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Effects of Enzymic Starch Degradation During Storage of Cooked Beef Sausages

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ABSTRACT

Enzymic starch degradation ability of cooked beef sausages was studied, using irradiation to extend storage life of vacuum-packed sausages. With cooking temperatures between 60 and 75°C, starch degradation increased significantly during storage. Variation in beef cuts or freezing of meat prior to processing did not influence the extent of starch degradation. The main product from starch hydrolysis was glucose, while small amounts of oligosaccharides containing two to four glucose units were detectable. An accumulation of glucose corresponding to 10% of the initially added starch did not significantly influence firmness or the amount of liquid exuded from sausages at the end of the storage period.

INTRODUCTION

Starch in meat products may degrade during processing (Dahl, 1958; ten Cate, 1963; Bell & Gill, 1983; Skrede, 1983*b*; Ekström *et al.*, 1984). The degradation is caused by amylolytic enzymes in the meat and is found to take place at relatively high temperatures where starch gelatinizes and the enzymes have not yet been heat-inactivated. Starch-degrading activity has been demonstrated in the temperature range of 60–70°C, while heating above 80°C inactivates the enzymes of interest (ten Cate, 1963; Bell & Gill, 1983; Skrede, 1983*b*). At 37°C, Bell & Gill (1983) found a 4-h lag period before any effect of enzyme action was registered in pork-starch slurries.

The starch-degrading ability varies with meat sources (Dahl, 1958). Bell &

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Gill (1983) reported activity in pork but not in beef and mutton. Skrede (1983*b*) and Ekström *et al.* (1984) detected starch-degrading enzyme activity both in pork and beef. The activity in pork was about twice that of beef. Also, liver extracts (ten Cate, 1963) and bovine blood plasma (Skrede, 1983*b*) have been shown to have starch-degrading enzyme activity.

Starch degradation results in the accumulation of glucose (Bell & Gill, 1983; Skrede, 1983*b*) and an increase in reducing power (Dahl, 1958; Skrede, 1983*b*). Ekström *et al.* (1984) reported the accumulation of maltose in sausages made with meats from pig heads. Experiments with iodine colouring of reaction mixtures from liver extracts and starch solutions, also showed formation of various higher dextrins (ten Cate, 1963). The knowledge of the ultimate products of the enzymatic hydrolysis may give information about which enzymes are involved in the starch degradation (Skrede, 1983*b*).

The starch-degrading ability of meat products is likely to continue beyond the process of cooking if this is insufficient for complete inactivation of the enzymes. During storage of vacuum-packed commercial meat products, starch degradation may therefore take place. To what extent starch degradation influences texture and water-holding capacity of meat sausages is not known.

The present experiments were undertaken to study the extent of starch degradation in beef sausages during storage. Further, the effect of degradation on texture and water-holding capacity of vacuum-packed sausages was investigated. Formation of oligosaccharides during starch degradation was studied by high-pressure liquid chromatography.

MATERIALS AND METHODS

Experimental design

In one series of experiments, sausages containing 9% protein and 25% fat were prepared from a commercial beef cut (15% fat). In addition, pork fat trimmings, 4·0% starch from Norwegian-produced potato flour, 1·8% sodium chloride and oleoresins were included. Sausages were cooked in a cooking/smoking cabinet up to core temperatures of 60, 65, 70, 75 and 80°C. Sausages were cooled in ice-water, stored at 4°C overnight and vacuum-packed (1 mm Hg) in plastic bags (Cryovac BB-I, low gas and vapour permeability) and stored at 4°C for 4 weeks. Glucose content, firmness and liquid exuded from sausages were determined at the day of production and after 4 weeks of storage. Colony forming units (CFU) and amylase-

producing bacteria (Mitrica & Granum, 1979) were determined in the batter, in sausages immediately after cooking and in sausages after 4 weeks.

In another series of experiments, three beef sources were used for the preparation of sausages with 11% protein, 23% fat, 4·5% starch from potato flour, 1·8% sodium chloride and natural spices. Meat from raw and frozen commercial beef cut (15% fat) and beef shoulder cut (20% fat) were used. The meat was obtained from a cutting line the day after slaughtering. Both commercial beef cuts originated from the same batch; frozen meat was prepared by freezing at −25°C prior to processing. Thawing and storage of unfrozen meat were done at 4°C.

