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the 6th national student conference

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ON FOOD SCIENCE AND TECHNOLOGY

ASSURING FOOD SAFETY AND FOOD QUALITY ALONG THE PRODUCTION CHAIN

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PROCEEDING

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PREFACE

This is the proceeding of the 6th National Student Conference on Food Science and Technology held on June 15, 2006 and organized by Department of Food Technology Soegijapranata Catholic University, Semarang. The conference with theme "Assuring Food Safety and Food Quality along the Production Chain illustrated that safety and quality assessment need to be developed and implemented in vertically coordinated supply chains rather than on single divisions. In this conference we also were informed by experts from international food factories, which are PT. Firmenich Indonesia and PT. SMART about the newest trend on food and business issues.

The Conference was designed for students of food science and technology and other related fields to improve their presentation capability in English. Simultaneously, they could share their research findings, experiences, and knowledge in a scientific and professional setting. Participants of the conference included a wide national spectrum of audience (students, lecturers, researchers) from food related academic circles.

The platform presentations covered following topics:
1. Food Quality and Safety
2. Food Processing and Engineering
3. Nutrition and Functional Food
4. Food Microbiology and Biotechnology

The organizing committee is grateful to all honorable speakers, participants, sponsor companies and all parties that cannot be mentioned one by one for joining this gathering and their valuable contributions to the Conference.

Semarang, October 2006
Ir. Bernadetha Soedarini, MP
Dipl.-Ing. Fifi Sutanto-Darmadi
Dr. Ir. Lindayani, MP
Ir. Sumardi, MSc

TABLE OF CONTENT

NUTRITION AND FUNCTIONAL FOOD

FOOD MICROBIOLOGY AND BIOTECHNOLOGY

FERMENTED ACETIC ACID AND LACTIC ACID AS ALTERNATIVE COAGULANT APPLIED TO TOFU

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ABSTRACT

Recently, the use of formalin, a substance used as corpse preservative in the food industry had aroused to national food issue. One of given food materials type addition of formalin is tofu. Tofu is food type made of soybean and the most consumed by societies. In addition of acetic acid and lactic acid are result of fermentation of microorganism as coagulant is one of the alternatives it is also potential to replace formalin as preservative in making tofu. Fermented media which will be used in this research "tajin", and the microorganism will instant yeast to yield acetic acid, and *Lactobacillus bulgaricus* to yield lactic acid. The treatment was made by adding acetic acid and lactic acid in a range of concentrations. This research aimed to determine the influence of acetic acid and lactic acid usage, as the result of fermentation of microorganism, as coagulant in making non formalin tofu. The evaluation was made to the aspects of physical, chemical, microbiological, and sensory characteristics. The parameters employed in physical analysis that would be texture analysis, in chemical analysis would be water content analysis, using the method of thermogravimetry, in microbial analysis would be shelf life analysis using the method of TPC (Total Plate Count), and in sensory analysis would be texture, flavor, and color and liking.

Keywords: tofu, formalin, coagulant, acetic acid, lactic acid, fermentation

INTRODUCTION

Containing 35% protein and being faction of Leguminosa (Moehyi, 1992), soybean is one of the food-stuff types which has highest vegetative protein from all legume types. The soybean (*Glycine max*) is an important source of edible vegetable oil (20%) and high-quality vegetable protein (48-50%). The protein from soybean is complete and has all eigth essential amino acids. The major proteins in soybean are globulins (glycinin and β- and γ-conglycinin), which represent 80% of total proteins. It constants at pH 7.6 and 0.5 ionic strength (Prestamo *et al.*, 2000) on the basis of their sedimentation

Tofu is a food product made by soybean milk coagulating, and rich in protein content. It has high water content that give good condition for spoilage

microbe to grow. To extend the shelf life, many tofu industries add formalin as preservative to the processing. That addition can improve end product texture and shelf life, but it is indicated to be hazardous to human's health if it is consumed (Mudjajanto, 2005).

