

# The Relations of Volatile Compounds, Some Parameters and Consumer Preference of Commercial Fermented Milks in Thailand

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**Abstract**—The aim of research was to define the relations between volatile compounds, some parameters (pH, titratable acidity (TA), total soluble solid (TSS), lactic acid bacteria count) and consumer preference of commercial fermented milks. These relations tend to be used for controlling and developing new fermented milk product. Three leading commercial brands of fermented milks in Thailand were evaluated by consumers (n=71) using hedonic scale for four attributes (sweetness, sourness, flavour, and overall liking), volatile compounds using headspace-solid phase microextraction (HS-SPME) GC-MS, pH, TA, TSS and LAB count. Then the relations were analyzed by principal component analysis (PCA). The PCA data showed that all of four attributes liking scores were related to each other. They were also related to TA, TSS and volatile compounds. The related volatile compounds were mainly on fermented produced compounds including acetic acid, furanmethanol, furfural, octanoic acid and the volatiles known as artificial fruit flavour (beta pinene, limonene, vanillin, and ethyl vanillin). These compounds were provided the information about flavour addition in commercial fermented milk in Thailand.

**Keywords**—Fermented milk, volatile compounds, preference, PCA.

## I. INTRODUCTION

FERMENTED milk drinks are regionally adapted dairy products. In Asia, a diluted yoghurt-based drink is very popular type of fermented milk drinks, such as drinking yoghurt in Thailand. Generally, drinking yoghurt is mainly offer to younger generation as targeted consumer group. Adding sugar content, addition of special combinations of aroma and flavour, and designed packaging are used for attraction young people. The others targeted consumer group is the health lover consumer. They are interested in probiotic bacteria and prebiotic compounds in drinking yoghurt products. Whereas drinking yoghurt is mainly consumed as a tasty, healthy and refreshing drinks [1].

Drinking yoghurts or known as culture yoghurts which consisted *Lactobacillus* sp. are remarkable products in Thailand. The market value of these product were rose to 4,000-5,000 million bath and growth more than 10% during past few years [2]. The driving factor may come from the health benefit of consisted living microorganism in those products. Another driving factor that affected on popularity of drinking yoghurt is aroma and flavour attribute. In which play

the important role on acceptance, desire and popularity of those product. [3]

Multivariate analysis of flavour attribute from market products is tool for generated a market analysis of products such yoghurt or drinking yoghurt that can be used as information of flavour trends. From those received flavour trends, new product development can be achieved to create the prototype products [4]. However, human perception cannot quantify stimulus separately. Aroma and flavours attributes from volatile compounds can be correlated to other attributes or other factor e.g. pH and acidity [5]. Form these knowledge lead to aim of this study which to define the relations between volatiles compounds, pH, TA, TSS, and LAB count with consumer preference in commercial fermented milk to use as information for the new product development.

## II. MATERIALS AND METHODS

### A. Fermented Milk Samples

Five samples from three market leaders brands of culture milks in Thailand were chosen including 1 normal sample (Y), 2 fat free samples (B-O and D-O), and 2 Low sugar samples (B-L and D-L). All products were purchased from local retail shop in Nakhon Pathom, Thailand and stored under refrigeration conditions before further analysis. All samples were conducted to analyze TA, pH, TSS, volatile compounds, LAB count and sensory evaluation.

### B. Composition Analysis

#### 1. TA Measurement

Total acidity was analyzed according to the method AOAC (1990) [6] and expressed in term of %lactic acid. The samples were titrated with 0.1 N NaOH by using phenolphthalein (Merck, Germany) as indicator.

#### 2. pH Measurement

The pH of samples was measured with a pH meter (Hanna, Italy).

#### 3. TSS Measurement

The total soluble solid was analyzed with digital hand held refractometer (Atago, Japan).

#### 4. Determination of Volatile Compounds

Volatile compounds in the samples were measured by using headspace-solid phase microextraction (HS-SPME) and GC-MS.

