EFFECTS OF SUGARS ON POSTMORTEM GLYCOLYSIS IN BOVINE MUSCLE MINCES

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SUMMARY

Sternomandibularis muscles were minced prerigor with sugars and enzymes, and held anoxically at 25°C. Glucose (1.5%) retarded the postmortem pH fall due to glycolysis (final pH 6.1 against the control's 5.5). Other sugars were inactive. Metabolic analyses indicated that glucose was not phosphorylated by endogenous hexokinase and that glucose inhibited phosphorylase. Amylases added at mincing also retarded pH fall, apparently by competing for glycogen. The work shows how modification of glycolysis is not limited to salt addition.

INTRODUCTION

Maintenance of the good processing properties of hot-boned meat can be achieved by mincing prerigor with salt (Hamm, 1977). Glycolysis is inhibited so the pH remains above 6 rather than falling to about 5.6. Because of the high pH and the interaction of salt with actomyosin at a time when the ATP concentration exceeds 1 μmole per gram, the water holding capacity of these minces and their binding strength after cooking are both very high.

We have found that addition of glucose or amylases to prerigor muscle minces will mimic some of the salt effects. This paper examines the phenomena.

MATERIALS AND METHODS

Sternomandibularis muscles from unstimulated steer carcasses were trimmed, cut into pieces, mixed, manually coated with sugar, salt or enzyme, and minced at room temperature in two passes (8 and 4 mm plates),all within 75 minutes of slaughter. The minces were maintained anaerobically at 25°C and after 30 hours at 2°C. Samples for analyses were periodically taken for pH (iodoacetate method), metabolite analyses, and for electronmicroscopy (Murphy and Weberg 1979).

For metabolite analyses, frozen mince (-35°C) was blended in perchloric acid at 0°C. The slurry was centrifuged and the supernatant filtered into flasks. Filtrates were titrated to neutrality, held to precipitate perchorate salts and finally filtered. Extracts were frozen. Controls were similarly processed. Analyses were: inorganic phosphate (Murphy and Riley 1962); lactate (Sigma procedure No.826 UV); hexose monophosphates (Michal 1984); purines (Holliss et al. 1984); free glucose and glucose as glycogen(Sigma No. 510; Dalrymple and Hamm 1973).

RESULTS

NaCl (2%) and glucose (1.5%) both retarded pH fall,although not identically (Fig.1). When either 1.5, 1.0 or 0.5% glucose was added, the pH fall was retarded. Between six and 140 hours, the pH values were maintained at approximately 6.2, 6.1 and 5.9, respectively, while the control pH was 5.4.

Of the monosaccharides, glucose, fructose, mannose and galactose, each tested at 1.5%, only glucose significantly retarded pH fall. The disaccharides, maltose, lactose and sucrose had little or no effect.

Preliminary experiments showed there were few differences between a mince treated with fructose and a control receiving no sugar. Therefore, the effects of adding 1.5% glucose are here compared with those for the iso-osmotic control, 1.5% added fructose. Seven hours after mincing the respective pH values were 6.18 and 5.50. Free glucose analyses showed a high glucose concentration in glucose-treated minces, as expected, but no significant change over the next seven hours. Although glycogen levels declined in the presence of glucose, the decline was less than for the fructose control (Fig.2). This difference was parallelled by lactate formation.

The hexose monophosphates (Scheme 1) were profoundly affected by glucose (Fig.3). With fructose, glucose-6-phosphate concentration initially fell, then increased. With glucose, the initial concentration was much lower and later decreased. The same differences and changes were observed for fructose-6- phosphate and glucose-1-phosphate, but at far lower concentrations.

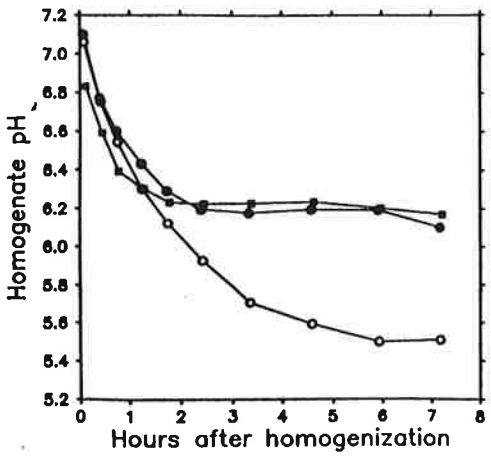


Fig. 1. Effects of glucose (●), NaCl (■) on pH fall. O, No addition.