Lactic acid bacteria are being used in probiotic preparations currently, either singly or in combination. *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* are the most commonly used in fermented dairy products since these bacteria comprise a portion of the desirable intestinal microflora and have beneficial effects, including stimulation of the immune response, anticarcinogenic activity and inhibition of the growth of pathogens (Lin *et al.*, 2004).

Homofermentative of *Lactobacillus bulgaricus* is lactic acid bacteria, its yielding lactic acid and aldehida from anaerobic fermentation with sugar substrat. *Lactobacillus bulgaricus* is positive gram bacterium which is not dangerous (non patogen) and is used much for the fermentation of food products. Optimal growth temperature for the *Lactobacillus bulgaricus* is 43-45° C (Rehm *et al.*, 1995). Lactic acids are naturally produced by lactic acid bacteria, such as *Lactobacillus bulgaricus* and several other bacteria strains such as *Streptococcus thermophilus*. Generally, lactic acid bacteria will alter sugar into alcohol during the fermentation process, which it happens under anaerobic condition. Because they produce alcohol in the presence of sugar naturally, lactic acid bacteria are survival under low pH condition. The optimum growing condition of lactic acid bacteria is under pH of 5.5, while their lowest tolerable pH is 3. Lactic acid bacteria may also survive under a great range of temperature (Just, 2004).

Dry yeast is a live inactive microorganism. It consists of dried yeasts mixed with substrate. Yeast's optimum growth temperature rangt ranges from 32 to 40°C when the relative humidity is about 86 - 90%. Like lactic acid bacteria, yeast will also break down anaerobically complex sugar into such as polysaccharides in its simpler form, such as glucose if inoculated into carbohydrate rich media. Broken down sugar form, i.e. glucose will then be used to produce carbon dioxide as well as ethanol. The following is the fermentation reaction using yeast as the culture :

$C_6H_{12}O_6 \longrightarrow CO_2 + 2C_2H_5OH +$ Energy (Widiastuti, 1997).

Yeasts are capable of deriving many types of substrates. Generally, yeasts grow optimally when the pH ranges from 3.5 - 6 and temperature ranges at 25 - 30°C. *Acetobacter aceti* is a microorganism that produces acetic acid. *Acetobacter aceti* grows optimally when the pH ranges from 3.5 to 4.5. Yeast and *Acetobacter aceti* are proven to be able to work synergically. The use of a mixed culture of these two microorganisms is expected to improve results obtained from the fermentation process. When mixed culture is used, first yeast will degrade the substrate into ethanol, carbon dioxide, and energy. Afterwards, the ethanol produced by the yeasts will be used as a substrate by *Acetobacter aceti* to produce acetic acid (Peppler & Perlman, 1979); (Rehm & Reed, 1995).

In this research, tajin is used as the medium (substrate) for growing the cultures. Tajin itself is defined as thick water from cooked rice. Tajin that is made from 150 gr of rice and 3 litres of water contains 9.5 ml of reducing sugar per 100 ml. Tajin majorly contains amilose type of starch. This amilose will then be broken down into glucose, maltose and alcohol or other acids by microorganisms during the fermentation process (Nugerahani *et al.*, 2000). So the aim of this research was expected to produce tofu with similar texture and shelf life using of mixed natural lactic and acetic acid as coagulant to the ones made with formalin addition.

MATERIALS AND METHODS

Materials

For the experiment would be used rice; water; starter inoculums which are *Lactobacillus bulgaricus, Streptococcus thermophilus, Acetobacter aceti,* and baker's yeast.

Methods

Because the research is still in the phase of preliminary study, this chapter will cover the process just till fermentation. The process is first, mixing rice with a certain amount of water then it is boiled and then the liquid is separated from solid material then it is cooled for about 25 minutes. The obtained liquid is called tajin. The tajin is sterilized. Sterile tajin is inoculated with the starter inoculums which are L. *bulgaricus*, S. *thermophilus*, baker's yeast, and A. *aceti*. Then it is fermented using batch and fed batch method for 5 days. It is analyzed until pH 4.

