International Journal of Advanced Technology in Engineering and Science www.ijates.com
Volume No.02, Issue No. 10, October 2014 ISSN (online): 2348 – 7550

EFFECT OF STORAGE ON VOLATILE OIL OF THE COMMERCIAL BLACK AND WHITE PEPPER

S.K. Shiva Rani[1], Neeti Saxena[2]

[1, 2] *Department of Botany, Mycology and Plant Pathology Lab,*
Osmania University College for Women, Koti, Hyderabad, Andhra Pradesh, (India)

ABSTRACT

Black pepper (Piper nigrum L.) is the native of south India popularly known as "king of spices". Pepper is mostly used in the curry recipes as masalas and also as an ingredient in the prescriptions of folk medicine, Modern and traditional medicinal systems. The chemical constituents like essential oils and piperamides are responsible for the pharmacological properties. In the present study black and white pepper samples were collected from both Post harvest conditions and storage conditions. Post harvest seeds were collected from tribal farmers of Paderu and Peddapally villages of Vishakhapatnam and stored seeds were collected from wholesaler and retailers of Vishakhapatnam. Essential oils are extracted from the above samples by hydrodistallation method by using Clevenger-type apparatus The Pepper essential oils were obtained from the black and white pepper samples with 2.6%, 1.8%, 1.4%, 1.5%, 0.8% and 0.4%.The amount of essential oil isolated from Post harvest seed samples in higher quantity compared to storage samples (wholesaler and retailers) of both black and white pepper samples. Chemical constituents of the essential oils of the samples were determined by using the gas chromatography-mass spectrometry method. Experimental results revealed that there was a qualitively and quantitative differences in chemical composition of the essential oils of the samples. The main and common constituents among all the samples wereα-Pinene, Cyclohexene, 1-methyl-4-(1-methylidene), 3-Cyclohexen-1-ol, 4-methyl-(methylethyisocaryophyllene; Bicyclo (3.1.0) hexane, 2-methyl-5-(1-methylethyl), α-Terpinene, α-Phellandrene, α-Salinene, α-fellandrene, Trans-caryophyllene. Components like β-caryophyllene, Limonene, Trans-caryophyllene components are in higher quantity followed by Caryophyllene oxide,Copane, Sabienene and 1,4- Cyclohexadiene 1-methyl-4-(1-methylethyl)- . Monoterpene hydrocarbons like Mycene α-Terpinolene, Limonene, α-Phellandrene, and Sabienene are in higher amount in Post harvest seed samples compared to wholesaler and retailer samples. Caryophyllene oxide is found to be in higher amount in stored samples of both black and white pepper storage samples compared to Post harvest samples.

Keywords: ***Black Pepper, White Pepper, Essential Oils and Gas Chromatogram-Mass Spectrometry.***

I. INTRODUCTION

Black pepper (*Piper nigrum L*) commonly known as kali mirch is a flowering vine of the Piperaceae family that is cultivate for its fruit, which is usually dried and used as a spice and seasoning. In dried from the fruit is referred to as peppercorns. It is a native of South India and popularly known as "King of spices. Peppercorns are described as black pepper (unripe green berries), white pepper (fully ripe red berries where the skin is removed), green pepper and red pepper depending upon time of collection. Among the varieties black pepper and white pepper has wide utility and export potential. Where as red and green peppercorns has marginal commercial value. Pepper is most commonly used in curry recipes, as masalas and also included in the prescriptions of

Ayurveda, Siddha, Unani medicines and also used as a folk medicine. In Ayurvedic formulations like Vatis-Gutika (pills),Rasa yoga (mineral based herbal formulations) Tailas (oil based herbal formulations)etc[1]. Pepper is also used as aphrodisiac, carminative, stomachic, antiseptic, diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neropathy,melanoderma and leprosy due to the presence of volatile compounds and alkaloids. [2,3,3,4&5]. Inhalation of black pepper oil increase the reflexive swallowing movement[6].Essential oils and piperamides are considered as major chemical constituents of pepper. The amount of essential oils present in black peppercorns ranges from 2-3.1% and in white pepper corns 0.95%-1.5%. Essential oils are odoriferous and volatile substances which are present in the aromatic plants. The chemical natures of the aromatic substances mainly depend upon the variety of chemical substances. Essential oils are comprised of combination of alcohols, phenols, aldehydes, ketones, acids ,esters, oxides and lactones which occurs in root, wood, bark, foliage, flower and fruit of the plants and found application as flavour, fragrances and medicinal aroma. Today, the essential oils are sought after for innumerable applications starting from markers for plant identifications and base for semi synthesis of highly complex molecules. The aromatic odor of Black pepper is due to the presence of essential oils in the pericarp of the fruit. Pepper essential oil is dominated by cyclic Terpenes and Sesquiterpenes but oxygenated Terpenes are relatively in minor constituents and they contribute to the characteristic odor of pepper oil [7].Due to its compounds like Trans-Caryophyllene, Limonen-6-ol, pivalate and (E)-3(10)-Caren-4-ol, pepper is considered as more effective antioxidant than many fruits and vegetables[8].Pepper is used for curing and preservation of meat, pork and beef as pepper retards the development of rancidity in oils and fats due to the presence of α-tocopherols in essential oils[9].There is a huge demand for pepper essential oils in food processing and pharmaceutical industries. The current work is an attempt to investigate the difference in chemical composition of the essential oils of the pepper varieties collected from post harvest and from storage conditions (wholesaler and retailers).

