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Comparison of solid-phase microextraction and purge-and-trap methods for the analysis of the volatile fraction of butter

Milena Povolo, Giovanna Contarini*
Istituto Sperimentale Lattiero-Caseario, Via A. Lombardo 11, 26900 Lodi, Italy

Abstract

The volatile fraction of butter stored at three different temperatures was investigated to monitor quality during commercial shelf-life (90 days). Two different extraction techniques were compared: dynamic headspace (purge-and-trap), and static headspace (solid-phase microextraction, SPME). As expected, the dynamic extraction provided a generally higher amount of volatile compounds than that obtained by SPME, but, with reference to individual compounds, SPME seemed to provide better extraction for volatiles having a higher molecular mass. Despite the different performances, both methods were able to detect volatiles useful for evaluating changes during storage.

Keywords: Solid-phase microextraction methods; Purge-and-trap; Headspace analysis; Butter; Food analysis; Volatile organic compounds

1. Introduction

Various techniques have been applied to evaluate volatile compounds in dairy products [1]. Analysis of the headspace composition, because of its simple procedure and ability to preserve the natural characteristics of the sample, is the most frequently used method. Headspace analysis can be carried out in either a static or a dynamic way. Static headspace is based on sampling of a gas phase aliquot over the sample, after equilibration. Dynamic headspace results in enrichment of volatiles in a cold trap or an inert support (e.g. Tenax) by continuously stripping with an inert gas flow through the matrix. In recent years, automatic systems performing dynamic extraction, cryofocusing and direct GC injection have been developed and applied to evaluate the flavor of dairy products [2–11].

SPME (solid-phase micro-extraction), developed in 1990 [12] to evaluate volatile compounds from waste waters, represents an advancement in the static headspace approach. In this technique volatiles are not sampled by a gas tight syringe, but are adsorbed onto a stationary phase coating a fused-silica fiber. If the matrix only contains substances consistent with gas chromatographic analysis, this fiber can be immersed directly into the liquid sample; otherwise, it should be exposed to the headspace above the sample. Headspace SPME is based on the equilibrium of analytes between the following three phases of the system: polymeric liquid coating, headspace and sample matrix. The amount of analyte adsorbed by the fiber is affected by both the thickness of the stationary phase and the distribution constant. The distribution constant generally increases with increasing molecular mass and boiling point of the

*Corresponding author. Fax: +39-0371-355-79.
E-mail address: gcontarini@ilclodi.it (G. Contarini).

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analyte [13]. Some recent examples of the application of SPME in the dairy field have concerned milk [14–16], cheese [17,18] and butter [19].

As far as the typical flavor of good-quality butter is concerned, the raw material and the metabolic activity of the bacteria used as starters (e.g. *Lactococcus lactis* subsp. *lactis* and subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetilactis* and *Leuconostoc*) play an important role [20].

Butter flavor results from the contribution of several compounds, belonging to different chemical classes, in a delicate balance. Research carried out on this subject by different authors [21–26] showed the presence of short chain fatty acids, aldehydes, ketones, alcohols, esters, lactones, terpenes, and sulfur compounds. Diacetyl has been recognized as the key compound for the typical butter flavor [22,23]; it, together with acetoin, is produced by the starter metabolism of pyruvic and citric acids; in particular, following the pathway of sugar metabolism, pyruvic acid can be changed into acetic acid, acetaldehyde, ethanol, acetoin, diacetyl and 2,3-butanediol [20,27].

If the refrigerating temperature is not maintained, butter flavor can be subjected to significant changes during storage as a result of lipolysis and oxidation processes. Both acidity value and peroxide content are the most used parameters to determine the severity of these degradations and, consequently, the conditions of butter during shelf-life [28]. Fat degradation causes a general increase in volatile compounds, especially methylketones, alcohols, aldehydes and free fatty acids [25,29,30]. Christensen and Holmer [29] used a dynamic headspace technique to study the volatile compounds related to oxidative rancidity in butter stored at different temperatures for 14 weeks and chose hexanal as an indicator for lipid oxidation. SPME technique, applied to evaluate the reduced sulphur compounds of butter, was able to detect the relationship between season and concentration of methanethiol and dimethyldisulphide [19].

