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# Identification of characteristic flavour precursors from enzymatic hydrolysis-mild thermal oxidation tallow by descriptive sensory analysis and gas chromatography–olfactometry and partial least squares regression

Xiaoxia Shi [a], Xiaoming Zhang [a,*], Shiqing Song [b], Chen Tan [a], Chengsheng Jia [a], Shuqin Xia [a]

[a] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, PR China
[b] Department of Biology and Food Engineering, Shanghai Institute of Technology, 200235 Shanghai, PR China

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ABSTRACT

The "enzymatic hydrolysis-mild thermal oxidation" method was employed to obtain oxidized tallow. Nine beeflike flavours (BFs) were prepared through Maillard reaction with oxidized tallow and other ingredients. Volatile compounds of oxidized tallow and beeflike flavours were analysed by SPME/GC–MS. Six sensory attributes (meaty, beefy, tallowy, simulate, burnt and off-flavour) were selected to assess BFs. Thirty four odour-active compounds were identified to represent beef odour through GC–O analysis based on detection frequency method. GC–MS profiles of oxidized tallow were correlated with GC–O responses and sensory attributes of BFs using partial least squares regression modelling (PLSR). Twenty nine compounds were considered as the potential precursors of oxidized tallow. Among them, tetradecanoic acid, D-limonene, 1,7-heptandiol, 2-butyltetrahydrofuran, (Z)-4-undecenal, (Z)-4-decenal, (E)-4-nonenal and 5-pentyl-2(3H)-furanone were unique products generated from enzymatic hydrolysis-mild thermal oxidation of tallow, while hexanal, heptanal, octanal, nonanal, decanal, pentanal, acetic acid, butanoic acid, hexanoic acid, 1-heptanol, 1-octanol, 3-methylbutanal, 2-pentylfuran, γ-nonalactone, 2-undecenal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, (E)-2-nonenal, (E)-2-octenal, (E)-2-decenal and (Z)-2-heptenal were common products generated from thermal oxidation of tallow.



## 1. Introduction

Meat flavour has been widely used as an important food additive in snack foods, including instant noodles, meat products, frozen food, condiment, etc. Based on preparation methods, meat flavour is usually divided into two classes, namely confecting flavour and thermal reaction flavour. The most common type is the latter one, and the primary reaction in this process is Maillard reaction. An extensive number of researches have investigated that Maillard reaction plays an important role in the formation of meat flavour.

Considerable researches suggest that the basic meaty aroma is the same, the species-specific differences are mainly caused by the flavour substances derived from lipid decomposition or oxidation [1]. Large amounts of volatile compounds are produced during lipid decomposition, including aldehydes, ketones, alcohols, lactones, free fatty acids, nitrogen containing compounds and oxygen containing compounds. Particularly, a high level of unsaturated lipids has been reported to contribute to desirable flavours in freshly cooked meat [2]. Animal fats can be oxidized by means of thermally controlled oxidation or enzymatic hydrolysis-mild thermal oxidation, and different flavours are produced based on the diverse treatments of animal fats. Considerable studies have revealed that species-specific flavour can be enhanced by heating animal fats in air [1,3–6]. However, thermally controlled oxidation needs high temperature, which is high energy-intensive and not easy to control in preparation, and the similarity to natural beef flavour needs further improvement. To solve these problems, an "enzymatic hydrolysis-mild thermal oxidation" method is proposed to prepare characteristic flavour precursors of oxidized tallow, which means initial hydrolysis of tallow is conducted by lipase firstly, then proceeding with further thermal oxidation under mild conditions to obtain oxidized tallow. Enzymatic pretreatment of tallow plays a significant role in the formation of desirable aroma during lipid oxidation and subsequent Maillard reaction. The main reason is that more amino groups in phospholipid and free fatty acids are released during enzymatic hydrolysis, and more pyrolysates, such as carbonyl or alcoholic compounds, are derived during tallow degradation, leading to the different pathways of Maillard

*Abbreviations:* BFs, beeflike flavours; GC–O, gas chromatography–olfactometry; SPME/GC–MS, solid-phase microextraction/gas chromatography–mass spectrometry; PLSR, partial least squares regression; PC, principle component; HVP, hydrolysed vegetable protein; PV, peroxide value; AV, acid value; p-AV, p-anisidine value.

* Corresponding author. Tel.: +86 510 85919106; fax: +86 510 85884496.
*E-mail address:* xmzhang@jiangnan.edu.cn (X. Zhang).

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reaction. However, to date enzymatic hydrolysis-mild thermal oxidation method was still not used in beeflike flavour preparation and no related report was found.

Consumers generally take aroma as one of the most important determinants of meat flavour quality. The volatile components of meat flavour have been analysed time and again in previous literature [7–9]. Yet, in recent years, more great efforts have been made to identify aroma-active compounds in cooked beef via GC–O [10–12]. GC–O is an efficient tool to select and evaluate aroma-active compounds from a complicated mixture.

The principal objectives of present study are to apply descriptive sensory analysis to describe the aroma attributes of beeflike flavours (BFs), determine the aroma-active compounds through GC–O analysis based on detection frequency method and identify volatile compounds in oxidized tallow samples by SPME/GC–MS analysis. The correlation between GC–MS profiles of oxidized tallow and quantitative descriptive sensory data as well as GC–O responses of BFs are analysed to understand which compounds in oxidized tallow samples have significant effects on aroma-active compounds and sensory attributes of BFs. Through above analyses, the characteristic flavour precursors from enzymatic hydrolysis-thermal oxidation tallow are identified and the main differences between enzymatic hydrolysis-thermal oxidation tallow and simple thermal oxidation tallow are elucidated.

## 2. Materials and methods

### 2.1. Materials

Refined tallow (Batch number: 110929) was purchased from Anhui Muyang Oil and Fats Co., Ltd. (Anhui, China). Lean beef was purchased from Wal-Mart supermarket in Wuxi, China. Hydrolysed vegetable protein (HVP) was purchased from Wuxi Xiehe Foodstuffs Co., Ltd. (Wuxi, China). L-Cysteine, glucose, D-xylose, thiamine, L-glutamic acid, L-proline, DL-methionine and taurine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Alkaline protease (activity 2.4 AU/mL) and flavourzyme (activity 500 LAPU/g) were purchased from Novozymes (Bagsvaerd, Denmark). Lipase (activity 20,000 U/g) was provided by Yiming Biological Products Co., Ltd. (Taizhou, China). Benzylalcohol, methanol and 1,2-dichlorobenzene were of chromatography grade from TCI Development Co., Ltd. (Shanghai, China). Other authentic reference compounds were obtained from commercial sources and Sigma–Aldrich Co. Ltd. (Shanghai, China).

