Open Access Journal

International Journal of Medical Research and Pharmaceutical Sciences

Volume 3 (Issue 12) : December 2016 ISSN: 2394-9414

DOI- 10.5281/zenodo.221015 Impact Factor- 3.109

SOLID PHASE MICRO EXTRACTION (SPME) FLAVOR ANALYSIS OF APPLE JUICE AND COFFEE MIXTURES USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Keywords:
Apple juices, coffee, Gas chromatography-mass spectrometry (GC-MS), Solid phase micro extraction (SPME), Sensory evaluation.

Abstract

This research was conducted to evaluate the flavor of apple juice and coffee mixtures and the sensory quality of SPME extracts using gas chromatography-mass spectrometry (GC-MS). Three samples with different compositions were examined. Sample A1 contained85% apple juiceand 15% coffee, sample A2 had87.5% apple and 12.5% coffee, and sample A3 had90% apple juiceand 10% coffee. The sensory analysis involved 100 panelists and a sequential monadic test. Sample presentation orders were balanced in each serving position using the Williams Latin Square design. The eleven components were odorants perceived in the samples. 2-methyl-1-butanol, butyl acetate, (e)-2-hexenal, hexanol, 3-methylbutyl acetate, hexyl acetate, 2,3-pentanedione, pyrazine, pyridine, 2-furanmethanol, and 5-methylfurfural were found in the aromatic composition of the apple juice and coffee mixtures.

INTRODUCTION

Fruit juices are often concentrated to reduce their weight and volume and thus their packaging, transportation, and storage costs (Hicks D, 1990). Apples produced in Korea are mostly consumed fresh and only 2.4% are processed. Considering the market price fluctuationof raw apple, it is very valuable to increase the proportion of apples processed for both producers and consumers. The major processed product of apples is natural juice, which has a complex flavor (O.S. Lukanin et al., 2003). Apple juice is one of the most popular juice beverages worldwide, and its qualityis greatly dependent on the characteristic “fresh apple juice” flavor. Flavor, including odor and taste, is an important food quality attribute.Coffee is also one of the most widely consumed beverages globally. In addition to caffeine, coffee contains more than 1000 different chemical compounds, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds (Higdon and Frei, 2006). Aroma is an important attribute that defines the consumer acceptance of coffee products. The reactions involved in coffee aroma formation are very complex and includeMaillard and Streckerreactions and the degradation of sugar, trigonelline, chlorogenic acids (C and A), proteins, and polysaccharides as some examples (Clifford, 1985; Dart and Nursten, 1985).

Mixing is commonly used in the food industry but is one of the least understood process operations. Some aspects of mixing can be measured for help in the planning and designing of mixing operations (Earle, 1966). Mixing apple juice and coffee could establish new odors and tastes to characterize a juice product.

Solid phase micro extraction (SPME) is a relatively new method of sample preparation in food analysis, along with membrane separation techniques, pressurized fluid extraction, supercritical fluid extraction, and microwave-assisted extraction (Buildini et al., 2002). SPME was developed by Pawliszyn et al. (Zhang and J. Pawliszyn, 1993;Pawliszyn, 1997). It represents a new approach to accumulate analytes by diffusion and combines the advantages of both static and dynamic headspace (HS). SPME is the first sampling technique based on analyte diffusion that has successfully been applied in a number of fields.

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SPME is simple, robust, low-cost, and very popular as a fast screening method for qualitative analysis. As shown in many applications, it is also a reliable method in quantitative analyses. It is characterized by high sensitivity and is often compared in this aspect to dynamic HS. In several works, both techniques were used in the analysis of food products such as cola (J.S. Elmore et al., 1997), milk, butter, and cheeses (R.T. Marsili, 1999; G. Contarini and M. Povolo, 2002; M. Povolo and G. Contarini, 2003; S. Mallia, E. Fernández-Garcia, and J.O. Bosset, 2005). For flavor compound isolation, SPME has potent advantages over distillation/extraction (SDE) methods in that isolation is performed at lower temperatures and usually for a shorter period of time, which prevents decomposition of thermally labile compounds. The formation of artifacts in the injection port is caused by fiber coating reactions and depends on the inertness of the coating material (H.H. Jelen, 2003).

This research investigated the flavor ofmixtures of apple juice and coffee.The sensory quality of SPME extracts was evaluated using gas chromatography-mass spectrometry (GC-MS).Focus was centered on aliphatic ester analysis, especially 3-methylbutyl acetate.

