

COMPARATIVE ANALYSIS OF THE CHEMICAL COMPOSITION AND PROPERTIES OF SPICE EXTRACTS 1. BLACK PEPPER (*PIPER NIGRUM* L.)

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Abstract. *The aim of this study was to compare the chemical composition, antioxidant and antimicrobial activities of a freon extract (extraction with C₂H₂F₄ (1,1,1,2-tetrafluoroethane)) and a dry encapsulated extract from black pepper (*Piper Nigrum* L.). The chemical composition was analyzed using GC/MS and the main compounds (concentration higher than 3 %) of the extracts were limonene (23.53 % and 19.07 %, respectively), β -caryophyllene (22.59 % and 18.79 %, respectively) and sabinene (18.18 % and 12.18 % respectively). The studied extracts demonstrated antioxidant activity against DPPH radical and antimicrobial activity against Gram-positive and Gram-negative bacteria.*

Keywords: *Black pepper, chemical composition, antimicrobial and antioxidant activities.*

INTRODUCTION. Fats and oils present in many foods and cosmetic products may easily deteriorate due to oxidation, in a chain of reactions in which free radicals are formed, propagated, and finally converted into stable oxygenated compounds, which are responsible for off-flavors and other undesirable characteristics [1]. Antioxidants, when added to lipid-containing foods, can increase shelf life by retarding the process of lipid peroxidation. Thus, there is a need for identifying alternative natural and safe sources of food antioxidants [2,3]. Herbs and spices, which are important part of the human diet, have been used for thousands of years in traditional medicine and to enhance the flavour, colour and aroma of foods. In addition to boosting flavour, herbs and spices are also known for their preservative [4], antioxidative [5], and antimicrobial [6] roles.

Black pepper (*Piper nigrum* L.) is one of the most popular spice products in oriental countries (mostly in Southeast Asia). *P. Nigrum* is a plant of the *Piperaceae* family, largely used as a flavouring agent in foods. Its characteristic aromatic odour is due to the volatile oils in the cells of the pericarp [7]. The essential oil of pepper is a mixture of a large number of volatile chemical compounds [8]. More than 80 components have been reported in pepper essential oil [9]. The components which are present in pepper essential oil include: monoterpene hydrocarbons (mainly α -pinene, β -pinene, sabinene and limonene), oxygenated monoterpene compounds (about 43 are known), sesquiterpene hydrocarbons (about 25 and the most important one is β -caryophyllene) and oxygenated sesquiterpene compounds [8].

Two methods for obtaining black pepper extracts were selected in the present study: extraction with liquefied gas freon (134a) and extraction with 96 % ethanol, followed by spray drying. Extraction with liquefied gas was performed at increased pressure and reduced temperature, which allows to obtain extracts containing valuable thermolabile substances. Freon extracts are clear, yellow or brown colored liquid with the characteristic odor of the raw material. Their chemical composition is similar to essential oils. These extracts are soluble in polar and non-polar solvents. The solvent wasn't detected in these extracts [10]. Drying is generally applied in nutraceutical processing, aiming the reduction of the product water activity to a safe level, which assures its microbial stability and minimizes physical and chemical changes during storage. The concept of spray drying is based on the increase in the surface area of the contact area between the material to be dried and the drying medium promoted by the atomization [11].

The aim of the present study is the comparative analysis of the chemistry, antioxidant and antimicrobial activities of a freon extract and a dry encapsulated extract from black pepper to find new sources of natural antioxidants.

MATERIAL AND METHODS

Plant material. Black pepper dry fruits were purchased from "Pimenta Bulgaria" Ltd, Sofia (Bulgaria).

Obtaining of freon extract. The used solvent was non-polar food grade liquefied gas Freon 134a - tetrafluoroethane (CAS number 811-97-2). The black pepper fruits were ground in an attrition mill to a size of 0.15-0.25 mm and the extract was obtained in a 1 dm³ volume laboratory extractor [10] under the following conditions: temperature 20-25 °C, pressure 5,7-6,5 bar, extraction time 50-60 min.

