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Enzymic Starch-Degrading Ability of Meat and Blood Plasma in Products After Processing

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ABSTRACT

The starch-degrading activity of beef muscles, commercial beef and pork cuts and of bovine blood plasma was studied. Beef sirloin and shoulder cuts were shown to contain enzymes capable of degrading starch. The activity corresponded to an increase in reducing equivalent of about 400–600 μequiv per 100 g fat free muscle per minute. In the commercial meat cuts, the starch-degrading activity of pork was higher than that of beef.

In bovine blood plasma and potato flour mixtures starch degradation was observed in samples that were heated and subsequently stored. The most extended starch degradation was found in samples heated to 60°C prior to storage.

In meat sausages the maximum starch degradation during storage was obtained when samples were heated to 70°C. The increase in reducing equivalents during 7 days' storage at 6°C corresponded to the hydrolysis of about one-third of the starch content of the sausage. Most of the increase in the reducing ability was detectable as glucose, indicating that a major part of the enzymic starch degradation resulted in free glucose. No decrease in the overall number of glucose equivalents was observed in the meat sausages during storage. The implication of starch degradation for the textural quality of sausages is discussed.

INTRODUCTION

Mammalian muscle tissue, body fluids and the gastrointestinal system contain several enzymes capable of splitting the glucosidic bonds of

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starch (Banks *et al.*, 1973). The enzymes serve important functions in the living organism, i.e. glycogenolysis (Karpiak *et al.*, 1977) and degradation of starch in the digestive process (Manners, 1979). Among these enzymes are the phosphorylating enzyme glycogen phosphorylase (EC 2.4.1.1) and the hydrolyzing enzymes α-amylase (EC 3.2.1.1), neutral glycoamylase (EC 3.2.1.3) and α-glucosidase (EC 3.2.1.20). These enzymes degrade starch or fragments of starch to molecules of shorter chain lengths, according to reaction patterns specific for each enzyme (Banks *et al.*, 1973; Kaczmarek & Rosenmund, 1977).

The activity of the enzymes of muscle tissue, organs and glands, as well as the activity of the enzymes of residual blood, may remain intact after the slaughtering of an animal. The enzymes may exert a starch-degrading effect in meat products as, for example, in cooked meat sausages which contain starch as a binder, and for improving textural quality.

Dahl (1958) showed that some decomposition of starch may occur in cooked sausages. The starch degradation was restricted to gelatinized starch. The phenomenon was ascribed to enzymic hydrolysis of starch by amylases present in the meat ingredients of the sausages.

In a sausage batter containing native starch, heat will cause a nearly simultaneous gelatinization of starch and heat inactivation of meat enzymes. When mixtures of starch-degrading enzymes and potato flour are heated at temperatures as high as 70 °C, extensive starch degradation is observed (ten Cate, 1963).

The present work was undertaken to study the starch-degrading ability of beef and pork cuts intended as ingredients for sausage production. It was attempted to study starch degradation during the heating and storage conditions normally used in sausage production.

MATERIALS AND METHODS

Biological material

Meat was obtained from a slaughterhouse the day after slaughtering. The study involved cuts from the sirloin and shoulder of individual beef carcasses, and commercial beef and pork cuts. The commercial beef cuts were: Cow I (2·5 % fat), Ox II (5·0 % fat), Cow II (15·2 % fat). The pork cuts were: Pork II (30·9 % fat) and Sow II (29·7 % fat). Two batches of each cut were analyzed. The samples were frozen at −40 °C and stored at

−25 °C during the 3-month experimental period. Further, two batches of industrially prepared bovine blood plasma, obtained in the frozen state, were included in the experiments.

Preparation of sausage batter, model sausages and heated blood plasma/potato flour mixtures

One batch of Pork II and one of blood plasma were used to formulate a sausage batter with 10% protein (9·25% from meat and 0·75% from plasma), 25% fat and 1·5% salt. Spices were natural pepper and nutmeg. Terramycin (10 mg/kg) and Fenoxypen (85 000 IE/kg) were added to inhibit microbial growth. Potato flour was added to a starch content of 3·5%. As a control, a starch-free batter was prepared by adding water instead of potato flour.

Model sausages were prepared in glass tubes. The heat treatment was programmed by using a thermostatted water bath with a linear temperature increase of 1·0 °C/min. Samples were removed from the water bath as the desired temperatures were obtained. The glass tubes were sealed with aluminium foil and stored at 6 °C. Similarly, blood plasma and potato flour mixtures (10:1, v/w) were heated to various temperatures prior to storage at 20 °C or at 6 °C.

