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Local Malaysian Isolates as Potential Culture for Fermented Chili Mash

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Abstract: *Lactic acid bacteria (LAB) are known as flavor producer microorganisms. This characteristic could be utilized to produce a fermented chili mash that has unique odour profile. The purpose of this work was to screen flavor producer LAB isolated from local foods to be used as bacterial culture in chili mash. Two sources of LAB starter, autochthonous (isolated from two months pre-fermented chili mash) and allochthonous (isolated from raw milk and home-made yoghurt) were evaluated for their odour characteristics and panelist acceptability using qualitative descriptive analysis (QDA) and hedonic test on chili mash fermented for two weeks. QDA revealed that the ten panelist preferred fermented chili mash (FCM) that has an intense sourness, sweet, flower and fruity smell, while yeasty, moldy, stinky and raw chili smell were rated low by the panelists. Steaming the chili mash for 15 min prior to LAB inoculation significantly ($p<0.05$) affect changes in pH but not the odour profile. LAB with desirable odour characteristics were identified using API 50CHL and PCR 16S rDNA as Lactobacillus plantarum Au2, L. plantarum Alo1, and Pediococcus pentosus Alo2. This study demonstrates that inoculating chili mash with selected LAB can produce odour that are desirable for fermented chili sauce.*

Key words: *fermented chili, lactic acid bacteria, odour profile, Lactobacillus plantarum, Pediococcus pentosus*

I. Introduction

Chilies are agricultural crop belonging to the *Capsicum annum* L. has been used as condiments in foods since ancient time and has become one of great important crops in Malaysian industry. Chili is processed as chili powder, chili puree, chili sauce and hot sauce and pasta sauces (Hall and Skaggs, 2003; Hector, 2011), salsa, and as flavoring a variety of food preparations. Product acceptability is based on several characteristics such as the color, viscosity, spiciness, sweetness, sourness and product taste (Kulvadee, 2002). Since chili is hot in taste, it would be difficult to be assessed by sensory taste as its active ingredient capsaicin causes irritation in the mouth a burning fiery sensation that the body perceives as pain. Therefore assessing of specific attributes in chili sauce would be very challenging as the specific product uniqueness being masked by hot and spicy taste. Sniffing is one of popular method being applied in sensory analysis. This is because olfaction is the sense of smell and the first stage of tasting. Odour of fermented chili can be determined as volatile components (gases) emanating from the fermented mash stimulate the nerve endings in the nasal cavity at the olfactory bulb region (Hervin, 2008).

Humans can distinguish between thousands of smells, which are perceived by neurons in the olfactory epithelium (skin cells) of the upper respiratory passages. The sense of smell, or olfaction, is even more complex than that of taste. Whether or not a volatile compound produces a stimulus depends on the size, shape, and degree of ionization of the molecule. Sniffing is required to draw a significant number of air molecules into this upper nasal cavity. Once detected, the stimulus passes directly from bundles of nerve cells called *glomeruli* into the olfactory lobe of the brain. Some molecules also reach this olfactory ephithelium by way of the back of the mouth during swallowing; this is known as gustatory aroma perception. This sniffing sensory method provides great deal of information in brewing industry of coffee and alcoholic beverages as compared to chemical and physical instrumental analysis (Igor et al., 2012). No instrument has been able to replace a trained tester even though the physiochemical properties fit with laboratory specification but it could be meaningless if the flavor is not acceptable to the consumers. Due to this reason, most large-scale breweries invite panels of trained tasters to assist in quality control (Scott, 1997).