MATERIALS & METHODS

Raw material

Freshly picked apples (Malusdomestica) were cultivated in Mungyeong city, Gyeongsangbuk Province, South Korea. The apples were washed with tap water and stored in a cold room for 2 days. Apples with a measured value of 13-15% Brix and pH 3-4 and were used for this study. Coffeewas purchased from a local market ***and stored at room temperature until use. The coffee had Brix values of 1-2% with pH 4-5.***

Sample preparation

Juice was extracted from the apples by a juicer machine (HD-WWF09, Hurom Group Corporation, South Korea). Excessive pulp and foam were removed from the juice by a 100-mesh filter. To prevent browning, 0.05% ascorbic acid ($C_6H_8O_6$)was immediately added to the 500 ml of apple juice.The coffee and apple juice were mixed with the ratios inTable 1in 500-ml quantities.

Table 1 Experimental condition for the mixing apple juice and coffee

Sample	Apple (%)	Coffee (%)
A1	85	15
A2	87.5	12.5
A3	90	10

Identification of the volatiles

Volatiles from the mixed apple juice and coffee were extracted using SPME fibers(50μm, 30μm DVB,Carboxen ™, PDMS StableFlex ™; Supelco, Supelco Park, Bellefonte, PA, USA). The fibers were conditioned in asplit GC injector port (2 h at 300 °C for CAR/PDMS; 4 h at 270 °C for DVB/CAR/PDMS SPME). Before each extraction, they were held at 260 °C for 5 min and then at room temperature for 2 min. SPME extraction was performed on 1 ml of stirred juice (40°C) contained in a 4-ml vial sealed with a PTFE-lined screw cap.

GC-MS analysis

The aroma profile of the apple juice samples was investigated by headspace solid-phase micro-extraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS).GC-MS was carried out using an HP 5975B quadruple mass selective detector (Agilent Technologies, USA). The mass spectrometer was operated in

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electron impact ionization mode at a voltage of 70 eV and electron voltage of 1000V. Mass spectra wastaken over the *m/z* range of 40–350.The flow rate of the helium carrier gas on the DB-5capillary column (30 m×0.25 mmI.D, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was 0.8 mL/min. The analysis was performed in splitless mode, and the injector temperature was 250 °C. The column was held at 35 °C for 5 min then increased to 220°C at 2.5 °C/min for 310 min.

Volatile components were identified by comparing their mass spectra.MS identifications were confirmed by comparing GC retention times of the analyticswith those from pure standards. The identification was confirmed using retention indices (RI), and the value was compared with those reported in the literature(Table 2). Linear retention indices (RI) of the compounds were calculated using a series of C8–C20 (*n*-alkanes, Aldrich, Milwaukee, MN, USA) injected in the same conditions. The volatile substance analyses were repeated three times for all samples.

Sensory evaluation

For the sensory panel,100people were recruited from Pusan National University, Pusan University of foreign studies, and Pusan Catholic University. Tests occurred in individual booths of the Sensory Analysis Laboratoryin the Department of Food Science and Nutrition at Pusan National University. The samples were served at a room temperature (18-20°C) in cups coded with three-digit numbers and covered with a piece ofpaper.The test was a sequential monadic test, and sample presentation orders were balanced in each serving position using the Williams Latin Square design (Williams, E. J, 1949). Consumers were asked to evaluate the appearance, flavor, sweetness, acidity, purchase intention, and overall acceptability of each sample on a 9-point hedonic scale (1 = ‘‘dislike extremely”, 9 = ‘‘like extremely”). Each product was evaluated using the 5-point ‘‘Just About Right” (JAR) scale. Statistical analyses were done with the Statistic Analytical System (SAS Institute Inc., Cary, NC, USA). ANOVA was performed on similarity rates considering the sampling method, the judge, and the repetition effects.