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#### a) Headspace Solid-Phase Microextraction (HS-SPME)

The method was adapted from Ning et al. [7], A 20 ml clear glass vial was added 8 ml of sample, 2 g sodium chloride, cyclohexanone (Sigma-Aldrich, USA) as internal standard and mixing the samples with a magnetic stirrer. The vial was tightly capped with septum. Then, sample was stirred at  $40 \pm 1^\circ\text{C}$  for 20 min. After that, the 50/30  $\mu\text{m}$  Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME (Supelco, USA) was inserted and exposed in the headspace for another 20 min incubation. The finished incubated fiber was thermally desorbed in the GC-MS injector port at  $250^\circ\text{C}$  for 5 min in splitless mode.

#### b) Gas Chromatography – Mass Spectrometry (GC-MS)

Volatile compounds were identified on GC-MS (Hewlett-Packard-HP 6890) (Agilent Technology Inc.). Helium was used as the carrier gas with a constant flow of 1.4 ml/min. The sample went through a DB-WAX (30m x 0.25 $\mu\text{m}$  x 0.25  $\mu\text{m}$ ) capillary column (Agilent Technology Inc.). The oven temperature was programmed from  $35^\circ\text{C}$ , and maintained for 5 min, and ramped to  $200^\circ\text{C}$  at  $4^\circ\text{C}/\text{min}$  and finally, maintained at  $200^\circ\text{C}$  for 15 min. The transfer line temperature was  $280^\circ\text{C}$ . The mass detector was operated at  $150^\circ\text{C}$  with electron impact mode at 70 eV. The total ion currents were monitored in a mass range of 29-300 m/z with 5 scan/s. The compounds were identified by comparison with mass spectra from NIST95 library database (NIST98, USA).

#### C. Microbial Analysis

The numbers of viable lactic acid bacteria were counted (LAB) by using MRS agar (Merck, Germany). The results were reported as logarithm of colony forming units per gram of sample (logCFU/g).

#### D. Sensory Evaluation

All fermented milk samples were served in small plastic cup and the lid was closed to retain volatiles. Samples were kept at refrigerated temperature before served. In each session, all five samples were served to consumer panels ( $n=71$ ) with random three-digit codes and in balanced presentation order. Consumer panels were requested to use a straw without opening lid to test the sample. Each consumer evaluated each sample for sweetness, sourness, flavour, and overall liking using a 9-point hedonic scales with 9= "like extremely" and 1= "dislike extremely".

#### E. Statistical Analysis

All instrument analyzed parameters were performing in triplicate. Data were examined statistically by Analysis of variance (ANOVA) and using Duncan's multiple range tests for the post hoc tests with SPSS 16.0 for windows (SPSS Inc, USA). The analyses were conducted at the 95% confidence level. For the determination of the relatives between volatiles compounds, testing parameters and consumer preference were performed by Principal Component Analysis (PCA) using XLSTAT 2006.5 (Addinsoft, USA).

### III. RESULTS AND DISCUSSION

The 5 commercial fermented milks consisting of B-L, B-O, D-L, D-O, and Y were evaluated by consumers using 9-point hedonic scales. The result was shown in Table I, the attributes liking scores of all products were in the level range of like slightly to like moderately. Only sweetness was not significantly different ( $p>0.05$ ) among 5 testing fermented milk samples. For others attributes, including sourness, flavour, and overall liking, B-O seemed to get the highest scored.

TABLE I  
CONSUMER PREFERENCE SCORED FOR COMMERCIAL FERMENTED MILK SAMPLES

Brand	Sweetness	Sourness	Flavour	Overall liking
B-L	$5.97 \pm 1.37^a$	$5.90 \pm 1.35^c$	$5.92 \pm 1.33^b$	$6.31 \pm 1.29^b$
B-O	$6.36 \pm 1.60^a$	$7.10 \pm 1.31^a$	$6.69 \pm 1.32^a$	$7.05 \pm 1.23^a$
D-L	$6.21 \pm 1.46^a$	$6.31 \pm 1.41^{bc}$	$6.21 \pm 1.43^{ab}$	$6.51 \pm 1.34^b$
D-O	$6.30 \pm 1.63^a$	$6.56 \pm 1.44^b$	$6.64 \pm 1.59^a$	$6.75 \pm 1.33^{ab}$
Y	$6.23 \pm 1.76^a$	$6.31 \pm 1.74^{bc}$	$6.33 \pm 1.58^{ab}$	$6.66 \pm 1.58^{ab}$

