitated a separate calibration curve, for accurate results. Both cali-

bration curves were straight lines.

Organoleptic Evaluation

Sour cream (15% fat) was used as the food system for taste threshold determination using the paired comparison technique, Larmond (1970). Each pair consisted of a spiked and unspiked sample of sour cream and panelists from our department pretrained for styrene were asked to find the spiked sample. The samples were prepared from a stock mixture of styrene and sour cream by successive dilutions with fresh sour cream to the desired styrene level. The range of styrene concentrations tested was 0.0005-0.01 ppm, the upper limit having been established by preliminary evaluation of the team as the one above which a strong and disagreeable off-taste is observed. Two sets of tests were carried out. In the first set, the team consisted of eight panelists for the two highest styrene levels (0.005 and 0.01 ppm) and eighteen for the lower ones; in the second set, eighteen panelists were employed in evaluating all five levels. Samples were presented in random order. Rinsing water and crackers were also presented to the panelists and an unlimited time was allowed for the evaluation. Results were analyzed according to the procedure outlined by Larmond (1970).

RESULTS AND DISCUSSION

The residual styrene was found to be 0.194% and 0.138% in the cheese and yogurt cups, respectively by the extraction method, and somewhat lower — 0.183% and 0.112%, respectively — by the FDA method. These values are well below the maximum level of 0.5% styrene in polystyrene allowed by the FDA for fatty-food packaging.

Figure 1 is a plot of the residual styrene in five polystyrene samples as determined by the two methods. The points represent averages of two determinations by each of the methods, the difference between the duplicates was however, minor. The straight line obtained (with some scatter) has a slope of 45° C, showing that results by the two methods are comparable. The FDA method has two disadvantages. One is that methylene chloride is highly volatile, and extensive precautions must be taken in order to prevent its evaporation and the attendant distortion of the results; the other is that polystyrene is injected into the separating column, together with the methylene-chloride solution, and its accumulation on the column may interfere with the separation process. On the other hand, the method is very fast and simple and if the appropriate precautions are taken, it is superior to the relatively lengthy extraction method.

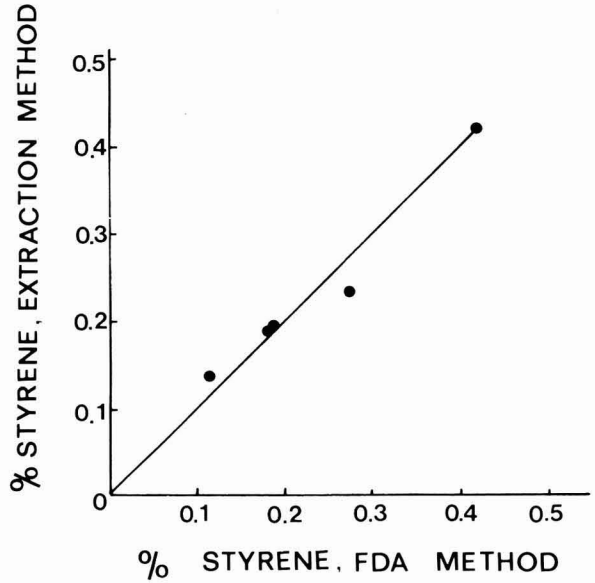

FIG. 1. RESIDUAL STYRENE IN POLYSTYRENE ACCORDING TO THE FDA AND EXTRACTION METHODS

Figure 2 is a representative plot of the amount of styrene found in the food simulants as function of contact time between them and the polymer. The general shape of the curve is similar to those obtained by Davies (1974) who described the migration of styrene from polystyrene sheets by means of a computerized curve fitting technique based on the following equation:

$$M_t = M_{inf}\left[1-\exp\frac{Dts^2}{M_{inf}^2\,a^2}\,\mathrm{erfc}\left(\frac{Dts^2}{M_{inf}^2\,a^2}\right)\right]^{0.5}$$

where M_t is the amount of styrene transferred after time t; s- the styrene content of the polystyrene (in ppm); D- the (adjustable) diffusion coefficient; M_{inf}- a parameter (likewise adjustable) representing the amount transferred in an infinite time; a- the ratio of solution volume to surface area of both sides of the sheet sample. The curves in Fig. 2 could therefore be described by an equation of the above mentioned type. No attempt was made, however, at this stage to determine its parameters, or to find a satisfactory alternative equation as further work on this aspect is under way and will duly be reported.

