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Effect of Organic Fertilization Treatment, Frying Oil and Cultivar Variety on the Volatile Profile of Potato Tubers

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ABSTRACT

The effect of organic fertilization and frying oil (olive, soybean and corn oil) on the volatiles' profile of three potato cultivars (Voyager, Spunta and Lady Rosetta) was studied. During cultivation, nine treatments (T) involving the combination of nitrogen, phosphorus and potassium fertilization were applied: T1: control treatment; T2: N1, P1, K1; T3: N1, P1, K2; T4: N1, P2, K1; T5: N1, P2, K2; T6: N2, P1, K1; T7: N2, P1 K2; T8: N2, P2, K1; and T9: N2, P2, K2, where N1=1.3 g N per plant; N2=2.0 g N per plant; P1=3.1 g P_2O_5 per plant; P2=5.2 g P_2O_5 per plant; K1= 4.0 g K_2O per plant; and K2= 6.6 g K_2O per plant. The main volatile compounds identified by HS-SPME GC/MS analysis were esters, alcohols, carbonyl compounds and hydrocarbons. Principal Component Analysis revealed that nitrogen fertilization affected the volatiles' profile only when high fertilization of P_2O_5 (5.2 g/plant) and K_2O (6.6 g/plant) was applied.

Keywords: HS-SPME, GC/MS, Analysis, Potato, Frying Oil.

INTRODUCTION

Potato chips are one of the most popular snacks worldwide. It is well-known that their acceptability is strongly influenced by their quality and hence their flavor is of increasing importance to consumers.

The aroma development in potato tubers is related to the high number of changes that occur during potato growth and heat-treatment (frying). There are a number of potential precursors responsible for the flavor, which may be produced by lipid hydrolysis and autoxidation, proteolysis and transformation of amino acids to aromatic compounds and carbohydrate metabolism. Therefore, several studies have been focused on the investigation of volatiles' profile and sensory characteristics of potatoes[1,2]. Identification of volatiles' profile has been also used for characterizing the geographical origin[3] and detecting the onset of disease[4,5]. However, very little is known about the effect of organic fertilization, frying oil, as well as cultivar variety on the generation of aroma compounds.

Voyager, Spunta and Lady Roseta are considered among the most popular and widely consumed chipping varieties (http://varieties.ahdb.org.uk/). Voyager is a new long white potato variety, with excellent results for boiling, baking and French fries that boasts high yield potential. Spunta is a very conventional and well-known table potato, suitable for production in many climates. It produces big, long, slightly kidney-shaped tubers with a pale skin and light yellow flesh at high yields. Lady Rosetta is a specialist crisping variety, with high dry matters and low reducing sugars. Lady Rosetta comes from Holland from the crossing variety Cardinal X SVP (VTN2) 62-33-3. It is a premature variety and the tubers have a round shape, red epidermis and yellowish flesh. It has very high quality characteristics, is suitable for the production of potato chips and is very resistant to humidity.

Thus, the effect of organic fertilization on the volatile metabolic profile of potato tubers of three chipping varieties (Voyager, Spunta and Lady Rosetta) after frying with three different oils (olive oil, soybean and corn oil) was of interest in the present study.