### 2.2. Sample preparation

#### 2.2.1. Preparation of enzymatic hydrolysis-mild thermal oxidation tallow

*Enzymatic hydrolysis of tallow*: Refined tallow and phosphate buffered solution (pH 6.5) were placed in the enzyme reactor at a ratio of 7:3 with mechanical stirring at 150 rpm. Lipase was added to the enzyme reactor with enzyme/substrate ratio (E/S) of $7.0 \times 10^{-3}$ (g lipase/g tallow) when the mixture was judged to be isothermal to the water bath (45 °C). After reacting for 8 h, the sample was heated at 95 °C for 10 min to deactivate the enzyme and then stored at −18 °C for further analysis.

*Thermal oxidation of tallow*: The enzymatic hydrolysis tallow mixture (100 g) was placed in a 250 mL 4-neck roundbottom flask and heated at a temperature range of 80–100 °C in a thermalstatic oil bath with mechanical stirring at 150 rpm, and feeding air at a rate of 25–125 L/h. After heating for 1–6 h, the samples were immediately cooled in ice-water and stored at −18 °C for further analysis.

Nine oxidized tallow with different oxidation states were chosen for further Maillard reaction, and their corresponding PV, AV and p-AV were listed in Table 1.

#### 2.2.2. Preparation of beeflike flavour samples (BFs)

A mixture of HVP (0.8 g), L-glutamic acid (0.05 g), L-cysteine (0.1 g), L-proline (0.05 g), DL-methionine (0.04 g), glucose (0.2 g), D-xylose (0.1 g), taurine (0.1 g), thiamine (0.08 g) and oxidized tallow (2 g), was dissolved in 16.0 g solution of the beef base [12]. The solution was transferred into 25 mL screw-sealed tubes. The pH was adjusted to 6.0 with 6 mol/L NaOH, and the tubes were tightly capped and then heated in a thermostatic oil bath with mechanical stirring (150 rpm) at 110 °C for 2 h. After reaction, the tubes were immediately cooled in ice-water and nine Maillard reaction products named BF1-9 were sampled for further analysis.

### 2.3. Analysis methods

#### 2.3.1. Quantitative descriptive sensory analysis

BF samples were evaluated by a well-trained sensory panel composed of 8 members at the age of 22–40, five females and three males. All panellists were familiar with the assessment of Maillard reaction products and had sensory evaluation experience. Sensory profiling was carried out in an air-conditioned room with isolated booths, which complied with ISO international standards [13]. The sensory attributes in this research were derived through sniffing, tasting and intensive discussions by panel members [14]. At last, six sensory attributes were used in the quantitative descriptive sensory analysis, which were meaty, beefy, tallowy, simulate, burnt and off-flavour. The reference materials were as follows: defatted beef brisket (0.5 kg, 2.5 cm thick) boiled in water for 2 h was labelled "meaty" note; pot roast (round bottom roast, approximately 200 g, wrapped with aluminium foil and baked for 1 h at 150 °C) was labelled "beefy" note; stewed beef product, purchased from Wal-Mart supermarket, the similarity degree of aroma was labelled "simulate" note [12]; refined tallow, purchased from Anhui Muyang Oil and Fats Co., Ltd., was labelled "tallowy" note; defatted beef brisket (100 g, 1 cm thick) roasted on a barbecue for 1 h was labelled "burnt" note; rancid tallow (100 g tallow, placed in a thermotank at 30 °C for 2 days to promote the tallow oxidation corruption) and rotten eggs (broken eggs, placed in a thermotank at 30 °C for 2 days) were labelled "off-flavour" note.

In order to avoid temperature influence, the samples were kept in 60 °C water bath before sensory evaluation. Each sample was given a three-digit number in a randomized design to avoid a so-called order effect. The intensity of the descriptive terms was scored on 10 cm unstructured line scales anchored "none" to the left and "extreme" to the right [15]. Every panel member individually quantified strengths of each attribute on the line scale, and the average of all the panellists was calculated for each BF sample.

#### 2.3.2. GC–MS analysis

In order to identify and quantify volatile compounds, a gas chromatography (Finnigan Trace GC/MS, Finnigan, USA) analysis was carried out on oxidized tallow samples and BFs according to the methods described by Song et al. [12,16].

#### 2.3.3. GC–O analysis

The GC–O system integrated a Finnigan trace GC (Finnigan, Perkin Elmer, USA) with a flame ionization detector and an OP275 sniffing port (GL Sciences Inc., Japan). And the detailed GC–O analysis was referred to Song et al. [12].

#### 2.3.4. Statistical analysis

Data from the descriptive analysis were evaluated by analysis of variance (ANOVA) using SPSS 13.0. ANOVA with Duncan's multiple

**Table 1**
Oxidized tallow information with nine different oxidation states.

| Oxidized tallow | Temperature (°C) | Time (h) | Air flow (L/h) | PV (meq./kg) | p-AV | AV (mg KOH/g) |
|---|---|---|---|---|---|---|
| T1[a] | – | – | – | 6.84 | 1.21 | 0.14 |
| T2 | 60 | 3 | 75 | 17.89 | 5.55 | 150.95 |
| T3 | 90 | 1 | 75 | 36.25 | 10.96 | 134.97 |
| T4 | 90 | 2 | 75 | 70.45 | 23.79 | 134.32 |
| T5 | 100 | 3 | 75 | 80.43 | 49.11 | 154.19 |
| T6 | 90 | 3 | 75 | 89.36 | 33.66 | 134.28 |
| T7 | 90 | 4 | 75 | 100.17 | 41.22 | 137.22 |
| T8 | 90 | 5 | 100 | 110.16 | 62.11 | 139.08 |
| T9 | 90 | 5 | 75 | 118.36 | 55.05 | 131.78 |

[a] Nine samples were denoted by the T in abbreviation followed by Arabic numbers.
T1 was the control sample.

**Table 2**
Average intensity scores of the 6 attributes for the BF samples by sensory panel.

| Sample[x] | Meaty[y] | Beefy | Tallowy | Simulate | Burnt | Off-flavour |
|---|---|---|---|---|---|---|
| BF1 | $3.22^{a} \pm 0.18$ | $3.73^{a} \pm 0.24$ | $3.33^{a} \pm 0.16$ | $2.78^{a} \pm 0.23$ | $5.55^{f} \pm 0.18$ | $5.51^{e} \pm 0.18$ |
| BF2 | $5.45^{b} \pm 0.23$ | $4.86^{b} \pm 0.20$ | $4.45^{b} \pm 0.26$ | $4.45^{b} \pm 0.24$ | $7.55^{i} \pm 0.29$ | $6.67^{g} \pm 0.42$ |
| BF3 | $7.65^{c} \pm 0.33$ | $6.02^{d} \pm 0.14$ | $5.55^{c} \pm 0.31$ | $5.62^{c} \pm 0.13$ | $6.79^{h} \pm 0.44$ | $7.22^{h} \pm 0.33$ |
| BF4 | $8.50^{f} \pm 0.22$ | $7.55^{g} \pm 0.28$ | $7.78^{g} \pm 0.44$ | $7.81^{g} \pm 0.18$ | $3.66^{d} \pm 0.32$ | $1.27^{b} \pm 0.17$ |
| BF5 | $8.53^{g} \pm 0.40$ | $7.21^{f} \pm 0.35$ | $7.15^{e} \pm 0.50$ | $7.45^{e} \pm 0.28$ | $2.28^{c} \pm 0.14$ | $7.69^{i} \pm 0.41$ |
| BF6 | $8.75^{h} \pm 0.46$ | $8.40^{i} \pm 0.43$ | $8.67^{i} \pm 0.27$ | $8.45^{i} \pm 0.11$ | $1.12^{b} \pm 0.09$ | $0.67^{a} \pm 0.11$ |
| BF7 | $9.12^{i} \pm 0.21$ | $8.01^{h} \pm 0.41$ | $8.12^{h} \pm 0.37$ | $8.00^{h} \pm 0.31$ | $0.55^{a} \pm 0.09$ | $2.11^{c} \pm 0.13$ |
| BF8 | $8.14^{d} \pm 0.19$ | $5.13^{c} \pm 0.25$ | $6.12^{d} \pm 0.26$ | $6.54^{d} \pm 0.56$ | $6.11^{g} \pm 0.15$ | $6.12^{f} \pm 0.37$ |
| BF9 | $8.41^{e} \pm 0.42$ | $7.12^{e} \pm 0.33$ | $7.71^{f} \pm 0.19$ | $7.79^{f} \pm 0.47$ | $3.89^{e} \pm 0.19$ | $2.30^{d} \pm 0.21$ |

