Food Chemistry 192 (2016) 724–728

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phytosterols in onion contribute to a sensation of lingering of aroma, a *koku* attribute

Toshihide Nishimura [a,*], Ai Saiga Egusa [a], Akira Nagao [a], Tsutomu Odahara [b], Takeshi Sugise [b], Noriko Mizoguchi [b], Yasuharu Nosho [b]

[a] *Department of Food Science, Faculty of Applied Life Science, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino-shi, Tokyo 180-8602, Japan*
[b] *Osaka Head Office, Kaneka Co., 2-3-8 Nakanoshima, Kita-ku, Osaka 530-8288, Japan*

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form 7 June 2015
Accepted 22 June 2015
Available online 23 June 2015

Keywords:
Phytosterol
Onion
Aroma persistence
Koku attribute

ABSTRACT

We aimed to examine the substance in a precipitate of heat-treated onion concentrate (HOC) that contributes to a sensation of lingering of aroma, a *koku* attribute induced by the sensing of richness and persistence in terms of taste, aroma and texture. Adding precipitate, separated from HOC, to consommé enhanced the lingering sensation of aroma in the consommé more than adding the supernatant from HOC. After the precipitate was washed with hot water and ethanol its enhancing effect disappeared. Analysis of the HOC precipitate showed that it contained phytosterols, such as beta-sitosterol and stigmasterol. Tests of binding to aroma compounds showed that both sterols, as well as the washed precipitate, were able to bind methyl propyl disulfide and *N*-hexanal. Thus phytosterols in the HOC precipitate seemed to bind and hold the aroma compounds and gradually release them, inducing a lingering sensation of aroma under the *koku* concept during consumption.

1. Introduction

It is well known that food palatability is controlled by many factors, including taste, aroma, texture, color, physical condition and circumstances. In Japan, the concept of *koku*, as well as these factors, is commonly used to assess palatable foods. Although "koku" has not been defined clearly yet, even in Japan, it was proposed as the sensation caused through their richness and lingering (persistence) in terms of taste, aroma and texture. To date, there have been some reports on compounds that can enhance *koku* attributes. Ueda, Sakaguchi, Hirayama, Mijajima, and Kimizuka (1990) and Ueda, Tsubuku, and Miyajima (1994) have shown that glutathione, alliin in garlic, and propenyl-L-cysteine sulfoxide enhance *umami* intensity even if they are added to *umami* solutions at concentrations below their thresholds. These compounds have been called *koku* enhancers. A8 (*N*-(1-methyl-4-hydroxy-3-imidazoline-2,2-ylidene) alanine) in consommé (Shima, Yamada, Suzuki, & Harada, 1998), maillard peptides in lengthily aged miso paste (Ogasawara, Katsumata, & Egi, 2006) and gouda cheese (Ogasawara, Yamada, & Egi, 2006), phthalide compounds in celery (Kurobayashi, Katsumi, Fujita, Morimitsu, & Kubota, 2008), and *kokumi* compounds (Dunkel, Koster, & Hofmann, 2007; Toelstede, Dunkel, & Hofmann, 2009; Ohtsu et al., 2010; Maruyama, Yasuda, Kuroda, & Eto, 2012; Kuroda et al., 2013) all enhance taste in terms of *koku* attributes, which increase the intensity of the *umami* taste or flavor of foods. All of these compounds contribute only to taste sensation as a *koku* attribute. In contrast, to our knowledge, there have been no reports on compounds that enhance aroma or texture in terms of *koku* attributes.

Onion is one of the most popular ingredients used all over the world for cooking foods of many different styles, including French, Chinese and Japanese. In French foods, especially, onions are essential for the preparation of consommés and sauces, because they are well known to improve food palatability. Raw onions contain various taste compounds, such as free amino acids, sucrose and organic acids, that are gradually extracted by heating during cooking (Ninomiya et al., 2010). Concentrated onion juice prepared from raw onion by heating is also used as a seasoning in food preparation. Many of the aroma compounds in this juice are produced by the maillard reaction between amino acids and sugars extracted from the onion tissue during heating (Tokitomo, 1995; Kubec, Drhova, & Velisek, 1998, 1999). Examples are the furan, pyrrole and pyrazine compounds that contribute to the palatability of cooked foods. Some sulfide compounds also have characteristic of onion flavor. These compounds are produced from propenyl-L-cysteine sulfoxide, which is a major compound that has no taste (Calvey et al., 1997; Block, Naganathan, Putman, & Zhao,

* Corresponding author.
E-mail address: toshixy@nvlu.ac.jp (T. Nishimura).

http://dx.doi.org/10.1016/j.foodchem.2015.06.075
0308-8146/

1992; Block, Putman, & Zhao, 1992). These taste and aroma compounds improve the palatability of food when onions are added.

Foods containing cooked onions are also well known to have both persistence and complexity of taste compounds and aroma compounds, such as furan, pyrazine and sulfide compounds. Both aroma and taste in onion are also thought to be associated with *koku* attributes of sensation, such as richness and lingering. Although liposome, nano-emulsion, nanoparticle, micro-emulsion and so on comprised from lipids including phytosterols have been reported to improve the performance of a cosmetic product by the regulation of the targeted release of fragrance and aromas (Chanchal, Pharm, & Swarnlata, 2008), to our knowledge there have been no reports on the aroma compounds involved in *koku* attributes in food.

Here, we tried to examine the role of a precipitate in heat-treated onion concentrate (HOC) in the persistence of food aroma and identified the compounds involved in the sensation of lingering as a *koku* attribute.

