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Investigating the optimum conditions for minimized 3-chloropropane-1,2-diol esters content and improved sensory attributes during savory beef flavor preparation

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ABSTRACT

In this study, the effects of enzymatic hydrolysis of tallow and addition of sodium chloride (NaCl) were evaluated on the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters and sensory characteristic of beef flavors. The enzymatic hydrolysis condition had significant effects on 3-MCPD mono/di-esters formation during the beef flavor preparation. Considering the safety and sensory characteristics of beef flavors, the optimal enzymatic hydrolysis conditions were selected as: lipase concentration 75 U/g tallow, tallow concentration 80% (w/v) and pH 7.0 at 47.5 °C for 9.5 h. Using the optimal enzymatic hydrolysis conditions, no 3-MCPD monoesters were detected and 3-MCPD-diesters concentration was strongly dependent on NaCl concentration and its addition moment (before or after thermal reaction) at different temperatures. In conclusion, beef flavor was prepared using the optimal hydrolysis conditions and heated at 110 °C for 100 min, then 10% NaCl was added when the system was cooled to 60 °C.

1. Introduction

3-Monochloropropane-1,2-diol (3-MCPD) esters generated mainly through high temperature treatment during the deodorization process of refined oils are potential hazardous contaminants and have become a worldwide safety issue in food processing (Franke, Strijowski, Fleck, & Pudel, 2009; Matthäus, Pudel, Fehling, Vosmann, & Freudenstein, 2011; Zelinková, Svejkovská, Velíšek, & Doležal, 2006). The most important precursors of 3-MCPD esters identified in food are chloride ions and acylglycerols including triacylglycerols (TAGs), diacylglycerols (DAGs) and monoacylglycerols (MAGs) (Franke et al., 2009; Freudenstein, Weking, & Matthäus, 2013; Hamlet et al., 2011; Shimizu, Vosmann, & Matthäus, 2012; Svejkovská, Doležal, & Velíšek, 2006; Zelinková et al., 2006).

During the last decade, different researches were mainly focused on 3-MCPD ester formation during oil processing (Craft, Nagy, Sandoz, & Destaillats, 2012; Freudenstein et al., 2013; Li et al., 2016; Wong et al., 2017; Yamazaki et al., 2013; Zhou, Jin, Wang, & Xu, 2013). However, the natural beef flavor production process was found to be more favorable to generate 3-MCPD esters, as compared to the deodorization of refined oils (Chai et al., 2016). Meat flavors are generally produced through Maillard reaction using various amino acids, sugars, products of lipid oxidation and protein hydrolysates (Mottram, 1998; Song & Xia, 2008; Zamora & Hidalgo, 2005). They have widely been used as important food additives in snack foods, including instant noodles, meat products, frozen foods, condiments, etc. (Sun, 2004). The safety of food is determined by the quality of various ingredients. Hence, the hazard of 3-MCPD esters (monoesters and diesters) cannot be ignored whether the meat flavor was used as a food additive in snack foods at low concentration or used at large concentration in sauces. The published data revealed that the chemical structure of 3-MCPD esters played a key role in determining its toxicity (Bakhiya, Abraham, Gurtler, Appel, & Lampen, 2011; Buhrke, Weisshaar, & Lampen, 2011; Liu et al., 2012; Schilter, Scholz, & Seefelder, 2011; Seefelder, Scholz, & Schilter, 2011; Seefelder et al., 2008). Liu et al. (2012) reported that 3-MCPD monoesters have a greater oral toxicity than diesters. Therefore, it is important to control the formation of 3-MCPD mono/di-esters during the savory flavors production process.

