Flavour Analysis of Stirred Yoghurt with Cheddar Cheese Adding into Milk

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Yoghurt has the strong flavour produced by adding Cheddar cheese into the milk and cold storage process before the fermentation. The flavour analysis of yoghurt was investigated by e-nose, e-tongue and gas chromatography-mass spectrometry (GC-MS). Yoghurt samples with adding Cheddar cheese or whole milk powder showed significant differences in their e-nose flavour profiles, but their e-tongue flavour profiles were roughly similar. Besides, neither e-nose nor e-tongue could accurately distinguish the yoghurt samples which were subjected to cold storage process before the homogenization or not. Among the detected volatile compounds, pentanal and dimethyl trisulfide were the most highly correlated with the cold storage process, and pentanal, ethyl butanoate, methanethiol, dimethyl disulfide and dimethyl trisulfide discriminated yoghurt samples adding Cheddar cheese or whole milk powder. This study showed the potential of adding Cheddar cheese in the yoghurt production to accelerate the flavour formation and increase the flavour compounds.

Keywords: stirred yoghurt, cheddar cheese, flavour analysis, cold storage process

Introduction

Yoghurt is the most popular fermented dairy product in Asian countries. Growth in the concerning of food and health has been reflected in the increased demand for yoghurt with distinctive characteristics, such as favorable flavour and abundant nutrients (Pohjanheimo and Sandell, 2009). The yoghurt is a kind of fermented dairy product through the action of Streptococcus thermophilus and Lactobacillus bulgaricus, which can produce flavour compound (Condoorso et al., 2008; Sieuwerts et al., 2008). Acceptability of yoghurt by the consumer is largely determined by their sensory properties (Isleten and Karagul-Yuceer, 2006). The flavour of yoghurt is mainly affected by yoghurt starter cultures, so we should exploit and design considerable yoghurt starters with the different fermentation performance (Guzel-Seydim et al., 2005; Skriver et al., 2003). That is a large and complicated project. Therefore, some researchers try to find another way to develop flavoured yoghurt. In addition, in recent years, diet has been linked to various diseases such as diabetes, obesity, cardiovascular disease, osteoporosis and cancer. Hence, the focus of flavoured yoghurt research has been shifted towards specific food ingredients contributing to increasing nutrition, granting healthy functions and accelerating flavour development (Walther et al., 2008).

Recently, there had some studies on adding food ingredients into the milk to develop flavoured yoghurt and cater consumers. Saint-Eve et al. (2006) investigated the impact of sodium caseinate and whey protein on the sensory properties of yoghurt and found that high ratio of caseinate would decrease the flavour intensity and the fruity notes. Cliff et al. (2013) explored the sensory characteristics of carrot juice yoghurt. Ye et al. (2012) investigated the effect of hawk tea on yoghurt and found that the amounts of some characteristic volatile compounds were significantly increased. However, there have been few studies on flavour
As an important source of proteins and amino acids with favorable flavour, cheese is used as a functional food ingredient in a wide range of food industrial in order to accelerate the flavour and improve nutritional values and biological effects on health (Hannon et al., 2006; Varming et al., 2013). El-Salam and Mohamed (2009) studied the effect of processed cheese on plasma lipid profile and lipid peroxidation and found that it could significantly reduce the lipid peroxidation. There is evidence to suggest that two bioactive tripeptides, valyl-prolyl-proline (VPP) and isoleucyl-prolyl-proline (IPP), found in cheese (Bütikofer et al., 2007). These peptides can lower the blood pressure. Cheese contains high concentrations of proteins, bioactive peptides, free amino acids, free fatty acids and other catabolic products typically associated with cheese flavour. In addition, there are many kinds of lactobacilli in ripened cheese, such as Lactobacillus casei, Lactobacillus plantarum and Lactobacillus acidophilus (Madureira et al., 2013; Karimi et al., 2012). The vigorous microflora can rapidly decompose protein and is largely responsible for development of the flavour.

