CHAPTER IV

PHYSIOCHEMICAL AND MICROBIOLOGIAL CHANGES OF CHILI MASH INNOCULATED WITH LACTIC ACID BACTERIA

4.1 INTRODUCTION

Lactic acid fermentation of vegetables is a traditional bio-preservation method for the manufacture of finished and half-finished foods. Traditional fermentation of vegetables is frequently characterized by a succession of hetero- and homo-fermentative lactic acid bacteria, together with or without yeasts, which are responsible for multi-step fermentation processes (Di Cagno et al., 2008b). Traditional lactic acid fermentation produced desirable flavor may be responsible which gives the unique characteristics of fermented vegetables. Very little sophisticated equipment is needed either to undertake or subsequently store the fermented product. The key practice involve in traditional fermentation is using salt at 2 to 8% concentration (Holzapfel, 2002) and modify the environment that allow growth and proliferation of naturally existing LAB. Lactic acid bacteria are normally in small numbers (2.0 to 4.0 log cfu/g) of the autochthonous microbiota that lead into spontaneous fermentation when vegetables are brined (Anderson, 1984). Fermentation of food is an art where at times the changes in fermentation conditions encourage the growth of undesirable microorganisms that changed the sensory properties or unsuccessful fermentation that could not inhibit the growth of spoilage and pathogenic microorganism (Di Cagno et al., 2008a; 2008b).

The practice of using starter cultures in fermented milk, sausage, cheese and leavened well known. The application of using starter culture in fermented vegetables is increasing only recently (Molin, 2001; Montet et al., 2006). Applying starter culture has become attractive especially to manufacturers. It leads into reduction of costs (eg. Energy), reduced fermentation times, reduced of spoilage, improved process control and sensory quality, improved safety attributes and reduced preparation of final products (Holzafel el al., 2002). Petäjä et al. (2000) inoculated *L. alimentarius* and *P. pentosacues* strain POHK to ferment sour cabbage. Their study observed that inoculated LAB lead into effective fermentation as the pH decreased significantly compared to control group within 48 h fermentation. Inoculation of *L. mesentroides* and *P. dextrinicus* lead to production of low salt sauerkraut (Wiander & Korhonen, 2002). LAB starter strains consisted of *L. curvatus, L. mesentroides*, *L. plantarum* and *W. confusa* inoculated into red and yellow chili peppers slices and stored in 1% sodium chloride (w/v) for 30 days at 25°C produced acceptable and shelf stable product (Di Cagno et al., 2009).

Lactic acid bacteria can be introduced as starter culture in controlled lactic acid fermentation of vegetables and fruits. They can be either from autochthonous or allochthonous starter. The selected LAB must have the ability to be adapted to the intrinsic characteristics of the new raw materials (Di Cagno et al., 2008a, 2009). A strong starter culture could minimize dry matter loses, avoid contamination with pathogenic and toxigenic bacteria and moulds, and minimizes the risk of incidental microflora that cause off-flavor. Di Cagno et al. (2011b) and Bourdichon et al. (2012) observed that autochothonous survive better than the allochothonous suited for vegetable fermentation.

The common attributes of chili are hot and spicy taste and they are made into products such as chili paste, chili sauce, chili powder which are then made from dried or fresh chili. Fermented chili mash prepared under anaerobic condition was observed to produce desirable and acceptable flavor as evaluated by sensory panelists as discussed in Chapter III. This Chapter reports the effect of three LAB strains (*L. plantarum* ALO1, *L. pentosus* ALO2, and *L. plantarum* AU2), and fermentation time on physiochemical and microbiological profile of fermented chili mash.

4.2 MATERIALS AND METHODS

4.2.1 Preparation of Bacterial Strain

The LAB used were *L. plantarum* ALO1, *L. pentosus* ALO2, and *L. plantarum* AU2 isolated from different sources as described in section 3.2.2.

4.2.2 Preparation of LAB Inoculated Chili Mash

Cilibangi fruits were obtained from Tanjung Karang, and then selected free from blemishes, defects and insect damages. The pericarps were removed and the chilies were washed with portable water to remove any impurities then ground using a food blender (Panasonic), packed 500g in Scott bottles with 6% rock salt added. The samples were subjected to three treatments three namely, (a) pre-pasteurized (80°C, 10 min) then with and without LAB inoculation, and (b) non-pasteurized samples with and without LAB inoculation. LAB was inoculated at 1% (v/v) of 24 h culture LAB in

phosphate buffer saline (PBS) to 500 g of chili mash. All the treatments were layered with 10% (v/v) sunflower oil to create anaerobic environment. All samples were fermented at 30°C for 28 days.

4.2.3 pH Measurement

The changes in pH during 28 days fermentation were monitored described in section 3.2.3.

4.2.4 Titratable Acidity Measurement

The total amount of acid present during the 28 days fermentation was evaluated described in section 3.2.7.2.

4.2.5 Microbial Measurement

The microbial measurement for 28 days fermentation was assessed explained in section 3.2.7.3.

4.2.6. Statistical Analysis

The analysis of variance (ANOVA) was conducted to study the effect of experimental factors (fermentation time, heating and LAB isolates) and their interaction on the microbial count (Log_{10}cfu/ml), pH and titratable acidity. The ANOVA analysis (Minitab16 Statistical Software., USA) was carried out to determine the statistical differences at ($p<0.05$) (APPENDIX D).