MATERIALS AND METHODS

Treatments and samples

During cultivation of three potato cultivars (Voyager, Spunta and Lady Rosetta), the following nine treatments (T) involving the combination of nitrogen, phosphorus and potassium fertilization under organic farming were applied: T1: control treatment; T2: N1, P1, K1; T3: N1, P1, K2; T4: N1, P2, K1; T5: N1, P2, K2; T6: N2, P1, K1; T7: N2, P1 K2; T8: N2, P2, K1; and T9: N2, P2, K2, where N1=1.3 g N per plant; N2=2.0 g N per plant; P1=3.1 g P_2O_5 per plant; P2=5.2 g P_2O_5 per plant; K1= 4.0 g K_2O per plant; and K2= 6.6 g K_2O per plant (Table 1). Fertilization was performed by using the organic fertilizers PatentKali (containing 30% potassium oxide (K_2O), 10% magnesium oxide (MgO) and 17% sulphur), Agrobiosol (6% total nitrogen, 0.5% (P_2O_5), 0.5% (K_2O) and 85% organic matter), Phosphorite (27% (P_2O_5) and Acadian (1% nitrogen, 1% phosphorus and 1% potassium). Seed tubers were planted in 11-L pots containing a mixture 1:1 (vol/vol) of peat (pH=6, no additional inorganic nutrient elements) and perlite. Each treatment was applied to five replicates of four plants each and the experiments were carried out at the Technological Educational Institute of Peloponnese (Kalamata, Greece) between March and May 2013.

The potato tubers (250 g) were then fried using olive, soybean or corn oil at 180^{0}C for 8 min.

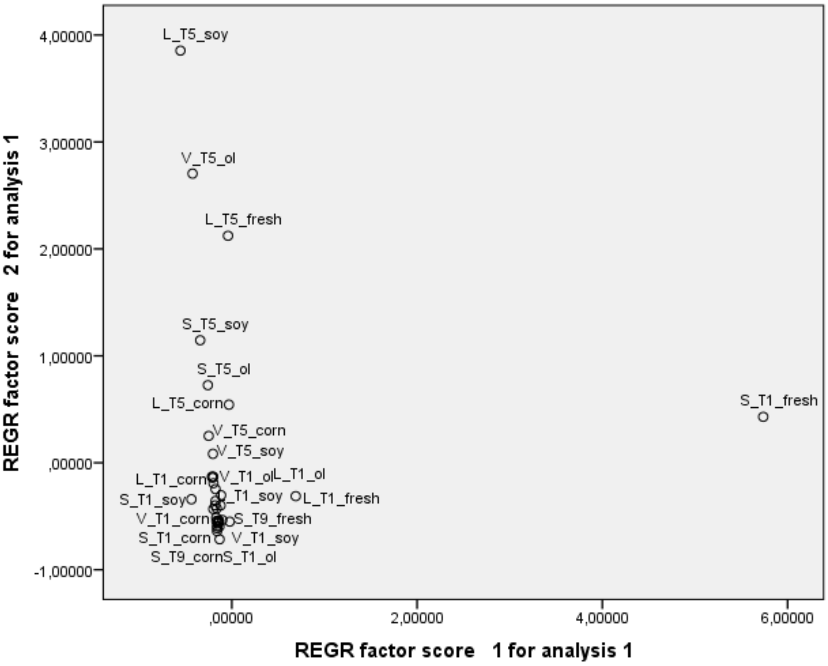

Fig. 1: PCA analysis of samples under treatments 1, 5 and 9. T1: treatment 1, T5: treatment 5, T9: treatment 9, S: Spunta cultivar variety, V: Voyager cultivar variety, L: Lady Roseta cultivar variety, ol: olive oil, soy: soybean oil, corn: corn oil, fresh: fresh samples (not fried)

