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Aroma Active Components in Aqueous Kiwi Fruit Essence and Kiwi Fruit Puree by GC-MS and Multidimensional GC/GC-O

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Gas chromatography−mass spectrometry (GC-MS) and multidimensional gas chromatography olfactometry (GC/GC-O) were utilized to study the aroma profile and the aroma active components of commercial kiwi essence and the initial fresh fruit puree. Totals of 29 and 33 components were identified and quantified in the essence and the puree, respectively. Ten components were quantified for the first time as constituents of the kiwi fruit including 3-penten-2-ol, 3-hydroxy-2-butanone, 3-methyl-2-butenal, 2-hexanol, nonanal, 3-methyl-1-butanol, 2-methyl-1-butanol, 3-methyl-2-butanone, 3-methyl 3-buten-2-one, and octane. Analysis of these samples by multidimensional gas chromatography−olfactometry (GC-O) allowed for the identification of >80% of the aroma active components present at level traces in this fruit. A total of 35 components appear to contribute to the aroma of kiwi fresh puree and its aqueous essence. Components described for the first time as constituents of the aroma profile in this fruit were 2-ethylfuran, 3-methyl-1-butanol, 2-cyclohexen-1-one, (*E*,*E*)-2,6-nonadienal, diethyl succinate, and hexyl hexanoate.

KEYWORDS: Tropical fruit; GC/GC; olfactometry

INTRODUCTION

The flavor of kiwi fruit [*Actinidia deliciosa* (A Chev) Liang et Ferguson *var*. *deliciosa* cv. Hayward] appears to be a subtle blend of several volatile components. The fruit softens considerably during ripening, which produces a large number of volatile compounds (*1*). The composition of this volatile fraction has been the subject of several studies (*2*−*22*), with >80 components identified. The main components identified in these reports include methyl and ethyl butanoate, hexanal, (*Z*)- and (*E*)-2-hexenal, hexanol, (*Z*)-, and (*E*)-3-hexenol, and methyl benzoate, all of which are typical degradation products of unsaturated fatty acids (*23*).

Different analytical techniques have been utilized to isolate and concentrate volatile components of kiwi fruit flavors: simultaneous distillation extraction (*2*, *20*), vacuum distillation (*3*, *5*, *7*, *19*), dynamic headspace sampling (*4*, *11*), and solid phase microextraction (SPME) (*22*).

A sensory and instrumental analysis of the aroma of kiwi fruit by Pfannhauser yielded a total of 52 compounds (*7*). His results showed a rapid change in volatile composition from fresh/mature to the overripe or frozen state with a decrease in C6 compounds such as hexanal, hexenol, and ethyl butyrate and an increase in terpene esters. These changes occur in parallel to changes in the sensory impression of kiwi aroma from a fresh/green to an undesired ester note.

Studies by Bartley and Schwede identified 27 components in kiwi fruit and declared that 2-hexenal was the major component in mature fruit but that on further ripening ethyl butanoate began to dominate the profile (*11*). These results were confirmed years later by Jung and Young, who studied the effect of storage period on the flavor components of Korean kiwi fruit (*20*). An investigation carried out by Young et al. (*3*) studied the volatile components in kiwi juice and found that, of the 73 compounds identified, (*E*)-3-hexenal appeared to be a major contributor to the "old cut grass" and hay aroma notes found in kiwi fruit juice (*14*).

Years later, Young and Patterson studied the characterization of bound flavor components in kiwi fruit (*16*). Volatile components were released by enzymatic hydrolysis with β-glucosidase. Major compounds found and identified were (*E*)-2-hexenal and benzaldehyde. Compounds not previously identified include octan-3-ol, camphor, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, neral, geranial, methyl 2-hydroxybenzoate, nerol, geraniol, and 2-phenylethanol. Pino defined a total of 48 volatile components of which ethyl benzoate, hexanal, and (*E*)-2-hexenal were the major contributors to the aroma (*19*).