4.3 RESULTS

4.3.1 Changes in Microbial Count, pH and Titratable Acidity of LAB Inoculated in Fermented Chili Mash in Non-Pasteurization Treatment

Changes in LAB, TPC and yeast and mold count during 28 days fermentation of *L. plantarum* ALO1 inoculated unpasteurized fermented chili mash is shown in Figure 9. The total count of *L. plantarum* ALO1 was significantly ($p<0.05$) reduced from initial $\log_{10}$ 10.02 to $\log_{10}$ 7.16 after 28 days fermentation. The TPC was variable in the ranged of $\log_{10}$ 7.31 but was at $\log_{10}$ 7.87 after 21 days. The mold and yeast count showed slight increased from $\log_{10}$ 4.65 to $\log_{10}$ 7.01 after 21 days, then decreased to $\log_{10}$ 6.34 after 28 days fermentation.

The initial pH value of *L. plantarum* ALO1 inoculated fermented chili mash was 4.56 then significantly ($p<0.05$) decreased to 3.2 after 7 days and remained unchanged until 28 days. The concentration of lactic acid significantly ($p<0.05$) increased from 0.938 initially then increased to 3.3 after 7 days but decreased to 1.65 after 21 days, and slightly increased to 1.86 after 28 days fermentation (Figure 10).

FIGURE 9: Microbial Changes in *L. plantarum* ALO1 Inoculated Fermented Chili Mash

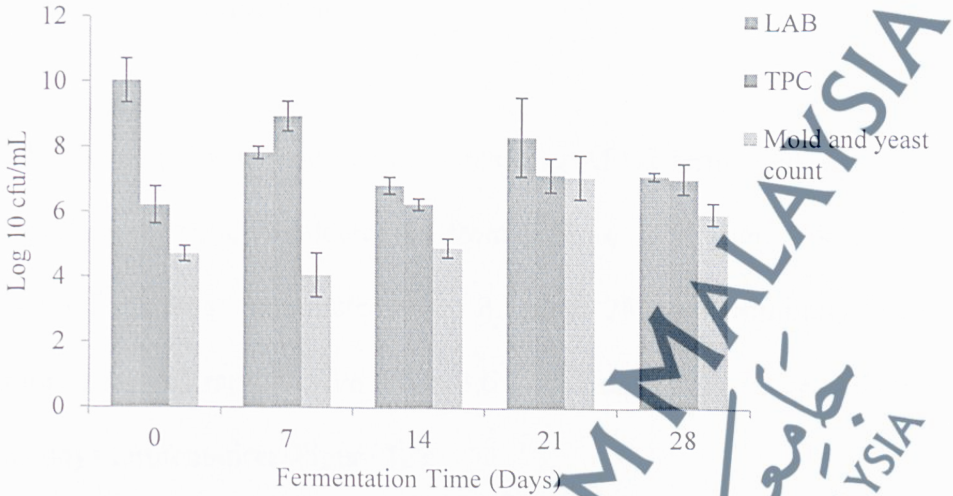


FIGURE 10: pH and Titratable Acidity of *L. plantarum* ALO1 Inoculated Fermented Chili Mash

A different trend was observed in non-pasteurized fermented chili mash inoculated with *L. pentosus* ALO2. Initial LAB count was $\log_{10}$ 10.00 then decreased significantly ($p<0.05$) to $\log_{10}$ 7.60 after 21 days followed by an increased to $\log_{10}$ 8.00 after 28 days fermentation (Figure 11). TPC showed slight increased to $\log_{10}$ 7.06 after 14 days was observed to achieve $\log_{10}$ 7.86 almost similar to fermented chili

mash inoculated with *L. plantarum* ALO1 after 28 days. Fermenting chilli mash with *L. pentosus* ALO2 was able to maintain the yeast and mold count at $\log_{10}$ 5.64 throughout the 28 days fermentation.

A rapid reduction in pH was observed in *L. pentosus* ALO2 fermented chili mash. The pH was significantly ($p<0.05$) decreased from 4.48 to 3.21 after 7 days, remained unchanged until 21 days, then increased to 3.5 after 28 days. Similarly, lactic acid concentration increased rapidly from 0.9 to 3.6 after 7 days, then decreased slightly to 3.2 after 28 days fermentation (Figure 12).

FIGURE 11: Microbial Changes in *L. pentosus* ALO2 Inoculated Fermented Chili Mash.

