Thesis for the Degree of Master of Engineering

A comparative study on flavor profiles and sensory properties of onion extracts prepared by different hydrothermal treatments

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향미 프로파일 및 관능적 특성에 대한 비교 연구

Advisor: Prof. Suengmok Cho

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A dissertation

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요 약

양파는 전 세계적으로 가장 대표적인 향미 소재 중 하나로, 과자, 드레싱, 빵 등의 다양한 가공식품에 널리 사용되고 있다. 본 연구에서는 열수추출법(HE), 증기추출법(SE), 고온고압추출법(AE)의 3가지 방법을 적용한 양파추출액을 제조하여 생양파즙(OJ)과 관능적 특성을 비교 및 분석하였다. 모든 추출물은 열수처리 온도, 압력, 시간에 따라 수율, 색상 및 향미 성분의 차이를 보였다. 온도, 압력의 상승에 따라 양파추출물의 수율이 증가하였고 황색도와 적색도 모두 유의적으로 증가하였다. 생양파즙은 관능적 기호도에 부정적인 영향을 미치는 쓴맛, 매운맛 및 떫은맛 성분들이 많은 반면, 열수처리 추출물은 관능적 기호도에 긍정적인 영향을 미치는 단맛 및 익힌 양파맛 성분이 감지되었다. 생양파즙에서는 총 54개, 열처리 양파추출물에서는 총 28개의 휘발성 화합물이 동정되었는데, 이는 열수처리로 인해 향미 화합물이 소실된 것으로 추정된다. 그러나, 악취를 함유하는 황화합물의 감소와 가열 풍미 성분인 알데히드류 및 퓨란류의 생성으로 인해, 열수처리 양파추출물의 선호도가 유의적으로 높은 결과를 보였다. 다변량분석에 의해, 전자코, 전자혀에서 검출된 향미 성분은 생양파즙과 열처리추출물들을 두 가지 그룹으로 확연히 분리하였다. 본 연구의 결과는 양파 추출시 열수처리를 함으로써 풍미 성분의 변화가 발생하여 관능적 특성이 향상됨을 확인하였다. 각 추출물들이 특징적으로 가지는 향미 성분 결과는 추후 양파 향미 제조의 기초 자료로 활용될 수 있을 것으로 기대된다.

Introduction

Onion has been highly consumed worldwide for a long time. Onion possesses unique flavors, so it is well-known as a representative flavoring ingredient all over the world (Pareek et al., 2017). It is widely used in a variety of commercial food products such as onion-flavored snacks and dressings.

As the flavor ingredient for foods, concentrated onion juice or extract prepared from raw onion is used (Nishimura et al., 2016). Onion juice contains an unpleasant flavor due to the concentration of strong pungency caused by pyruvic acid and off-flavor generated by various sulfur compounds (Kubec et al., 2018; Schwimmer et al., 1962). Therefore, several studies have been conducted for the development of onion processing methods by adding chemical products or adopting new technologies to improve sensory properties (Shim et al., 2019). However, these processes usually accompany high costs, making them difficult to apply to commercial manufacturing, and residual chemical components occurred during the process are harmful to the environment and health.

Hydrothermal treatment is an effective method to accelerate the decomposition of flavor compounds by promoting the breakdown of tissues and cells of vegetables (Putriani et al., 2022). In addition, it not only destroys the off-flavor of vegetables but also increases the complex effect of flavor by forming new flavor compounds produced by the Maillard reaction (Bi et al., 2021). Also, it is easy to apply in medium-sized factories and is cost-effective and eco-friendly as it does not require any chemical treatments (Azwanida, 2015). In a previous study, it was found that hot water extraction of ginger improved flavor by removing bitterness (Nam et al., 2022). In particular, when heating is applied to onions, it contributes to sensory improvement by changing pungency to sweetness (Clark et al., 2018; Nishimura et al., 2016b).

Until now, there have been many studies on the flavor profiles of raw or heat-treated onions (Boelens et al., 1971; Choi et al., 2017). However, most studies on onion flavor have focused on the effect of heat treatment such as roasting and frying or various drying methods (Edith et al., 2018; Maftoonazad et al., 2022; Villière et al., 2015). These papers are missing a more comprehensive approach to both taste and flavor analysis. Although research on onion flavor has been conducted for a long time, there is no comprehensive study on flavor profile analysis of onion extracts by hydrothermal treatments.

In this study, we prepared three onion extracts using different hydrothermal treatments (hot water extract, HE; steaming extract, SE; autoclave extract, AE) and compared their flavor profiles and sensory properties. The flavor profiles were observed by measurement of taste and aroma compounds, and analysis of electronic nose and electronic tongue. In addition, a comprehensive analysis was conducted by examining the correlation between sensory attributes and flavor profiles.

Materials and Methods

1. Materials

Onion (*Allium cepa L.*) used in this study was cultivated by Changnyeong, Gyeongsangnamdo, and stored at room temperature or in a cool place until the experiment. All chemicals and reagents used in this study were analytical grades.

2. Preparation of different onion juice and extracts

Onions were cut into approximately 3 cm horizontally and vertically, and 1 kg of raw sliced onion was used. Onions were prepared by different methods: onion juice (OJ), hot water extract (HE), steaming extract (SE), and autoclave extract (AE). The manufacturing procedure is presented in Figure 1. The onion was squeezed using a juicer (OJ). Onion was extracted by boiling 1,000 mL of distilled water at 100°C for 2 h (HE). The onion was placed in a steamer with boiling water and steamed at 100°C for 2 h, and all the liquid that fell due to the steam was recovered (SE). Onion was extracted in an autoclave with 1 L of distilled water at 120°C at 1.2 Pa for 1 h (AE). After extraction, all residues were squeezed with a juicer and centrifuged at 9,000 rpm for 20 min at 4°C. After that, it was concentrated to 36.5°brix through a rotary evaporator at 45°C.

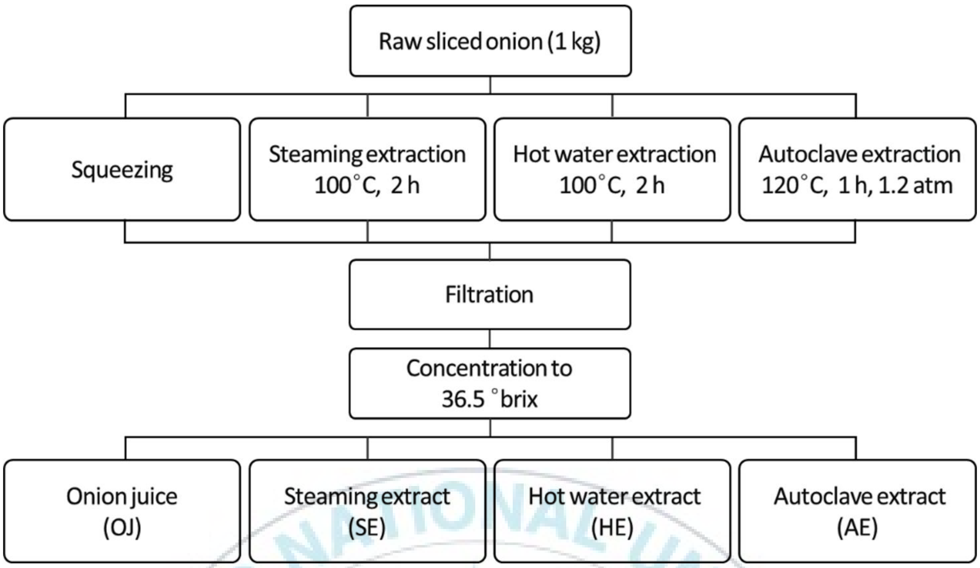

Figure 1. The manufacturing process of onion juice and extracts.

