International Food Research Journal 16: 581-588 (2009)

Some physico-chemical qualities and acceptability of fermented curry paste

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Abstract: The effects of lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus bulgaricus*) inoculation on the sensory attributes and consumers acceptance of fermented curry paste compared with uncultured sample were assessed. pH, titratable acidity (TA) and color changes, during four-month storage were monitored. Hedonic test was utilized to evaluate consumer perception and acceptability of fermented and ordinary curry pastes. Rapid pH drop was observed in inoculated sample with *Lb. plantarum* presenting better performance than the *Lb. bulgaricus*. Titratable acidity increased significantly ($p<0.05$) in both fermented samples due to production of up to 1.7 % lactic acid which leads to a decrease in pH from 5.5 to 3.8. Color analysis showed in fermented samples, the lightness (L) and yellowness (b*) increased significantly while redness (a*) decreased, concurring with the consumer preference of fermented samples for color attribute. The consumer survey and sensory evaluation results showed there were no significant difference ($p>0.05$) in most of the attributes of original recipe and fermented curry paste except for color and sweetness. In summary, this study showed fermented curry paste with *Lb. plantarum* and *Lb. bulgaricus* exhibited new sensory attributes encouraging acceptability by consumers.

Keywords: *Lactobacillus plantarum.*, *Lactobacillus bulgaricus.*, fermented curry paste

Introduction

Curry paste is wet blend of spices, herbs and chilies that are used as the basis of curry. These perfect blends of fresh, dried spices or extractives that are balanced with acids, salt, sugar and oil can be used to develop emulsions, topical seasonings, glazes and tumbling or injection marinades for foods and beverage (Uhl, 2000). It has a big economic value, originated from Southern India and traveled to all parts of the world (Farrel, 1985). Malaysian cuisine is a fusion of the different cooking styles and dishes of three nationalities mainly Malay, Chinese and Indian. The major spices are used in most Malaysian curry include black pepper, cardamom, chilly, clove, coriander, cumin, ginger and turmeric. Their curry pastes are incorporated with many fresh spices such as onion, garlic, galangal and other fresh herbs. Malay curry tends to be aromatic, slightly pungent with spicy note and reddish whereas Malaysian Indian curry tends to be very hot and spicy with sour note (Siaw *et al*., 2005). Due to increasing ethnic diversity and the influence of ethnic foods, the demand for the creation of the new line of curry paste has increased. Lactic acid bacteria may influence the flavor of curry paste in a variety of ways and the most obvious being the production of lactic acid from fermentable substrate and concurrent increase in sourness. However there are other end products of microbial metabolism, such as volatile compounds, which may affect the organoleptic properties of curry paste, especially flavor and aroma. By definition, lactic acid bacteria are bacteria that ferment a sugar predominantly to mainly lactic acid (lactate) and some byproduct. Lactate, the major end product of LAB fermentation, has application as a preservative, acidulant and flavorant in food processing (Liu, 2003). In this study, the objective was to produce a product with new sensory attributes and meet consumers' increasingly adventurous tastes.

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Materials and Methods

Spices

All spices, common sugar, salt and cooking oil were purchased from a local hypermarket, Carrefour, in Putra Jaya, Malaysia. Cardamom, chili, cinnamon, clove, cumin, coriander, ginger, mustard and turmeric were in powder form while garlic and shallot were bought as fresh ingredients a day before experiment. Chili, cumin, coriander and turmeric powders were Babas brand and the other spices were unpacked products. Spice powders were kept in glass bottles with screw cap at ambient temperature (25°C).

