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Effect of Molasses on Golden Apple Snail Silage Production

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ABSTRACT

The production of golden apple snail silage was carried out by fermenting the minced golden apple snail with locally screened lactic acid bacteria; L1/2, at ambient temperature using molasses as the carbon source for bacterial growth. The pH value rapidly decreased to 5.0 after 3 days of fermentation, allowing an increase of free amino acid that was released from protein hydrolysis. The highest free amino acid was not only attained at a low molasses concentration, but the TVB-N was also the highest as a result of the growth of undesirable organisms after the stationary growth of lactic acid bacteria. In contrast, the bacterial growth was inhibited at a high golden apple snail to molasses ratio. The optimal ratio of golden apple snail to molasses for golden apple snail silage production was 1: 0.15 (kg: litre). Consequently, after 10 day incubation, the following results were obtained: the pH of 4.97, total tritratable acidity of 1.47 % w/w, and free amino acid concentration of 1.47 mg/g of sample.

Key word: golden apple snail, silage, molasses, feed

INTRODUCTION

The golden apple snail (*Pomacea caniculata*), locally known as "Hoy Cherry", was introduced to Thailand in 1982 as an aquarium pet from Japan, Taiwan, and the Philippines (Srinivet, n.d.). It grows and reproduces very rapidly and one snail can produce 300-3000 eggs per month with 80 % hatchery in 7-14 days (Srinivet, n.d.; Gonzales and Olive, 2001). After the introduction, some snails escaped to the wild and turned into a serious pest in rice fields. In 1990, the newly transplanted rice seeding was firstly damaged in 8 provinces in the central Thailand and it rapidly spreaded over the rice fields around Thailand within a few years. Although entrepreneurs envisioned a mass production of golden apple snail as human food, the snail did not become a great gastronomic success in Thailand either (Gonzales and Olive, 2001). From the proximate analysis, the minced golden apple snail mainly consists of 57.28 % protein (Srinivet, nd.). It could be suitably used as a protein substitute for aquaculture feed production. However, golden apple snail easily spoils thereby proving to be an excellent source of nutrients for microbial growth. An alternative use of golden apple snail in order to improve their quality for a feed ingredient is the fermentation by lactic acid bacteria. The golden apple snail silage is the product of a controlled fermentation process in which carbohydrates added to minced snail are fermented with lactic acid bacteria.

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Similar fish silage production was also reported by Bello *et al.* (1993). Furthermore, the ensilation process is rapid and efficient in tropical countries because the technology is simple. Moreover, the equipment is inexpensive and the production scale may be varied at will. The objective of this work was to investigate the production of the golden apple snail silage and the effect of molasses, a carbon source for lactic acid bacteria, on golden apple snail silage production.

MATERIALS AND METHODS

Golden apple snail

Golden apple snails (*Pomacea caniculata*), obtained from rice field in Thasala district, Nakorn Si Thammarat, Thailand, were dipped into boiling water for 2 minutes. The meat was removed from shell and then minced using a Hobart mincer. The minced meat was blanched for 5 minutes and stored at –20 °C.

Microorganism and preparation of inoculum

A lactic acid bacterium, L1/2, was obtained from the Biotechnology laboratory, Walailak University. In order to prepare a starter culture, a loopful of cells from stab of MRS agar was transferred into 10 ml of inoculum medium containing sucrose 50 g/l, yeast extract 5 g/l, peptone 5 g/l, K_2HPO_4 5 g/l and $MnSO_4$ 0.03 g/l and incubated at 37 °C for 24 h. To prepare the inoculum for fermentation, the starter culture was subsequently transferred to 90 ml steriled inoculum medium and was incubated at 37 °C for 24 h.

Fermentation of golden apple snail

Minced golden apple snail was thoroughly mixed with molasses and 10% of the prepared inoculum. The molasses concentration was varied in terms of the golden apple snail to molasses ratio of 1: 0.05, 1: 0.15, and 1: 0.25 (kg : litre). The mixture was incubated at ambient temperature for 15 days. During incubation, the silages were stirred daily by swirling and 50 g of sample was taken for analysis on days 0, 3, 5, 10 and 15. Sample was incubated in boiling water for 30 minutes before analysis.

