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Effect of Fresh Spices on the Fermentation, Colonization of Lactic acid Bacteria and Sensory Acceptability of Fermented Clams (*Mercenaria mercenaria*)

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Effect of Fresh Spices on the Fermentation, Colonization of Lactic acid Bacteria and Sensory Acceptability of Fermented Clams (*Mercenaria mercenaria*)

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Abstract. Fermentation is one of the common methods used for food preservation in South-Eastern Asia. However, the fermented food products were not well accepted especially by young generations due to its unique taste and distinct aroma. In this study, clams (*Mercenaria mercenaria*) had been used to investigate the effect of different fresh spices on the fermentation process, colonization of microflora of lactic acid bacteria and sensory acceptability. The clams were fermented for 8 days with addition of different fresh spices such as garlic, ginger and lemongrass. Fermented clam without any addition of fresh spices was served as control The effects on fermentation were analysed in terms of physico-chemical changes such as pH, color and texture.The colonization of lactic acid bacteria were observed on the De Man, Ragosa and Sharpe (MRS), M17 and tomato juice agar.The sensory acceptability of fermented clams with addition of fresh spices were also carried out. The changes of physico-chemical of all parameters were decreased over fermentation period. The CFU of lactic acid bacteria by using MRS, M17 and tomato juice agar were increased for all different fresh spices over fermentation period. Sensory evaluation showed that fermented clam with the addition of lemongrass had the highest acceptability score in terms of aroma, color, taste, texture and overall acceptability.

1. Introduction

Fermentation process is the oldest and economical methods of preserving the safety and quality of foods [1]. It may be defined as any process for the production of product by mass culture of microorganism to break down complex compounds. During fermentation process, microorganism will convert the chemical composition of raw material, enhance nutritional value of product, improve texture and flavour, fortify the product with health promoting bioactive compound, degrade anti nutritive factors and undesirable compounds, produce antioxidant component and antimicrobial compounds and also stimulate the probiotic function [2].

The shellfish including clam is one of the protein source which easily spoiled after being caught. So, in order to prevent the spoilage from occur, preservation method is required. The spoilage occur due to the breakdown of immune system of shellfish which allowed the bacteria to proliferate freely.

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Preservation of food product can be done by applying the principles of chemistry, biological, engineering and other branches of sciences in order to extend the shelf life of raw material so that they can be stored longer because certain type of shellfish only available during certain seasons of the year and it spoils quickly.Therefore, there are several method of preservation technique which had been introduced including salting, cooling, freezing, drying, canning, and also fermentation [3].

Lactic acid bacteria (LAB) play a significant role in the production of most of the fermented food and beverages [4]. The function of LAB in fermented food are to prolong the shelf life of perishable raw material, enhance the unique taste, texture, nutritional properties, health attributes and improve the commercial value of traditionally and industrially fermented food product [5]. Initially, LAB present in low number in the raw material used, but soon it will increase as other organism are inhibited due to the addition of salt and the continuous growth of LAB will reduced the pH of fermented food making less possibility for other organism to grow. As a result, fermented product commonly have longer shelf life compared to their original raw material and their ultimate spoilage is differ in characters [6].

2. Materials and methods

2.1. Sample source and preparation

Leucaena stem was obtained from *Leucaena* tree located in Padang Besar, Perlis, Malaysia. The stem was chopped manually prior to size reduction. The sample was washed using tap water to remove any impurities and dried at 80 ºC in an oven for 24 hours. The dried sample was ground using grinder and sieved between sizes of 1 mm- 1.7 mm. Prior to the experiment, the sample was kept in air tight container.

2.2. Fermentations ingredients

The ingredients that are used for fermentation are salt, sugar, cooked rice and fresh spices (garlic, ginger, and lemongrass).

2.3. Production of fermented clam (Mercenaria mercenaria

The ingredients which include freshly cooked rice (5%), salt (10%), sugar (5%) and fresh spices (5%) will be mixed in 4 different containers according to the groups which are A (control), B (garlic), C (ginger), D (lemongrass). After the addition of the ingredients, the flesh of clam were stored in airtight containers in dark condition and fermented at room temperature for 8 days.

2.4 Physico-chemical analyses

Physico-chemical analyses include determination of pH and texture (firmness)

2.4.1. pH determination. Determination of pH were carried out by mixing 100 ml of distilled water with 10 g flesh of fermented clam by using food blender to make it like suspension. pH meter calibrated with pH buffer 4 and 7was used to measure the pH level of this suspension. The measurement of pH was conducted every 2 days intervals which on day 0, 2, 4, 6 and 8.

