ORIGINAL ARTICLE

The content of active constituents of stored sliced and powdered preparations of turmeric rhizomes and zedoary (bulb and finger) rhizomes

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Abstract

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The content of active constituents of stored sliced and powdered preparations of turmeric rhizomes and zedoary (bulb and finger) rhizomes
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The stability of active constituents (curcuminoids and volatile oil) in turmeric (*Curcuma longa* Linn.) rhizomes and zedoary [*Curcuma zedoaria* (Berg.) Roscoe] bulb and finger rhizomes during storage have been investigated. They were prepared as sliced and powdered and separately packed, either in black polyethylene bags or in paper bags, and stored at room temperature (28-31°C). Samples at initial and three monthly intervals were examined over 12-15 months storage to determine the contents of curcuminoids, volatile oil and moisture. The results showed that storage of rhizomes in black polyethylene bags could prevent samples from taking up moisture better than those stored in paper bags. The sliced and powdered turmeric rhizomes exhibited no decrease in curcuminoids content after 15 months of storage irrespective of the nature of the packing material. However, the slices of zedoary (bulb and finger) rhizomes lost curcuminoids to a lesser extent than powdered rhizomes during storage period. Volatile oil content of turmeric

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rhizomes, zedoary (bulb and finger) rhizomes decreased slower when stored as slices rather than as powders. The result from the present study suggested that in order to maintain the quality of turmeric and zedoary rhizomes as raw material for food and medicinal uses, they should be prepared in sliced form and stored in black polyethylene bags in order to maintain their content of active constituents during storage period.

Key words : turmeric, zedoary, curcuminoids, volatile oil, stability, storage conditions

บทคัดย่อ

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ปริมาณสารออกฤทธิ์ของเหง้าขมิ้นชันและเหง้าขมิ้นอ้อย (ชนิดหัวและชนิดแง่ง) ที่เก็บรักษาในรูปแบบแว่นและแบบผง

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ศึกษาความคงตัวของสารออกฤทธิ์ (เคอร์คูมินอยด์และน้ำมันหอมระเหย) ในเหง้าขมิ้นชันแห้งและเหง้าขมิ้นอ้อยแห้งชนิดหัวและชนิดแง่ง ในรูปแบบแว่น และแบบผงในถุงพลาสติกสีดำ หรือในถุงกระดาษ ที่อุณหภูมิห้อง (28-31 องศาเซลเซียส) ดำเนินการโดยวิเคราะห์หาปริมาณสารเคอร์คูมินอยด์ ปริมาณน้ำมันหอมระเหย และปริมาณความชื้น ของตัวอย่างตั้งแต่เริ่มการเก็บรักษา และทุก 3 เดือน เป็นเวลาติดต่อกันในระยะการเก็บรักษา 12-15 เดือน พบว่า ถุงพลาสติกสีดำสามารถป้องกันการดูดความชื้นของตัวอย่างได้ดีกว่าถุงกระดาษในระหว่างการเก็บรักษา และพบว่าปริมาณสารเคอร์คูมินอยด์ไม่ลดลงในเหง้าขมิ้นชันที่เก็บทั้งแบบแว่นและแบบผงไม่ว่าจะเก็บในถุงพลาสติกสีดำหรือในถุงกระดาษตลอดระยะเวลา 15 เดือนของการเก็บรักษา แต่ขมิ้นอ้อยแบบแว่นทั้งชนิดหัวและชนิดแง่งมีการลดลงของสารเคอร์คูมินอยด์ช้ากว่าที่เก็บแบบผง ปริมาณของน้ำมันหอมระเหยในขมิ้นชันและขมิ้นอ้อยที่เก็บแบบแว่นลดลงช้ากว่าที่เก็บแบบผง จากผลการวิจัยจึงมีข้อเสนอแนะว่าการเก็บรักษาขมิ้นชันและขมิ้นอ้อยที่ยังคงไว้ซึ่งคุณภาพของปริมาณสารออกฤทธิ์ในการที่จะนำไปใช้เป็นยาหรืออาหารควรเก็บสมุนไพรเหล่านี้ไว้ในรูปแห้งแบบแว่นในถุงพลาสติกสีดำ

