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INFLUENCE OF STEAM WATER STERILIZATION PROCESS ON THE CONTENT OF VOLATILE AROMA COMPOUNDS IN MARJORAM (*ORIGANUM MAJORANA* L.) ESTIMATED WITH GC/MS AND GC/O

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Key words: marjoram, volatile aromatic compounds, sterilization, GC/MS, GC/O, sensory analysis

The study analysed volatile aromatic compounds of marjoram (*Origanum majorana* L.*)*, steam sterilized using a method developed and implemented at the Institute of Agricultural and Food Biotechnology in Warsaw, at the Division of Food Concentrates in Poznań (Poland). Contents of aromatic compounds were determined in production tests of marjoram before and after sterilization. Fourteen aromatic compounds of marjoram were identified and assayed. Using olfactometry it was found that the dominant aroma both before and after sterilization was the aroma defined as balsamic, whose equivalent is cis-sabinene hydrate, in spite of the fact that linalol was a quantitatively dominant compound. After sterilization cis-sabinene hydrate still remained the main odorant, although the intensity of its aroma decreased. It was found that the process of marjoram sterilization resulted in losses of total volatiles, amounting to approx. 49%, with individual compounds being lost to varying degrees. Sensory analysis showed significant changes in the aroma of marjoram before and after sterilization in most of the analysed production batches. Results suggest that the analysis of contents of the main odorant, cis-sabinene hydrate, may be a criterion in assessing changes in the aroma of sterilized marjoram and the assessment of the sterilization process.

INTRODUCTION

Marjoram is a popular spice, widely used to aromatize foodstuffs, which due to its content of essential oil is found attractive for its aroma. Contents of volatile aromatic compounds in marjoram may change during sterilization, required because of microbial contamination. The addition of microbially contaminated spices to foodstuffs may prove dangerous for the health of the consumer. For this reason several methods of spice sterilization are applied. Methods are being constantly searched for, which would effectively reduce microflora, and at the same time would not affect the sensory quality of processed spices. Selection criteria of spice sterilization methods include also cost-effectiveness of the process and consumer acceptance. Studies have been undertaken on the sterilization of spices at the laboratory scale using ozone, carbon dioxide, a mixture of ethanol and methanol, high hydrostatic pressure, as well as ultraviolet, infrared and microwave radiation. Sterilization methods currently widely used on the commercial scale are steam sterilization and irradiation. The application of radiation is feared by consumers, thus at present steam sterilization is being frequently used.

Decontamination using thermal processing and combined thermal and pressure processing caused changes in the composition of volatiles in black pepper, including a decrease in the contents of most monoterpene compounds and an increase in the contents of α- and γ-terpinene and terpinene-4-ol, while the action of high pressure resulted in the intensification of these changes [Skąpska *et al.*, 2002]. In studies on coriander and caraway the components found most significant quantitatively, *i.e.* linalol in coriander and carvone in caraway, did not change considerably [Skąpska *et al.*, 2004]. In the case of radiation sterilization studies showed a slight effect of this method on chemical changes in spices [Marcotte, 1993; Kamiński *et al.*, 1991; Piggeott & Othman, 1993].

At the Institute of Agricultural and Food Biotechnology in Warsaw, at the Division of Food Concentrates in Poznań (Poland) an original technology of constant steam sterilization of spices was developed and implemented. The method effectively destroys microflora, although information is lacking on the effect it has on aroma contents in analysed raw materials. This problem is essential from the point of view of both food producers and consumers.

Influence of sterilization process on volatile aroma compounds in marjoram (*Origanum majorana* L.*)* was a theme of this research work.

MATERIALS AND METHODS

Materials. Samples of marjoram from four different production batches of marjoram (P1, P2, P3, P4) prepared at the Institute of Agricultural and Food Biotechnology in Warsaw, at the Division of Food Concentrates in Poznań (Poland).

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TABLE 1. Identification of volatile compounds in the samples of marjoram.

Volatile compound	GC-FID		GC-MS	
	IR	+/-	IR	+/-
Sabinene	970	-	988	+
β-Pinene	981	+	992	+
Myrcene	1008	+	1001	+
α-Terpinene	1023	+	1027	+
p-Cymene	1033	+	1036	+
Limonene	1038	+	1042	+
Cyneol	1041	+	1050	+
γ-Terpinene	1071	+	1074	+
Sabinene cis-hydrate	1080	+	1116	+
Linalool	1113	+	1125	+
Camphor	1156	+	1168	+
Borneol	1175	+	1185	+
Terpine-4-ol	1185	+	1193	+
α-Terpineol	1196	+	1204	+
Bergamol	1266	+	1253	+
Carwone	1255	+	1268	+
Thymol	1304	+	1308	+
Carvacrol	1314	+	1316	+
Caryophyllene	1430	+	1438	+
α-Phellandrene		-	1014	+
3-Carene		-	1018	+
2-Carene		-	1112	+

IR- Kovats' retention index; + identified; - unidentified; parameters of GC/FID and GC/MS separation were specified in the text.