2.4.2. Texture (firmness) determination. The firmness of fermented clam was determined by using Texture Analyzer TAX. TPlus (Stable Micro System) with a P/5 cylindrical steel probe. It was conducted every 2 days interval starting from day 0 to day 8. The parameters were set as folows: Test mode: Compression; Pre-test speed: 3.0 mm/s; Test speed: 0.5 mm/s; Post-test speed: 10 mm/s; and the distance: 10 mm. The firmness value was then determined at the highest positive peak of compression.

2.5 Sample preparation for lactic acid bacteria studies

25 g flesh of fermented clam and 25 ml of sterile distilled water were blend together using food blender in aseptic condition .Then it was diluted up to 10^{-5} concentrations. 1 ml of diluted sample

were pipette into each agar plates by using micropipette. This process was carried out in a laminar flow to maintain aseptic condition.

2.5.1. Determination of lactic acid bacteria using De Man, Ragosa and Sharpe (MRS) Agar. MRS agar is a selective media for *Lactobacillus spp*. 51 g per litre of distilled water were added together with 1 mL of Tween 80 (Cat. No. P8074). The mixture were heated within several minutes so that the media can dissolve completely. The media was poured into a container and proceed by sterilized it using autoclave at 121°C for 15 minutes. MRS agar which are selective for was mixed with 1 mL of homogenized sample and proceed by incubation for 3 days at 35 ° C. The agar was cultivated under anaerobic conditions by placing the container inside black plastic. After 3 days, the CFU of lactic acid on MRS agar was counted and recorded.

2.5.2. Determination of lactic acid bacteria using M17 agar
M17 agar is a selective agar for *Streptococcus spp.* An amount of 25 g of M17 were added into 95 mL of distilled water and mixed together. The mixture were heated for several minutes so that the media is completely dissolved. The media was poured into a container and proceed by sterilizatiion process using autoclave at 121 ° C for 15 minutes. After sterilization, the media was cool down to 50 ° C before adding 50 mL of sterile lactose solution (10% w/v). M17 agar was mixed with 1 mL of homogenized sample from section and proceed by cultivated it for 3 days at 35 ° C. The agar was cultivated under anaerobic conditions by placing the container inside black plastic. After 3 days, the CFU of lactic acid on M17 agar was counted and recorded.

2.6. Sensory Evaluation
On day 6 of fermentation process, the fermented clam (*Mercenaria mercenaria*) were prepared and served together with sliced onion, chilli and lime juice. 30 respondents selected randomly from lecturers and students in Uniciti Alam. They were served with 4 sample of fermented clam which are control, garlic, ginger and also lemongrass. Sensory evaluation form was distributed to all the respondents that involve to get the feedback of the products. They will evaluate the fermented products based aroma, colour/appearance, taste, mouthfeel/texture and overall acceptability.7 point hedonic scales was used to determine the acceptance of respondents towards this product. Score 1 indicate ‘dislike very much’, score 7 ‘indicates like very much’ whereas score 4 indicate ‘neither like nor dislike’.

2.7. Experimental Design and Statistical Analyses
Basically, the design of this experiment is based on the Completely Randomized Design (CRD) with four treatments represent by three sample per treatment. All the data obtained from the physical and chemical analysis were analyzed by using Analysis of Variance (ANOVA) and significant different among the treatments was determined using Turkey Multiple Comparison Test at $p < 0.05$ from SAS software

3. Results and discussion

3.1. Changes in physic-chemical properties
Figure 1 shows the changes in pH of fermented clam with addition of different fresh spices over the fermentation period. It showed that the pH of the fermented clam with and without addition of fresh spices showed a decreasing trend over the fermentation period of 8 days. Among all the treatments, fermented clam with addition of ginger showed more drastic decrease in pH compared to control. The decreasing of the pH values over fermentation period may due to the dissociation of large polypeptides and free amino acids that presence in the salt [7]. According to Ana et al. (2014), [8] the decreasing of pH values over fermentation period coincide with the increasing of lactic acid bacteria population and with the raise on lactic acid production.