3. Yields

The moisture content of samples was measured at 105°C using a moisture analyzer (MA 35, Sartorius AG, Goettingen, Germany) (Hu et al., 2019). After weighing 2 g of the sample in an aluminum pan containing glass fiber, all samples were measured three times. The extraction yield was calculated using the following equation:

$$Yield(\%) = \frac{Solid\ contents\ of\ extracts\ (g/mL) \times\ Total\ volume\ of\ extracts\ (mL)}{Total\ weight\ of\ raw\ onion\ (g)}$$

4. Colors

The CIE colors of onion juice and extracts were carried out using a color meter (SP60, Lovibond Co., Amesbury, UK). The sample was stirred at 200 rpm for 1 min, and 8 mL of the homogenized sample was placed in a petri dish (55×12 mm) to measure the chromaticity. L^* (lightness), a^* (redness), and b^* (yellowness) values of each sample were repeatedly measured 10 times and calculated as an average value. A standard white plate was employed to calibrate the equipment (L^* = 94.62, a^* = -0.780, b^* = 1.362).

5. Sensory evaluation

Sensory evaluation was conducted by 15 trained panelists (7 males and 8 females, 22-30 years old) at Pukyong National University (Busan, Korea). Each sample was subjected to a blind test in randomization. A total of 9 attributes for aroma (onion aroma and sweet aroma), taste (sweetness, bitterness, pungency, astringency, and cooked onion flavor), and overall acceptance(aroma and taste) were performed on samples using a 9-point scale (1 point = no sensation, 5 points = moderate, 9 points = very strong). The panelists opened the lid of the sample container and sniffed the sample three times to evaluate the intensity of the odor. To evaluate the taste intensity, each sample was tasted with a spoon of 1 ml volume. The panelists took a break between each sample and rinsed their mouths with distilled water.

6. Free sugars

Five grams of each homogenized sample was diluted with 25 mL of distilled water. The diluted samples were extracted by heating them in a water bath at 85°C for 25 min. Samples were centrifuged at 2,000 rpm for 10 min and filtered through a 0.45 μm membrane filter. Sugars were analyzed by HPLC (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector. The column used for separation was ZORBAX Carbohydrate (4.6 × 250 mm, 5 μm, Waters, Milford, MA, USA), and the column temperature was maintained at 40°C. The sample (10mL) was injected at a flow rate of 1.0mL/min, and the mobile phase was composed of acetonitrile and water (80:20 w/w). All analyses were performed in triplicate.

7. Free amino acids

To obtain the contents of free amino acids, 3 mL of samples were dissolved in an equal volume of 16% (v/v) TCA, and shaken at ambient temperature for 15 min. After centrifugation at 3,000 rpm for 15 min, the supernatant was filtered using a 0.24 μm filter. The free amino acids were quantified by an automatic amino acid analyzer (L-8900, Hitachi High Technologies Co.). All analyses were performed in triplicate.

8. Volatile compounds

The samples were prepared by the volume headspace vial method. A 10 mL liquid sample is placed in a 250 mL brown bottle and sealed with PTEE septum (Qmx Laboratories Ltd, Essex, UK) fibers. Volatile compounds are collected by heating in an oven at 60°C for 30 min, and placed to cool at room temperature for 30 min. It is adsorbed to the triple-layer adsorption tube (Tenax-TA, USA) for 5 min using a mass flow controller (MFC) and vacuum pump. The compound collected in the tube is injected into the GC-MS in a two-stage injection by an automatic thermal desorber (ATD 650, Perkin-Elmer, Boston, MA). In the first step, desorption occurs by heating of 350°C, and then in the second step, it is concentrated in a new tube at -40°C below, and then heated to 330°C again and injected into the GC-MS (QP 2010 Plus, Shimadzu Co., Kyoto, Japan). Volatile compounds are detected using a helium carrier gas as a mobile phase on an HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

Each compound is obtained from Wiley 221 and Nist 107 mass spectrum databases (John Wiley & Sons, Inc.).

9. Electronic tongue

The taste intensity of samples was analyzed using an electronic tongue system (ASTREE II, Alpha MOS, Toulouse, France). The electronic tongue system combines multiple sensors to detect individual taste components including five primary taste sensors (UMS: umami, BRS: bitter, STS: salty, SWS: sweet, SRS: sour). For electronic tongue analysis, each sample was prepared by mixing between samples (5 mL) and purified water (95 mL). The sample solution (100 mL) was mounted on the electronic tongue sampler, and the sensor was immersed in the sample solution for 120 s to measure the intensity of taste components related to the sensor. Each sensor was thoroughly rinsed with purified water to reduce errors due to sample-to-sample contamination. The analysis was repeated 5 times per sample, and taste patterns were represented using principal component analysis (PCA) based on the results of taste sensors (Lee et al., 2020).

10. Electronic nose

Volatile compounds in each sample were analyzed using an electronic nose system attached with a flame ionization detector (FID) and MXT-5 column (HERACLES Neo, Alpha MOS). 5 mL of the sample was placed in a headspace vial. After stirring at 500 rpm for 10 min at a temperature of 50°C, volatile compounds were collected on headspace in a vial. Volatile compounds were collected using an automatic sampler installed in the electronic nose system, and volatile compounds were injected into an inlet of the electronic nose system. The trap absorption and desorption temperatures were 40°C and 250°C, respectively. The oven temperature condition was started at 40°C for 5 s, increased to 270°C at a rate of 4°C/s, and held for 30 s. Analysis was repeated 3 times per sample to confirm volatile patterns. The retention index was based on Kovat's index library and used AroChemBase (Alpha MOS) to identify individual compounds through the time at which peaks were separated. In addition, volatile patterns were represented using principal component analysis (PCA) based on the results of volatile profiles (Lee et al., 2021).

11. Statistical analysis

All data were expressed as the means ± standard deviation (SD). Results were statistically analyzed using a one-way analysis of variance, followed by Tukey’s multiple comparison test using the statistical software, SPSS 22.0 (SPSS, Inc., USA). Significant differences between samples were set at the significant level of 0.05. The heat map of volatile compounds and VIP scores were drawn by MetaboAnalyst v5.0.