Lactic acid bacteria are among the most important microorganisms typically associated with fermented food.This bacterium is widely used in fermentation industry as it recognized as beneficial microbes. Manipulating the potential usage of LAB in chilies sauce industry might be beneficial as this bacterium is known as flavor producer microorganisms (Andreja et al., 2012; Di Cagno et al., 2008). Lactic acid bacteria isolated from natural sources has always been the most powerful means for industrially fermented product such as sauerkraut, kimchi, table olives, pickles product and mixed vegetable drinks (Carmen et al., 2012; Suree et al., 2012). Two types of starter that were commonly used were autochthonous and allochthonous. Many researchers focus on developing of autochthonous starter as they proposed that autochthonous isolate has better adaptability inside fermentation system. An example of autochthonous starter that were able to preserve chili under room temperature was successfully developed by Raffaella et al. (2008).

LAB that was isolated from dairy food sources was proven to modify the flavor of products. These isolates were found to produce nice fermented flavor in yoghurt, cheese and sour cream products (Smitt et al., 2005; Vinderola et al., 2002). In accordance to wine fermentation, the accepted odour that most preferred by consumers were intense in sour, sweet, perfume, and fruity odour while non-accepted odour were intense in yeasty, moldy, stinky and flat smell (Mariana et al., 2014; Loureiro & Malfeito-Ferreira, 2003). The idea of producing a fermented chili with unique odour characteristics might be achieved by utilizing the allochothonous and the autochthonous starter that may modify the flavor and odour attributes of chilies mash that could later be used to make chili sauce with unique fermented odour characteristics. It is therefore purposeful to initiate fermentation of chili mash via introduction of two types of starter culture autochthonous and allochthonous. This work was done to evaluate the effect of pre-heating on fermented chili mash (FCM) and to select the potential starter from local food that would generate desirable odour that are mostly preferred by sensory panels.

II. Material And Methods

Isolation of Lactic Acid Bacteria from Food Sources

Lactic acid bacteria from autochthonous and allochthonous sources were used. The autochthonous LAB was from two months pre-fermented Cilibangi while allochthonous LAB were isolated from home-made yoghurt, raw cow milk and commercial yoghurt. 10 g of sample was diluted in 90 mL PBS solution. Appropriate serial dilution was made until 10^5 with phosphate buffer saline (PBS) solution and spread plated on modified MRS agar (Oxoid) added with 0.1% calcium carbonate, and incubated anaerobicaly at 37^oC for 48 h. The isolates that developed clear zone on modified agar plate were presumptive LAB and further checked with Gram staining and catalase test. Six selected LAB strains were purified, subculture in MRS broth and kept frozen -80^oC in 10% glycerol (Asma et al., 2012).

Preparation of Lacto-Fermented Chili Mash

Chilies fruits were selected to remove their pericarps, free from blemishes, defects, and insect damage. The chilies were washed with portable water to remove any impurities then ground using a food blender (Panasonic) with 8% rock salt added. The ground sample were pre-heat treated (steamed in 80^oC, 10 min) or not-steamed. Fermentation was conducted using 100 ml universal bottles each containing 100 g of pepper mash inoculated with 24 h cultures of LAB (1% v/v). All samples were fermented at 37^oC at room temperature for 14 days.

pH Measurement and Sensory Analysis

FCM were evaluated for the odour profile by 5-score qualitative descriptive analysis (QDA Ten panelists who were familiar with evaluation of food sensory attributes were selected from USIM students. FCM were evaluated based on the following characteristics: raw chili, sour, sweet, perfume, yeasty, fungi, stinky, fruity and flat using the scale between 1 (worst) to 9 (best). Hedonic test was conducted to evaluate the acceptability of fermented mash; results were scored as good 6-10), acceptable (5) and worst (1-4) (Wiander & Korhonen, 2011). pH of the fermented Cilibangi mash was measured using a pH-meter (Metler Toledo, Germany).