[x] Nine beeflike flavours (BFs) were denoted by the BF1-9.
[y] Means (listed in ascending order) for each attribute with a column with different letters showed significant differences ($p \leq 0.05$) using Duncan's multiple comparison tests.

comparison tests were performed to determine whether there were differences among individual samples for each sensory attribute. The differences were considered to be significant at $p \leq 0.05$.

To get an overview of potential connections between GC–MS profiles of oxidized tallow, aroma-active compounds and sensory attributes of BFs, multivariate analyses were performed using the Unscrambler version 9.7 (CAMO ASA, Oslo, Norway). Partial least squares (PLS) regression analysis was believed to be the most powerful multivariate calibration technique in chromatography, spectroscopy and sensory sciences [17]. PLS1 and PLS2 prediction models were calculated for comparison, as PLS1 showed only one response variable at a time, and PLS2 handled several responses simultaneously. All variables were centred and standardized (1/Sdev) so as to make each variable have a unit variance and zero mean before applying PLS analysis in order to obtain unbiased contribution of each variable to the criterion, *Y*. By applying PLS analysis to standardized data, importance of peaks for each attribute could be compared quantitatively based on regression coefficients and loading weights for each predictor or *X* variable used in PLS models [18]. A full cross-validation was applied to the regression models. An uncertainty test was performed, where the approximate uncertainty variance of the regression coefficients was estimated by modified jack-knifing [19] (the significance level at $p \leq 0.05$). Ellipse on the figures represented $r^2 = 50\%$ and 100%, respectively.

## 3. Results and discussion

### 3.1. Descriptive sensory analysis

The obtained sensory data were shown in Table 2, and different letters in Table 2 showed significant differences ($p \leq 0.05$) using Duncan's multiple comparison tests. The resulting sensory scores in the six attributes were significantly different ($p \leq 0.05$) among the nine BF samples, which indicated that the oxidation state of tallow was of prime importance in the formation of beeflike flavours. In particular, BF6 showed very strong meaty, beefy, tallowy and simulate attributes and was very weak in burnt and off-flavour notes compared to other samples.

As shown in Table 2, BF1, BF2, BF3, BF8 and BF9 were very strong in burnt and off-flavour notes. Among them, BF1 was the control sample with unoxidized tallow. Tallow added to prepare BF2 and BF3 were in low oxidation states. Due to lacking characteristic flavour precursors and degradation products of oxidized tallow, the aroma of those three BF samples was unacceptable. A significant increase in perceived beefy and meaty aroma intensity was observed when mildly oxidized tallow, BF4, BF5 or BF6, was added to prepare BF samples, mainly because mildly oxidized tallow contained plentiful flavour precursors which contributed to the formation of characteristic beef aroma. However, BF5 showed evident rancidity and sulphurous aroma because of abundant supervenient volatile acids. When high oxidized tallow used to prepare BF7, BF8 and BF9, more undesirable flavours came into being, such as burnt and off-flavour.

As can be seen from sensory evaluation results, beeflike flavours generated from Maillard reaction of mildly oxidized tallow and other ingredients were more coordinated and full of characteristic beef aroma. In order to obtain desirable flavours, the suggested chemical parameters of oxidized tallow should be PV 70.45–100.17 meq./kg, AV 134.28–137.22 mg KOH/g, and p-AV 23.79–49.11.

### 3.2. Analysis of volatile compounds of oxidized tallow samples

During oxidation of tallow, plentiful volatile compounds were identified, such as aldehydes, ketones, alcohols, carboxylic acid, lactones, furans and others, as listed in Table 3. These volatile compounds were mainly formed during the oxidation of the primary unsaturated fatty acids in tallow, i.e. linoleic, oleic and palmitoleic acid [20], and contributed to the overall aroma of BFs.

The major volatiles in oxidized tallow included 21 aldehydes, 4 ketones, 7 alcohols, 13 carboxylic acid, 5 esters, 1 lactone, 4 furans