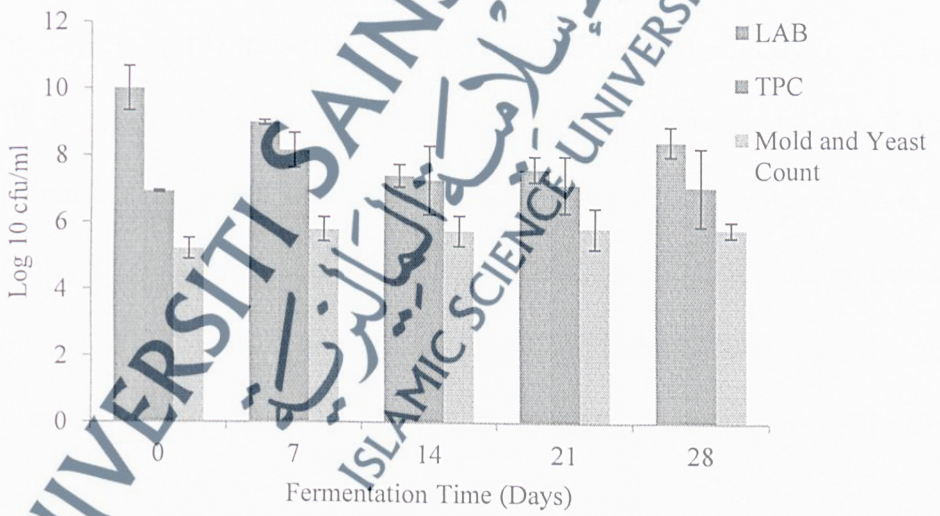
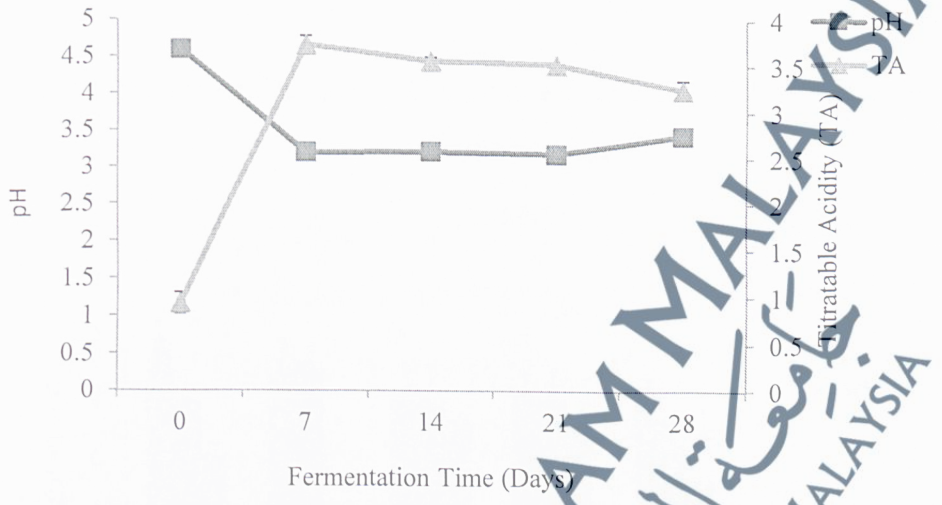


FIGURE 12: pH and Titratable Acidity of *L. pentosus* ALO2 Inoculated Fermented Chili Mash



In the non-pasteurized fermented chili mash inoculated with *L. plantarum* AU2 a different trend in total LAB count was observed. A gradual decreased in LAB count was observed (Figure 13) in which the LAB count decreased slightly ($p > 0.05$) from $\log_{10}$ 10.08 to $\log_{10}$ 8.05 after 28 days. Initial TPC on 0 day was $\log_{10}$ 7.82 then increased significantly ($p<0.05$) to $\log_{10}$ 9.12 after 7 days, then decreased gradually to $\log_{10}$ 7.54 after 28 days fermentation. Although the initial count of mold and yeast was $\log_{10}$ 6.06 on 0 day, the count decreased to $\log_{10}$ 4.72 after 7 days, then the number increased to $\log_{10}$ 7.21 after 14 days and remained unchanged until 28 days fermentation.

Reduction in pH was similar to non-pasteurized fermented chili mash inoculated with *L. plantarum* ALO1and *L. pentosus* ALO2. pH of fermented chili mash inoculated with *L. plantarum* AU2 decreased significantly ($p<0.05$) from 4.56 to 3.20 after 7 days, then remained unchanged until 28 days fermentation (Figure 14). Titratable

acidity of fermented chili mash inoculated with *L. plantarum* AU2 increased significantly ($p<0.05$) from 1.0 to 3.2 g/L lactic acid equivalent after 14 days and decreased slightly to 2.7 after 28 days (Figure 14).

FIGURE 13: Microbial Changes in *L. plantarum* AU2 Inoculated Fermented Chili Mash.

