



Phytochemicals and Antioxidant Activity of Clove (*Syzygium aromaticum* L.) as Affected by Various Moisture Levels

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Moisture content in a plant matrix is an important factor as the nature of phyto-constituents present in spices may be altered by the presence of moisture. The present study was conducted to observe the effect of moisture levels viz. 5 % moisture level (more than normal), 10 % moisture level (more than normal) and normal moisture level (control) on phyto-constituents and antioxidant activities of clove buds. Contents of total phenols, flavonoids, sugars, DPPH free radical scavenging activity and antioxidant activity decreased under the influence of moisture conditions during storage. The contents of total phenols in samples with 10 % and 5 % and normal moisture level after 64 days of storage were 70.89, 73.76 and 96.07 mg GAE/g, flavonoids (21.02, 23.37 & 38.24 mg CE/g), total sugars (12.73, 13.88 & 23.01 mg/g), reducing sugars (12.59, 13.73 and 22.77 mg/g), non-reducing sugars (0.14, 0.15 and 0.24 mg/g), IC₅₀ values from DPPH free radical scavenging activity (253.0, 231.7 and 124.9 µg/mL) and antioxidant activity (51.05, 55.19 and 84.67 %). The contents of various phytochemicals and antioxidant activity decreased more at 10 % moisture level in comparison to 5 % moisture level.

Keywords: *Syzygium aromaticum*, Moisture levels, Total phenols, Flavonoids, Sugars, Antioxidant activity.

INTRODUCTION

Spices are common food adjuncts, which have been used as flavouring, seasoning and colouring agents throughout the world [1]. They are also used as traditional medicines and possess potent antioxidant properties [2]. Phenolic compounds in these plants are responsible for their antioxidant activity. The nature of many phyto-constituents such as phenolics and flavonoids present in spices may be altered due to the presence of moisture, which further affects the antioxidant activity of spices. Moisture content in a plant matrix is an important factor as it affects the shelf life of plant material due to microbial growth during storage. Most of the plant materials gain or lose moisture in the presence of various humidity conditions due to their hygroscopic nature. This leads to chemical and physical alterations in the material [3]. Moisture content of 9 % or lower prevents fungus growth and infestation. Fungal growth takes place at about 14 % or slightly higher moisture level [4]. Atmosphere relative humidity, temperature and sanitation should be regulated to maintain quality of plant materials [5]. Moisture and other vapor migration produce changes in texture, colour, flavor, ingredients, nutritional value and quality of food items.

Cloves (*Syzygium aromaticum* L.) are aromatic buds of medium sized evergreen tree belonging to family Myrtaceae.

Dried clove buds are rich in steam-volatile oil, carbohydrates, total sugars, tannins, sterols, triterpenes, flavonoids, proteins and mineral matter [6,7]. The major component of clove oil is a phenol, namely eugenol. Survey of the literature reveals that no systematic work has been done on the comparative study of various moisture levels on phyto-constituents and antioxidant activity of clove. Thus, the objective of present study was to observe the effect of moisture levels on phyto-constituents and antioxidant activity of powdered samples of clove buds.

EXPERIMENTAL

Highest purity chemicals were used throughout the experiment. Folin-Ciocalteu reagent, Tween 20, aluminium chloride, sodium nitrite and concentrated sulphuric acid were obtained from Merck Specialities Pvt. Ltd. Sodium hydroxide, sodium sulphate, sodium carbonate, sodium potassium tartrate, sodium bicarbonate, gallic acid, β-carotene and methanol were supplied by SISCO Research Laboratories Pvt. Ltd. (SRL). Chloroform, phenol, copper sulphate pentahydrate and ammonium molybdate were procured from Qualigens Fine Chemicals. 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), catechin and linoleic acid were sourced from Sigma-Aldrich.

Plant material: Dried clove buds of germplasm material were obtained for study. Good quality clove buds were selected and a fine powder was obtained by grinding in warring blender.