**Table 3**
Volatile compounds identified in oxidized tallow by SPME-GC/MS.

| No. | Volatile compounds | RI[a] | ID[b] | No. | Volatile compounds | RI | ID |
|---|---|---|---|---|---|---|---|
| | Aldehydes | | | | Carboxylic acid | | |
| 1 | Pentanal | 884 | B | 33 | Formic acid | 866 | A |
| 2 | Hexanal | 1085 | B | 34 | Acetic acid | 885 | A |
| 3 | Heptanal | 1198 | B | 35 | Butyric acid | 1176 | A |
| 4 | (Z)-2-heptenal | 1313 | B | 36 | Hexanoic acid | 1458 | A |
| 5 | Octanal | 1350 | B | 37 | Heptanoic acid | 1584 | A |
| 6 | (E,E)-2,4-heptadienal | 1432 | A | 38 | Octanoic acid | 1691 | A |
| 7 | (E)-2-octenal | 1478 | B | 39 | Nonanoic acid | 1771 | A |
| 8 | (E)-4-nonenal | 1499 | B | 40 | Decanoic acid | 1835 | A |
| 9 | Nonanal | 1505 | A | 41 | Lauric acid | 1933 | C |
| 10 | (E)-2-nonenal | 1615 | B | 42 | Tetradecanoic acid | 2011 | A |
| 11 | (Z)-4-decenal | 1631 | B | 43 | Hexadecanoic acid | 2079 | A |
| 12 | Decanal | 1637 | A | 44 | Oleic acid | 2170 | A |
| 13 | (E,E)-2,4-nonadienal | 1697 | B | 45 | Stearic acid | 2176 | A |
| 14 | (Z)-2-decenal | 1706 | B | | Esters | | |
| 15 | (E)-2-decenal | 1725 | B | 46 | Diethyl phthalate | 1959 | C |
| 16 | (Z)-4-undecenal | 1732 | B | 47 | Methyl palmitoleate | 2034 | B |
| 17 | Z-citral | 1718 | C | 48 | Diisobutyl phthalate | 2065 | C |
| 18 | E-citral | 1745 | C | 49 | Methyl oleate | 2097 | B |
| 19 | (E,E)-2,4-decadienal | 1784 | B | 50 | Octadecanoic acid methyl ester | 2101 | B |
| 20 | 2-Undecenal | 1805 | B | | Lactone | | |
| 21 | 5-(Hydroxymethyl)furfural | 1829 | C | 51 | Nonanolactone | 1867 | A |
| | Alcohols | | | | Furans | | |
| 22 | 4-Ethynyl-4-methyl-1,5-hexadiene-3-ol | 868 | C | 52 | 2-Pentylfuran | 1265 | A |
| 23 | 1-Heptanol | 1331 | A | 53 | 4-Methyl-2-propylfuran | 1417 | C |
| 24 | 1-Octen-3-ol | 1336 | A | 54 | 2-Butyltetrahydrofuran | 1543 | B |
| 25 | 1-Octanol | 1485 | A | 55 | 5-Pentyl-2(3H)-Furanone | 1756 | C |
| 26 | Glycerin | 1596 | A | | Thiophenes | | |
| 27 | 1,7-Heptanediol | 1845 | C | 56 | 2-(Dimethylamino)-3-phenylbenzo[b]thiophene | 1337 | C |
| 28 | 4,4,6-Trimethyl-2-cyclohexen-1-ol | 1858 | C | | Alkanes | | |
| | Ketones | | | 57 | D-Limonene | 1290 | B |
| 29 | 7-Ethyl-4-nonanone | 1871 | C | 58 | β-Bisabolene | 1840 | C |
| 30 | 4-(Benzoyloxy)-2H-pyran-3-one | 1127 | C | | Others | | |
| 31 | 2-Isopropylcyclohexan-1-one | 1763 | C | 59 | 2-Amino-6-methoxypurine | 1245 | C |
| 32 | 2-Methoxy[1]benzothieno[2,3-c]quinolin-6(5H)-one | 1655 | C | 60 | Methoxy-phenyl-oxime | 1405 | C |
| | | | | 61 | 3,5-Dithiahexanol 5,5-dioxide | 1023 | C |

[a] Retention indices were determined by using a series of hydrocarbons in range of C6–C26 on the DB-WAX column.
[b] ID, identification method: A, mass spectrum and RI agree with that of the authentic compound run under similar GC–MS conditions; B, mass spectrum and RI agree with literature data; and C, compare mass spectrum and RI with the reference compounds on the basis of MS spectra (NIST 98 & Wiley 130K).

and 1 thiophene (Table 3). From a quantitative point of view, aldehydes were the most abundant compounds among a total of 61 volatile compounds tentatively identified in the oxidized tallow. C6–C10 n-aldehydes with low threshold values were the major volatiles and mainly associated with tallowy aroma. Unsaturated n-2-alkenals may play a more important role in the formation of species-specific flavour. Mottram [2] reported that (E,E)-2,4-decadienal generated from linoleic acid oxidation was a possible precursor of 2-pentylpyridine that had been identified in thermally controlled oxidation tallow. (E)-2-decenal was the most abundant oxidation compound, Rochat and Chaintreau [21] reported that (E)-2-decenal was related to a tallowy, orange and spicy odour. In addition, aldehydes were believed not only to contribute to the odour of food, but also to react with other compounds to provide species-specific flavour through amino-carbonyl reactions.

Unsaturated alcohols such as 1-penten-3-ol and 1-octen-3-ol were reported as components of boiled beef flavour [22]. Only 1-octen-3-ol was identified in the present study. Mottram [23] reported that 1-octen-3-ol generated from arachidonic acid oxidation had a mushroom flavour and made an important contribution to pork flavour.

Furans could be produced from sugar caramelization and carbohydrate degradation [24]. Even though none of the various furans had been accounted as being crucial to meaty flavour, they had been regarded to contribute to the overall odour of boiled meat [22]. 2-Pentylfuran with beany and grassy flavour was commonly found in several beef products such as canned beef [25], boiled beef [26], roasted beef [9] and also found in our study.

It was well known that lipase could partly or entirely hydrolyse fat into free fatty acids, which could exactly explain the fact that why much more hexadecanoic acid, oleic acid and stearic acid were identified in the enzymatic hydrolysis-mild thermal oxidation tallow than thermally controlled oxidation tallow. Besides, lauric acid, tetradecanoic acid, 1,7-heptanediol, Z-citral, E-citral, 5-(hydroxymethyl)furfural, 2-butyltetrahydrofuran, (Z)-4-undecenal, (Z)-4-decenal, (E)-4-nonenal, 5-pentyl-2(3H)-furanone, D-limonene and 3,5-dithiahexanol-5,5-dioxide were not identified in thermally controlled oxidation tallow [16], illustrating that these compounds might be produced during the enzymatic hydrolysis-mild thermal oxidation of tallow.

**Table 4**
The characteristic volatile compounds of the optimal BF sample, determined by GC–O analysis, the compounds' detection frequencies and odour descriptors, and their potential flavour precursors.

