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Research Article

Assessment of microbiological and modified aroma profiles of lactic acid bacteria (LAB) fermented curry paste

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Abstract. This study was conducted to determine the effects of lactic acid bacteria (LAB) inoculation on the shelf life and aroma profile of curry paste. The effect of fermentation on the aroma profile of curry paste was studied using gas chromatography mass spectrometer with solid phase micro-extraction (GC/MS-SPME) headspace technique. Fermented curry paste was able to reduce the survival period of yeast and mold by 2 days, minimizing the likelihood of spoilage. Total volatile compounds of the fermented and control samples were not significantly different ($p>0.05$). This study showed the possibility of producing fermented curry paste with *L. plantarum* and *L. bulgaricus* with at least 120 days shelf life followed by production of a slight amount of new organic compounds.

Keywords: *L. plantarum, L. bulgaricus,* fermentation, food storage, Malaysia

Introduction

Curry paste is a popular wet blend of spices and herbs in Malaysia and most of Asia which is consumed as chicken, meat or fish gravy and as a vegetable seasoning. Curry paste is primarily known as an important ingredient in Thai cuisine and it can also be a generic commercial product which substitutes curry powders or spice blends used in other cuisines. It has a significant economic value, originating from Southern India and travelling to all parts of the world [1, 2]. The characteristics of individual curry paste from each country depend solely on the balance or ratio of herbs to spices used in blending. Curry in Malaysia is a mixture of 8 to 15 spices to create a seasoning blend effect with an "authentic" flavour. As the curry pastes contain fresh ingredients they have a limited shelf life and need to be preserved or made fresh as required [3]. Due to increasing ethnic diversity and the influence of ethnic food, the demand for the creation of a new line of curry paste has increased. The creation of a new paste line has to consider consumer insight and response to today's key purchase motivators: health and taste [4].

Lactic Acid Bacteria (LAB) comprise a clad of gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod or cocci that are associated by their common metabolic and

physiological characteristics. These bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. Lactate, the major end product of LAB fermentation, has application as a preservative, acidulant and flavouring in food processing [5]. This trait has, throughout history, linked LAB with food fermentation, as acidification inhibits the growth of spoilage agents. Proteinaceous bacteriocins are produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic microorganisms. Furthermore, lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item [6].

In this study, in order to produce a more shelf stable curry paste, an effort was made to preserve curry paste by fermentation (biopreservation) with lactic acid bacteria (LAB). The intention was to also produce a product with new aroma profile to meet the increasingly adventurous tastes of consumers.

Materials and Methods

All spices, common sugar, salt and cooking oil were purchased from a local hypermarket, Carrefour, in Putra Jaya, Malaysia in January 2007.

Curry Paste Preparation

The formulation and preparation of curry paste was based on the Puttrajappa *et al.* [7] recipe with some modification. The composition of curry paste ingredients is given in Table 1. Fresh shallots and garlic were peeled and mixed 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 colour. Chili powder was added while 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 until the resulting mass became thick and viscous. 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.

Bacterial Strain

Two strains were used in this study for fermentation of curry paste. Bacterial cultures were freeze-dried *Lactobacillus plantarum*, Lp 115 400B (mesophilic and hetrofermentative bacteria) which were obtained from DANISCO, Malaysia and *Lactobacillus bulgaricus* (thermophilic and homofermentative bacteria) was isolated from commercial natural yogurt by using the plating method and incubation under anaerobic conditions as described by Papamanoli *et al*. [8]. Twenty-five grams of commercial natural yogurt (Nestle) was taken aseptically, transferred to a stomacher bag, 225 ml Buffered Peptone Water (BPW, CM0509, Oxoid Ltd., Basingstoke, UK), (1:10) was added and the mixture homogenized for one minute using a Stomachers Laboratory Blender 400 (Seward Medical, London, UK). An aliquot (1 ml) of the homogenate was serially diluted with 9 ml BPW in aseptic conditions up to 10^{-7} dilution. An aliquot (0.1 ml) of each dilution was aseptically 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 [9]. Stock cultures were prepared by inoculating a loopful of *Lb. bulgaricus* isolated onto Nutrient Agar (NA, CM 3, Oxoid Ltd., Basingstoke, UK) slant in a screw-capped bottle and incubating at 45°C for 24 hours.

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

Inoculation of Curry Paste

To prepare inoculate, 1g of freeze dried cultures of *Lb. plantarum* and one loopful of isolated *Lb. bulgaricus* from NA slant were transferred into 10 mL MRS broth and incubated at 30°C and 45°C, respectively for 48 hours in order to obtain 10^9 cfu/g (stationary phase). After 48 hours, the cells were harvested by centrifugation at 6000 rpm at 25°C for 10 min (Refrigerator Centrifuge, Eppendrof, Germany) and the supernatant was discarded. The cells were washed twice in sterilized distilled water and re-suspended in sterilized distilled water.