II. MATERIALS AND METHODS

The seeds of both varieties Black and white peppercorn post harvest seed samples were collected from tribal farmers of Paderu & Peddapally villages of Vishakhapatnam district and storage samples from wholesaler & retailer of Vishakhapatnam district of Andhra Pradesh.

2.1 Extraction of Essential Oil

The sample was submitted to hydrodistallation for 4 hours, using a Clevenger- type apparatus, according to the European Pharmacopoeia (European Pharmacopoeia).A 50g of powered sample along with 500ml of distilled water was taken in a round bottom flask. The sample was heated to 60^0c for 4 hours on a heating mantle. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated until time of analysis. The pepper was extracted until no more essential oil was obtained. The steam-distilled essential oil of *Piper nigrum* L. is a strong-smelling spicy essential oil and can range in colour from greenish-yellow to brown and the oil is thin in consistency.

2.2 Quantities Analysis of Essential Oils from the Samples by Using Gas Chromatogram-Mass Spectrometry

Samples of essential oils were diluted in methyl tertbutylether (MTBE) (1:100) and analyzed in a trace GC/MS apparatus (Thermo Finnegan, Manchester, America) equipped with an SLB-5MS fused-silica capillary column (‘Resets’) (30-mx0.25-mm internal diameter, 0.25-um film thickness).Helium (1.0 ml/min) was used as a carrier

gas. Samples were injected in the split mode at a ratio of 1:40.The injector was kept at 250^{0}C and the transfer line at 280^{0}C. The column was maintained at 50^{0}C for 2 min and then programmed to 260^{0}C at 5^{0}C for 2 min and then programmed to 260^{0}C at 5^{0}C per min and held for 5 min at 260^{0}C.The MS was operated in the EI mode at 70 Ev, in the m/z range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in the literature (Liang Renjie *et al.*, 2010) and supplemented by the Wiley &QuadLib 1607 gas chromatography-mass spectrometry (GC-MS) libraries. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one. The Kovat indices were determined from the retention times after co injection with n-alkanes.

Total Ion Spectrum Of Essential Oil Of Black And White Pepper Collected From Post Harvest And Storage Conditions With GC-MS