Results and Discussions

1. Extraction yields

The different hydrothermal treatments affected the yield of the onion extracts. Yield is determined by several factors including heating method, temperature, time, and pressure (Ela et al.,2020). The yield of SE, HE, and AE were 4.48%, 5.73%, and 6.53%, respectively (Table 1). In particular, AE has the highest yield in the hydrothermal treatment extracts. This is related to the previous study that the yield increases as the high temperature and pressure increase (Khan et al., 2019). Since AE has high yields despite the shortest extraction time, so it is the potential for efficient industrial production with appropriate pressure temperature and time control. On the other hand, SE with the lowest yield is considered to be difficult to utilize in the food industry if it does not have characteristic sensory advantages.

2. Colors

The colors of samples were clearly distinguished (Table 1). This clear difference in colors was also confirmed by the measurement of color values. All samples showed a significant difference (p<0.05) in color value. The unheated OJ showed the highest L^* value and the lowest b^* values. Among hydrothermal treated samples, the color darkened as the yield increased, which is consistent with significantly lower L^* (Yang

Table 1. Extraction yields and colors of onion juice and extracts

Sample name		OJ	SE	HE	AE
Appearance					
Color value	L^* (lightness)	36.59 ± 0.09 ^{a}	35.98 ± 0.17 ^{b}	31.23 ± 0.19 ^{c}	31.22 ± 0.04 ^{c}
	a^* (redness)	-0.20 ± 0.21 ^{c}	-1.92 ± 0.30 ^{d}	0.23 ± 0.24 ^{b}	3.30 ± 0.08 ^{a}
	b^* (yellowness)	11.04 ± 0.16 ^{d}	15.32 ± 0.11 ^{c}	17.29 ± 0.12 ^{b}	21.39 ± 0.04 ^{a}
Yield (%)		4.58 ± 0.04 ^{c}	4.48 ± 0.03 ^{d}	5.73 ± 0.03 ^{b}	6.53 ± 0.05 ^{a}

Data represent means ± standard deviation (SD). Different letters are significantly different according to Tukey's multiple comparisons test (p<0.05). OJ, onion juice; SE, steaming extract; HE, hot water extract; AE, autoclave extract.

et al., 2022) and higher b^* (Khajehei et al., 2015). In particular, AE had significantly the highest a^* and b^* values, which was consistent with the darkest brown in appearance. The color darkens as the Maillard reaction, which occurs as a result of the condensation of a carbonyl and an amine at high temperature, forms a brown high molecular weight compound known as melanoidin (Starowicz et al., 2019). AE with brown color can be used as the flavoring for foods with dark color.

3. Sensory evaluation

Sensory evaluation of aroma was performed for three attributes (onion aroma, sweet aroma, and overall acceptance of aroma), and the results are shown as a radial chart (Figure 2A). As expected, OJ had a high onion aroma intensity and low sweet aroma intensity, but the hydrothermal treated extracts represented distinctly different sensory characteristics with high sweet aroma and low onion aroma. The flavor of raw onions is considered to be pungent and sulfur flavors. According to previous studies, many aroma compounds, such as furan, pyrazine, and pyrrole, are generated by the Maillard reaction between amino acids and sugars extracted from onion tissues when onion juice is heated, resulting in a sweet aroma (Kubec et al., 1998, 1999; Yukiko et al., 1995). Within the hydrothermal treatment extracts, the sweet flavor increased as the condition of heat treatment became more severe (SE < HE < AE). The overall acceptance of the aroma in samples was proportional to the intensity of the sweet aroma. Taste evaluation

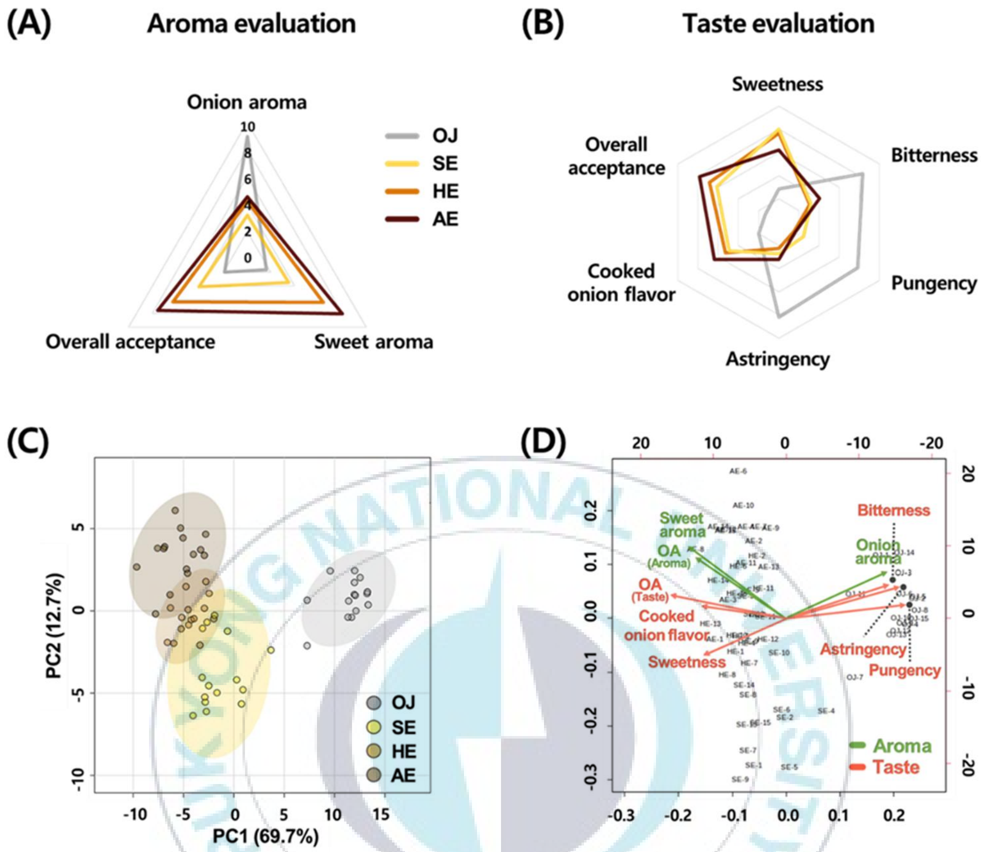

Figure 2. Sensory evaluation data of onion juice and extracts: (A) radar chart of aroma evaluation score; (B) taste evaluation score; (C) distribution of sample based on the sensory attribute scores; (D) correlation between samples and sensory attributes by PCA. OJ, onion juice; HE, hot water extract; SE, steaming extract; AE, autoclave extract.

was performed for six attributes (sweetness, bitterness, pungency, astringency, cooked onion taste, and overall acceptance), and the results were shown as a radial chart (Figure 2B). Similar to the aroma evaluation results, taste evaluation also showed clearly distinguished data between OJ and hydrothermal treated extracts. OJ had significantly highest bitterness, pungency, and astringency, whereas sweetness and cooked onion taste were significantly highest in the hydrothermal treated extracts. Menuel (2011) reported that various sulfur compounds in onions without heat treatment contributed to pungency and bitter flavor. In addition, when the onion is heated, propyl allyl disulfide and allyl sulfide with pungency flavors are decomposed to form propyl mercaptan with strong intensity of sweetness (Kim et al., 2010). The intensity of sweetness of SE and HE was higher than that of AE. On the other hand, the cooked onion taste intensity was highest in AE, followed by HE and SE. The cooked onion flavors include furan, pyrazine, and sulfide compounds, which have both persistence and complexity of taste and aroma (Nishimura et al., 2016). OJ, which had highly unfavorable characteristics of bitterness, pungency, and astringency, had a significantly lowest overall preference. In our study, the overall acceptance of the hydrothermal treated samples increased as the intensity of sweetness decreased, which was different from a previous study. In a previous study comparing the characteristics of thermal treated onion juices at different temperatures and times, the overall acceptance was high when the off-flavor, off-taste, and bitterness were low, and the sweetness was relatively high (Lee et al., 2020). Our results suggest that excessive sweetness negatively affects the overall preference. When it has a certain amount of sweetness, the sweetness can have a negative impact on sensory due to its concentrated taste.

