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Volatile Profile of Minimally Processed Herbs during Cold Storage

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Abstract

Herbs contain high amounts of volatile aroma compounds, but improper handling and processing decrease this content. The study aimed to assess the changes in aroma volatiles during the cold storage of three herbs: parsley (Petroselinum crispum (Mill.) Fuss var. neapolitanum*), dill (Anethum graveolens* L.*) and lovage (Levisticum officinale* Koch.*). The samples were minimally processed and stored at 4°C for 12 days. GC-MS profiling was used to determine the content of volatile aroma compounds; the results were compared to previously determined quality attributes: chlorophylls, total polyphenols, total plate count and total yeast and mould count. Parsley contained mainly 1,3,8-p-menthatriene, β-phellandrene and β-myrcene; the main volatile of dill was α-phellandrene, while lovage had high amounts of β-phellandrene, α-terpinolene and β-myrcene. Aroma maps showed that parsley and lovage had a diversity of olfactory notes (turpentine, spice, balsamic, must, mint, citrus), while dill presented a narrower range (dill, mint, turpentine, citrus). The physiological processes that continued in tissues during storage altered significantly the content of volatile aroma compounds. The maps eased the identification of the major volatiles of each herb, but also portrayed the changes caused by storage. Principal component analysis showed the aroma profile of each herb and confirmed the relation between quality attributes and storage.*

Keywords: aroma maps, dill, lovage, parsley, principal component analysis, volatile compounds

1. Introduction

Culinary aromatic herbs add complexity to our dishes, but they have also been long used in phytotherapy for their health benefits. Plant secondary metabolites are responsible for these properties and include different chemicals: vitamins, flavonoids, terpenoids, carotenoids and phytoestrogens with essential functions in plant metabolism and defense mechanisms (1, 2). Secondary metabolites exert potentially beneficial effects on human health by preventing chronic diseases: cancer, type II diabetes and obesity (2-8). Aliphatic C17-polyacetylenes (falcarinol and falcarindiol) from parsley, dill and lovage have anti-inflammatory and anti-platelet aggregation (9, 10). Essential oils protect the cardiovascular system by preventing lipid peroxidation, lowering cholesterol and triglyceride levels and be reducing blood pressure (11-14).Volatile aroma compounds (VAC) contain high amounts of terpenoids (8, 15) that enhance the antioxidant and antimicrobial activity of essential oils (12, 15-22).

But improper handling, storage and mechanical operations applied to herbs decrease the quality and the content of bioactive compounds – VAC included – proportionally to the number of processing steps (2). Cutting has the highest impact because it breaks the tissues structure of vacuoles which come into contact with cytoplasmic oxidases generating oxidative damage (2). This affects the minimally processed fresh herbs that have a growing market worldwide (23). These products are treated mildly by washing, cutting, grating, shredding, are free of additives (24) and provide the convenience demanded by consumers (23). They are stored refrigerated – other methods are not allowed – leading to a short shelf-life. Past research focused primarily on the study of VAC from herbs with few references to the changes that appear during storage (25-30). Herbs from *Lamiaceae* family (basil, lemongrass, marjoram, mint, oregano, rosemary, thyme) have been thoroughly studied (12), while *Apiaceae* family (coriander, dill, lovage, parsley) has been less considered (31). Consequently, the aim of this study was to identify and evaluate the changes that VAC from minimally processed herbs suffer during cold storage. Parsley (*Petroselinum crispum* (Mill.) Fuss var. neapolitanum), dill (*Anethum graveolens* L.) and lovage (*Levisticum officinale* Koch.) were chosen as representatives of *Apiaceae* family. A specific objective was to create aroma profiles of the herbs – aroma maps – as a visual approach to the study of VAC that could represent their variation during cold storage. A second specific objective was to apply a chemometric approach to the study of VAC by using the multivariate similarities and differences among the herbs to graph the experimental outcome. Principal component analysis (PCA) was employed because it reduces the spatial dimensions of the results and gives a simple view of the basic structure and the correlations of experimental data (32).