Obtaining of dry encapsulated extract. The black pepper fruits were ground. The used solvent was 96 % ethanol and extraction under following parameters was performed: temperature 50 °C, static process, a single extraction for 5h and hidromodul 1:5. After concentration maltodextrin and distilled water were added. The dry extract was obtained in a laboratory spray dryer installation produced by company "TCT" Ltd., Plovdiv (Bulgaria) under following the parameters: inlet temperature 200-220°C and outlet temperature 90-100°C [12].

Determination of chemical composition. Gas Chromatography-Mass Spectrometry Analysis was performed using an AGILENT 7890A gas chromatograph equipped with MSD 5975 C detector and HP-5MS (5% Phenyl Methyl Silox) column (30 m x 250 μ m; film thickness 0,25 μ m); temperature: 40 °C for 3 min then 5 °C/min to 300 °C for 5 min; Run time: 60 min; carrier gas helium, 0.8 ml/min constant flow; injector split, 250 °C; MSD, 150 °C, transfer line; pressure: 6.9999 psi.

Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). DPPH[•] is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. The radical scavenging capacity was determined according to the method described in [13]. 2.75 ml from methanol solution of DPPH (with concentration 0.04 mg/ml) was added to 150 μ l from the samples with different concentration of black pepper extracts. The samples were kept at 35 °C in the dark and after 15 min

the optical density was measured at 517 nm. The optical density of the samples, the control and the empty samples were measured in comparison with methanol. The experiment was performed in triplicate and the results were statistically evaluated. The data of different samples were analyzed independently by ANOVA software (Excel 5.0). Mean values and standard errors of the mean were reported. Significance of differences was defined at $p \leq 0.05$.

The percentage inhibition values of DPPH – radical were calculated using Eq. (1):

$$I (\%) = [(A_c - A_s / A_c) \times 100] \quad (1)$$

Where: A_c – absorbance of the control sample, A_s – absorbance of the sample with extract.

Determination of antimicrobial activity. Antimicrobial activity of extracts was determined against Gram-positive and Gram-negative bacteria. Preparation of the extracts: the dried encapsulated extract was dissolved in saline solution and the freon extract in 50% solution of DMSO (dimethylsulfoxide, Sigma - Aldrich Co.). Analyzed concentrations: dry encapsulated extract of black pepper – from 3000 $\mu\text{g/ml}$ to 23 $\mu\text{g/ml}$; freon extract of black pepper – from 1250 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$. Test microorganisms: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Salmonella enterica ssp. enterica* ATCC BAA-2162 and *Listeria monocytogenes* (clinical isolate). Culture medium TSB (Tryptic Soy Broth) (Scharlau) with pH - 7.3 was used. Obtaining of test culture: 5 ml TSB was inoculated with 3-4 colonies from the test bacteria culture after 24 h cultivation on slanting PCA agar (Scharlau). The tubes with liquid medium were cultured in a thermostat at 37 °C for 18-20 h until optical density corresponding to a standard 0.5 McFarland ($1.0/1.5 \times 10^8$ cfu/ml). The absorbance at 600 nm was measured against a control sample of the inoculated media without extract. The Minimal Inhibitory Concentration (MIC, %) and Minimal Bactericidal Concentration (MBC) of the extracts were determined by reference method of serial dilutions in accordance with the recommendations of the NCCLS [14]. MIC and MBC were defined as the lowest concentration of the sample causing 50% and over 99% reduction of the absorbance in comparison with the control, respectively. The experiment was performed in triplicate and the results were statistically evaluated. The data for the different samples were analyzed independently by ANOVA software (Excel 5.0). Mean values and standard errors of the mean were reported. Significance of differences was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION. The chemical composition of the extracts is listed in Table 1. In the black pepper extracts were identified 30 compounds (with 23.7 % more in the freon extract compared to the dry encapsulated extract). As listed, the major constituents (concentration higher than 3 %) of the extracts were limonene (23.53 % in freon extract and 19.07 % in dry extract), β -caryophyllene (22.59 % and 18.79 % respectively) and sabinene (18.18 % and 12.18 % respectively).