The sensory evaluation results for each sample were classified by PCA (Figure 2C). PCA is a multivariate analytical statistical technique that reduces the multivariate data sets to one dimension, making them easier to distinguish differences in the sensory profile of samples (Elhaik, 2022). The sensory score for each sample was marked as isolated dots on the PCA score plot. PC1 represents the contribution rate of 69.7%, whereas PC2 represents the contribution rate of 12.7%. The samples by PCA were divided into two clusters, and OJ was clearly distinguished from the other samples (HE, SE, and AE).

Figure 2D shows the correlation between samples and sensory attributes by PCA. The OJ was positively correlated with the characteristics of onion aroma, bitterness, pungency, and astringency, which have an unfavorable effect on sensory, so it is negatively correlated with overall acceptance. Hydrothermal treatment extracts showed different results compared to OJ; especially, AE showed a high correlation with a sweet aroma, which is explained to be the formation of many aroma compounds due to the Maillard reaction caused by heating at the highest temperature (Starowicz & Zieliński, 2019; Wei et al., 2019). Similar to the previous study, the overall acceptance was driven positively by the sweet aroma (Pimentel et al., 2015). The sweetness was highly correlated with HE and SE, which seems to be influenced by total sugar contents. Meanwhile, cooked onion taste had a high correlation with AE and HE, which showed the highest correlation with overall acceptance. Our results showed that AE with a cooked onion taste and sweet aroma had high overall acceptance.

4. Free sugars

Onion accounts for most of the sugar content except for about 80-90% of the moisture content (Bhattacharjee, 2013). Free sugars in onions include glucose, fructose, and sucrose, which have a sweet taste (Galdón et al., 2009). Compared to OJ, the hydrothermal treated extracts showed higher sucrose, lower glucose, and fructose contents (Table 2). The total sugar content of SE was the highest of 26.57 g, whereas the total sugar content of AE was the lowest at 20.97 g. This affected the sweetness score of sensory evaluation. To compare the degree of sugar extraction based on raw onion (1 kg), the sugar yield was calculated. The total sugar yield is higher in hydrothermal treated extracts than OJ. The yields of glucose and fructose were the highest in AE, followed by the highest total sugar yields in AE. This result is supported by a similar study that the content of free sugar in heat-treated onion juices was increased upon heat treatment (Lee et al., 2020). In previous studies, sucrose content differs according to temperature, pressure, and time, it breaks down into fructose and glucose at high temperatures (Woo et al., 2009). When heated at a high temperature, the decrease in sugar content may occur due to Maillard reaction products, which are formed by the reaction between sugars, amino acids, and proteins (Kousar et al., 2008).

Table 2. Sugar contents (g/ 100 g extracts) and sugar yields (g/100 g raw sliced onion)

Sample		sucrose	glucose	fructose	Total sugar
OJ	sugar content	2.30 ± 0.00 [c]	12.10 ± 0.20 [a]	10.07 ± 0.06 [a]	24.47
	sugar yield	0.11	0.56	0.46	1.13
SE	sugar content	5.30 ± 0.17 [a]	11.40 ± 0.26 [b]	9.87 ± 0.56 [a]	26.57
	sugar yield	0.23	0.5	0.45	1.18
HE	sugar content	5.57 ± 0.15 [a]	10.13 ± 0.3 [c]	8.23 ± 0.15 [b]	23.93
	sugar yield	0.32	0.6	0.48	1.40
AE	sugar content	4.40 ± 0.00 [b]	9.10 ± 0.26 [d]	7.47 ± 0.15 [d]	20.97
	sugar yield	0.29	0.61	0.5	1.40

Data represent means ± standard deviation (SD). Different letters are significantly different according to Tukey's multiple comparisons test (p<0.05). OJ, onion juice; SE, steaming extract; HE, hot water extract; AE, autoclave extract.

5. Free amino acids

Free amino acids contribute to sour, bitter, sweet, and umami tastes (Chen & Zhang, 2007). The total free amino acid content was highest in AE and lowest in SE (Table 3). Arginine and glutamic acid are the highest free amino acids in all samples, which were the major free amino acids consistent with the previous study (Fredotović et al., 2020). Arginine is a bitter and sweet component that contributes to a pleasant overall preference (Chen et al., 2012). AE had significantly highest arginine contents than others. Glutamic acid, found in many foods such as cheese, mushrooms, tomatoes, and onions, enriches the taste with umami (Zhang et al., 2013). When calculated as the value of taste activity divided by the threshold, arginine and glutamic acid are the highest, so it may affect the taste of samples. Methionine was not detected in OJ, but in hydrothermal treatment extracts, the content increased as the condition of heat treatment became more severe (SE < HE < AE). A previous study has revealed that methionine yields high when heated in an aqueous solution at 100°C temperatures for several hours (Lawson et al., 1962). Methionine is a substance that emits a cooked beef aroma, which makes it possible to enrich the taste (Lee et al., 2019; Mottram, 1998). When a reaction flavor technology (RFT) was performed by adding glutamic acid, arginine, methionine, and glycine, various flavors such as roasted onion/garlic-like/meaty odor were formed (Wang & Cha, 2018). Considering the above study, we suggest that AE with high contents of glutamic acid, arginine, and methionine will synergize to form a cooked onion flavor.

