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Comparative Study of the Quality Factors of Two Different Cultivars of Pepper (*Piper nigrum* L.)-Karimunda and Panniyur-I

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Pepper is known for centuries for its taste and flavour and aptly called the king of spices. There are hundred cultivars of black pepper cultivated in various parts of Kerala. In the present study, we compare the quality factors of two different cultivars of *Piper nigrum* L.-Karimunda and Panniyur-I. The moisture and volatile oil content of both fresh and dry pepper samples and refractive index of the volatile oils was determined. Marked differences in chemical composition of volatile oils from fresh and dry pepper was noticed. The percentage of monoterpenes and sesquiterpenes varied in the two cultivars. The fresh peppery note of the Karimunda can be correlated with the higher content of volatile constituents. Knowledge of the variation in the composition of pepper oils from different cultivars is essential when pepper is used as a component of food flavourings.

Key Words: Piperine, Karimunda, Panniyur-I, *Piper nigrum* L.

INTRODUCTION

The genus piper belongs to the family piperaceae, comprising more than 700 species distributed throughout the tropical and subtropical regions of the world, the most important economic spice being pepper-*Piper nigrum*. Some of the most popular cultivars of pepper are Karimunda, Aimpiriyan, Neelamundi, Narayakodi, Arakulamunda, Kalluvally, Malligesara and Uddakarae. These cultivars exhibit great variability with regard to various morphological, agronomic characters and yield. Research efforts in the last three decades have resulted in identification of high yielding varieties of black pepper which when popularized will contribute substantially to increase the production and productivity of this spice. Out of these varieties Panniyur-I, Panniyur-II, Panniyur-III, Panniyur-IV, were developed by Kerala Agricultural University while the rest of Sreekara, Suchakara, Panchami and Pournami were developed by the National Research Centre for Spice.

The tropical plant Piperaceae has provided many past and present civilizations with a source of diverse medicines and food grade spice. Piper extracts offer unique and useful source of biopesticide material for controlling small-scale insect outbreaks and reducing the likelihood of resistance development when applied as a synergist with other botanical insecticides such as pyrethrum[1]. The chemistry of piper species has been widely investigated and the phytochemical investigations from all parts of the world have led to the isolation of a number of physiologically active compounds *viz.*, alkaloids/amides, prophenyl phenols, lignans, neolignans, terpenes, steroids, piperolides, chalcones, dihydrochalcones, flavones and flavannones. According to Kato and Furlan[2], the chemistry of members of the family piperaceae is of great interest owing to the variety of biological properties displayed. A survey of structural diversity and bioactivity reveals that groups of species specialize in the production of amides, phenyl propanoids, lignans, neolignans, benzoic acid and chromenes, alkaloids, polyketides and a plethora of compounds of mixed biosynthetic origin.

Parmar *et al.*[3] reported 38 compounds of different types from 12 *Piper* species. Parmar *et al.*[4] also reviewed the secondary metabolites isolated from Piper species and nearly 600 chemical constituents belonging to different classes of bioactive compounds are listed. Boll *et al.*[5] have reported the isolation of long-chain amides, aristolactams, cepharadiones and wax esters and especially a large number of lignans and neolignans. In the present study we compare the quality factors of two different cultivars of *Piper nigrum* L.- Karimunda and Panniyur-I.

EXPERIMENTAL

Two cultivars of pepper (*Piper nigrum* L.) namely Karimunda and Panniyur-I were procured from a garden at Nilambur, Kerala. The berries were dried in a cross-flow drier at 50 ºC. Moisture of the samples was determined by Dean and Stark method and volatile oils were extracted by Clevenger distillation method[6]. Refractive index was measured using Abbe refractometer.

General procedure

Dean and stark method: A known weight of the sample placed in a flask with an organic solvent (xylene). The flask containing the sample and xylene was attached to a condenser by a side arm and the mixture heated. The water in the sample evaporates and moves up into the condenser where it is cooled and converted back into liquid water, which then trickles into the graduated tube. When no more water is collected in the graduated tube, distillation stopped and the volume of water read from the tube.

Analysis of oils by gas liquid chromatography (GLC): Hewlett Packard 5890 series II provided with electronic integrator with a fused silica capillary column, 30 m length, 0.53 mm internal diameter and film thickness 0.15 m was used for the GLC analysis of the oils. GLC conditions were: nitrogen as carrier gas (1 mL/min), split ratio 1/50, injection temperature 250 ºC, flame ionisation detector (FID) temperature 300 ºC and temperature programmed from 80-200 ºC at the rate of 5 ºC/min. Quantitative data was obtained from FID area percentage without the use of correction factors.