Sausages were prepared 3 days after slaughtering and cooked for 20 min in water at two different temperatures (70 and 85°C). After storage overnight (4°C), sausages were packed in plastic bags and evacuated (1 mm Hg). Five days after production, the bags were irradiated with 5·8 KGy, using a ^{60}Co source of approximately 10 KCi (Institute of Energy Technology, Norway). The dose was controlled with a perspex dosimeter. After irradiation sausages were stored at 4°C. Carbohydrate content, firmness and liquid exuded from sausages were analysed 1 week, 1, 2, 4 and 8 months after processing of the sausages.

Analyses

Oligosaccharides

These were determined by high performance liquid chromatography (HPLC). An Aminex Carbohydrate HPX-42A column, 300 mm × 7·8 mm (Bio-Rad Laboratories) for the analysis of oligosaccharide (degree of polymerization, DP, up to 11) was used. The column was equipped with a de-ashing guard system (Bio-Rad Laboratories) and operated at 85°C using water (0·6 ml/min) as the eluant. Quantification was performed by measuring peak heights. Glucose and maltose were used as external standards (1 mg/ml). Oligosaccharides were calculated as maltose.

Samples were prepared by homogenizing 10·00 g of sausages in 10 ml distilled water. After centrifugation, 5 ml supernatant was mixed with 0·1 ml each of 15% $K_4(Fe(CN)_6)$ and 30% $ZnSO_4$ solutions (Stoya, 1969), centrifuged, filtered and injected into the chromatographic column.

Glucose was also determined enzymatically as described by Skrede (1983*a*).

Firmness

Firmness of sausages was determined using an Instron Universal Testing Instrument, model TM-SM (Instron Ltd, Great Britain) equipped with a conical penetrometer of 7 mm upper width and 10 mm height (Andersson &

Hansson, 1979). The firmness was calculated as the maximum force needed to penetrate a cross-section of the sausage.

Liquid
Liquid exuded from sausages stored in evacuated plastic bags was determined by weight.

Statistical analysis

A three-way analysis of variance (ANOVA, meat source × cooking temperature × storage time), all fixed effects, was used to determine statistically significant differences at the 5% level ($P < 0{\cdot}05$).

RESULTS

In sausage batter from the first experiment series, analysis revealed 10^6 CFU per g. Cooking reduced the number to < 10 per g. During 4 weeks of storage, CFU increased in sausages heated to the lower temperature (Table 1). Before cooking, 2×10^5 amylase-producing bacteria per gram were found. The number was reduced during cooking and remained low during storage of sausages (< 10 per gram).

In sausages cooked to a core temperature of 60°C, the glucose content increased nearly five times during 4 weeks of storage (Fig. 1). At higher cooking temperatures, the effect of storage upon glucose formation decreased. At 75 and 80°C, the glucose content remained unchanged throughout the storage period.

TABLE 1
Colony Forming Units (CFU) per Gram, Firmness (g) and Liquid Exudation (%) of Vacuum-Packed Sausages Cooked to Various Temperatures and Stored at 4°C for 4 Weeks. Firmness (Twelve Replicates) and Liquid Exudation (Four Replicates) are Given as Average ± Standard Deviation

Temperature (°C)	*CFU/g after 4 weeks*	*Firmness (g)*		*Liquid exuded (%) after 4 weeks*
		At production	*After 4 weeks*	
60	$>10^5$	97 ± 8	132 ± 9	2·9 ± 0·6
65	7×10^3	162 ± 7	185 ± 8	2·3 ± 0·4
70	5×10^2	163 ± 13	217 ± 10	2·3 ± 0·4
75	<10	185 ± 16	234 ± 13	2·2 ± 0·3
80	<10	182 ± 14	220 ± 18	1·4 ± 0·3