Table 3. Free amino acid contents of onion juice and extracts

Free amino acids	Contents (mg/100 g)				Taste attribute	Taste threshold (mg/100 mL)	Taste activity values			
	OJ	SE	HE	AE			OJ	SE	HE	AE
Glutamic acid	94.64±0.12	95.28±0.05	88.72±0.23	94.21±0.11	Umami (+)	30	3.15	3.18	2.96	3.09
Aspartic acid	ND	ND	ND	ND	Umami (+)	100	ND	ND	ND	ND
Glycine	5.17±1.03	ND	3.44±0.65	4.85±0.43	Sweet (+)	130	0.04	ND	0.03	0.04
Serine	15.21±2.01	ND	14.86±1.31	14.24±0.12	Sweet (+)	150	0.10	ND	0.10	0.09
Alanine	6.28±0.12	5.88±0.34	5.45±0.04	6.01±0.56	Sweet (+)	60	0.10	0.10	0.09	0.10
Threonine	73.78±3.46	81.49±0.78	85.17±2.42	84.99±0.98	Sweet (+)	260	0.28	0.31	0.33	0.33
Proline	0.69±0.03	0.80±0.34	1.21±0.45	1.43±0.29	Sweet/bitter (+)	300	ND	ND	ND	ND
Lysine	47.13±1.75	47.43±0.19	42.84±0.21	41.86±0.16	Sweet/bitter (-)	50	0.94	0.95	0.86	0.84
Valine	1.75±0.02	1.39±0.02	1.23±0.04	1.36±0.14	Bitter (-)	40	0.04	0.03	0.03	0.03
Isoleucine	6.38±0.25	6.46±0.31	7.24±0.23	7.32±0.23	Bitter (-)	90	0.07	0.07	0.08	0.08
Leucine	20.98±0.01	18.95±0.17	19.64±0.28	20.51±0.12	Bitter (-)	190	0.11	0.10	0.10	0.11
Phenylalanine	14.81±0.06	14.77±0.23	15.99±0.12	16.64±0.13	Bitter (-)	90	0.16	0.16	0.18	0.18
Histidine	13.40±0.04	14.02±0.18	13.71±0.17	17.15±0.21	Bitter (-)	20	0.67	0.70	0.69	0.86
Arginine	169.96±0.43	156.04±0.34	167.66±0.59	196.39±0.28	Bitter/sweet (+)	50	3.40	3.12	3.35	3.93
Methionine	ND	3.80±0.13	6.64±0.10	10.10±0.23	Bitter/sweet/ sulfurous (-)	30	ND	0.13	0.22	0.34
Tyrosine	14.48±0.11	14.14±0.13	14.12±0.02	14.25±0.13	Bitter (-)	ND	0.00	0.00	0.00	0.00
Tryptophan	21.65±0.09	21.13±0.23	21.44±0.03	27.21±1.21	Bitter (-)	90	0.24	0.23	0.24	0.30
Aspartic acid	ND	ND	ND	ND	Sour	3	ND	ND	ND	ND
Asparagine	ND	ND	ND	ND	Sour	100	ND	ND	ND	ND

Data represent means ± standard deviation (SD). OJ, onion juice; SE, steaming extract; HE, hot water extract; AE, autoclave extract.

6. Volatile compounds identified by GC-MS

Analysis of volatile compounds in samples was measured using GC-MS, and relative evaluation was performed by comparison of peak value (Figure 3). Figure 3A is the comparison result of volatile compounds of onion juice and onion extracts shown as a heat map. A total of 53 types of compounds were identified including 3 acids and esters, 4 alcohols, 8 aldehydes, 11 hydrocarbons, 1 furan, 5 ketones, and 21 sulfur-containing compounds. OJ has overwhelmingly higher volatile compounds than that of other samples, and it seems that a lot of the existing onion flavor disappeared due to the heating. Previous study indicates that thermal reactions may have caused the degradation of volatile compounds that are abundant in fresh onions (Gao et al., 2019). Sulfur compounds, known as the most abundant component in onions, were detected the most among all volatile compounds (Ueda et al., 1994). These sulfur compounds generally have very low thresholds compared to other compounds, so when converted to odor value, it is thought to be involved as the main odor component (Cha et al., 1998). Sulfur compounds were significantly higher in OJ than in hydrothermal treated samples, which can be explained by the disappearance of sulfur compounds when the onion is heated. These authors suggested that the decrease in the organosulfur compounds during the heat treatment could be due to the formation of intermediate compounds (Moreno et al., 2020). In aldehydes, the volatile compound that observed the highest peak area in OJ was propionaldehyde, followed by benzaldehyde, which all had a pungent odor, and this compound was not detected in hydrothermal treated samples. In

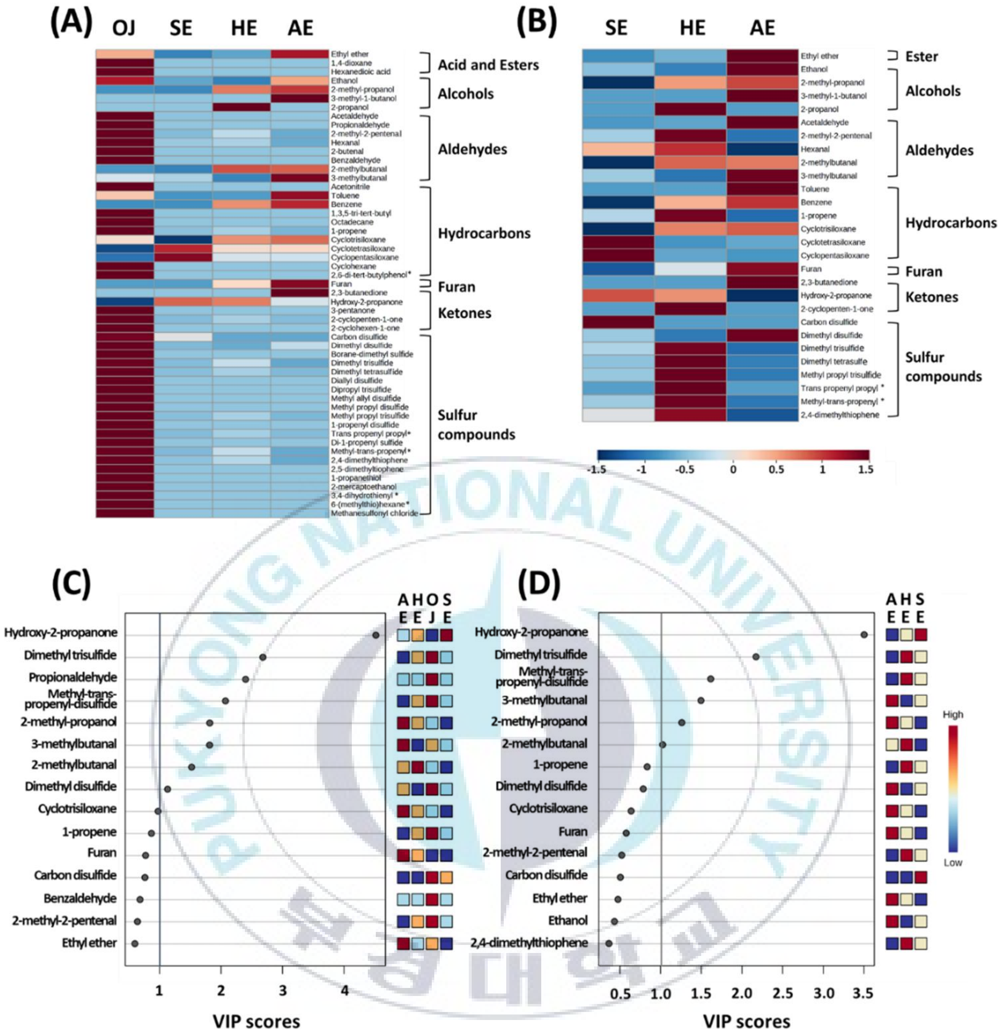

Figure 3. Volatile compounds of onion juice and extracts based on GC-MS: (A) heatmap of volatile concentration including OJ, (B) excluding OJ, (C) variable importance in projection (VIP) scores of volatile compounds including OJ, and (D) except for OJ. OJ, onion juice; HE, hot water extract; SE, steaming extract; AE, autoclave extract.

