HS-SPME/GC-MS DETERMINATION OF ITALIAN RICE FLAVOUR

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Abstract

Quality control of agricultural crops has traditionally been based on human sensory perception; in this context rice is generally considered as a staple food because of its flavour and texture.

Food flavour, due to a large number of volatile compounds, influences consumers widely and, when food safety and nutritional contribution are guaranteed, sensory parameters become the discriminating factor in defining the food quality. For these reasons, flavour is today considered an important issue in order to add value to a product and differentiate it on the market.

This study was focused on the identification of some compounds which could be used as markers of the product quality and be able to characterize the aromatic profile of different rice cultivar.

Rice flavour was analyzed by using headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS) in order to compare the flavour profile richness of rice samples from three different national cultivar (*Carnaroli, Arborio e Roma*).

Seventeen volatile compounds were identified and confirmed with standards mass spectra acquisition. The reproducibility of the identified compounds was studied by means of RSD showing very good results ranged from 1.54 to 15.84.

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Riassunto

La valutazione della qualità delle colture agricole si basa tradizionalmente sulla percezione sensoriale; in tale contesto, il riso è generalmente considerato come alimento di base per il suo flavour. L'aroma di un prodotto alimentare, dovuto alla presenza di un elevato numero di sostanze chimiche volatili, esercita una notevole influenza sul consumatore e, quando la sicurezza e l'apporto nutrizionale sono garantiti, i parametri sensoriali diventano il fattore discriminante nella definizione della qualità. Per questi motivi, l'aroma rappresenta oggi un aspetto su cui puntare per dare valore aggiunto ad un prodotto differenziandolo da altri presenti sul mercato.

Il lavoro ha riguardato l'individuazione di composti potenzialmente utilizzabili come marker della qualità del prodotto finito e in grado di caratterizzare il profilo aromatico di differenti qualità di riso. E' stato analizzato il flavour di campioni di riso utilizzando la tecnica di microestrazione in fase solida in spazio di testa (HS-SPME), seguita da determinazione gascromatografica accoppiata alla spettrometria di massa (GC-MS), per confrontare la ricchezza dei profili aromatici di campioni di riso provenienti da tre differenti cultivar (*Carnaroli, Arborio e Roma*) del territorio nazionale. Diciassette composti volatili sono stati identificati e confermati con l'acquisizione degli spettri di massa dei relativi standards. La riproducibilità dei composti in esame, valutata calcolando gli RSD, ha mostrato ottimi risultati nell'intervallo 1.54÷15.84.

Keywords: HS-SPME, rice flavour, food quality, volatile compounds.

Introduction

Flavour is often the most pertinent quality attributes of a food product. Currently, only a rudimentary understanding of the relationship between chemical composition and sensory quality exists. This is primarily due to the presence of compounds that impact flavour when present at concentrations approaching the parts-per-billion range (1). Unfortunately no one instrumental technique has yet been developed that can simulate the response of the human nose. However, with recent technological advances, new efforts have been directed toward applying modern analytical instrumentation to the assessment of sensory quality. In this context, headspace analysis yields a gas chromatographic profile that can be used for testing the relationship between the relative concentration of sensory impact compounds to overall sensory quality.

The flavour of Italian rice samples was analyzed in this study by means of the headspace solid phase microextraction followed by the gas chromatography coupled with mass spectrometry (ion trap) determination (HS-SPME/GC-MS).

The HS-SPME technique has proven to be a simple, rapid and sensitive method for collecting the volatile compounds from the headspace of a sample (2-3). In a sample consisting of a complex matrix such as food, the ideal extraction conditions are not readily achieved because the absolute concentration of a given compound is not fixed.

Compounds are continually formed and degraded through myriad complex chemical reactions as a function of temperature, moisture, and pressure. However, relative quantitative information can be obtained by treating the samples under consistent conditions and measuring the resultant production/ release of the volatile compounds (4).

Rice matrix was chosen as target considering its importance as staple food in many countries. Italian rice, besides represents part of the traditional national cuisine, has been historically a source of livelihood for many people in Italy and has become one of the highest quality products of the country. Rice is one of the main crops in Italy and Italy is the main rice grower in Europe, with paddy production of 1.388.900 t in 2008 (5). Italian rice differs in many valuable cultivars (6), *Carnaroli, Roma and Arborio* varieties were considered in this study for a comparison analysis.