Genotype Identification of Lactic Acid Bacteria by 16S rDNA

Total genomic DNA of selected LAB was extracted from overnight culture in 20 mL MRS broth incubated at 30^oC using BReal Genomics^{TM} gram positive DNA purification kit (USA). One mL of overnight culture was centrifuged at 2 min 13,400 rpm at 25^oC (Eppendorf centrifuge 5804 R). 200 µl lysozyme buffer was added and cell pellet was re-suspended for 10 min. After that 200 µl of GB buffer was added and incubated until clear lysate was obtained. Ethanol was added to sample lysate in order to bind the DNA. For washing, 400 µl of WI buffer was added into lysate and final DNA was obtained after 100 µl pre-heated lysate solution was added DNA was kept at -20^oC for further study. PCR setting was set at 95^oC for 2 min annealing, 92^oC for 45 sec melting, 54^oC for 1 min elongation and 72^oC for 1 min final extension with the total of 35 cycles using two primers (16S forward: (5-AGAGTTTGATCCTGGCTC-3) and 16S reverse: (5-CGGGAACGTATTCAC-CG-3). The partial 16SrDNA sequences were sequenced by public databases (GeneBank) (Asma et al., 2012).

III. Results

pH Changes during Fermentation

The initial pH of chili was 5.3. pH of both pre-heated and non- heated FCM decreased significantly ($p<0.05$) during the first 3 d fermentation (Figure 1), and then level off until 14 d fermentation except to non-heated LAB non-inoculated FCM. Final pH of LAB inoculated pre-heated FCM reached pH lower than 3.5

whereas pH of non-heated chili only reached 3.6. pH 3.2 was the lowest pH recorded observed in non-heated control sample as well as heated FCM inoculated with LAB Au2 and Alo2.

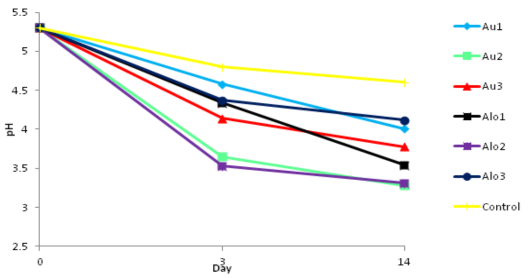

Figure 1: pH of fermented pepper mash within 14 days fermentation under pre-heat

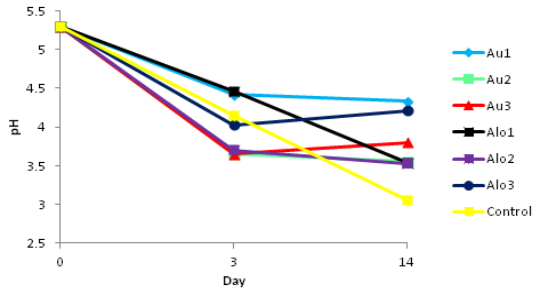

Figure 2: pH of fermented pepper mash within 14 days fermentation under no heat treatment

The general accepted odour for developing FCM were characterized as sour, sweetie, flowery while the undesirable odour were set to be yeasty, fungi, stinky, flat and raw in chilies aroma following the criteria set by Mariana et al. (2014); Loureiro & Malfeito-Ferreira (2003). Five-scale QDA were conducted and presented as spider web diagram (Figure 3 & Figure 4). Non heated FCM scored higher for rawness and perfume order. Alo1 and Alo2 produced almost similar odour trend in both heat treatments. Alo1produced FCM with scores ranged 4.3-4.4 sour, 4.0-4.2 sweet, 1.7-1.8 flower and 4.5-4.6 fruity smell. Alo2 produced FCM with highest perfume, sweet and fruity odour in pre-heat treated chili (Figure 3).