Sample preparation and extraction: Powdered sample of clove buds (4 g) was weighed, 0.2 g *i.e.* 0.2 mL and 0.4 g *i.e.* 0.4 mL of distilled water was added to the powdered samples with vigorous and uniform mixing using a mortar and pestle, to achieve 5 and 10 % moisture levels, respectively in powdered samples. The samples having moisture level were sealed in zip-lock polythene bags and weighed immediately. This weight was referred to as initial weight. All the zip-lock polythene bags were double sealed and placed in an airtight box, which was kept in refrigerator at 4 °C. Control samples (4 g) *i.e.* powdered samples with normal moisture level were also sealed in zip-lock polythene bags and stored in similar conditions along with powdered samples having 5 and 10 % moisture levels. All bags were weighed initially daily and latter on at 2-3 days interval to observe the loss in weight with respect to initial weight. In case of loss in weight, calculated amount of distilled water was added by 10 µL syringe and bags were zip-locked properly. The powdered samples of clove buds having 5 and 10 % moisture levels and control samples that were stored in zip-lock polythene bags used for analysis. One zip-lock polythene bag of each treatment was taken out for analysis initially at one-week interval *i.e.* 7, 14, 21, 28 and 35 days after maintaining the moisture level and final sample was taken out at nine weeks interval *i.e.* 64 days after maintaining the moisture levels. The samples collected periodically were extracted using soxhlet extraction technique and acetone as a solvent. Each extraction was performed in triplicate.

Estimation of total phenols content: Total phenols content was estimated by Folin-Ciocalteu method [8,9] using gallic acid as standard. The total phenols content present in various extracts was calculated and results were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g).

Estimation of flavonoids content: Flavonoids content was determined by colorimetric assay [9,10] using catechin as standard. The flavonoids content present in various extracts was calculated and results were expressed as mg catechin equivalents per gram (mg CE/g).

Estimation of total sugars content: Total sugars content was determined by phenol sulphuric acid method [11,12] using glucose as standard. The total sugars content present in various extracts was calculated and results were expressed as milligrams per gram (mg/g).

Estimation of reducing sugars content: Reducing sugars content was determined by Nelson-Somogyi method [12,13] using glucose as standard. The reducing sugars content present in various extracts was calculated and results were expressed as milligrams per gram (mg/g).

Estimation of non-reducing sugars content: Non-reducing sugars content was calculated as the difference of total sugars content and reducing sugars content.

$$\begin{aligned} \text{Non-reducing sugars content} = \\ \text{Total sugars content} - \text{Reducing sugars content} \end{aligned}$$

DPPH free radical scavenging activity: The antioxidant activity was estimated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [14]. The concentration of extracts was adjusted between 25-500 µg/mL by appropriate dilution. DPPH free radical scavenging activity was evaluated for each concentration [9].

Antioxidant activity: Antioxidant activity was estimated by β-carotene bleaching method [9,15] based on the antioxidant ability of extract to inhibit lipid per oxidation.

RESULTS AND DISCUSSION

Total phenols content: Total phenols content (mg GAE/g) in powdered samples of clove buds with 5 % moisture level (more than normal) was 93.18 after 7 days of storage followed by 88.86, 82.11, 77.56, 75.25 and 73.76 after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 91.58, 87.84, 81.23, 76.49, 73.57 and 70.89. On comparing the total phenols data of samples with 5 and 10 % moisture level (more than normal) with the data of normal moisture (control), it was found that overall decrease in total phenols content was 23.22 and 26.21 %, respectively after 64 days of storage. The decrease in total phenols content may be probably due to degradation of phenols into their derivatives under the influence of moisture during storage. Results of present study well matched with the previous study that the moisture content could affect the extraction ability because of high water content in mushroom samples, which could dilute the concentration of total phenols content in plant tissues resulting in lower contents of total phenols [16].