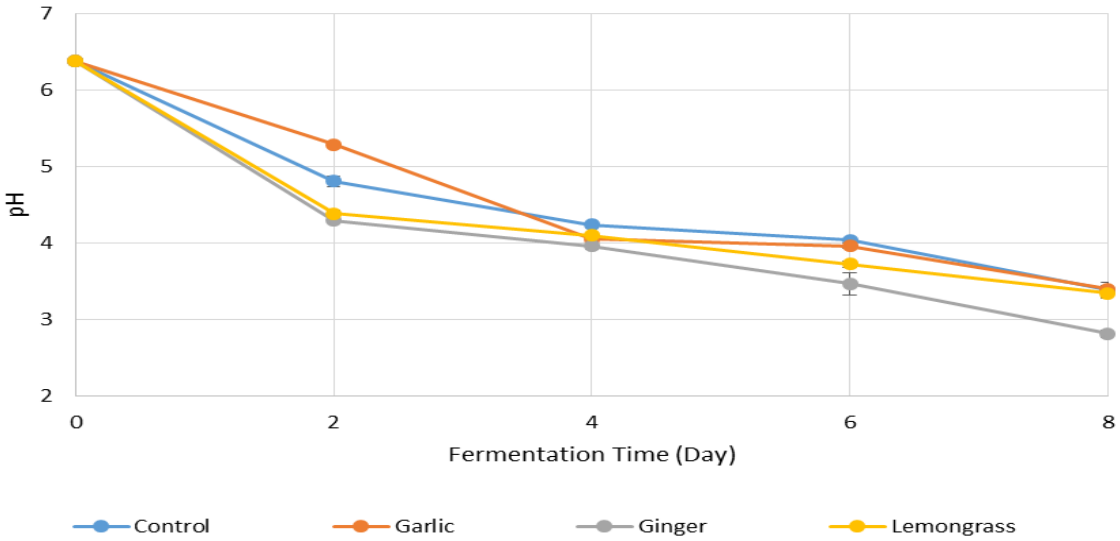

Figure 1. pH of fermented clam with addition of fresh spices during fermentation. Vertical bars represent standard errors..

The changes in texture (firmness) of fermented clam with the addition of different fresh spices during fermentation were evaluated and presented in Figure 2. Firmness is defined as the force needed to penetrate the flesh. It is clear from the result in the Figure 2 where the firmness of fermented clam with addition of fresh spices decreased as fermentation progressed. During fermentation, control showed significantly ($P<0.05$) the most firm texture followed by garlic, ginger and lemongrass. This proves that the addition of fresh spices had a great influence on the textural properties of fermented clam. Decrease in texture during fermentation process might due to the fermentation activity and hydrolysis of the compound. The protein in the fish product and carbohydrate source from the addition of cooked rice during fermentation were hydrolyzed by enzyme produced by microorganism and broken down into simple compound [6].

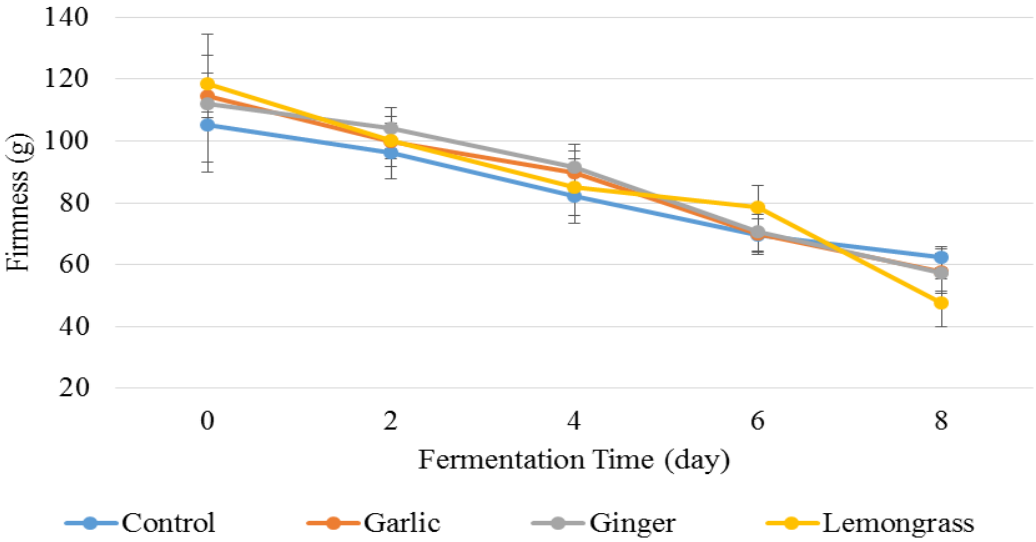

Figure 2. The texture (firmness) of fermented clam with addition of different fresh spices during fermentation. Vertical bars represent standard errors.