Cheeddar cheese is a natural and most popular cheese. As a living system, it is rich in bioactive enzymes, lactobacilli and flavour precursors. Research on the possible accelerative role of Cheddar cheese on yoghurt flavour is rarely reported. In this study, Cheddar cheese was added into the milk to enhance the flavour, enrich the nutrition and increase acceptance of yoghurt. The flavoured yoghurt was evaluated using electronic nose and electronic tongue. The volatile compounds were investigated by GC-MS.

Materials and Methods

Preparation of Stirred Yoghurt The stirred yoghurt was prepared according to Lucey (2004) with slight modifications. The formulas and process of yoghurt samples are shown in Table 1. Cheddar cheese (Kerrygold, Evanton, USA) was cut into pieces and then added into fresh cow milk with sugar (commercially available) in sample 2 and sample 3. Whole milk powder (Fonterra Ltd, Auckland, New Zealand), cream (Bright Dairy & Food Co., Ltd., Shanghai, China) and sugar were added into cow milk in sample 1. The protein and fat content were consistent in three samples. The compositions of three samples were sheared by FLUKO FM300 high shear emulsifier (FLUKO, Shanghai, China) at 10000 rpm for 20 min at 45°C, and then the base mix of sample 3 was homogenized using the high pressure of 18 MPa at 60°C and processed by high-temperature short-time (HTST) at 95°C for 5 min immediately. Cooled to 42°C, sample 3 was inoculated with 0.05‰ (w/w) YoFlex Express CN yoghurt starter cultures (Chr. Hansen, Hørsholm, Denmark). It was maintained at 42°C in MIR-253 incubator (Sanyo, Osaka, Japan) until the pH reaching to 4.6. After stirred at 500 rpm for 5 min, the sample 3 was stored at 4°C. However, the base mix of sample 1 and 2 should be added cold storage process before homogenization, storing at 4°C for 12 h, and then were processed in accordance with sample 3.

Electronic Nose Yoghurt (200 g) samples were weighted into a 20 mL crimp-seal sample vial, respectively. They were refrigerated at 4°C until being measured with the e-nose.

An e-nose (FOX4000, Alpha M.O.S., Toulouse, France) with 18 metal oxide sensors and automatic headspace sampling system was used in this study. The e-nose conditions included heating the sample at 35°C for 1200 s with agitation at 500 rpm before injection. Then, a 2500 μL sample was withdrawn from the headspace, injected into the sensor chambers and flushed over the sensors with a flow rate of 150 mL min⁻¹. Data were recorded for 120 s, and the delay between the sample injection and the next injection was 300 s (Gutiérrez-Méndez et al., 2008). For each sample, 4 replicates were prepared for analyzing and the average values of the replicates were used in the data evaluation.

Electronic Tongue Yoghurt (200 g) was centrifuged (10000 × g, 10 min) after being frozen 2 h at −20°C, and then the supernatant was filtered. The filtrate of 100 mL was measured in a gas cup and refrigerated at 4°C until being measured with the e-tongue.

An e-tongue (ASTREE II, Alpha M.O.S., Toulouse, France) with 7 sensors was used in this study. After each testing, the sensors were rinsed by using de-ion water for 15 s (Wei et al., 2013). For each sample, 7 replicates were prepared for analyzing and the average values of the replicates were used in the data evaluation.

Gas Chromatography- Mas Spectrometry Headspace solid-phase micro-extraction was used to extract the volatile compounds of the yoghurt samples according to Ye et al. (2012). The fiber used for manual extraction was DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA).

Three samples were prepared for extraction by weighting 10 g of yoghurt into a 20 mL crimp-seal sample vial, respectively. The

### Table 1. Formulas and process for stirred yoghurt

<table>
<thead>
<tr>
<th>Formulas and process</th>
<th>Sample 1⁺</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow milk (g)</td>
<td>896.3</td>
<td>900.0</td>
<td>900.0</td>
</tr>
<tr>
<td>Cheddar cheese (g)</td>
<td>0.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Whole milk powder (g)</td>
<td>20.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cream (g)</td>
<td>3.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>80.0</td>
<td>80.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Cold storage process</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

⁺: adding cold storage process before homogenization; –: not adding cold storage process before homogenization.