| Code[a] | Compound | Detection frequency | Odour description[b] | ID[c] | Flavour precursors (regression coefficient)[d] |
|---|---|---|---|---|---|
| A1 | 3-Methyl butanal | 3 | Fresh, fermented | C | |
| A2 | Hexanal | 5 | Green, floral | B | |
| A3 | Furfural | 4 | Caramel, herby, nutty | B | 37(0.02) |
| A4 | Heptanal | 4 | Tallowy, fresh | B | |
| A5 | 3-(Methylthio)propionaldehyde | 6 | Boiled meat, umami, fried potato | B | |
| A6 | (Z)-2-heptenal | 3 | Fried potato, popcorn | B | |
| A7 | Benzaldehyde | 4 | Nutty, savoury | B | |
| A8 | Octanal | 5 | Nutty, roasted meaty | B | |
| A9 | Phenylacetaldehyde | 4 | Honey, buttery | B | 22(0.05) |
| A10 | Nonanal | 6 | Nutty, buttery, beefy | B | |
| A11 | Decanal | 4 | Buttery, sweet, nutty | B | |
| A12 | 2-Undecenal | 5 | Tallowy, nutty, almond | B | |
| A13 | 2-Nonen-1-ol | 3 | Mushroom, boiled potato | B | |
| A14 | 2,3-Pentanedione | 5 | Pungency, rancid, sour | B | |
| A15 | Acetol | 3 | Buttery, boiled eggs | B | |
| A16 | 3-Hydroxy-2-butanone | 4 | Buttery, fruity | C | |
| A17 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | 5 | Meaty, sulphurous | B | |
| A18 | Acetic acid | 6 | Sour, vinegar | B | |
| A19 | Butanoic acid | 5 | Rancid, fermented | B | |
| A20 | Hexanoic acid | 4 | Mushroom, sour | B | |
| A21 | γ-Hexalactone | 5 | Roasted meat, lemon | B | 7(0.03), 8(0.03), 10(0.03), 11(0.03), 13(0.04), 15(0.04), 16(0.04), 20(0.03), 24(0.05), 28(0.04), 54(0.06) |
| A22 | γ-Nonalactone | 5 | Herby, bitter | B | |
| A23 | 2-Pentylfuran | 4 | Metallic, fresh, fruity | B | |
| A24 | 2-Hexylfuran | 5 | Meaty, savoury, oil | B | 7(0.03), 10(0.03), 37(0.04), 42(0.03), 54(0.03), 59(0.02) |
| A25 | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | 5 | Roasted meat, nutty, almond | B | 57(0.13) |
| A26 | 2-Acetylpyrrole | 3 | Lemon, nutty, sour | B | |
| A27 | 2-Pentylthiophene | 3 | Metallic, rubber tubing | C | 33(0.09) |
| A28 | 2-Hexylthiophene | 5 | Sulphurous, rancid, meaty | C | 32(0.11), 58(0.08) |
| A29 | 5-Methyl-2-thiophenecarboxaldehyde | 3 | Sweet, fruity | C | |
| A30 | 3-Ethyl-2-thiophenecarboxaldehyde | 5 | Beefy, sulphurous, rancid | B | |
| A31 | 2-Methyl-3-furanthiol | 6 | Tallowy, meaty, sulphurous, rancid | C | |
| A32 | Furfuryl mercaptan | 3 | Fruity, nutty | B | |
| A33 | Bis(2-methyl-3-furyl)disulphide | 6 | Boiled meat, roasted meat, ham, sulphurous | B | |
| A34 | D-Limonene | 4 | Fruity, sweet | B | |

[a] Code representing the 34 aroma-active compounds observed in GC–O.
[b] Odour descriptor expressed by panellists at a given retention index in GC–O which was the same as that in GC–MS.
[c] ID, Identification method: B, identified by comparing it with the reference compounds on the basis of MS spectra (NIST 98 & Wiley 130K), LRI, odour quality and authentic compounds; and C, identified tentatively by comparing it with literature data on the basis of LRI and odour quality.
[d] Arabic numbers correspond to the volatile compounds in Table 3.

### 3.3. Determination of odour-active compounds

The volatile compounds extracted from beeflike flavours by SPME were isolated and detected by panellists at the sniffing port of GC–O. Compounds with detection frequency greater than 50% were believed to be characteristic flavour components. As was shown in Table 4, a total of 34 aroma-active compounds were identified, mainly consisting of heterocyclic sulphur or nitrogen compounds and aldehydes. Among the 34 aroma-active compounds, acetic acid, nonanal, 3-(methylthio)propionaldehyde, 2-methyl-3-furanthiol and bis(2-methyl-3-furyl)disulphide were the most potent odourants.

In our foregoing statements, we knew that hexanal (green, floral), heptanal (tallowy, fresh), (Z)-2-heptenal (fried potato, popcorn), octanal (nutty, roasted meaty), decanal (buttery, sweet, nutty), 2-undecenal (tallowy, nutty, almond), acetic acid (sour, vinegar), butanoic acid (rancid, fermented), hexanoic acid (mushroom, sour), 2-pentylfuran (metallic, fresh, fruity) and D-limonene (fruity, sweet) were produced during oxidation of saturated or unsaturated fatty acids from tallow, which were also found in the GC–O profiles. It can be speculated that these compounds may not participate in amino-carbonyl reactions, but they played a crucial role in the formation of overall beeflike flavours. Particularly, lipid-derived aroma-active aldehydes may contribute to the tallowy note of BFs.

It was reported that thiol, sulphide or disulphide group substituted furans at the 3-positon had been regarded to be associated with typical meat-like aroma [12]. In this study, 2-methyl-3-furanthiol and bis(2-methyl-3-furyl)disulphide were identified. 2-Methyl-3-furanthiol responsible for beeflike aroma had been demonstrated by several research groups. For example, Baek et al. [27] reported that 2-methyl-3-furanthiol was the most intense compound in the beeflike process flavour, which was produced from hydrolysed vegetable proteins. 2-Methyl-3-furanthiol was found by Mottram [2] having high aroma values in cooked lean beef, along with methional and bis(2-methyl-3-furyl)disulphide. Kerscher and Grosch [28] proposed 2-methyl-3-furanthiol as one of the most potent odourants in boiled beef. Moon et al. [29]

also indicated that 2-methyl-3-furanthiol was responsible for beef broth or roasted meat odour.

Most of the thiophenes found in meat were identified to be substituted at the 2-position [29]. Four thiophenes were found in this study, such as 2-pentylthiophene, 3-ethyl-2-thiophenecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde and 2-hexylthio-phene. All of them were substituted at the 2-position, which was consistent with previous conclusions. 5-Methyl-2-thiophenecarboxaldehyde was reported to be found in cooked beef [30]. In addition to the aforementioned compounds, 2-acetylpyrrole was reported to be identified in cooked ground beef [31], fresh, frozen beef stew and canned beef stew [32] and boiled beef [29]. Wu and Cadwallader [33] indicated that furaneol was an important odorant in the overall aroma of the meatlike process flavouring produced from hydrolysed vegetable protein.

Compared with our previous study [12], nearly half of the characteristic flavour compounds (Table 4) were not identified in the BF samples generated from the Maillard reaction of thermally controlled oxidation tallow, such as 2,3-pentanedione, γ-hexalactone, furfural, 3-hydroxy-2-butanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 3-(methylthio)propionaldehyde, 2-pentylthio-phene, acetol, 2-hexylthiophene, furfuryl mercaptan, 2-acetylpyrrole and D-limonene. Enzymatic hydrolysis-mild thermal oxidation tallow changed the reactants composition and pathways of Maillard reaction, leading to generation of different Maillard reaction products. These different odour-active compounds in BFs brought about the final flavour differences.

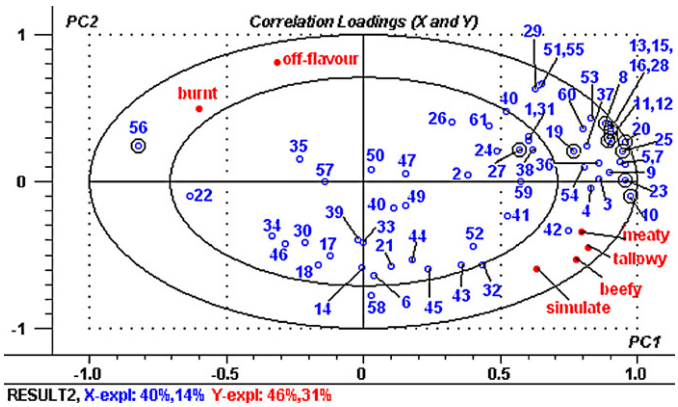


**Fig. 1.** An overview of the variation found in the mean data from partial least squares regression (PLSR) correlation loadings plot for tallow oxidation products (*X*-matrix) and sensory attributes (*Y*-matrix). Significant variables are marked. Ellipses show $r^2$ = 50% and 100%, respectively.