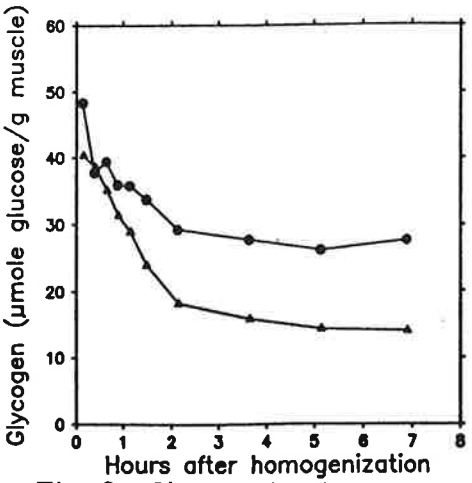
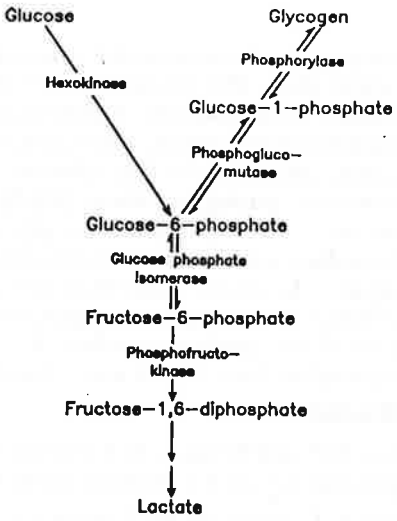


Fig. 2. Changes in glycogen. ●/▲, 1.5% glucose/fructose.



Scheme 1. The glycolytic path.

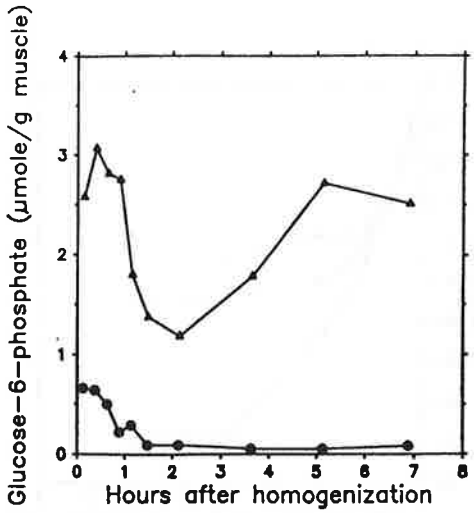


Fig. 3. Changes in glucose-6-phosphate. Symbols in Fig. 2.

Concentrations of ATP, ADP and AMP differed little between the two minces. ATP concentrations, initially about 4 μmole per gram,fell to less than 0.1 μmole per gram. AMP concentrations increased to 0.5 μmole per g during the first two hours, then fell steadily to less than 0.1 μmole per gram.

Other purine metabolites (Scheme 2) showed marked differences. Degradation of AMP to inosine (Fig.4b), and to (hypo)xanthine (Fig.4c) was greater in the glucose mince, at the expense of IMP (Fig.4a). Adenosine did not accumulate in either mince.

Electron micrographs of the fructose control (or no-addition minces also examined), showed a decrease in glycogen over seven hours, and gaps between myofibrils were seen where glycogen deposits are normally found. By contrast, glycogen deposits were evident in glucose-treated minces at all times.

After seven hours, glucose-treated minces had better fluid retention, were more cohesive, and were darker red than no-addition minces or minces treated with inactive sugars. But the excellent cohesiveness and absence of drip loss characteristic of prerigor salt treatment was not duplicated by glucose.

While glucose alone retarded pH fall, glucose plus added hexokinase from yeast accelerated the fall and produced a mince with low pH. When amyloglucosidase was included in a prerigor mince the pH fall was retarded (Fig.5). α-Amylases (pancreas; *Rhizopus*) and the β-amylase (barley) had similar effects.

DISCUSSION

From the inhibition of pH fall (Fig.1) and the retention of glycogen, it is clear that glucose inhibited glycolysis. Dalrymple and Hamm (1975) and Newbold and Scopes (1967) showed that phosphofructokinase and phosphorylase controlled metabolite concentrations in post-mortem glycolysis. Hexose monophosphates accumulated as glycolysis slowed, as confirmed here by the fructose control (Fig.3). With glucose, however, little hexosemonophosphate was produced, evidence that glucose inhibits the phosphorylase complex (Scheme 1). Two influences might explain the effect. First, glucose is a competitive inhibitor of phosphorylase (Cori et al. 1943). Second, Holmes and Mansour (1968) showed that glucose promotes the conversion of phosphorylase a to phosphorylase b the less active form by binding to phosphorylase a, so making it a better substrate for the phosphatase catalyzing the conversion (Bailey and