Analytical methods

Samples were analyzed for pH, total acidity, free amino acid content, Total Volatile Base Nitrogen (TVB-N). The pH of sample was measured using pH meter Orion model 420 A. Total Tritratable Acidity (TTA) was measured by tritrating against 0.1 N NaOH to a final pH of 8.4 and calculated as lactic acid (Cira *et al.*, 2002). The free amino acid content was determined by Ninhydrin's reaction, modified from Sawhney and Singh (2000) and glycine was used as a standard. The total volatile base nitrogen (TVB-N) analysis was determined by the modified Kjeldahl method (Velho, 2001).

RESULTS AND DISCUSSION

Characteristics of golden apple snail fermentation by lactic acid bacteria

The fermentation of golden apple snail with lactic acid bacteria L1/2, with the addition molasses of 0.15 litre per kg of minced golden apple snail, was successful in stabilizing and preventing it from spoilage. During the course of fermentation, the liquid level increased and the color became more brownish. The pH value sharply dropped from 8.18 to 5.07 within the first 3 days of fermentation and varied with a narrow range of 4.0-5.0, corresponding to the preparation of lactic acid fermented shrimp (Fegbenro and Bello-Olusoji, 1997). The stability of pH at the end of fermentation could be the effect of amino acid buffering action and other salts in the silage (Fegbenro, 1996). In addition, changes in TTA corresponded to the changes in pH as shown in Figure 1. However, the TTA was not related to pH that suddenly decreased at day 3 because of the

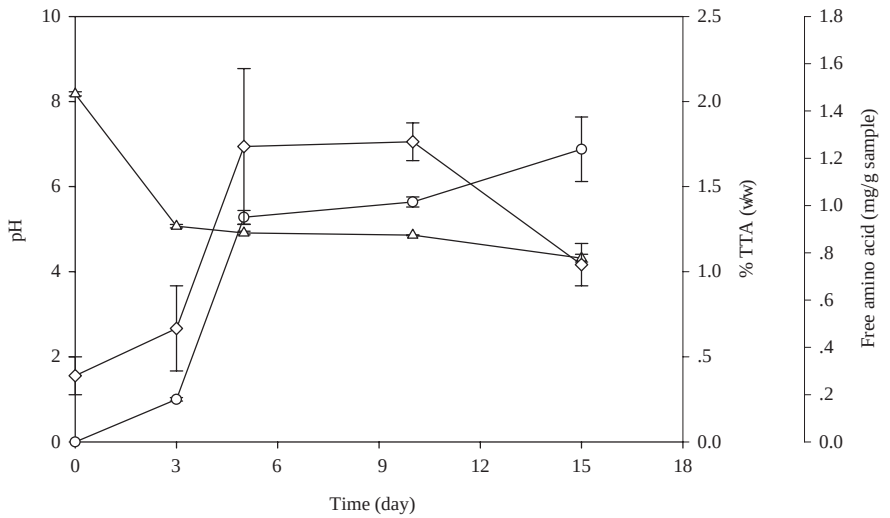

Figure 1 pH (-△-), total tritratable acidity (-○-) and free amino acid production (-◇-) during the fermentation of golden apple snail silage.

buffering capacity of the silage. The highest TTA was achieved with a value of 1.72 % at the end of incubation. Generally, acidification of silage was an important factor in the inhibition of spoilage microorganisms. Furthermore, lactic acid bacteria are known to synthesize anti-microbial compounds such as bacteriocin, acetoin, and hydrogen peroxide (Lindgren and Dobrogosz, 1990). These would affect the growth of spoilage microorganisms. In addition, free amino acids increased steadily with the maximum value of 1.27 mg/g sample and then decreased to 0.75 mg/g sample after 10 days. This could be explained that the production of lactic acid in the silage enhanced protein hydrolysis, resulting in an increasing of free amino acid contents. Nevertheless, a decrease of free amino acids at the end of fermentation, occurred from the growth of undesirable organisms in silage, could be reflected by a continual decrease of pH. Moreover, the interactions between free amino acids and sugars in the unutilized molasses also caused the reduction of free amino acids. Some amino acids, such as tryptophan, are labile under acid conditions and also affected by the temperature and the duration of acid treatment (Fegbenro and Bello-Olusoji, 1997). From the result, the fermentation time in the further study would be terminated at 10 days because the highest free amino acid content in the golden apple snail silage was attained.