Figure 2 shows that the migration of styrene into oil was higher from

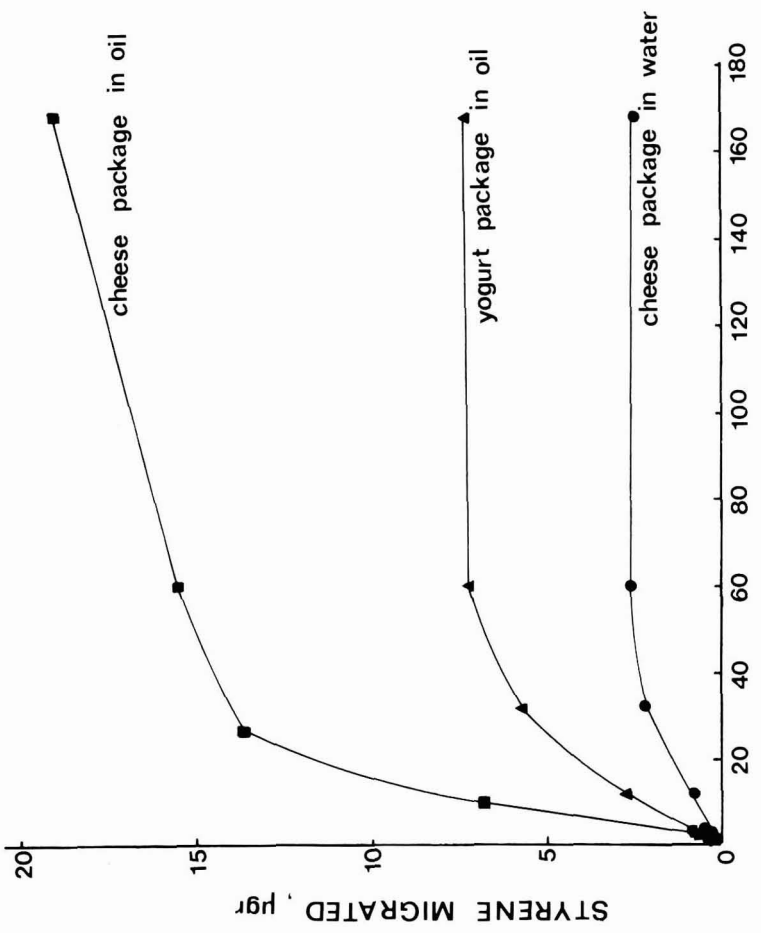

FIG. 2. AMOUNT OF STYRENE FOUND IN THE FOOD SIMULANT AS A FUNCTION OF CONTACT TIME

cheese than from the yogurt package. This was expected since the styrene content in the cups was higher. Moreover, the concentration of styrene in the oil was much higher than that in water — also as expected, as the solubility of styrene in water is much lower than in oil and its partition coefficient[1] in the polystyrene/water system is higher than the corresponding coefficient for the polystyrene/oil system. It is also seen that while for the cheese cups in oil an asymptotic styrene level was not reached even after a week of extraction, asymptotic levels of 2.5 μg and 6.6 μg, respectively were obtained for the cheese cups in water and the yogurt cups in oil after a contact period of approximately 60 h. Recalling that the amounts of the liquid media were very close to 50 cc and that the measured density of the oil was 0.92 g/cc, the above values correspond to 50 ppb (w/v or w/w) of styrene in water and 132 (w/v) or 143 ppb (w/w) of styrene in oil.