addition, OJ accounted for most of 2-methyl-2-pentenal, which was found to be a large component in raw onions in a previous study (Lee et al., 2008). The previous report indicated that 2-methyl-2-pentenal was formed by aldol condensation and dehydration of 2 molecules of propanal, the main aromatic component of raw onion produced from the lachrymatory factor (Boelens et al., 1971). In contrast, 2-methylbutanal and 3-methylbutanal, which show cocoa flavor and coffee flavor, generally had higher peak areas in the hydrothermal extraction samples than OJ. All alcohol components showed higher peak values in the hydrothermal extraction samples than OJ, which emits a sweet flavor. These results suggest that hydrothermal treatment on onions not only reduces pungent and off-flavor generated by sulfur compounds but also increases the sweet flavor such as alcohols and several aldehydes.

To better understand the volatile compounds within only the hydrothermal treatment samples, a relative comparison was performed in three samples (Figure 3B). A total of 28 compounds were detected among the hydrothermal treated extracts, and the concentrations of volatile compounds in AE were higher than those in the SE, and HE. The aroma compound with the highest peak area in all samples was hydroxy-2-propanone with a fruity and sweetish flavor, which was not identified in OJ. 1-hydroxy-2-propanone has been identified as the main intermediate for the generation of the roasted odor (Cardinal et al., 2020; Hofmann et al., 1998). In the sulfide group, HE had a higher concentration than AE and SE, and AE was the lowest overall. In AE, 2-methyl-propanol and 3-methyl-1-butanol were the highest in alcohols compared to other samples, which have a sweet scent. These compounds affect the sweet aroma intensity of the sensory evaluation, resulting in high scores of the AE. We found that the composition of volatile compounds changed according to the hydrothermal treatment method.; particularly, HE had the most sulfur compounds, whereas, AE had more favorable flavors such as sweet.

Variable importance in the projection (VIP) scores was performed to screen the major aroma compounds in each sample (Figure 3C). Generally, when the VIP score of flavor compounds was equal to or greater than 1.0, it was considered marker compounds in samples (Wu et al., 2019). There were 8 volatile compounds with a significant effect on flavor with VIP scores > 1.0 in OS. Hydroxy-2-propanone was the most characteristic variable to separate the samples, which was high in SE. It has a sweet and caramel-type odor, which is reported a volatile compound in meat (Elmore & Mottram, 1997). Dimethyl trisulfide, methyl-trans-propenyl-disulfide, and dimethyl disulfide were the most important discriminatory sulfur compounds in OJ, these compounds were known as unpleasant odors with foul odors (Shirasu et al., 2009). In addition, propionaldehyde, a characteristic compound in OJ, has a pungent odor, which negatively affects sensory attributes. 2-methyl-propanol, 3-methylbutanal, and 2-methylbutanal, which are fatty acid oxidation products, were identified as the marker compounds of AE and HE, respectively, and form cacao, malty, and sweet flavors (Giri et al., 2010). In particular, when 2-methylbutanal and 3-methylbutanal, are together, it yielded the strongest synergistic effects (Chen et al., 2020).

Figure 3D represents VIP scores of volatile compounds in the hydrothermal treated samples except for OJ. The HE-specific marker compounds were dimethyl trisulfide and methyl trans propenyl disulfide. This indicates that among the hydrothermal treated samples, sulfur compounds remain the most in HE, which may negatively affect sensory properties.

7. Correlation of sensory attributes and flavor compounds

Pearson correlation was investigated to determine the relationship between sensory attributes and flavor compounds of onion samples, including 19 taste compounds and 15 volatile compounds (Figure 4). The taste compounds consisted of 3 free sugars and 16 free amino acid components (Figure 4A), and the aroma compounds were performed on 15 marker compounds selected from the VIP score (Figure 4B). Some of the flavor compounds showed opposite behavior with respect to different sensory properties. For example, sugar correlated positively with sweetness but negatively with bitterness, pungency, and astringency. In sugar, sucrose showed a positive correlation with sweetness, but glucose and fructose showed a negative correlation. The factor that affects the sweetness of onions is not only sugar but also sweet ingredients such as propyl mercaptan. It is thought that the effect of masking the sweetness of sugars by the pungent flavor compounds of onion influenced the results (Lee et al., 2009). Among the free amino acids with sweetness, threonine and proline showed a positive correlation with the sweetness property, but other sweet free amino acids showed a

