Assessment of Flavor Volatiles of Iranian Rice Cultivars during Gelatinization Process

N. Amiri Khorheh ^a , M. H. Givianrad b, M. Seyyedain Ardebili c , K. Larijani b*

^a M. Sc. of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran. ^b Assistant Professor of the Department of Chemistry, Science and Research Branch, Islamic Azad University,

Tehran, Iran. ^c Assistant Professor of the College of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received 16 June 2010; Accepted 10 October 2010

ABSTRACT: A combined gas chromatography-mass spectrometry (GC-MS) with headspace solid-phase micro extraction (SPME) method has been employed for the analysis of the flavor volatiles of three different rice cultivar, including two modified Iranian rice cultivars and Hashemi rice cultivar during gelatinization. The proposed combination provided a powerful system for easy and rapid screening of a wide range of flavors in fragrant rice samples. In order to optimize the different experimental parameters, the effect of fiber composition, water content of rice samples and equilibrium time were investigated. As a result, while the gelatinization was in progress, the amount of the volatile compounds was increased as well. All of the free flavor volatiles, the bound flavor components and the compounds formed by the thermal decomposition of the non-volatile constituents existing in rice could be liberated during the gelatinization process. Therefore, a broad range of the flavor volatiles of rice could be extracted, concentrated and identified. Altogether, 75, 55 and 66 components were identified for Hashemi, HD5 and HD6 rice samples, respectively, which 58 unique compounds were not detected previously. The identified volatile components in the three Iranian rice cultivars belong to the chemical classes of aldehydes, ketones, alcohols and heterocyclic compounds, as well as fatty acids and esters, phenolic compounds and hydrocarbons. **Continuistation Process**
 *K. Amiri Khorheh", M. H. Givianna¹⁹, M. Seyredain Ardohli', K. Larijani¹

¹M. Sevrel Fourist controllers with Columbian 2⁹, M. Seyredain Ardohli', K. Larijani¹

¹M. Secret of the Co*

Keywords: *Flavor Volatiles Components, Gas Chromatography (GC), Mass Spectrometry (MS), Rice (Oryza sativa L.), Solid-Phase Microextraction (SPME).*

Introduction

 \overline{a}

Rice is the major part of people's diet in many countries, which contributes 21% of the calorie intake. In this aspect, one of the most significant factors in market business and expenses is the aroma of 'fragrant rices'. This is a trait, which distinguishes it from ordinary rice (Fitzgerald *et al*., 2009; Laohakunjit & Kerdchoechuen, 2007).

There are many different volatile components in cooked rice grains, including those, which are the result of biochemically distinct pathways, and are very rich sources of hydrocarbons, organic acids, alcohols,

aldehydes, ketones, esters and phenols. In some cases, there are chemically synthesized components, which are stored during the process of rice development. However, there are some other compounds, which are extracted because of chemical breakdowns. Among the latter, fatty acids are good samples (Bergman *et al*., 2000).

Aromatic compounds can have both positive and negative influences on people's tastes. The former can be flavor and fragrance components such as 2AP and the aromatic alcohols, and the latter can be offflavors of hexanal and 2-pentylfuran (Lam & Proctor, 2003).

^{*} Corresponding Author: givianradh@yahoo.com

Among those aromatic compounds, which have been identified in many studies, 2-acetyl-1-pyrroline (2AP) is known as the most important key flavor component of rice aroma (Buttery *et al*., 1983b; Jezussek *et al*., 2002; Laksanalamai & Ilangantileke, 1993; Paule & Powers, 1989). Its structure consists of a five-carbon N-heterocyclic ring, which was first determined in cooked rice and the volatile oil of freeze-dried Pandan leaf with a threshold value of 0.1nl/l water, which was very low comparing other components (Buttery *et al*., 1983a; Buttery *et al*., 1988; Wongpornchai *et al*., 2003).

The concentration value was high up to 0.09 mg/kg, which was 10 times more than non-fragrant rice value (<0.006-0.008 mg/kg). This amount was identified in all parts of fragrant rice, but not in roots (Buttery *et al*., 1983b).