2. Materials and Methods

2.1. Sample preparation

The samples were prepared as follows: fresh parsley (*Petroselinum crispum* (Mill.) Fussvar. neapolitanum), dill (*Anethum graveolens* L.) and lovage (*Levisticum officinale* Koch.) were purchased in June from a local market. Batches were homogenized, foreign bodies, yellow and withered leaves were removed. The stems were cut to 6 cm. The herbs were cleaned, washed with tap water, drained for about 1h, divided into samples of 50g and thermo-sealed in polyethylene bags (Krups Vacupack Plus F380) (33, 34). Samples were stored at 4°C up to 12 days. Samples from the 1st, 5th, 8th and 12th day of storage were analyzed.

2.2. Volatile aroma compounds (VAC) determination

Gas-chromatography coupled with mass spectrometry (GC-MS) protocols proposed by M. TOFANĂ et al. (35), S.A. SOCACI et al. (36), (37) were used, with some modifications. A GC-MS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph - mass spectrometer equipped with a CombiPAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland) was used. The VAC were extracted using headspace technique by incubating the herb samples at 60°C for 15 min. An aliquot of the gaseous phase was injected in the GC injector and VAC were separated on a Zebron ZB-5 ms capillary column of 50 m x 0.32 mm i.d and 0.25 μm film thickness. The column oven temperature program was: 40°C (3 min) to 240°C with 5°C/min and maintained for 5 min. The carrier gas was helium at a flow rate of 1ml/min, the ion source temperature and interface temperature were set to 250°C and the MS mode was EI. The mass range scanned was 50-550m/z. The identification of separated VAC was made by comparing the obtained mass spectra with the ones in the NIST27 and NIST147 mass spectra libraries.

2.3. Aroma profile determination

The perceived aromas for each VAC were obtained from Flavornet database (38). The percent of each odor was calculated as:

$$I = \frac{\sum pi}{\sum p} \cdot 100\ (\%)$$

where: pi=amount of odorant "i"
p=amount of all odorants

2.4. Statistical interpretation of data

Statistical analyses were performed using XLSTAT (Addinsoft, New York, USA, Version 2012.4.03) statistical software. Pearson coefficient was used to identify and quantify correlations among VAC and chlorophylls, total polyphenols, total plate count and total yeast and mold count, previously determined (33, 34). Principal component analysis (PCA) was also applied to highlight the correlations among VAC, quality attributes and storage.

3. Results and Discussions

Twelve volatile aroma compounds (VAC) were identified for parsley. Beta-myrcene, 1,3,8-p-menthatriene, β-phellandrene and d-limonene accounted for 80.4% of VAC (table 1). The 1,3,8-p-menthatriene is specific to *Apiaceae* family (26) and it confers the characteristic aroma (25, 28-30). The β-phellandrene and β-myrcene are also typical to parsley leaves (28-30). Several studies found 4-isopropenyl-1-methylbenzene, apiol, linalool and myristicin (28, 29), but research shows that VAC vary greatly with pedo-climatic factors, season and extraction method (26, 27, 39).

Table 1. Volatile profile of minimally processed parsley during storage

Volatile compound	Volatile compounds (expressed as % from total peaks area)				Perceived aroma*
	Storage day				
	1	5	8	12	
β-myrcene	27.0	5.9	11.0	17.0	balsamic, spice, must
1,3,8-p-menthatriene	23.4	35.0	26.8	22.9	turpentine
β-phellandrene	22.5	9.7	31.4	28.7	mint, turpentine
d-limonene	6.4	12.5	8.0	7.8	citrus, mint
2-methyl-1-propenyl-benzene	4.2	5.3	4.0	3.8	phenol, spice
terpinolene	4.0	11.0	5.4	4.6	must
α-pinene	3.3	9.7	4.4	7.3	turpentine, mint, spice
α-phellandrene	2.4	3.2	3.9	2.5	dill
o-cymene	2.4	2.9	2.5	1.6	solvent, gasoline, citrus
p-cymene	1.6	1.7	1.1	0.5	solvent, gasoline, citrus
β-pinene	1.2	1.9	0.6	1.5	pine, resin, turpentine
γ-terpinene	0.2	0.2	0.1	0	gasoline, turpentine