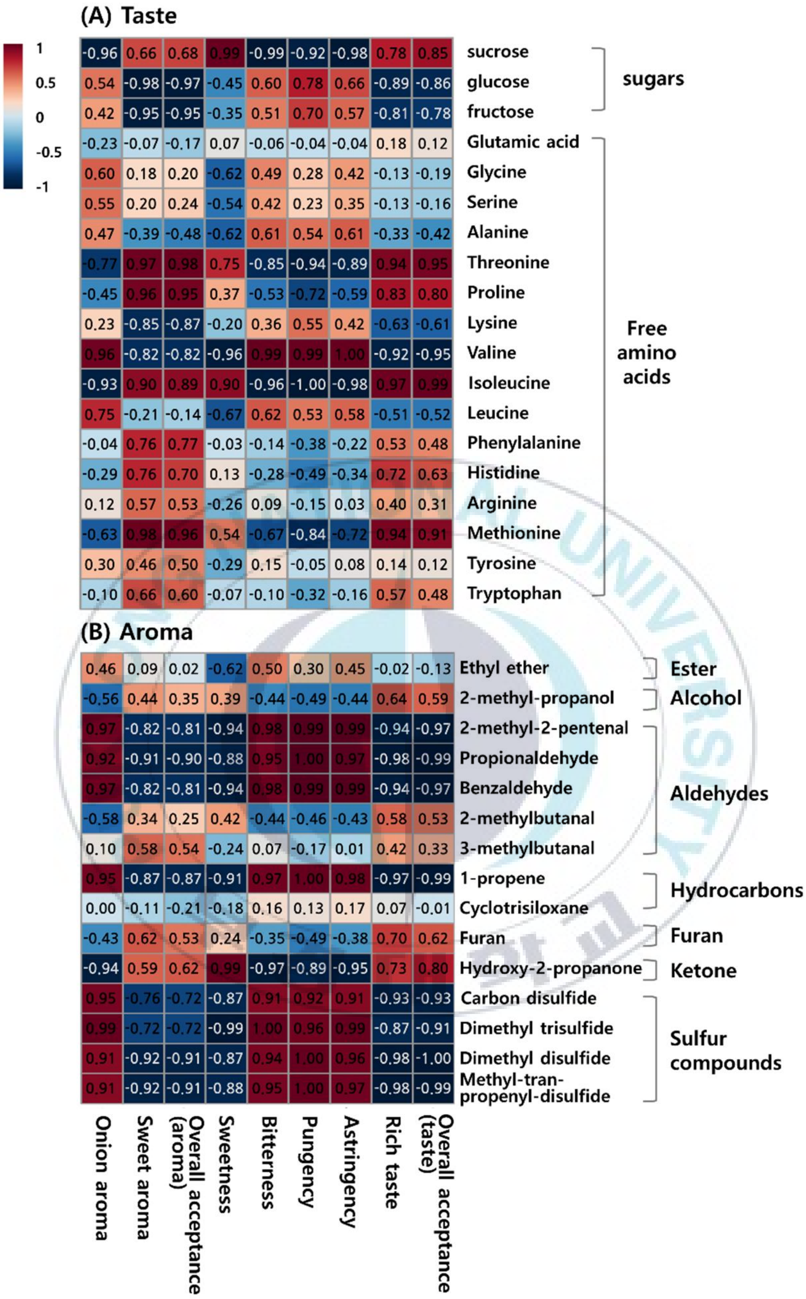

Figure 4. Correlation of sensory attributes and flavor compounds of onion juice and extracts.

negative correlation. Threonine had the highest taste activity values among free amino acids with a sweetness attribute (Table 3). Among the free amino acids representing bitterness, valine showed a negative correlation with sweetness, and a positive correlation with bitterness, pungency, and astringency. Methionine showed a high correlation with a sweet aroma, rich taste, and overall preference. Methionine has the effect of enriching the taste, bringing a positive effect on taste. Flavor compounds that positively affected the overall acceptance of taste were sucrose, glutamic acid, threonine, proline, isoleucine, phenylalanine, histidine, arginine, methionine, tyrosine, and tryptophan.

In aldehyde, all 2-methyl-2-pentenal, propionaldehyde, and benzaldehyde showed a high positive correlation with onion aroma, pungency, bitterness, and astringency attributes. These are abundant components in raw onions with off-flavor and pungency. According to a previous study, off-flavors occur when aldehydes are high (Petersen et al., 1999.). Volatile compounds that have a positive correlation with sweet aroma attributes include 2-methyl-propanol, 2-methylbutanal, 3-methylbutanal, furan, and hydroxy-2-propanone. They are substances that have nutty, sweet, and cacao flavors. Furans are generated by Maillard reactions when foods are subjected to various heat treatments (Cho & Lee, 2014). Sulfur compounds showed positive relationships with onion aroma, bitterness, pungent, and astringency, and negative correlations with the remaining sensory attributes. Flavor compounds that positively affected the overall acceptance of aroma were 2-methyl-propanol, 2-methylbutanal, 3-methylbutanal, furan, and hydroxy-2-propanone.

8. Electronic tongue and nose

Figure 5A shows the result of taste intensities of onion juice and onion extracts prepared by different extraction using E-tongue analysis. Relative sensor intensities were measured for the basic five taste components. The OJ significantly showed the highest bitterness (8.5), umami (8.0), saltiness (8.5), and lowest sourness sensor (2.8). Most of the taste sensors were strongly detected in OJ, but in human sensory evaluation, five basic tastes may not be properly recognized due to the masking of pungent flavor in raw onions (Lee et al., 2009). The high bitterness intensity of the electronic tongue is consistent with the result of bitterness, which received high scores in the sensory evaluation, due to the presence of more sulfur compounds in OJ than in other samples (Menuel, 2011). Except for OJ, there was no significant difference in sweetness, but AE was slightly lower. In the sensory evaluation, there was a difference in sweetness, but there was little difference detected by the electronic tongue. In the SRS sensor represented by sourness, HE received 8.0, which was higher than SE and AE (6.6). In umami, HE was the highest score, followed by AE, and SE. The result of the electronic tongue may be different from actual sensory evaluation, which has a complex and delicate human taste receptor system explaining subtle differences that the device cannot detect (Bleibaum et al.,2002; Xu et al., 2020). However, it is possible to measure objective and consistent information using an electronic sensor, different from sensory evaluation, which is affected by various factors such as race, age, and gender (Sipos et al., 2012).

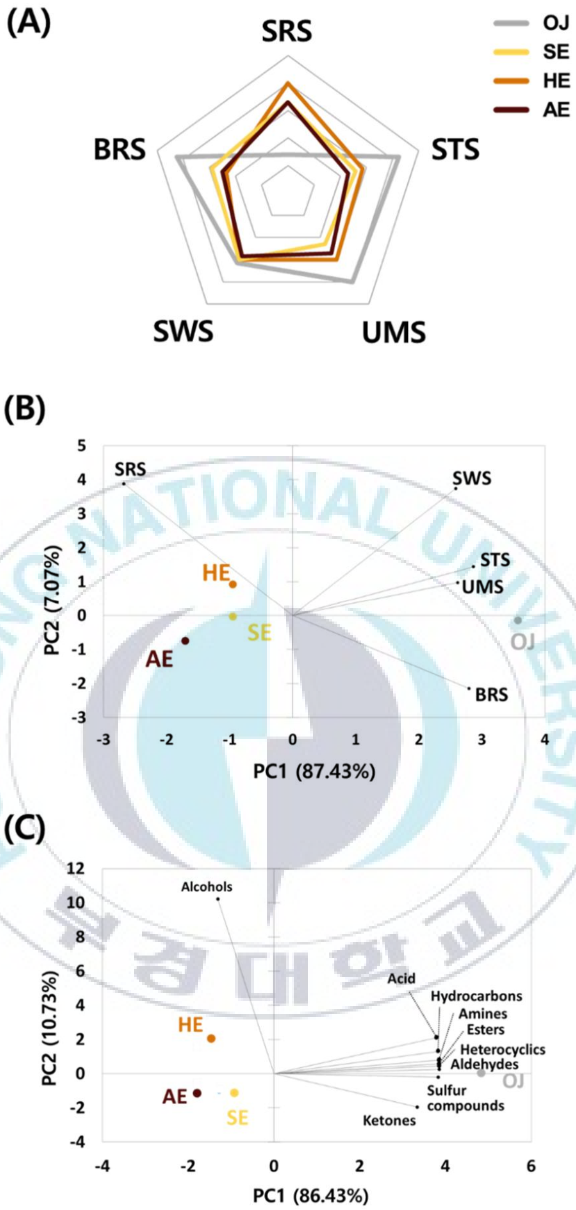

Figure 5. Electronic tongue and nose data of onion juice and extracts: (A) Radar plot of taste sensor responses in the electronic tongue, (B) PCA analysis of electronic tongue, and (C) electronic nose. OJ, onion juice; HE, hot water extract; SE, steaming extract; AE, autoclave extract.