Table 2 Characteristics of volatilecompounds

Volatile Compounds	Odor characteristics	Reference
Aliphatic Alcohols		
Hexanol	Resin, flower, green	Adedeji, Hartman, Rosen & Ho, 1991
2-Methyl-1-butanol	Malt	Rychlik, Schieberle&Grosch Compilation of Odor Thresholds, 1998
Aliphatic Aldehyde		
Hexanal	Grass, tallow, fat	Bravo, Hotchkiss &Acree, 1992
(E)-2-Hexenal	Green, leaf	Chisholm, M. G., M. A. Wilson, et al., 2003
Aliphatic Ester		
Ethyl butyrate	Apple	Acree, Barnard & Cunningham, 1984
Butyl acetate	Pear	Cunnigham, Acree, Barnard, Butts &Braell, 1986
3-Methylbutyl acetate	Fruit	Fuhrmann, E. and W. Grosch, 2002

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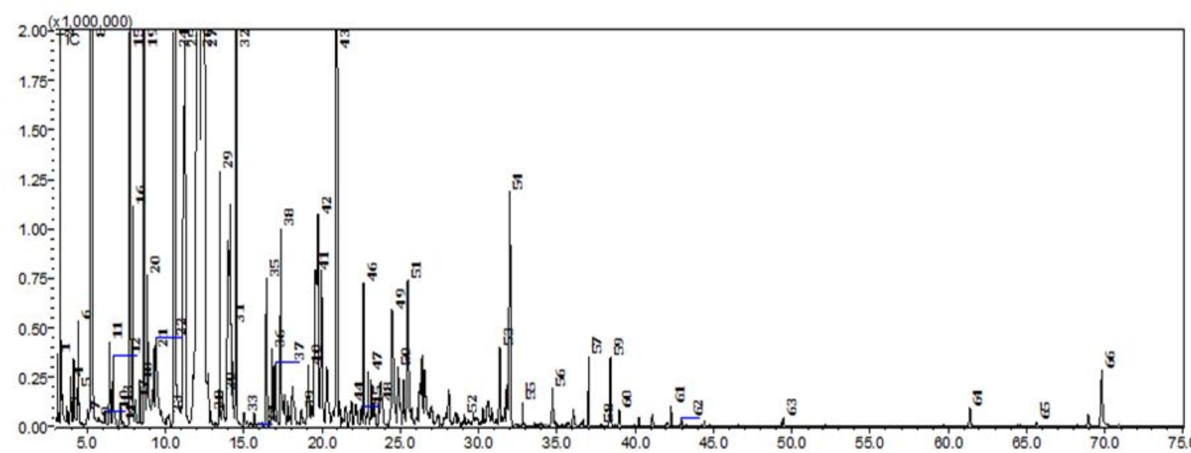
Hexyl acetate	Fruit, herb	Fuhrmann, E. and W. Grosch, 2002
Ketones		
2,3-Pentanedione		
Pyrazine		
Pyridine		
Pyridine	Unpleasant fish-like odor	Pyridine MSDS, fishersci.com
Furans		
2-Furanmethanol	Resin, adhesive, wetting agent	Stamm, Alfred, 1977
5-Methylfurfural	Almond, caramel, burnt sugar, coffee	Schieberle&Grosch, 1987

RESULTS & DISCUSSIONS

Volatile components in the mixing juices

A total of 11 compounds were identified in the volatile component analysis of the juice mixtures(Figure 2):2-methyl-1-butanol, butyl acetate, (e)-2-hexenal, hexanol, 3-methylbutyl acetate, hexyl acetate, 2,3-pentanedione, pyrazine, pyridine, 2-furanmethanol, and 5-methylfurfural. Figure 2showsthat the juice mixture samples all have higher amounts of 3-methhyl acetate than other volatile compounds. This indicates that the coffee and apple juice mixtures had high aroma intensity.Sample A2 (87.5% apple juice +12.5% coffee) has a bigger value than sample A1 (85% apple juice +15% coffee) and sample A3 (90% apple juice +10% coffee). Figure 1 shows the chromatograms of the juice samples. Peaks corresponding to aliphatic esters and aliphatic alcohols can clearly be seen. The peak identities were assigned based on retention times and chromatographic patterns and confirmed by GC-MS.