Sample 1 had mean fat of 3.91%, mean protein of 3.17%, mean total solids of 20.76%; sample 2 and 3 had mean fat of 3.91%, mean protein of 3.17%, mean total solids of 20.71% detected by Milko Scan FT120.
vials were sealed with an aluminum crimp-seal containing a polytetrafluoroethylene silicone septum, and then were placed in a 60°C stirring water bath for 30 min. Then the prepared fiber was inserted through the septum and fully exposed to the headspace for 15 min. The exposed fiber was removed and inserted into the GC-MS where the volatiles were thermally desorbed.

Analysis of the volatile compounds was performed using an Agilent 7890 (II) gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5975 series mass selective detector. The SPME fiber was inserted into the injection port that was held at 250°C, and the compounds were thermally desorbed for 2 min under the splitless conditions (Welty et al., 2001). A DB-17MS column 30 m × 0.25 mm × 0.25 μm (Agilent Technologies, Santa Clara, CA, USA) was used to separate the volatile compounds.

The temperature of the column was held at 45°C for 5 min, ramped at 10°C min⁻¹ to 80°C, and then further heated to 240°C at the rate of 5°C min⁻¹. The carrier gas was helium (1 mL min⁻¹). The mass spectrometer was run in the electron impact mode at 70 ev. The mass scan range was 25 to 400 m/z (Condurso et al., 2008; Haque and Aryana, 2002; Iwasawa et al., 2014; Ye et al., 2012).

Statistical analysis Two pattern recognition techniques: principal component analysis (PCA) and discriminant function analysis (DFA) were applied to analyze the data of e-nose and e-tongue in this paper. PCA could decompose the experimental data matrix into latent variables and explain the variables in the data by 2D and 3D loading plot that could elucidate the relationships between the original variables and their influence on the system (Tian et al., 2007). DFA is a parametric learning classifier, which can be used for both qualitative and quantitative analysis (Wei et al., 2013).

The data of e-nose and e-tongue were analyzed by PCA and DFA which is the part of the software integrated in the e-nose and e-tongue (Alpha MOS). ANOVA using SAS was used to determine significant ($P < 0.05$) differences among the treatments. Means were separated by using Duncan’s multiple range test.

**Results and Discussion**

*e-nose* Volatile molecules are absorbed at the surface of the semiconductor where they react with the dissolved oxygen species, and this produces a change in resistance, which can be amplified and analyzed through a database capture software system (Ampuero and Bosset, 2003; Trihaas et al., 2005). Identification and classification of an analyte mixture is accomplished through recognition of this unique aroma signature (electronic fingerprint) of collective sensor responses (Wilson and Baietto, 2009).

The responses of e-nose sensors are shown in Figure 1. There were different responses among 18 sensors of e-nose. In addition, the types of LY2/G, LY2/AA, LY2/GH, T30/1, PA/2, P30/1, P40/2 and P30/2 sensor could distinguish sample 1 with sample 2 and sample 3 ($P < 0.05$), but they couldn’t distinguish sample 2 and sample 3 accurately ($P > 0.05$), that is, the eight types of sensors were able to characterized the yoghurt samples with adding the Cheddar cheese or whole milk powder into the fresh milk. The results indicated that adding Cheddar cheese into fresh milk could change the flavour of yoghurt and affect volatile flavour compound formation.

Principal component analysis based on the electronic nose data produced by three types of stirred yoghurt is shown in Figure 2. Principal component analysis transforms original dependent variables into new uncorrelated variables. As seen in Figure 2, the first two principal components accounted for 82.40% and 9.25%,
respectively. They represented 91.65% of the total variance and almost reflected the overall fingerprint information of volatile compounds of samples. Sample 1 could be separated well from sample 2 and sample 3 based on differences in their general flavour profile of e-nose.

Discrimination index (DI) provided by software integrated in the e-nose is used to characterize the degree of difference of samples. The maximum value of DI is 100. The higher the value of DI is, the more differences the two samples have. As seen in Table 2, the value of DI between sample 1 and sample 2 was 69.56%, and the value between sample 1 and sample 3 was 67.17%, that is, sample 1 could be distinguished with sample 2 and sample 3. But the value of DI between sample 2 and sample 3 was 13.79%, suggesting that the flavour profile of two samples were roughly similar.