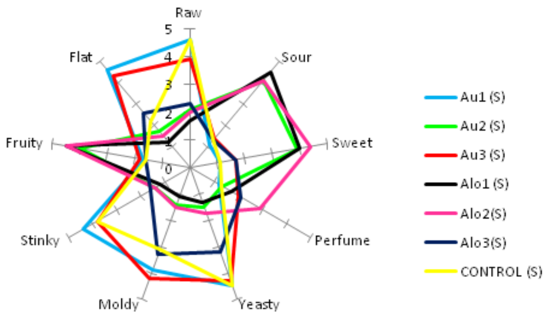

Figure 3: Spider web plot showing the mean intensity of smell for 7 different samples under pre-heat treatment

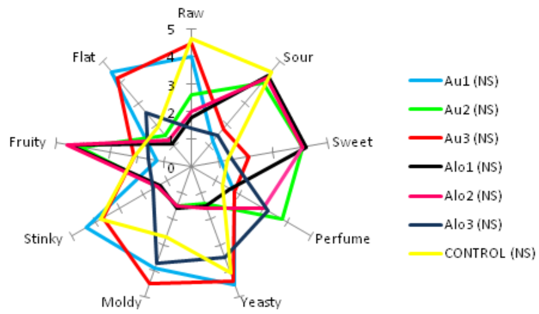

Figure 4 Spider web plot showing the mean intensity of smell for 7 different samples under no heat treatment

Score of Hedonic Test

Three strains Au2, Alo1and Alo2 were pre-identified to produce desirable fermented chili smell under both steam and non-steam treatment were concentrated odour of sourness, sweetness and fruitiness. This was supported by the mean hedonic score which ranged from 5.556-7.44 as presented in Table 1. Non-desired smell of FCM scored low in hedonic test with ranged from 3.222-4.556 consisted of inoculated FCM with LAB isolates Au1, Au3and Alo3 as well as uninoculated control sample.

Table 1: Score of hedonic test with standard deviation

Sample	Steam[a]	Non-Steam[a]
Au1	4.222±5.118[a]	3.667±3.674[a]
Au2	5.556±8.156[a]	6.000±8.426[a]
Au3	4.667±4.746[a]	3.778±4.381[a]
Alo1	6.444±7.955[a]	5.444±5.570[a]
Alo2	5.778±8.212[a]	7.444±8.618[a]
Alo3	4.556±4.746[a]	4.333±5.074[a]
Control	3.222±2.682[a]	3.333±4.031[a]

Values represent means ± standard deviation; n=30; Hedonic rating based on 9-hedonic scales with the descriptors: 9=like extremely,8=like very much,7=like moderately,6=like slightly,5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately,2=dislike very much, and 1=dislike extremely

Genotypic Identification of LAB

The best three LABs (Alo1, Alo2 and Au2) that have the ability to produce desirable smell characteristic were identified by phenotypic and genotypic identification. Phenotypically the isolates were pre-identified as *Lactobacillus plantarum1* with T. index ranged 0.69-0.73 by API CHL method. The phenotyphic identification was further confirmed utilizing genotypic identification. After PCR amplification and sequencing it was confirmed that allochothonous isolates Alo1 (home-made yoghurt) was identified as *Lactobacillus plantarum* where as Alo2 (raw milk) was identified as *Pediococcus pentosus.* Au2, autochthonous isolates that was accepted as potential flavor producer was identified as *Lactobacillus plantarum* (Figure 5).

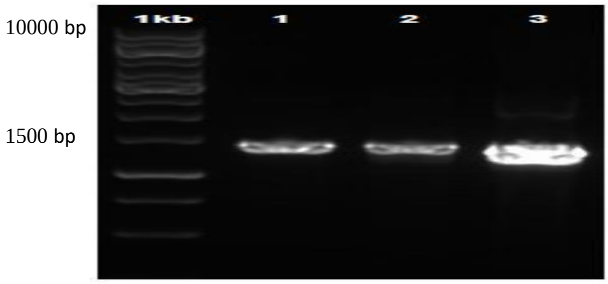

Figure 5: The DNA bands of LAB on the 1.5% agarose ge using primers 16S.S:((5 AGAGTTTGATCCTGGCTC-3) and 16S reverse: (5-CGGGAACGTATTCAC-CG-3). DNA ladder, Alo1, ALo2 and Au2.