On the basis of the obtained results was found qualitatively overlapping but quantitative difference in the compounds identified in both extracts, mainly due to the different selectivity of both the solvent and extraction method.

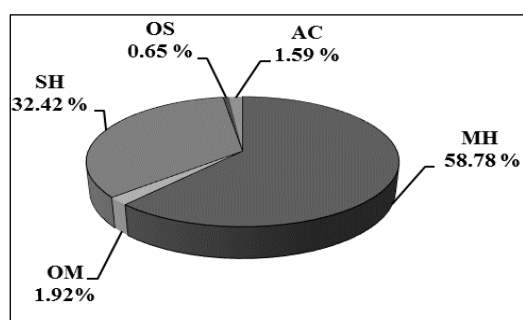


Fig. 1. Groups of components in the Freon extract, % (MH – monoterpene hydrocarbons; OM – oxygen hydrocarbons; SH – sesquiterpene OS – oxygen sesquiterpenes; AC – aromatic compounds)

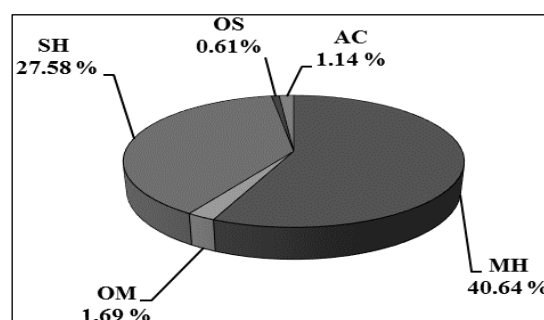


Fig. 2. Groups of components in the dry encapsulated extract, % (MH – monoterpene OM – oxygen hydrocarbons; SH – sesquiterpene OS – oxygen sesquiterpenes; AC – aromatic compounds)

The distribution of the different groups of aromatic compounds, contained in the black pepper extracts is shown on fig. 1 and fig. 2. Monoterpene hydrocarbons were the dominant group in both black pepper extracts - 58.78 % in freon and 40.64 % in dry extract, followed by sesquiterpenes (32.42 % and 27.58 % respectively).

Table 1. Chemical composition of black pepper extracts

RI	Compounds	Freon extract	Dry encapsulated extract
		%	
928	α -Thujene	0.27	0.05
939	α -Pinene	4.65	2.10
954	Camphene	0.11	0.07
971	Sabinene	18.18	12.18
979	β -Pinene	7.24	3.88
991	β -Myrcene	1.61	1.03
1003	α -Phellandrene	2.78	1.91
1024	p-Cymene	1.59	1.14
1027	β -Phellandrene	0.11	0.09
1030	Limonene	23.53	19.07
1032	1,8-Cineole	0.09	0.08
1055	γ -Terpinene	0.09	0.06
1088	Terpinolene	0.21	0.20
1092	β -Linalool	0.35	0.30
1144	Camphor	0.66	0.59
1179	Terpinene-4-ol	0.14	0.12
1189	α -Terpineol	0.68	0.60
1352	α -Longipinene	1.36	1.20
1419	β -Caryophyllene	22.59	18.79
1435	α -trans-Bergamotene	0.74	0.42
1454	α -Humulene	1.60	1.56
1483	γ -Curcumene	1.18	1.13
1485	Germacrene D	1.60	1.57
1498	α -Muurolene	0.36	0.33
1506	β -Bisabolene	0.82	0.61
1513	γ -Cadinene	0.56	0.49
1524	δ -Cadinene	1.21	1.17
1560	Germacrene B	0.40	0.31
1619	(-)-Spathulenol	0.59	0.56
1675	α -Bisabolol	0.06	0.05
Total %:		95.36	71.66

The qualitative composition of both extracts in the present study corresponded to those for ethanol and freon extracts [15, 16] but the quantitative differences in the identified compounds could be due to differences in origin, harvest, weather conditions and obtaining method.

The antioxidant activities of the black pepper extracts are shown on fig. 3 and fig. 4.