The latest research involving the study of the aroma profile in this fruit was made by Wan et al. (*22*). These authors identified 42 volatile components in kiwi fruit by SPME-GC-MS, with 4-pentenal, (*E*,*E*)-2,4-nonadienal, 2-nonanone, ethyl octanoate, butyrolactone, and 2-propenyl butanoate reported for the first time in this fruit. However, the real role of each component in the aromatic quality of the fruit can be known

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only by studying their aroma activity. One of the major difficulties in studying aroma is the determination of those components that have a real contribution to the flavor of food (*24*). Thus, Young et al. (*3*) addressed the olfactometric study of the kiwi fruit character impact compounds, where (*E*)-2-hexenal and the butanoate esters, with some contributions from the C6 alcohols, were reported as the most aroma active components. Similar results were obtained by Fischboeck et al. (*8*), who investigated an extract from deep-frozen kiwi puree by GC-MS and GC-O to correlate this chemical information with sensory impression. In addition to (*E*)-2-hexenal and cyclohexanone, three terpene esters, linalyl acetate, isobornyl acetate, and α-terpenyl acetate, were identified and found to be responsible for an overripe, terpene-like off-flavor in stored kiwi puree. Nevertheless, Paterson et al. (*13*) declared that the volatile components identified in kiwi fruit are commonly found in other fruits, and there do not appear to be any character impact flavor compounds.

The research conducted to date has largely been concerned with the volatile components found in the whole fruit, puree, or juice. No research related to the aroma profile of the aqueous essence of kiwi fruit has been completed so far. The aqueous essences of tropical fruits are being used by the flavor industry in a wide range of applications to enhance product acceptance. An integrated approach utilizing such technologies as GC-MS and GC-O will provide useful information about the most important active contributors to the aroma of kiwi essence and fresh fruit puree. The objective of the present work is to determine the flavor active compounds in aqueous kiwi essence and fruit puree.

MATERIALS AND METHODS

Kiwi Samples. Unpasteurized fresh kiwi fruit puree and commercial aqueous essence were obtained from a local manufacturer of aqueous essences located in Florida. The samples were taken to the laboratory within 2 h after the industrial processing and frozen at −20 °C for 2 weeks. Both the essence and the puree were derived from the same fruit source. The aqueous essence was obtained by a proprietary process that utilizes a low-temperature, short-time steam distillation.

Extraction of Volatile Compounds. *Fresh Kiwi Fruit Puree.* Isolation of volatile compounds was made using a liquid extraction technique. Cyclohexanone (10 ppm) was added to 120 g of fresh puree, and the volatile components were then extracted with 80 mL of methylene chloride (Sigma Chemical Co., St. Louis, MO) by stirring for 1 h with a magnetic stirrer at ∼2 °C. The resulting mixture was centrifuged for 10 min (3000*g* at 5 °C), and the organic layer was dried with sodium sulfate and concentrated to ∼1 mL using a distillation−rectification system, followed by further concentration to 0.1 mL using a flow of nitrogen.

Commercial Kiwi Essence. Volatile components present in 3 mL of essence with cyclohexanone at 10 ppm were extracted twice using 2 mL of methylene chloride for each extraction and mixed between two glass syringes connected by a stainless steel luer lock adaptor and combined. The final emulsion was centrifuged as above, and the organic layer was recovered and dried. This fraction was concentrated to 0.1 mL using a flow of nitrogen. The extraction efficiencies for the commercial kiwi essence and fresh kiwi fruit puree were 98% as measured by successive extractions.

GC-MS Analysis. The qualitative and quantitative analyses of the volatile compounds were conducted using an Agilent 6890/5973N gas chromatograph−mass spectrometer (GC-MS) (Palo Alto, CA) with a 7683 autosampler equipped with a 30 m × 0.25 mm HP-5 (cross-linked phenyl-methyl siloxane) column with 0.25 μm film thickness (Agilent) using a 1 mL/min flow rate. The oven temperature was initially held at 40 °C for 6 min, then increased at 2.5 °C/min to 150 °C, and finally increased at 90 °C/min to 250 °C. The injection port and ionizing source were kept at 250 and 280 °C, respectively. The split ratio was 10:1 with 2 μL of sample injected. After a 2 min solvent delay, mass spectra (*m*/*z* 35−300) were collected at 5.27 scans/s. Compound identifications were made by comparison of the mass spectra and retention times with those of corresponding reference standards (Aldrich Chemical Co., St. Louis, MO; Bedoukian Research, Inc., Danbury, CT) for all compounds except methyl propionate, 3-methyl-3-buten-2-one, and (*E*,*E*)-2,4-heptadienal, which were identified by the NIST98 library (NIST, Gaithersburg, MD) and published retention indices (*25*).