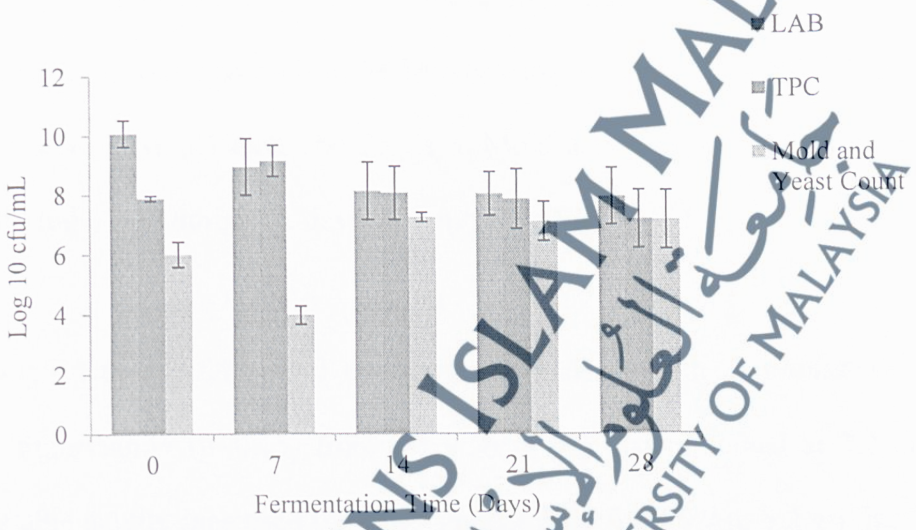
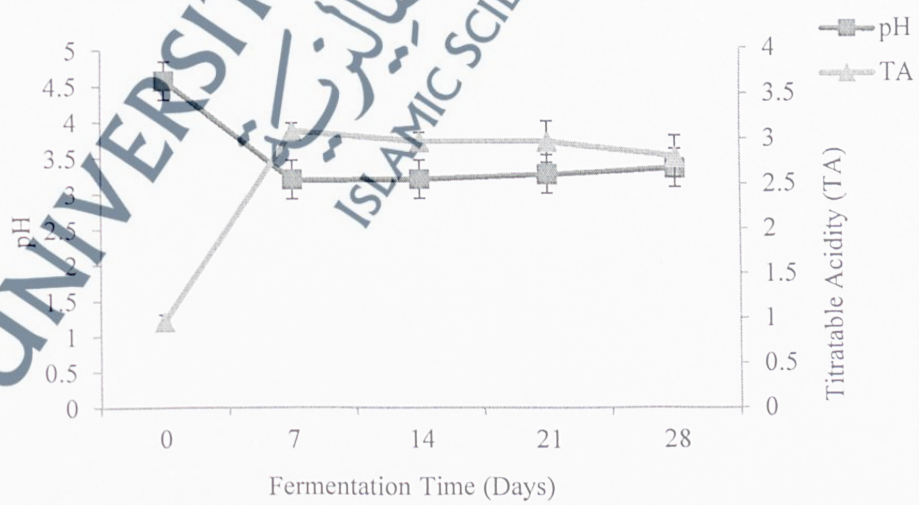


FIGURE 14: pH and Titratable Acidity of *L. plantarum* AU2 Inoculated Fermented Chili Mash.



4.3.2 Changes in Microbial Count, pH and Titratable Acidity of LAB Inoculated in Fermented Chili Mash in Pasteurization Treatment.

A similar trend in total LAB count was observed between non-pasteurized and pasteurized fermented chili mash inoculated with *L. plantarum* ALO1, however, the LAB count was significantly ($p<0.05$) reduced from initial $\log_{10}$ 10.04 to $\log_{10}$ 6.07 after 28 days. TPC was observed to be between $\log_{10}$ 7.12 to $\log_{10}$ 8.76, higher than LAB count at 14 days, 21 days and 28 days. Mold and yeast count was inhibited at $\log_{10}$ 1.26 to $\log_{10}$ 1.40 during 28 days fermentation (Figure 15).

The pH of pasteurized fermented chili mash inoculated with *L. plantarum* ALO1 decreased significantly ($p<0.05$) from pH 4.56 to 3.2 and remained at 3.2 until 28 days. Titratable acidity increased ($p<0.05$) significantly from 0.9 to 3.2 g/L lactic acid after 7 days then decreased slightly to 2.8 after 14 days and increased slightly to 3.5 g/L lactic acid after 28 days different to that observed in non-pasteurized fermented chili mash inoculated with *L. plantarum* ALO1 (Figure 16).

FIGURE 15: Microbial Changes in *L. plantarum* ALO1 Inoculated Fermented Chili Mash