* Source: http://www.flavornet.org/flavornet.html

The samples of parsley contained β-myrcene; 1,3,8-p-menthatriene and β-phellandrene above 20%. The values determined differed slightly from other reports (26, 27). The content of β-phellandrene; 1,3,8-p-menthatriene and β-myrcene varied from 3.6% to 33.5%; 20.1% to 68.8% and 15.7% to 16.4% respectively, dependent on geographic areas (26). Summer grown parsley had higher concentrations of myristicin and 1,3,8-p-menthatriene (42.7% and 10.0%) compared with winter grown parsley (30.7% and 5.4%). But, the content of β-phellandrene and β-myrcene was higher in winter grown parsley (35.9 % and 8.7%) than in summer grown parsley (21.8% and 4.5%) (27).Dill contained α-phellandrenemore than 75% of the total VAC (table 2). The β-phellandrene and *o*-cymene were identified and together with α-phellandrene summed over 90% of VAC. Other authors also determined α-phellandrene (47.7–62.5%), β-phellandrene, (7.4–7.5%), limonene (3.7–3.8%), myristicin (1.7–28.2%) and dill ether (0.9–14.8%) (27, 30). They reported significant seasonal differences. Lovage contained high levels of β-phellandrene, α-terpinolene, β-myrcene, d-limonene and α-phellandrene (table 3). They accounted for more than 83% of the total VAC. Sabinene and α-pinene were determined in considerable amounts and together with the major VAC reached 90% of total VAC. Lovage has a maximum content of 0.5% essential oils (31).

Table 2. Volatile profile of minimally processed dill during storage

Volatile compound	Volatile compounds (expressed as % from total peaks area) Storage day				Perceived aroma*
	1	5	8	12	
α-phellandrene	74.6	76.1	81.8	80.7	dill
β-phellandrene	9.5	9.1	8.0	8.1	mint, turpentine
o-cymene	6.5	5.0	2.5	4.4	solvent, gasoline, citrus
d-limonene	3.3	3.3	3.0	3.0	citrus, mint
α-pinene	3.0	3.9	2.9	2.2	turpentine, mint, spice
β-myrcene	0.7	0.7	0.6	0.5	balsamic, spice, must
β-trans-ocimene	0.2	0	0	0	sweet, herb
β-pinene	0.1	0.2	0.1	0	pine, resin, turpentine
terpinolene	0.1	0.1	0.1	0	must

* Source: http://www.flavornet.org/flavornet.html

Table 2. Volatile profile of minimally processed lovage during storage

Volatile compound	Volatile compounds (% of the total) Storage day				Perceived aroma*
	1	5	8	12	
β-phellandrene	56.0	67.7	60.3	63.7	mint, turpentine
α-terpinolene	12.8	0.5	6.4	0.2	must
β-myrcene	10.9	9.5	11.3	23.9	balsamic, spice, must
d-limonene	4.8	5.1	4.8	3.2	citrus, mint
α-phellandrene	2.9	3.0	2.8	2.7	dill
sabinene	2.3	2.4	2.5	1.6	pepper, turpentine, wood
α-pinene	2.0	2.7	3.0	1.6	turpentine, mint, spice
β-pinene	0.4	0.4	0.4	0.4	pine, resin, turpentine
camphen	0.2	0.6	0.6	0.2	camphor
o-cymene	0.2	0.2	0.2	0.3	solvent, turpentine, spice
γ-terpinene	0.2	0.2	0.1	0	gasoline, turpentine

* Source: http://www.flavornet.org/flavornet.html

Significant differences appeared in the content of VAC during storage: β-myrcene decreased by 37% in parsley, being the main difference compared to the first day of storage, *o*-cymene by 33%, *p*-cymene by 68% while γ-terpinene was lost entirely. The content of β-phellandrene and α-pinene increased, however, by 27% and 121%, respectively. Negative correlations between storage period and VAC were determined only for *p*-cymene (r=-0.89; p<0.05) and γ-terpinene (r=-0.90; p<0.05).The content of α- and β-phellandrene from dill increased until the 8thday of storage, generating a positive correlation with storage ($r_{α\text{-}phellandrene}$=0.86; $r_{β\text{-}phellandrene}$=0.90; p<0.05). At the end of the trial, the content of d-limonene and β-myrcene decrease by 92% and 73%, respectively, producing a negative correlation with storage ($r_{d\text{-}limonene}$=-0.80; $r_{β\text{-}myrcene}$=-0.99; p<0.05). Some minor VAC, such as a β-trans-ocimene, were lost entirely. VAC from lovage varied during storage. The content of β-phellandrene increased by 21% in the 5th day of storage. The α-terpinolene decreased by