Quantification. Linear regression models were generated using standard dilution techniques with cyclohexanone as internal standard. Target ions were used in the identification and quantification of each component by the mass spectrometry system. Standard reference compounds were used in all cases if available. For the quantification of the three compounds that were not available, linear regressions of similar components were used. The substitutions were methyl propionate by methyl isobutyrate, 3-methyl-3-buten-2-one by 3-penten-2-one, and (*E*,*E*)-2,4-heptadienal by (*E*,*E*)-2,4-hexadienal.

GC/GC—Olfactometry. Two HP-5 (5% cross-linked phenyl-nethyl siloxane) columns (30 m × 0.25 mm i.d. with 0.25 μm film thickness)

Table 1. Volatile Components in Commercial Kiwi Essence and Fresh Kiwi Puree by GC-MS

component	RI	concn, ppm (± SD) commercial essence	concn, ppm (± SD) fresh puree
methyl propionate	604	1.57 ± 0.33	nd[a]
ethyl acetate	613	tr[b]	nd
1-butanol	653	1.08 ± 0.1	nd
3-methyl-3-buten-2-one	653	nd	0.76 ± 0.01
3-methylbutanal	654	0.40 ± 0.08	nd
1-penten-3-one	680	1.29 ± 0.03	0.78 ± 0.03
1-penten-3-ol	683	1.25 ± 0.3	nd
methyl isobutyrate	685	0.16 ± 0.06	nd
3-methyl-2-butanone	707	nd	1.12 ± 0.28
ethyl propanoate	709	1.75 ± 0.08	0.18 ± 0.01
3-hydroxy-2-butanone	711	18.64 ± 0.39	18.55 ± 0.35
methyl butyrate	723	19.61 ± 1.07	0.52 ± 0.12
3-methyl-2-butenal	730	tr	2.00 ± 0.01
3-methyl-1-butanol	737	0.74 ± 0.08	nd
2-methyl-1-butanol	744	0.69 ± 0.11	nd
(*E*)-2-pentenal	754	1.34 ± 0.01	1.01 ± 0.01
1-pentanol	766	nd	0.31 ± 0.01
(*Z*)-2-penten-1-ol	767	0.93 ± 0.06	nd
toluene	770	nd	0.02 ± 0.01
3-penten-2-ol	774	2.07 ± 0.1	0.53 ± 0.08
ethyl butyrate + hexanal	800	36.07 ± 1.2	0.75 ± 0.06
octane	800	nd	0.80 ± 0.02
2-hexanol	803	0.36 ± 0.06	0.46 ± 0.02
(*E*)-2-hexenal	857	141.83 ± 2.98	1.85 ± 0.54
(*E*)-2-hexen-1-ol	862	44.79 ± 4.71	0.72 ± 0.18
hexanol	865	11.07 ± 0.74	nd
methyl 2-(methylthio)acetate	889	1.13 ± 0.23	0.74 ± 0.01
γ-butyrolactone	891	nd	0.09 ± 0.03
β-pinene	934	nd	0.87 ± 0.01
methyl hexanoate	934	0.50 ± 0.01	nd
ethyl 3-hydroxybutyrate	949	nd	3.99 ± 0.02
α-pinene	982	nd	0.50 ± 0.7
ethyl (methylthio)acetate	996	nd	0.13 ± 0.01
ethyl hexanoate	997	0.63 ± 0.02	nd
2-pentylfuran	1001	nd	0.02 ± 0.00
(*E*,*E*)-2,4-heptadienal	1009	nd	0.17 ± 0.09
limonene	1031	nd	0.04 ± 0.01
hexanoic acid	1085	nd	tr
methyl benzoate	1091	0.59 ± 0.12	0.32 ± 0.01
linalool	1101	1.36 ± 0.06	nd
nonanal	1104	0.40 ± 0.05	0.40 ± 0.09
phenylethyl alcohol	1116	nd	2.03 ± 0.01
ethyl benzoate	1170	0.82 ± 0.08	0.15 ± 0.01
octanoic acid	1179	nd	tr
α-terpineol	1198	1.01 ± 0.09	0.95 ± 0.01
geraniol	1240	25.20 ± 0.64	1.40 ± 0.23

[a] nd, not detected. [b] tr, traces.