3.2. The microflora of lactic acid bacteria

Figure 3 represent the CFU of lactic acid bacteria (*Lactobacillus* spp) on fermented clam with addition of different fresh spices by using MRS agar during fermentation process. These findings signify that the sample with the addition of fresh spices had the highest CFU of *Lactobacillus* spp compared to control. It was observed that ginger had the highest CFU of *Lactobacillus* spp with value of 164×10^5 CFU at day 8 of fermentation. The significant differences ($P<0.05$) between different fresh spices can be seen on day 0 and day 4 where the control had the lowest CFU of *Lactobacillus* spp The increasing and decreasing of CFU are related to the changes in pH of fermented clam over the fermentation period. When the pH decrease, the CFU will eventually increase due to the acidic condition caused by formation of lactic acid bacteria.

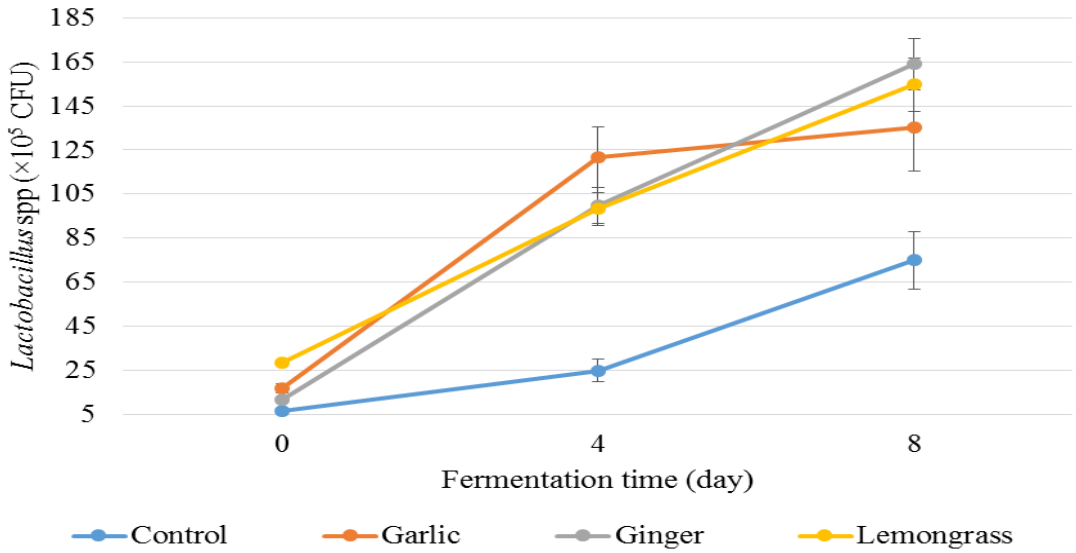

Figure 3. CFU of lactic acid bacteria (*Lactobacillus* spp) on fermented clam with addition of different fresh spices by using MRS agar.

Figure 4 showed the CFU of lactic acid bacteria (*Streptococcus* spp) on fermented clam with addition of different fresh spices by using M17 agar during fermentation process. An increasing trend of CFU of *Streptococcus* spp on fermented clam with addition of fresh spices by using M17 agar were observed in Figure 4. Ginger had the highest CFU of *Streptococcus* spp with the value of 159.6×10^5. Although there were high number in CFU of *Streptococcus* spp among all treatments, there were no significant difference ($P>0.05$) found on day 0 of fermentation process. However, there were significant differences ($P<0.05$) among day 4 and day 8 where ginger had the highest value of CFU and garlic had the lowest value. The difference of microbes number among each treatment might due to the different number of microbes present in raw material, quality of ingredients used in the fermentation process, sanitation and hygienic application during processing [9].