Standards of volatile compounds originated from Sigma-Aldrich Co.: β-pinene, myrcene, α-terpinene, p-cymene, limonene, γ-terpinene, cis-sabinene hydrate, linalool, terpinene-4-ol, α-terpineol, bergamol, carvacrol, and caryophyllene.

Isolation of volatile compounds. A marjoram sample (1 g) was performed using steam distillation in a Deryng apparatus. Prior to distillation 1 mg internal standard of tetradecane was used with 0.5 mL of 97% xylene by Sigma-Aldrich Co. as a solvent. Distillation was run for 3 h.

Gas chromatography (GC/FID), mass spectrometry (GC/MS). A Hewlett-Packard HP 6890 gas chromatograph with a FID detector, a HP-5 capillary column with the dimensions of 30 m, 320 μm, 0.25 μm with the Ph Me Siloxane packing (Hewlett-Packard) were used in the study. Helium was used as carrier gas at a flow rate of 1 mL/min, temperature of the column was: 5 min at 35°C, increment of 30°C/min to 60°C, next increment of 6°C/min to 200°C and 30°C/min to 280°C. For mass spectrometry a Hewlett-Packard HP 5890 II gas chromatograph coupled with a HP 5971MDN-5 quadruple mass spectrometer was used.

Gas chromatography and olfactometry (GC/O). A Hewlett- Packard HP 5890 gas chromatograph equipped with a flow splitter and a smelling port using a DB-5 column with the dimensions of 30 m, 0.53 mm and 0.25 μm were used. Helium was used as carrier gas at a flow of 1 mL/min, column temperature was: 1 min at 40°C, increment of 8°C/min to 200°C, next increment of 20°C/min to 280°C and 5 min at 280°C. Separated fractions were smelled at successive dilutions of the analysed marjoram distillate until the disappearance of the last detectable aroma. In this way the dilution factor (FD) was obtained for fractions [Jirovetz, 2001; Zawirska-Wojtasiak, 2004].

Sensory analysis. The analysis was conducted by the triangle method. Spice samples, suspended on starch gel being a neutral carrier, were presented to the assessing panel members in sealed containers [Zawirska-Wojtasiak *et al.*, 1998]. Prior to analysis, the samples were heated to 40°C. Analysis was performed by twelve trained panelists with sensory sensitivity. Results were found in statistic tables at significance levels of $\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$.

RESULTS AND DISCUSSION

A total of 22 aromatic compounds, known from literature sources as aromatic compounds of marjoram, were identified in essential oils from marjoram samples [Jimenez-Carmona *et al.*, 1999; Novak *et al.*, 2000; Raghavan, 2000; Vagi