Whitey's (1976) studies on styrene migration into yogurt as well as into cold and hot water from polystyrene cups gave the following results: for cups with an average residual styrene concentration of 632 ppm (w/w) an average level of 12.1 ppb (w/v) of styrene in yogurt was found; for cups with a residual styrene concentration in the range of 1333 to 1855 ppm, average values of 326 and 52 ppb were determined after leaching for 24 h with hot and cold water, respectively. The latter value is comparable with our own value of 50 ppb. The values of M_{inf} obtained by Davies (1974) for water ranged from 50 to 900 ppb, depending on the initial styrene concentration, s, in the polystyrene: M_{inf} = 50 ppb for s = 0.05% and M_{inf} = 350 ppb for s = 0.24%. Our own result of 50 ppb for s = 0.19% is thus lower than could have been expected according to Davies, but direct comparison is inappropriate because of the fact that his M_{inf} is a curve fitting parameter, not the concentration at equilibrium. Moreover, there is no mention in Davies' treatment as to whether M_{inf} given in ppm, refers to the concentration in the contacting phase or is the transferred mass of styrene divided by the weight of the polystyrene used in the migration studies.

Table 1, summerizes the organoleptic threshold evaluations and shows that 0.005 ppm (5 ppb) was the lowest concentration at which a positive identification was obtained (at 95% confidence) while at the lower level of 2.5 ppb, one set of tests yielded a significant result and the other — an insignificant one. It can therefore be stated that off-taste is detectable when the styrene concentration in sour cream is 5 ppb or more — a surprisingly low level.

Figure 2 shows, for the cheese cup in oil after 60 h and one weeks's contact, levels of 15.5 μg (337 ppb) and 19 μg (413 ppb) styrene, respec-

[1] defined as: styrene conc. in PS/styrene conc. in water

Table 1. Organoleptic Threshold Evaluation of styrene in sour cream

Styrene Concen. (ppm)	Test No. 1			Test No. 2		
	No. of Panelists	No. of True Answers	Significance Level	No. of Panelists	No. of True Answers	Significance Level
0.0100	8	8	99%	18	14	95%
0.0050	8	8	99%	18	14	95%
0.0025	18	14	95%	18	11	Insignificant[1]
0.0010	18	12	Insignificant[1]	18	7	Insignificant[1]
0.0005	18	11	Insignificant[1]	18	6	Insignificant[1]

[1] At the 95% confidence level

tively-approximately 70-85 times higher than the organoleptic threshold. As stated before, a concentration of 50 ppb styrene was found after 60 h leaching with water. Bearing in mind that the effective surface in our migration studies was approximately double the actual exposed surface inside the cups and that the capacity of a yogurt cup is approximately three times the volume of the simulant used in the present studies, a ratio of about only 2 between styrene concentration and threshold level should have been anticipated if the surface area were reduced and the volume of simulant increased. On the other hand an increase in food volume, with a view to a reduced styrene concentration, would increase the driving force for leaching, which in turn could increase the styrene content of the simulant. A concentration of two to three times the threshold level can therefore be expected in a low-fat product packaged in polystyrene cups of the type investigated in the present study. For high fat products, on the other hand, a much higher styrene level can be expected. The levels of styrene in yogurt found by Whitey for polystyrene cups with low residual styrene are, on the average, two and a half times the threshold level. However, in the cases of polystyrene cups with high residual styrene, and where hot water was used as leaching medium, significantly higher styrene concentrations were observed.

The present study showed that when the residual styrene in a polystyrene container is of the order of 0.1-0.2% (much lower than the levels of 1% for non-fatty foods, and 0.5% for fatty foods, allowed by the FDA), significant monomer migration will occur which may impart an off flavor to the contents. The problem may become more serious if styrene is found to undergo changes during metabolism, with the potential risk of carcinogenicity as suggested by Milvy and Garro (1976) and Loprieno *et al.* (1976).

SUMMARY

The FDA method for styrene determination in polystyrene food containers was compared to an extraction method, and comparable results were obtained. A threshold concentration of styrene in sour cream of 5 ppb was determined, at the 95% confidence level by an organoleptic evaluation. Migration studies from commercial polystyrene containers, with oil and water used as food simulants, revealed that the level of styrene transferred were much higher for oil as compared to water. In the latter case, styrene levels double the sensory threshold were found after 60 h of contact. With soybean oil, however, the styrene concentration was almost two orders of magnitude higher.