\*a-c means different letters are significantly different ( $p<0.05$ ) in a row

Different pH can be affected the flavour perception, sourness, and sweetness [4]. Ott et al. [5] demonstrated that pH had linear relationship with acidity perception and significantly influence on odor attributes in yoghurts. In this research, we observed acidity of fermented milk in 2 terms (pH and TA) as shown in Table II. The pH and TA values of commercial fermented milks showed in narrow range, but still significantly difference ( $p\leq 0.05$ ). When compared the relationship between pH and TA values with sourness and flavour liking scores (Table I), TA was closer to these attribute than pH in our research. Therefore, TA measurement could be more appropriate parameter for controlling acidity in fermented milk than pH. The measured TSS and living LAB were also varying in testing samples. These may be due to different compositions and production of each company. However, the relationships between these parameters and attributes liking scores were re-concluding by Principal component analysis (PCA) later.

TABLE II  
pH, TSS, TA AND VIABLE LAB CELL OF COMMERCIAL FERMENTED MILK

Brand	pH	TSS ( $^\circ\text{brix}$ )	TA (%lactic acid)	Numbers of LAB (log CFU/g)
B-L	$4.02 \pm 0.02^a$	$11.53 \pm 0.23^c$	$0.59 \pm 0.03^c$	$7.65 \pm 0.18^c$
B-O	$3.80 \pm 0.00^d$	$18.13 \pm 0.03^c$	$0.82 \pm 0.02^a$	$9.07 \pm 0.01^a$
D-L	$3.94 \pm 0.02^b$	$17.42 \pm 0.17^d$	$0.63 \pm 0.01^d$	$7.78 \pm 0.17^c$
D-O	$3.89 \pm 0.00^c$	$19.99 \pm 0.13^b$	$0.76 \pm 0.01^b$	$7.52 \pm 0.01^c$
Y	$3.73 \pm 0.02^c$	$20.84 \pm 0.13^a$	$0.72 \pm 0.01^c$	$8.74 \pm 0.21^b$

\*a-e means different letters are significantly different ( $p<0.05$ ) in a row

We mainly observed that the volatile compounds consisting in commercial fermented milks in Thailand could be separated into 2 groups. The first group was produced from fermentation by lactic acid bacteria used as starter culture. The mostly founded volatile compounds were shown in Table III. However, the volatile compounds had different profile in each brand (data not showed).

TABLE III  
THE FIRST GROUP OF VOLATILE COMPOUNDS IN COMMERCIAL FERMENTED MILK SAMPLES; PRODUCED BY FERMENTATION

Brand	ethanol	acetic acid	furfural	decanal	octanol	furanmethanol	octanoic acid	nonanoic acid
B-L	15.29±0.74 <sup>a</sup>	1.62±0.25 <sup>bc</sup>	0.32±0.02 <sup>b</sup>	0.64±0.03 <sup>a</sup>	2.35±0.18 <sup>b</sup>	nd	0.64±0.06 <sup>b</sup>	0.11±0.01 <sup>b</sup>
B-O	16.40±1.01 <sup>a</sup>	1.90±0.07 <sup>b</sup>	0.31±0.10 <sup>b</sup>	0.63±0.06 <sup>a</sup>	2.87±0.14 <sup>a</sup>	1.14±0.10 <sup>c</sup>	0.53±0.06 <sup>b</sup>	0.12±0.02 <sup>b</sup>
D-L	3.71±0.73 <sup>d</sup>	1.49±0.13 <sup>c</sup>	0.45±0.02 <sup>a</sup>	0.10±0.01 <sup>c</sup>	2.78±0.16 <sup>a</sup>	1.88±0.07 <sup>a</sup>	0.67±0.13 <sup>b</sup>	nd
D-O	9.26±0.32 <sup>c</sup>	3.14±0.21 <sup>a</sup>	0.54±0.04 <sup>a</sup>	0.17±0.01 <sup>b</sup>	2.58±0.35 <sup>b</sup>	1.56±0.12 <sup>b</sup>	1.04±0.08 <sup>a</sup>	0.18±0.03 <sup>a</sup>
Y	nd	0.24±0.04 <sup>d</sup>	0.20±0.03 <sup>c</sup>	nd	0.37±0.06 <sup>c</sup>	0.99±0.05 <sup>d</sup>	0.37±0.03 <sup>c</sup>	nd