Materials and Method

Samples

Rice samples from three different cultivar, *Carnaroli, Arborio and Roma*, were selected from local markets. The method optimization was performed with samples of rice *Carnaroli*. The samples were stored in their unopened packages at 4 °C until their use. For the analysis, rice was milled in a mixer until its reduction in a homogeneous flour. This exhaustive treatment was preferred in order to obtain an homogeneous matrix and for the better recovery achieved in terms of peaks area.

Fibre

According to literature, a 50/30 μm DVB/CAR/PDMS Stableflex (2 cm) was chosen to carry on the analysis, this kind of fibre combines the characteristics of three different coatings giving good results for all of the examined compounds (7).

The fibre was purchased from Supelco (Bellefonte, PA). The fibre was conditioned at 270 °C for 1 h in the GC injector port before its use. Furthermore, it was cleaned between each analyses to prevent contamination and a blank desorption was performed to check possible carry-over.

Chemicals

Thirty four selected standards were used to build up a mass spectra library for identification of volatile compounds in rice samples determined by GC-MS, seventeen of them were found in rice samples. Hexanal, octanal, heptanal, nonanal, decanal, limonene, 2-pentylfuran, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 3-pentanol, 1-octen-3-ol, 2-methyl-propanol, 2-hexanol, benzaldehyde, acetic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, malthol, tetrahydrofurfuryl alcohol, phenylethyl alcohol, methyl nonanoate, methyl butirrate, ethyl butirrate, methyl propionate and vanillin were supplied by Sigma-Aldrich (St. Louis, MO, USA).

The mass spectra were acquired by HS-SPME of 1µL of several standard mix of analytes at 600 mg/L in 20 mL glass vials. The standard solutions were prepared in water (ultrapure Milli-Q water) or diethyl ether (Carlo Erba, Milan, Italy). Low amount of standard solution at high concentration was processed in order to avoid fiber active sites saturation by the solvent.

HS-SPME procedure

The HS-SPME procedure was carried out by means of a Triplus Autosampler (Thermo Scientific). All extractions were performed using 10.5 g of sample in a 20 mL glass vials sealed tightly with crimp cap and PTFE/silicone septum. The vials were incubated in the autosampler oven at 40, 55, 70 and 80 °C; after 20 min the SPME fibre was exposed to the headspace above the surface of the solid sample for 30 min at the same temperature.

After the extraction time, the autosampler immediately desorbed the fibre into the injector of the GC/MS system with a desorption time of 5 min. Because of the solid nature of the matrix, no internal standard was used.

Chromatographic conditions

Thermal desorption was carried out in splitless mode (splitless

time: 5 min) inside a PTV injector with thermal program from 60 to 230 °C at 14.5 °C/s and held for 5 min. A Merlin microseal septum and a SPME inlet liner (0.8 mm i.d., 2.75 o.d. × 120 mm, Restek) were used. All samples were processed with a Trace GC-Ultra/Ion Trap Polaris Q GC-MS system (Thermo-Finningan), equipped with a HP-INNOWAX capillary column (30 m × 0.25 mm i.d., 0.15 μm, Agilent). Carrier gas was helium with a flow rate of 1 mL/min.

The oven temperature was programmed as 40 °C for 5 min, ramped at 6 °C/min to 150 °C, then at 15 °C/min to 230 °C and held for 3 min. GC/MS ion trap operated in the electron-impact mode (70 eV) and in the scan range (m/z) from 30-200 amu, with an ion source temperature of 250 °C and the transfer line at 250 °C.

Results and Discussion

The complex equilibrium established during the SPME process make absolute quantification extremely difficult. As each volatile substance has a different equilibrium partition constant between the headspace and SPME fiber, the relative GC peak areas do not reflect the true proportion of these analytes in the headspace.

Most of the literature describing SPME technique reported mainly qualitative analysis. However, quantification in food products is becoming more and more prevalent. For liquid samples, the quantitative analysis is performed by means of calibration curve. In our case, using of standard addition for heterogeneous or solid samples, the mass-transfer mechanism can be different for the standards added and the native analytes, and thus the pre-equilibrium approach is not suitable (8).

Nevertheless, headspace analysis yields a gas chromatographic profile that can be used for testing the relationship between the relative concentration of sensory impact compounds to overall sensory quality (9). SPME procedure may be, therefore, suitable for the relative comparison of volatile compounds concentrations between rice varieties. This was indeed the final objective of our work.

