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Effect of enzymatic hydrolysis with subsequent mild thermal oxidation of tallow on precursor formation and sensory profiles of beef flavours assessed by partial least squares regression

Shiqing Song [a], Qi Tang [a], Khizar Hayat [c], Eric Karangwa [b], Xiaoming Zhang [b,*], Zuobing Xiao [a]

[a] School of Perfume and Aroma Technology, Shanghai Institute of Technology, 200235 Shanghai, PR China
[b] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, 214112 Jiangsu, PR China
[c] Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan

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ABSTRACT

Effects of different pretreatments of tallow on flavour precursor development and flavour profiles of beef flavours (BFs) were evaluated. Analysis of free fatty acids and volatiles of tallow by GC and GC–MS indicated that the enzymatic hydrolyzed–thermally oxidized tallow formed the most characteristic flavour precursors compared with others. The results of descriptive sensory analysis confirmed that beef flavour 4 from enzymatic hydrolyzed–thermally oxidized tallow had the strongest beefy, meaty and odour characteristics, followed by beef flavour 2 from oxidized tallow. Electronic nose data confirmed the accuracy of the sensory analysis results. The correlation analysis of 51 volatile compounds in tallow and sensory attributes of BFs showed that some compounds, especially aldehydes, made a significant contribution to sensory attributes. Correlation analysis of free fatty acids and sensory attributes through partial least squares regression (PLSR) confirmed that the moderate enzymatic hydrolysis-thermal oxidation pretreatment of tallow was necessary to achieve the characteristic beef flavour.

1. Introduction

Over the years, meat flavours have increasingly found application in meat analogues and processed instant foods. The special-specific meat flavours added in foods are necessary to stimulate people's appetite.

Generally, two methods are employed to enhance the special-specific flavour, one is to add flavouring substances (i.e. top note) to the meat flavours after thermal reaction, and the other one is the lipid involved in the Maillard reaction (Qiu, Zhao, Sun, Zhou, & Cui, 2013). However, although the first method can offer a rich top note, flaws such as the impermanent meat odour and flavour existed. If animal lipid is added directly to the reaction system without any pretreatment, it is very difficult to form typical flavour precursors and thus lacks the characteristic flavour of different meats (beef, pork, chicken and mutton) because the process is not enough to cause lipid oxidization.

The characteristic meat flavour can be enhanced by adding the oxidized lipid to the Maillard reaction. Oxidation of unsaturated fatty acids, especially when induced by heat, results in volatile compounds which can produce the characteristic odours of specific meats (Wood et al., 2008). Previous research also demonstrated that oxidized tallow, containing more degradation products, especially aldehydes, were able to participate in Maillard reactions and modify flavour formation to a greater extent than the non-oxidized control (Song et al., 2012).

The moderate oxidation of lipids is a prerequisite to achieve the characteristic flavour. If lipid oxidation is inadequate, the characteristic meat flavour will not develop; but excessive oxidation will lead to unpleasant smells. The controlled oxidization of the lipids can be done by thermally controlled oxidation or catalytic oxidation by lipoxygenase, and different flavours are produced based on the treatments. However, thermally controlled oxidation needs high temperature, which is energy-intensive and not easy to control, and the similarity to natural beef flavour needs improvement. The second technique overcomes some drawbacks, but lipoxygenase mainly catalyses the oxidation of free polyunsaturated fatty acids in the fat. However, it is well known that animal fat is low in unsaturated fatty acid and mainly contains triglycerides, which leads to lower efficiency of oxidation.

To solve these problems, an "enzymatic hydrolysis–thermal oxidation" method is proposed to prepare characteristic flavour precursors from oxidized tallow, which means initial hydrolysis of tallow by lipase, then thermal oxidation under mild conditions to obtain oxidized tallow. The main reason is that more amino groups in phospholipids 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 different pathways of the Maillard reaction. Recently, the enzymatic hydrolysis–thermal oxidation method was used to prepare beeflike flavour, and eight unique compounds were generated from enzymatic hydrolysis–thermal oxidation of tallow

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

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compared with pure thermal oxidation of tallow (Shi et al., 2013). However, it is still unknown how enzymatic hydrolysis pretreatment of lipid influences the formation of flavour precursors and aroma characteristics of meat process flavours.

The principal objectives of the present study were to analyse the free fatty acids of pre-treated tallow using GC analysis; identify volatile compounds released from tallow samples by SPME/GC–MS analysis and investigate the impact of various pre-treatments on the formation of major volatile compounds; compare the influence of different participants in Maillard-reactions, the control, oxidized, enzymatic hydrolyzed and enzymatic hydrolyzed–thermally oxidized tallow, on the flavour of the final beef flavour (BF) products by descriptive sensory analysis and use of an electronic nose (e-nose), which has objective and real-time analytical capability, to gain an overall understanding of the BFs. The correlation among free fatty acids and volatile compounds of tallow, quantitative descriptive sensory data and e-nose response of BFs were investigated using PLSR to identify which free fatty acids significantly influence individual sensory attributes. Through the above analyses, the main differences between enzymatic hydrolysis–thermal oxidation, simple thermal oxidation and simple enzymatic hydrolysis were elucidated, and the best pretreatment method of tallow was then determined for preparing characteristic beef flavour precursors.