HS-SPME GC/MS analysis

The SPME GC/MS analysis was carried out as described recently[1] with some modifications. In brief, grated fresh or fried samples (≈ 4 g each) from all treatments (108 samples in total) were placed into a 20 mL headspace vial fitted with a Teflon-lined septum sealed with an aluminum crimp seal, through which the SPME syringe needle (bearing a 2 cm fiber coated with 50/30 mm Divinylbenzene/Carboxen on poly-dimethyl-siloxane bonded to a flexible fused silica core, Supelco, Sigma-Aldrich, UK) was introduced. The container was thermostated at 70°C for 20 min. The absorbed volatile analytes were then analyzed by GC/MS (6890N GC, 5973 Networked MS MSD, Agilent Technologies, USA) using an HP-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a constant pressure of 70 kPa. Oven temperature was set at 40°C for 1 min, followed by a temperature gradient of 20°C/min to 120°C, held for 8 min, then increased to 260 C/min at a rate of 20°C and held for 2 min. The injector was operated in splitless mode. Injector and detector temperatures were 260°C. The mass spectrometer was operated in the electron impact mode with the electron energy set at 70 eV. Results were processed by ChemStation integrated software (Agilent Technologies). The identification was carried out by comparing the retention times and mass spectra of volatiles to those of authentic compounds generated in the laboratory (in-house libraries), by mass spectra obtained from NB575K, stack and Wiley275 libraries, and by determining Kovats' retention indexes and comparing them with those reported in the literature. Kovats' retention indexes were determined by injection of a standard mixture containing the homologous series of normal alkanes (C8-C24) in pure hexane under exactly the same experimental conditions, as described above. 4-methyl-2-pentanol diluted in pure ethanol was used as an internal standard (IS) at 0.8 mg/kg of sample. The volatile compounds were semi-quantified by dividing the peak areas of the compounds of interest by the peak area of the IS and multiplying this ratio by the initial concentration of the IS (expressed as mg/kg). The peak areas were measured from the full scan chromatograph using total ion current (TIC). Each experiment was carried out in duplicate and the mean data are presented (standard deviation for all values was about ±10% in most cases).

Statistical analysis

Principal component analysis (PCA) of data was computed using SPSS (v. 15.0).

RESULTS AND DISCUSSION

The present study constitutes a preliminary investigation on the effect of organic fertilization, frying oil and cultivar variety on the volatile metabolic profile of potato tubers. The strategy adopted was to apply fertilization treatments during cultivation on three chipping potato varieties (Voyager, Spunta, and Lady Rosetta). Subsequently, the outcome of frying using three different oils (extra virgin olive, soybean or corn oil) separately on the profile of volatile compounds was monitored.

HS-SPME GC/MS analysis

For the evaluation of the aromatic profile, the fried potato tubers were analyzed using the HS-SPME GC/MS technique. In total, 108 duplicate samples were analyzed and 46 compounds were detected. The main volatile compounds identified were esters, alcohols, carbonyl compounds and hydrocarbons (Table 2).

Sugars, aminoacids and fatty acids are the major precursors of the volatile compounds formed when potatoes are cooked by various means. Esters, mainly the short chain acids, are responsible for fruity aromas and have been identified in oils, such as olive oil[1,6]. Carbonyl compounds mainly result from a degradative reaction of lipid oxidation of unsaturated fatty acids, such as oleic, linoleic and linolenic acids, and contribute to reactions which yield volatile flavor compounds. In potato crisps unsaturated fatty acids may come from the oil used for frying[1]. On the other hand, hydrocarbons have a less pronounced effect on potatoes' flavor.

Most of the compounds identified were also detected in previous studies. Ethyl acetate, ethanol, acetone, chloroform, heptane, toluene, ethyl benzene, *p*- and *m*-xylene, *a*-pinene and 1,3,5-trimethyl-benzene are usually present in potato tubers[7,8,9]. Likewise, benzaldehyde, tridecane, hexadecane, 2-ethyl-1-hexanol, 2-methyl-butanal, and 3-methyl-butanal have been identified in Italian potatoes[3] and in potatoes after microwave baking[10] in addition to hexane, hexanal, heptanal, 2-heptenal, nonanal, 6-methyl-5-hepten-2-one and limonene. Of note, 2-ethyl-1-hexanol has been also found in significant amounts in the headspace of tubers deliberately infected by *Phytophthora infestans* and in *Fusarium coeruleum*-inoculated tubers[7]. However, in our study, no such infection was noticed. Hexanal, heptanal, 2-heptenal, and nonanal may derive from oxidative degradation of unsaturated acids present in frying oils[11]. These compounds contribute to the aroma of oxidised crisps, due to their low odour threshold and the content of hexanal, heptanal and nonanal is usually increased during storage[1,2]. Alkylpyrazines, like 3-ethyl-2,5-dimethyl-pyrazine, are formed by the Maillard reaction.