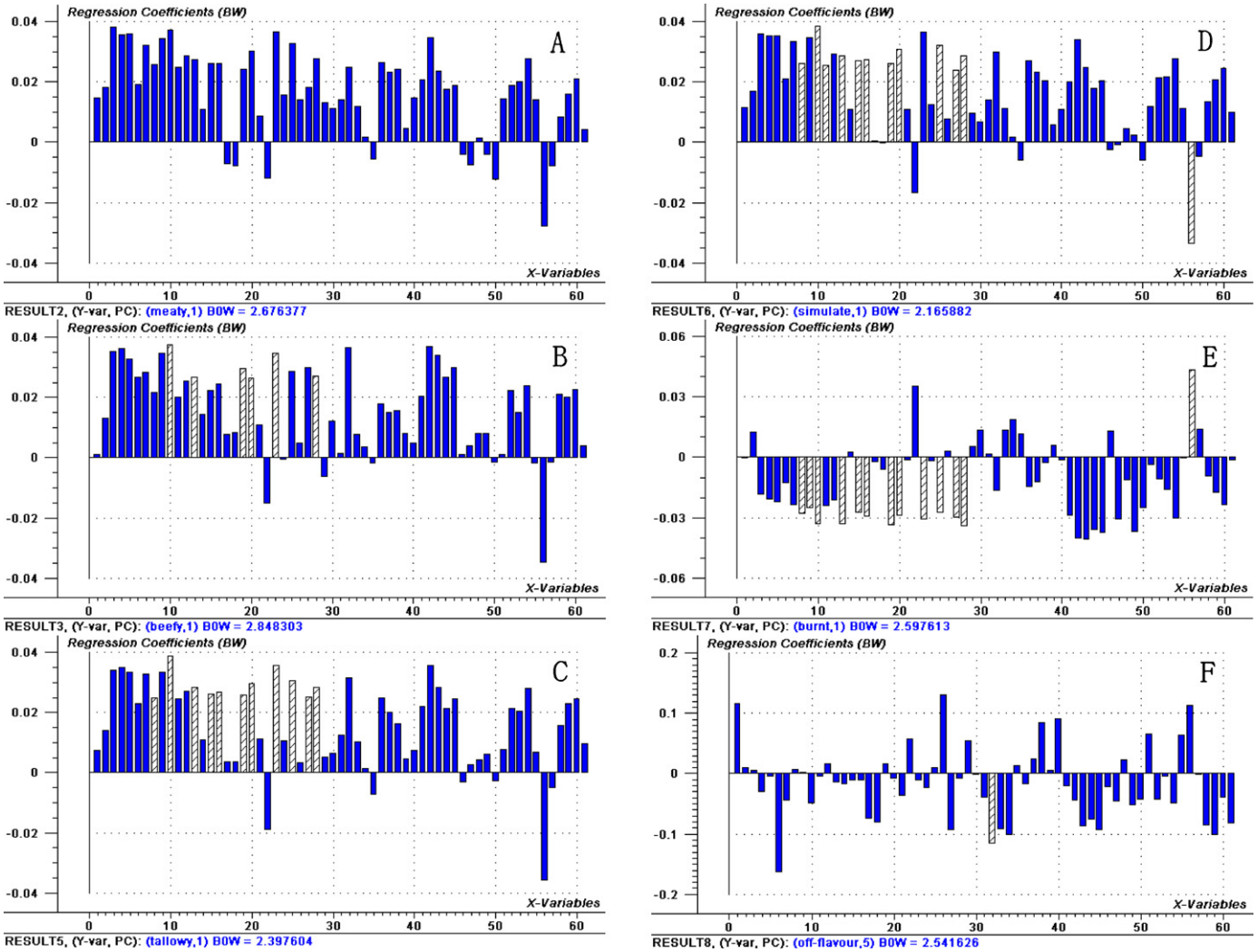


**Fig. 2.** Standardized, estimated regression coefficients and significance indications (streaked bars) from PLS1 prediction models for the sensory attributes variables meaty (A), beefy (B), tallowy (C), simulate (D), burnt (E) and off-flavour (F) from tallow oxidation products.

### 3.4. Relationship between chemical composition of oxidized tallow samples and sensory attributes of BF samples

To study overall relationship between sensory profiles by the panel and GC–MS data of oxidized tallow samples, two data sets were analysed by PLS2. More than 90 peaks were observed in purge and trap GC–MS profiles but only 61 peaks (Table 3) were used as variables in the subsequent PLS analysis, which commonly detected at least in three samples. The *X*-matrix was designated as GC–MS profiles. The *Y*-matrix was designated as sensory attributes (Fig. 1). The derived PLSR model with 3 principal components (PC) explained 72% of the validated variation. Further PCs did not provide any predictive improvement in the *Y*-matrix obtained. Thus PC1 vs. PC2 and PC2 vs. PC3 were explored. We did not show PC2 vs. PC3 here because no additional information was gained through their examination.

The variables marked with small circles were determined to be significant. The big circles indicated 50% and 100% explained variance, respectively. For the variation in PC1, it can be noted that all the GC–MS profiles except Z-citral, E-citral, 4-ethynyl-4-methyl-1,5-hexadiene-3-ol, 4-(benzoyloxy)-2H-pyran-3-one, acetic acid, butyric acid, diethyl phthalate, 2-(dimethylamino)-3-phenylbenzo[b]thiophene and D-Limonene were located on the right side as well as four sensory attributes (meaty, beefy, tallowy, and simulate), while the variation in PC2 was explained by all the GC–MS profiles and sensory attributes (meaty, beefy, tallowy and simulate placed in the lower right corner, burnt and off-flavour placed in the upper left corner). In addition, all the sensory variables were outside of the $r^2 = 50\%$ ellipse, indicating that the sensory attributes were well explained by the PLSR model [34]. Besides, two sensory clusters were located in the opposite sides, four sensory attributes (meaty, beefy, tallowy and simulate) out of six were quite closely located, which meant these four sensory attributes covaried with some GC–MS variables simultaneously.