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GLYCOALKALOID STABILITY DURING THE EXTRUSION OF POTATO FLAKES

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ABSTRACT

The total glycoalkaloid (TGA) of non-extruded potato flakes was found to be 17.2 *mg/*100 *g of flakes on the dry weight basis. Extrusion at* 70, 100, 130 *and* 160° *C of flakes containing* 59 *and* 48% *added moisture did not result in a significant reduction of TGA. However, product containing* 38% *added moisture and extruded at* 160° *did result in lower TGA levels. Likewise, extrusion at* 130° *and* 160° *of product containing* 25% *added moisture also resulted in lower TGA levels. Thus, it would appear that under certain extrusion conditions, TGA is not stable.*

INTRODUCTION

Tubers of the potato plant contain a class of naturally-occurring toxicants that are commonly called glycoalkaloids. The primary compounds of this type in potatoes are α-solanine and α-chaconine, which are glycosides of the steroidal alkaloid solanidine. A total glycoalkaloid (TGA) content of 20 mg/100 g of raw potato has been proposed by Jadhav and Salunkhe (1975) as being the acceptable upper limit for these compounds due to their potential health hazard.

Extensive literature exists relative to the numerous factors that influence TGA synthesis and levels in raw potatoes (Maga 1980). However, few data are available relative to TGA levels in processed potatoes. Jadhav and Salunkhe (1975) reported, without the support of data, that potato glycoalkaloids are not destroyed by cooking, baking or frying. Porter (1972) reported that these compounds decompose in the neighborhood of 260° C which is approximately 70° above normal commercial potato frying temperature. Earlier, Baker *et al.* (1955) concluded that solanine was stable to cooking although their preliminary data did not completely indicate this to be the case. Recently, Sizer *et al.* (1980) found the process of chipping, under the conditions employed, to be non-destructive to

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TGA. Actually the concentration of TGA occurred during the frying step due to product moisture loss.

Recent trends in the snack food industry indicate that snacks formed by extrusion processing are becoming more popular (Maga and Sizer 1978). Certain of these extruded snacks are made from dehydrated potatoes. Since no published data are available on the fate of TGA during the extrusion of potato products, this study was designed with this question in mind.

MATERIALS AND METHODS

Potato Flake Reconstitution

Freshly prepared commercially available drum-dried potato flakes were obtained for this study. Potato flakes were mixed in a Paterson-Kelley Model LB-P-8 twin shell blender for 10 min with tap water to yield added moisture contents of 25, 38, 49 and 59%. The resulting potato meal was rapidly transferred after mixing to an air-tight container to minimize evaporative losses.

A Brabender Plasticorder Extruder Model PL-V500 was used. The barrel had a diameter of 1905 mm with a 20:1 length to diameter ratio and was rifled with eight 0.79 mm × 3.18 mm logitudinal grooves. The screw had a diameter of 19.0 mm with a helix angle of 17.7°. The flight depth was 3.81 mm in the feed section and tapered to a minimum depth of 0.76 mm in the metering section, thus having a 5:1 compression ratio. Extrusion temperatures of 70, 100, 130 and 160° C were maintained by use of variable power electrical heating jackets and air-cooled jackets. The die had a diameter of 1.98 mm.

The extruder was started cold and the screw speed adjusted to 100 rpm by means of a tachometer. Product was introduced and the unit heated to the desired temperature for a minimum of 10 min before samples were taken. This insured that the unit had reached operating equilibrium.

Retention time was determined by measuring the time in seconds for a one gram plug of reconstituted potato flake at the appropriate moisture level containing green food coloring to first begin exiting the extruder. The time from injection until a distinctive green color appeared in the exiting product was designated as retention time.

Pretreatment of Samples Before TGA Analysis

Extruded samples were permitted to cool at room temperature and

then were frozen. To minimize heat buildup, samples were ground in the frozen state to pass through a 50 mesh screen, then freeze-dried and stored at −30° C until analyzed. To serve as a control, 6 samplings of nonextruded potato flakes were also freeze-dried and subjected to TGA analysis.

TGA Analysis

TGA analyses were performed utilizing the non-acqueous titration method with bromphenol blue introduced by Fitzpatrick and Osman (1974) as modified by Fitzpatrick *et al.* (1978). Three samples were taken from each trial composite for analysis. According to MacKenzie and Gregory (1979), the above method has limitations and may not be useful for detecting small differences in TGA levels between treatments.