Figure 1 Chromatographic profiles of SPME juice mixtures



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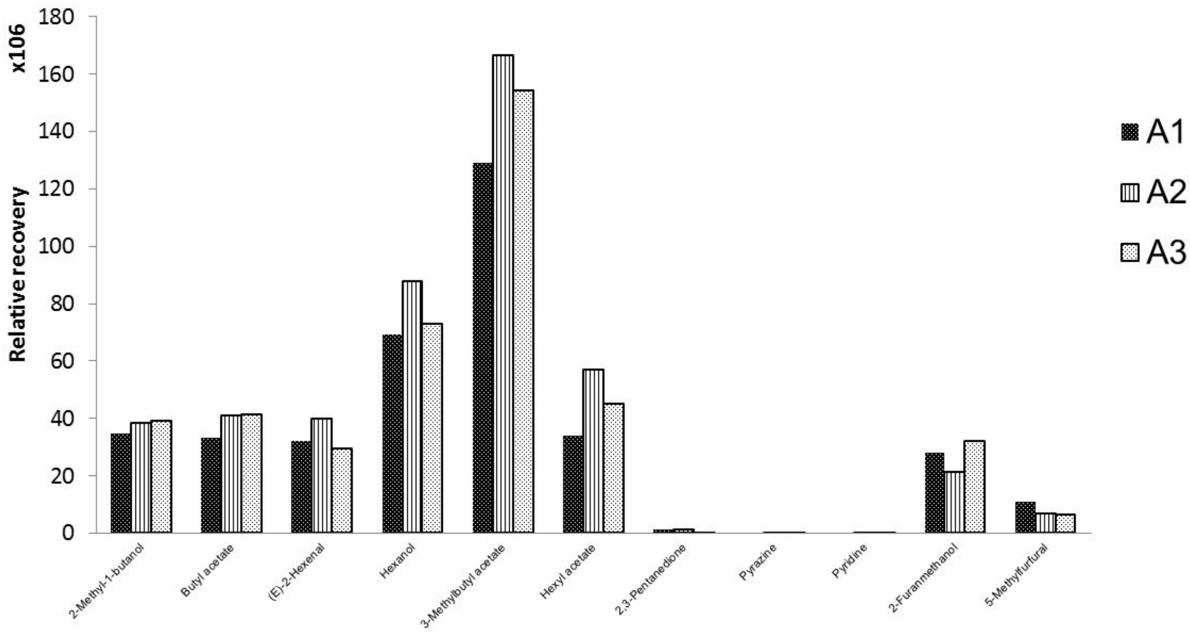

Figure 2 Characteristics of the volatile compounds in the juice mixtures

Sensory evaluation

Figure 3shows a spider-web representation of the consumer testing analysis scores (1 to 9) given by 100 consumers to the samples of juices. The parameters of flavor, sweetness, purchase intention, and overall acceptability are higher for sample A3 (90% apple juice +10% coffee) than sample A2 (87.5% apple juice +12.5% coffee) and sample A1 (85% apple juice +15% coffee). Sample A1 (85% apple juice +15% coffee) has a better appearance. The acidity of sample A2 (87.5% apple juice +12.5% coffee) was the highest.

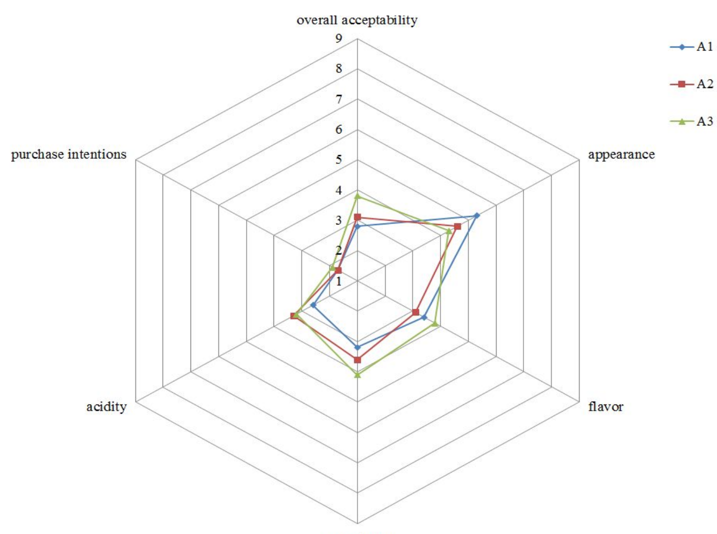

Figure 3 Spider-web representation of consumer testing analysis

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CONCLUSION

This study provided the first comprehensive methodology for odor SPME and evaluated sensory quality based on GC-MS.The samples all had more 3-methhyl acetate than other volatile compounds. The mixtures of coffee and apple juice have a fruit aroma. Overall, the sensory test panelists preferred sample A3, which had 90% apple juice and 10% coffee.GC-MS will enable future investigations to further improve SPME performance.

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