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Curcuma longa Linn. (commonly called turmeric) and *Curcuma zedoaria* (Berg.) Roscoe (commonly called zedoary) have been cultivated as vegetables, spices and sources of perfume in South and Southeast Asian countries (Saralamp *et al.*, 2000). The main active constituents of these rhizomes are coloring matters and volatile oil (Evan, 2002). Coloring matters are composed mainly of curcumin, demethoxycurcumin and *bis*-demethoxycurcumin. The volatile oil contains mainly aromatic principles, i.e. turmerone, *ar*-turmerone and zingiberene (Martins *et al.*, 2001, Mau *et al.*, 2003). Their rhizomes are widely used in foods as a condiment. They have been known for their coloring and flavoring properties and widely used in food industries (Souza *et al.*, 1997). They have also been used for centuries as traditional remedies such as stimulant, stomachic, carminative, diuretic, anti-diarrhea, anti-emetic, anti-inflammatory, antipyretic, anti-microbial and antioxidant agents. (Yoshioka *et al.*, 1998; Araujo and Leon, 2001; Martins *et al.*, 2001; Jayaprakasha *et al.*, 2005). They have also been used to treat and cure ulcers, wounds and other kinds of skin disorders, and as anticarcinogenic agents (Ammon and Wahl, 1991).

Since the qualities of turmeric and zedoary rhizomes for food and medicinal uses are based directly on the content of the curcuminoids and volatile oil, it is important that rhizomes are

handled and stored correctly in order to maintain the levels of the active principles in the harvested raw material. Turmeric and zedoary rhizomes are exposed to a variety of conditions during processing, packaging and storage, and some of these may have detrimental effect on the stability of the active constituents and consequently on their quality. Some factors affecting the stability of active constituents of turmeric and zedoary have previously been investigated (Pfeiffer *et al.*, 2003; Ansari *et al.*, 2005). The content of active constituents could be affected by environmental factors such as temperature, pH, light, moisture and air (Chosdu *et al.*, 1995; Price and Buescher, 1997; Wang *et al.*, 1997; Chatterjee *et al.*, 1998; Chatterjee *et al.*, 2000; Bernabe-Pineda *et al.*, 2004; Sowbhagya *et al.*, 2005).

Little information is available on the effect of storage conditions on the raw and processed rhizomes on the quantity of active constituents of these rhizomes. The old-styled drugstores and commercial manufacturers usually keep turmeric and zedoary rhizomes in containers such as paper or plastic bags, and as sliced or powdered forms in order to prescribe or utilize for products formulation. Therefore, it is of interest to study the effect of storage conditions on the active constituents. The results of this study will be beneficial for developing appropriate industrial production processes, which hopefully will result in better maintenance of the active constituents in commercially available rhizomes. Hence, in the present study, the determination of the contents of curcuminoids and volatile oil in turmeric and zedoary rhizomes stored under various conditions was performed.

Materials and Methods

Materials

Fresh rhizomes of turmeric and zedoary (bulb and finger) (12-month old) were collected from Phang-Nga and Songkhla provinces, Thailand respectively. The packaging materials used in this study were: black polyethylene bags (BPB) and paper bags (PB) purchased from Chalermsak Trading, Thailand. Unit pouches of the above packing materials were 8 inches wide and 16 inches long.