Chemical analyses

Starch-degrading enzyme activity was determined by a slightly modified version of the method for α-amylase activity described by Fossum & Whitaker (1974). Meat samples were ground twice through a 5 mm grinder plate prior to extraction. Enzyme extracts were prepared by stirring sample/water mixtures (1:1 w/w) for 10 min at 4 °C before centrifuging at 10 000 × *g* for 40 min. The extracts were stored at −25 °C prior to analysis. Starch solutions (4%) were prepared daily by boiling soluble starch in water for 10 min.

For the enzyme assay, 0·5 ml extract and 0·5 ml starch solution were incubated in a water bath at 30 °C. After exactly 3 min, 3·0 ml dinitrosalicylic acid reagent were added. The solution was then heated for exactly 5 min in a vigorously boiling water bath. After cooling to room temperature and addition of 8 ml water, the absorbance was measured against water at 540 nm.

The results were calculated as reducing equivalents generated in 100 g

sample per minute, using maltose as a standard. Corrections were made for the absorbance of the reagent blank and the reducing equivalents of the extract and the starch solution. The enzyme activity of meat samples was calculated on a fat-free basis.

The starch-degrading ability of blood plasma in plasma/starch mixtures and of starch-containing sausages was determined as the increase in reducing equivalents during storage. At the end of the storage period the number of reducing equivalents in the plasma/starch mixtures and in water extracts of the sausages (1:1, w/w) was determined. The method was as described for starch-degrading activity except that the addition of starch and the 3 min incubation at 30 °C were omitted. The number of reducing equivalents of each sample was corrected for the reducing equivalents of their respective blanks containing only blood plasma, potato flour or starch-free sausage. The blanks were given identical heating and storage conditions.

Starch and free glucose determinations were accomplished according to methods published previously (Skrede, 1983).

Statistical analysis

Analysis of variance was used to determine statistically significant differences at the 5 % level ($p < 0{\cdot}05$).

RESULTS AND DISCUSSION

The method used for estimating enzymic starch degradation measures increases in the number of reducing groups during starch hydrolysis. The method is frequently used to determine α-amylase activity (Anderegg *et al.*, 1976). However, when glucosidic bonds are broken by different enzymes, as will be the case in crude muscle extracts (Karpiak *et al.*, 1977), this enzyme assay will not be specific for α-amylases. Thus, the enzyme activity determined in the present study has been referred to as starch-degrading activity rather than α-amylase activity (Banks *et al.*, 1973).

Starch-degrading activity of various muscle groups of beef, commercial meat cuts and blood plasma

Starch-degrading activity was detected in cuts from the muscle groups sirloin and shoulder of four individual beef carcasses (Table 1). Some

TABLE 1
Starch-Degrading Enzyme Activity[a] of Sirloin and Shoulder Cuts of Different Beef Carcasses

Carcass	*Enzyme activity*	
	Sirloin	*Shoulder*
Cow (10 years)	630	503
Cow (4 years)	480	613
Cow (4 years)	553	360
Ox	640	547

[a] Enzyme activity: reducing equivalents (μequiv) per 100 g fat-free muscle per min.

variations in the enzyme activity of the muscle groups from the different carcasses were observed. However, the differences were not statistically significant.

The data presented in Table 2 give the starch-degrading activity of the commercial meat cuts and the industrially prepared bovine blood plasma included in this study. There were significant differences between enzyme activities of different meat cuts. The enzyme activity of one of the pork cuts was more than twice the average enzyme activity of the beef cuts. Within batches (especially bovine blood plasma) there appeared to be

TABLE 2
Starch-Degrading Enzyme Activity[a] of Commercially Available Meat Cuts and Blood Plasma

Sample	*Enzyme activity*	
	$\bar{x}$	*SEM*
Beef:		
Cow I	527	75
Cow II	465	39
Ox II	523	90
Blood plasma	459	207
Pork:		
Pork II	1 070	53
Sow II	723	10

[a] Enzyme activity: reducing equivalents (μequiv) per 100 g (ml) fat-free sample per min.

greater discrepancies. The difference between batches was significant. The results clearly demonstrated the presence of a variable but easily detectable starch-degrading enzyme activity in commercial cuts of beef and pork and in bovine plasma subjected to normal freezing and storage conditions.