Discriminant function analysis based on electronic nose data produced by three types of stirred yoghurt is shown in Figure 3. DF1 accounted for 99.424% and was able to discriminate the yoghurt samples adding Cheddar cheese or whole milk powder. In the vertical direction, DF2 accounted for 0.576%. And there was an unobvious discrimination between sample 2 and sample 3. Discriminant function analysis applied to the e-nose data was

### Table 2. Discrimination index of volatile compounds of three yoghurt samples

<table>
<thead>
<tr>
<th>Discrimination index (%)</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>69.56</td>
<td>67.17</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.00</td>
<td>13.79</td>
</tr>
</tbody>
</table>

![Fig. 2. Principal component analysis based on electronic nose data produced by different yoghurt samples](image1)

![Fig. 3. Discriminant function analysis based on electronic nose data produced by different yoghurt samples](image2)
particularly useful to classify the yoghurt samples adding Cheddar cheese or whole milk powder, but was unable to distinguish the yoghurt that was subjected to be stored at 4°C for 12 h before the homogenization or not.

E-tongue The responses of e-tongue sensors are shown in Figure 4. There were few differences of responses among three samples for 7 sensors of e-tongue. The results indicated that neither adding Cheddar cheese into the fresh milk nor storing yoghurt bas mix at 4°C for 12 h before the homogenization could change the flavour profile of e-tongue. E-tongue based on conducting polymers had been used to evaluate acid, sweet, bitter, salty and astringent tastes (Baldwin et al., 2011; Deisingh et al., 2004). The same pH and sucrose content of three samples may account for roughly similar flavour profiles of e-tongue.

Principal component analysis based on the electronic tongue data produced by three types of stirred yoghurt is shown in Figure 5. The first 2 principal components accounted for 94.346% and 4.194%, respectively. They represented 98.540% of the total variance and almost reflected the overall fingerprint information of yoghurt. However, principal component analysis applied to the e-tongue data was unable to distinguish the three samples.

Discriminant function analysis based on the electronic tongue data produced by three types of stirred yoghurt is shown in Figure 6. DF1 and DF2 accounted for 97.64% and 2.36%, respectively. Discriminant function analysis applied to e-tongue data was particularly useful to classify the three samples. DFA assumes replicate samples are clustered, but PCA treats each replicate samples as individual data (Wei et al., 2013). That’s why DFA is
superior to PCA in the discriminating samples capacity.

**Volatile Compounds** The volatile compounds detected in the three samples are given in Table 3. Twenty-one compounds, consisting of 3 alcohols, 4 aldehydes, 5 carboxylic acids, 2 esters, 4 ketones, and 3 sulfur compounds, were identified in the three samples. All of them have been identified in dairy products previously (Alonso and Fraga, 2001; Condurso et al., 2008; Hannon et al., 2006; Ye et al., 2012).

As seen in Table 3, among the 21 volatile compounds, pentanal, ethyl butanoate, methanethiol, dimethyl disulfide and dimethyl trisulfide were not detected in the sample 1. Further, besides 2-pentanol and 2-nonanol, the amounts of other volatile compounds were significantly different between the samples.

**Table 3. Results of volatile compounds analysis of three yoghurt samples, showing the averaged peak areas (in arbitrary unit ×1000)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Averaged peak area × 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>Butanol</td>
<td>23.5 ± 0.3 (^b)</td>
</tr>
<tr>
<td>2-Pentanol</td>
<td>34.6 ± 0.8 (^b)</td>
</tr>
<tr>
<td>2-Nonanol</td>
<td>145.2 ± 2.6 (^b)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>297.9 ± 8.3 (^b)</td>
</tr>
<tr>
<td>Butanal</td>
<td>218.3 ± 2.8 (^b)</td>
</tr>
<tr>
<td>Pentanal</td>
<td>ND</td>
</tr>
<tr>
<td>Heptanal</td>
<td>1254.9 ± 10.2 (^b)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>354.9 ± 1.5 (^b)</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>17.2 ± 0.3 (^b)</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>25.6 ± 0.4 (^b)</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>15.4 ± 0.2 (^b)</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>390.2 ± 2.8 (^b)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>15.8 ± 0.5 (^b)</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>ND</td>
</tr>
<tr>
<td>Acetone</td>
<td>584.2 ± 2.5 (^b)</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>1854.6 ± 22.1 (^a) (^b)</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>677.3 ± 5.6 (^b)</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>257.8 ± 3.5 (^b)</td>
</tr>
<tr>
<td>Methanethiol</td>
<td>ND</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>ND</td>
</tr>
<tr>
<td>Dimethyl trisulfide</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\)Values are expressed as mean ± SD. Different superscript capital letters (A, B) in a row denote significant differences between sample 1 and sample 2 (\(P < 0.05\)); different superscript lower-letters (a, b) in a row denote significant differences between sample 2 and sample 3 (\(P < 0.05\)).