96% in the 5th day, followed by a 50% increase and reached at the end of the study only 1.5%of the content determined in the 1st day. It was correlated negatively with storage (r=-0.73; p<0.05), similar to d-limonene and α-phellandrene (r=-0.79; p<0.05). The β-myrcene decreased by 13% in the 5th day, but reached doubled in the 12th day, leading to a positive correlation with storage (r=0.79; p<0.05). Similar trends were reported for VAC in fresh cilantro: the maximum content was reached on the 3rd day of storage, followed by a decrease from the 5th to the 15th day (40). A non-linear variation was observed for VAC in pomelo: some VAC increased until the 3rd day, followed by a decreasing trend (41).

The physiological processes that continue in tissues during storage generate these variations. Plants use oxygen in respiration and terpenoids may be formed by mevalonic acid pathway (41). Other compounds such as alcohols, aldehydes, acids and esters may result from redox reactions. Thus, some VAC increase towards the end of the storage period while other decrease because of tissue degradation caused by ageing and water losses. Aroma profile maps were proposed as an original contribution to the study of VAC during storage (figure 1). Parsley and lovage had a wide range of olfactory notes: turpentine, spice, balsamic, must, mint and citrus. Dill had a narrow range of aromas: dill, mint, turpentine and citrus. Turpentine and mint notes intensified for parsley; must notes decreased in intensity for dill; spicy and balsamic aromas increased for lovage after 12 days of storage. Research showed that terpenes behaved differently in strong correlation to aromas. Wood, citrus, spice and floral notes decreased on the 4th day of storage while must, earthy and fresh notes became stronger (41).

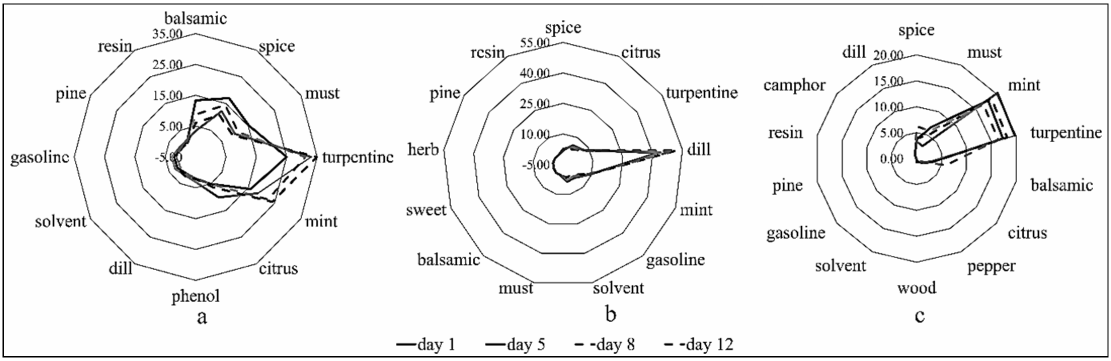

Figure 1. Aroma profile during the storage of minimally processed herbs
a – parsley, **b** – dill, **c** – lovage

The PCA showed the correlation between VAC and storage (figure 2). The two factors selected represented 79.91% of the variability. Factor 1 (F1) consisted of 1,3,8-p-menthatriene, d-limonene, 2-methyl-1-propenyl-benzene, terpinolene, α-pinene, *p*-cymene, β-pinene, γ-terpinene. Each contributed proportionally, between 9.03% and 12.18%. All VAC were correlated positively with F1 ($r_{\alpha\text{-pinene}}$=0.85 to $r_{1.3.8\text{-p-menthatriene}}$=0.99; p<0.05). Factor 2 (F2) contained two groups: VAC positively correlated with F2 ($r_{\alpha\text{-phellandrene}}$=0.88; $r_{o\text{-cymen}}$=0.95; p<0.05) and negatively correlated (β-phellandrene, sabinene, camphen, β-myrcene and α-terpinolene; $r_{\alpha\text{-terpinolene}}$=-0.54 to $r_{\beta\text{-phellandrene}}$=0.90; p<0.05). They contributed to F2 with proportions between 6.10% and 18.72%.

