INFLUENCE OF TYPE OF CARBOHYDRATE ON THE NITRATE REDUCTASE ACTIVITY OF SELECTED MICROCOCCAL STRAINS

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ABSRACT

Influence of different carbohydrates on the nitrate reductase activity of few selected micrococcal strains isolated from raw-dried sausage was studied. Results indicated that maximum activity was achieved in presence of sucrose. Addition of glucose to the sausage mix led to the development of somewhat undesirable flavour in the finished product.

INTRODUCTION

Micrococci are used either singly or as a component of mixed meat starter cultures mainly due to their ability to reduce nitrate/nitrite to its simpler forms which ultimately take part in the development and stability of the desired colour in fermented meat products (Lücke and Hechelmann, 1987; Niinivaara, 1991; Geisen *et al.*, 1992; Hammes and Knauf, 1992). In meat mass, this conversion of nitrate/nitrite takes place through a complex biochemical process. The nitrate reductase activity of micrococci is influenced by many factors, such as the initial microbial load, water activity, temperature of ripening, pH of the meat mass and other growth conditions (Teruya *et al.*, 1976). Few researchers have also observed that formation of colour in fermented sausages was influenced by the sugar added to the sausage formulations (Tändler, 1963; Coretti and Tändler, 1965). However, reports on the effect of different types of carbohydrates on the nitrate reductase activity of micrococci are scanty and hence, this work was undertaken to study the effect of three different types of sugar on the nitrate reductase activity of selected micrococcal strains as well as their effect on the formation of desirable and stable colour and flavour of fermented pork sausages.

MATERIALS AND METHODS

Micrococcal starter cultures: Two strains of *Micrococcus varians* namely, M160 and M483 isolated from Bulgarian raw-dried sausage were used in the study (Borpuzari and Boschkova, 1993). Stock cultures were maintained in slants of Chapman's medium (Chapman, 1945).

Determination of nitrate reductase activity: The method described by Kuusela *et al.* (1978) with certain modifications was used for determination of the nitrate reductase activity of the strains. Bacterial suspensions were prepared by adding 1% NaCl solution to the young slant cultures of the strains grown on Chapman's agar. Concentration of cells in the bacterial suspension was adjusted to 10^6cfu/ml spectrophotometrically by adding the required volume of NaCl solution. Two millilitres of bacterial suspension were pipetted out to a test tube containing 1ml each of buffered solution of sodium formate (pH 6.0), 0.01% solution of sodium nitrate and 2% solution of respective carbohydrates, i.e., glucose, sucrose and fructose. For the control samples , the sugar solution was replaced by additional 1ml of NaCl solution. The tubes were kept in water bath (30±1°C) for upto 12h. After 2, 6, and 12h of incubation, the reaction was stopped by adding 1ml of 10% solution of uranylacetate and the entire volume was centrifuged for 10 min at 7000 rpm. Two millilitres of the supernatant were transferred to a test tube to which 0.5ml of sulphanilamide (1g sulphanilamide dissolved in 100ml of 25% HCl) and 0.5ml of naphthyl-1-amine (20mg naphthyl-1-amine dissolved in 100ml of distilled water) were added. The solutions were mixed properly and the tubes were kept undisturbed in a dark room for 40 min and absorbance was recorded spectrophotometrically at 570nm. The quantity of reduced nitrate was calculated by using the formula suggested by Kuusela *et al.* (1978).

Preparation of sausage: Pork sausages were prepared as per method and recipe of Sharma and Mukhopadhyay (1992). However, in two instances sucrose was replaced by glucose and fructose. Liquid starter cultures of the selected micrococcal strains were added to the sausage mix before mixing to a concentration of 10^6cfu/g.

Sensory evaluation: The colour and flavour qualities of the finished product were evaluated by a 5-membered semi-trained panelists using the 9 point hedonic scale.

RESULTS AND DISCUSSION

Results of the *in vitro* study on the influence of glucose, sucrose and fructose indicated that all these three sugars exerted varying degrees of influence on the nitrate reductase activity of the selected micrococcal strains (Table 1) and the effect of sucrose was maximum. Coretti and

Table 1 : **Influence of Carbohydrates on nitrate reductase activity of micococcal strains (μgNaNO$_3$/ml)***

Strains	Sugars	Time (h)		
		2	6	12
M160	Glucose	2.77	7.44	4.29
	Sucrose	4.71	12.20	6.57
	Fructose	3.50	9.12	5.24
M483	Glucose	1.89	5.24	3.78
	Sucrose	5.96	12.74	7.00
	Fructose	4.18	11.57	8.02

* n=7

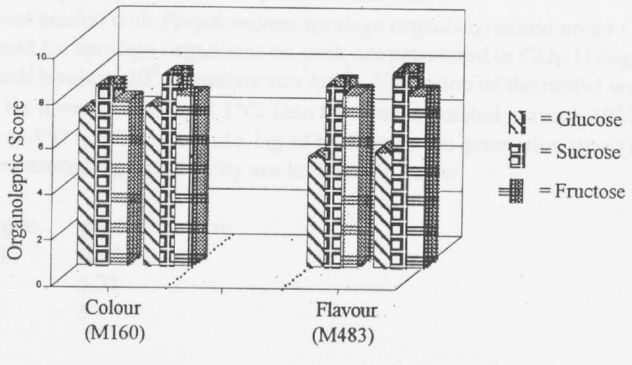

Figure 1. **Influence of Carbohydrates on eating quality parameters of pork sausage fermented with micrococcal starter cultures.**

Tändler (1965) also observed in their study on the influence of different carbohydrates added to the sausage mix and prepared with mixed starter culture containing micrococci as one of the culture components that addition of fructose to the sausage mix was undesirable as it was difficult to be broken down by the microbial cultures and advocated the use of sucrose instead.

It was interesting to note that the amount of nitrate reduced was highest at 6h of incubation whereafter the values gradually declined upto 12h. This may probably be due to the fact that these two strains of micrococci possess both nitrate- and nitrite reductase enzyme systems. From technological point of view this ability of the micrococcal strains may be considered to be desirable as these could reduce the added nitrate and nitrite more effectively and quickly to its simpler forms helping in the formation of nitrosomyoglobin for better colour formation in the fermented meat products.

The effect of addition of different types of sugars on the organoleptic qualities of the finished product, viz., colour on the cut surface and flavour indicated that both in relation to colour and flavour, the sausage prepared with sucrose and micrococcal starter culture containing the strain M483 scored the best results (Fig. 1).

In regard to the colour formation, the performance of the other two sugars were also quite satisfactory. However, the flavour score of the sausage prepared with added glucose scored poor indicating that glucose is not a desirable carbohydrate for sausage fermentation as the micrococcal cultures could break down and utilise glucose very quickly giving rise to the possibility of formation of undesirable flavour components during the process of colour development of the sausage.

CONCLUSION

From the results of the above study it may be concluded that out of the three sugars tested, sucrose appeared to be the best both in regard to *in vitro* nitrate reductase activity of micrococcal strains and formation of stable and desirable colour and flavour of fermented pork sausages.

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