The enzymatically hydrolyzed tallow has been added to the Maillard reaction system to enhance the beef flavor characteristics (Shi, Zhang,

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Song, Jia, & Xia, 2013; Song et al., 2014). Tallow hydrolysates could be the major source of acylglycerols including TAGs, DAGs and MAGs, which are the effective 3-MCPD mono/di-ester precursors (Freudenstein et al., 2013; Shimizu et al., 2012; Svejkovská et al., 2006). Besides, chloride ions (organic and inorganic chlorine) are generally considered as crucial precursors for the 3-MCPD esters formation (Freudenstein et al., 2013; Shimizu, Weitkamp, Vosmann, & Matthäus, 2013a; Svejkovská et al., 2006; Zhang et al., 2015). Svejkovská et al. (2006) reported that the formation of 3-MCPD esters was directly proportional to the concentration of sodium chloride (NaCl). However, there was no significant difference in the concentration of 3-MCPD esters when amount of NaCl added in oil substrate varied from 1 to 10% (Zhou et al., 2013). These results could be attributed to the hard dissolution of NaCl in the oil substrate. On the contrary, Svejkovská et al. (2006) attributed the formation of 3-MCPD esters to the excellent emulsifying properties of Tween 80. Due to its function in regulating the flavor, color, and bacteriostatic properties, NaCl is generally used as seasoning and preservative in food industry. During beef flavor production, NaCl was added to achieve two effects including controlling temperature by adding together with amino acids, reducing sugars, yeast extract, hydrolyzed vegetable protein (HVP), etc. to increase of the solid content and boiling point of the system; and enhancing the taste and extending the shelf-life by adding NaCl after thermal reaction which was to control temperature without NaCl by the increase of pressure with the use of high-pressure vessels.

The relationship between 3-MCPD esters and their potential health hazards accelerated the development of strategies for the reduction of these esters in foods (Bornscheuer & Hesseler, 2010; Craft, Chiodini, Garst, & Granvogl, 2013; Franke et al., 2009; Matthäus et al., 2011; Strijowski, Heinz, & Franke, 2011; Zulkurnain et al., 2012; Zulkurnain, Lai, Tan, Abdul Latip, & Tan, 2013). Earlier studies demonstrated that it was an effective way to modulate the formation of 3-MCPD esters by controlling their precursors (Franke et al., 2009; Freudenstein et al., 2013; Matthäus et al., 2011; Shimizu et al., 2013a; Zulkurnain et al., 2012). Hence, the effect of NaCl concentration, its addition moment and temperature on 3-MCPD mono/di-esters formation in beef flavor production process was evaluated in this paper. On the best of authors' knowledge, the effects of the degree of hydrolysis and its hydrolysate at different hydrolysis conditions on the formation of 3-MCPD mono/di-esters have not yet been well documented. Although, the optimum enzymatic hydrolysis conditions of tallow have been studied in consideration of the sensory characteristics, however, the quality of savory flavors will change with the changing preparation conditions. Therefore, in order to ensure the safety and quality of beef flavors, both 3-MCPD mono/di-esters concentration and sensory characteristics were simultaneously evaluated during the enzymatic hydrolysis of tallow in this study. The melting point of tallow is 47.5 °C, and the lipase is almost inactive when the temperature is above 50 °C. Therefore, the main factors of enzymatic hydrolysis investigated in this work included pH, tallow concentration, lipase concentration and enzymolysis time. The aim of this study was to control the concentration and distribution of 3-MCPD mono-/di-esters in beef flavors without altering the quality of savory flavors to meet the demand of customers.