Three types of curry paste samples were prepared: control sample without inoculation, inoculated with *Lb. plantarum* and inoculated with *Lb. bulgaricus*. *Lb. plantarum* and *Lb. bulgaricus* inocula from the stationary phase were added to 1 kg of curry paste to give a final concentation of 10^6 cfu/g and incubated at 30°C and 45°C, respectively during the fermentation period. Control samples without inoculation were kept at ambient temperature. Experiment was conducted in universal bottles each containing 31±1 g of curry paste with screw cap.

Microbiological Analyses

On sampling days 0, 1, 2, 3, 10, 40, 80 and 120 each curry paste sample was evaluated for microbial quality including the total plate count (TPC), LAB count, coliform, *Staphylococcus aureus* and yeast and mold enumeration. The samples (10 g) were aseptically transferred to Maximum Recovery Diluent (MRD; CM 733, Oxoid Ltd., Basingstoke, UK). Decimal dilution in the same MRD were prepared and an aliquot (0.1 ml) of each dilution was

aseptically transferred to Plate Count Agar (PCA; CM 325, Oxoid Ltd., Basingstoke, UK) for total plate count, de Man Rogosa and Sharpe agar (MRSA; CM01361, Oxoid Ltd, Basingstoke, UK) for LAB, Violet Red Bile agar (VRBA; BD211695, Difco™, USA) for coliform, Baird-Parker agar (BPA; CM0275, Oxoid Ltd., Basingstoke, UK) for *S. aureus*, and Dichloran Rose Bengal Chloramphenicol agar (DRBC; BD 258710, Difco™, USA) for yeast and mold counts for each duplicate, using surface spread method [9]. The PCA plates were incubated at 30°C ± 1ºC, VRBA and BPA plates at 37°C ± 1ºC for 48h and DRBC plates at 25°C ± 1ºC for 1 week. The MRSA plates for mesophilic *L. plantarum* and thermophilic *L. bulgaricus* were incubated anaerobically at 30ºC and 45ºC ± 1ºC respectively for 48h. Anaerobic jars with gas generating kit (BR056A, Oxoid Ltd, Basingstoke, UK) were used for anaerobic condition. Colonies were then counted and recorded as colony forming units per gram (cfu/g) sample.

Analysis of Volatile Compounds of Curry Paste

The volatile organic composition was monitored by sampling the headspace of curry paste using the solid phase microextraction (HS-SPME) technique [10]. An aliquot (4 g) of each sample was transferred into a 20 ml flat bottom sample vial and capped with aluminum crimp cap. The sample vial was held in a warm water bath at 50°C for 15 min to allow equilibrium between the sample and headspace, prior to SPME sampling. After 15 minutes, fibre was inserted into the sample container through the cap and exposed to the headspace for 15 min.
The fibre used in this study was polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 µm. Finally, the volatile compounds were desorbed by exposing the treated fibre to the environment of the GC injector port for 5 min. The aroma profile of samples was analyzed with a HP 5890 gas chromatography (GC) instrument coupled to HP 19091J-33 mass spectrometry (MS). The column oven temperature was programmed to rise from an initial temperature of 50°C (held for 2 min) to 230°C at 10°C /min and held for 5 min at 230°C. The injection temperature was 240°C. The total time was 25 min. Helium was used as carrier gas with a flow rate of 1.0 ml/min. Compounds were identified using the Wiley-275 mass spectral library and retention time (RT).

Statistical Analyses

All experiments were carried out in duplicate from two different batches. Average values (means) and standard deviations were reported using the SAS (SAS 9.1, 2002-2003) and significant differences ($P < 0.05$) was done using the Duncan's multiple test range.

Results and Discussion

The data in Table**s** 2, 3 and 4 illustrate the two species of lactic acid bacteria count, total plate count (TPC) and yeast and mold enumeration in curry paste samples during four months storage.

LAB Count

The two species of lactic acid bacteria, *L. plantarum* and *L. bulgaricus*, were found capable of growing well on curry paste (Table 2). The mean of the initial load of *L. plantarum* was 1.9×10^6 cfu/g which increased sharply to 3.8×10^8 cfu/g 24 hours after incubation at 30°C. Extending the fermentation resulted in a gradual increase of viable cell count which reached the peak value of 1.8×10^{10} cfu/g in 10 days. The initial number of *L. bulgaricus* was 1.5×10^6 cfu/g and increased gently during 10 days of incubation at 45°C the peak value of 1.1×10^9

Table 2. Changes in lactic acid bacteria (LAB) count of control, *L. plantarum* and *L. bulgaricus* fermented curry paste samples during 120 days storage.