The antioxidant activity against DPPH[•] was the intensified with increase of the extracts concentration. The freon extract demonstrated stronger radical-scavenging activity, compared to the dry encapsulated extract. It was found that the freon extract at concentration 10 μ g/ml lead to 51.71% inhibition (fig. 3), while the dry extract from black pepper inhibited DPPH[•] with 59.30% at concentration 300 μ g/ml (fig. 4).

The antioxidant activities of the black pepper essential oil and extracts varies depending on the content of limonene, β -caryophyllene and sabinene. An essential oil and ethyl acetate extract from black pepper at a concentration 20 μ l/ml inhibited DPPH[•] approximately 90% and 80%, respectively [15]. This activity was stronger than that of the freon extract in this study, which inhibits DPPH[•] with 72.84% at the same tested concentration (fig. 3). The activity of the freon extract at 10 μ g/ml was similar to the one found by Kapoor et al., [15] for ethanol extract.

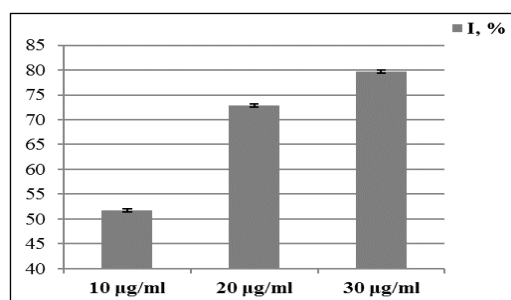


Fig. 3. Antioxidant activity of the freon extract against DPPH radical, %

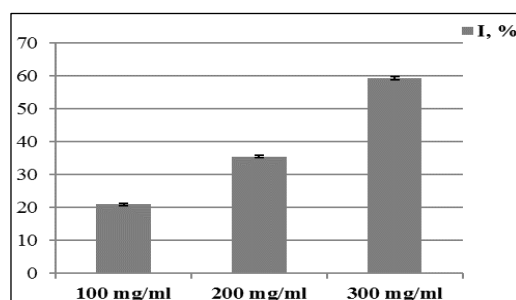


Figure 4. Antioxidant activity of the dry encapsulated extract against DPPH radical, %

Freon extract at a concentration of 30 µg/ml demonstrated stronger radical-scavenging activity (79.71%) compared to those found by Zarai et al., [17] for an ethanol extract, which inhibits DPPH[•] with 65.59% at a concentration 50 µg/ml.

Table 2: Antimicrobial activity of black pepper extracts against Gram-negative bacteria

Dry encapsulated extract from black pepper			Freon extract from black pepper		
Optical density (600 nm)					
Concentration	<i>Escherichia coli</i> ATCC 8739	<i>Salmonella enterica</i> ssp. <i>enterica</i> ATCC BAA-2162	Concentration	<i>Escherichia coli</i> ATCC 8739	<i>Salmonella enterica</i> ssp. <i>enterica</i> ATCC BAA-2162
0 h	0.325 ^a ± 0.012	0.334 ^a ± 0.015	0 h	0.325 ^a ± 0.012	0.334 ^a ± 0.015
24 h	2.513 ^g ± 0.175	0.892 ^f ± 0.020	24 h	2.513 ^f ± 0.175	0.892 ^f ± 0.020
3000 µg/ml	0.798 ^b ± 0.013	0.469 ^b ± 0.019	1250 µg/ml	0.814 ^b ± 0.059	0.333 ^a ± 0.059
1500 µg/ml	0.915 ^c ± 0.047	0.480 ^b ± 0.029	625 µg/ml	1.102 ^c ± 0.133	0.425 ^b ± 0.033
750 µg/ml	1.179 ^d ± 0.005	0.557 ^c ± 0.026	313 µg/ml	1.307 ^d ± 0.114	0.526 ^c ± 0.018
375 µg/ml	1.213 ^e ± 0.044	0.691 ^d ± 0.021	156 µg/ml	1.350 ^d ± 0.004	0.606 ^d ± 0.010
188 µg/ml	1.217 ^e ± 0.089	0.679 ^d ± 0.030	78 µg/ml	1.442 ^d ± 0.100	0.706 ^e ± 0.059
94 µg/ml	1.210 ^e ± 0.078	0.664 ^d ± 0.040	39 µg/ml	1.498 ^d ± 0.100	0.704 ^e ± 0.035
47 µg/ml	1.222 ^e ± 0.010	0.696 ^d ± 0.016	20 µg/ml	1.507 ^d ± 0.088	0.701 ^e ± 0.033
23 µg/ml	1.567 ^f ± 0.138	0.752 ^e ± 0.023	10 µg/ml	1.533 ^e ± 0.094	0.716 ^e ± 0.033