Detection method: Constituents of the oils were identified by comparing the retention time of the references. GC-MS analysis was carried out in Shimadzu GC-MS QP 5050A and components identified by comparing the mass spectra of components by NIIST Library as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature[7].

Thin layer chromatography (TLC) for separation of piperine: Thin layer chromatographic (TLC) method was used to separate piperine from the extracts of pepper[8]. A small quantity of the pepper extracts dissolved in methanol and spotted on a TLC plate. A small quantity of authentic sample of piperine was also spotted, the plate developed with hexane, ethyl acetate (80:20) system. After drying the plate was sprayed with 10 % methanolic H_2SO_4 and heated in an oven at 110 ºC. Piperine separated out from the extracts and R_f value was found to be 0.28. The odor profile was also noted.

RESULTS AND DISCUSSION

The analytical data are presented in Tables. Table-1 shows the moisture and volatile oil content of both fresh and dry pepper samples and refractive index of the volatile oils. Fresh pepper yielded a colourless pleasant, aromatic oil with a fresh pepper note. Fresh Karimunda oil gives 3.89 % oil and Panniyur-I gave 3.67 % dry Karimunda pepper contains 3.33 % oil and Panniyur-I 3.35 % with pleasant aromatic peppery smell. The slight loss in the volatile content may due to the loss of low volatile compounds on drying. Refractive indices of both the fresh and dry pepper oils of the two cultivars show that the fresh oils have got a slight lower value than dry oils. This may be due to the compositional variation of the oil.

TABLE-1
ANALYSIS OF MOISTURE AND VOLATILE OIL

S. No.	Sample	Moisture % (v/w)	Oil (%)	Refractive index
1	Karimunda (fresh)	64.0	3.89	1.4750
2	Karimunda (dry)	13.0	3.33	1.4830
3	Panniyur-I (fresh)	70.0	3.67	1.4780
4	Panniyur-I (dry)	10.5	3.35	1.4875

Table-2 shows chemical composition of volatile oils from green and black pepper obtained from Karimunda by capillary GC and GC-MS. Twenty seven compounds were identified in the oils. The volatile oil of green pepper constituted about 6.53 % monoterpenes and 37 % sesquiterpenes and other polar sesquiterpenes and other polar compounds constituted about 55 %. The major monoterpene identified in green pepper oil is Δ carene + *o*-cimene which constitutes about 18.7 %, where as in dry pepper oil it is only 10.8 %. The second major monoterpene is β pinene + myrcene (15.4 %) in green pepper oil whereas in dry pepper oil it is only 9.3 %. The third major monoterpene in green pepper oil is d-limonene (12.4 %) and it is 11.1 % in dry pepper oil which is almost equal. In case of dry pepper oil d-limonene (11.1 % and Δ carene + *o*-cimene (10.8 %) constitute the major monoterpene compounds. α-Pinene content is 8 % in green pepper oil and it is only half (4 %) in dry pepper oil. The major monoterpene constituent is about 55 % in green pepper oil and 35 % in dry pepper oil.

TABLE-2
COMPOSITION OF VOLATILE OIL OF KARIMUNDA

S. No.	Constituents	Retention time (min)	GC peak area (%)	
			Fresh pepper oil	Dry pepper oil
1	α-Thujene	2.57	0.01	0.01
2	α-Pinene	3.07	8.01	3.98
3	Camphene	3.30	0.18	0.06
4	β-Pinene + myrcene	3.68	15.40	9.34
5	Δ-Carene + *o*-cimene	4.15	18.75	10.84
6	d-Limonene	4.53	12.37	11.1
7	Sabinene hydrate	4.87	0.12	0.03
8	γ-Terpinolene	5.10	0.51	0.11
9	*p*-Menth-1, 8-diene	5.73	4.20	2.87
10	Linalool	6.14	0.01	0.14
11	Terpinene-4-ol	7.62	0.02	0.09
12	α-Terpineol	7.79	0.42	0.51
13	δ-Elemene	11.64	0.34	0.18
14	Cubebene	11.99	0.37	0.34
15	α-Copaene	12.60	0.40	0.47
16	β-Elemene	12.91	1.67	2.33
17	β-Caryophyllene	13.93	19.36	15.58
18	Humulene	14.35	2.73	4.67
19	β-Bisabolene	15.07	4.45	7.53
20	γ-Cadinene	15.29	3.21	7.01
21	Longifolene	15.50	1.12	0.12
22	Z-nerolidol	16.33	0.02	0.58
23	Elemol	16.61	0.92	1.17
24	E-nerolidol	17.03	0.75	10.45
25	Cedrol	17.61	0.14	0.41
26	Caryophyllene oxide	17.95	0.19	0.46
27	δ-Cadinol	18.53	0.23	0.48

This can be explained that these low volatile monoterpenes are lost during drying. It is observed earlier that the aroma profile of the oil changes during drying. The pleasant fresh, peppery and pinery aroma of the fresh pepper oil may be due to the presence of large content of these monoterpenes in the fresh pepper oil which are decreased during drying. A monoterpene *p*-menth-1, 8-diene is found to be 4.2 % in green pepper oil and 2.9 % in dry pepper oil. It is observed that the oxygenated monoterpenes are less in both green (2.5 %) and fresh pepper oils (5.5 %). Oxygenated monoterpenes identified are α terpineol, terpinen-1-ol and linalool.