In order to provide sample preparation methods and analytical techniques, Solidphase microextraction (SPME) is introduced as a rapid, sensitive and reliable technique for the extraction and concentration of volatile compounds from different sample matrices. SPME consists of two separate steps. The first step deals with partitioning the target analytes between the sample matrix and the fiber surface, whereas the second step deals with the direct desorption of absorbed analytes into injection port by means of chromatographic techniques (Djozan & Ebrahimi, 2008). Paule & Powers, 1989). Its sinceture consists reliable system for effective tapping and the strained in order of the components of

This technique has many advantages including less time for extraction, ease of use, preventing the loss of analytes and field sampling with portable field sampler (Djozan *et al*., 2009; Mehdinia *et al*., 2006).

This technique has a great sensitivity particularly in the study of identifying flavor indicators of rice, since the extracted fraction on the fiber is statistically introduced into the gas chromatography (GC) by thermal desorption (Bergman *et al*., 2001; Grimm *et al.*, 2004). For instance, Laguerre *et al*., (2007) suggested an analytical technique for the analysis of the volatile fraction using SPME-MS (Laguerre *et al*., 2007).

In our current study, our main purpose is to use HS-SPME-GC-MS device as a reliable system for effective trapping and screening of a wide range of volatile flavor compounds in the headspace of Iranian rice samples during the gelatinization process.

Materials and Methods

Rice Samples

Three Iranian fragrant rice samples were used in this study, including two new modified varieties (HD5, HD6) and one Hashemi variety (HD1), which are predominantly consumed in Iran.

These samples were collected from Rice Research Institute. All samples were harvested in July 2009 and contained 20% moisture. After 24 h from harvesting, the samples were sun-dried to about 12-13% moisture content, then they were dehulled at the growing area and were transported to the laboratory and stored in nylon bags and placed in the refrigerator at 4ºС until the experiments were completed.

Gelatinization process

Some glass balls were poured into a glass of 275ml water and put under primary heating until they reach the boiling point. 50g of the tested sample was added into the boiling water and stirred. After 7min, 10 rice grains were chosen randomly and put on a glass plate with equal distances, and covered, slipped and gelatinized. The number of gelatinized rice grains were observed and counted after 8min. The experiment was repeated every one minute to determine all the ten gelatinized grains. This method was used for all the samples in this study. This method was performed for the first time.

Rice cooking

The traditional Iranian rice cooking was used, by which 150g of white rice and 400ml of distilled water were added. The whole process was divided into 4 stages, including all the steps from the beginning until the end of gelatinization process. The cooking time of this process was determined by the temperature at which crystalline structure melting occurred. Rice with high GT required more time to cook, whereas rice with low GT, required less time; usually up to 4 min. The former had an unacceptable texture (Fitzgerald et al., 2009).

Headspace Solid-phase micro extraction sampling

Using SPME, a sampling device was designed in order to collect the volatiles during the course of gelatinization. The volatiles during gelatinization were released out through the first side arm, while SPME fiber was located in the second side of the sampling device with a flexible septum. In all stages of the gelatinization, the fiber was placed in the manually operated SPME holder, and the septum was covered with a Teflon-coated silicone in order to prevent the release of volatile compound from the septum. The fiber was lowered in the sampling port to adsorb flavor volatile compounds of rice and desorb them thermally in the injection port of GC-MS instrument for 10 min at 250°С. Afterwards, the fiber was exposed to the SPME fiber conditioner at 250°С for 1hr for reconditioning before being subjected to the next volatile samples. unid the end of gelatinization process. The column The injector is
mynching that the most set and particle and particle is a certification at 98,99% was
structure melting cocurred. Received as a certification is certifica

SPME fiber with PDMS (100µm, nonbonded), CAR/PDMS (75µm, bonded) and DVB/CAR/PDMS (65µm, bonded) coating, provided by Supelco (Bellefonte, PA, USA) was used as commercial fiber and was preconditioned in an SPME fiber conditioner (GL Sciences) at 250°С for 1hr before the first measurement.

GasChromatography-Mass Spectrometry

Gas chromatography separation was performed on a HP-6890 GC system (HEWLETT PACKED, USA), equipped with a mass detector (HP-5973, USA), and a HP-5MS (5% Phenyl dimethyl siloxan) column. The injector temperature was set at 250°С and purified Helium at 99.99% was selected as a carrier gas at a flow rate of 1ml/min. Temperature programming was performed at 60°С for 3min, and increased to 220ºС at the rate of 5°С/min and maintained at 220°С.