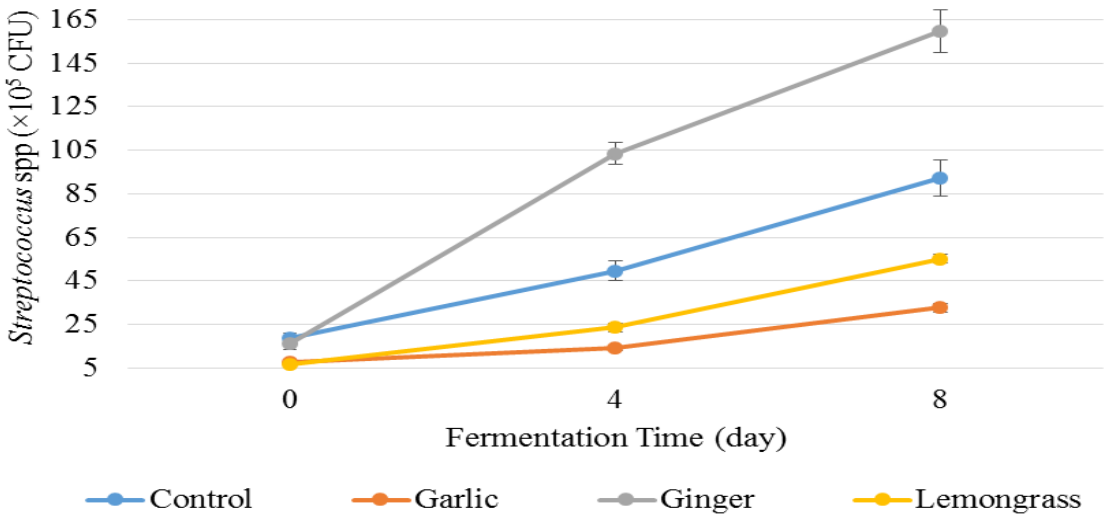

Figure 4. CFU of lactic acid bacteria (*Streptococcus* spp) on fermented clam with addition of different fresh spices by using M17 agar during fermentation. Vertical bars represent standard errors.

3.3. Sensory acceptability

The sensory evaluation test of fermented clam with addition of different fresh spices was carried out by 30 panelist and the result are shown in Figure 5. From the result obtained, lemongrass had the highest mean score for aroma, taste, texture or mouthfeel and overall acceptability (5.333, 5.500, 5.100, 5.300) respectively. Color, texture and overall acceptability showed no significant diffrent ($P>0.05$) among all treatments. However, lemongrass had the highest significant different ($P<0.05$) for aroma and taste attributes with value of 5.333 and 5.300 respectively. The fermentation process in fish preservation was recommended in the study by Nwabuezo (2010) as it can enhace the flavor of end product and make it more acceptable to the consumer.

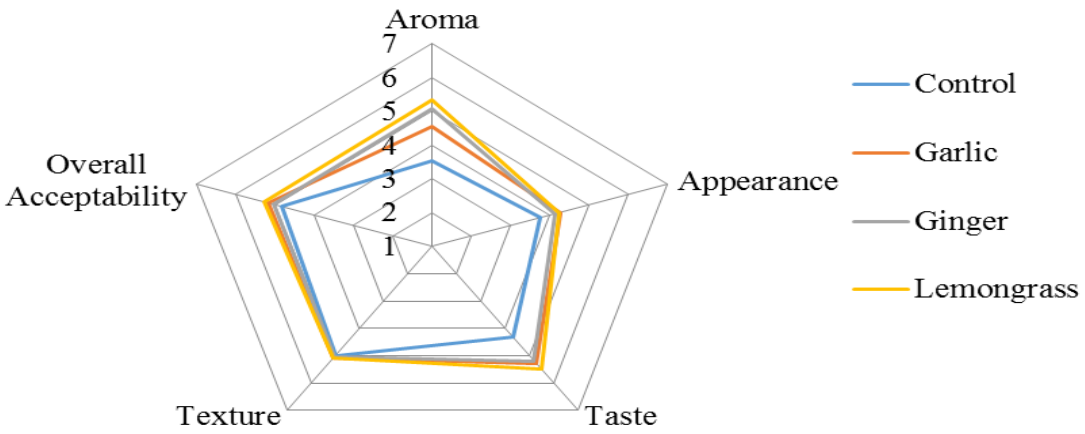

Figure 5. Sensory acceptability of fermented clams with addition of different fresh spices.

4. Conclusion

In conclusions, over the fermentation period, the pH, and texture were decreased. Meanwhile the Colony Forming Unit (CFU) were increased in selective media used which are MRS and M17. Sensory evaluation showed that samples prepared with the addition of lemongrass are the most acceptable by the panelist. The highest significant difference($P<0.05$) for pH showed by garlic, texture (the most firmness) by control, CFU of *Lactobacillus* spp on MRS agar by garlic, CFU of *Streptococcus* spp on M17 agar showed by ginger and sensory acceptability by ginger. The result of this study have showed that the addition of fresh spices over the fermentation process of clam (*Mercenaria mercenaria*) have the great potential where it can give added value to the product in terms of aroma, color or appearance, taste, mouthfeel or texture and also overall acceptability. This fermentation process produce the safe products since this products does not involve any chemical used over the process.This fermentation process can increase the shelf life of clam since it inhibit the growth of pathogenic microorganism. The obtained result of this study can conclude that the use of fresh spices could enhance the flavour of fermented food product thus solving the problem of taste complained by many **consumer.**

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