Table 2. Descriptors of the Volatile Components Perceived by GC-O in Kiwi Essence and Kiwi Fruit Puree

component	Kovats index	descriptors	frequency of detections	
			essence	puree
3-penten-2-ol	712	herbal, green, vinyl, burnt rubber	9	9
2-ethylfuran	728	rubber, pungent, acid		3
3-methyl-1-butanol	742	ripe onion		3
(*E*)-2-pentenal	754	fruity, strawberry	9	8
(*Z*)-2-penten-1-ol	767	green, plastic, burnt rubber		6
hexanal	785	green, herbal, grass	8	8
ethyl butanoate	788	fruity, strawberry	4	9
(*E*)-2-hexenal	835	fruity, strawberry, cherry	9	9
(*E*)-2-hexen-1-ol	854	walnut, medicinal, cooked butter	9	7
propyl butanoate	880	moldy orange, solvent	6	
heptanal	885	dry fish, pesticide, solvent, smoky	9	6
methyl 2-(methylthio)acetate	894	mash potato, acid, cooked nut, roasted oil	6	7
2-cyclohexen-1-one	914	weak pesticide	3	3
5-methylfurfural	945	spicy, acid, coffee	3	3
6-methyl-5-hepten-2-one	974	mushroom, earthy, vinyl, rubber	9	9
1-octen-3-ol	977	garlic, mushroom, spicy, rubbery, carrots	8	9
(*E*,*E*)-2,4-heptadienal	998	orange oil, oily		4
eucalyptol	1030	sweet, mint	4	3
(*E*)-2-heptenal	1041	pesticide, onion	3	3
NPI[a]	1059	fresh sunflower seeds	3	3
octanol	1087	burnt matches, toasted bread	7	3
methyl benzoate	1103	lettuce, herbal, watermelon	7	9
linalool	1110	lemon, parsley	4	6
phenylethyl alcohol	1132	floral, spicy, herbal, rose		6
NPI	1155	plastic, herbal, moldy, wet herb	4	8
(*E*,*E*)-2,6-nonadienal	1162	banana candy, green, herbal	8	8
diethyl succinate	1167	fabric, fruity, watermelon	7	6
ethyl benzoate	1185	chamomile flower, celery		3
α-terpineol	1197	anise, toothpaste, fruity		8
NPI	1250	toothpaste, anise, fruity	6	6
carvone	1253	basil leaves, herbal, sweat, spicy, fennel	9	6
NPI	1277	cinnamon, pine, potato skin, oat	3	6
NPI	1317	sweet, anise		6
NPI	1324	spicy, sesame		8
hexyl hexanoate	1379	fruity, peach, prune	9	9

[a] NPI, not positively identified.

were used as both the preparative column and main column with a 1 mL/min flow rate. The columns were installed in Hewlett-Packard 5890 and 5890 series II plus chromatographs and coupled via a CTS1 cryotrapping device (Gerstel, Inc., Baltimore, MD), which was maintained at −150 °C during repeated preparative runs to collect and concentrate the aromatic fraction. Preparative GC effluent was split 1/99 between the FID and the analytical column, whereas the analytical GC effluent was split 1/1 between the mass spectrometer and the sniffing port. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The transfer line to the GC-O sniffing port was held at 300 °C. The volume of extract analyzed and oven program temperatures were the same as those described above for the GC-MS. Humidified air was added in the sniffing port at 100 mL/min. Compounds were identified using mass spectra, retention times, and Kovats indices (as determined relative to alkanes from pentane to pentadecane) of the volatile components in both extracts with those of reference standards. The mass spectrometer retention times were compared with the retention times measured in the olfactometry runs, and both were compared to reference standards. To identify the components that were perceived by the panelists, but did not present any detectable peak in the mass spectra, several preparative runs were performed to concentrate the sample in the cryotrap. Data were collected using the ChemStation G1701AA data system (Hewlett-Packard, Palo Alto, CA).

Olfactometry Global Analysis (Frequency Response). GC-O frequency analysis was performed by using a modified version of the method reported by Pollien et al. (*26*). Three panelists analyzed each sample in triplicate for the detection of aroma active compounds, and verbal descriptors were recorded for both extracts. Aroma active components were considered to be those that were detected and described by at least one panelist three times using identical terms.