The sesquiterpenes and high polar oxygenated compounds accounts for 37 % in green pepper oil whereas it is 55 % in dry pepper oil. β-Caryophyllene is the major sesquiterpene hydrocarbon present in both oils, 19 % in green and 16 % in dry oil. Other major sesquiterpenes present are β-bisabolene (4.5%). γ-Cadinene (3.32 %) and humulene (2.7 %) in green pepper oil. Except for β-caryophyllene these major sesquiterpenes are high in dry pepper oil. The composition to the dry pepper oil is β-bisabolene (7.5), γ-cadinene (7 %) and humulene (4.7 %). β-Elemene content is slightly high in dry pepper *i.e.*, 2.3 % and it is 1.8 % in green pepper oil. Longifolene is 1.1 % in green pepper oil and it is only found to be 0.1 % in dry pepper oil. E-Nerolidol is found to be the major sesquiterpenes oxygenated compound (10.5 %) in dry pepper oil but it is present in very small quantity in green pepper oil. Dried fruits had predominantly sesquiterpenoids while fresh fruits contain both mono and sesquiterpenoids. Other oxygenated sesquiterpenes identified are elemol, Z-nerolidol, cedrol, caryophyllene oxide and δ-cadinol in both the oils. Mono-sesquiterpene and other polar compounds ratio of Karimunda fresh oil is 1.4 and for dry oil it is 0.8.

Analysis of oil from green and dry pepper oil of Panniyur-I variety is shown in Table-3. Twenty eight compounds have been identified by GC and GC-MS. Fresh pepper oil contains 43 % monoterpene compounds and 57 % sesquiterpene compounds. But dry pepper oil constitutes about 34 % monoterpenes and 66 % sequiterpenes and other polar compounds. Sabinene + β pinene was found to be the major monoterpenes hydrocarbons in both oils 19.8 and 13.8 %, respectively in green and dry pepper oils. Second major monoterpenes was d-limonene 14.3 % in green and 10.6 % in dry pepper oil. α-Pinene constitutes about 3.4 % in green and 5 % in dry oils. Oxygenated monoterpenes idenfitied are α-terpineol, linalool and terpinene 4-ol. The major monoterpenes constitute about 37.5 and 29.3 % in green and dry pepper oils, respectively. The fresh note of green pepper oil noted may be due to the presence of higher content of low volatile monoterpenes and minor oxygenated compounds. This may be due to the loss of these low volatile monoterpene during drying. The observations are in conformity with the studies of Ntonifor *et al.*[9] which showed that there were large differences in the composition of dried and fresh piper fruits. Dried fruits had predominantly sesquiterpenoids while fresh fruit contain both mono and sesquiterpenoids.

Sesquiterpene and other polar compounds constitute about 57 % in green pepper oil and 66 % in dry pepper oil. The major sesquiterpene hydrocarbon identified is β-caryophyllene 23.4 and 21 %, respectively in green pepper oil and dry pepper oil. Second major sesquiterpene identified is β-bisabolene and present to the extent of 7.4 % in green pepper and 13.3 % in dry pepper oil. Third major sesquiterpene hydrocarbon identified is a copaene (5.6 %) in green pepper oil and 9.4 % in dry oil. δ-Elemene and γ-cadinene content in fresh pepper oil are 3.8 and 2.6 %, respectively. These compounds are found to be less in dry pepper oil. Among the oxygenated sesquiterpenes E-nerolidol constitutes about 7.8 % in dry oil. γ-Cadinol analyzed for 3.2 % in green and 4.4 % in dry oils. Other oxygenated compounds like z-nerolidol, elemol, cedrol and caryophyllene oxide are present only in small qualities in both the oils. Mono/sesquiterpenes and other polar compounds ratio of Panniyur fresh oil is 0.75 % for dry oils it is 0.5 %.

