EFFECT OF GLUTAMINASE FROM BACILLUS SUBTILIS GT STRAIN ON SENSORY EVALUATION OF PROCESSED MEAT PRODUCTS

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SUMMARY

Recently, extracellular glutaminase of Bacillus subtilis GT strain was purified by ion-exchange, gel-filtration and hydrophobic chromatographies. The enzyme completely converted L-glutamine into L-glutamic acid (Glu). This study was conducted to determine the effect of glutaminase from Bacillus subtilis GT strain on sensory evaluation of processed meat products. Model sausage was prepared as follows: ground pork meat was mixed with 2% NaCl, 100 ppm $NaNO_2$, 0.5% white pepper, 0.25% polyphosphates and 10% distilled water. PH was adjusted to 6.0. One group of samples was cooked at 75°C for 1 hr immediately after being stuffed into collagen casing, another group was cooked under similar conditions after curing for 3 days at 4°C. Glutaminase was added 0.001, 0.05, 0.1 unit (U) per gram meat following the addition of 10% distilled water to the sausage at mixture. Glu was determined using a Yamasa L-Glu Assay Kit (Japan). Sensory evaluation was carried out by the ranking method and the results were analyzed with Kramer's method. Glu content in model sausage increased with the amount of glutaminase preparation. In a sample after curing for 3 days, it was higher than that immediately after curing. The 0.1 U glutaminase sample immediately after curing and 0.05 U glutaminase sample after curing for 3 days showed significantly excellent sensory evaluation. These results suggested that the sensory evaluation of processed meat products is improved by addition of this glutaminase preparation.

Introduction

It has been well-known that in addition to the four basic tastes, sweetness, bitterness, sourness, and saltiness, another primary taste has the ability to enhance or improve the flavor of foods. This is described by the word umami, derived from the Japanese for deliciousness (Kawamura and Kare, 1987). This basic taste mainly responds to amino acids, especially L-glutamic acid (Glu). Because, the receptor for Glu is different from the receptors for the four basic tastes, Glu does not affect the taste of the four primary tastes, the taste quality of Glu is different from that of other primary tastes and umami cannot be reproduced by mixing any of the four basic tastes (deMan, 1990). In general, monosodium glutamate (MSG) has long been used as a flavor enhancer and is now being considered as a primary taste, umami, in meat products manufacture. However, throughout developed countries, there has been a tendency to avoid the addition of MSG in processed meat products. Therefore, in manufacturing meat products of good flavor, it is necessary that endogenous umami components are maximally extracted from the raw meat materials during preparation.

Recently, extracellular glutaminase of Bacillus subtilis GT strain was purified by ion-exchange, gel-filtration and hydrophobic chromatographies (Shimizu et al., 1991). The enzyme specifically converts free L-glutamine (Gln) into Glu. The glutaminase preparation has already been used in Japanese traditional fermented foods such as miso (Harayama and Yasuhira, 1991) and soy sauce (Nakadai and Nasuno, 1989; Tomita, et al., 1989), and as a result of a sensory evaluation, the majority of panelists pointed out increased umami in the miso by the addition of the glutaminase preparation (Harayama and Yasuhira, 1991). This study was conducted to determine the effect of glutaminase from Bacillus subtilis GT strain on sensory evaluation of processed meat products.

Materials and Methods

1. Purification and characterization of glutaminase

Glutaminase was purified by the following six steps: step 1, preparation of Bacillus subtilis GT strain culture filtrate, steps 2 to 6 were DEAE-Cellulose, Hydroxylapatite, DEAE-Toyopearl 650M, Sephacryl S-200 and Phenyl Sepharose CL-4B column chromatographies, respectively (Shimizu et al., 1991). For the enzyme purified by the above steps, only free L- and D-glutamine were hydrolyzed. The enzyme was most active at pH 6.0, and stable in a pH range of 5.0 - 8.0. This enzyme was stable up to 50°C, and the optimum temperature for enzyme reaction was 50°C. This enzyme manifested more than 85% of its original activity even in the presence of 25% NaCl at pH 5.5. The enzyme completely converted Gln into Glu. One unit (U) of glutaminase activity is defined as the amount of enzyme that forms 1 μmol of Glu per minute in the initial stage of reaction from 0.5% of Gln at 37°C.