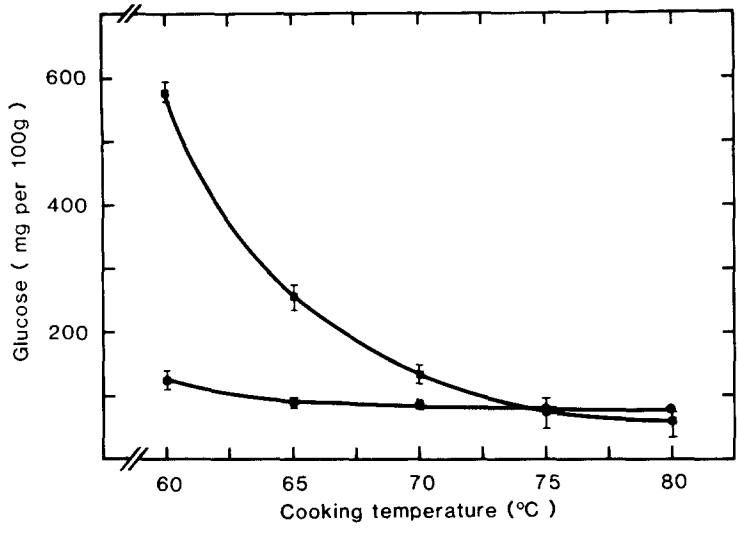

Fig. 1. Glucose concentration at production (●—●) and after 4 weeks' storage at 4°C (■—■) of vacuum-packed sausages cooked at various temperatures. Standard errors of the means (SEM) are given.

Cooking temperature influenced firmness of the sausages (Table 1). Sausages cooked at 60°C were significantly less firm than sausages cooked at the higher temperatures both at the time of production and after 4 weeks. In addition, significantly more liquid exuded from sausages cooked at 60°C compared with those cooked at 80°C (Table 1).

In the second series of experiments, irradiation allowed vacuum-packed sausages to be stored without freezing for an extended period of 8 months. During this period, sausages cooked to a core temperature of 70°C increased their free glucose content about four times (Fig. 2). This corresponds to the hydrolysis of about 9% of the initially added starch content. In sausages cooked at the higher temperature of 85°C, glucose formation during storage was rather limited. No significant effect of the applied meat sources upon glucose formation in sausages cooked either at 70°C or at 85°C was found.

Through chromatography, oligosaccharides with a degree of polymerization (DP) ranging from 1 to 4, were found in extracts from the sausages (Fig. 3). The detection limit was 2 mg per 100 g sausages. In all samples, the glucose concentration was highest among the carbohydrates detected. The dominating role of glucose was especially evident in sausages heated to 70°C, where about 90% of all detectable carbohydrates were glucose. In sausages heated to 85°C, the glucose content represented about 60% of the carbohydrates detected. These relationships between glucose and higher oligosaccharides were maintained throughout the experimental period, despite the increase in free glucose upon storage.

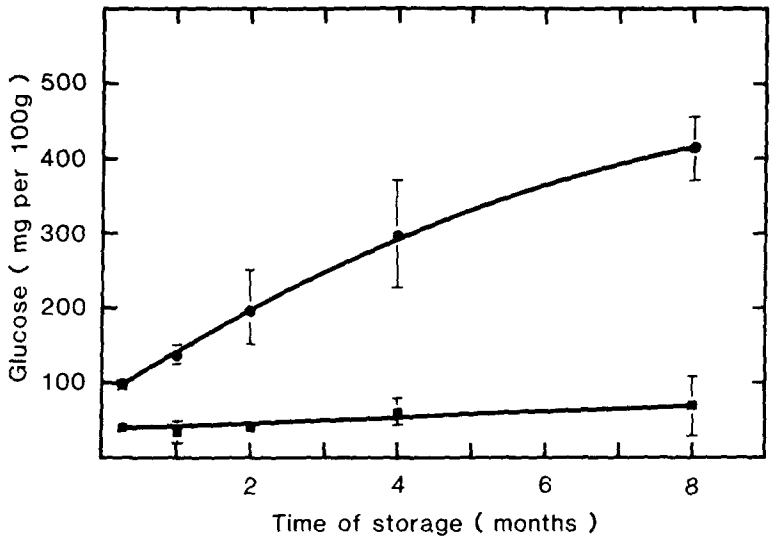

Fig. 2. Glucose concentration during storage at 4°C of irradiated vacuum-packed sausages cooked at 70°C (●—●) or 85°C (■—■). Results are average of sausages from three different meat sources, standard errors of the means (SEM) are given.