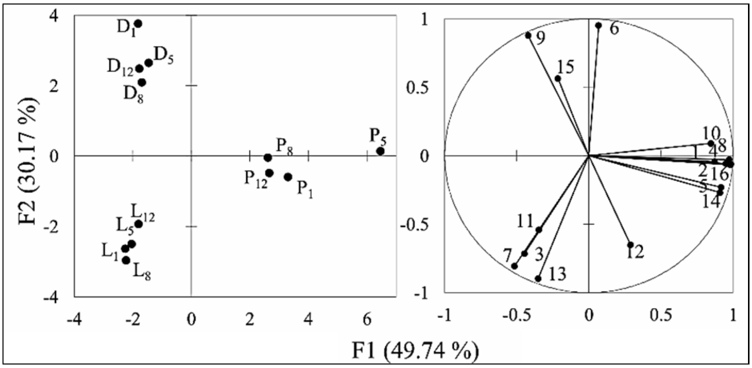

Figure 2. PCA of volatile compounds in the minimally processed herbs during storage
P = parsley; D = dill; L =lovage; the subscripts = the storage in days; 1 = 1,3,8-p- menthatriene; 2 = 2-methyl-1-propenyl-benzene; 3 = camphen; 4 = *p*-cymene; 5 = d-limonene; 6 = *o*-cymene; 7 = sabinene; 8 = terpinolene; 9 = α-phellandrene; 10 = α-pinene; 11 = α-terpinolene; 12 = β-myrcene; 13 = β-phellandrene; 14 = β-pinene; 15 = β-trans-ocimene; 16 = γ-terpinene

Parsley was placed between the 1st and 2nd quadrant because of the high variety of identified VAC. Dill occupied the 4th quadrant for its high content of α-phellandrene and dill-specific β-trans-ocimene. The samples aligned by storage due to the negative correlation between α-phellandrene and storage (r=-0.86; p<0.05). Lovage was assigned to the 3rd quadrant characterized by α-terpinolene, sabinene and camphene, unique to lovage. The samples were grouped chronologically because α-terpinolene was negatively correlated with storage (r=-0.73; p<0.05). The aromatic profile was compared to other quality attributes to have a deeper insight of the changes that occur in during cold storage. VAC were correlated to previously determined chlorophylls, vitamin C, total polyphenols, total plate count (TPC) and total yeast and mould count (TYMC) of the samples (33, 34). The PCA emphasized the relation between quality attributes and storage (figure 3). Three factors were chosen: F1 and F2 gave 70.35% of the variability, while F2 and F3 43.87%. F1 consisted of 1,3,8-p-menthatriene, 2-methyl-1-propenyl-benzene, terpinolene, *p*-cymene, α- and β-pinene, d-limonene, TPC and γ-terpinene with proportion between 6.68% and 9.86%. All attributes were correlated with F1 positively ($r_{\gamma\text{-terpinene}}$=0.79 to $r_{1.3.8\text{-p-menthatriene}}$=0.95; p<0.05). F2 contained *o*-cymene, α-phellandrene, β-phellandrene (10.00-12.34%) correlated positively ($r_{\beta\text{-phellandrene}}$=0.79, $r_{o\text{-cymene}}$=0.93; p<0.05) and vitamin C, sabinene, total polyphenols and camphen (5.40-9.05%) correlated negatively ($r_{vitamin\ C}$=-0.61; $r_{camphen}$=-0.83). F3 grouped total chlorophylls, chlorophylls a and TYMC (14.13-21.08%) correlated positively (r_{TYMC}=0.64, $r_{total\text{chlorophylls}}$=0.82) and chlorophylls b (12.83%) correlated negatively ($r_{total\text{chlorophylls}}$=-0.67). The quality attributes were placed according to their correlation, while the herbs corresponding to the characteristic of each quadrant. The chart F1 to F2 shows the particularity of each herb whereas the chart F2 to F3 the biochemical changes produced by storage. Parsley had a wide variety of VAC, dill a high content of chlorophylls, vitamin C and α-phellandrene, while dill was rich in total phenols and β-phellandrene (33). Chlorophylls and vitamin C decreased during storage for all herbs but TPC and TYMC increased (33, 34). The content of VAC was affected by storage. Some VAC decreased: β-myrcene, p-cymene and γ-terpinene in parsley; β-phellandrene, *o*-cymene and β-myrcene in dill; α-phellandrene in lovage, while β-phellandrene and α-pinene increased in parsley and α- and β-phellandrene in dill.