Flavonoids content: Flavonoids content (mg CE/g) in powdered samples of clove buds with 5% moisture level (more than normal) was 35.58 after 7 days of storage followed by 33.68, 30.60, 28.68, 25.46 and 23.37 after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 34.96, 32.27, 28.39, 26.31, 23.11 and 21.02. On comparing the flavonoids data of samples with 5 and 10 % moisture level (more than normal) with the data of normal moisture (control), it was found that overall decrease in flavonoids content was 38.89 and 45.03 %, respectively after 64 days of storage. The decrease in flavonoids content may be probably due to degradation of flavonoids into their derivatives under the influence of moisture during storage. Results of present study are in agreement with other studies on different crops. Major flavonoids in tea powder were degraded under the presence of moisture [17]. Flavonoid content in dried tomato halves with 18.24 and 30 % moisture decreased and the degradation in samples with higher level of moisture was more than samples containing lower level of moisture [18].

Total sugars, reducing sugars, non-reducing sugars: Total sugars content (mg/g) in powdered samples of clove buds with 5 % moisture level (more than normal) was 20.84 after 7 days of storage followed by 19.44, 18.40, 17.35, 16.29 and 13.88 after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 19.93, 18.43, 16.85, 15.89, 15.13 and 12.73. Reducing sugars content (mg/g) in powdered samples of clove buds with 5 % moisture level (more than normal) was 20.61 after 7 days of storage followed by 19.24, 18.21, 17.17, 16.12 and 13.73 after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 19.72, 18.24, 16.68, 15.73, 14.98 and 12.59. Non-reducing sugars content (mg/g) in powdered samples of clove buds with 5 % moisture

TABLE-1
PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF CLOVE BUDS (*Syzygium aromaticum* L.) AT DIFFERENT MOISTURE LEVELS

Days	Normal moisture level (control)	5 % Moisture level (more than normal)		10 % Moisture level (more than normal)		Normal moisture level (control)	5 % Moisture level (more than normal)		10 % Moisture level (more than normal)	
		Total phenols (mg GAE/g)	Degrad. (%)	Total phenols (mg GAE/g)	Degrad. (%)		Flavonoid (mg CE/g)	Degrad. (%)	Flavonoid (mg CE/g)	Degrad. (%)
7	96.03±0.02	93.18±0.08	2.97	91.58±0.04	4.63	38.21±0.04	35.58±0.05	6.88	34.96±0.04	8.51
14	96.00±0.03	88.86±0.06	7.44	87.84±0.03	8.50	38.25±0.03	33.68±0.06	11.95	32.27±0.07	15.61
21	96.06±0.03	82.11±0.03	14.52	81.23±0.05	15.44	38.18±0.05	30.60±0.09	19.85	28.39±0.06	25.64
28	96.08±0.01	77.56±0.07	19.28	76.49±0.07	20.39	38.16±0.03	28.68±0.07	24.84	26.31±0.05	31.05
35	96.04±0.02	75.25±0.09	21.65	73.57±0.08	23.40	38.22±0.02	25.46±0.03	33.39	23.11±0.04	39.53
64	96.07±0.03	73.76±0.05	23.22	70.89±0.05	26.21	38.24±0.03	23.37±0.06	38.89	21.02±0.04	45.03
Days	Normal moisture level (control)	Total sugars (mg/g)		Total sugars (mg/g)		Normal moisture level (control)	Reducing sugars (mg/g)		Reducing sugars (mg/g)	
		Degrad. (%)	Degrad. (%)	Degrad. (%)	Degrad. (%)		Degrad. (%)	Degrad. (%)	Degrad. (%)	Degrad. (%)
7	23.02±0.03	20.84±0.06	9.47	19.93±0.04	13.42	22.77±0.04	20.61±0.03	9.49	19.72±0.07	13.39
14	23.06±0.04	19.44±0.03	15.70	18.43±0.06	20.08	22.81±0.05	19.24±0.06	15.65	18.24±0.09	20.04
21	23.00±0.06	18.40±0.08	20.00	16.85±0.07	26.74	22.75±0.03	18.21±0.04	19.96	16.68±0.05	26.68
28	23.05±0.03	17.35±0.04	24.73	15.89±0.08	31.06	22.80±0.02	17.17±0.07	24.69	15.73±0.08	31.01
35	23.07±0.02	16.29±0.05	29.39	15.13±0.05	34.42	22.83±0.06	16.12±0.08	29.39	14.98±0.04	34.38
64	23.01±0.05	13.88±0.07	39.68	12.73±0.09	44.68	22.77±0.03	13.73±0.04	39.70	12.59±0.07	44.71
Days	Normal moisture level (control)	Non-reducing sugars (mg/g)		Non-reducing sugars (mg/g)		Normal moisture level (control)	Antioxidant activity (%)		Antioxidant activity (%)	
		Degrad. (%)	Degrad. (%)	Degrad. (%)	Degrad. (%)		Degrad. (%)	Degrad. (%)	Degrad. (%)	Degrad. (%)
7	0.25±0.01	0.23±0.01	8.00	0.21±0.01	16.00	84.50±0.02	77.13±0.06	8.72	73.32±0.12	13.23
14	0.25±0.01	0.20±0.01	20.00	0.19±0.00	24.00	84.65±0.03	72.09±0.04	14.84	69.26±0.05	18.18
21	0.25±0.01	0.19±0.00	24.00	0.17±0.01	32.00	84.59±0.01	67.21±0.09	20.55	63.99±0.07	24.35
28	0.25±0.00	0.18±0.01	28.00	0.16±0.01	36.00	84.54±0.04	61.95±0.03	26.72	59.22±0.10	29.95
35	0.24±0.02	0.17±0.00	29.17	0.15±0.00	37.50	84.63±0.04	58.26±0.07	31.16	55.17±0.04	34.81
64	0.24±0.01	0.15±0.02	37.50	0.14±0.01	41.67	84.67±0.02	55.19±0.11	34.82	51.05±0.08	39.71