\(^b\)ND: not detected.

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**Fig. 6.** Discriminant function analysis based on electronic nose data produced by different yoghurt samples.
compounds were significantly higher than that in the sample 1 (p < 0.05). The results indicated that adding Cheddar cheese into the milk could affect the volatile compound formation. The results of e-nose were confirmed here. At the same time, pentanal and dimethyl trisulfide were not detected in sample 3. Besides 2-nonanal and 2-nonanone, the amounts of other volatile compounds in sample 2 were significantly higher than that in the sample 3. The results indicated that adding cold storage process before the homogenization was also beneficial to volatile compound formation.

Volatile flavour compounds are created by a variety of mechanisms, in which amino acid catabolism is a major process for flavour formation (Yvon and Rijnen, 2001). Aromatic amino acids (phenylalanine, tyrosine and tryptophan), branched-chain amino acids (leucine, isoleucine and valine) and methionine are the major precursors of these volatile flavour compounds. Cheddar cheese contains high concentrations of proteins, bioactive peptides, free amino acids and lactobacilli (Hannon et al., 2006; Karimi et al., 2012). This may account for the differences of volatile compounds between sample 1 and sample 2.

As the characteristic flavour compounds in the cheese, dimethyl disulfide and dimethyl trisulfide are produced from methionine (Mahajan et al., 2004; Yvon and Rijnen, 2001). These sulfur compounds were detected in sample 2 and sample 3, so the yoghurt adding the Cheddar cheese had a unique favourable cheese flavour.

The difference between sample 2 and sample 3 was that sample 2 was stored at 4°C for 12 h before the homogenization, so there was adequate time for the activation of the enzymes and vigorous microflora existed in Cheddar cheese to degrade proteins and amino acids (Karimi et al., 2012; Yvon and Rijnen, 2001). This was beneficial to form the volatile flavour compounds.

Aldehydes contributed strongly to the volatile flavour of yoghurt. Acetaldehyde with high level was detected in sample 2. It is an important flavour compound in yoghurt (Alonso and Fraga, 2001). It can be formed by decomposition of threonine by the action of threonine aldolase (Wouters et al., 2002). The straight-chain aldehydes can be formed during β-oxidation of unsaturated fatty acids and contribute to the green-grass-like and herbaceous aromas (Hannon et al., 2006). The derived ester also participates in the yoghurt flavour, especially compounds such as ethyl butanoate, formed by the esterification of fatty acids with ethanol.

Conclusions

Flavour analysis of yoghurt was investigated by e-nose, e-tongue and GC-MS. Yoghurts adding Cheddar cheese or whole milk powder showed significant differences in their e-nose flavour profiles, but their e-tongue flavour profiles were roughly similar. In addition, neither e-nose nor e-tongue could accurately distinguish the yoghurts subjected to cold storage process before the homogenization or not. Among the detected volatile compounds, pentanal and dimethyl trisulfide were the most highly correlated with cold storage process before the homogenization, and pentanal, ethyl butanoate, methanethiol, dimethyl disulfide and dimethyl trisulfide discriminated among yoghurts containing Cheddar cheese or whole milk powder. The results showed that adding Cheddar cheese into the milk during yoghurt manufacture had a beneficial effect on accelerating flavour formation and increasing flavour compounds. Besides, adding the cold storage process before the homogenization would also affect volatile compound formation, but the effect was subtle. Therefore, the composition of yoghurt base mix may be a major reason for affecting flavour of yoghurt.

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