RESULTS AND DISCUSSION

Volatile Components in Kiwi Essence and Kiwi Fruit. Totals of 30 and 33 compounds were identified and quantified by GC-MS in the volatile fraction of commercial kiwi essence and fresh puree kiwi fruit, respectively (**Table 1**).

The aromatic fraction of commercial kiwi essence contained 12 alcohols, 10 esters, 6 aldehydes, 2 ketones, and 1 sulfur compound. In fresh fruit puree 7 alcohols, 6 esters, 5 aldehydes, 4 ketones, 2 sulfur compounds, 1 lactone, 2 acids, 1 furan, 3 terpenic hydrocarbons, and 2 hydrocarbons have been identified and quantified as constituents of the aroma profile of this fruit.

Major components found in the commercial essence of the kiwi fruit were 3-hydroxy-2-butanone, methyl butyrate, ethyl butyrate, hexanal, (*E*)-2-hexenal, (*E*)-2-hexenol, hexanol, and geraniol. Meanwhile, major compounds found in fresh kiwi fruit puree were 3-methyl-2-butanone, 3-hydroxy-2-butanone, (*E*)-2-hexenal, ethyl 3-hydroxybutyrate, phenylethyl alcohol, α-terpineol, and geraniol. These results agree with those found by Winterhalter (*23*), which affirm that methyl and ethyl butanoate, (*E*)-2-hexenal, hexanal, and hexanol were the major volatile components identified in this fruit. However, (*Z*)- and (*E*)-3-hexenol were described by Young et al. (*14*) as the major contributor to the "old cut grass" aroma in kiwi fruit juice, but they were not detected as components of the aroma profile of the kiwi essence or kiwi fresh fruit puree in the present study. The most recent study regarding the aroma profile of kiwi fruit was by Wan et al. (*22*), who reported a total of 42 volatile components in the analysis of the headspace of the kiwi fruit at

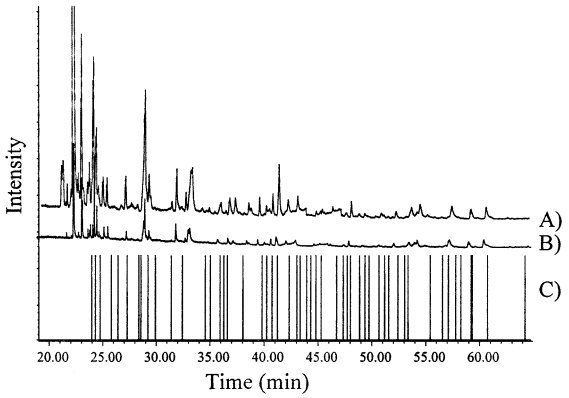

Figure 1. Chromatogram (B) with corresponding aromagram (C) and a chromatogram generated from a 10 times larger injection using GC/GC for comparison (A).

various stages of ripeness. In the present work, 10 components are reported for the first time as constituents of the aroma profile of this fruit. Five of these components were identified in the essence and the fruit puree samples and correspond to 3-penten-2-ol, 3-hydroxy-2-butanone, 3-methyl-2-butenal, 2-hexanol, and nonanal, whereas 3-methyl-1-butanol and 2-methyl-1-butanol were quantified only in the essence and 3-methyl-2-butanone, 3-methyl 3-buten-2-one, and octane were identified only in the fresh fruit puree.

The aroma profile of the kiwi essence is richer in alcohols and esters with low molecular weight than the fruit puree. Methyl and ethyl butanoates are the esters found in highest concentrations in the essence, whereas ethyl 3-hydroxybutanoate is found only in the puree and is the most abundant ester quantified there.

(*E*)-2-Hexanol, geraniol, and hexanol are the alcohols present in greatest concentration in the commercial essence. Only six alcohols were quantified in the fresh fruit puree with phenylethyl alcohol being the most abundant. Nevertheless, it was not detected in the commercial essence. Geraniol is the next most abundant alcohol in the puree, and hexanol, which was quantified as one of the most abundant alcohols in the essence, was not detected in the puree. α-Terpineol has been quantified in both samples at similar levels.