The mass selective detector was applied in an electron impact ionization mode at 70ev.

The interface temperature was 230°С. An alkane mixture with C8-C20 alkane and concentration of 40mg/ml in hexane was purchased from Fluka. The mixture was used to estimate retention indices (RI), and it was injected into the fiber for 5min by the headspace extraction from a 10ml SPME vial, including 1ml HPLC-grade water spiked with 10µl of the mixture.

The volatile compounds were positively identified by matching their mass spectra, with the spectra of reference compounds in Adams Mass Spectra Library (9th edition) and verified based on mass spectra and RI values reported in the literature (Buttery & Ling, 1998; Buttery *et al*., 1999; Fan & Qian, 2006).

Results and Discussion

Cooking time

The cooking time of rice is determined by the temperature at which the crystalline structure of the starch begins to melt. This is called gelatinization temperature (GT). In the samples, GT was 55-85˚C. Hashemi rice sample with high GT required more time to cook. Lowering the GT of the modified (HD5, HD6) rice grains could decrease the average cooking time.

Regarding tables 1, 2 and 3 the figures 1, 2 and 3 were designed and gelatinization time for 0%, 50%, 90% and 100% of the whole grains was identified and during this time, SPME fiber was injected. The whole process was divided into four stages: Ι, 17'; II, 20':49"; III, 24':37"; IV, 28' which were assigned to Hashemi rice sample leading to the gelatinization of 0, 50, 90, and 100

percent of rice grains. For HD5, variety at cooking stages: Ι, 15'; II, 18':12"; III, 20':28"; IV, 23' which could lead to the gelatinization of 0, 50, 90 and 100 percent of rice grains. In HD6, variety at cooking stages: Ι, 15'; II, 17':38"; III, 21':28"; IV, 24' which could lead to the gelatinization of 0, 50, 90 and 100 percent of rice grains.

Table 1. Hashemi rice samples

J. FBT, IAU, 1, 41 -54, 2011

Fig. 1. Gelatinization profile of Hashemi rice samples

N. Amiri Khorheh et al.

Fig. 2. Gelatinization profile of HD5 rice samples

J. FBT, IAU, 1, 41 -54, 2011

Fig. 3. Gelatinization profile of HD6 rice samples

Flavor volatiles in the three Iranian rice cultivars

Using headspace SPME method, the flavor volatiles in the three Iranian rice cultivars were extracted during the gelatinization, and analyzed by GC-MS. These compounds were determined by comparing their mass spectra and RI values with an authentic compound, whereas others were identified by their corresponding mass spectra (Adams Mass Libraries) and RI values, when RI values on the HP-5MS capillary column were available in the literature (Buttery & Ling, 1998; Buttery *et al*., 1999; Fan & Qian, 2006). A whole range of 75, 55 and 66 compounds were identified for Hashemi, HD5 and HD6, respectively. For HD5, 23, 11, 23 and 36 compounds were identified at stages Ι, II, III and IV, respectively. Also regarding HD6, 14, 19, 47 and 32 compounds were identified at the stages as HD5, and 14, 42, 37 and 43 compounds for Hashemi.

Altogether, the volatile compounds in the three Iranian rice samples during the gelatinization belong to the chemical classes of aldehydes, ketones, hydrocarbons, esters and phenols, etc., which corresponds with those chemical classes of compounds previously mentioned in non- Iranian rice using various methods of extraction.

58 new and unique compounds were detected and identified in the samples as presented in the tables 4, 5 and 6.

Variation in flavor volatiles of rice during four different cooking stages

Significant differences were investigated in the volatile compounds of rice during the four different gelatinization stages.

Two major compounds were detected at stage Ι known as nonanal and hexanal. The latter is known as an important lipid oxidation product in rice. However, there are other components, which have been identified only at stage Ι, such as ethanol, in contrast with hexadecanoic acid, which was identified as a predominant compound at stage II. Moreover, pentacosane were detected at stage III & IV.