Days	LAB* count		
	Control curry paste	*L. plantarum* inoculated curry paste	*L. bulgaricus* inoculated curry paste
0	ND[a]	1.9×10^{6A}	1.5×10^{6A}
1	ND	3.8×10^{8A}	3.3×10^{6A}
2	ND	1.1×10^{9A}	2.8×10^{8A}
3	ND	2.0×10^{9A}	3.1×10^{8B}
10	ND	1.8×10^{10A}	1.1×10^{9B}
40	ND	1.8×10^{8A}	1.3×10^{7B}
80	ND	1.6×10^{7A}	0^{B}
120	ND	3.1×10^{6A}	0^{B}

*Values are means for n=4.
[A-C] Means with different letters within the same row are statistically significant ($p<0.05$).
[a] ND-Not Detected

cfu/g. LAB could not be detected from uncultured samples. *L. plantarum* tended to decrease slightly during further storage and finally reached to 3.1×10^6 cfu/g in 120 days. Yoon *et al.* [11] reported that *L. plantarum* could survive the high acidity and low pH in fermented cabbage juice. In contrast, the viable LAB count of *L. bulgaricus* sample decreased after 40 days to 1.3×10^7 cfu/g and thereafter samples lost cell viability completely in 80 and 120 days. Shah & Jelen [12] pointed out that one of the factors for loss of availability of probiotic organisms (LAB) was attributed to the decrease in pH of the medium and accumulation of organic acids as a result of growth and fermentation.

Total Plate Count, Yeast and Mold Enumeration

Growing number of colonies, in both MRSA and PCA media of fermented samples, showed an almost similar pattern of changes. It is interesting to note that most of the microorganisms presented in PCA are LAB. In contrast, total plate count in control samples showed colony number of about 10^3 cfu/g with significant difference ($p<0.05$) during four months storage (Table 3). This thus indicated that growth and activity of LAB in fermented samples inhibited the growth of non lactic acid bacteria which were grown in control.

The growth of yeast and mold were significantly ($p<0.05$) affected in fermented samples of both specimens. The survival of yeast and mold were inhibited by *L. bulgaricus* and *L. plantarum* growth activity after only 2 days of fermentation and cells completely lost viability (Table 4). One of the most important contributions of LAB is the extended shelf life of the fermented product due to competition for nutrients and the presence of stated-derived inhibitors such as lactic acid, hydrogen peroxide and bacteriocins. The fermentation process relies on the rapid colonisation of the food by lactic acid producing bacteria, which lower the pH and make the environment unsuitable for the growth of spoilage organisms. However,

Table 3. Total plate count (TPC) of control, *L. plantarum* and *L. bulgaricus* fermented curry paste samples during 120 days storage.

Days	TPC*		
	Control curry paste	*L. plantarum* fermented curry paste	*L. bulgaricus* fermented curry paste
0	1.9×10^{2B}	3.2×10^{7A}	8.1×10^{6B}
1	2.1×10^{3B}	1.9×10^{9A}	3.2×10^{7B}
2	2.7×10^{3B}	1.2×10^{10A}	1.9×10^{9AB}
3	3.9×10^{3B}	2.7×10^{10A}	2.2×10^{9B}
10	5.2×10^{3B}	1.9×10^{11A}	9.8×10^{9B}
40	1.8×10^{3B}	2.2×10^{9A}	4.6×10^{7B}
80	1.6×10^{3B}	2.2×10^{8A}	7.5×10^{2B}
120	1.2×10^{3B}	5.3×10^{6A}	0^{C}

*Values are means for n=4.
$^{A-C}$ Means with different letters within the same row are statistically significant ($p<0.05$).

Table 4. Changes in yeast and mold of control, *L. plantarum* and *L. bulgaricus* fermented curry paste samples.

Days	Mold and Yeast*		
	Control curry paste	*L. plantarum* fermented curry paste	*L. bulgaricus* fermented curry paste
0	1×10^{4A}	8.2×10^{3A}	1.2×10^{3A}
1	1.3×10^{4A}	3.9×10^{3B}	2.2×10^{3B}
2	1.8×10^{4A}	3.5×10^{2B}	7.8×10^{2B}
3	1.5×10^{4A}	0^{B}	0^{B}
10	3.1×10^{4A}	0^{B}	0^{B}
40	2.5×10^{4A}	0^{B}	0^{B}
80	3.3×10^{4A}	0^{B}	0^{B}
120	3.6×10^{4A}	0^{B}	0^{B}

*Values are means for n=4.
$^{A-C}$ Means with different letters within the same row are statistically significant ($p<0.05$).

very few lactic acid bacteria have been reported which produce antibacterial agents that can inhibit the growth of mold. Lavermicocca *et al.* [13] reported *L. plantarum* isolated from sourdough produces organic acids such as phenyl lactic acid and 4-hydroxy-phenyllactic acids which act as antibacterial agents, inhibiting the growth of mold as well. During fermentation oxygen is replaced by producing CO_2 as the Lactobacilli favour an anaerobic atmosphere. Thus the restriction of oxygen can ensure that yeasts do not grow. The presence of yeast and mold in uncultured samples (control) during 120 days can be another reason to support this idea. There was no significant change in yeast and mold count in uncultured samples.