RESULTS AND DISCUSSION

Based on the pH analyzing is to see that fed batch fermentation method is more effective than batch fermentation. It is

showed that the pH in fed batch fermentation decreased faster. This was due to the nutrition from the medium, in this case was tajin, especially when the nutrition started to run out. This describes the term of fed batch fermentation, in which the cultures wer fed continuously or sequentially with the medium without removal of culture fluid (Figure 1 and Figure 2). This shows that fed batch fermentation was more effective than simple batch fermentation in order to decrease pH (as seen in Table 1 and 2).

Table 1. pH value during fermentation of Tajin using L. *bulgaricus* with batch and fed batch fermetation

Day	Batch fermentation	Fed Batch Fermentation
0	8,97	8,97
1	7,34	7,13
2	5,78	5,5
3	5,16	4,96
4	4,78	4,63
5	4,65	4,52

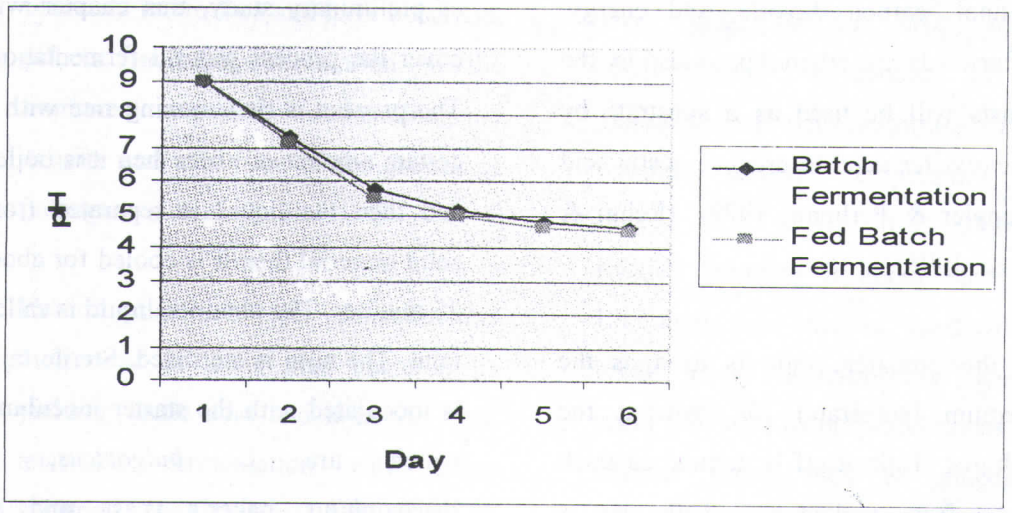

Figure 1. Profile of "tajin" fermentation by L. *bulgaricus* using batch and fed batch fermentation based on pH and time of fermentation (day)

Table 2. pH value during fermentation of Tajin using bakers yeast with batch and fed batch fermetation

Day	Batch fermentation	Fed Batch Fermentation
0	8,97	8,97
1	7,32	7,16
2	5,96	5,72
3	5,43	5,28
4	4,91	4,75
5	4,67	4,55

