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Metabolomic approach for determination of key volatile compounds related to beef flavor in glutathione-Maillard reaction products

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ABSTRACT

The non-targeted analysis, combining gas chromatography coupled with time-of-flight mass spectrometry (GC–TOF/MS) and sensory evaluation, was applied to investigate the relationship between volatile compounds and the sensory attributes of glutathione-Maillard reaction products (GSH-MRPs) prepared under different reaction conditions. Volatile compounds in GSH-MRPs correlating to the sensory attributes were determined using partial least-squares (PLS) regression. Volatile compounds such as 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol, 2-propylpyrazine, 1-furan-2-ylpropan-2-one, 1*H*-pyrrole, 2-methylthiophene, and 2-(furan-2-ylmethyldisulfanylmethyl)furan could be identified as possible key contributors to the beef-related attributes of GSH-MRPs. In this study, we demonstrated that the unbiased non-targeted analysis based on metabolomic approach allows the identification of key volatile compounds related to beef flavor in GSH-MRPs.

1. Introduction

Metabolomic approaches have been proven to be an important tool providing valuable application in many scientific fields, such as biochemistry, drug discovery, and agricultural and food science [1–3]. Several experimental techniques, including nuclear magnetic resonance spectroscopy (NMR), gas chromatography (GC), or liquid chromatography (LC) coupled to mass spectrometry (MS), are routinely applied to investigate a whole range of metabolites [2,4]. In particular, GC–MS has been used for analysis of volatile and semivolatile metabolites in agricultural and food products, as it provides high selectivity, resolution, precision, and sensitivity [5]. Additionally, GC coupled with time-of-flight MS (GC–TOF/MS) allows a notable amount of chemical information to be obtained using non-targeted analysis as well as targeted approach, mainly due to its advantages such as fast analyte detection and efficient deconvolution process [6,7]. In general, two different methodologies, targeted and non-targeted approaches, can be applied for analyzing a complex set of metabolites [1,6]. In targeted analysis, the metabolomics data are scanned for specific compounds normally identified in a reference library. That is, targeted analysis means the quantitative determination of a selected number of predefined and identified metabolites using reference libraries [1,6]. In contrast, metabolites are not identified and the spectral data of all potential compounds are not preselected in non-targeted analysis [6]. Some non-targeted approaches require deconvolution functions such as unbiased mass peak extraction and alignment of peaks over all samples [8]. Multivariate statistical analysis can then be used for clustering and visualization of mass spectrometry-based metabolomic data through the dimensional reduction of numerous metabolites [9–11].

The compositions of volatiles and non-volatiles directly influence the organoleptic characteristics of foodstuffs, and particularly their sensory perception during food consumption [12]. Some studies related to flavor and taste of foods have been performed combining sensory evaluation and instrumental analysis. In particular, the assessment of the relationship between sensory properties and constitutive components by multivariate analysis has been applied to investigate compositional differences in various kinds of foods including wine [9], pine-mushrooms [10], and coffee [13]. Skogerson et al. [9] identified compositional differences in various wines using GC–TOF/MS and proton NMR, which were correlated with the wine sensory property of “body” and the viscous mouthfeel, using multivariate statistical methods. In addition, Cho et al. [10] demonstrated the potent odorants in pine-mushrooms by correlating GC-olfactometry and sensory data sets using partial least-squares (PLS) regression. However, these studies focused only on targeted analysis, which employed the quantitative determination of a selected number of predefined and preidentified components. It is difficult to obtain a comprehensive understanding

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of the relationship between entire chemical compositions and sensory characteristics using targeted analysis because most foodstuffs contain potentially hundreds of components that influence their flavor and taste characteristics. In addition, the identification and quantification of all of the compounds that influence the sensory attributes of foods can be practically impossible. On the other hand, the holistic non-targeted analysis, which is commonly used for metabolomic studies, may be a potential tool to gain knowledge on the components related to sensory attributes as a whole. Therefore, non-targeted analysis could be employed to obtain a comprehensive characterization of food properties. In particular, the non-targeted analysis has revealed a correlation between volatile component (ethyl formate) and the perceived fermented off-note in coffee, showing that ethyl formate can be used as a new quality-trait marker [13]. The main advantage of the non-targeted approach is that it is possible to identify novel compounds related to specific characteristics of foods without any bias [13].

The Maillard reaction between amino acids and reducing carbohydrates is one of the most important reactions leading to the generation of diverse odorants and tastants that contribute to the sensory characteristics of thermally processed foods [14–16]. During the thermal processing, reactive intermediates, such as hydrogen sulfide, cysteamine, and mercaptoacetaldehyde, are liberated from sulfur-containing amino acids and subsequently participate in the Maillard reaction and Strecker degradation to form volatile and non-volatile sulfur-containing compounds [17,18]. In addition to these precursors, glutathione [γ-L-glutamyl-L-cysteinylglycine, (GSH)], which is a tripeptide that contains a cysteine residue, can form diverse volatile sulfur-containing compounds that are related to savory and meaty-type flavor notes [16,17,19]. Kim and co-workers observed that beef-related sensory attributes were significantly stronger in beef soup samples containing GSH or GSH-Maillard reaction products (MRPs) than in beef soup containing more beef but no GSH or GSH-MRPs. This study clearly indicates that the addition of GSH and its MRPs enhances the beef flavor and beef odor in beef soup systems [20–22].