Lactic acid bacteria cultures

Bacterial cultures were freeze-dried *Lactobacillus plantarum*, Lp 115 400B (mesophilic and hetrofermentative bacteria) which obtained from DANISCO, Malaysia and *Lactobacillus bulgaricus* (thermophilic and homofermentative bacteria) was isolated from commercial natural yogurt (Nestle) by using plating method and incubation under aerobic condition which described by Papamanoli *et al.* (2003). Twenty-five grams of yogurt was taken, added with 225 ml Buffered Peptone Water (BPW, CM0509, Oxoid Ltd., Basingstoke, UK), (1:10) and homogenized using a Stomachers Laboratory Blender 400 (Seward Medical, London, UK). An aliquot (1 ml) of the homogenate was serially diluted with 9 ml BPW up to 10^{-7} dilution. An aliquot (0.1 ml) of each dilution was spread in respective media for LAB, de Man Rogosa and Sharpe agar (MRSA, CM01361, Oxoid Ltd, Basingstoke, UK), in duplicate. The plates were incubated anaerobically at 45°C for 48 hours in anaerobic jars with gas generating kit (BR056A, Oxoid Ltd, Basingstoke, UK). Representative LAB strains were isolated from MRS plates and cultivated in MRS broth (Oxide, CM0359, England) at 25°C. The purity of the isolate was checked by repetitive streaking and sub-culturing on fresh MRS agar and characterized using Gram stain, to observe cell morphology and catalase reaction test (Roberts and Greenwood, 2003). Stock cultures were prepared onto Nutrient Agar (NA, CM 3, Oxoid Ltd., Basingstoke, UK) slant and incubating at 45°C for 24 hours.

Curry paste preparation

Curry paste samples were prepared according to Puttrajappa *et al.* (1990) recipe with some modification. The composition of curry paste is given in Table 1. Fresh shallots and garlic were peeled and blended with a blender (Philips Comfort Blender) to make a uniform crushed mix. The oil was heated in a pot and the blended shallots and garlic were fried to light brown color. Chili powder was added with stirring over a low heat, followed by coriander, cumin, clove, ginger and cardamom powders. Water was added concurrently. Turmeric powder and salt were added while mixing. Mustard powder and sugar were added at the end and heating was continued till the resulting mass became thick and viscose. When the spices were cooked to the right point to eliminate the raw taste, the oil began to separate from the thick paste. The cooking time was about 15 min.

Fermentation of curry paste

Lactobacillus plantarum and *Lactobacillus bulgaricus* were grown in MRS broth (Oxide, CM0359, England) at 30°C and 45°C respectively for 48h. 1 kg curry paste were inoculated with a

Table 1. Formulation of curry paste using selected spices

No.	Ingredients	Percentage (%)
1.	Shallot	20
2.	Coriander	6
3.	Chili powder	3
4.	Cumin	3
5.	Turmeric	1.5
6.	Cardamom	0.7
7.	Cinnamon	0.7
8.	Clove	0.7
9.	Garlic	0.7
10.	Ginger	0.7
11.	Mustard	0.5
12.	Sugar	7
13.	Salt	2
14.	Oil	10.5
15.	Water	43
	Total	100

48h culture (~ 10^9 cfu/ml) of *Lb. plantarum* or *Lb. bulgaricus* and mixed. Experiment was conducted in universal bottles each containing 31±1 g of curry paste with screw cap. Samples inoculated with *Lb. plantarum* were kept in an incubator at 30° C and *Lb. bulgaricus* were incubated at 45° C during the fermentation period.

Physico-chemical analysis

Sample of control and fermented curry paste were taken at 0, 24, 48, 72h, 10, 40, 80 and 120 days for physico-chemical analysis. All the assays were carried out in duplicate and repeated at least twice.

pH

pH of all samples was measured by using the method introduced by Simsek 2007. Five grams of each sample was blended and homogenized with 45 ml distilled water. pH of samples was measurd by using a pH meter (Radiometer analitycal 210pHmeter, France).

Acidity

Lactic acid serves as a major organic acid in fermented curry paste due to the production of the acid during fermentation. The method used to determine % lactic acid was based on Simsek (2007). Five grams of each sample was blended and homogenized with 45 ml of distilled water. Then, 5 drops of 1% phenolphthalein were added and mixed. The samples were titrated with 0.1 M NaOH. The formula to calculate % lactic acid (LA) is as follows:

$$\%\text{Lactic Acid (LA)} = \frac{\text{ml alkaline} \times \text{molarity of NaOH} \times \text{molecular mass of LA}}{\text{ml sample} \times 10}$$

Where,
Molarity of NaOH = 0.1
Molecular mass of LA = 90.08

Color

Minolta colorimeter (CR-400; Japan) was used to determine color values of the curry paste samples in terms of the L, a* and b* as measures of brightness, redness and yellowness, respectively. Each sample was put in a Petri dish and colorimeter was dipped it to determine the color value.