The first aim of this research was to evaluate the performance of purge-and-trap (PT) and SPME for analysis of the volatile fraction of butter. As the Tenax used for PT analysis is not a selective adsorptive material, a multiple component fiber was used for SPME analysis. The second aim of our study was to evaluate the effectiveness of the volatile fraction as a parameter of quality of butter stored at different temperatures during the shelf-life.

2. Experimental

2.1. Samples

A 10-kg sample of butter was collected from one manufacturer immediately after production and subdivided into ten sub-samples, each stored in a hermetically sealed glass jar. One sub-sample was analysed on the day after production (time 0), while the others were stored at different temperatures and sampled at different times, as reported in Table 1.

The maximum storage time (90 days) corresponded to the usual shelf-life indicated on the label by the manufacturer. The temperatures selected for sampling were derived from the real occurrence of three situations:

(i) storage in industrial refrigerators (4 °C);
(ii) normal storage in home refrigerators (10 °C)
(iii) storage at room temperature (21 °C) simulating a possible breakdown of the logistic chain.

Each sample was analyzed in duplicate.

2.2. PT–GC–MS analytical method

A butter sample (20 g) was weighed in a 40-ml vial. The vial was equilibrated at 45 °C in a thermostatic bath for 5 min and connected to a nitrogen source (flow-rate: 30 ml/min) and a Tenax trap (TA

Table 1
Sampling plan

Sample code	Time of sampling (days from production)	Storage temperature (°C)
0	1	
40A	40	4
40B	40	10
40C	40	21
70A	70	4
70B	70	10
70C	70	21
90A	90 (End of shelf-life)	4
90B	90 (End of shelf-life)	10
90C	90 (End of shelf-life)	21

60–80 mesh, Chrompack, Middelburg, The Netherlands) through two separate outlets. Volatile compounds were stripped for 60 min. Nitrogen at the same flow-rate as that used for the analysis was then blown through the trap for 5 min to remove moisture. The Tenax trap was fitted to a thermal desorption cold trap injector (TCT, Chrompack), directly connected to a gas chromatographic system. The compounds were desorbed from Tenax by heating at 250 °C for 10 min and then transferred by a carrier gas stream (He) to a fused-silica trap, cooled at −120 °C by liquid nitrogen. Injection into the capillary system was performed by flash-heating (250 °C for 1 min) the cold trap, on which volatile compounds had been reconcentrated. After each run TCT was automatically cleaned by a 10-min back-flush cycle. A Hewlett-Packard (Palo Alto, CA, USA) 5890 series II gas chromatograph, equipped with a Chrompack TCT CP 4010 injector and coupled with a Hewlett-Packard HP 5980A mass spectrometer, was used. An HP-Innowax (Hewlett-Packard) capillary column (60 m×0.32 mm I.D., 0.5 μm film thickness) was chosen. The oven temperature was held at 40 °C for 8 min, programmed to 210 °C at a rate of 4 °C/min and held at 210 °C for 10 min. The interface temperature was 220 °C; the flow-rate of He carrier gas was 1 ml/min. The mass spectra were acquired as full scans from m/z 35 to m/z 270 (1.6 scans/s), with a source temperature of 200 °C under a 70-eV ionization potential. Peak recognition was performed by comparison with mass spectra from both the Wiley library [31] and authentic standards analysed under the same conditions.

2.3. SPME–GC–MS analytical method

A sample (10 g) was weighed in a 20-ml vial provided with a hole cap and a Teflon-faced silicone rubber septum. The vial was equilibrated at 45 °C for 5 min in a thermostatic bath.

A 2-cm long fiber, coated with a 30-μm thick divinylbenzene–carboxen–polydimethylsiloxane film was used (Supelco, Bellefonte, PA, USA). The SPME fiber was conditioned according to the manufacturer's recommendations (280 °C for 30 min in a GC injector). The fiber was exposed to the headspace above the sample for 30 min, and the vial was maintained under stirring at 45 °C. After this time, the fiber was drawn into the needle and introduced into the gas chromatograph split/splitless injector. The injector was fitted with an inlet liner (0.75 mm I.D.; Supelco). During the injection phase, splitless mode was applied for 3 min, and the injector temperature was held at 270 °C. The same GC–MS equipment and conditions as those used for the purge-and-trap analysis were applied. Results obtained from both PT and SPME were expressed as area units.