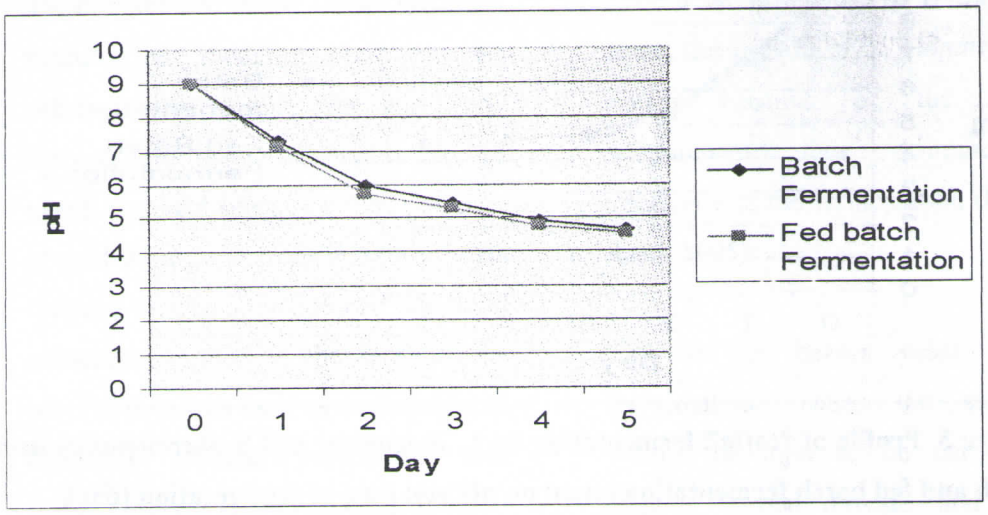

Figure 2. Profile of "tajin" fermentation by baker's yeast using batch and fed batch fermentation based on pH and time of fermentation (day)

The use of mixed culture had proven to be more effective than the use of single culture to reduce pH in a 5 days fermentation (as seen in table 3,4 and figure 3,4). As shown in the tables and figures, the use of mixed culture of L. *bulgaricus* and S. *thermophilus* and the mixed culture of bakers yeast and A. *aceti* reduced pH more effectively than the use of single culture of L. *bulgaricus* or bakers yeast alone. The following figures and tables also show that fed batch fermentation was more effective than simple batch fermentation in reducing pH.

Table 3. pH value during fermentation of Tajin using L. *bulgaricus* and S. *thermophilus* with batch and fed batch fermetation

Day	Batch fermentation	Fed Batch Fermentation
0	8,95	8,95
1	7,06	6,85
2	5,41	5,27
3	4,74	4,55
4	4,52	4,4
5	4,45	4,31

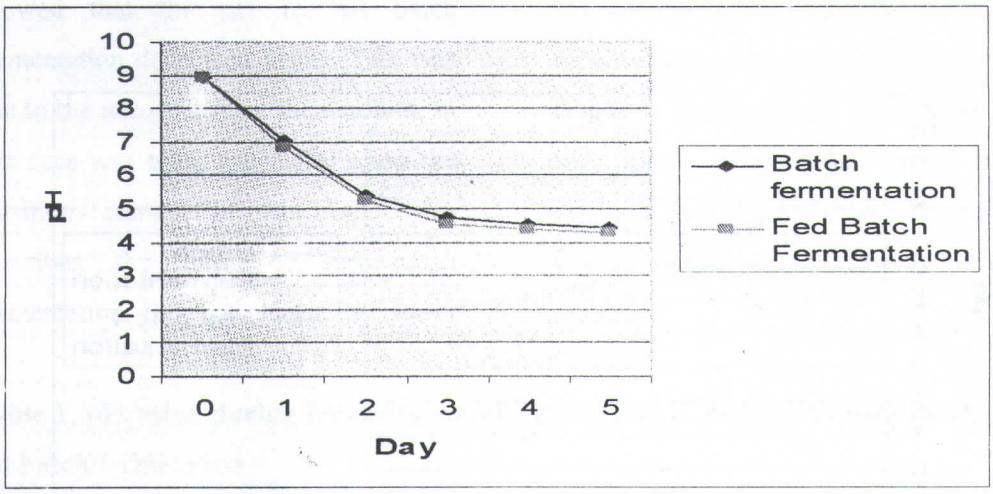

Figure 3. Profile of "tajin" fermentation by L. *bulgaricus* and S. *thermophilus* using batch and fed batch fermentation based on pH and time of fermentation (day)

Table 4. pH value during fermentation of Tajin using bakers yeast and A. *aceti* with batch and fed batch fermetation

Day	Batch fermentation	Fed Batch Fermentation
0	8,98	8,98
1	7,05	6,89
2	5,68	5,46
3	5,17	4,92
4	4,73	4,56
5	4,54	4,35