mean ±SD, ^{a,b,c,d,e,f,g} - index showing data with statistical different value in columns (p<0.05)

Freon extract at a concentration of 30 µg/ml demonstrated stronger radical-scavenging activity (79.71%) compared to those found by Zarai et al., [17] for an ethanol extract, which inhibits DPPH[•] with 65.59% at a concentration 50 µg/ml.

The differences in antioxidant activity of the tested spice extracts between this study and reference literature data could be attributed to the different origin of the spices and the different methods for obtaining extracts.

The antimicrobial activity of the black pepper extracts against Gram-negative bacteria: *Escherichia coli* ATCC 8739 and *Salmonella enterica* ssp. *enterica* ATCC BAA-2162 and Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* (clinical isolate) is presented in tables 2 and 3.

Both black pepper extracts inhibited the growth of Gram-negative bacteria *Escherichia coli* ATCC 8739 but in the tested concentrations complete inhibition was not found. These extracts exhibited stronger antimicrobial activity against *Salmonella enterica* ssp. *enterica* ATCC BAA-2162. The dry extract demonstrated inhibitory activity with MIC=3000 µg/ml against *Salmonella enterica* ssp. *enterica* ATCC BAA-2162 and for the freon extract MIC was at 625 µg/ml and complete inhibition with MBC at 1250 µg/ml determined (tab. 2). The extracts had a more pronounced antimicrobial activity against Gram (+) microorganisms: *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* (clinical isolate). Both extracts of black pepper exhibited activity against *S.*

aureus but MBC was not determined at the tested concentrations. The freon extract inhibited *L. monocytogenes* with MIC at 313 µg/ml. (tab. 3).

Table 3: Antimicrobial activity of black pepper extracts against Gram-positive bacteria

Dry encapsulated extract from black pepper			Freon extract from black pepper		
Optical density (600 nm)					
Concentration	<i>Staphylococcus aureus</i> ATCC 6538	Concentration	<i>Staphylococcus aureus</i> ATCC 6538	Concentration	<i>Staphylococcus aureus</i> ATCC 6538
Concentration	<i>Staphylococcus aureus</i> ATCC 6538	<i>Listeria monocytogenes</i> (clinical isolate)	Concentration	<i>Staphylococcus aureus</i> ATCC 6538	<i>Listeria monocytogenes</i> (clinical isolate)
0 h	0.297 ^a ± 0.014	0.302 ^a ± 0	0 h	0.297 ^a ± 0.014	0.302 ^a ± 0
24 h	2.475 ^g ± 0.122	0.793 ^f ± 0.005	24 h	2.475 ^f ± 0.122	0.793 ^h ± 0.005
3000 µg/ml	0.567 ^b ± 0.021	0.456 ^b ± 0.013	1250 µg/ml	0.391 ^b ± 0.026	0.341 ^b ± 0.023
1500 µg/ml	0.753 ^c ± 0.011	0.498 ^c ± 0.006	625 µg/ml	0.468 ^b ± 0.036	0.365 ^b ± 0.037
750 µg/ml	0.873 ^d ± 0.045	0.526 ^d ± 0.020	313 µg/ml	0.652 ^c ± 0.018	0.394 ^c ± 0.010
375 µg/ml	0.881 ^d ± 0.019	0.564 ^d ± 0.055	156 µg/ml	0.687 ^c ± 0.041	0.404 ^c ± 0.010
188 µg/ml	1.023 ^e ± 0.071	0.656 ^e ± 0.070	78 µg/ml	0.803 ^d ± 0.049	0.438 ^d ± 0.003
94 µg/ml	1.032 ^e ± 0.033	0.661 ^e ± 0.038	39 µg/ml	0.841 ^d ± 0.018	0.483 ^e ± 0.018
47 µg/ml	1.075 ^e ± 0.071	0.709 ^e ± 0.029	20 µg/ml	0.857 ^d ± 0.076	0.585 ^f ± 0.026
23 µg/ml	1.209 ^f ± 0.040	0.714 ^e ± 0.049	10 µg/ml	1.194 ^e ± 0.030	0.739 ^g ± 0.040