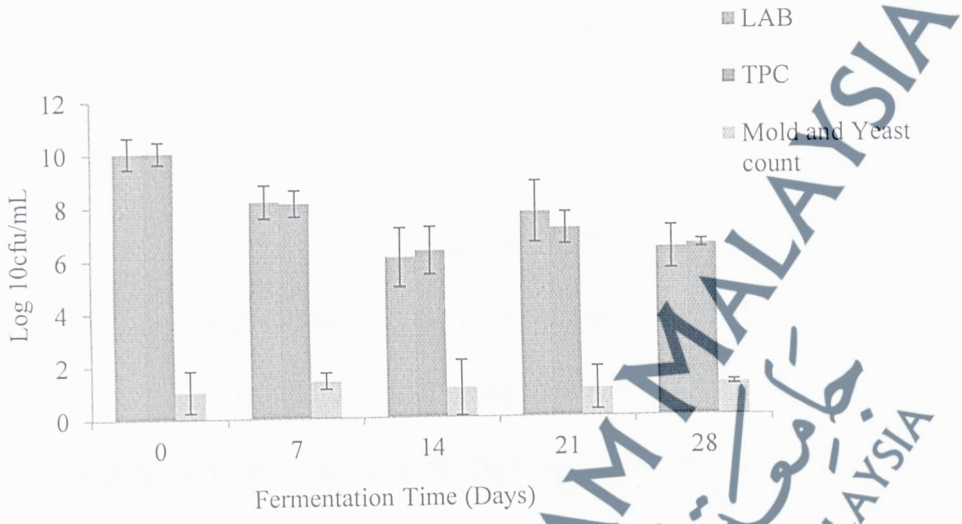
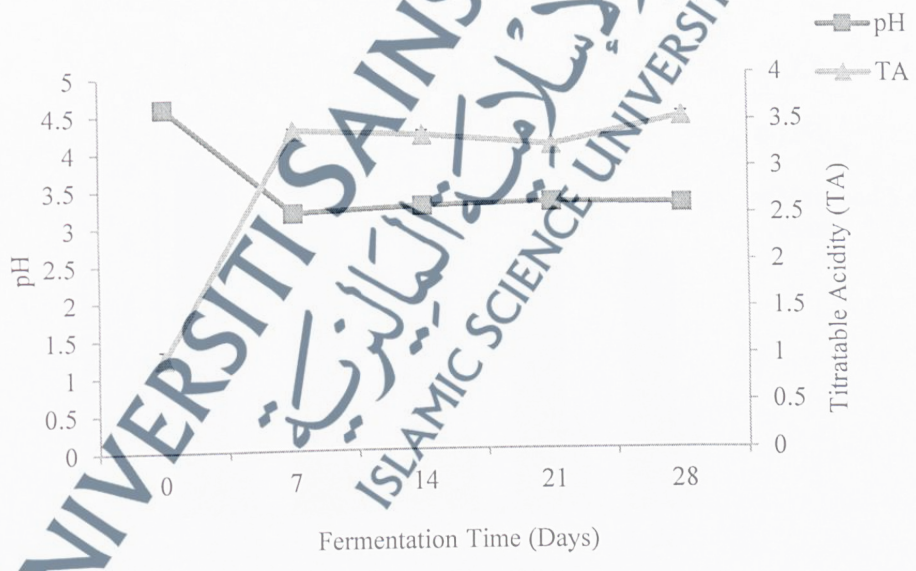


FIGURE 16: pH and TitratableAcidity of *L. plantarum* ALO1 Inoculated Fermented Chili Mash



Pasteurized fermented chili mash inoculated with *L. pentosus* ALO2 showed different trend of LAB, TPC and mold and yeast count as compared to non-pasteurized fermented chili mash inoculated with *L. pentosus* ALO2. LAB decreased gradually from $\log_{10}$ 10.52 to $\log_{10}$ 8.96 after 14 days and remained stable at $\log_{10}$ 7.43 after 28 days. TPC count was slightly lower than the LAB count started at 14 days to 28 days

with LAB count around $\log_{10}$ 8.51 to $\log_{10}$ 7.13 with gradual decreased trend differ with non-pasteurized fermented chili mash inoculated with *L. pentosus* ALO2. Conversely, mold and yeast count gradually increased from $\log_{10}$ 1.29 to $\log_{10}$ 2.85 after 28 days (Figure 17).

Pasteurized fermented chili mash inoculated with *L. pentosus* ALO2 showed persistent trend of pH and titratable acidity as compared to unpasteurized fermented chili mash with *L. pentosus* ALO2 inoculated. pH decreased rapidly ($p<0.05$) from 4.56 to 3.20 and remain persisted until 28 days. The titratable acidity increased from 0.9 g/L lactic acid equivalent to 3.4 during 7 days, remained until 28 days (Figure 18).

FIGURE 17: Microbial Changes in *L. pentosus* ALO2 Inoculated Fermented Chili Mash.

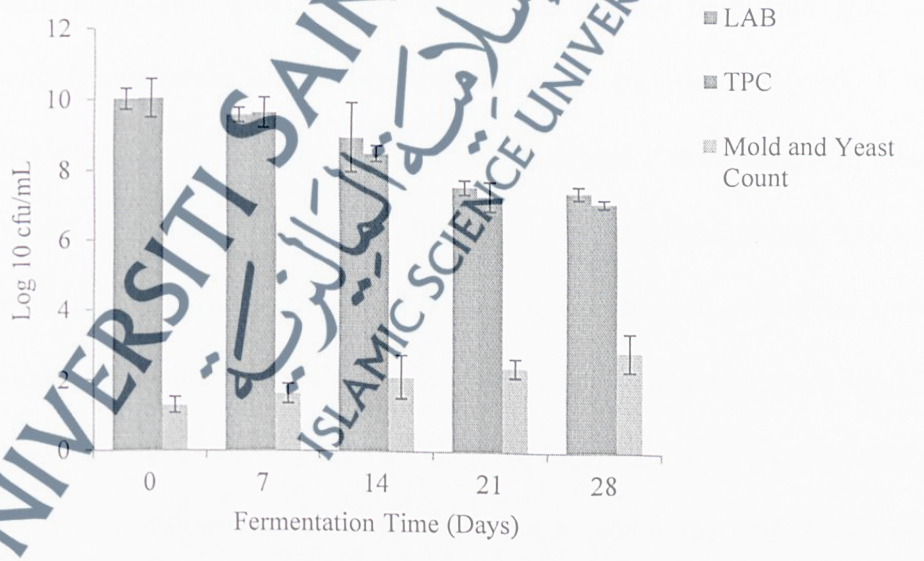
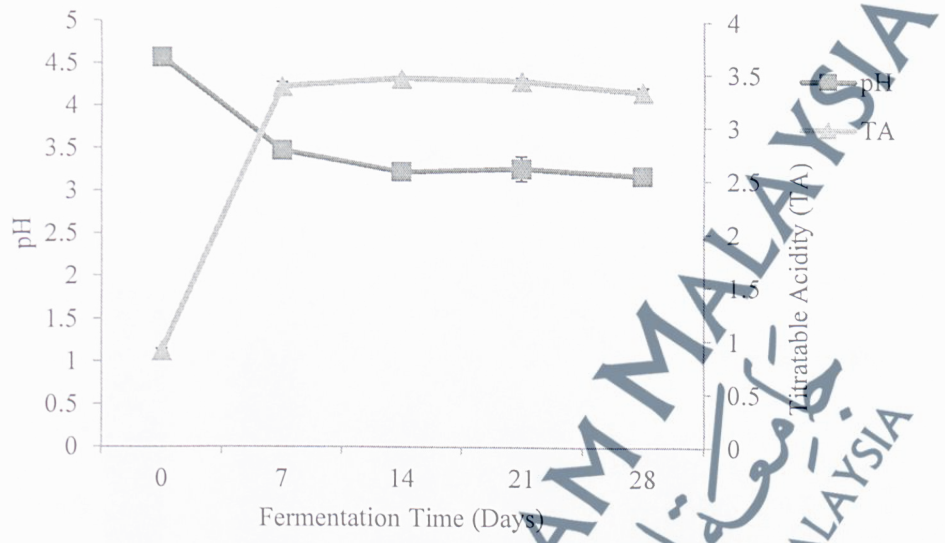