Sensory analyses

Hedonic test was carried out by 50 student panelists from Faculty of Science and Technology in Universiti Kebangsaan Malaysia (UKM). Testing was performed in a sensory laboratory. For each type of samples, curry gravy was prepared by adding 1000 ml water and 150 g coconut milk to 500 g curry paste and boiled for 5min. One tablespoon of each boiled gravy sample was served in 25 ml transparent glass cup labeled with three digit codes. Three samples were served with three slices of bread at the

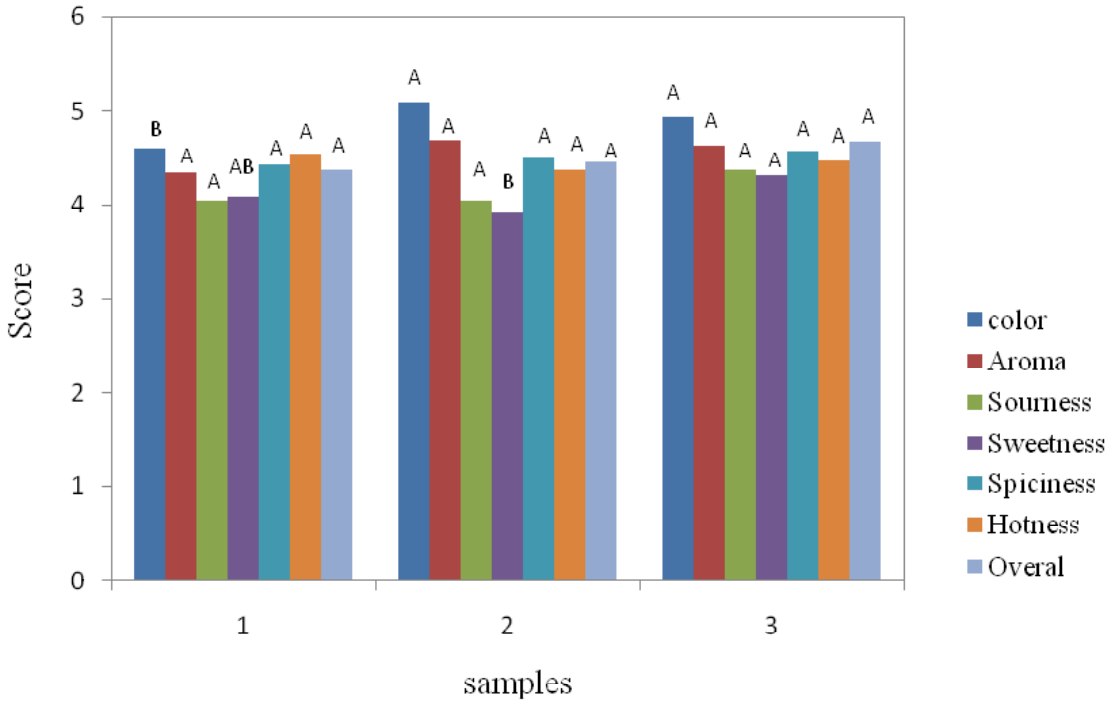

Figure 1. Mean of hedonic scores of 1: control, 2: *Lb. plantarum* and 3: *Lb. bulgaricus* samples (n=100)

same time and the presentation of the samples was randomized. The panelists were asked to dip a bread slice completely into each gravy sample, taste it and circle evaluation on the scale for each attribute in a specific form given. Scores were awarded on a scale of 1–7, in which 1 indicated dislikes extremely and 7 like extremely (Aminah 2004). The sensory attributes evaluated were color, aroma, sourness, sweetness, spiciness, hotness and overall acceptances.