2. Materials and methods

2.1. Materials

Refined tallow (Lot No. 130215) was purchased from Anhui Muyang Oil and Fats Co., Ltd. (Anhui, China). Lipase AY "Amona" 30G, activity 30,000 u/g, was purchased from Japan Amano Co., Ltd. Beef hydrolysate (degree of hydrolysis 29.13%) was prepared according to Song et al. (2010). Hydrolyzed vegetable protein (HVP) was provided by Tianning Flavour & Fragrance Co., Ltd. (Shanghai, China). D-Xylose, glucose, DL-alanine, L-cysteine hydrochloride, glycine, taurine, thiamine and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 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 hydrolyzed tallow

Refined tallow in phosphate buffered solution (pH 6.5) was placed in the enzyme reactor at a ratio of 1:1 with mechanical stirring at 150 rpm. Lipase AY "Amona" 30G was added to the reactor with enzyme/substrate (E/S) ratio of 5.0×10^{-3} (g lipase/g tallow) when the mixture was judged to be isothermal to the water bath (45 °C). After reacting for 5 h, the sample (named T3) was heated to 95 °C for 10 min to deactivate the enzyme. The quality parameters of the sample were as follows: peroxide value (PV) 7.51 meq/kg, acid value (AV) 96.5 mg KOH/g tallow and degree of lipolysis 73.05%. It was stored at −18 °C prior to use. The experiment was repeated three times.

2.2.2. Preparation of oxidized tallow

2.2.2.1. Preparation of enzymatic hydrolyzed–mild thermally oxidized tallow. The abovementioned enzyme treated tallow (100 g) was placed in a 250 mL 4-neck round-bottom flask and heated at 80 °C in a thermostatically controlled oil bath with mechanical stirring at 200 rpm, and feeding air at a rate of 60 L/h. After heating for 3 h, the sample (named T4) was immediately cooled in ice-water and stored at −18 °C for analysis. The peroxide value (PV) and the acid value (AV) of the sample were 102 meq/kg and 49 mg KOH/g tallow, respectively. The experiment was repeated three times.

2.2.2.2. Preparation of pure thermally oxidized tallow. The oxidized tallow (named T2) was prepared according to Song et al. (2010). The oxidation state of the sample was as follows; peroxide value (PV) 105 meq/kg, p-anisidine value (p-AV) 38, and acid value (AV) 1.9 mg KOH/g tallow. The experiment was repeated three times.

2.2.3. Preparation of beef flavours (BFs)

A mixture of HVP (0.4 g), DL-methionine (0.02 g), D-xylose (0.1 g), glucose (0.1 g), L-cysteine (0.1 g), L-glutamic acid (0.025 g), L-proline (0.025 g), thiamine (0.06 g), taurine (0.05 g), tallow (T1) or pre-processed tallow (T2, T3 or T4) (1 g) was dissolved in an 8.12 g solution of the beef hydrolysate. The solution was transferred into 50 mL screw-sealed tubes. The pH was adjusted to 6.5 with 6 mol/L NaOH or 1 mol/L HCl; the tubes were tightly capped and then heated in an oil bath with magnetic stirring (150 rpm) at 110 °C for 120 min. After reaction, the tubes were immediately cooled in ice-water and based on the treatment four Maillard reaction products named BF1–4 were sampled for further analysis.

2.3. Analysis methods

2.3.1. Free fatty acid (FFA) analysis

The free fatty acids were separated from the fat by adsorption on an aminopropyl column (500 mg/3 mL Agilent AccuBOND NH2 −), then converted to their methyl esters, and identified and determined by gas chromatography, in duplicate.

2.3.1.1. Separation of FFA. The tallow samples were dissolved with an aliquot of hexane to make 0.04 g fat/mL hexane. Glycerides were eluted with 4 mL of $CHCl_2$:isopropanol (2:1) (eluting twice, each 2 mL) and free fatty acids were eluted with 8 mL of acetic acid–methyl tertbutyl ether solution (2%).

2.3.1.2. FFA analysis. The separated FFA was placed in a 20 mL tube with plug and incubated in a 45 °C water bath until the solvent was evaporated, using nitrogen to obtain oily liquid. Then 2 mL of methanol and 1 mL of concentrated sulphuric acid were added and incubated for 30 min in a 60 °C water bath for methylation. After cooling to room temperature, 2 mL of hexane and 3 mL of water were added and mixed thoroughly. In addition, an internal standard, 1 mL of tridecanoic methyl–methanol solution (3.6 mg/mL) was added to each sample and left overnight at room temperature for phase separation. The top hexane layer containing methylated fatty acids was analysed for fatty acid composition using a GC (Shimadzu GC-2010). A CP-WAX column (30 m, 0.32 mm i.d.) was used to separate the fatty acids. A ramped oven temperature condition (120 °C for 3 min, ramped to 190 °C at 10 °C/min and then to 230 °C at 2 °C/min, and maintained at 230 °C for 15 min) was used. Temperatures of both inlet and detector were 250 °C and helium was the carrier gas at a linear flow of 3 mL/min. Detector (FID) air, hydrogen gas, and make-up gas (He) flows were 400, 35, and 43 mL/min, respectively.

2.3.1.3. Identification and quantification. Fatty acids were identified by the retention time of known standards. Tridecanoic methyl was selected as the internal standard and the relative quantitative correction factor and contents of FFA were determined. The calculation formula of FFA is as follows:

$$w_i(mg/g) = f' * \frac{A_i * m_s}{A_s}/m$$

$$f' = \frac{A_i * m_{ix}}{A_{ix} * m_s}$$

where w_i denotes the concentration of each FFA; m_s and m denote the content of internal standard and sampling weight, respectively; A_i and