\*a-d means different letters are significantly different ( $p < 0.05$ ) in a row

\*\*nd = not detected.

The second group (Table IV) was the volatile compounds that normally were not founded in fermented milks. Due to these volatile compounds were not founded in reported volatile compounds of plain yoghurt by many researchers [8]-[13]. These compounds were artificial fruit flavours added, such as citrus flavour (limonene, citral, and etc.) and vanilla flavour (vanillin and ethyl vanillin). The second group compounds were similarly profile in all testing samples (data not showed).

The relationships among commercial fermented milk samples and variables (sensory attributes, pH, TA, TSS, and LAB) were determined using PCA. The scree plot of eigenvalues showed that a model including the first two factors explained 90.2% of total variance. As shown in Fig. 1, all testing sensory attributes were related to each other and also related to TA and TSS. Sensory attribute such as sweetness level affected on overall liking and flavour liking as evidence by [14]. Sweetness also affect on sourness by sucrose concentration [15]. B-O seemed to be the suitable prototype for new product development due to its closeness to satisfied attributes in the principal component plot.

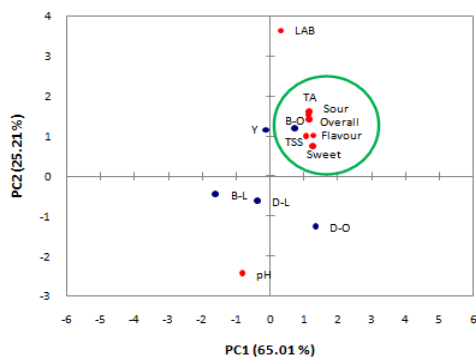


Fig. 1 Principal component biplot of commercial fermented milk samples and variables (sensory attributes, pH, TA, TSS, and LAB); PC = principal component

The association between volatile compounds and sensory attributes using PCA was indicated in Fig. 2. As seen in the principal component plot, all sensory and a half of volatile compounds were separated by factor 2 (explaining 30% of total variable, negative loading). Thereby, the related volatile compounds with the sensory attributes were acetic acid, furanmethanol, furfural, and octanoic acid from fermented compounds group and beta pinene, ethyl vanillin, eucalyptol,

limonene, and vanillin from artificial flavour group. Barnes [16] suggested that, for consumers, the overall liking of plain yoghurt was based on fruit flavour, sweetness, sourness, and balance between sweetness and sourness liking. It was supported by our studies, the volatile compounds related to sensory attributes were mainly founded from artificial fruit flavour such as citrus flavour (beta pinene and limonene). Whereas vanilla flavour significantly increased sensory attributes liking scores in strawberry yoghurt [4].