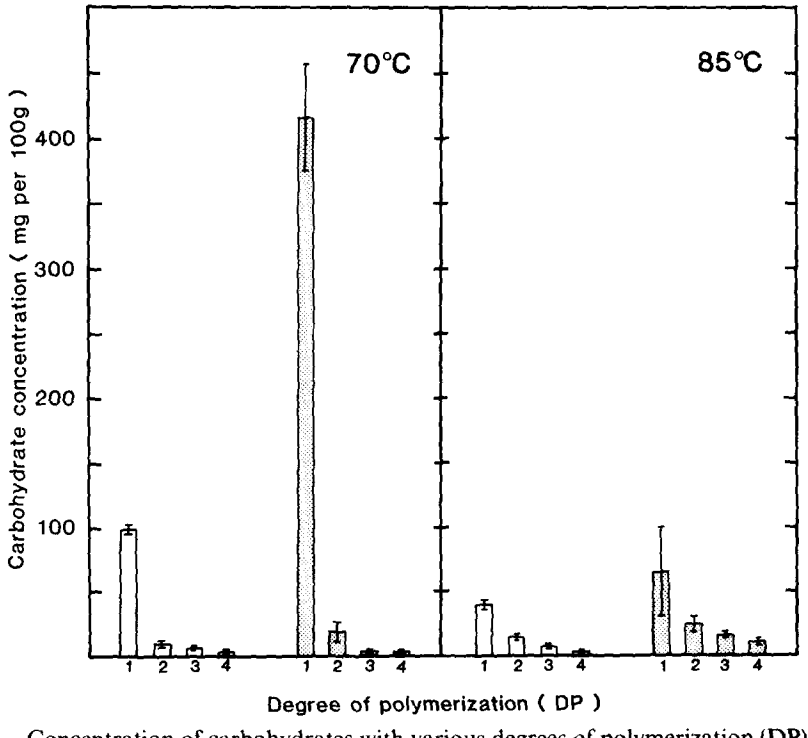

Fig. 3. Concentration of carbohydrates with various degrees of polymerization (DP) after storage at 4°C for 1 week and 8 months (dotted columns) of irradiated vacuum-packed sausages cooked at 70°C or 85°C. Standard errors of the means (SEM) are given.

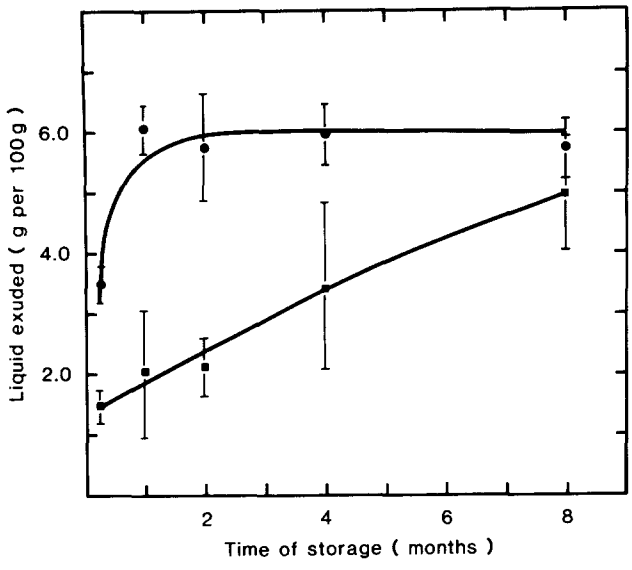

Fig. 4. Amount of liquid exuded during storage at 4°C of irradiated, vacuum-packed sausages cooked at 70°C (●—●) or 85°C (■—■). Standard errors of the means (SEM) are given.