Primary heating of rice at gelatinization stages Ι and II resulted in the evaporation of aldehydes in rice, and fatty acids in steam distillation, respectively. However, excess steam and heat have debilitative influence on the extraction of low-boiling-point volatiles. Thus, further heating at stages III and IV, increased the rate of evaporation of a broad range of the flavor volatiles of rice.

Similarities and differences among the three different Iranian rice cultivars

By comparing the volatile matters shown in tables 4, 5 and 6, one might have a better understanding of the similarities and differences in the rice samples.

There were no significant differences in the profiles of flavor volatiles; however, less volatile compounds were identified in two modified rice samples as compared to Hashemi. For instance, some components such as Phthalic acid, Farnesol, Ethanol, alpha-Pinene and Camphene were detected in two modified samples, whereas Hashemi major compounds were beta.Bisabolene, Cyclosativene, Methyl isoeugenol, Isobutyl salicylate, Turmerone, lilia, <cis-2-tertbutyl->Cyclohexanol acetate, 2-phenyl- 2 methyl- Aziridine, 4-ethyl- 3,4- dimethyl-Cyclohexanone, n- Octyl- Cyclohexane, 9 methyl- Nonadecane, 2- methyl- Tridecane, (E,E)-2,4-Decadienal, Indol.

Optimization of different experimental parameters

Hexanal was employed as a target to optimize the conditions of flavor compounds.

Initially, three types of commercial fibers (PDMS, CAR/PDMS and DVB/CAR/PDMS) were selected in order to extract volatiles from the headspace above the rice samples in the beginning of the

experiment. The DVB/CAR/PDMS fiber was used in all applications and is known as the most effective compound in the extraction of flavor volatile (Fig. 4).

In order to estimate the effect of water content of rice samples on the SPME, different volumes of water were added to 150g rice samples and SPME experiments were performed. The results in figure 1 indicate that increasing water content up to 400ml was in agreement with the extraction efficiency and consequently an increase in hexanal. However, addition of higher amount of water showed a great decrease in extraction efficiency, since this would result in producing an adhesive mixture which did not easily agitate and made the diffusion more difficult. On the other hand, addition of lower quantity of water was not sufficient for cooking rice. Therefore, 400 ml of water was selected as a suitable amount for the rest of the experiment (Fig. 5). volatiles. Thus, further heating at stage iii diribtent volumes of variet were added to the samely and AIV, increased the rate of evaporation of 150g rice samples and SFME experiments a broad range of the flavor volatiles

To investigate the effect of time, the rice samples were extracted for 5-30min. As figure 6 indicates, with an increase in equilibrium time, the extracted amounts of hexanal were increased into a maximum threshold after 30 min. Therefore 30 min was considered as the optimum period to reach the equilibrium for hexanal (Fig. 6).

Conclusion

The volatiles in the three rice cultivars during gelatinization were directly extracted using an HS-SPME method and analyzed by GC-MS. Altogether, 75, 55 and 66 compounds were identified for Hashemi, HD5 and HD6 rice samples, respectively.

Further studies should be carried out to consider future separation and identification on these compounds, as well as comparing these compounds among Iranian, non-Iranian and modified samples

Fig. 5. Comparison of the extraction efficiencies of the various additions of water

Fig. 6. Equilibrium time profile

N. Amiri Khorheh et al.

Table 4. Hashemi rice sample

J. FBT, IAU, 1, 41 -54, 2011

N. Amiri Khorheh et al.

Table 6. HD6 rice sample

References

Bergman, C. J., Delgado, J. T., Bryant, R. & Grimm, C. (2001). Screening for 2 acetyl-1-pyrroline in the headspace of rice using SPME/GC-MS. Journal of Agricultural and Food Chemistry, 49, 245- 249.

Bergman, C. J., Delgado, J. T., Bryant, R., Grimm, C., Cadwallader, K. R. & Webb, B. D. (2000). Rapid gas chromatographic technique for quantifying 2-acetyl-1 pyrroline and hexanal in rice(*Oryza sativa* L.). Cereal Chemistry, 77, 4, 454-458.

Buttery, R.G., Ling, L.C. 1998. Additional studies on flavor components of corn tortilla chips. Journal of Agricultural and Food Chemistry, 46, 2764-2769.