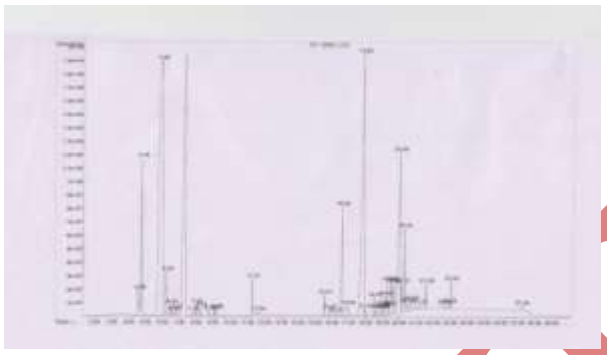

Fig.1

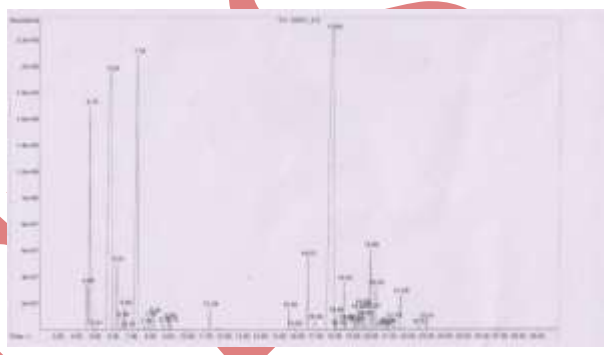

Fig.2

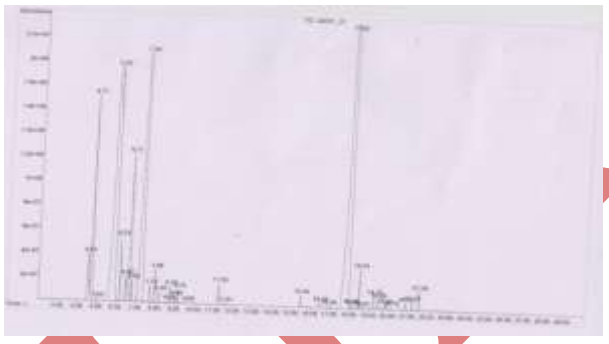

Fig.4

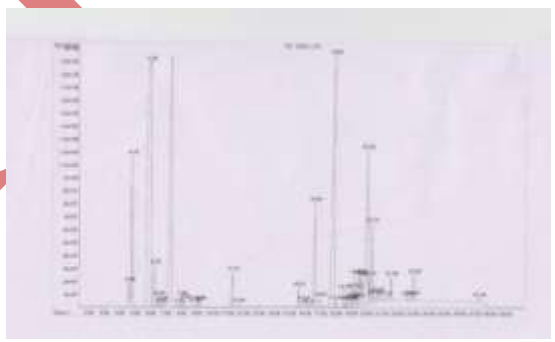

Fig.3

Fig.5

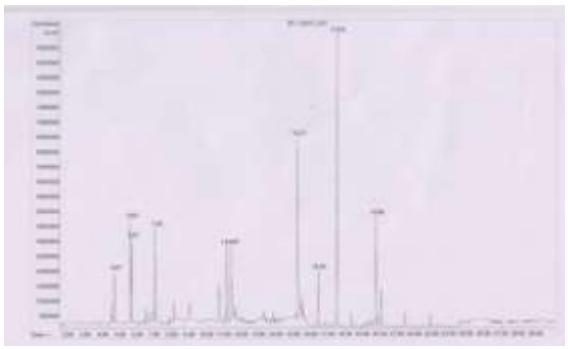

Fig.6

Fig.1 (post harvest black pepper), Fig.2&3(wholesaler &retailer black pepper)

Fig.4 (post harvest white pepper), Fig.5&6(wholesaler &retailer white pepper)