Effects of molasses on golden apple snail silage production

Generally, agricultural waste, such as shrimp waste, fish waste or even golden apple snail, has a low concentration of fermentable sugars. A carbon source like glucose, lactose, cassava or molasses must be added for bacterial growth (Fegbenro and Bello-Olusoji, 1997; Shirai *et al.*, 2001). The effects of molasses concentration on apple snail fermentation was investigated by varying the amount of golden apple snail and molasses at the ratio of 1: 0.05, 1: 0.15, and 1: 0.25 (kg : litre). From Figure 2a, the pH value suddenly dropped to 4.0-5.0 after 3 days of fermentation. For the behavior of silage with the added molasses, it was similar to that with added sucrose and lactose, which was reported by Cira *et al.* (2002) on lactic acid fermentation of shrimp wastes for chitin recovery. However, the silage

behavior was also depended on the characteristic of lactic acid bacteria (Cira *et al.*, 2002). The TTA as a function of molasses concentration during the fermentation was shown in Figure 2b. It was found that the TTA suddenly increased at the first 3 days and slightly increased until the end of fermentation. Nonetheless, at a ratio of 1: 0.05, the TTA steadily increased and then became constant whereas TTA at a high golden apple snail to molasses ratio continuously increased. The result indicated that the growth of lactic acid bacteria had reached the stationary phase and allowed undesirable organisms to grow.

A gradual increase of free amino acid content was consistent to the TTA change in silage. From Figure 2c, free amino acid concentration increased with a decrease of golden apple snail to molasses ratio. At the high ratio of golden apple snail to molasses, the catabolic repression of sucrase production may be induced; therefore, the growth of lactic acid bacteria was inhibited. Sucrase is a key enzyme in sucrose metabolic pathway of sucrose-consuming lactic acid bacteria (Steinmeitz, 1993). Moreover, lactic acid bacteria was also inhibited by a high osmotic pressure. At low ratio of golden apple snail to molasses, both the highest free amino acid and TVB-N were attained at the end of fermentation. Figure 3 shows that at each ratio, the TVB-N at the end increased with a decrease of golden apple snail to molasses ratio. Total volatile base nitrogen (TVB-N) is an indicator of protein spoilage in the fermentation process. These total volatile base nitrogen compounds are amine and ammonia, which are the products of protein decomposition in the fermentation process. In addition, the TVB-N of more than 25 mg/ 100 g sample is not acceptable in feed (Suttawat, 2005). The results reveal that the optimal molasses content in golden apple snail silage production is the ratio of 1 kg of minced golden apple snail to 0.15 litre of molasses and free amino acid concentration was attained at 1.47 mg/g sample.