2.4. Determination of acidity value

The FIL-IDF method 6B:1989 [32] was applied.

2.5. Determination of peroxide value

The provisional FIL-IDF method 74A:1991 [33] was applied.

2.6. Statistical analysis

The results obtained from PT and SPME (the mean value of the two replicates) were submitted to multivariate statistical analysis, by applying principal component analysis (PCA) to autoscaled data [34]. The data set was composed of ten objects (butter samples) and nine variables (volatile compounds).

3. Results and discussion

3.1. Qualitative comparison between PT and SPME

The compounds detected by using PT and SPME are listed in Table 2. It is worth noting that the listed compounds were not detected in all butter samples, but their presence was dependent upon the time/temperature of storage.

Fig. 1 shows total ion chromatograms for the same butter sample obtained from PT and SPME. The same scale of abundance was used for both profiles. Although the same GC conditions were applied, differences between the retention times were found as a result of the difficulty of setting up identical flow-rates.

In accordance with the results obtained from

Table 2
Occurrence of volatile compounds identified by PT and SPME

Chemical class	Compound	PT	SPME	Ref.
Ketones	Acetone[a]	+	+	[2,21,22]
	2-Butanone	+	+	[21,22]
	2-Pentanone[a]	+	+	[2,21,22]
	Diacetyl[a]	+	+	[2,21–24,26]
	2,3-Pentanedione	+	–	[24]
	2-Hexanone	–	+	
	2-Heptanone[a]	+	+	[2,21,22,24]
	Acetoin[a]	+	+	[22–24]
	2-Octanone	–	+	[24]
	2-Nonanone[a]	+	+	[21,22,24]
	8-Nonen-2-one	–	+	
	2-Undecanone	–	+	[24]
Aldehydes	Acetaldehyde	–	+	[22]
	2-Methylpropanal	+	–	[2,23]
	Butanal	+	–	[22,23]
	2-Methylbutanal	+	–	[2,22]
	3-Methylbutanal	+	–	[2,23]
	Hexanal	+	+	[2,23,24,26]
	Heptanal	+	–	[22,23]
	2-Heptenal	+	–	[23]
	Octanal	+	–	[23]
	2-Octenal	+	–	[23]
	Nonanal	+	–	[22,23]
	Decanal	+	–	[22,23]
	2-Decenal	+	–	[23]
	Benzaldehyde	+	–	[21,23]
Alcohols	Ethanol	–	+	[22]
	2-Propanol	+	–	[23]
	Isobutanol	+	–	[22,24]
	2,3-Butylene glycol	+	–	[20]
	1-Butanol	+	–	[22,23]
	3-Methyl-1-butanol	+	+	[24]
	1-Pentanol	+	–	[22,23]
	1-Hexanol	+	+	[22–24]
	1-Octen-3-ol	+	–	[24]
	1-Octanol	+	–	[23,24]
Esters	Ethyl acetate[a]	+	+	[2,22]
	Ethyl butanoate	+	–	[22,23]
	Butyl acetate	+	–	
	Ethyl hexanoate	+	–	[22]
Acids	Acetic acid	+	+	[21–23,26]
	Butyric acid[a]	+	+	[21–23,26]
	Hexanoic acid[a]	+	+	[21–23,26]
Sulfur compounds	Dimethyl sulfide	+	–	[2,17,23]
	Dimethyl disulfide	+	–	[17]
Hydrocarbons	2,2,4,6,6-Pentamethylheptane	+	+	
Terpenes	α-Pinene	+	–	
	Limonene	+	–	

[a] These compounds were selected for PCA analysis.