Nevertheless, aroma perception in food products depends not only on the concentration and odour thresholds of volatile compounds, but also on their interactions with other food components and among volatile compounds.

Chemometrics

PCA is used in exploratory analysis, as it gives graphical representations of inter-sample and inter-variable relationships and provides a way to reduce the complexity of the data. PCA was considered as a suitable and valuable tool to identify potential significant effects due to the very high number of samples.

The results revealed that nitrogen fertilization affected the volatiles' profile only when high fertilization of P_2O_5 and K_2O was applied (treatments 5 and 9). As shown in Figure 1, samples under treatment 5 clearly distinguished from samples under treatments 1 or 9. These results were in accordance with a previous study[3], reporting that nitrogen can be used as an indicator of the geographical origin of potato, due to the different regional agricultural practices adopted and types of fertilizer used. No other effect or correlation was observed (data not shown).

Table 1: Samples, organic fertilization and frying treatments applied at Voyager, Spunta and Lady Rosetta potato cultivars

Treatment codes	Fertilization treatments			Voyager cultivar				Spunta cultivar				Lady Rosetta cultivar			
	Nitrogen (g/plant)	P_2O_5 (g/plant)	K_2O (g/plant)	No frying	Olive oil frying	Soybean oil frying	Corn oil frying	No frying	Olive oil frying	Soybean oil frying	Corn oil frying	No frying	Olive oil frying	Soybean oil frying	Corn oil frying
T1: control (no fertilization treatment)	0	0	0	V_T1 _fresh	V_T1 _ol	V_T1 _soy	V_T1 _corn	S_T1 _fresh	S_T1 _ol	S_T1 _soy	S_T1 _corn	L_T1 _fresh	L_T1 _ol	L_T1 _soy	L_T1 _corn
T2: N1, P1, K1	1.3	3.1	4.0	V_T2 _fresh	V_T2 _ol	V_T2 _soy	V_T2 _corn	S_T2 _fresh	S_T2 _ol	S_T2 _soy	S_T2 _corn	L_T2 _fresh	L_T2 _ol	L_T2 _soy	L_T2 _corn
T3: N1, P1, K2	1.3	3.1	6.6	V_T3 _fresh	V_T3 _ol	V_T3 _soy	V_T3 _corn	S_T3 _fresh	S_T3 _ol	S_T3 _soy	S_T3 _corn	L_T3 _fresh	L_T3 _ol	L_T3 _soy	L_T3 _corn
T4: N1, P2, K1	1.3	5.2	4.0	V_T4 _fresh	V_T4 _ol	V_T4 _soy	V_T4 _corn	S_T4 _fresh	S_T4 _ol	S_T4 _soy	S_T4 _corn	L_T4 _fresh	L_T4 _ol	L_T4 _soy	L_T4 _corn
T5: N1, P2, K2	1.3	5.2	6.6	V_T5 _fresh	V_T5 _ol	V_T5 _soy	V_T1 _corn	S_T5 _fresh	S_T5 _ol	S_T5 _soy	S_T5 _corn	L_T5 _fresh	L_T5 _ol	L_T5 _soy	L_T5 _corn
T6: N2, P1, K1	2.0	3.1	4.0	V_T6 _fresh	V_T6 _ol	V_T6 _soy	V_T6 _corn	S_T6 _fresh	S_T6 _ol	S_T6 _soy	V_T6 _corn	L_T6 _fresh	L_T6 _ol	L_T6 _soy	S_T6 _corn
T7: N2, P1, K2	2.0	3.1	6.6	V_T7 _fresh	V_T7 _ol	V_T7 _soy	V_T7 _corn	S_T7 _fresh	S_T7 _ol	S_T7 _soy	S_T7 _corn	L_T7 _fresh	L_T7 _ol	L_T7 _soy	L_T7 _corn
T8: N2, P2, K1	2.0	5.2	4.0	V_T8 _fresh	V_T8 _ol	V_T8 _soy	V_T8 _corn	S_T8 _fresh	S_T8 _ol	S_T8 _soy	V_T8 _corn	L_T8 _fresh	L_T8 _ol	L_T8 _soy	S_T8 _corn
T9: N2, P2, K2	2.0	5.2	6.6	V_T9 _fresh	V_T9 _ol	V_T9 _soy	V_T9 _corn	S_T9 _fresh	S_T9 _ol	S_T9 _soy	V_T9 _corn	L_T9 __fresh	L_T9 _ol	L_T9 _soy	S_T9 _corn

