THE INFLUENCE OF PROCESSING PARAMETERS ON LEUCINE CATABOLISM BY A STRAIN OF *STAPHYLOCOCCUS SIMULANS*

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Introduction

In the production of fermented sausages, various *Staphylococcus* spp. are widely used as starter cultures and participate in the development of typical flavour. Numerous flavour compounds have been identified in previous studies (Berdagué *et al.*, 1993; Stahnke, 1995). Among them, more attention has been paid to the formation of the branched – chain aldehyde, especially 3-methylbutanal, which is derived from the microbial catabolism of L-leucine and has a strong impact on sausage aroma (Stahnke, 1995). *Staphylococcus xylosus* and *S. carnosus* are popular species used in commercial starter cultures, and the volatiles originating from branched-chain amino acid degradation by these different strains have been studied (Søndergaard and Stahnke, 2002; Olesen and Stahnke, 2003). However, other species such as *S. simulans* were also proposed, as starters (Coppola *et al.*, 1997). As it is generally accepted that the ability for leucine catabolism is highly strain dependent, and processing conditions may have a great influence on the growth of strains and on their aroma formation. Therefore, it is necessary to select strains with high aroma-producing potential as starter culture to improve the sensory quality of fermented meat products. A strain referred to as S52 was screened from Chinese dry-cured bacon and identified as *S. simulans* on the basis of its biochemical characteristics and 16S rRNA analysis (data unpublished). It was catalase positive, coagulase negative, DNase negative and urease positive. It possessed nitrate reductase activity and produced acetoin. Moreover, it had moderate lipolytic and proteolytic activities, and did not produce pigment and haemolytic ring. Therefore, it was thought to be safe after primary tests and was chosen as a potential strain as a starter. The objective of the present study was to investigate the effect of various processing parameters on the capacity of *S. simulans* S52 to catabolize leucine into volatile compounds.

Materials and Methods

S. simulans S52 was grown in MC medium described by Talon *et al.*, (1999). After overnight incubation at 30°C under shaking (100 rpm), cells were harvested by centrifugation (10,000×g, 10 min, 4°C) and washed twice with sterile saline solution (0.9% NaCl), then resuspended in sterile saline solution. The optical density of the cell suspension was measured at 600 nm and adjusted to $OD_{600} \approx 1.0$. The reaction mixture was composed of leucine (2 mM), pyridoxal-5 phosphate (2 mM), α-ketoglutaric acid (10 mM), and resting cells (0.5 ml). It was prepared with sterile phosphate buffer KH_2PO_4-Na_2HPO_4 (0.067 M, pH 6.5) and the reagents were sterilized by a filter. Fifteen-ml screw-top clear vials, hole cap with PTFE/Silicone septa (Supelco, Bellefonte, PA, USA) containing 3.5 ml of liquid were incubated for 3d and 9d, respectively, at 30°C under static conditions. The control was prepared with 0.5 ml sterile saline solution instead of resting cells. All the practices were performed under aseptic conditions. To study the effects of different factors on the production of 3-methybutanal, a factorial experi-mental design with four factors was set up. The factors were temperature, pH, the concentration of NaCl and nitrite, respectively. The experimental data were analyzed in triplicate by variance analysis with SPSS 11.5 software. The volatile compounds of the reaction mixtures were extracted and analyzed according to Song *et al.*, (2005). Standard solutions of 3-methylbutanal and 3-methlybutanoic acid were extracted and analysed under the same conditions.

Results and Discussion

The result showed the metabolites of leucine produced by *S. simulans* S52 during the incubation of nine days. 3-methylbumtanal was the only degradation product after three days, and reached the highest quantity at the fifth day when 3-methylbutanoic acid was also produced. After a week, the amount of 3-methylbuantanal decreased, meanwhile, the 3-methylbutanoic acid increased. On the ninth day, there was only 3-methylbutanoic acid. The sequence of aldehyde and acid production revealed that *S. simulans* S52 first metabolised leucine into branched-chain aldehyde, which was then oxidised into acid. The ability of *S. simulans* S52 to catabolize leucine was strongly affected by changing the processing factors ($P<0.01$). Increasing the temperature and pH resulted in an increased generation of 3-methylbutanal, however, high level of NaCl and $NaNO_2$ inhibited its production. The optimal temperature and pH for the production of 3-methylbutanal were 40°C and 7.0, without NaCl and $NaNO_2$. Our work in the future aims to find out the prevailing factor influencing the production of 3-methylbutanal as well as the interaction of different factors.

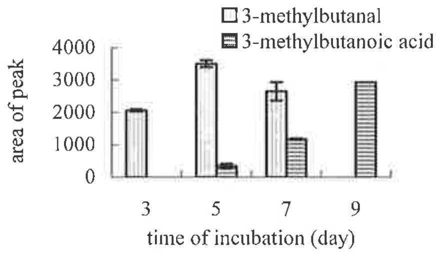

Figure 1: Mean peak area of methyl-branched aldehyde and acid produced by *S. simulans* S52 during the incubation.

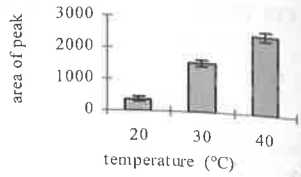

Figure 2: Effect of temperature on the production of 3-methylbutanal by *S. simulans* S52 incubated for 3 days.

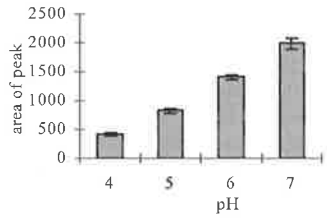

Figure 3: Effect of pH on the production of 3-methylbutanal by *S. simulans* S52 incubated for 3 days.

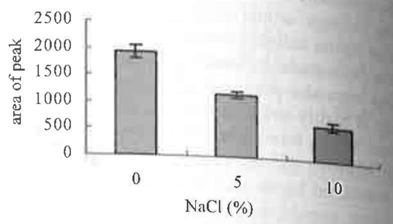

Figure 4: Effect of NaCl on the production of 3-methylbutanal by *S. simulans* S52 incubated for 3 days.

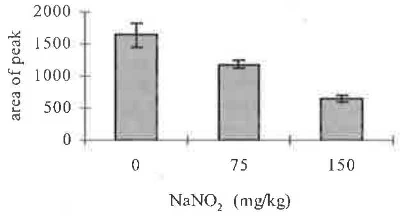

Figure 5: Effect of nitrite on the production of 3-methylbutanal by *S. simulans* S52 incubated for 3 days.

Conclusions

S. simulans S52 could catabolize L-leucine into 3-methylbutanal and 3-methylbutanoic acid, and processing parameters such as temperature, pH, NaCl and $NaNo_2$ influenced differently its capability.

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