Statistical analyses

Data obtained were subjected to an Analysis of Variance (ANOVA; SAS 9.1, 2002-2003) and Duncan Multiple Range Test (DMRT) at $\alpha = 5\%$ for comparison between treatment means.

Results and Discussion

pH

The pH decrease is an important parameter for assessing fermentation process and how fast the process reach conditions (pH lower than 4.5) which can hinder or inhibit the growth of food-borne pathogens. The regulation requires that foods preserved by acidity have a pH of 4.6 or less. At these levels, production of deadly toxins by the organism that causes botulism is inhibited (Rushing and& Curtis 2000). The changes in pH of uncultured and cultured samples during four months storage are shown in Table 2. The average of initial pH value of samples was 5.52 which at the end of fermentation, decreased to the lowest pH, 3.82-3.89, in curry paste produced with *Lb. plantarum* and *Lb. bulgaricus* respectively. The pH of *Lb. plantarum* fermented samples decreased sharply after 24 h of fermentation, and then slightly decreased until the end of fermentation, whereas in *Lb. bulgaricus* fermented samples, the pH decreased rapidly after 48 h of fermentation and then gradually decreased until the end of fermentation. The lowest pH (3.8) in both samples was attained after ten days of fermentation and subsequently remained stable during four months storage at ambient temperature (25°C). The fermentation and growth of the bacteria is self-limiting due to the sensitivity of lactic acid bacteria to such acidic pH (Dugas, 2004). The pH value of uncultured samples were rather constant ($P>0.05$) during 40 days storage at room temperature (25°C) but there was significant ($p<0.05$) change after 40 days and pH decreased to nearly 4.4. The reduction of pH in control sample after 40 days may be resulting from yeast and mold activity and growth.

Acidity

Preservation of fermented foods by lactic acid bacteria is due primarily to sugars being converted to organic acids, causing a reduction in pH and removal of carbohydrates as nutrient sources. Lactic acid is a major metabolic end product of carbohydrate fermentation by LAB (Wikipedia, 2007). Table 3 shows the content of titratable acidity (%TA) expressed as lactic acid during four months storage of samples. The average of %TA of curry paste before fermentation was 0.34 % which increased significantly ($p<0.05$) during the fermentation to nearly 1.7% in both fermented samples. The curry paste inoculated with *Lb. plantarum* contained higher amounts of lactic acid (1%) after 24 h of fermentation than curry paste inoculated with *Lb. bulgaricus* which contributed to the sharp decrease in pH after 24 h. Generally, there was significant difference ($p<0.05$) in lactic acid production between *Lb. plantarum* and *Lb. bulgaricus* which indicated *Lb. plantarum* produced more lactic acid (1.74%) compared to *Lb. bulgaricus* (1.68 %) during fermentation of curry paste. Papamanoli *et al.* (2003) reported that *Lb. plantarum* strains are more aciduric than the other species as all strains. In general, all species of lactic acid bacteria have their own particular reactions and niches, but overall, *Lb. plantarum* produces high acidity in all vegetable fermentations and plays the major role (Battcock and Azam-Ali, 1998). In vegetable fermentation, *Lb. plantarum* was found to tolerate a lower internal pH than other LAB and, therefore, would have a lower acid anion concentration (Sapers *et al.,* 2006). In both fermented samples lactic acid reduced slowly but not significantly during four months storage at 25°C and reached to 1.62 and 1.70 % in *Lb. bulgaricus* and *Lb. plantarum* fermented sample respectively. Although lactate is the end product of lactic acid fermentation, it can be catabolized under anaerobic condition to produce pyrovate, which is further catabolized to acetate, formate or carbon dioxide (Murphy *et al*., 1985; Viega-da-Cunha and Foster, 1992). Changes in LA content of uncultured samples were likely level off and were in agreement with absence of LAB in these samples.