Volatile compounds of Italian rice

Seventeen volatile compounds were identified by GC-MS in Italian rice *Carnaroli* (Table 1). The compounds were first tentatively identified by using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software.

The deconvoluted mass spectra of thirty four selected standards were used to build-up a library for further use on screening rice samples.

The compounds were classified, according to their functional groups, in furans, terpens, aldehydes, ketones, acids and alcohols.

The HS-SPME parameters were optimized considering the peaks area of 10 compounds selected in order to cover different ranges of functionality for a comparable study.

TABLE 1
IDENTIFIED VOLATILE COMPOUNDS IN THE FLAVOUR OF ITALIAN RICE CARNAROLI BY HS-SPME/GC-MS

N	RT	Compound name	Classification
1	5.79	hexanal	aldehyde
2	8.58	2-heptanone	ketone
3	8.88	limonen	terpen
4	10.00	2-pentylfuran	furan
5	11.54	octanal	aldehyde
6	13.09	1-hexanol	alcohol
7	13.97	nonanal	aldehyde
8	15.36	1-octen-3-ol	secondary alcohol
9	15.42	1-heptanol	alcohol
10	16.73	benzaldehyde	aromatic aldehyde
11	17.45	1-octanol	alcohol
12	23.09	hexanoic acid	carboxylic acid
13	24.01	phenylethyl alcohol	aromatic alcohol
14	24.70	heptanoic acid	carboxylic acid
15	25.88	octanoic acid	carboxylic acid
16	26.83	nonanoic acid	carboxylic acid
17	27.64	decanoic acid	carboxylic acid

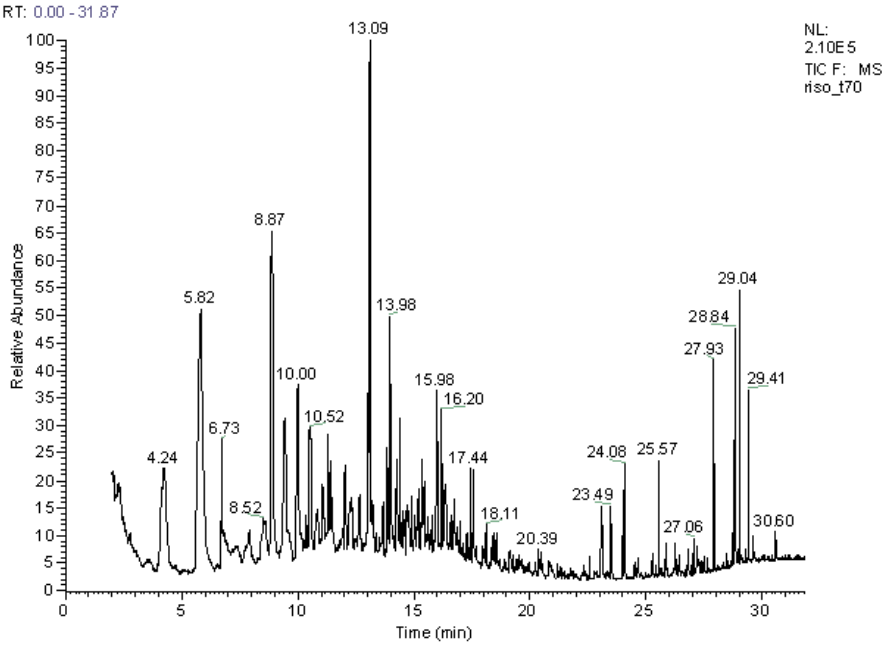

Fig. 1 - GC/MS chromatogram of Carnaroli flavour by HS-SPME (mass range 30÷200 amu): 1. hexanal; 2. limonene; 3. 2-penthylfuran; 4. 1-hexanol; 5. nonanal.

Effect of sampling temperature on the analytes extraction

The HS-SPME procedure was optimized considering the effect of different extraction temperatures.

The results for some analytes are reported in Figure 2.