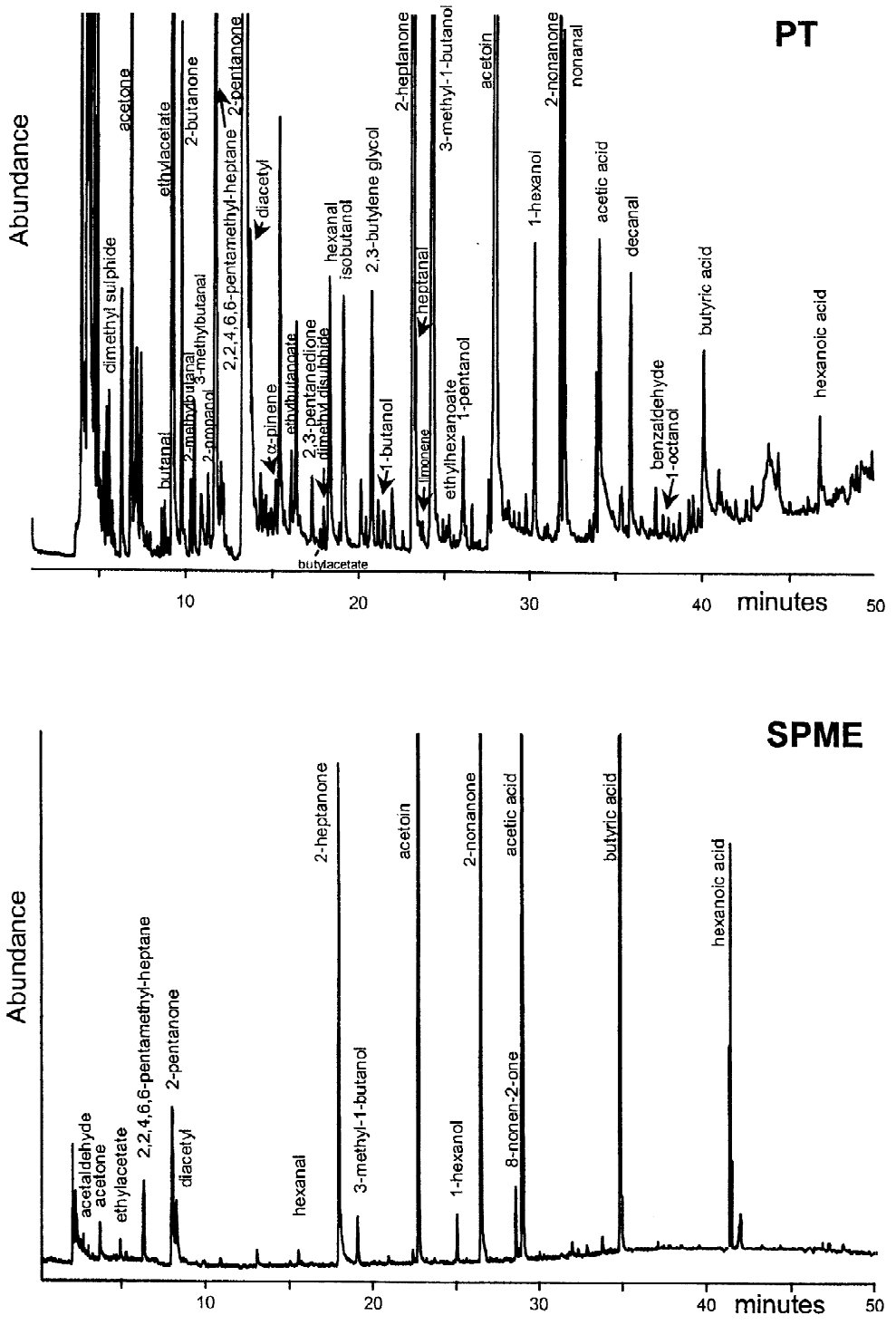

Fig. 1. Total ion profiles for volatile fraction of butter (the same abundance scale was adopted).

Elmore et al. [35] and Vercammen et al. [36] PT provided a richer qualitative-quantitative flavor profile than SPME. This result was mainly due to the following reasons: the dynamic headspace usually provides a higher yield of extraction; the amount of sample extracted by PT was twice that obtained from SPME; and Tenax had a much larger surface than fiber. The largest test portion of the PT analysis was adopted to detect compounds present in low amounts (e.g. terpenes) as well. The same approach could not be applied to SPME due to the small fiber surface. In this case, the results obtained from several tests using increasing amounts of sample, showed that the limit of the extraction yield strictly depended on the fiber adsorption ability. The increase in test portion did not result in an increase in extraction.