2. Materials and methods

2.1. Chemicals and samples

Hydrolyzed vegetable protein (HVP) and yeast extract were provided by Tianning Flavour & Fragrance Co., Ltd. (Shanghai, China). Bovine bone was provided by Anhui QiangWang Seasoning Co., Ltd. (Anhui, China). Lipase (Yiming, 10 U/mg) was obtained from Yiming Biological Products Co., Ltd (Jiangsu, China). Papain (600 U/mg) was purchased from Novo Co., Ltd. (Novozyme Nordisk, Bagsvaerd, Denmark). Tallow was purchased from Tianjin Muyang Oil and Fats Co., Ltd. (Tianjin, China). D-Xylose, glucose, L-cysteine, DL-methionine, sodium glutamate (≥99%), taurine and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The 3-MCPD ester standards were purchased from Toronto Research Chemicals Inc. (Toronto, ON, Canada), including 1, 2-dipalmitoyl-3-chloropropanediol (98%), 1-palmitoyl-3-chloro-1, 2-propanediol (98%), 1,2-dioleoyl-3-chloropropanediol (98%), 1-palmitoyl-2-oleoyl-3-chloropropanediol (98%), 1-oleoyl-3-chloro-1, 2-propanediol (98%), 1-palmitoyl-2-stearoyl-3-chloropropanediol (98%), 2-disteartoyl-3-chloropropanediol, (98%), 1-oleoyl-2-stearoyl-3-chloropropanediol (95%) and 1-stearoyl-3-chloro-1,2-propanediol (98%). HPLC grade of methanol, hexane, 2-propanol (IPA), ammonium acetate and dichloromethane (DCM), and analytical grade of ethyl acetate and methyl *tert*-butyl ether (MTBE) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CNWBOND NH_2 SPE Cartridges (1000 mg) were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China).

Seven commercial edible beef flavor samples (BF1-7) were purchased from three different companies: Yuanpeng Food Ingredients Co., Ltd. (Tianjin, China), Shandong HaoJiaWang Biological Technology Co., Ltd. (Shandong, China) and Wanhui Flavors & Fragrances Co., Ltd. (Guangzhou, China), respectively.

2.2. Sample preparation

2.2.1. Preparation of enzymatically hydrolyzed tallow

Crude tallow in phosphate buffer solution (pH, 5.0–9.0) was placed in the enzyme reactor with mechanical stirring. Different tallow concentrations of 50, 60, 70, 80 and 90% (w/v) were used. Lipase concentration (15–115 U/g tallow) was added to the reactor and the mixture temperature was adjusted at 47.5 °C for 2–11 h. After hydrolysis, the samples were heated at 95 °C for 10 min to inactivate the enzyme, then quickly cooled in ice water and stored at −18 °C prior to further analysis.

2.2.2. Preparation of enzymatically hydrolyzed bovine bone

Bone cement (45 g; water content, 65.38%; protein content, 9.75%) and deionized water (55 g) were placed in enzyme reactor with magnetic stirring for thermal denaturation at 50 °C for 5 h. Papain was added to the reactor with enzyme/substrate (E/S) ratio of 1.5×10^{-2} (g papain/g protein), then the pH of the mixture was adjusted to pH 6.0 and heated at 55 °C. After 5 h reaction time, the sample was heated at 95 °C for 10 min to deactivate the enzyme, then quickly cooled in iced water and stored at −18 °C prior to further analysis.

2.2.3. Preparation of beef flavors (BFs)

A mixture of HVP (1.25 g), yeast extract (2.5 g), DL-methionine (0.375 g), L-cysteine (0.375 g), DL-xylose (0.125 g), glucose (0.375 g), taurine (0.125 g), sodium glutamate (2.0 g) and enzymatically hydrolyzed tallow (3.0 g), was dissolved in 15.0 g solution of the bovine bone hydrolysate. The solution was transferred into 50 mL screw-sealed tubes and then heated in a thermostatic oil bath with mechanical stirring (150 rpm) at 110 °C for 100 min. To evaluate the effect of NaCl on the sensory characteristics and 3-MCPD esters formation, 2–20% (w/w) NaCl concentration was added to the system before and after thermal reaction (110 °C). The effect of temperature during NaCl addition after thermal reaction was also studied at 60–110 °C. After reaction, the tubes were immediately cooled in iced-water and stored at −18 °C for further analysis.

2.3. Analytical methods

The qualitative and quantitative analysis of 3-MCPD esters was conducted using UPLC-TQ-MS (Waters, Milford, MA, USA) according to method reported in our earlier research (Chai et al., 2016). Beef flavour samples (1 ± 0.02 g) were accurately weighed and dissolved in a mixture of MTBE and ethyl acetate (4:1 v/v, 5 mL), vortexed for 5 min