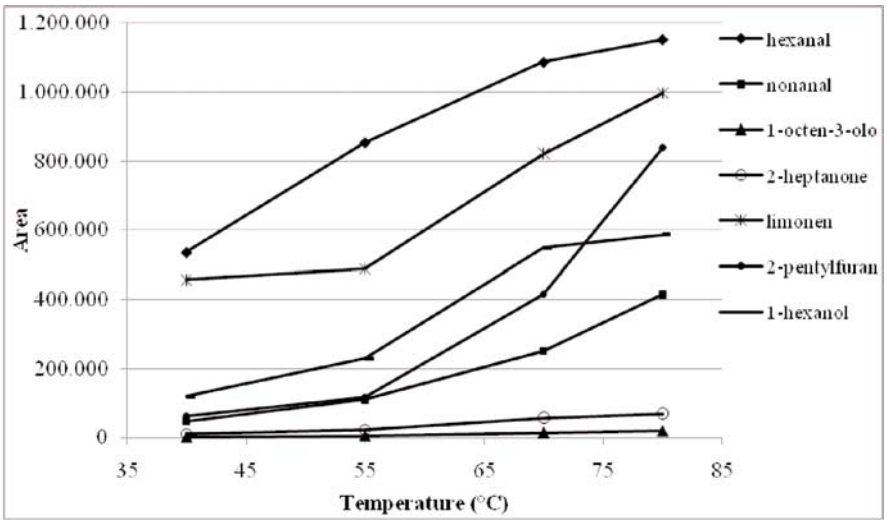

Fig. 2 - Extraction temperature profile for some volatile compounds. Sample weight: 10.5 g, incubation time: 20 min, extraction time: 30 min, extraction temperature: 40, 55, 70 and 80 °C.

The results showed a good extraction efficiency in term of recovery (peaks area) at 70 °C and 80 °C for all analytes. According to the HS-SPME theory, heating provide energy to the volatile molecules to overcome energy barriers that tie them to the matrix (10) enhancing the mass transfer process, increasing the vapour pressure of the analytes (11) and therefore facilitating the release of the analytes into the headspace (12).

From the other hand the adsorption of the volatile by the fibre coating is an exothermic process, therefore, since the high temperature is good for the release of volatiles from their matrix, it can adversely affect their adsorption by the coating due to decrease of partition coefficients.

An extraction temperature of 70 °C was chosen for further acquisitions considering the recoveries achieved.

Reproducibility of volatile compounds

In order to evaluate the reproducibility of SPME and of rice matrix volatiles release, the relative standard deviation (RSD) was calculated by processing a rice sample *Carnaroli* three times during the same day.

The RSD, of retention time and peak area, was calculated for each

analyte and the results showed a good reproducibility with RSD ranged from 1.54 to 15.84 as reported in Table 2.

TABLE 2
RELATIVE STANDARD DEVIATION OF RETENTION TIME AND PEAK AREA OF SEVENTEEN VOLATILES OF THE FLAVOR OF RICE *CARNAROLI* BY HS-SPME/GC-MS

Compound name	RSD	
	RT	Area
hexanal	0.01	8.37
2-heptanone	0.01	7.23
limonen	0.06	1.72
2-pentylfuran	0.06	8.81
octanal	0.05	8.28
1-hexanol	0.04	3.60
nonanal	0.04	1.54
1-octen-3-ol	0.01	5.38
1-heptanol	0.01	12.02
benzaldeyde	0.01	7.23
1-octanol	0.06	5.29
Phenylethyl alcohol	0.01	2.48
hexanoic acid	0.03	2.01
heptanoic acid	0.01	5.94
octanoic acid	0.02	6.58
nonanoic acid	0.02	15.84
decanoic acid	0.00	7.04

Comparison between Italian rice cultivar

After the optimization, the HS-SPME/GC-MS method was applied to samples selected from three different Italian rice cultivar: Carnaroli, Arborio and Roma, in order to verify flavour profiles. The profiles were summarized in the radar graphs reported in Figure 3.