The profile obtained from PT showed a major concentration of the most volatile compounds eluted within few minutes, while the extraction yield of those having a higher retention time appeared to be similar to that from SPME. Tenax seemed to have higher affinity for both the most volatile compounds and the compounds belonging to the class of aldehydes and alcohols. Conversely, SPME fiber provided a good recovery of ketones and acids, including those having a high molecular mass. The different extraction ability of the two techniques with respect to the molecular mass of the volatiles, was observed also by Demyttenaere et al. [37] for ethyl esters extracted from whiskey and by Nilsson et al. [38] for chlorinated volatile organic compounds extracted from drinking water.

3.2. Repeatability

The repeatability of both techniques was tested by performing three replicates on the same sample on the same day. To carry out this test, a sample of butter, stored at 4 °C for 40 days, was selected. Relative standard deviations for the major compounds detected by both techniques are reported in Table 3. Values for SPME and PT ranged between 0.1 and 16.6%, with a comparable mean value of 7.6 and 6.3 for PT and SPME, respectively. The mean values of the repeatability were in accordance with those reported by other authors [14,35,37,39], even obtained from different matrices, on different volatiles and by using different fibres. As a consequence, a more detailed comparison with the above-mentioned literature cannot be made.

Table 3
Repeatability of PT and SPME expressed as relative standard deviation (RSD%) of three replicates

Compounds	RSD (%, $n=3$)	
	PT	SPME
Acetone	11.4	10.5
Ethylacetate	4.6	16.6
2-Pentanone	14.4	13.1
Diacetyl	6.2	5.3
Hexanal	15.0	12.0
2-Heptanone	0.4	3.5
3-Methyl-1-butanol	3.6	3.2
Acetoin	8.2	1.0
1-Hexanol	3.0	3.4
2-Nonanone	4.5	0.1
Acetic acid	11.0	2.1
Butyric acid	13.9	1.1
Hexanoic acid	3.2	10.1
Mean value	7.6	6.3

3.3. Applications

In order to evaluate the effect of storage on the volatile fraction of butter, the data obtained from the two techniques were subjected to multivariate statistical analysis (PCA). Due to the high number of variables (volatile compounds), as compared to the number of objects (butter samples), the most significant volatiles were selected (Table 2). Selection was based on the following criteria: volatile compounds occurring in high amounts, detected by both techniques and playing a role in describing both flavor and degradation phenomena of butter.

Fig. 2 shows the biplot on the first and second eigenvector (90% of variance explained) of the ten objects and nine variables obtained from PT analysis. Two separate groups containing both variables and objects were observed along the axis of the first eigenvector. This separation seemed to be associated with the storage temperature. The samples stored at 4 °C throughout the shelf-life (40A, 70A, 90A), together with those sampled at 40 days (40B and 40C) and 70B, were placed in the left region of the graph, namely close to butter analyzed immediately after production (0). This behavior suggested that these samples, though scattered, were not signifi-