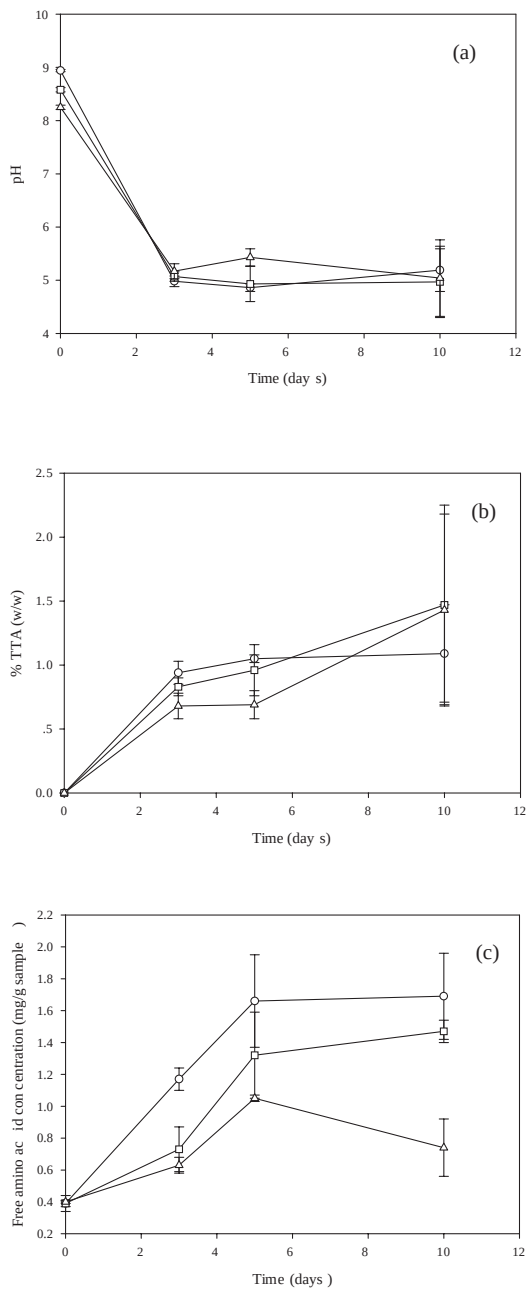

Figure 2 pH (a), TTA (b) and free amino acid concentration (c) during the fermentation of golden apple snail silage at various ratios of golden apple snail (kg) to molasses (litre).
: -○- 1:0.05, -□- 1:0.15, -△- 1:0.25

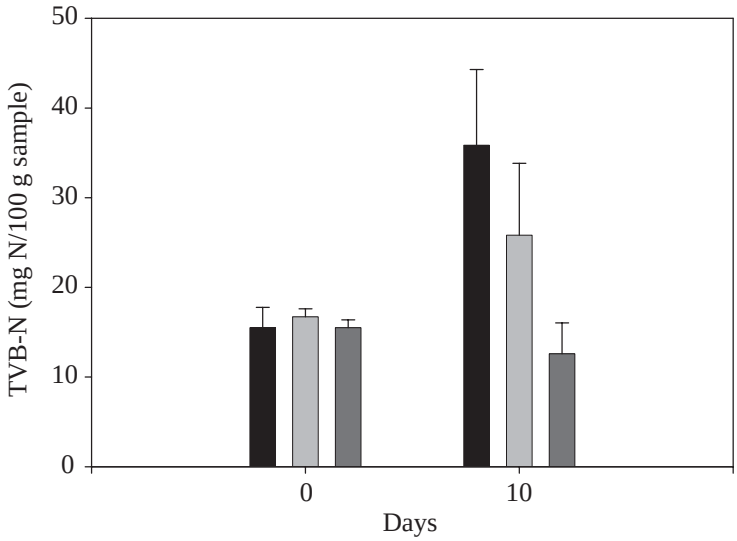

Figure 3 The TVB-N in the golden apple snail silage at the beginning and the end of fermentation with the various ratios of golden apple snail (kg) to molasses (litre).
: - ■ - 1:0.05, - □ - 1:0.15, - ■ - 1:0.25

CONCLUSIONS

Ensilation of minced golden apple snail with locally screened lactic acid bacteria helped improving the quality of the snail silage by increasing the free amino acids, which can readily be used by aquatic animals. The reduced pH, due to lactic acid produced, improved the silage storage time as it inhibited the growth of undesirable microorganisms. Furthermore, addition of molasses at low molasses to minced snail ratio was proven to be beneficial as it provided the extra carbon source to lactic acid bacteria. The optimal ratio of golden apple snail to molasses for golden apple snail silage production by lactic acid bacteria L1/2 at room temperature was 1: 0.15 (kg : litre). Consequently, after incubation for 10 days, the results were obtained: the pH of 4.97, total tritratable acidity of 1.47 % w/w, free amino acid concentration of 1.47 mg/g of silage, and the TVB-N of 25.81 mg nitrogen/ 100 g sample. The results suggested that the golden apple snail ensilation could be an efficient way for improving the quality of golden apple snail used as a protein substitute in aquaculture feed and to prolong feed.

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