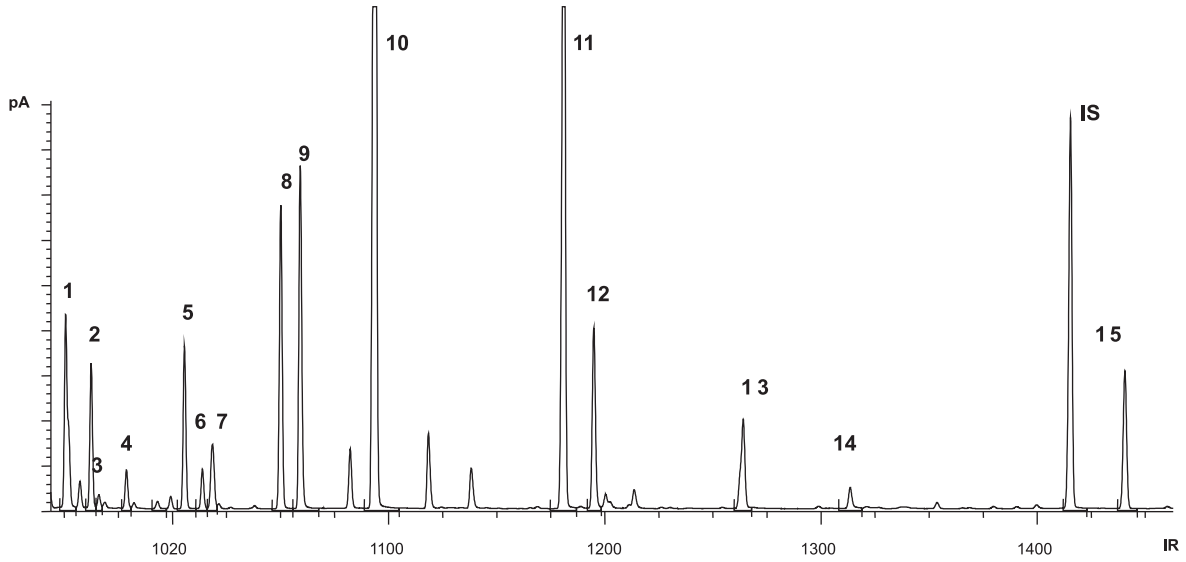

FIGURE 1. Separation of volatile compounds in the sample of marjoram (P1) before the sterilization process. IS- internal standard, IR- Kovats' retention index, parameters of GC separation were specified in the text. 1. sabinene, 2. unidentified, 3. β-pinene, 4. myrcene, 5. α-terpinene, 6. p-cymene, 7. limonene, 8. γ-terpinene, 9. sabinene cis-hydrate, 10. linalool, 11. terpinene-4-ol, 12. α-terpineol, 13. bergamol, 14. carvacrol, 15. caryophyllene

et al., 2005;Vera & Change-Ming, 1999]. There were: sabinene, β-pinene, myrcene, α-terpinene, p-cymene, limonene, cyneol, γ-terpinene, sabinene cis-hydrate, linalool, camphor, borneol, terpinene-4-ol, α-terpineol, bergamol, carvone, thymol, carvacrol, caryophyllene, α-phelandrene, 3-carene, and 2-carene (Table 1). Among them the quantitative content was determined for 14 aromatic compounds, separation of which is presented in Figure 1. Literature data show that contents of volatile compounds in marjoram oil markedly differ in the composition and quantitative composition of aromatic compounds, depending on the origin and variety [Vera & Chane-Ming, 1999; Raghavan, 2000]. Greek marjoram has more sabinene and cis- and sabinene trans-hydrate, while Indian and Turkish marjorams contain more linalool, caryophyllene, carvacrol and eugenol [Raghavan, 2000]. According to a study by Vagi *et al.* [2005], essential oil of marjoram contained the greatest amounts of linalool, terpinene-4-ol, and γ-terpinene. Similarly, in the analysed marjoram samples it

TABLE 2. Volatile compounds content of marjoram (P1) before and after sterilization process.

Volatile compound	Kovats' retention index (IR)	Content (mg/g)	
		before sterilization	after sterilization
Sabinene	970	0.52±0.02	0.57±0.01
Unidentified	978	0.27±0.01	0.14±0.01
β-Pinene	981	0.03±0,01	0.02±0.01
Myrcene	988	0.08±0.01	0.04±0.01
α-Terpinene	1023	0.36±0.01	0.16±0.1
p-Cymene	1033	0.06±0,01	0.06±0.01
Limonene	1041	0.15±0.01	0.10±0.01
γ-Terpinene	1071	0.67±0.01	0.27±0.01
Sabinene cis-hydrate	1081	0.77±0.01	0.27±0.01
Linalool	1113	3.12±0.02	1.33±0.04
Terpine-4-ol	1185	1.97±0.02	0.97±0.02
α-Terpineol	1196	0.45±0.01	0.30±0.01
Bergamol	1266	0.30±0.02	0.11±0.01
Carvacrol	1314	0.08±0.01	0.07±0.01
Caryophyllene	1430	0.37±0.01	0.57±0.03
TOTAL		9.21±0.03	4.98±0.01

parameters of GC separation were provided in the text

TABLE 3. Olfactometric dilution factors for flavour compounds in marjoram distillate (P1).

Volatile compound	Kovats' retention index IR	Odour descriptor	Dillution factor FD before sterilization process	Dillution factor FD after sterilization process
Unidentified	923	sickly	4	4
β-Pinene	951	mushroom	8	2
Limonen	991	lemon-like	8	2
Sabinene cis-hydrate	1035	balsamic	128	32
Linalool	1076	flowery	32	8
Terpine-4-ol	1146	earthy	32	8
α-Terpineol	1156	floral	4	2
Carvacrol	1263	spicy	4	4
Caryophyllene	1369	herbs	4	2

parameters of GC/O separation were specified in the text

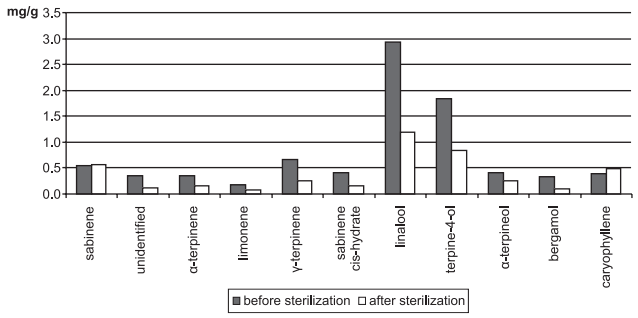

FIGURE 2. The average (for 4 batches) content of main volatile compounds in marjoram before and after the sterilization process.

was found that quantitatively the dominant compounds were linalool, whose content was on average 33%, and terpinene-4--ol, whose content was on average 23%. Contents of the other volatile compounds in the analysed samples amounted to less than 8%of the total assayed volatile compounds.