Effect of heat on the starch-degrading ability in plasma/starch mixtures

As can be seen from Fig. 1, the action of the starch-degrading enzymes of bovine plasma on potato starch during storage is greatly influenced by the previous heat treatment of the enzyme/substrate mixture, i.e. by the

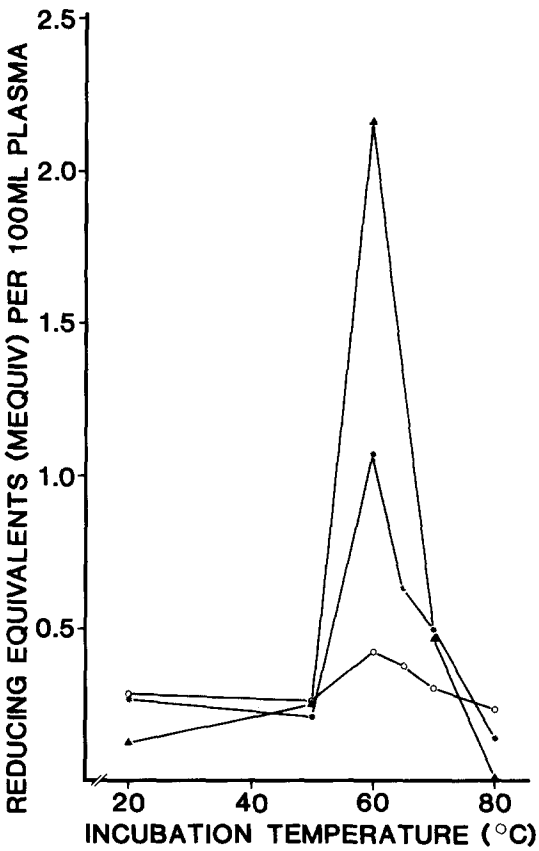

Fig. 1. Increase in reducing equivalents in bovine blood plasma/potato flour mixtures heated to various temperatures prior to storage. ○, Plasma A stored for 24 h at 20 °C. *, Plasma A stored for 7 days at 6 °C. ▲, Plasma B stored for 7 days at 6 °C.

combined opposite effect of starch gelatinization and enzyme denaturation. In the experimental series with bovine plasma and potato flour the effect of the starch-degrading enzymes, given as the increase in reducing equivalents during the heating and storage period, reached a maximum in samples that were heated to 60 °C prior to storage at 30 °C or at 6 °C.

The two experimental series with plasma A (Fig. 1) were given identical heat treatment. As can be seen, there was an increase in reducing equivalents during a storage period for samples which had intitially been heated to 60–75 °C. This implies that starch-degrading enzyme activity was actually present in the plasma after the heat treatment. In samples heated to 80 °C this effect of storage was not demonstrated, and it is likely that the limited enzyme attack observed took place mainly during the heating period.

The present results confirm those of ten Cate (1963) with liver and muscle extracts of pork. In his experiments starch gelatinization was extensive enough in the temperature interval (60–75 °C) to allow enzymic attack, while the enzymes had not yet been completely heat denatured. At 80 °C, extensive heat denaturation of the enzymes occurred and the enzyme activity disappeared. Donaldson *et al.* (1977) have shown that about half of the amylase activity of human sera was retained after heating at 65 °C for 15 min.

The results further indicate that even native starch granules were susceptible to a certain enzymic degradation (Fig. 1). In the plasma/starch mixtures heated to 20 °C the increase in reducing equivalents due to enzyme activity during 7 days of storage amounted to about 20 % of the increase detected in samples heated to 60 °C. Enzyme attack on uncooked sausages containing potato flour was not found by Dahl (1958). However, in agreement with the present study, a limited hydrolysis was observed in the experiments of ten Cate (1963). The latter finding was explained by the presence of traces of gelatinized starch in unheated potato flour. More recently, α-amylases have been found also to degrade native starch granules, although to a limited extent (Manners, 1979).

Effect of heat on starch-degrading ability and starch/glucose content in sausages

The pork and bovine blood plasma used for sausage production were shown to contain starch-degrading enzyme activities of 1123 and

665 μequiv per 100 g (ml) per min, respectively. When sausages were heated to various temperatures and then stored for 1 week at 6 °C, increases in reducing equivalents were demonstrated (Fig. 2). The increases were due to starch hydrolysis alone, since corrections were made for the reducing level in starch-free sausages stored under identical conditions. Thus, the results showed that the enzyme activity present in the raw material (Table 2) was at least partly retained in the sausages.