FIGURE 18: pH and TitratableAcidity of *L. pentosus* ALO2 Inoculated Fermented Chili Mash



Rapid reduction of LAB count was observed in pasteurized fermented chili mash inoculated with *L. plantarum* AU2 differ to non-pasteurized fermented chili mash inoculated with *L. plantarum* AU2. The count decreased significantly ($p<0.05$) from $\log_{10}$ 10.22 to $\log_{10}$ 6.29 after 14 days and continue to decrease until $\log_{10}$ 5.52 after 28 days. TPC mimic the LAB count in which the number of $\log_{10}$ cfu/ml count almost to be the same. Mold and yeast count showed gradual increased which its number remained at $\log_{10}$ 1.51 until 14 days. Then, it increased ($p>0.05$) slightly to $\log_{10}$ 2.42 after 21 days and increased significantly ($p<0.05$) to $\log_{10}$ 4.00 after 28 days achieved similar count as non-pasteurized fermented chili mash inoculated with *L. plantarum* AU2 (Figure 19).

Initially, pH reduced significantly ($p<0.05$) from 4.56 to 3.20 after 14 days, decreased slightly ($p>0.05$) to 2.6 after 14 days and persisted until 28 days. Concomitantly, titratable acidity increased from 0.9 g/L lactic acid equivalent to 3.4 after 14 days,

then decreased slightly ($p>0.05$) to 2.8 g/L lactic acid equivalent after 28 days have similar trend as non-pasteurized fermented chili mash inoculated with *L. plantarum* AU2 (Figure 20).

FIGURE 19: Microbial Changes of *L. plantarum* AU2 Inoculated Fermented Chili Mash

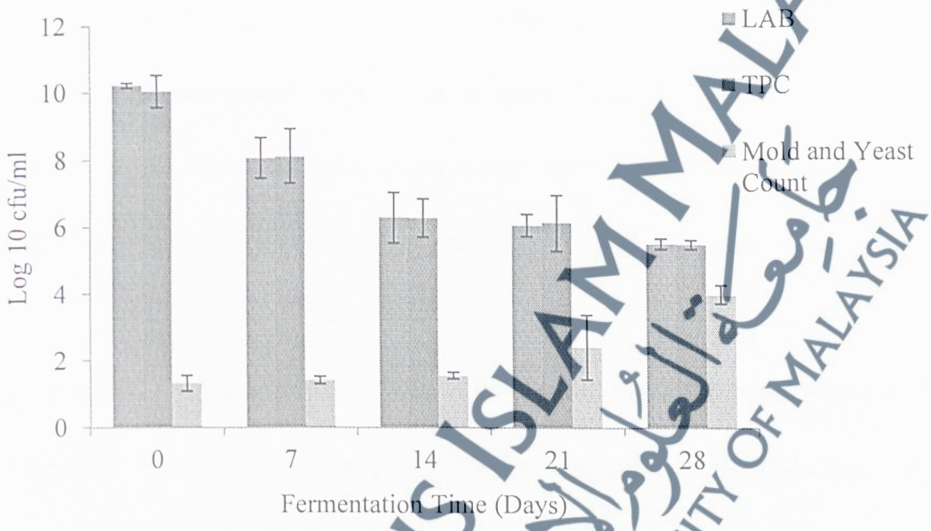
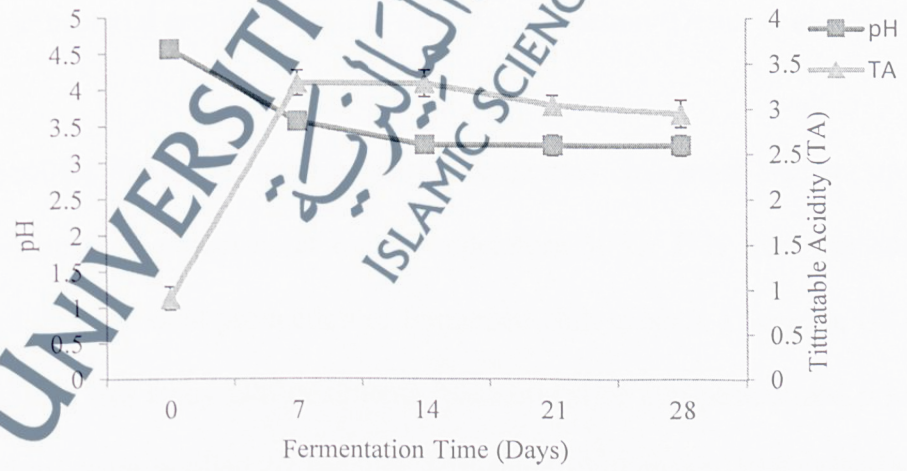