level (more than normal) was 0.23 after 7 days of storage followed by 0.20, 0.19, 0.18, 0.17 and 0.15 after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 0.21, 0.19, 0.17, 0.16, 0.15 and 0.14. The decrease in contents of total sugars, reducing sugars and non-reducing sugars in powdered samples of clove buds with respect to samples having normal moisture level (control) was 39.68, 39.70 and 37.50 %, respectively at 5 % moisture level (more than normal) in comparison to 44.68, 44.71 and 41.67 %, respectively at 10 % moisture level (more than normal) after 64 days of storage period. A study on liberica coffee beans showed that sucrose content decreased during storage under relative humidity (66.13 to 74.11%) and the decrease in sucrose content increases with the increase in relative humidity during storage [19]. Total sugars content decreased in samples of herbal drugs with 4.56 to 6.50 % moisture and the samples with maximum moisture had minimum amount of total sugars content [20].

DPPH free radical scavenging activity: DPPH free radical scavenging activity of powdered samples of clove buds with 5 and 10 % moisture level (more than normal) and normal moisture level (control) at various concentrations is given in Table-2. The data showed that DPPH free radical scavenging activity decreased under the influence of moisture during storage. The IC₅₀ values for powdered samples of clove buds were between 141.9 to 231.7 µg/mL at 5 % moisture level (more than normal) in comparison to 149.8 to 253.0 µg/mL at 10 % moisture level (more than normal) and 124.6 to 124.9 µg/mL at normal mois-

ture level (control) during 7 to 64 days of storage period. The higher IC₅₀ values at 5 % moisture level (more than normal) and 10 % moisture level (more than normal) in comparison to control clearly shows that DPPH free radical scavenging activity decreased under the influence of moisture and the decrease was more at 10 % moisture level in comparison to 5 % moisture level. The probable reason for lower DPPH free radical scavenging activity (*i.e.* higher IC₅₀ values) in the samples at 10 % moisture level may be due to the presence of lower amount of antioxidant compounds *i.e.* total phenols and flavonoids in the samples at 10 % moisture level. Similar findings were reported in a study on dried peppers that the DPPH free radical scavenging activity was proportional to the sample's moisture and decreased from 45.25 % to 35 % and 28 % at moisture levels of 4 % and 12 %, respectively during three months of storage [21].