mean ±SD, ^{a,b,c,d,e,f,g,h} - index showing data with statistical different value in columns (p<0.05)

The higher resistance of Gram (-) bacteria could be explained by the presence of external lipopolysaccharide wall, which hindered the diffusion of the extracts to the cells protoplasm [18]. This fact explains the lower antimicrobial activity of tested extracts against Gram (-) pathogens. The high potential for antimicrobial activity is closely associated with the presence of compounds with antimicrobial action in the spices. The chemical analysis of extracts showed dominating presence of monoterpene hydrocarbons and their derivatives (tab.1). The antimicrobial activity of black pepper was related to the content of monoterpene hydrocarbons: sabinene, β - pinene, limonene, monoterpene derivatives: borneol, 1,8 - cineol and linalool, and sesquiterpene hydrocarbons - mainly β - caryophyllene [19].

The mechanism of terpenes action is not fully clarified but it is supposed that lipophilic compounds were involved in the destruction of the microorganism cell membrane [20]. According to Oka [21] the adsorption of active ingredients on the cell wall of microorganisms is depended on their concentration.

Some researchers suggest that terpenes such as thymol, carvacrol, linalool, eugenol, α-pinene and β-pinene exhibit antimicrobial activity against widespread pathogens [22, 23, 24]. The cyclic terpenes caused destruction of the cell membrane and scattering of proton driving force [25]. The activity of spices was explained by the disbalance of various enzyme systems, which complicated the production of energy or the synthesis of structural components in the microbial cells [26].

According to Ram Kumar and Pranay, and Ali et al., [27,28] water, ethanol and methanol black pepper extracts exhibited antimicrobial activity against *S. aureus* and *E. coli*.

In our study dry encapsulated extract of black pepper demonstrated stronger antimicrobial activity against *E.coli* and *Salmonella* compared to those found by Zarai et al., [17] for an ethanol extract. The freon extract activity against *Salmonella* at a concentration 625 µg/ml was similar to that according to Zarai et al., [17]. It was found that the dry encapsulated extract and the freon extract from black pepper exhibited stronger antimicrobial activity against *S. aureus* and *E.coli*, in comparison with that found by Ertürk [29] for 95% ethanol extract.

CONCLUSION. From the present study may be concluded that both extracts can be used as a potential source of natural antimicrobial and antioxidant compounds and can applied to food products.