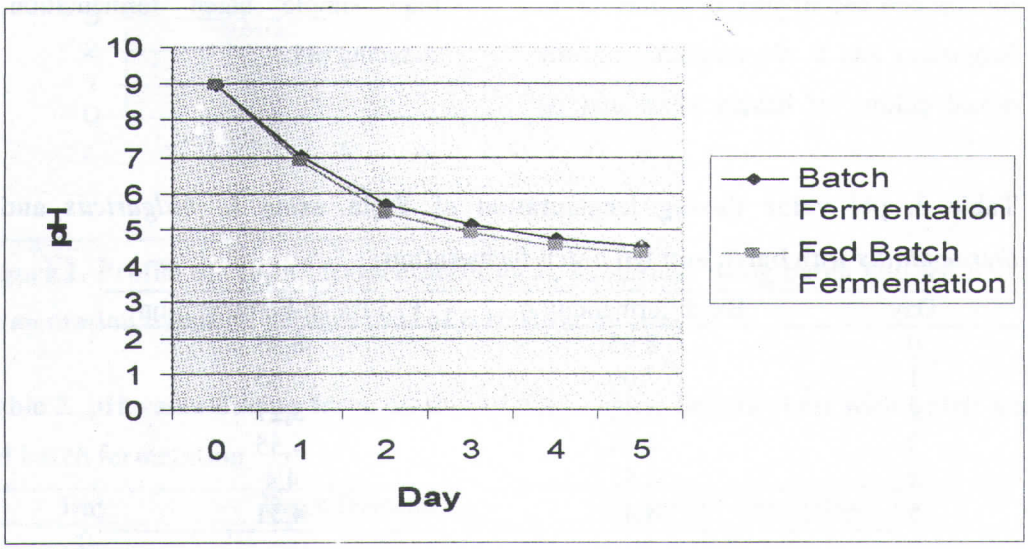

Figure 4. Profile of "tajin" fermentation by baker's yeast and A. *aceti* using batch and fed batch fermentation based on pH and time of fermentation (day)

During fermentation, inoculated microorganisms grow and degraded further the medium used as the substrate, the tajin. The tajin was derived into many substrates, one of which was acid substance. The presence of acid during the fermentation process resulted the reduction of pH. The acid substance resulted in the fermentation process then was used as a coagulant to coagulate soybean protein in tofu making process (Rehm & Reed, 1995).

The reduction of pH during the fermentation process was mainly due to the production of acids by the microorganism(s) used in the fermentation. The use of mixed culture of L. *bulgaricus* and S. *thermophilus* was more effective to reduce pH in the fermentation than using the L. *bulgaricus* alone. This could mean that more acid was produced by the mixed culture of L. *bulgaricus* and S. *thermophilus* than the single culture of L. *bulgaricus* alone. In the use of mixed culture, S. *thermophilus* first worked by deriving several substances and produces acid. The acid produced caused decreasing of environment's pH, thus inhibiting the growth of S. *thermophilus* itself. Meanwhile, the produced acid promoted the growth of L. *bulgaricus*. L. *bulgaricus*, then replaced S. *thermophilus* to derive substances in the substrate to produce lactic acid. In the use of L. *bulgaricus* alone, the growth of L. *bulgaricus* was slower because of the alkaline environment, thus decreased the effectivity of the fermentation (Rehm & Reed, 1995).

The use of bakers yeast in tajin fermentatione cause the substances within the tajin to be derived into ethanol, carbon dioxide, and energy. When a single culture of bakers yeast was used in the fermentation, the end result would include ethanol. When a mixed culture of bakers yeast and A. *aceti* was used, the ethanol produced by bakers yeast would then be used by A. *aceti* to produce acetic acid. Both acetic acid and ethanol were acids, however acetic acid had lower pH and higher acidity than ethanol, and therefore it made the use of mixed culture be more effective than the use of bakers yeast alone (Rehm & Reed, 1995).

CONCLUSIONS

- Fed batch fermentation method is more effective than batch fermentation method
- The use of mixed culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) may increase the effectiveness of the fermentation process to produce

lactic acid. And mixed culture (baker's yeast and A. *aceti)* effective to produce acetic acid.

ACKNOWLEDGEMENTS

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