Color

The changes in color values of uncultured and cultured samples during four months storage are summarized in Table 4.5. The lightness (expressed as L value) and yellowness (expressed as b* value) of both inoculated samples increased during fermentation period and the four months of storage. Although, the lightness of both inoculated samples showed the same pattern, there was significant difference ($p<0.05$) in lightness between *Lb. plantarum* and *Lb. bulgaricus* samples from the initial stage until the end of storage.

Table 2. Changes in pH value of control, *Lb. plantarum* and *Lb. bulgaricus* fermented curry paste samples during 120 days storage

Days	pH*		
	Control curry paste	*Lb. plantarum* inoculated curry paste	*Lb. bulgaricus* inoculated curry paste
0	5.52^{A}	5.52^{A}	5.52^{A}
1	5.52^{A}	4.18^{C}	5.38^{B}
2	5.50^{A}	4.11^{B}	4.20^{B}
3	5.52^{A}	3.94^{B}	3.93^{B}
10	5.50^{A}	3.81^{C}	3.89^{B}
40	5.47^{A}	3.83^{C}	3.88^{B}
80	5.43^{A}	3.83^{C}	3.90^{B}
120	5.42^{A}	3.82^{C}	3.88^{B}

*Values are means for n = 8.
$^{A-C}$ Means with different letters within the same row are statistically significant ($p<0.05$).

Table 3. Changes in Acidity (% LA) of control, *Lb. plantarum* and *Lb. bulgaricus* fermented curry paste samples during 120 days storage

Days	% LA*		
	Control curry paste	*Lb. plantarum* inoculate curry paste	*Lb. bulgaricus* inoculate curry paste
0	0.34^{A}	0.34^{A}	0.34^{A}
1	0.34^{C}	1.00^{A}	0.58^{B}
2	0.33^{C}	1.33^{A}	1.08^{B}
3	0.35^{B}	1.66^{A}	1.63^{A}
10	0.35^{C}	1.74^{A}	1.68^{B}
40	0.37^{C}	1.70^{A}	1.65^{B}
80	0.34^{C}	1.71^{A}	1.61^{B}
120	0.37^{C}	1.70^{A}	1.62^{B}

*Values are means for n = 8.
$^{A-C}$ Means with different letters within the same row are statistically significant ($p<0.05$).

Lb. plantarum fermented sample showed the higher L value compare with *Lb. bulgaricus* fermented sample. It may be resulted from faster pH drop and production of more lactic acid in *Lb. plantarum* samples. The higher b* value indicated that curry paste tended to be more yellow in color. Turmeric (curcumin) and chili (carotenoids) powder are the main colorant in red curry paste formulation. Hirasa *et al.* (1998) reported curcumin appears as yellow at pH range from acid to neutral and reddish-brown in alkaline pH. As well, quercetin which belongs to the flavonoid family is brown to reddish-brown in shallot, does not change from alkaline to natural pH but tends to be insoluble in acid solution. Accordingly, reduction of pH as a result of production of lactic acid during curry paste fermentation can explain this observation in fermented samples. On the other hand, fermentation process decreased redness (expressed as a* value) from 20.8 to 13.05-15.23 in curry paste inoculated with *Lb. plantarum* and *Lb. bulgaricus* respectively. The a* value of *Lb. bulgaricus* sample decreased gradually compared with *Lb. plantarum* sample. As reported by Talon *et al*. (2000) *Lb. plantarum* is able to produce H_2O_2, which is an oxidation agent and it is often suggested that this lactic acid bacteria could be involved in color and flavor faults in sausages. The highly unsaturated structure of carotenoid molecule is susceptible to isomerization and oxidation (Rodriguez-Amaya, 1997). Therefore, carotenoids oxidation of chili pepper could partly explain this observation in fermented samples. Compared with untreated curry paste, fermented samples maintained a scarlet color during the four months of storage. L, a* and b* value of preferred curry paste reported by

Table 4. Changes in color value of control, *Lb. plantarum* and *Lb. bulgaricus* fermented curry paste samples during 120 days storage