Buttery, R. G., Ling, L. C. & Juliano, B. O. (1983a). Identification of rice aroma compound 2-acetyl-1-pyrroline in Pandan leaves. Chem. Ind. (London), 20, 478-485.

Buttery, R. G., Ling, L. C., Juliano, B. O. & Turnbaugh, J. G. (1983b). Cooked rice aroma and 2-acetyl-1-pyrroline. Journal of Agricultural and Food Chemistry, 31, 823- 826.

Buttery, R. G., Ling, L. C. & Turnbaugh, J. G. (1988). Contribution of volatiles to rice aroma. Journal of Agricultural and Food Chemistry, 36, 1006-1009

Buttery, R. G., Orts, W. J., Takeoka, G.R. & Nam, Y. (1999). Volatile flavor components of rice cakes. Journal of Agricultural and Food Chemistry, 47, 4353- 4356.

Djozan, D. & Ebrahimi, B. (2008). Preparation of new solid phase micro extraction fiber on the basis of atrazine – molecular imprinted polymer: Application for GC/MS screening of triazine herbicides in water, rice and onion. Analytica Chimica Acta, 6I6, I52-I59.

Djozan, D., Makham, M. & Ebrahimi, B. (2009). Preparation and binding study of solid-phase microextraction fiber on the basis of ametryn-imprinted polymer:

Application to the selective extraction of persistent triazine herbicides in tap water, rice, maize and onion. Journal of Chromatography A, 1216, 2211-2219.

Fan, W. & Qian, M. (2006). Chracterization of aroma compounds of Chinese \cdots Wuliangye, and \cdots Jiannanchun, liquors by aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, 54, 2695-2704.

Fitzgerald, M. A., Couch, S. R. M. & Hall, R. D. (2009). Not just a grain of rice: the quest for quality. Trends in Plant Science, 14, 133-139.

Grimm, C., Lioyd, S. W., Godshall, M. A. & Braggins, T. J. (2004). Screening for sensory quality in food using solid phase micro-extraction tandem mass spectrometry. Advance in Experimental Medicine and Biology, 542, 167-174.

Jezussek, M., Juliano, B. O. & Schieberle, P. (2002). Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, 50, 1101-1105.

Laguerre, M., Mestres, C., Davrieux, F., Ringuet, J. & Boulanger, R. (2007). Rapid discrimination of scented rice by solid-phase microextraction, mass spectrometry, and multivariate analysis used as a mass sensor. Journal of Agricultural and Food Chemistry, 55, 1077-1083. 24).

Deegman, C. J., Delgado, J. T., Bryant, Cinnese w Warman extract dibition analysis.

R. Grimm, C. Codwalidotar K. R. & Wobb, Journal of Agricultural and Food Chemistry,

R. D. (2000). Rapid gas eluoronatic pipe: \frac

Laksanalamai, V. & Ilangantileke, S. (1993). Comparison of aroma compound (2 acetyl-1-pyrroline) in leaves from Pandan (Pandanus amaryllifolius) and Thai fragrant rice (Khao Dawk Mali-105). Cereal Chemistry, 70, 381-384.

Lam, H. S. & Proctor, A. (2003). Milled rice oxidation volatiles and odor development. J. Food Sci, 68, 2676-2681.

Laohakunjit, N. & Kerdchoechuen, O. (2007). Aroma enrichment and the change during storage of none-aromatic milled rice coated with extracted natural flavor. Food Chemistry, 101, 339-344.

Mehdinia, A., Mousavi, M. F. & Shamsipur, M. (2006). Nano- Structured lead dioxide as a novel stationary phase for solid-phase microextraction. Journal of Chromatography A, 1134, 24-31.

Paule, C. M. & Powers, J. (1989). Sensory and chemical examination of aromatic and nonaromatic rices. Journal of Food Science, 54, 343-347.

Wongpornchai, S., Sriseadka, T. & Choonvisase, S. (2003). Identification and quantification of the rice aroma compound, 2-acetyl-1-pyrroline in bread flowers (Vallaris glabra Ktze). Journal of Agricultural and Food Chemistry, 51, 457- 462. Chronical Comply A_1 , 1134, 24-31.

The C. M. & Powers, J. (1989). Agricultural and Food Chemistry, 51, 457.

Sensory and elemical examination of 462.