IV. Discussion

pH is the general attributes indicating the successtibility of fermentation. In this study, it was observed that pH of inoculated FCM reduced significantly ($p<0.05$) faster than non-inoculated FCM (control) under pre-heated FCM. This phenomenon suggesting that inoculation of LAB under heat-treatment resulted to controlled fermentation as discussed by (Holzapfel, 2002). In contrast, non-heated FCM resulted unpredictible pH profile. Rapid pH decreament was observed in all inoculated FCM and non-inoculated FCM sample (control). The differences of pH decreased in heated and non-heated FCM may be resulted by types of microorganism that present in FCM. Heating at 80^{o}C for 15 min caused natural flora reduction in vegetable surfaces (Aamir et al., 2013). Thus, inoculated LABs may have lower competition with microorganisms that naturally present in FCM. In addition, it is suggested that spontaneous fermentation process may happen in non-heated FCM due to pH that decreased rapidly in uninoculated sample (control). Spontaneus fermentation caused successive fermentation resulted by different types of microorganisms under un-controlable condition (Chen et al., 2006).

Sensory analysis is a good tool for selection of product with good attributes and acceptibility (Hervin, 2008). Flavour of fermented foods are dependent on the type microbes used to initiate the fermentation (Andreja et al., 2012; Di Cagno et al., 2008). Selected of fermented food that based on odour profile was done in this study. Acceptable odour profile was observed in LAB inoculated FCM than without inoculation (control) in both heated and non-heated FCM. This study was parallel with other studies proving that LAB inoculation may modify the odor of fermented food (Chao et al., 2008; Smitt et al., 2005). High score of sourness odour may be explained by the pH profile. As example, uninoculated FCM (control) in non-heat treated chili scored highest in sourness odor (Figure 4) had the lowest pH value (Figure 2). The presence of sourness odour can be generated by the presence of both volatile and non-volatile acid which can be pre-determined by pH measurement (Marené, 2013).

Potential LABs were identified by phenotypic and genotypic identification. The LABs were genotypically identified as *Lactobacillus plantarum* and *Pediococcus pentosus.* These two isolates were knows as common microorganisms that responsible for changes in pH and flavor development in fermented vegetables. Lactic acids produced by these microorganisms generate the sour flavor (Chen et al., 2006). It is proposed that the LAB was the particular microbe that contributes to the smell production parallel with Chao et al., (2008) who found 7 different genera that consist of 32 species of indigenous *Lactobacillus*, *Lactococcu*s, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weisella* responsible for flavor development on stinky tofu brines. *Lactobacillus plantarum* and *Lactobacillus brevis are the* main strain that produce lactic acid as indispensible constituent of particular flavor (Shi & Zou, 2011). Marshall, 1987 pointed other types of LAB such *Leuconostoc mesentroides* ssp. Cremoris and *Leoconostoc lactis* were the aroma producer microorganism. This evidences really confirmed that LAB were beneficial microbes that contribute to food organoleptic attributes.

V. Conclusion

Sources of starter either authocthonous or allocthonous starter can be used for fermenting chili since both sources of isolates have their own specialty smell profile and pH reduction. Production of fermented chili mash did not require any pre-heat treatment as the pre-steamed chili did not produce any significant different with the non-heated samples. Descriptive and hedonic test can further clarify samples that grab consumer's preferences most. Descriptive statistic revealed that fermented chili mash that is acceptable by consumers should have an intense smell in sour, sweetie, flower and fruity smell. Samples that intense in yeasty, fungi, stinky and raw smell were not desirable based on consumers' preferences. Authochothonous starter (Au2) and allocthonous starter (Alo1 and Alo2) were presumptively chosen as the best isolates to conduct chili fermentation as these isolates grab the most preferred samples and cause efficient pH reduction in chili fermentation system. The responsible LAB that contribute to smell development were *Lactobacillus plantarum* and *Pediocooccus pentosus.*

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