To further study which tallow oxidation compounds had significant contribution to each sensory attribute of BFs, PLS1 regression analysis was carried out by calculating estimated regression coefficients from jack-knife uncertainty test (Fig. 2). Most tallow oxidation compounds positively correlated to meaty, beefy, tallowy and simulate notes (Fig. 2A–D). No compounds were significantly correlated to meaty note. Beefy note was significantly correlated to (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, 2-undecenal, 1-heptanol and 4,4,6-trimethyl-2-cyclohexen-1-ol and 67% of the variation was explained. The compounds (E)-4-nonenal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E)-2-decenal, (Z)-4-undecenal, (E,E)-2,4-decadienal, 2-undecenal, 1-heptanol, 1-octanol, 1,7-heptanediol and 4,4,6-trimethyl-2-cyclohexen-1-ol had significant influence on tallowy note and 69% of the variation was explained. The compounds (E)-4-nonenal, (E)-2-nonenal, (Z)-4-decenal, (E,E)-2,4-nonadienal, (E)-2-decenal, (Z)-4-undecenal, (E,E)-2,4-decadienal, 2-undecenal, 2-(dimethylamino)-3-phenylbenzo[b]thiophene, 4,4,6-trimethyl-2-cyclohexen-1-ol, 1-octanol and 1,7-heptanediol showed significant correlation with simulate note, which regression coefficients were positive except 2-(dimethyl-amino)-3-phenylbenzo[b]thiophene, and 69% of the variation was explained. Different from the four aforementioned attributes, most compounds were correlated to burnt note and had significant and negative regression coefficients, e.g. (E)-4-nonenal, nonanal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E)-2-decenal, (Z)-4-undecenal, (E,E)-2,4-decadienal, 2-undecenal, 1-heptanol, 1-octanol, 1,7-heptanediol and 4,4,6-trimethyl-2-cyclohexen-1-ol. Only 2-(dimethylamino)-3-phenylbenzo[b]thiophene showed significant and positive correlation with burnt note. As to off-flavour attribute, 2-methoxy[1]benzothieno-[2,3-c]quinolin-6(5H)-one was the only compound which showed significant and negative correlation, and 65% of the variation was explained. These correlation results were consistent with the findings of many researches. Rochat and Chaintreau [21] reported that saturated and unsaturated aldehydes in the range of C6–C10 along with n-alka-2,4-dienal were believed to play an important role in characteristic tallowy aroma. Cerny and Grosch [35] indicated that (E)-2-nonenal and (E,E)-2,4-decadienal provided the tallowy note in the roasted beef flavour. According to Grosch [36], aldehydes, especially olefine aldehydes and n-alka-2,4-dienal were the key compounds with tallowy aroma note in thermal process beef flavourings. Besides, aldehydes were important intermediate products which may react with other compounds to produce flavour through amino-carbonyl reactions.

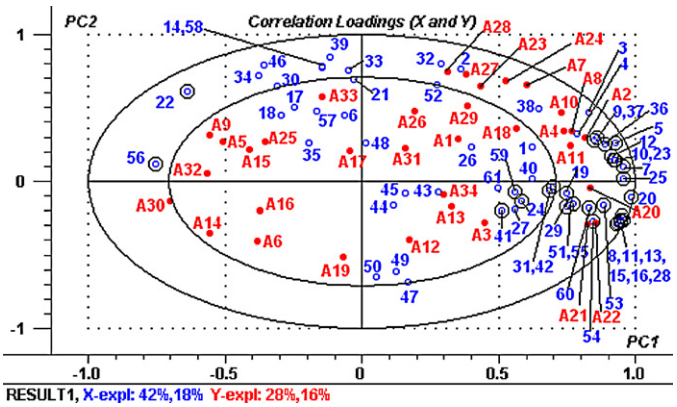


**Fig. 3.** An overview of the variation found in the mean data from partial least squares regression (PLSR) correlation loadings plot for tallow oxidation products (*X*-matrix) and aroma-active compounds (*Y*-matrix). Odour-active compounds of A1-34 correspond to the code compounds in Table 4.

### 3.5. Relationship between chemical composition of oxidized tallow samples and odour-active compounds of BF samples

To examine the relationship between GC–MS data from oxidized tallow and GC–O profiles from BFs, a PLS2 regression analysis was carried out. The derived PLSR model included three significant PCs explaining 71% of the cross-validated variance. Fig. 3 shows the correlation loadings for 61 GC–MS peaks (*X*-matrix) and 34 GC–O profiles (*Y*-matrix). PC2 versus PC3 was not presented here, because no additional information was gained through their examination. Due to the quantity and complexity of GC–MS and GC–O profiles, it was not easy to figure out the significant variables. To further study which compounds made greater contribution to each aroma-active compound, PLS1 regression analyses (figures not shown) were carried out, and the correlating significant compounds identified in oxidized tallow were listed in Table 3.

Among these 34 aroma-active compounds, A1: 3-methyl butanal, A2: hexanal, A4: heptanal, A6: (Z)-2-heptenal, A8: octanal, A10: nonanal, A11: decanal, A12: 2-undecenal, A18: acetic acid, A19: butanoic acid, A20: hexanoic acid, A22: γ-nonalactone, A23: 2-pentylfuran and A34: D-limonene were tallow oxidation products. As can be seen in Table 4, heptanoic acid showed significance to A3: furfural. However, this was only the mathematic correlation, and it could not be considered that heptanoic acid was the flavour precursor of furfural. In fact, it was accepted that furfural was generated from the dehydration of deoxyglycosones. 4-Ethynyl-4-methyl-1,5-hexadiene-3-ol had a significant influence on A9. (E)-2-octenal, (E)-4-nonenal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E)-2-decenal, (Z)-4-undecenal, 2-undecenal, 1-octen-3-ol, 4,4,6-trimethyl-2-cyclohexen-1-ol and 2-butyltetrahydrofuran showed significant and positive correlation with A21. (E)-2-octenal, (E)-2-nonenal, heptanoic acid, tetradecanoic acid, 2-butyltetrahydrofuran and

2-amino-6-methoxypurine were positively and significantly correlated to A24, which was consistent with the findings of many researches that furans were generated from oxidation of unsaturated aldehydes [36]. D-Limonene was significantly correlated to A25. Formic acid showed positive correlation to A27. 2-Methoxy[1]benzothieno[2,3-c]quinolin-6(5H)-one and β-bisabolene positively correlated to A28. Meanwhile, some volatile compounds in oxidized tallow showed significant and negative correlation with aroma-active compounds. For example, 2-(dimethylamino)-3-phenylbenzo[b]thiophene, which was only found in low oxidation tallow T1, T2 and T3, was negatively correlated to A2, A4, A8, A10, A11 and A20. All detected volatile compounds in oxidized tallow showed non significance to A25: 2,5-dimethyl-4-hydroxy-3(2H)-furanone, A26: 2-acetylpyrrole, A27: 2-pentylthiophene, A31: 2-methyl-3-furanthiol and A33: bis(2-methyl-3-furyl) disulphide. The reasons may be like that some of them were produced from the sugars and amino acids degradation. For example, some researchers had reported that 2,5-dimethyl-4-hydroxy-3(2H)-furanone was produced from hydrolysed vegetable protein [33]; 2-methyl-3-furanthiol, along with bis(2-methyl-3-furyl)disulphide which was a dimmer of 2-methyl-3-furanthiol, was formed via thermal degradation of thiamine or as the thermal product of pentoses and cysteine [37].