RESULTS AND DISCUSSION

The TGA levels found in 6 samplings of commercial nonextruded potato flakes used in this study are shown in Table 1. As can be seen, the samples ranged from a low of 16.9 to a high of 17.5 mg/100 g with the average value being 17.2 mg/100 g. Assuming little or no loss occurred in the manufacture of these flakes, the raw potatoes utilized would have had approximately 3.5 mg/100 g fresh weight, which is an acceptable consumption level (Jadhav and Salunkhe 1975). Since the potatoes utilized for the manufacture of the flakes used in this study were no longer available, it is not known with certainty if TGA level was influenced by processing.

Table 1. TGA levels in nonextruded potato flakes

Sample	TGA Level (mg/100g flakes — dry weight basis)
1	17.4
2	16.9
3	17.5
4	17.3
5	17.3
6	17.0
Average:	17.2

Table 2. TGA levels in extruded potato flakes

% Water Added	Temperature of Extrusion (°C)	Retention Time (Sec)	TGA Level[1]	Statistical Significance[2]
59	70	45	17.1 ± 0.2	1, a
	100	35	17.2 ± 0.9	1, a
	130	32	17.1 ± 0.5	1, a
	160	30	17.0 ± 1.0	1, a
48	70	52	16.5 ± 0.7	1, a
	100	50	16.5 ± 1.1	1, a
	130	47	16.1 ± 0.5	1, a
	160	42	16.0 ± 0.9	1, a
38	70	44	15.2 ± 0.6	1, a
	100	47	15.1 ± 1.1	1, a
	130	53	14.3 ± 0.7	1, b
	160	52	13.7 ± 0.5	2, b
25	70	100	11.4 ± 0.7	1, c
	100	105	11.2 ± 0.5	1, c
	130	120	10.3 ± 0.9	2, c
	160	135	8.1 ± 0.8	3, d

[1] mg/100g dry product

[2] Different numbers within each group are significantly different within group ($\alpha = 0.05$). Different letters among groups are significantly different ($\alpha = 0.05$)

However, in the case of extrusion processing of these potato flakes, it can be seen from Table 2 that TGA losses did occur under certain conditions. It should be noted that as the amount of moisture in a formulation decreased because of an increase in viscosity, which gave it a longer residence time, the product would undergo more severe thermal treatment especially at higher temperature. In addition, since the green colored product exited for some time after its first appearance due to backmixing of product within the extruder, the residence time within the extruder for each formulation variable was actually longer than measured retention time.

As can be seen from Table 2, no apparent losses in TGA were noted with the high moisture level (59 and 48%) formulations over an extruder temperature range of 70-160°. However, when the formulation contained 38% added moisture, a statistically significant TGA loss was detected at 160° as compared to lower extrusion temperatures. This same trend was observed at the two higher extrusion temperatures employed with the 25% added moisture formulations. Thus, these data would indicate that under certain formulation and extruder operation conditions TGA losses can occur.

Perhaps it can be argued that the observed losses could be due to inefficient TGA extraction of the more adversely extruded samples. Originally, this concept was to be evaluated by adding known quantities of a nonpotato glycoalkaloid, such as tomatine, before extrusion. However, a preliminary trial demonstrated that at low moisture-high temperature extrusion conditions, lower tomatine levels were measured. Indirect evidence refuting the belief that extrusion may be binding TGA in some manner and thus result in lower measured values can be presented by viewing the TGA changes associated with specific moisture-temperature combinations among the groups shown in Table 2. For example, combinations of 59 and/or 48% added moisture and an extrusion temperature of 160° did not result in TGA change. However, at the same temperature but using only 38 and/or 25% added moisture, changes were observed. Other factors that could serve as possible explanations for lower levels of TGA under certain extrusion conditions include product residence time and internal pressure, which could result in TGA destruction through physical shearing as well as possible thermal degradation.

Not considering the possible influence of pressure and shear, another possible reason for TGA destruction at low moisture/high temperature extruder variables is a concentration effect of reactants due to the lowering of the water content at a high water activity (Labuza 1970).

Thus, although the method of TGA analysis employed may have limitations, changes were significantly large enough to statistically dem-

onstrate loss of TGA during the extrusion of potato flakes.

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