Days	L			a*			b*		
	Control curry paste	*Lb.plantarum* inoculated curry paste	*Lb.bulgaricus* inoculated curry paste	Control curry paste	*Lb.plantarum* inoculated curry paste	*Lb.bulgaricus* inoculated curry paste	Control curry paste	*Lb.plantarum* inoculated curry paste	*Lb.bulgaricus* inoculated curry paste
0	29^{A}	29^{A}	29^{A}	20.8^{A}	20.8^{A}	20.8^{A}	16.7^{A}	16.7^{A}	16.7^{A}
1	29^{A}	32.88^{B}	29.2^{B}	20.8^{A}	20.77^{B}	20.67^{A}	16.7^{B}	19.08^{A}	16.98^{B}
2	29.3^{C}	34.08^{A}	30.72^{B}	20.91^{A}	16.88^{C}	19.98^{B}	16.83^{C}	20.68^{A}	17.62^{B}
3	29.08^{C}	34.96^{A}	30.78^{B}	20.69^{A}	16.88^{C}	19.54^{B}	17.06^{B}	21.78^{A}	17.96^{B}
10	29.06^{C}	36.3^{A}	32.14^{B}	20.96^{A}	16.45^{C}	18.81^{B}	17.2^{C}	23.58^{A}	21.16^{B}
40	29.18^{C}	38.08^{A}	34.67^{B}	20.66^{A}	15.6^{C}	17.03^{B}	17.13^{B}	27.02^{A}	26.46^{A}
80	28.71^{C}	38.49^{A}	36.52^{B}	20.56^{A}	14.24^{C}	16.35^{B}	17.64^{B}	28.44^{A}	28.45^{A}
120	28.92^{C}	41.13^{A}	37.14^{B}	20.51^{A}	13.05^{C}	15.23^{B}	17.69^{B}	29.87^{A}	30.1^{A}

*Values are means for n = 8.

$^{A-C}$ Means with different letters within the same row are statistically significant ($p<0.05$).

Fah (2005) was 32.37, 16.45 and 22.28 respectively. The results showed there was a slight decrease in the yellowness of control sample from 16.7 in the first day to 17.7 at the end of storage. The lightness and redness of control samples maintained relatively stable during four months.

Sensory evaluation

Sensory evaluation was carried out with intention to compare consumer acceptance of fermented curry paste with the original recipe (uncultured). The results from sensory evaluation of fermented and original sample of curry paste are presented in Figure 4.1. The organoleptic evaluation indicated that there was no significant difference ($p>0.05$) in most of the attributes between fermented and non-fermented samples such as aroma, sourness, spiciness, hotness and overall acceptance. Aroma of *Lb. plantarum* and *Lb. bulgaricus* fermented samples were more preferred (4.69-4.64) by consumers compared with original recipe (4.34) but it was not significantly different. This finding is in agreement with the result of slight volatile compounds being detected from fermented samples by GC-MS. Overall acceptance of *Lb. bulgaricus* sample followed by *Lb. plantarum* sample and original recipe were respectively the most favorite by consumer. Analysis of results obtained from the Duncan test showed significant difference ($p<0.05$) only in color and sweetness attributes. Fermented samples were more preferred by panelists for color attribute compared with original recipe. As reported before, significant color difference between color of fermented and uncultured samples detected by instrumental method may explain consumer color acceptance. According to instrumental measurement of color, fermented samples were lighter; more yellow, less reddish and maintained a scarlet color compared with uncultured samples. In contrast, the sweetness of *Lb. bulgaricus* fermented sample received highest score (4.31) of acceptability by consumer and *Lb. plantarum* fermented samples obtained the lowest score (3.92). This result may be explained by lower acidity of *Lb. bulgaricus* sample compared with *Lb. plantarum* sample which could be the result of less conversion of sugar to lactic acid in *Lb. bulgaricus* fermented sample. On the other hand, more acceptability of the sourness of *Lb. bulgaricus* sample (4.38) compared with *Lb. plantarum* sample (4.04) could support this idea.

Acknowledgements

This work was financial supported through IRPA grant: (03-02-02-0072-EA262).

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