TABLE-3
COMPOSITION OF VOLATILE OIL OF PANNIYUR I

S. No.	Constituents	Retention time (min)	GC peak area %	
			Fresh pepper oil	Dry Pepper oil
1	α-Thujene	2.78	0.01	0.32
2	α-Pinene	3.17	3.35	4.95
3	Camphene	353	0.17	0.07
4	Sabinene + β-Pinene	4.17	19.84	13.75
5	Myrcene	4.73	0.39	0.05
6	α-Phellandrene	4.99	0.13	0.19
7	Limonene	5.21	14.31	10.62
8	Sabinene hydrate	5.53	0.23	0.06
9	γ-Terpinolene	5.79	0.77	0.44
10	*p*-Menth-1,8-diene	6.44	0.17	0.05
11	Linalool	7.07	0.05	0.14
12	Terpinene-4-01	8.35	0.66	0.11
13	γ-Terpineol	8.60	0.20	0.13
14	d-Elemene	12.56	3.79	0.32
15	Cubebene	12.83	0.24	0.46
16	α-Acopaene	13.07	5.63	9.43
17	β-Elemene	14.04	23.43	20.99
18	β-Caryophyllene	14.04	23.43	20.99
19	Humulene	1532	2.63	3.28
20	Cedrene	15.71	0.01	0.14
21	Germacrene	16.33	0.06	0.12
22	β-Bisabolene	16.55	7.42	13.33
23	γ-Cadinene	16.83	2.62	0.94
24	Z-Nerolidol	17.23	0.10	0.77
25	Elemol	17.57	0.02	0.08
26	E-Nerolidol	18.03	0.24	7.80
27	Caryophyllene oxide	18.93	0.38	1.42
28	d-Cadinol	19.36	3.22	4.76

Above description of the oils shows the difference between the oils from green and dry cultivars of Karimunda and Panniyur-I. Among the two cultivars Karimunda fresh analyzed for 63 % monoterpenes and Panniyur fresh for 42.9 %. The major monoterpene constituent constitutes 54.5 % and in Karimunda fresh and 37.5 % in Panniyur-I fresh oil. Karimunda fresh oil contains Δ-carene and *o*-cimene (18.8 %) as major compounds and β-pinene and myrcene constitute 15.4 % Panniyur-I fresh constitutes sabinene and β-pinene as major constituents about 19.8 %. Limonene is present to the extent of 14.3 %. α-Pinene content is high in Karimunda fresh oil 8.0 and 3.4 % in Panniyur-1. Such type of variation is also noticed in the sesquiterpene content of Karimunda and Panniyur dry oils. Caryophyllene content of Panniyur (23.4 %) is higher than in Karimunda (19.3 %) β-bisabolene content is also higher in Panniyur than in Karimunda 7.4 % and 4.5 %, respectively. Humulene content is same for both oils. γ-Cadinene analyzed for 3.2 % in Karimunda and 2.6 % in Panniyur-I. E-Nerolidol content is 1.5 % in Karimunda and 7.3 % in Panniyur. Gopalakrishnan *et al.*[10], have reported oil constituents from three Panniyur genotypes-α-pinene (5.07-6.18 %), sabinene (8.50-17.16 %), β-pinene (9.16-11.08 %), myrcene (2.20-2.30 %), limonene (21.06-22.71%), *p*-cymene (0.0-0.18 %), β-cardyophyllene (21.59-27.70 %) and oxygenated constituents (3.39-5.68 %).

In case of dry oils Karimunda analyzed for 44 % mono terpene and Panniyur for 33 %. Major monoterpenes are d-limonene and Δ-carene + *o*-cimene in Karimunda dry oil where as sabinene + β-pinene constitute the major constituent in Panniyur. Among the sesquiterpene hydrocarbons, caryophyllene is the major constituents in both fresh and dry samples of pepper oils. β-Bisabolene content of fresh oil of Panniyur is more (7.4 %) than in Karimunda 4.5 % dry sample of Panniyur contains 13.35 % and Karimunda 7.5 % γ-cadinene content of Karimunda, green and dry oils are higher than Panniyur samples, humulene analyzed for more or less than same.

In general, chemical composition of the dry oil samples varied widely. This is due to variations in geographical origins and agro climatic conditions. Mono/sesquiterpene ratio is found to be 1.7 % for Karimunda fresh oil whereas it was only 0.8 for Panniyur-I and for dry oil it is 0.8 and 0.5, respectively. Odor characteristics show that Karimunda oil has got moderately intense odour notes of refreshing pinelike, spicy, peppery and sharp note. The odor profile of Panniyur-I has less of the fresh green peppery, pungent and spicy note.

Conclusion

A marked differences is observed between the two cultivars of dry oils. The oil distilled from the fresh Karimunda pepper oil is superior in odour profile than dry oil and Panniyur I oil.

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