Table 2 presents contents of volatile aromatic compounds in one batch (for sample) of marjoram before and after sterilization. It was found that the sterilization of marjoram resulted in losses of total volatiles, ranging from 46 to 54% (on average 49%), with individual compounds being lost to varying degrees. Losses of linalool, quantitatively the main component, ranged from 57 to 61%, while losses of cis–sabinene hydrate ranged from 46 to 63%. Losses of the other volatile aromatic compounds were as follows: for β-pinene 33-50%, myrcene 50-71%, α-terpinene 50-66%, p-cymene 20-44%, limonene 61-65%, γ-terpinene 57-65%, terpinene-4-ol 49-57%, α-terpineol 33-47%, bergamol 59-74%, and carvacrol 0-25% (Figure 2). No losses of sabinene nor caryophyllene were recorded. While investigating the effect of drying temperature on the aroma of basil Barbieri *et al.* [2004] found that the temperature of drying air already within the range of 40-60°C significantly affected individual aromatic compounds. The effect of temperature resulted in an increase or decrease of aromatic compound contents.

Using olfactometry (Table 3) it was found that the dominant aroma both after and before sterilization was the aroma defined as balsamic, which is the equivalent of cis-sabinene hydrate, despite the fact that linalol was quantitatively the dominant compound. After sterilization cis-sabinene hydrate still remained the main odorant, although the intensity of its aroma decreased. Marjoram subjected to sterilization exhibited lower FD values for most detectable aromatic compounds except for carvacrol; however, the highest dilution factor was always recorded for cis-sabinene hydrate.

Sensory analysis was conducted as well. For batch P1 11 positive responses were recorded, while for batches P2, P3 and P4 there were 9 positive responses each (n = 12). Thus in all analysed marjoram samples a statistically significant difference was found between spice aroma before and after sterilization. In one case this difference was found even at a significance level of α=0.001.

CONCLUSIONS

1. Quantitatively the dominant compounds in the analysed marjoram were linalool and terpinene-4-ol.

2. As a result of marjoram steam sterilization losses of total volatile compounds were observed, ranging from 46 to 54%. Not all volatiles in the analysed spices were subjected to identical rates of losses.

3. Using GC, GC/O and sensory analysis it was found that steam sterilization had a significant effect on sensory attributes of marjoram.

4. The main odorant of marjoram, both before and after sterilization, was the aroma defined as balsamic, corresponding to cis-sabinene hydrate, in spite of the fact that quantitatively the dominant compound was linalool.

5. Results suggest that the analysis of contents of the main odorant, *i.e.* cis-sabinene hydrate, may be a criterion in the assessment of changes in the aroma of sterilized marjoram and in the assessment of the sterilization process.

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WPŁYW STERYLIZACJI PARĄ WODNĄ NA ZAWARTOŚĆ LOTNYCH ZWIĄZKÓW ZAPACHOWYCH MAJERANKU (*ORIGANUM MAJORANA* L.*)* OCENIANY METODAMI GC/MS I GC/O

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Poddano ocenie lotne związki zapachowe majeranku (*Origanum majorana* L.*)* sterylizowanego parą wodną metodą opracowaną i wdrożoną w Instytucie Biotechnologii Przemysłu Rolno-Spożywczego w Warszawie, w Oddziale Koncentratów w Poznaniu (Polska). Oznaczono zawartość 14 związków lotnych majeranku (rys. 1, tab. 2). Stwierdzono, że prowadzony proces sterylizacji majeranku powodował straty sumy oznaczanych związków lotnych, które wynosiły średnio 49%, przy czym poszczególne związki ubywały w różnym stopniu (rys. 2). Za pomocą olfaktometrii stwierdzono, że dominującym zapachem zarówno przed jak i po sterylizacji był zapach określony jako balsamiczny, któremu odpowiada związek wodzian cis-sabinenu, pomimo, że przeważającym ilościowo związkiem był linalol (tab. 3). We wszystkich próbach majeranku wystąpiła statystycznie istotna różnica pomiędzy aromatem przyprawy przed i po sterylizacji. Uzyskane wyniki sugerują, że badanie zawartości głównego odoranta wodzianu cis-sabinenu może być kryterium oceny zmian aromatu sterylizowanego majeranku i oceny procesu sterylizacji.