Table 1: CHEMICAL COMPOSITION OF ESSENTIAL OF BLACK AND WHITE PEPPERS

	Compounds	Retention time (min)						(%)					
s. no		Black pepper(p.h)	Black pepper(wsl)	Black pepper(rtl)	White pepper(p.h)	White pepper(wsl)	White pepper(rtl)	Black pepper(p.h)	Black pepper(wsl)	Black pepper(rtl)	White pepper(p.h)	White pepper(wsl)	White pepper(rtl)
1.	α-Pinene	4.56	4.56	3.07	4.55	4.55	4.67	6.31	5.31	3.04	6.05	2.05	0.13
1	Bicyclo(3.1.0)hex-2-ene,2-methyl-5-(1-methylethyl)-	4.77	4.76	3.36	4.76	4.79	5.61	6.6	6.6	0.05	0.03	0.03	0.04
2	Bicyclo(3.1.1)hept-2-ene,2,6,6-trimethyl-	5.01	5.01	4.58	5.21	5.02	5.66	0.04	0.12	0.09	0.11	0.08	0.02
3	Bicyclo(2.2.1)heptane,2,2-dimethyl-3-methylene-	5.92	5.89	4.78	5.90	5.95	7.01	0.03	0.27	0.07	0.06	0.08	0.09
4	Bicyclo(3.1.0)hexane,4-methylene-1-(1-methylethyl)-	6.23	6.20	5.04	6.56	6.24	7.23	0.04	1.09	0.09	0.09	0.11	0.10
5	1,6-octadiene,7-methyl-3-methylene-	6.50	--	--	6.78	6.50	7.88	0.08	---	--	0.17	0.74	0.68
6	1,3cyclohexadiene,2-methyl-5-(1-methylethyl)-	6.71	6.61	6.24	6.79	6.69	7.90	1.43	0.32	1.65	2.76	1.12	1.53
7	1,3cyclohexadiene,1-methyl-4-(1-methylethyl)-	6.82	6.78	6.50	6.88	6.82	8.09	1.43	0.05	2.76	1.12	1.33	1.78
8	1,3,6-octatriene,3,7-dimethyl-	7.34	7.70	6.62	7.56	7.43	8.18	0.08	0.17	0.98	0.74	0.88	0.06
9	1,4-cyclohexadiene,1-methyl-4-(1-methylethyl)-	7.46	7.94	6.82	7.74	7.74	8.30	9.23	9.02	8.56	10.05	7.89	8.88
10	Cyclohexene,1-methyl-4-(1-methylethylidene)-	7.46	8.20	7.74	8.06	8.02	8.67	0.51	0.54	0.34	0.65	0.87	0.66
11	1,6-octadien-3-ol,3,-dimethyl-	7.73	8.72	7.98	8.54	----	---	0.08	0.32	0.56	0.45	---	---
12	3-cyclohexen-1-ol,4-methyl-1-(methylethyl)-	7.98	9.03	8.24	8.91	8.76	----	1.02	1.18	1.12	2.23	2.79	----
13	copaene	8.67	9.12	8.76	9.09	9.06	9.88	11.01	10.03	9.89	8.09	7.77	7.56
14	β-caryopyllene	8.75	11.23	9.07	9.23	9.13	10.73	29.91	13.40	19.09	18.90	25.56	16.78
15	γ-elemene	9.03	11.59	9.19	10.32	10.20	-	0.44	0.58	0.34	0.11	0.05	-
16	α-guaiene azulene,1,2,3,4,5,6,7,8-octahydro-4-dimethyl-7-(1-methylethenyl)-	9.17	15.55	9.68	10.66	11.37	-	0.12	0.05	0.13	0.33	0.13	-
17	β-selinene	9.66	15.82	11.31	11.31	---	-	0.89	1.16	1.12	1.45	---	1.12
18	β-bisabolene	11.30	16.93	11.64	13.07	---	-	1.23	1.12	0.34	0.65	---	-
19	Delta-cadinene	11.60	---	---	15.55	15.54	-	0.16	---	---	0.11	0.17	-
20	Germacrene 1,5-cyclodecadiene	15.51	18.04	15.86	15.84	15.83	11.17	0.40	0.40	0.34	0.78	0.66	0.31
21	Bicyclo(3.1.0)hexan2-ol,2methyl-5-(1-methylethyl)-	16.47	18.13	16.21	16.01	16.11	11.43	0.63	0.63	0.62	0.16	0.91	0.42
22	limonene	10.12	10.65	10.66	10.14	10.14	10.42	25.04	21.04	21.06	20.79	18.07	13.05
23	Cyclohexene,4-ethyl-4-methyl-3(1-methylethyl-3-trans)	17.93	18.63	16.92	16.77	16.94	--	0.06	0.06	0.14	0.56	0.33	---
24	α-caryophyllene	18.05	19.06	17.39	17.31	17.29	15.27	12.75	12.75	12.75	12.67	11.75	11.34
25	Sabienene	18.15	19.13	17.89	17.89	17.97	16.44	3.54	4.52	1.43	3.67	2.55	1.78
26	1,6,10-Dodocatrien-3-ol,3,7,11-trimethyl	18.53	19.27	17.97	18,02	18.97	--	0.98	0.97	0.56	0.77	0.19	---

27	Caryophyllene oxide	18.62	19.49	18.52	18.52	19.06	22.07	1.06	2.02	6.07	1.55	3.81	6.28
28	β-myrcene	19.12	---	---	19.14	---	---	0.04	---	---	0.79	----	---
29	α-terpinolene	19.27	19.71	18.93	19.23	---	---	0.13	0.13	0.09	0.23	---	---
30	α-copaenetri cyclo(4,4,0,02,7)dec-3-ene.	19.49	---	---	20.13	---	----	0.87	---	--	0.13	---	---
31	Eudesma-4(14),11-diene	19.70	20.07	19.07	20.22	---	---	0.02	0.24	0.89	0.81	---	---
32	α-fellandrene	19.84	20.23	19.40	20.43	20.35	17.45	0.04	0.04	0.15	0.15	0.95	0.08
33	α-phellandrene	19.97	20.39	20.45	20.56	20.45	19.89	1.13	1.13	0.19	0.24	0.17	1.08
34	β-trans-ocimene	20.90	20.64	20.06	20.76	20.67	---	0.03	0.03	0.24	0.32	0.89	---
35	Camphene.	21.22	20.81	---	20.80	---	----	0.13	0.08	---	0.77	---	---
36	α-cubebene	21.58	---	---	20.86	20.85	18.34	0.03	---	----	0.05	0.01	0.17
37	α-copaene	22.58	21.22	---	21.09	21.21	---	0.02	0.02	---	0.02	0.07	---
38	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-3-(1-methylethylidene)-	21.31	21.31	21.19	21.34	21.59	18.81	0.02	0.02	0.09	0.09	0.09	0.47
39.	Trans-Caryophyllene	26.67	26.87	27.05	25.39	28.56	28.80	32.9	34.8	39.81	35.37	32.1	39.81
40.	α-Copaene-8-ol	27.07	28.09	27.24	26,77	22.64	---	2.09	2.7	1.09	1.65	1.99	---