FIGURE 20: pH and Titratable Acidity of *L. plantarum* AU2 inoculated Fermented Chili Mash



4.4 DISCUSSION

Natural or spontaneous fermentation of vegetables involved the naturally present microorganisms. It is dependent on trial and error, traditional skills and often time consuming (Holzapfel, 2002; Plengvidhya et al., 2004). To overcome failures during fermentation, therefore, the application of starter culture to get reproducible and improved quality of fermented vegetables is considered. The idea of starter culture was ideally to prepare material containing large number of microorganisms that can accelerate the fermentation process (Ruiz-Barba et al., 2012; Di Cagno et al., 2008).

The main criteria for selecting the starters that suitable for vegetable fermentation are (i) rate of growth; (ii) changes in pH and (iii) rate of total acid production (iv) color profile (Karovičová et al., 2003; Gardner et al., 2001). Extrinsic factor such as temperature, degree of substrate exposure to air, concentration of fermentable carbohydrate, buffering capacity, natural inhibitor in raw vegetable can affect the lactic acid bacterial growth as well as rate of acidification (Demir et al., 2006).

A protocol for the manufacture of started fermented chili mash was set up under 28 days fermentation processes at room temperature 30°C. This step was adapted and modified by traditional production of fermented chili mash in Lousiana, USA (Koh et al., 2005). In this study two treatments (pasteurized or non-pasteurized prior to LAB inoculation) were applied to produce fermented chili mash. Pasteurization of chili mash reduced the mold and yeast count until $\log_{10}$ 1.03 as compared to non-pasteurized fermented chili mash ($\log_{10}$ 5.12) during 0 day (Figure 15, 17 and 19). Similarly, growth of yeast and mold in pasteurized fermented chili mash inoculated

with *L. plantarum* ALO1, *L. pentosus* ALO2 and *L. plantarum* AU2 was kept at low number throughout 28 days fermentation (Figure 15, 17 and 19). Pre-treat by blanching (85°C, for 2 min) of red and yellow chill mash and inoculated with *L. plantarum* before fermentation inhibit the growth of yeast and mold in chili mashed during processing and storage (Di Cagno et al., 2009). In this study layering the fermented chili mash with the sun-flower oil create anaerobic environment that favor the growth of LAB and maintains the counts $>\log_{10}6$ (Figure 9,11,13,15,17 and 19) during the 28 days fermentation. Layering the vegetable surface with oil is one of popular traditional method used in pickling of vegetable to prevent growth of spoilage yeast during storage (Lee et al., 1997; Di Cagno et al., 2009; Malfeito-Ferreira et al., 2001).

Presence of yeast and mold in vegetable fermentation can cause detrimental effect. Beall. et al. (2012) reported that the presence of yeast resulted in spoiled fermented chilli mash from the production of excess alcohol production. Yeast and mold are dominant microorganisms in non-pasteurized fermented chili mash and the counts increased with LAB count through 28 days fermentation (Figure 9 and 13). Similarly, Albert et al. (2010) observed that sauerkraut fermentation is dominated with both LAB and yeast. Peng et al. (2001) suggested a possible synergistic effect between yeast and LAB in which the autolysis of yeast after alcoholic fermentation released nutrient that are favorable for bacterial growth.

However, the growth of yeast alone or interaction with LAB and yeast are highly desirable for different flavor profile; a wide range of volatile and non-volatile flavor compounds are produced as end products of sugar fermentation (Romano et al., 2003).

This only can be achieved if the fermentation is conducted under controlled environment where the yeast population can be controlled during the fermentation process. Otherwise, fermentation failure could happen.