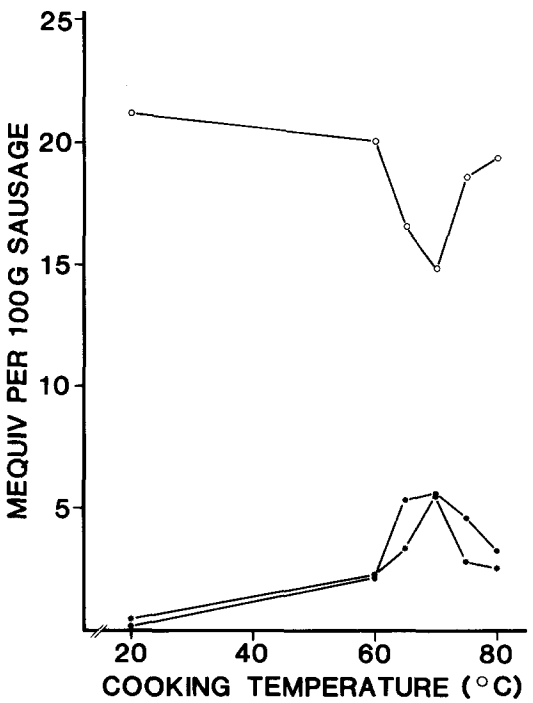

Fig. 2. Changes in reducing equivalents (●), free glucose (*) and glucose in starch (○) of meat sausages heated to various temperatures followed by 7 days' storage at 6 °C.

As with plasma/starch mixtures, maximum enzyme action in stored sausages was observed after a specific heating temperature (Fig. 2). However, the maximum increase in reducing power was obtained in samples heated to 70 °C, compared with 60 °C in the plasma/starch mixture. In both experimental series the samples were heated according to the same temperature/time programme. Whether this change in temperature optimum was due to better conditions for heat stabilization

of the enzymes in the sausages than in the plasma, or to less susceptible starch granules in the sausages at the lower temperatures, cannot be concluded from the present data.

The method used for glucose determination differentiates between free glucose and glucose bound by α-glucosidic bonds as found in starch and fragments of starch (Skrede, 1983). The amount of glucose in starch in the sausages varied in accordance with changes in reducing equivalents (Fig. 2). Simultaneously, changes in the amount of free glucose were found, and the overall content of glucose equivalents remained constant during the experiment. Analyses of sausages without starch added revealed no changes in free or bound glucose with varying temperature. The implication is that the changes in free and bound glucose observed in the starch-containing sausages were due to degradation of added starch only, rather than to an enzymic effect on glycogen or other sausage ingredients.

In sausages heated to 70 °C, about 30 % of the initial starch content was found as free glucose (Fig. 2). In a sausage with 3.5 % starch added this corresponds to a decrease in detectable starch content to less than 2·5 %. To what extent the α-bound glucose fraction was composed of various starch degradation products was not investigated. However, the finding of from 60 to 100 % of the generated reducing equivalents as free glucose (Fig. 2) indicates that a main proportion of the starch degradation resulted in free glucose.

Considering the enzymes that may have been involved in the starch degradation, free glucose will be the product of glucoamylase and α-glucosidase whereas the reaction products from α-amylase largely are maltose and smaller amounts of glucose and maltotriose in addition to α-dextrin (Karpiak *et al.*, 1977; Manners, 1979). However, glucose may also be a more important reaction product from α-amylase under the actual conditions.

Thus, Banks *et al.* (1973) have demonstrated maltose-splitting enzyme activity associated with α-amylase of bovine serum. The end product from glycogen phosphorylase activity is glucose 1-phosphate. This metabolite was not detectable under the actual analytical conditions (Skrede, 1983) and is not included in the amount of free glucose liberated upon degradation of starch.

The specific enzymes responsible for the observed degradation of starch were not identified. However, it can be stated that starch-degrading enzyme activity may be important for the starch content of sausages

heated to temperatures in the range 60–75 °C, i.e. the temperatures commonly used in sausage making. To what extent starch degradation may influence textural quality and liquid exudation from sausages during vacuum packaging and storage remains to be elucidated. It is likely, however, that the extent of starch degradation observed in sausages in these experiments will influence the water-holding capacity of the starch as it is well known that hydrolyzed starch is less capable of binding water than intact starch.

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