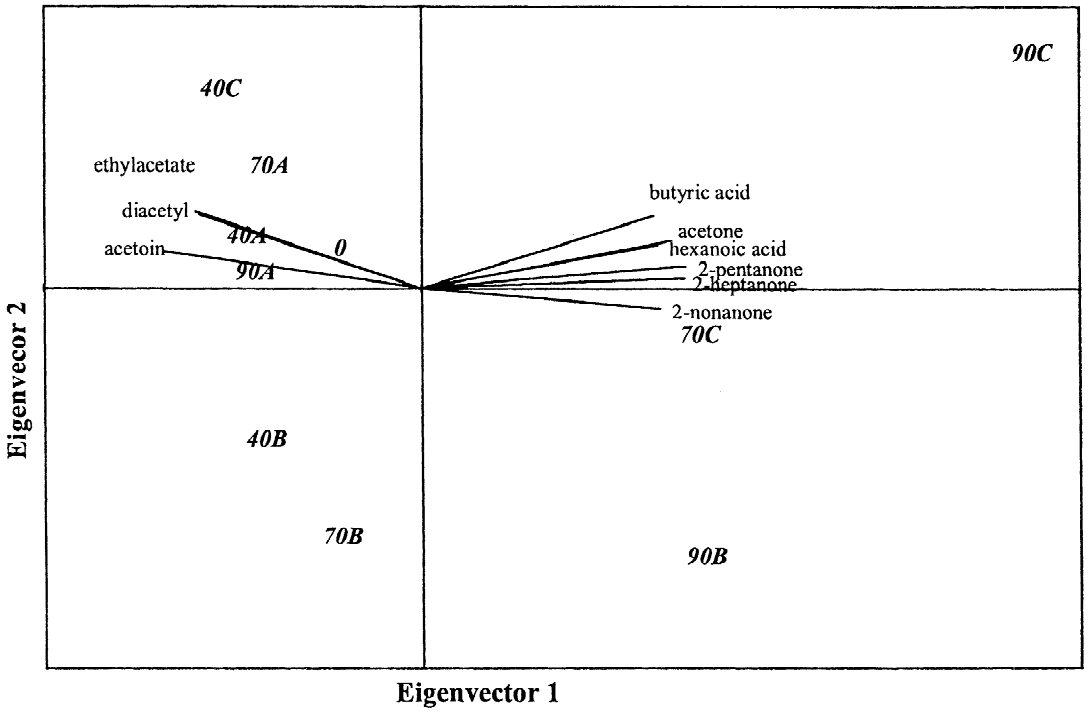

Fig. 2. PCA for results obtained from PT analysis.

cantly affected by storage and maintained their initial characteristics. The position of sample 40C led to the hypothesis that significant degradation did not occur up to the 40th day of storage at 21 °C, and, at these conditions, growth of starter bacteria was probably promoted.

The samples stored at 21 °C for over 40 days (70C, 90C) and that stored at 10 °C for 90 days (90B) were well separated in the right region of the plot. This discrimination was mainly caused by a simultaneous increase in volatile compounds resulting from oxidative and lipolytic processes (ketones and acids) and a decrease in those deriving from the metabolism of starter bacteria (diacetyl and acetoin).

The same statistical evaluation was applied to the results from SPME analysis (Fig. 3). The biplot on the first two eigenvectors (92% of variance explained) showed the same trend obtained from PT data. In this case, volatile compounds seemed to be able to better discriminate between the effects of storage temperature. Samples maintained at 4 °C throughout the shelf-life (40A, 70A, 90A) and at 10 °C for 40 days (40B), together with the 0 time butter sample, were grouped in a very small region. From observation of sample 70B, it can be supposed that storage at 10 °C for 70 days was also responsible for an initial degradation.

Finally, SPME analysis confirmed the behavior of sample 40C.

In order to demonstrate the differences detected by volatile compounds, acidity and peroxide values were determined. Results were compared with an acidity value of 1.5 mmol/100 g and a peroxide value of 0.5 mequiv. O_2/kg, (dotted lines) which, according to other authors [40,41], are considered to be the threshold limits, beyond which defects caused by lipolysis and oxidation are perceptible (Fig. 4).

Up to 40 days of storage, the acidity value of all the butter samples was below this limit. During the following 30 days, only the samples stored at 4 and 10 °C appeared to be below this limit and, at the end of shelf-life, the sample stored at 10 °C exceeded this limit as well.

Results for peroxides with respect to the limit of 0.5 mequiv. O_2/kg were comparable with those of