Both cooking temperature and storage time significantly influenced the amount of liquid exuded from the vacuum-packed sausages (Fig. 4). More exudate was initially found in sausages heated to the lower temperature of 70°C compared with heating to 85°C. At 70°C, the entire increase in the amount exuded was found during the first month of storage. At 85°C, however, there was a nearly linear increase in the amount exuded throughout the 8-month storage period. At the end of the storage period, no difference in the amount of liquid exuded from sausages cooked at 70°C or at

TABLE 2

Firmness (g) During Storage of Vacuum-Packed Beef Sausages Cooked at 70°C or 85°C. Results are Average ± Standard Error of the Mean from Three Productions with Different Meat Sources

Storage period	*Firmness (g)*	
	70°C	*85°C*
1 week	320 ± 25	312 ± 20
1 month	440 ± 22	452 ± 28
2 months	393 ± 28	425 ± 27
4 months	468 ± 32	505 ± 15
8 months	390 ± 15	424 ± 6

85°C was found. No effect of meat quality upon the amount of liquid exuded was found.

Firmness in sausages varied during the storage period and reached a maximum after 4 months of storage (Table 2). Meat quality and cooking temperature did not significantly influence firmness of sausages.

DISCUSSION

Storage of beef-based sausages with starch added caused an increase in glucose concentration when sausages were cooked at temperatures between 60 and 75°C. Thus, when the cooking process was insufficient for inactivation of the starch degrading enzymes, starch was continuously hydrolysed during storage.

Previously, Skrede (1983*b*) reported no significant differences in starch-degrading enzyme activity between various beef cuts. Also in the present study, no effect upon glucose accumulation in sausages during storage was found when using various beef raw materials. Further, the two experimental series revealed starch degradation of a comparable extent. A short period of freezing did not influence the enzyme activity significantly. Thus, the level of enzyme-activity seems to be relatively constant within beef.

The main product from starch hydrolysis in sausages was glucose. Especially at the lower cooking temperatures, where glucose accumulation was most extensive, glucose dominated the oligosaccharides with DP 2–4. As discussed previously (Skrede, 1983*b*), glucoamylase (EC 3.2.1.3) and α-glucosidase (EC 3.2.1.20) are the enzymes reported to give free glucose as the reaction products, while α-amylase (EC 3.2.1.1) produces maltose mainly. The lack of maltose accumulation in the present experiments may rule out α-amylase as the enzyme of interest. However, Banks *et al.* (1973) reported a maltose-splitting enzyme activity associated with α-amylase of bovine serum. Ekström *et al.* (1984) found that when meat from pig heads but not from beef heads, was used, maltose accumulated in sausages. The enzyme activity was ascribed to enzyme secretion from the pig's parotid gland. Final conclusions on the enzymes involved in starch degradation from meats require isolation and characterization of the enzymes. The present results demonstrate that glucose formation was not caused by bacterial amylases produced during the storage period.

Radiation was used to extend the storage-life of sausages, so that samples heated to temperatures used under practical conditions could be studied after extensive starch-degradation. The radiation of food products may induce various chemical changes in both carbohydrates and proteins. The doses of about 6 KGy used in the present study are not likely to have altered

the enzyme activity greatly (Urbain, 1977). In vacuum-packed raw beef irradiated with doses up to 10 KGy, Risvik (1986) found perceived changes in smell, taste and texture when evaluated by a trained sensory panel. This indicates retained enzyme activities since similar changes were not found in irradiated bacon where enzyme activity is strongly depressed due to processing conditions. The irradiation may have caused a partial depolymerization of the starch (Dauphin & Saint-Lebe, 1977). The effect is likely to occur to the same extent at both cooking temperatures of the present experiments and should thereby not affect the relative ease of starch hydrolysis among the sausages.

Under the conditions of the present experiments, the enzyme activity retained in the sausages after cooking at 70°C corresponds to the transformation of 2·5% of the starch added into glucose during a 4-week storage period. During this period, corresponding to the shelf-life for commercial products, neither the amount of liquid exuded nor the firmness of sausages were significantly influenced by the starch degradation. The effect of storage on the amount of liquid exuded seemed to be caused by the different cooking temperatures, not the starch degradation.

The results showed a maximum of 10% transformation of starch into glucose. This did not have any impact on the amount of liquid exuded and the firmness of the sausages. Whether this is because the removal of some glucose from the starch chains has a very small influence upon the functionality of the starch or whether water-holding capacity and firmness of cooked sausages are caused by components other than starch, remains to be elucidated.

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