2. Materials and methods

2.1. Materials

Onions cultivated in Hokkaido (Japan) were bought from the farm, and mainly comprised of some yellow species, such as Kitamomiji 2000, Super Kitamomiji and Ohotsuku 222, which are major species as yellow onions in Hokkaido.

Consommé was prepared by using commercial consommé seasonings (Ajinomoto Co., Tokyo, Japan). Stigmasterol and beta-sitosterol were purchased from Wako Pure Chemical Ltd. (Tokyo, Japan) and Kanto Kagaku Reagent Division, respectively (Tokyo, Japan). Methyl propyl disulfide, *N*-hexanal, furfural and 2,6-dimethylpyrazine were purchased from Wako Pure Chemical Ltd.. The other chemicals used were all of reagent grade.

2.2. Separation of concentrated onion juice

Onion juice was obtained from onions by homogenization and squeezing. The onion juice was then concentrated until 70% Brix to prepare onion concentrate (OC). The OC was separated into supernatant and precipitate by centrifugation. Then, intact OC and the supernatant of OC (OC-sup) were heated and concentrated for 1 h at 160 °C to obtain HOC and heat-treated supernatant of OC (HOC-sup).

The HOC was also separated by centrifugation to obtain a precipitate.

2.3. Analysis of precipitate from OC

We analyzed the precipitates from unheated OC and HOC by pyrolytic gas chromatography–mass spectrometry (GC–MS) according to the method of del Rio, Gutierrez, and Martinez (2004), as follows. Approximately 1 mg of precipitate of OC was analyzed with a pyrolyzer (EGA/PY-2020iD, Frontier Lab. Ltd., Fukushima, Japan) coupled to a GC–MS system (GC–MS; Shimadzu GCMS-QP2010 Ultra, Kyoto, Japan). Pyrolysis was performed in a glass liner at 610 °C. GC was performed by using a 30 m × 0.25 mm i.d. UA-5 column (film thickness 0.25 μm, Frontier Lab. Ltd., Fukushima, Japan). Helium was used as the carrier gas at a constant linear velocity of 48.1 cm/s. The oven temperature was programmed to gradually increase from 40 to 300 °C at a rate of 6 °C/min until it reached 300 °C, with an initial hold time of 1 min and a final hold time of 10 min. GC/MS conditions were as followed: (1) injection temperature, 280 °C; (2) split ratio, 30:1; (3) interface temperature, 230 °C; (4) ion source temperature, 300 °C; (5) scan range, 29–800 *m*/*z*; and (6) scan rate, 2.0 scans/s.

2.4. Ability of HOC precipitate or phytosterols to bind to aroma compounds

The precipitate separated from HOC was washed with hot water and ethanol to remove aroma compounds bound to it, and then dried. Methyl propyl disulfide was added to 50 mg of washed HOC precipitate in a cold tube on ice and left for 10 min. Ten milliliters of water was then poured into the mixture and this solution in a tube was boiled at 90 °C for 4.5 h. After boiling, the solution was transferred to a headspace (HS)-GC tube and the amounts of aroma compounds released from the solution at 40 °C for 10 min were measured. The aroma compounds released were trapped on an HS sampler (HS; TurboMatrix 40, Perkin Elmer, Massachusetts, USA). GC was performed on a GC-2014 (Shimadzu Co., Kyoto, Japan) equipped with a TC-Wax capillary column (GL Sciences Inc., Tokyo, Japan) of 0.25 mm i.d. and 30 m long, with an FID at 260 °C. An initial oven temperature of 40 °C for 4 min was used; the temperature was then increased at 6 °C /min to 240 °C.

We used the same methods as described above to measure the capacity of phytosterols to hold the aroma compounds *N*-hexanal, furfural and 2,6-dimethylpyrazine, as well as methyl propyl disulfide. Each of the four kinds of aroma compound were placed separately with 50 mg of sterols (Wako Pure Chemical Ltd., Tokyo, Japan) in a cold tube on ice and left for 10 min. Ten milliliters of water was poured into the mixture, and this solution in the tube was boiled at 90 °C for 4.5 h. After boiling, the solution was transferred to an HS-GC tube and the amounts of aroma compounds released from the solution were measured with HS-GC as described above.

We also used static HS-GC to examine the ability of beta-sitosterol to bind and hold methyl propyl disulfide. Methyl propyl disulfide was added to 10 ml of water with or without 50 mg of beta-sitosterol in a 20-ml tube on ice. The methyl propyl sulfide released from the water and equilibrated with the headspace for 10 min was measured by using headspace (HS)-GC. The aroma compounds released were trapped on an HS sampler (HS; TurboMatrix 40, Perkin Elmer, Massachusetts, USA), and measured with HS-GC as described above.

2.5. Sensory evaluation of persistence of aroma

We performed a sensory evaluation of the effect of adding the unseparated HOC, the supernatant in HOC, the precipitate in HOC, or the HOC-sup on persistence of the aroma in consommé (Additional Fig. 1). Sensory evaluation was conducted by 12 well-trained panelists. Standard solutions with different persistences (scores of 1, 3 and 5) were prepared using the dilution of the unseparated HOC. Panelists judged aroma persistence (i.e. the sensation of lingering of an aroma) by comparison with these standard solutions.

We used Student's *t*-test and tukey to analyze the results of the sensory evaluation in Fig. 1 and those in Fig. 2, respectively.

3. Results

3.1. Effects of HOC and HOC-sup on aroma persistence inducing a sensation of lingering

Initially, we heated OC and OC-sup at 160 °C for 1 h to obtain HOC and HOC-sup, respectively. We then examined the effect of adding 2% HOC or HOC-sup on the aroma persistence of consommé using sensory evaluation by 12 trained panelists.