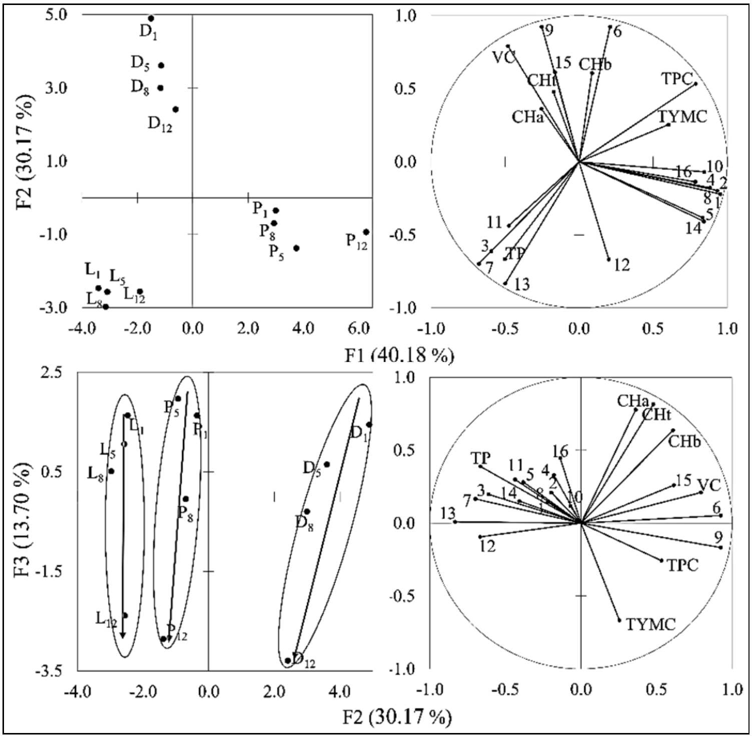

Figure 3. PCA of quality attributes of minimally processed herbs during storage
P = parsley; D =dill; L =lovage; the subscripts = the storage in days; 1 = 1.3.8-p- menthatriene; 2 = 2-methyl-1-propenyl-benzene; 3 = camphen; 4 = *p*-cymene; 5 = d-limonene; 6 = *o*-cymene; 7 = sabinene; 8 = terpinolene; 9 = α-phellandrene; 10 = α-pinene; 11 = α-terpinolene; 12 = β- myrcene; 13 = β-phellandrene; 14 = β-pinene; 15 = β-trans-ocimene; 16 = γ-terpinene; Cha = chlorophyll a; Chb = chlorophyll b; Cht = total chlorophylls; VC = vitamin C; TP = total polyphenols; TPC = total plate count; TYMC = total yeast and mould count

4. Conclusions

Parsley, dill and lovage are rich in VAC, but they vary during storage. Most of the VAC in parsley reached their maximum on the 5th day, except 1,3,8-p-menthatriene and α-phellandrene which attained the highest concentration on the 8th day. Similar, α-phellandrene in dill and lovage recorded its peak on the 8th day. The β-phellandrene, β-myrcene and *o*-cymene had, however, their maximum on the first day of storage. Aroma maps add a visual approach to VAC analysis while PCA can be successfully applied to determine the aromatic profile for culinary herbs. It helped identifying the effect of cold storage on VAC of minimally processed parsley, dill and lovage and some correlations between VAC and storage. PCA can be used to evaluate the connection among quality parameters and the evolution of the product along storage. The correlation between VAC and other biochemical parameters gave encouraging results on the importance of aroma perception in assessing the quality changes subsequent to minimal processing and during storage.

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