Although the Maillard reaction is a well known route for investigating diverse components related to sensory qualities in thermally processed foods, correlations between the sensory characteristics and constitutive components of MRPs using non-targeted analysis have not been performed yet. Also, it is generally accepted that the Maillard reaction and its products cannot be easily analyzed using targeted approach, due to their complexity and diversity. To overcome these limitations, we applied non-targeted analysis based on GC–TOF/MS to analyze the volatile compounds of GSH-MRPs. Therefore, the objectives of this study were to identify and quantify some of the critical volatile compounds related to beef flavor in GSH-MRPs. Then, the non-targeted approach to provide information about the correlation between the volatile compounds and the sensory attributes of GSH-MRPs in beef stock samples was applied. Finally, we discovered the volatile compounds identified as possible key contributors related to beef flavor in GSH-MRPs.

2. Experimental

2.1. Chemicals

GSH was purchased from Kyowa Hakko Kogyo (Tokyo, Japan). The following reducing sugars were obtained from commercial suppliers: glucose (Samyang Genex, Seoul, Korea), xylose (Zhejiang Huakang Pharmaceutical, Zhejiang, China), and ribose (Now Foods, IL, USA). *n*-Alkane standards (C_8–C_{22}) and anhydrous sodium sulfate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dichloromethane of high performance liquid chromatography grade was obtained from Fisher Scientific (Seoul, Korea). All authentic standard compounds (flavoring agents) selected for confirmation experiment were obtained from Penta Manufacturing (Livingston, NJ, USA).

2.2. Model Maillard reaction systems

In a preliminary test, 20 panelists who had previous experiences in descriptive analysis sorted the 24 samples produced under different reaction conditions (3 types of sugars, 2 pH levels, 2 temperature conditions, and 2 reaction times) according to their similarity, and then selected 12 GSH-MRPs with different flavor profiles.

GSH (0.005 M) and reducing sugar (0.005 M), such as glucose, xylose or ribose, were dissolved in 100 mL of ultrapure water (aquaMAXTM145-Ultra 350, Young Lin Instrument, Gyeonggi-do, Korea). The reaction mixtures were adjusted to pH 7 or 11 using 0.5 M sodium hydroxide (Youngjin Chemical, Gyeonggi-do, Korea), and then sealed in a 200 mL stainless-steel cylinder. The cylinder was heated to 120 °C (for 90 min) or 150 °C (for 120 min) in a drying oven (LDO-250N, Daihan Lab Tech, Gyeonggi-do, Korea) (Table 1). After the thermal reaction, the cylinder was cooled for 30 min in cold water (approximately 4 °C) before opening the cap. The final pH of the reaction mixtures was adjusted to 6.5 which is the typical pH of beef stock using either 0.5 M sodium hydroxide or 0.5 M hydrochloric acid (Youngjin Chemical, Gyeonggi-do, Korea).

2.3. Sensory analysis procedure

2.3.1. Preparation of beef stock samples for sensory evaluation

The beef stock samples were prepared following a procedure reported by Kwon et al. [22]. The beef stock samples for the descriptive analysis were prepared by boiling 250 g of beef in 5 L of water for 1 h at a maximum heat level (heat level: 12) on the hot plate (THL 1797, Rommelsbacher Elektrohaushaltgeräte GmBH, Dinkelsbühl, Germany), and then simmered for 1 h at the medium heat level (heat level: 8). The beef stock was cooled for 3 h at room temperature and strained with an 18-mesh metal sieve to remove the beef and solidified fat. The prepared stock was kept frozen at −20 °C for 1 day. Before the sensory test, the frozen beef stock was thawed and heated at a maximum heat level (heat level: 12, approximately 100 °C) on a hot plate (THL 1797, Rommelsbacher Elektrohaushaltgeräte GmBH, Dinkelsbühl, Germany) for 1 h, and GSH-MRPs prepared at different conditions were added at 0.1% (solid bases) level to the beef stock. The level of the GSH-MRPs in the beef stock was determined as described in a previous study [22].

The prepared beef stock samples containing GSH-MRPs were kept at 60 ± 2 °C in 0.25 L individual thermos (IB-020TPY, Sejongisoli, Daegu, Korea) until the evaluation. The samples were poured into individual tasting beakers just before the evaluation. All the samples were coded with 3-digit random numbers and the presentation order of the samples was randomized. Warm (40 ± 2 °C) filtered tap water (Ceramic Filter System, Fariey Industrial Ceramics, London, U.K.) was provided to the panelists to rinse their mouths between tasting samples.

2.3.2. Panel selection and training for sensory evaluation

Eight female panelists (24–31 years of age), who had experience in the descriptive analysis of various food products in the Department of Food Science and Engineering at Ewha Womans University (Seoul, Korea), were selected. Training sessions were held 4 times per week for 8 weeks, and each session took approximately 1 h. During the training sessions, the panelists developed and defined the sensory attributes and selected reference samples corresponding to each characteristic (Table 2). The training sessions continued until the panelists showed consistent results in