Coliforms and *S. aureus* could not be detected in all controlled and fermented samples, throughout the storage period of 4 months. Possibly, the lack of initial contamination, or the impact of competition and/or antagonistic reactions with LAB could have prevented their proliferation. The absence of Coliforms and *S. aureus,* even in uncultured samples gives rise to a second possible reason. It may also happen that curry paste does not offer a suitable environment for the proliferation of Coliforms and *S. aureus* as a result of the antimicrobial properties of spices.

Aroma Profile

The identified volatile compounds of fermented and non-fermented samples, their retention indices and percentage composition are given in Table 5 where the components are listed in order of their elution on the JW DB-5MS column. Analysis of 10 days old uncultured (control), *L. bulgaricus* and *L. plantarum* fermented samples led to identification of 47, 55 and 57 components respectively out of 70 identified compounds that represented more than 90% of the volatile oil. The results were nearly similar between fermented and non-fermented samples and there was no significant difference ($P>0.05$) in quantity of volatile compounds. Generally, the major compounds of the essential oil from all samples were found to be *para-cymene* (an alcohol from coriander, cumin and cinnamon), *γ-terpinene* (a terpene from cardamom and coriander), *linalool* (an alcohol from coriander and ginger), *cuminal* (an aldehyde from cumin), *eugenol* (an alcohol from clove) and *ß-caryophyllene* (an ether from clove and cinnamon). These findings of the current study are consistent with those of Fah *et al*. [14] who found between 45 and 62 volatile compounds in curry paste samples. The formation of volatile organic compounds in fermented samples was quantitatively slight. The small quantities of *acetic acid* (0.15%, pungent odor), *butane-2,3-diol* (0.18%, sweet-sour, succulent and fruity odor), *caproic acid* (0.04-0.05%, an unpleasant odor like that of goats or sweat), *benzyl alcohol* (0.06%, mild pleasant aromatic odor), *benzoic acid* (0.06%, repulsive odor), *nonyl alcohol* (0.11%, rose-orange odor), *benzothiazol* (0.1%, vanilla bean odor) and *α-Ethylcaproic acid* (0.12-0.31%, sweet, herbaceous and faint musty odor) were detected from fermented samples. These compounds were also detected from other fermented products such as *acetic acid* in wine, beer and cider, *butane-2,3-diol* in fermented milk and soybean, *benzyl alcohol*, *benzoic acid*, *nonyl alcohol*, *benzothiazol* in wine [15, 16]. Kenearly *et al.* [17] reported an alternative route to produce *caproic acid* that involves direct fermentation by microbial cultures. Generally, LAB might have exerted lipolytic enzymes that hydrolyzed the fat to fatty acids leading to esters that have strong flavors [18]. It was observed that *L. bulgaricus,* a homofermentative bacterium, generated more volatile compounds such as *acetic acid*, *butan-2,3-diol*, *benzyl alcohol* and *nonyl alcohol* in curry paste compared with *L. plantarum,* a hetrofermentative bacterium. These results were in contrast from Jay *et al*., [19] that heterolactics are more important than homolactics in producing flavour and aroma components (volatile compounds) and small amounts of alcohol. Ly *et al.* [20] found that

bacteria, through their great diversity of physicochemical surface properties, can interact directly with aroma compounds or in an indirect way, by modifying the emulsions characteristics leading to changes in the transfer of aromas. Accordingly the different aroma profile observed in *L. bulgaricus* and *L. plantarum* fermented samples could be dependent on the physicochemical properties of both bacterial surface and aroma compounds.

Conclusion

In this study, *Lactobacillus plantarum* (mesophilic and hetrofermentative) and *Lactobacillus bulgaricus* (thermophilic and hemofermentative) were examined for their ability to utilize curry paste for cell synthesis and lactic acid production in the course of fermentation. *L. plantarum* and *L. bulgaricus* grew well in curry paste and after 10 days the viable cell counts reached to the highest value of 1.8×10^{10} and 1.1×10^{9} cfu/g at 30°C and 45°C respectively. From the aspect of preservation, it was shown that an enhanced inhibition effect on yeast and mold survivorship can be obtained through fermentation. *L. bulgaricus* and *L. plantarum* were able to reduce the survival period of mold and yeast in 2 days, minimizing thus the likelihood of spoilage. Increase of the shelf life can be considered as the main point of this study, while flavour profile of volatile compounds of curry paste indicated some production of organic compounds. This slight increase in aromatic compounds may result in enhanced consumer acceptability.

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