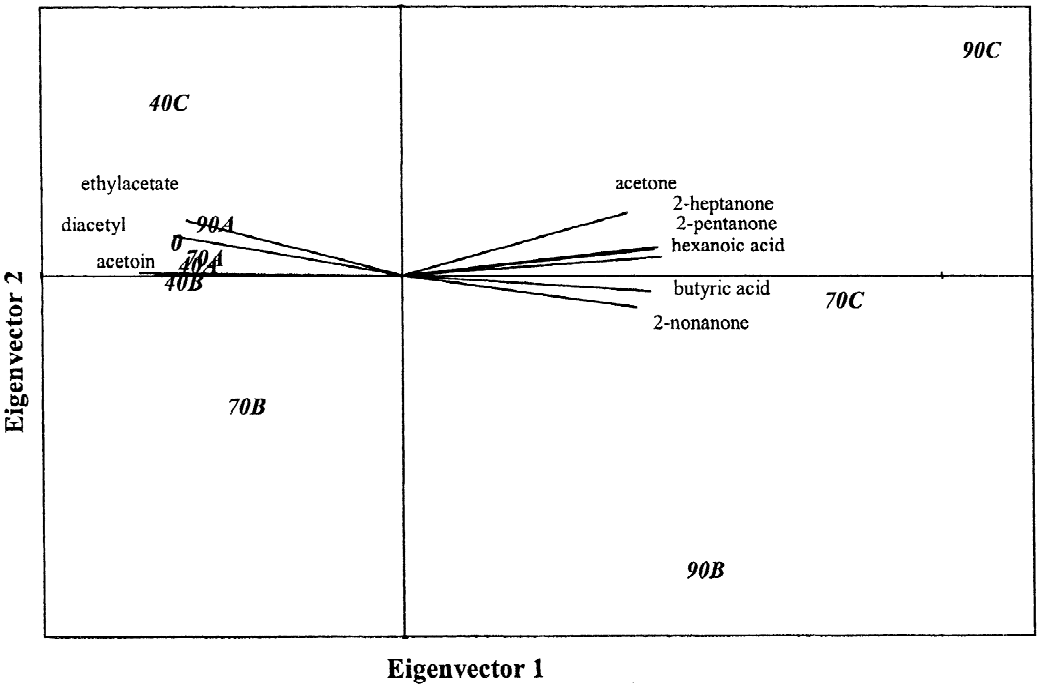

Fig. 3. PCA for results obtained from SPME analysis.

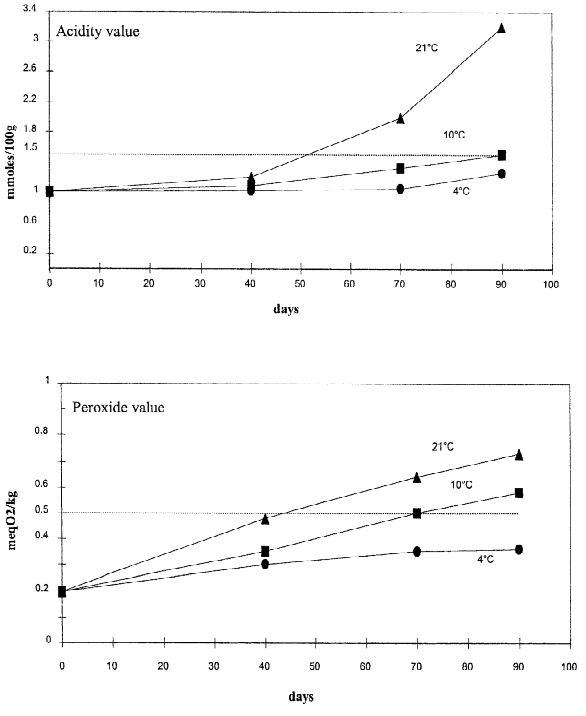

Fig. 4. Acidity and peroxide values.

acidity. From a comparison between these results and those obtained from the SPME analysis, it can be noticed that all the samples showing acidity and peroxide values under the limit corresponded to the samples well grouped in the right region of PCA results (Fig. 3). This result demonstrated that these samples maintained their initial characteristics. Acidity and peroxide values of samples 40C and 70B were very close to the limit, which confirmed that these samples did not belong to the above-mentioned group. The discrimination between samples stored at 21 °C for over 40 days (70C, 90C) and at 10 °C for over 70 days (90B) was also supported by acidity and peroxide results.

4. Conclusions

Despite the different performances showed in the extraction yield, both PT and SPME were able to detect volatile compounds useful for discriminating butter samples stored under different conditions. In this application, the SPME technique was shown to

be more sensitive to monitor butter quality, though it exhibited a lower extracting ability. As far as the influence of temperature on butter conditions is concerned, samples stored at 4 °C did not change their characteristics throughout the shelf-life. Conversely, the shelf-life of butter stored at 10 °C and that of the samples stored at 21 °C were reduced to 70 and 40 days, respectively. Finally, SPME did require a shorter time of analysis, a lower amount of sample and no additional instrumentation on the GC, but it also required immediate GC analysis of the volatile fraction. On the other hand, extraction step and gas chromatographic analysis can be carried out at two different times by using the PT technique, performed by the off-line system.

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