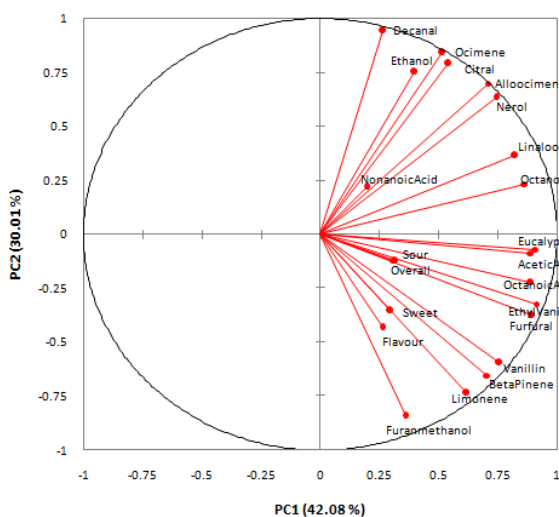


Fig. 2 Principal component biplot of volatile compounds and sensory attributes; PC = principal component

#### IV. CONCLUSIONS

B-O was the suitable prototype for new fermented milk development for Thailand market. The development process had to concern about the interaction between sensory attributes (sweetness, sourness, and flavour perception), TA, and TSS which affected the overall liking of product. The fruit flavour such citrus flavour (lemon and beta pinene) and vanilla flavour could be added to increase consumer liking. The main fermented volatile compounds were related to consumer liking were acetic acid, furanmethanol, furfural and octanoic acid.

TABLE IV  
THE SECOND GROUP VOLATILE COMPOUNDS IN COMMERCIAL FERMENTED MILK SAMPLES; ARTIFICIAL FLAVOUR ADDED

brand	beta pinene	limonene	eucalyptol	ocimene	allicimene	linalool	citral	nerol	ethyl vanillin	vanillin
B-L	1.05 ± 0.10 <sup>c</sup>	30.83 ± 5.04 <sup>d</sup>	8.31 ± 0.82 <sup>b</sup>	2.13 ± 0.20 <sup>a</sup>	0.41 ± 0.02 <sup>a</sup>	32.12 ± 1.43 <sup>a</sup>	5.32 ± 0.09 <sup>b</sup>	3.28 ± 0.05 <sup>a</sup>	0.22 ± 0.02 <sup>c</sup>	0.19 ± 0.01 <sup>c</sup>
B-O	8.54 ± 0.05 <sup>c</sup>	33.88 ± 3.10 <sup>d</sup>	6.38 ± 0.20 <sup>c</sup>	1.74 ± 0.14 <sup>b</sup>	0.39 ± 0.07 <sup>a</sup>	30.27 ± 1.68 <sup>ab</sup>	7.70 ± 1.67 <sup>a</sup>	2.83 ± 0.25 <sup>b</sup>	0.35 ± 0.11 <sup>b</sup>	0.18 ± 0.04 <sup>c</sup>
D-L	10.87 ± 1.02 <sup>b</sup>	62.80 ± 0.58 <sup>b</sup>	10.37 ± 0.76 <sup>a</sup>	0.92 ± 0.01 <sup>c</sup>	0.14 ± 0.01 <sup>b</sup>	31.07 ± 0.11 <sup>ab</sup>	1.36 ± 0.24 <sup>c</sup>	2.02 ± 0.04 <sup>d</sup>	0.55 ± 0.02 <sup>a</sup>	1.02 ± 0.06 <sup>b</sup>
D-O	12.42 ± 0.87 <sup>a</sup>	83.91 ± 0.87 <sup>a</sup>	11.26 ± 0.21 <sup>a</sup>	1.73 ± 0.02 <sup>b</sup>	0.35 ± 0.03 <sup>a</sup>	29.15 ± 0.86 <sup>b</sup>	4.70 ± 0.29 <sup>b</sup>	2.58 ± 0.15 <sup>c</sup>	0.59 ± 0.04 <sup>a</sup>	1.16 ± 0.08 <sup>a</sup>
Y	1.11 ± 0.22 <sup>c</sup>	40.46 ± 0.57 <sup>c</sup>	1.00 ± 0.18 <sup>d</sup>	0.81 ± 0.09 <sup>c</sup>	nd	5.80 ± 0.19 <sup>c</sup>	2.17 ± 0.22 <sup>c</sup>	0.30 ± 0.02 <sup>c</sup>	nd	nd

\*a-d means different letters are significantly different (p<0.05) in a row

\*\*nd = not detected.

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