Table 2: Volatiles identified in fresh and fried potato tubers

Compounds identified	KI	KI_lit	Concentration range(mg/kg)
Esters			
Ethyl acetate	<700	628[12]	0.0-4.8
Ethyl pentanoate	903	898[13]	0.0-0.7
Ethyl hexanoate	1001	1001[14]	0.0-4.5
Ethyl octanoate	1197	1195[15]	0.0-2.9
Ethyl nonanoate	1278	1275[16]	0.0-0.9
Ethyl decanoate	1397	1397[17]	0.0-0.4
Ethyl tetradecanoate	1795	1793[13,17]	0.0-1.0
Ethyl pentadecanoate	1900	1900	0.0-0.3
Ethyl hexadecanoate	1997	1193[13],1991[17]	0.0-24.1
Alcohols			
Ethanol	<700	-	3.5-80.6
1-octen-3-ol	980	975[14]	0.0-0.4
2-ethyl-1hexanol	1023	-	0.0-<0.1
Aromatic Hydrocarbons			
Toluene	764	762[14]	0.0-0.1
Ethylbenzene	865	868[18]	0.0-<0.1
p-xylene	873	888[14],873[19]	0.0-0.1
1,3-dimethyl-Benzene (*m*-xylene)	881	866[14], 862[18]	0.0-0.1
Styrene	899	890[17]	0.0-<0.1
Mesitylene (1,3,5-trimethyl-benzene)	973	970[20]	0.0-<0.1
Aldehydes			
2-methyl-propanal	<700	550[21]	0.0-0.1
Acetaldehyde	<700	427[16]	0.0-1.4
2-methyl-butanal	<700	593[15]	0.0-0.1
3-methyl-butanal	<700	654[15], 646[21]	0.0-0.2
Hexanal	801	802[18]	0.0-13.5
Heptanal	909	902[14]	0.0-0.1
2-Heptenal	963	951[14]	0.0-0.2
Benzaldehyde	968	966[22]	0.0-1.5
Nonanal	1104	1104[15]	0.0-0.2
Ketones			
Acetone	<700	-	0.0-0.7
2-Heptanone	895	889[23]	0.0-0.2
6-methyl- 5-hepten-2-one	988	988[24]	0.0-0.1
3-octen-2-one	1032	1036[25]	0.0-3.6
(E,E)-3,5-Octadien-2-one	1062	1068[26]	0.0-3.2

Alkanes			
Cyclopentane	<700	563[27]	0.0-1.3
2-methyl-pentane	<700	569[19]	0.0-6.7
3-methyl-pentane	<700	585[19]	0.0-2.4
Hexane	<700	600	0.0-6.9
Heptane	700	700	0.0-0.3
2,2,6-dimethyl-decane	1019	-	0.0-<0.1
Tridecane	1300	1300	0.0-0.6
Hexadecane	1600	1600	0.0-0.8
Octadecane	1800	1800	0.0-0.2
Alkenes			
a-pinene	940	940[24]	0.0-0.1
Limonene	1027	1027[28]	0.0-1.4
Others			
Chloroform	<700	-	0.0-1.1
Phenacyl thiocyanate	729	-	0.0-0.2
3-ethyl-2,5-dimethyl-pyrazine	1069	-	0.0-0.3

CONCLUSIONS

In conclusion, the results indicated that mainly nitrogen fertilization rather than phosphorus and potassium fertilization or frying oil and potato variety may affect significantly the volatiles' profile of potato chips. However, more research is still required to assess the role of each factor that might have a significant effect on the quality of fried potato tubers.