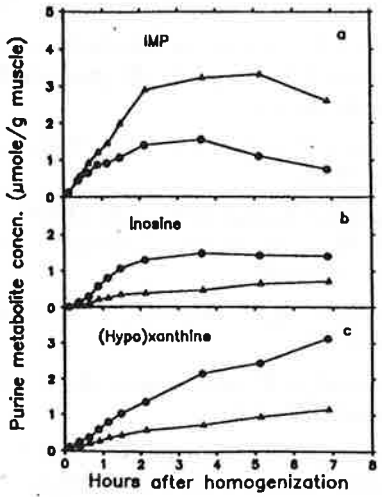
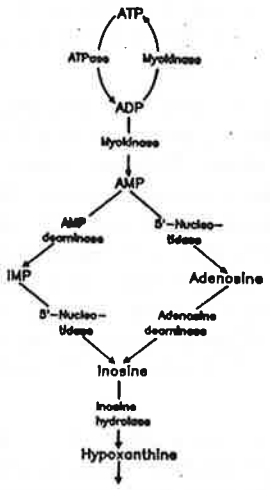


Fig. 4. Changes in purine metabolites. Symbols in Fig. 2.



Scheme 2. Purine catabolism.

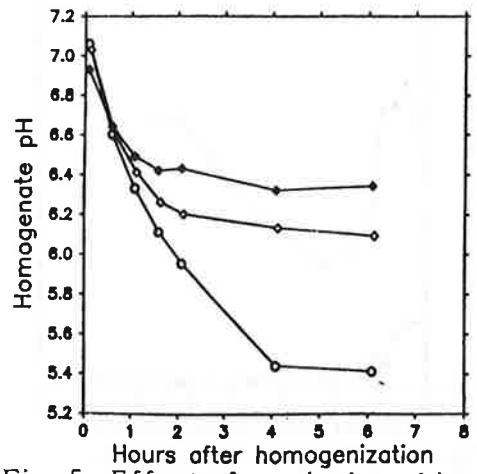


Fig. 5. Effect of amyloglucosidase. 54 units/g, ◆; 11, ◇; no addition, O.

Whelan 1972; de Barsy et al. 1972). The present metabolic environment would permit this effect.

The failure of endogenous hexokinase to initiate glycolysis with added glucose might be explained by concentrations of inhibitors. Concentrations of glucose-6-phosphate and inorganic phosphate in the present studyj exceeded the Ki values for rat-muscle hexokinase (Easterby and Qadri 1982). Yeast hexokinase is not affected by glucose-6-phosphate (Easterby and O'Brien 1973)perhaps explaining its effectiveness in initiating glycolysis. In older animals, the use of glucose by skeletal muscle is reduced and the use of glycogen enhanced (Beitner et al 1979). They attributed this to glucose-1,6-diphosphate, a potent hexokinase inhibitor and an intermediate of phosphoglucomutase catalysis (Leloir et al. 1948). Glucose-1,6-diphosphate would be present since phosphoglucomutase would be necessary (Scheme 1) to support the still significant glycogen metabolism in the glucose homogenate (Fig.2).

Newbold and Lee (1965) and Dalrymple and Hamm (1974) showed that salt affects phosphorylase or an associated regulatory enzyme. Salt inhibition might be explained by a nonspecific ionic effect on phosphorylase whereas glucose probably inhibits activity by stimulating the phosphorylase conversion. For either case (Newbold and Lee 1965, and present work), once hexose monophosphate is generated with a suitable hexokinase, pH fall proceeds normally.

Concentrations of adenosine phosphates were largely unaltered by glucose, indicating that ATPase, myokinase and AMP deaminase (Scheme 2) were not affected by glucose. Downstream purine metabolites were strongly affected by glucose. The lower IMP concentrations with glucose indicates that a 5'- nucleotidase is activated (Scheme 2). That adenosine did not accumulate can be attributed to the very high AMP deaminase activity in skeletal muscle (Ogasawara et al. 1982) and possibly to a specificity of the skeletal muscle 5'- nucleotidase for IMP (Scheme 2).

Beyond an increased shelf life, by providing the microflora a carbohydrate substrate (Shelef 1977), there is no obvious practical application of the glucose effect, except perhaps in the area of fermented meats. Rather, the effect shows that modification of meat properties is not limited to salt addition. Amylases are also capable of eliciting the glucose effect. Amyloglucosidase hydrolyses glycogen, ultimately to free glucose. Glycolysis was inhibited by amyloglucosidase (Fig.5), α-amylase and β-amylase. The last hydrolyses glycogen to maltose. Maltose does not inhibit glycolysis and is not a hexokinase substrate. It seems likely then that amylases inhibit glycolysis by competing for glycogen, and that any glucose produced (by amyloglucosidase for instance) is not phosphorylated and cannot enter glycolysis.

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