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REFERENCES

1. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society*, 1998; 75: 199-212.
2. Wanasundara UN, Shahidi F. Antioxidant and prooxidant activity of green tea extract in marine oils. *Food Chemistry*, 1998; 63: 335-42.
3. Zainol MK, Hamid A, Yousuf S, Muse R. Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) urban. *Food Chemistry*, 2003; 81: 575-81.
4. Neilsen PV, Rios R. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology*, 2000; 60: 219-29.
5. Shobana S, Naidu KA. Antioxidant activity of selected Indian spices. *Prostaglandins Leukotenes and Essential Fatty Acids*, 2000; 62: 107-10.
6. Salie F, Eagles PFK, Leng HMJ. Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of Ethnopharmacology*, 1996; 52: 27-33.
7. Murthy CT and Bhattacharya S. Cryogenic grinding of black pepper. *Journal of Food Engineering*, 2008; 85: 18-28.
8. Peter KV. *Handbook of herbs and spices*. 1st ed., Woodhead Publishing Ltd., Cambridge, England: 2001, pp. 319.
9. Gopalakrishnan M, Menon N, Padmakumari, KP, Jayalekshimi A, Narayanan CS. GC analysis and odour profiles of four new Indian genotypes of *Piper nigrum* L. *Journal of Essential Oil Res.*, 1993; 5: 247-53.
10. Nenov N. Low temperature extraction of essential oil bearing plants by liqueficate gases. 1. Laboratory installation. *Sci. Works of the University of Food Technologies*. 2006; 53(2): 195-200.
11. Oliveira WP, Souza CRF, Kurozawa LE, Park KL. Spray drying of food and herbal products. 1st ed., Woo MW, Mujumdar AS and Daud WRW. *Spray draying Technology*: 2010, pp. 113-56.
12. Djurkov T. Investigate the opportunities of obtaining powdered honey. *Sci. Works of the University of Ruse*, 2012; 51(9.2): 105-8.
13. Brand-Williams W, Cuvelier M and Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und –Technologie*, 1995; 28: 25-30.
14. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically – Ninth Edition: Approved Standard M7-A9*. NCCLS, Wayne, PA, USA, 2012.
15. Kapoor IPS, Singh B, Singh G, De Heluani CS, De Lampasona MP, Catalan C. Chemistry and in vitro antioxidant activity of volatile oil and oleoresins of black pepper (*Piper nigrum*). *Journal of Agricultural and Food Chemistry*, 2009; 57: 5358-64.
16. Stoyanova A, Denkova Z, Nenov N, Slavchev A, Jirovetz L, Buchbauer G, Lien HN, Schmidt E, Geissler M. C₂H₂F₄-SCFE-Oleoresins of black pepper (*Piper nigrum* L.) and ginger (*Zingiber officinale* (L.) Rosc.) from Vietnam: antimicrobial testings, gas chromatographic analysis and olfactive evaluation. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 2006; 5 (5): 1615-23.
17. Zarai Z, Boujelbene E, Salem NB, Gargouri Y, Sayari A. Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. *LWT - Food Science and Technology*, 2013; 50: 634-41.
18. Russel M. Filamentous phage assembly. *Molecular Microbiology*, 1991; 5 (7): 1607-13.
19. Harold M. *Black pepper and relatives*. On food cooking revised edition, 2004; pp. 427-9.
20. Ahmed V, Baqai FT and Ahmed R. A tigenin pentasaccharide from *Cestrum diurnum*. *Phytochemistry*, 1993; 34: 511-5.

21. Oka S. Mechanism of Antimicrobial effect of various food preservatives. In: Molin, N., Ed. *Microbial Inhibitors in Food*, The Fourth International Symposium of Food Microbiology. Stockholm, Sweden, Almqvist & Wiksell, 1964; pp. 3-16.
22. Hirasa K and Takemasa M. *Spice science and technology*. New York, Marcel Dekker Inc., 1998.
23. Kim J, Marshall MR and Wei C. Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 1995; 43 (11): 2839-45.
24. Juven BJ, Kanner J, Schved F and Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of Applied Bacteriology*, 1994; 76: 626-31.
25. Sikkema J, de Bont JAM and Poolman B. Mechanism of membrane toxicity of hydrocarbons. *Microbiological Reviews*, 1995; 59(2): 201-22.
26. Wilkins KM and Board RG. *Natural Antimicrobial Systems*. In: Gould, G.W., Ed. *Mechanisms of Action of Food Preservation Procedures*. London, Elsevier, 1989; pp. 285.
27. Ram Kumar P and Pranay J. Comparative studies on the antimicrobial activity of black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) extracts. *International Journal of Applied Biology and Pharmaceutical Technology*, 2010; 1(2): 491-501.
28. Ali MA, Alam NM, Yeasmin MS, Khan AMM and Sayeed A. Antimicrobial screening of different extracts of *Piper longum* Linn. *Research Journal of Agriculture and Biological Sciences*, 2007; 3: 852-57.
29. Ertürk Ö. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. *International journal of Biologia*, Bratislava, 2006; 61(3): 275-8.