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REFERENCES

1. Sanches-Silva, A., Lopez-Hernández, J., & Paseiro-Losada, P. Profiling flavor compounds of potato crisps during storage using solid-phase microextraction. *Journal of Chromatography A*, **1064**(2), 239-245: 2005.
2. Lee, J. H., & Pangloli, P. Volatile compounds and storage stability of potato chips fried in mid-oleic sunflower oil. *International Journal of Food Properties*, **16**(3), 563-573 2011.
3. Longobardi, F., Casiello, G., Sacco, D., Tedone, L., & Sacco, A. Characterisation of the geographical origin of Italian potatoes, based on stable isotope and volatile compound analyses. *Food Chemistry*, **124**(4), 1708-1713 2011.
4. Blasioli, S., Biondi, E., Samudrala, D., Spinelli, F., Cellini, A., Bertaccini, A., Braschi, I. Identification of volatile markers in potato brown rot and ring rot by combined GC-MS and PTR-MS techniques: Study on in vitro and in vivo samples. *Journal of Agricultural and Food Chemistry*, **62**(2), 337-347 2014.
5. De Lacy, C., Evans, Ewen, Gunson, Ratcliffe, & Spencer, P. Identification of volatiles generated by potato tubers (Solanum tuberosum CV: Maris Piper) infected by Erwinia carotovora, Bacillus polymyxa and Arthrobacter sp. *Plant Pathology,* **48**(3), 345-351 1999.
6. Olias, J. M., Perez, A. G., Rios, J. J., & Sanz, L. C. Aroma of virgin olive oil: Biogenesis of the

"green" odor notes. *Journal of Agricultural and Food Chemistry*, **41**(12), 2368-2373 1993.