From these correlation results, it was proposed that the characteristic beeflike flavour precursors from oxidized tallow might be hexanal, heptanal, octanal, nonanal, decanal, pentanal, acetic acid, butanoic acid, hexanoic acid, tetradecanoic acid, 3-methylbutanal, 2-pentylfuran, 1-heptanol, 1-octanol, D-limonene, 1,7-heptandiol, 2-butyltetrahydrofuran, γ-nonalactone, 2-undecenal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, (E)-2-nonenal, (Z)-4-undecenal, (E)-2-octenal, (E)-2-decenal, (Z)-4-decenal, (E)-4-nonenal, (Z)-2-heptenal and 5-pentyl-2(3H)-furanone. Among these, tetradecanoic acid, D-limonene, 1,7-heptandiol, 2-butyltetrahydrofuran, (Z)-4-undecenal, (Z)-4-decenal, (E)-4-nonenal and 5-pentyl-2(3H)-furanone were unique products generated from enzymatic hydrolysis-mild thermal oxidation of tallow. The others were common products generated from pure thermal oxidation of tallow in our previous study [16].

## 4. Conclusions

Enzymatic hydrolysis-mild thermal oxidation of tallow plays a significant role in the formation of the characteristic beeflike flavours during lipid oxidation and subsequent Maillard reaction. Thirty four compounds were selected as specific compounds to represent beef odour. The characteristic flavour precursors were identified through PLSR analyses between GC–O responses, quantitative descriptive sensory data and GC–MS profiles of oxidized tallow. Twenty nine compounds were believed to be the potential characteristic flavour precursors, among these, tetradecanoic acid, D-limonene, 1,7-heptandiol, 2-butyltetrahydrofuran, (Z)-4-undecenal, (Z)-4-decenal, (E)-4-nonenal and 5-pentyl-2(3H)-furanone were unique products generated from enzymatic hydrolysis-mild thermal oxidation of tallow. The existing of these precursors made BFs more similar to natural beef flavour. Therefore, "enzymatic hydrolysis-mild thermal oxidation" method is a recommendable technology and can be widely applied in future.

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## References

[1] A.M. Pearson, L.M. Wenham, W.A. Carse, K. McLeod, C.L. Davey, A.H. Kirton, J. Anim. Sci. 36 (1973) 511.
[2] D.S. Mottram, Food Chem. 62 (1998) 415.
[3] D.S. Mottram, R.A. Edwards, J.H.H. Macfie, J. Sci. Food Agric. 33 (1982) 934.
[4] F. Caporaso, J.D. Sink, P.S. Dimick, C.J. Mussinan, A. Sanderson, J. Agric. Food Chem. 25 (1977) 1230.
[5] I. Hornstein, P.F. Crowe, J. Agric. Food Chem. 11 (1963) 147.
[6] I. Hornstein, P.F. Crowe, J. Agric. Food Chem. 8 (1960) 494.
[7] G.J. Hartman, Q.Z. Jin, G.J. Collins, K.N. Lee, C.T. Ho, S.S. Chang, J. Agric. Food Chem. 31 (1983) 1030.
[8] L.J. Farmer, D.S. Mottram, F.B. Whitfield, J. Sci. Food Agric. 49 (1989) 347.
[9] D.B.S. Min, K. Ina, R.J. Peterson, S.S. Chang, J. Food Sci. 44 (1979) 639.
[10] D. Machiels, S.M. van Ruth, M.A. Posthumus, L. Istasse, Talanta 60 (2003) 755.
[11] J.C. Xie, B.G. Sun, F.P. Zheng, S.B. Wang, Food Chem. 109 (2008) 506.
[12] S.Q. Song, X.M. Zhang, K. Hayat, M.G. Huang, P. Liu, E. Karangwa, F.L. Gu, C.S. Jia, S.Q. Xia, Z.B. Xiao, Y.W. Niu, J. Chromatogr. A 1217 (2010) 7788.
[13] ISO-8589, Sensory Analysis – General Guidance for the Design of Test Rooms, International Organisation for Standardisation, Geneva, Switzerland, 2007.
[14] M. Morten, G.V. Civille, B.T. Carr, Sensory Evaluation Techniques, CRC Press, Boca Raton, Florida, 1999, p. 161.
[15] J.S. Vestergaard, M. Martens, P. Turkki, LWT – Food Sci. Technol. 40 (2007) 1095.
[16] S.Q. Song, X.M. Zhang, K. Hayat, P. Liu, C.S. Jia, S.Q. Xia, Z.B. Xiao, H.X. Tian, Y.W. Niu, Food Chem. 124 (2011) 203.
[17] H. Martens, M. Harald, Multivariate Analysis of Quality: An Introduction, Wiley, Chichester, UK, 2001.
[18] Unscrambler User Manual, CAMO ASA, Trondheim, 2006, p. 195.
[19] F. Westad, H. Martens, J. Near Infrared Spectrosc. 8 (2000) 117.
[20] D. Ladikos, V. Lougovois, Food Chem. 35 (1990) 295.
[21] S. Rochat, A. Chaintreau, J. Agric. Food Chem. 53 (2005) 9578.
[22] F. Shahidi, L.J. Rubin, L.A. D'Souza, R. Teranishi, R.G. Buttery, Crit. Rev. Food Sci. Nutr. 24 (1986) 141.
[23] D.S. Mottram, J. Sci. Food Agric. 36 (1985) 377.
[24] T. Shibamoto, J. Agric. Food Chem. 28 (1980) 237.
[25] T. Persson, E. von Sydow, J. Food Sci. 38 (1973) 377.
[26] C. Hirai, K.O. Herz, J.A.N. Pokorny, S.S. Chang, J. Food Sci. 38 (1973) 393.
[27] H.H. Baek, C.J. Kim, B.H. Ahn, H.S. Nam, K.R. Cadwallader, J. Agric. Food Chem. 49 (2001) 790.
[28] R. Kerscher, W. Grosch, Z. Lebensm. Unters. Forsch. 204 (1997) 3.
[29] S.Y. Moon, M.A. Cliff, E.C.Y. Li-Chan, Food Res. Int. 39 (2006) 294.
[30] R.A. Wilson, C.J. Mussinan, I. Katz, A. Sanderson, J. Agric. Food Chem. 21 (1973) 873.
[31] G.M. Leod, J.M. Ames, J. Food Sci. 51 (1986) 1427.
[32] R.J. Peterson, S.S. Chang, J. Food Sci. 47 (1982) 1444.
[33] Y.-F.G. Wu, K.R. Cadwallader, J. Agric. Food Chem. 50 (2002) 2900.
[34] S.Q. Song, X.M. Zhang, K. Hayat, C.S. Jia, S.Q. Xia, F. Zhong, Z.B. Xiao, H.X. Tian, Y.W. Niu, Sens. Actuators B 147 (2010) 660.
[35] C. Cerny, W. Grosch, Z. Lebensm. Unters. Forsch. 194 (1992) 322.
[36] W. Grosch, in: A.J. MacLeod (Ed.), Food Flavors, Elsevier, Amsterdam, 1982, p. 325.
[37] F.B. Whitfield, D.S. Mottram, S. Brock, D.J. Puckey, L.J. Salter, J. Sci. Food Agric. 42 (1988) 261.