P.h-Post harvest seed sample; wsl-wholesaler sample; rtl-retalier sample

III. RESULTS AND DISCUSSION

The Pepper essential oils were obtained from the black and white pepper samples with 2.6%, 1.8%, 1.4%, 1.5%, 0.8% and 0.4%.The amount of essential oil isolated from post harvest seed samples in higher quantity compared to storage samples (wholesaler and retailers) of both black and white pepper and the same was observed by the[9] that essential oil isolated from pepper samples stored in glass vessels for one year at temperature is decreased..According to the [10] stated that color and content of essential oils change during storage and these changes are linked to the temperature and moisture content.

By using the Gas chromatogram and mass spectroscopy chemical composition of essential oils were studied. The GC-MS chromatograms for hydrodistallation extraction of six peppers are illustrated in Figs1-6, respectively.Total forty compounds were indentified from black and white pepper through GC-MS studies. According to the [11] α-Pinene,Cyclohexene,1-methyl-4-(1-methylethylidene)-3-cyclohexene-ol,4-methyl-1-(1-methylethyl)-,β-Pinene,Limenen-6-ol pivalate(E)-3-(10)-caren -4-ol and Transcaryophyllene were in higher amount in the genus peppers.

In this study, the identified components from pepper essential oils, their retention indices, their percentages composition, and the concentration of the components were summarized in table1.α-Pinene,Bicyclo(3.1.0)hex-2-ene,2-methyl-5-(1-methylethyl),Bicyclo(3.1.1)hept-2-ene,2,6,6trimethyl-,Bicyclo(2,2,1)heptane,2.2-dimethyl-3-methylene,Bicyclo(3.1.0)hexane,4-methylene-1methylethyl-,Copaene,1,3cyclohexadiene,2-methyl-5-(1-methylethyl)-,1,3,6-octatriiene,3,7-dimethyl-3-methylene-)-,Cyclohexene,1-methyl-4-(1-methylethlidene)-,3-Cyclohexene-1-methyl-4(1-methylethyl)-,1,3Cyclohexadiene,1-methyl-4-(1-methylethyl)-,β-Pinene,Trans-caryophyllene1,6-octadiene, 7-methyl-3-methylene, Caryopyllene, α-Fellandrene, α-Phellandrene, α-Selinene were common components detected in the oils of all varieties. According to the literature Transcaryophyllene and Limone 6-ol pivalate were found to be the main components in the pepper essential oils [12]. β-

caryophyllene, Limonene, Trans-caryophyllene components are in higher quantity followed by Caryophyllene oxide,Copane, Sabienene and 1,4- Cyclohexadiene 1-methyl-4-(1-methylethyl)-.Due to the presence of triterpenes, alcohols, ketones acids monoterpenes and sesquiterpenes essential oils act against micro organisms by causing instability of the plasma membrane leading to rupture of cells[13].

Components like1,6-Octadiene,7-methyl-3-methylene-,a-Cubebene were not traced in the essential oils of storage samples of black pepper(wholesaler and retailers).1,6-Octadiene-3-ol,3-dimethyl-,β-Selinene,β-bisabolene,Germacrene1,5-Cyclodecadiene, a-Terpindene,Eudesma-4 (14)11-diene components were not found in the oils of storage samples of white pepper(wholesaler and retailers).γ-Elemene, α-Guaiene azulene,1,2,3,4,5,6,7,8-octahydro-4-methyl-7-(1-methyletheyl)-,Cyclohexene4-ethyl-4-methyl-3-(1-methylethyl-3-trans)1-6-10,Dodocatriene 3-ol 3,7,11-trimethyl, β-Trans-ocimene, α-Copane-8-ol components were not detected in the white pepper retailers samples.