LAB strain inoculated in this study survived well at >$\log_{10}$ 6 after 28 days fermentation (Figure 9,11,13,15 and 17). Other researchers reported that LAB survived well in vegetables substrate such as cabbage, radish, olive and chili after long fermentation time (Petaja et al., 2000; Hasan & Huseyin, 2013). High numbers of LAB ($\log_{10}$ 7 to 9) in early stages of fermentation of vegetables could accelerate the fermentation process as suggested by de Castro et al. (1998); peeled and blanched garlic inoculated with *L. plantarum* resulted to complete the fermentation after 2 days. Completeness of cabbage fermentation as evaluated by harmonic test on cabbage juice showed that fermentation was completed within 72 h fermentation in samples inoculated with *L. plantarum* (Kohajdová & Karovičóvá, 2004). In this study, *L. pentosus* ALO2 grew very well compared to other LAB in both pasteurized and unpasteurized fermented chili mash and the total LAB count was at log10_{10} 7 throughout the 28 days fermentation (Figure 11 and 17).

Additionally, strain of *L. pentosus* have been isolated from different food sources like cheese, yoghurt milk, sauerkraut and kimchi, and was found to be well adapted in vegetable substrate (Tamminen et al., 2004; Panagou et al., 2003). *L. plantarum* ALO1 showed good survivability in fermented chili mash even though fluctuation in total count was observed during the 28d fermentation (Figure 9 & 15). In contrast the growth pattern of *L. plantarum* AU2 was different, a gradual decrease in total count was observed and can be predicted (Figure 13 & 19). This difference suggested the

ability of microbe to convert lactose, sucrose, glucose and fructose at different conversion rate and its ability to utilize pectin in plant products (Fu & Mathews, 1999). *L. plantarum* is the most popular culture strain and are often used for fermented commodities such as sausage, pickles, olives, cucumber and silage (Carmen *et al.*, 2012; Albert et al., 2010).

This study used single LAB starter inoculation to the chili mash with 6% salt added, However, differences in TPC between pasteurized and non-pasteurized Fermented chili mash was observed (Figure 9 to 20). The TPC of pasteurized fermented chili mash was within the same values as LAB count, indicating that no other bacteria was involved (Figure 15,17 and 19). In contrast, non-pasteurized fermented chili mash has lower TPC value than the LAB count (Figure 9, 11, and 13). This is in accordance with Holzapfel (2002) where single cultures eventually lead to mixed-strain fermentation if the raw materials are not sterilized prior to inoculation. The TPC count in pasteurized fermented chili mash resembled the LAB population since the numbers are almost equal (Figure 19) suggesting that the inoculated LAB is responsible for the fermentation. LAB as facultative bacteria has the ability to grow under aerobic condition and grow faster and reach have high cell densities incubated under aerobic condition (Ji et al., 2013). Single culture fermentation under controlled fermentation offers consistent end product quality over extended period of times.

Lactic acid bacteria caused acid production in vegetables fermentation (Albert et al., 2010; Kohajdova et al., 2009; Sakai et al., 2008). The presence of acid in fermentation is highly desirable since it can be the key factor of preserving effect as well as producing laterally sour taste and atypical aroma (Kohajdová et al., 2009; Karovičová

& Kohajdová, 2003). Rapid acid production in chili mash can only be obtained by inoculating lactic acid producer starter whereas non-lactic acid producer starter will not result into acid production. Bozkurt et al., (2004) reported that traditional chili paste inoculated with *Saccharomyces spp.* and *Streptomyces griseus* obtained minimum pH value 4.08 and maximum titratable acidity value 1.37 within 46 days of fermentation, higher than the final pH of lactic acid fermented vegetables which obtain normal pH range 3.5 to 3.8 (Karovičová & Kohajdová, 2003).

The amount of salt added to ferment vegetables varies between 4 to 15% depending on type of vegetables used. Kimchi achieved the best quality with 4% salt concentration, sauerkraut with 0.5% salt and brine olive fermentation with 8 to 15% salt (Meen & Kwon, 1984; Viander et al., 2003; Romeo et al., 2010). The added salt in fermented vegetables draws water and sugar to initiate fermentation (Jay et al., 2005). LAB utilized the available carbohydrates, such as glucose and fructose, reducing the pH by lactic acid production (Breidt et al., 2007). A decreased in pH and increased in lactic acid concentration in all fermented chili mash inoculated with LAB was observed in this study (Figure 12, 14, 16 and 18). Rapid decreased in pH and increased in titratable acidity were observed in all inoculated pasteurized fermented chili mash suggesting fermentation is successful for all LAB inoculated sample similar to the observation made by Kohajdová et al. (2009). Presence of oxidative yeast accelerate the removal of lactic acid and lead to rapid increase in pH, decreased in titratable acidity and gas formation that cause bloating in the case of cucumber fermentation. (Wendy et al., 2011) Once microbial lactic acid is utilized by yeast, a higher pH developed and a variety of putrefactive microorganisms would grow, fetid odors and loss in color in fermented vegetable may happen (Binsted et al., 1962).

4.5 CONCLUSIONS

This study showed that rapid fermentation can be obtained by LAB inoculated fermented chili mash after 7 days fermentation. Pre-pasteurized fermented chili mash resulted to controlled fermentation. *L. pentosus* ALO2 is the best LAB strain to conduct chili mash fermentation. *L. pentosus* ALO2 grew well in the fermented chili mash with final count >$\log_{10}$ 6 and inhibit mold and yeast growth <$\log_{10}$ 2. In addition, it resulted to stable pH reduction and expectable titratable acidity increased during 28 days fermentation. The rapid fermentation process of fermented chili mash may useful for industrial chili paste production that can shorten the long fermentation period.