More aldehydes have been detected in the fresh fruit puree than in the essence. It is interesting to note that (*E*)-2-hexenal is the major compound identified in the commercial essence; meanwhile, in the fresh fruit puree the concentration is >10 times lower than in the essence, and it is not the component quantified in highest concentration. (*E*,*E*)-2,4-Heptadienal was identified only in the fresh puree, and 3-methylbutanal was one of the major aldehydes identified in the essence of the fruit, whereas it was detected only at level traces in the puree. Among the ketones quantified, 3-hydroxy-2-butanone was the ketone present in greatest concentration in both samples. Limonene, α-pinene, and *β*-pinene were the terpenic hydrocarbons identified only in the fresh fruit puree. 2-Pentylfuran and *γ*-butyrolactone were identified as aromatic constituents of the fresh fruit puree only. Only two sulfur components were identified: methyl (2-methylthio)acetate was quantitated in both the essence and puree, but ethyl (methylthio)acetate was identified only in the fresh fruit puree.

GC-O. *Olfactometric Global Analysis (Frequency of Detections)*. The aroma active compounds perceived by the three panelists are shown in **Table 2**. Only the components that were detected at least three times per one of the panelists were considered as contributors to the aroma of commercial kiwi essence and fresh fruit puree. As a result of this, olfactometric analysis allows for the detection of components that were not quantified by GC-MS [propyl butanoate, heptanal, 5-methyl furfural, 6-methyl-5-hepten-2-one, 1-octen-3-ol, eucalyptol, (*E*)-2-heptanal, octanol, and carvone] and even the identification of components described for the first time as constituents of the aroma profile in this fruit, including 2-ethylfuran, 3-methyl-1-butanol, 2-cyclohexen-1-one, (*E*,*E*)-2,6-nonadienal, diethyl succinate, and hexyl hexanoate.

A total of 26 components contribute to the aroma of kiwi commercial essence. Among them, 3-penten-2-ol, (*E*)-2-pentanal, (*E*)-2-hexanal, (*E*)-2-hexenol, heptanal, 6-methyl-5-hepten-2-one, carvone, and hexyl hexanoate are the most important contributors to the aroma of the essence because they were perceived by the three panelists in all of the replications. It is important to mention the contribution of other components that were not detected in all of the replications but were perceived by all of the panelists, including hexanal, 1-octen-3-ol, octanol, methyl benzoate, (*E*,*E*)-2,6-nonadienal, and diethyl succinate.

The olfactometric analysis of the fresh kiwi puree yielded a total of 35 aroma active components. Among them, 3-penten-2-ol, ethyl butanoate, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, 1-octen-3-ol, methyl benzoate, and hexyl hexanoate appear to be the major contributors to the aroma in the puree because they were perceived by the three panelists during all nine analyses. (*E*)-2-Pentenal, hexanal, (*E*)-2-hexenol, methyl (2-methylthio)acetate, (*E*,*E*)-2,6-nonadienal, α-terpineol, and two not positively identified components also have an important contribution to the desirable aroma of this fruit, although they were not perceived in all of the replications.

These results agree with those reported by Young et al. (*3*) and Gilbert et al. (*18*). They concluded that ethyl butanoate, (*E*)-2-hexenal, and some C6 alcohols have the most prominent effect on consumer acceptability of the attribute kiwi fruit flavor. 2-Ethylfuran, (*Z*)-2-penten-1-ol, (*E*,*E*)-2,4-heptadienal, phenylethyl alcohol, ethyl benzoate, α-terpineol, and two not positively identified components could be considered the cause of the different aromas perceived between the essence and the fresh fruit, because they were perceived only in the puree of the fruit.

Figure 1 is an example of the aromagram (C) and two chromatograms (A and B) obtained from fresh puree analyses. Chromatogram B corresponds to a normal injection (2 *μ*L of puree extract). There are a moderate number of volatile active components perceived in the puree (C) that do not have any corresponding peak in the chromatogram (B), because they are present at levels below the limit of detection of the MS. Multidimensional GC was utilized to concentrate the sample in a cryotrap and to improve the signal in the mass spectrometer. The sample was concentrated 10 times via multiple injections (A), and >80% of the aroma active components could then be identified.

Olfactometric and the multidimensional GC analysis are crucial in the study of the aroma composition of food, because not only are the presence and concentration of a specific compound important, but so it the odor level at which it is present. This determines whether it could play a central role in contributing to the overall flavor of the product.

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