2. Model sausage preparation and addition of glutaminase

Model sausage was prepared as follows: after fat and connective tissue had been removed from commercial pork meat (ham) as far as possible, the meat was ground twice through a plate with 3.2 mm diameter holes on a Meiko meat chopper No. 5. The ground meat (100 g) was thoroughly mixed with 2% NaCl, 100 ppm $NaNO_2$, 0.5% white pepper, 0.25% phosphates (40% sodium polyphosphate, 30% sodium pyrophosophate, 20% sodium metaphosphate, 10% potassium metaphosphate) and 10% distilled water. The pH was adjusted to 6.0 with lactic acid or NaOH. One group of samples was cooked at 75°C for 1 hr immediately after being stuffed into collagen casing (curing 0 day sample), another group was cooked under similar conditions after curing for 3 days at 4°C (curing 3 days sample). Glutaminase was added 0.001, 0.05, 0.1 U per gram meat following the addition of 10% distilled water to the sausage at mixture. The sample was analyzed for Glu content, and a sensory evaluation was performed.

3. Determination of Glu

Glu was determined using a Yamasa L-Glu Assay Kit (Japan). This kit is based on a colorimetric method for the determination of L-Glu with a new heat-stable enzyme, L-Glu oxidase, which exclusively acts on L-Glu. The absorbance was measured at 600 nm.

4. Sensory evaluation

Sensory evaluation was carried out by the ranking method (Institute of Food Technologists, 1981). This method is used to make simultaneous comparisons of several samples on the basis of a single characteristic, in our case umami (deliciousness). Samples (which may include a control or standard) are presented simultaneously and ranked according to intensity of the characteristic designated, in our case umami intensity. Rank totals or average ranks are obtained for each sample; differences are interpreted through statistical analysis of the data. We used 4 samples, each ranked by 9 panelists. The results were analyzed with Kramer's method (1963).

Results and Discussion

Glutamic acid content: The effect of added amount of glutaminase preparation on Glu content in pork sausages after curing for 0 and 3 days is shown in Fig. 1. Glu content in model sausages of both 0 and 3 days curing increased with the amount of glutaminase preparation. Glu content in all samples after curing for 3 days was higher compared to those after curing for 0 day samples. Glu content in the 0.1 U glutaminase sample after curing for 0 day and the 0.05 U glutaminase sample after curing for 3 days increased to about 300 μg/g meat. The production of Glu in samples after curing for 0 day to which 0.001, 0.05 and 0.1 U glutaminase was added increased by 1.3, 2.7 and 3.3 times that of the control, respectively. The production of Glu in samples after curing for 3 days after addition of the same amounts of glutaminase as above increased by 1.5, 3.4 and 3.9 times that of the control, respectively. Harayama and Yasuhira (1991) reported that the production of Glu increased by 1.5 to 1.6 times by adding 0.1 to 0.5 g of glutaminase preparation (Daiwa Kasei K. K., Japan) per 1 kg of miso at the beginning of fermentation. However, the glutaminase preparation contained 80 U glutaminase per gram enzyme, and in addition, this preparation contained ca. 2,700 AUlg bacterial a-amylase

and ca. 6,400 PU/g protease. In any case, it is suggested that Glu content in processed meat products increased with the amount of glutaminase preparation.

Sensory evaluation: The effect of added amount of glutaminase preparation on sensory evaluation of pork sausages after curing for 0 and 3 days is shown in Tables 1 and 2. We used 4 samples, each ranked by 9 panelists. Rank totals required for significance at the 5% level using Kramer's method (1963) are 15-30. That is to say, test samples with rank sums of less than 15 are superior, and test samples with rank sums greater than 30 are inferior. Since none of these rank sums exceeds 30 or is less than 15, we cannot conclude that any of these samples are superior or inferior. Table 1 shows that the rank sum of the 0.1 U glutaminase sample after curing for 0 day is less than 15, indicating it as being superior. The 0.001 U sample, however, has a rank sum greater than 30, so that it may be considered inferior. Table 2 shows that the rank sum of the 0.05 U glutaminase sample after curing for 3 days is less than 15, indicating it as being superior. Control has a rank sum greater than 30, so that it may be considered inferior. The 0.1 U glutaminase sample after curing for 0 day and the 0.05 U glutaminase sample after curing for 3 days showed excellent sensory evaluation. However, these results for the 0.001 U glutaminase sample after curing for 0 day was notably inferior (Table 1) and the 0.1 U glutaminase sample after curing for 3 days was not particularly superior (Table 2) in the present study, the reasons for this being unknown at the present time.

Further work will be needed to clarify the effect of glutaminase on sensory evaluation and on the composition of amino acids of processed meat products.

Conclusion

These results suggest that Glu content in model sausage increased with the amount of glutaminase preparation, and the 0.1 U glutaminase sample after curing for 0 day and the 0.05 U glutaminase sample after curing for 3 days showed excellent sensory evaluation. In conclusion, the presented results suggest that the sensory evaluation of processed meat products is improved by addition of this glutaminase preparation.

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