Antioxidant activity: Antioxidant activity of clove buds was determined by β-carotene bleaching method. Antioxidant activity (at 500 µg/mL concentration level) in powdered samples of clove buds with 5 % moisture level (more than normal) was 77.13 % after 7 days of storage followed by 72.09, 67.21, 61.95, 58.26 and 55.19 % after 14, 21, 28, 35 and 64 days of storage, respectively (Table-1). The corresponding values for samples with 10 % moisture level (more than normal) were 73.32, 69.26, 63.99, 59.22, 55.17 and 51.05 %. On comparing the antioxidant activity of samples with 5 and 10% moisture level (more than normal) with the data of normal moisture (control), it was found that overall decrease in antioxidant activity was 34.82 and 39.71 %, respectively after 64 days of storage. Lower antioxidant

TABLE-2
DPPH FREE RADICAL SCAVENGING ACTIVITY (%) AND IC₅₀ OF CLOVE BUDS AT DIFFERENT MOISTURE LEVELS

Days	Moisture level	DPPH free radical scavenging activity (%)					IC ₅₀ (µg/mL)
		Concentration (µg/mL)					
		500	250	100	50	25	
7	5 % moisture level (more than normal)	87.50 (7.36)*	70.45 (7.07)	41.67 (7.50)	27.77 (7.40)	15.53 (7.61)	141.9
	10 % moisture level (more than normal)	82.72 (12.42)	66.33 (12.50)	38.42 (14.72)	26.28 (12.37)	14.76 (12.20)	149.8
	Control	94.45	75.81	45.05	29.99	16.81	124.8
14	5 % moisture level (more than normal)	82.98 (12.10)	65.96 (12.92)	39.20 (12.89)	26.52 (11.42)	14.90 (10.94)	150.1
	10 % moisture level (more than normal)	81.23 (13.95)	65.28 (13.82)	37.65 (16.33)	24.84 (17.03)	14.38 (14.05)	154.0
	Control	94.40	75.75	45.00	29.94	16.73	124.9
21	5 % moisture level (more than normal)	77.40 (18.07)	62.29 (17.88)	35.88 (20.41)	23.59 (21.29)	13.58 (19.12)	166.5
	10 % moisture level (more than normal)	74.71 (20.92)	59.93 (20.99)	33.57 (25.53)	22.67 (24.36)	13.28 (20.91)	181.7
	Control	94.47	75.85	45.08	29.97	16.79	124.6
28	5 % moisture level (more than normal)	73.72 (21.88)	59.09 (21.97)	34.14 (24.07)	22.39 (25.12)	13.08 (21.72)	183.6
	10 % moisture level (more than normal)	69.69 (26.15)	55.95 (26.12)	32.87 (26.89)	21.07 (29.53)	12.44 (25.55)	202.3
	Control	94.37	75.73	44.96	29.90	16.71	124.7
35	5 % moisture level (more than normal)	68.97 (26.96)	55.35 (27.00)	32.86 (27.08)	21.94 (26.92)	12.22 (27.26)	206.2
	10 % moisture level (more than normal)	65.37 (30.77)	52.43 (30.85)	31.08 (31.03)	20.68 (31.11)	11.61 (30.89)	234.0
	Control	94.43	75.82	45.06	30.02	16.80	124.7
64	5 % moisture level (more than normal)	66.03 (30.05)	52.95 (30.12)	30.43 (32.42)	19.99 (33.26)	11.71 (29.96)	231.7
	10 % moisture level (more than normal)	60.94 (35.44)	50.37 (33.52)	28.97 (35.67)	18.02 (39.83)	11.19 (33.07)	253.0
	Control	94.39	75.77	45.03	29.95	16.72	124.9

*Values in parenthesis are degradation (%)

activity in the samples at 10 and 5 % moisture level may be due to the presence of lower amount of antioxidant compounds *i.e.* total phenols and flavonoids in the samples at 10 and 5 % moisture level.

Conclusion

Clove is found to possess marked antioxidant activity as it is a rich source total phenols and flavonoids. Phyto-constituents and antioxidant activity of clove decreased under moisture conditions during storage. Thus, the spices like clove should be stored properly in order to prevent ingress of moisture and oxygen. The presence of moisture conditions may deplete the nutritive value of the food items.

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