Volatile compounds in samples were identified using an electronic nose, the result was shown in Table 4. A total of 25 volatile compounds were detected, including 1 acid, 1 alcohol, 4 aldehydes, 4 esters, 2 amines, 3 hydrocarbons, 1 heterocyclic compound, 2 ketones, and 6 sulfur-containing compounds. Overall, OJ had a relatively high volatile component compared to the other samples, because the aroma components were lost when the onion was heated (Choi et al., 2017). In all samples, sulfur compounds were found the most among the volatile compounds (Colina et al., 2013), and there was no significant difference between hydrothermal treated samples, but OJ showed a higher content than other samples. Propanal, which elicits a pungent scent, was found overwhelmingly in OJ and was detected significantly less in other samples. Amines including trimethylamine with amine, fishy, and pungent flavor and butanamine with pungent, and acidic flavor were observed in OJ, but not in hydrothermal treated samples. HE had significantly higher levels of ethanol with alcoholic and pungent flavor, which was little detectable in other samples. Similar to GC-MS results, OJ had more aroma components than other hydrothermal treated extracts, but most emit a pungent and sulfurous odor. However, individual volatile compounds and detailed results showed different results. Since the electronic nose system measures the combination scent of a sample rather than only responding to a specific scent, it has limitations compared to GC-MS, which detected individual aromatic compounds (Cevoli et al., 2011).

The degree of separation between samples of taste intensity and aroma components measured by the electronic tongue and nose was analyzed through PCA. In Figure 5B, PC1 has shown 87.43% variance and PC2 has shown 7.07% variance, explaining 94.5%

Table 4. Volatile compounds in onion juice and extracts using electronic nose (Peak area × 10^3)

Volatile compounds	RT[1)](RI[2)])	Sensory description	OJ	HE	SE	AE
Acid (1)						
2-Methylpropanoic acid	42.25(789)	Acidic, butter, dairy	0.59±0.05	0.09±0.04	ND	ND
Alcohol (1)						
Ethanol	15.16(435)	Alcoholic, pungent	0.95±0.14	8.50±0.42	0.42±0.09	0.98±0.07
Aldehydes (4)						
Propanal	17.57(489)	Earthy, pungent	36.73±3.75	2.73±0.20	4.35±0.06	1.93±0.10
2-Methylbutanal	28.31(663)	Almond, apple	0.10±0.04	0.18±0.03	0.09±0.01	0.12±0.10
Methional	54.41(905)	Baked, creamy	0.22±0.06	0.20±0.05	0.19±0.02	0.19±0.05
Nonenal	74.15(1,181)	Green, melon	4.14±0.96	ND	0.21±0.14	0.16±0.05
Esters (4)						
Isopropyl acetate	27.29(651)	Banana, fruity, sweet	0.18±0.03	0.34±0.06	0.17±0.04	0.10±0.09
Methyl bu-2-enoate	36.93(744)	Blackcurrant, fruity	1.29±0.14	ND	ND	0.28±0.11
Diemthyl-methylphosphonate	53.28(893)	-	0.33±0.17	0.08±0.04	ND	ND
Butyl methacrylate	61.54(989)	-	17.76±2.73	0.42±0.14	0.34±0.12	0.58±0.20
Amines (2)						
Trimethylamine	13.94(408)	Amine, fishy, Pungent	0.89±0.19	ND	ND	ND
Butanamine	25.95(637)	Acidic, pungent	0.11±0.03	0.03±0.05	ND	ND
Hydrocarbons (3)						
3-Ethylpentane	31.55(698)	-	0.08±0.07	0.04±0.03	ND	ND
3-Ethylhexnae	40.43(773)	-	ND	0.07±0.02	0.04±0.03	ND
6-Methyl tridecane	82.55(1,339)	-	1.57±0.11	0.45±0.16	0.42±0.12	0.48±0.19
Hetrocyclic (1)						
2,6-Dimethylpyrazine	55.33(916)	Cocoa, nutty, roasted	5.27±0.55	0.10±0.04	0.18±0.03	ND
Ketones (2)						
3-Hexen-2-one	47.51(838)	-	1.14±0.16	0.09±0.08	0.31±0.12	ND
Heptene-3-one	50.98(871)	-	0.46±0.10	0.08±0.04	0.08±0.07	0.08±0.07
Sulfur-containing compounds (6)						
2-Mercaptoethnol	20.35(550)	Sulfurous	0.23±0.04	0.24±0.02	0.16±0.07	0.33±0.04
2-Methyl-2-propanethiol	22.55(599)	Sulfurous	0.43±0.07	0.34±0.06	0.31±0.04	0.39±0.05
Propanethiol	24.25(618)	Vinegar, pungent	2.07±0.32	0.12±0.16	0.12±0.01	0.18±0.16
Butanethiol	33.66(716)	Coffee, onion	1.09±0.24	0.08±0.03	ND	ND
Octanethiol	70.98(1,128)	Sulfurous	3.45±0.58	ND	0.14±0.07	0.28±0.08
Bis-(2-furylmethyl) disulfide	97.91(1,677)	Cabbage, onion	2.38±0.08	2.09±0.13	2.46±0.51	2.33±0.16

Data represent the mean ± SD in triplicate.
1)RT: retention time
2)RI: retention indices
3)ND: not detected.

of the total variation in the data set. OJ was positively correlated with the PC1 plot, whereas other thermal extraction samples were negatively correlated. In the score plot, OJ is associated with the BRS sensor, STS sensor, and UMS sensor, and HE was found to be correlated with the SRS sensor. In Figure 5C, the variances of PC1 and PC2 were respectively 86.43% and 10.73%, explaining a total of 97.16% variation in the data set. Within the samples, OJ was clearly distinguished, but there was no significant difference between the hydrothermal treated extracts.

Conclusion

For centuries, onion has been used in a wide variety of foods worldwide. We analyzed the flavor profiles of onion juice and extracts prepared by different hydrothermal treatments by measurement of taste and aroma compounds, and analysis of electronic nose and electronic tongue. All extracts have different yields, colors, and flavor profiles according to different hydrothermal treatment temperatures, pressures, and times. As the temperature and pressure increased, the yield of onion extract increased, and both yellowness and redness significantly increased. OJ was related to the negative flavor attributes such as bitterness, pungency, and astringency, whereas the hydrothermal treatment extracts were associated with positive flavors such as sweetness, and sweet aroma. A total of 54 volatile compounds were identified in OJ, but only 28 volatile compounds were detected in hydrothermal treated extracts due to the disappearance of aroma compounds by heat treatment. However, the sensory preference of the hydrothermal treatment extracts was significantly higher than that of OJ, due to the reduction of a pungent sulfur compounds and the formation of aldehydes and furans emitting roasted flavor. In electronic nose and tongue, OJ and hydrothermal treated extracts were clearly distinguished. Our results suggest that the hydrothermal treatment of onion can contribute to sensory improvement. In particular, AE is considered to be an efficient process with short extraction time and high yields, which is suitable for application to roasted onion flavor manufacturing. However, studies are needed to find the optimal pressure and temperature conditions for higher yield and efficiency using statistics programs such as response surface methodology (RSM).

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