Beta-Myracene and a-Copane tricycle (4, 0, 0, 0, 2, 7) dec-3-ene were not traced in both black and white pepper storage samples (wholesaler and retailers). α-Copane is not detected in the essential oils of both black and white pepper retailers samples. Monoterpene hydrocarbons like Myracene α-Terpinolene, Limonene, α-Phellandrene, and Sabienene are in higher amount in post harvest samples compared to wholesaler and retailer samples as it is also supporting the findings of Packiyosothy etal., 1983 that there is a loss of monoterpene hydrocarbons in essential oil content in stored black pepper which stored for six months in tinned cans. Caryophyllene oxide is found to be in higher amount in stored samples of both black and white pepper storage samples compared to post harvest seed samples is due to the oxidation of β-Caryophyllene during the storage. As Caryophyllene oxide is less volatile so it is retained during the storage period to some extent where as other hydrocarbons will volatilized [14].

IV. CONCLUSION

The differences in the chemical composition of the essential oils of post harvest and storage samples may depend on the time of harvest, age of the seed, processing process, prolonged storage and poor storage conditions. The loss of essential oil is parallel with the changes of the total amount of each compound, there is a difference in the change of the relative amounts of the compounds is due to their sensitivity towards temperature. Even ageing of seeds is indirectly proportional to the seed moisture and the high moisture content favours more changes to occur that is the reason for strong quality changes occur in the initial phase of storage. Essential oils do not become rancid, but they can oxidize, deteriorate and lose their beneficial therapeutic properties over time. The present discovery of the difference in chemical composition of pepper essential oils may be beneficial for the pharmaceutical industry and meat processing industries in selection of variety.

REFRENCES

Journal papers

[1] Sunita, S., S.N. Menon and A. Singh, 2010. Quantitative Analysis of Piperine form Ayurvedic Polyherbal formulations using Reverse Phase High Performance liquid chromatography. *Indian J. Pharma. Bio. Sci*., V1 (1):1-10.

[2] Chiranjib, B, V.S. Narayn, P.S. Variyar and C. Bandyopadhya, 1990. Phenolics of green pepper berries (*Piper nigrum*). *J. Agri. Food. Chem*., 38: 8-12.

[3] Algohary, M.E.M., B.M. Mahmoud, H.M. Ali and M.M. Homeida, 1994. Medicinal plants of North Africa, pp: 142-144.

Thesis

[4] Ali, W.E.M.M. 1995. Toxicological study on medicinal plants: piper abyssinica and *Indigofera oblongifolia*. Ph.D Thesis, University of Khartoum, Sudan.

Journal papers

[5] Park, J.E., H.J. Choi, S.H. Jung, N.J. Kim and D.H. Kim, 2004. East-West medicinal plants of Korea. *J. Pharmacognosy*., 32: 257-268

[6] Vijayakumar R.S.., Surya D., Nalini N. Antioxidant efficiency of black pepper and piperine in rats with high fat diet induced oxidative stress. *Redox Rep*. 2004; 9(2): 105-110.

[7] Andric.Kumoro, Maritah and Harcharan Singh, Extraction of Sarawak Black pepper essential oils using supercritical carbon dioxide; *The Arabian Journal of Science and Engineering*, Volume 35, Number 2B October 2010.

Book

[8] "The wealth of India" A dictionary of Indian raw materials and industrial products Vol. VIII: Ph-Re.

Journal papers

[9] Orav A,Stulova I, Kailas T, Muurisepp M. Effect of storage on the essential oil composition of *Piper nigrum L.* fruits of different ripening states. *J Agric Food Chem.* 5; 52(9) : 2582-6; may 2004.

[10] Arabhosseini.A, Huisman.W, Van Boxtel, J.Muller , Long –term effects of changing conditions on the essential oil and colour of tarragon leaves during storage. *Journal of food Engineering* 79(2007) 561-566.

[11] Barbero GF, Liazid A, Palma M, Barroso CG ;Fast determination of capsaicinoids from peppers by high-performance liquid chromatography using a reversed phase monolithic column. *Food Chem* 107: 1276-1282(2008).

[12] Liang Renjie. Shi Shidi. Ma Yongjun; Analysis of volatile oil composition of the peppers from different production areas *J.Med Chem Res* 19:157-165; 2010.

[13] Burt,Essential oils their antibacterial properties and potential applications in food –a review. *Int J Food Microbiol* 94:223-253 ;2004

[14] Packiyosothy E.V, S.Balachandran and E.R.Jansz, Effect of storage in small packages on volatile oil and piperine content of ground pepper, *J. Natn. Sci. Coon.* Srilanka 11(1): 111-122; 1983.