7. De Lacy Costello, B. P. J., Evans, P., Ewen, R. J., Gunson, H. E., Jones, P. R. H., Ratcliffe, N. M., & Spencer-Phillips, P. T. N. Gas chromatography–mass spectrometry analyses of volatile organic compounds from potato tubers inoculated with Phytophthora infestans or Fusarium coeruleum. *Plant Pathology*, **50**(4), 489-496: 2001.
8. Lui, L., Vikram, A., Hamzehzarghani, H., & Kushalappa, A. C. Discrimination of three fungal diseases of potato tubers based on volatile metabolic profiles developed using GC/MS. *Potato Research*, **48**(1-2), 85-96 2005.
9. Lui, L. H., Vikram, A., Abu-Nada, Y., Kushalappa, A. C., Raghavan, G. S. V., & Al-Mughrabi, K. Volatile metabolic profiling for discrimination of potato tubers inoculated with dry and soft rot pathogens. *American Journal of Potato Research*, **82**(1), 1-8 2005.
10. Oruna-Concha, M. J., Bakker, J., & Ames, J. M. Comparison of the volatile components of eight cultivars of potato after microwave baking. LWT – *Food Science and Technology,* **35**(1), 80-86 2002.
11. Belitz, H. D. and Grosch, W. Lipids. In Food Chemistry (Springer-Verlag Berlin: Heiderlberg, pp. 128-200: 1987.
12. Baek, H. H., Cadwallader, K. R., Marroquin, E., & Silva, J. L. Identification of predominant aroma compounds in muscadine grape juice. *Journal of Food Science*, **62**(2), 249-252 1997.
13. Adams, R. P. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, IL. pp. 1-550, USA 1995.
14. Engel, E., Baty, C., le Corre, D., Souchon, I., & Martin, N. Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *Journal of Agricultural and Food Chemistry,* **50**(22), 6459-6467: 2002.
15. Qian, M., & Reineccius, G. Potent aroma compounds in Parmigiano Reggiano cheese studied using a dynamic headspace (purge-trap) method. *Flavour and Fragrance Journal*, **18**(3), 252-259: 2003.
16 Jirovetz, L., Buchbauer, G., Ngassoum, M. B., & Geissler, M. Aroma compound analysis of Piper nigrum and Piper guineense essential oils from Cameroon using solid-phase microextraction–gas chromatography, solid-phase microextraction–gas chromatography–mass spectrometry and olfactometry. *Journal of Chromatography A*, **976**(1–2), 265-275 2002.
17. Pino, J. A., Mesa, J., Muñoz, Y., Martí, M. P., & Marbot, R. Volatile components from mango (Mangifera indica L.) cultivars. *Journal of Agricultural and Food Chemistry*, **53**(6), 2213-2223 2005.
18. Pino, J. A., & Quijano, C. E. Study of the volatile compounds from plum (Prunus domestica L. cv. Horvin) and estimation of their contribution to the fruit aroma. *Food Science and Technology (Campinas)*, **32**, 76-83 2012.
19. Lubeck, A. J., & Sutton, D. L. Kovats retention indices of selected hydrocarbons through C10 on bonded phase fused silica capillaries. *Journal of High Resolution Chromatography,* **6**(6), 328-332 1983.
20. Wang, Z., Fingas, M., & Li, K. Fractionation of a light crude oil and identification and quantitation of aliphatic, aromatic, and biomarker compounds by GC-FID and GC-MS, Part II. J*ournal of Chromatographic Science,* **32**(9), 367-382 1994.
21. Zehentbauer, G., & Reineccius, G. A. Determination of key aroma components of Cheddar cheese using dynamic headspace dilution assay. *Flavour and Fragrance Journal,* **17**(4), 300-305: 2002.
22. Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. Comparison of the volatile vomposition in Thyme honeys from several origins in Greece. *Journal of Agricultural and Food Chemistry*, **55**(20), 8152-8157: 2007.
23. Santos, A. S., Andrade, E. H. D. A., Zoghbi, M. D. G. B., & Maia, J. G. S. Volatile constituents of fruits of Annona glabra L. from *Brazil. Flavour and Fragrance Journal,* **13**(3),

148-150 1998.

24. Jalali-Heravi, M., Zekavat, B., & Sereshti, H. Characterization of essential oil components of Iranian geranium oil using gas chromatography–mass spectrometry combined with chemometric resolution techniques. Journal of Chromatography A, **1114**(1), 154-163 2006.
25. Triqui, R., & Bouchriti, N. Freshness assessments of Moroccan sardine (Sardina pilchardus): comparison of overall sensory changes to instrumentally determined volatiles. *Journal of Agricultural and Food Chemistry,* **51**(26)*,* 7540-7546 2003*.*
26. Beaulieu, J. C., & Grimm, C. C. Identification of volatile compounds in Cantaloupe at various developmental stages using solid phase microextraction. *Journal of Agricultural and Food Chemistry,* **49**(3), 1345-1352 2001.
27. Xu, X., Stee, L. L. P., Williams, J., Beens, J., Adahchour, M., Vreuls, R. J. J., Lelieveld, J. Comprehensive two-dimensional gas chromatography (GC × GC) measurements of volatile organic compounds in the atmosphere. Atmos. *Chem. Phys*., **3**(3), 665-682, 2003.
28. Asfaw, N., Licence, P., Novitskii, A. A., & Poliakoff, M. Green chemistry in Ethiopia: the cleaner extraction of essential oils from Artemisia afra: a comparison of clean technology with conventional methodology. *Green Chemistry,* **7**(5), 352-356: 2005.