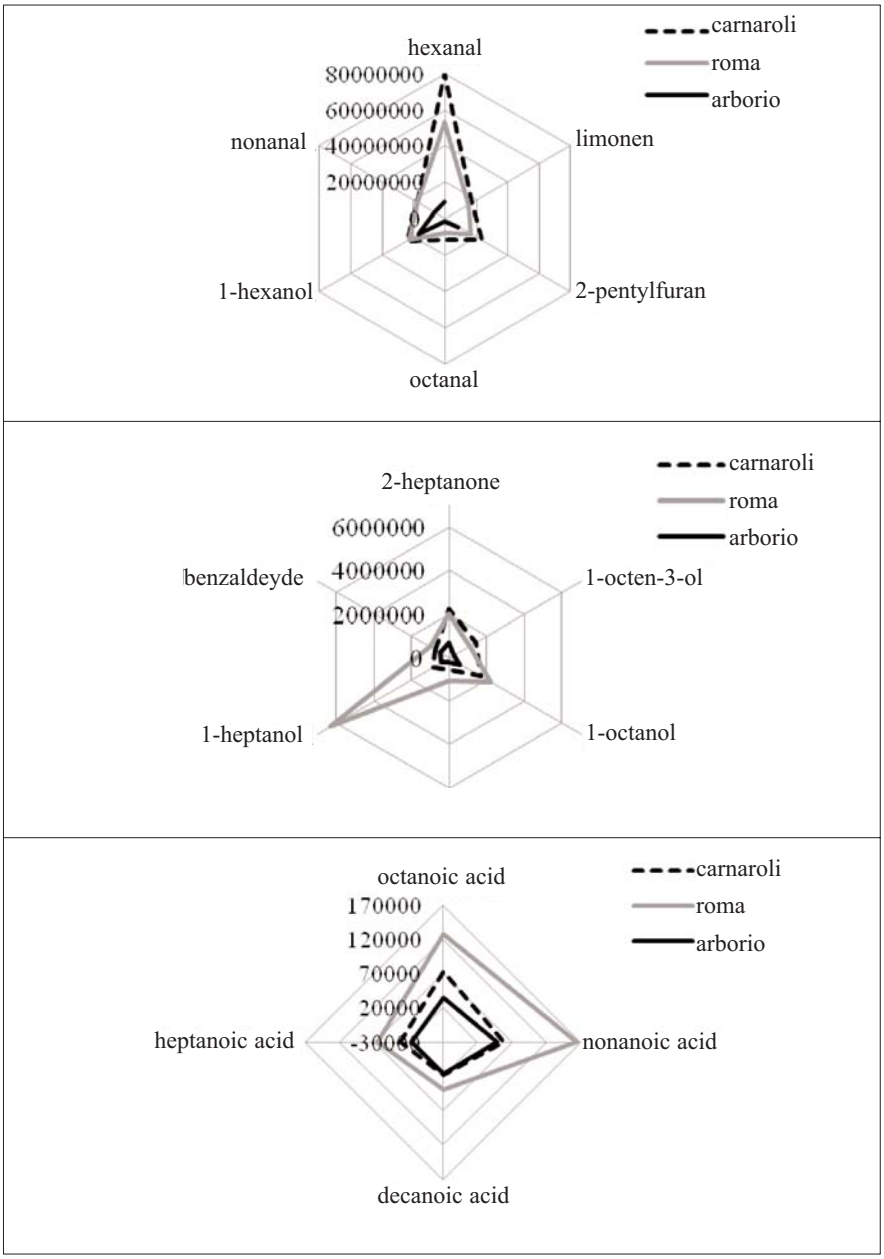

Fig. 3 - Comparison of the relative intensities of selected compounds between three different Italian rice quality: *Carnaroli, Arborio* and *Roma*.

The flavour of *Arborio* sample less rich in volatiles than *Carnaroli* and *Roma* cultivars, is showed in the graphs.

The latter shows higher content of carboxylic acids while the great amount of 1-heptanol could represent a marker for this cultivar as well as hexanal for *Carnaroli*.

Conclusion

The combination of HS-SPME with GC-MS allows sensitive analysis of rice samples flavour. The developed extraction method has proven to be simple, rapid and sensitive for collecting the volatile fraction from the headspace of the samples. Seventeen volatile compounds (aldehydes, ketones, terpens, furans, alcohols and carboxylic acids) were identified by the analysis of a rice sample of *Carnaroli* cultivar.

The best fibre extraction temperature was assessed at 70 °C. A very good reproducibility of the volatile compounds release was achieved with RSD ranged from 1.54 to 15.84. A first approach on the determination of the flavour distinctions between different Italian rice cultivar was carried out applying the developed method on three market samples: *Carnaroli, Arborio and Roma*.

The further step of this research will be the identification of other volatiles that characterize the rice flavour considering the richness of the chromatographic profile as shown in Figure 1 on a more wide market sampling. A further aspect of the research will be the monitoring of a secondary fragmentation pattern by MS-MS mode to complete the elimination of background noise and to get a better discrimination from matrix interferences.

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