Preparation and storage conditions of the plant materials

The rhizomes of turmeric, zedoary (bulb) and zedoary (finger) were washed with water, cut into slices about 0.5 cm thickness and dried in a hot air oven at 50°C for 4 days. About half of the dried rhizomes were pulverized and passed through a sieve No. 20. The dried sliced and powdered rhizomes were separately packed, either in the closed black polyethylene or paper bags and stored at room temperature (28-31°C) over a 12-15 month period. These rhizomes were sampled periodically, and analyzed for curcuminoids, volatile oil and moisture content. Turmeric samples were analyzed at the beginning of storage and at 3, 6, 12 and 15 month periods. Zedoary samples were analyzed at the beginning of storage and at 3, 6, 9 and 12 month periods. The dried slices were ground to a fine powder and passed through a sieve No. 20. prior to weighing and determining of total curcuminoids content.

Quantitative analysis of total curcuminoids

Curcuminoids concentration in the samples was determined using UV-spectrophotometer (Model Genesis 5, Miltonroy, USA.) according to the procedure described previously (Ministry of Public Health, Thailand, 1998). After withdrawal from the storage bags, the dried sliced rhizomes were pulverized and passed through a sieve No. 20; the stored powdered rhizomes were assayed without further treatment. Samples solutions were then prepared by accurately weighing about 300 mg of powdered turmeric or zedoary rhizomes, transferring to 10 ml volumetric flasks, and volume adjusted to 10 ml with tetrahydrofuran (Lab-Scan, Thailand). The mixture was set aside at room temperature for 24 hrs with frequent shaking. One ml of the clear supernatant solution was accurately transferred into a 25 ml volumetric flask, diluted to volume with methanol (Lab-Scan, Thailand) and mixed. Finally, 1.0 ml of this solution

was transferred into a 50 ml volumetric flask, diluted to volume with methanol and mixed. The absorbance was measured at 420 nm. Five standard curcumin solutions were prepared by using standard curcumin (Fluka, Switzerland) in methanol to obtain about 0.78, 1.56, 1.95, 2.34 and 3.12 x 10^{-3} mg/ml. The absorption intensities of the standard solutions were measured at 420 nm and methanol was used as a blank. The concentration of curcuminoids in the test samples was interpreted from the calibration curve and the percentage of total curcuminoids was calculated as total curcuminoids content in the samples and expressed as the weight of total curcuminoids per 100 g dried weight of stored sample (% w/w). The assay of each stored sample was performed in triplicate (n = 3).

Determination of volatile oil content

Volatile oil content was determined by a method described earlier (Ministry of Public Health, Thailand, 1998). Ten grams of the powdered turmeric or zedoary were accurately weighed and placed in a 500 ml round-bottomed flask, and one hundred ml of water was added into the flask with a few boiling chips. The flask was connected to a Dean stark apparatus. The Dean stark apparatus was filled with water to the standard line and 2.0 ml of xylene (Lab-Scan, Thailand) was added. The flask was heated at 130-150°C and the distillate was collected at a rate of 2-3 ml per minute for 5 hrs. After cooling (about 1 hr), the total volume of the distillate was measured and subtraction of the volume of added xylene gave the volume of volatile oil obtained from plant material. The volatile oil content was calculated and expressed as a volume of the oil per 100 g of sample (% v/w calculated on dried basis). The assay of each stored sample was performed in triplicate (n = 3).

Moisture content measurement

Moisture content was determined by the azeotropic distillation (Ministry of Public Health, Thailand, 1998). Two hundred ml of toluene (Lab-Scan, Thailand) and 2 ml of water were introduced into a dried 500 ml flask. The flask was then attached to the azeotropic apparatus and then heated at 130-150°C for 2 hrs and allowed to cool afterward for 1-2 hrs. The water volume was measured. The powdered rhizomes of turmeric or zedoary (25 g) were placed in the flask and heated gently for 15 minutes. The distillate was collected at the rate of 2 drops per second until most of water was distilled over and the rate of distillation was increased to about 4 drops per second. The inside of the condenser was rinsed with toluene and the flask was continuously heated for 5 minutes. After cooling (about 1 hr), the water volume was measured, and moisture content was then calculated and expressed as amount of water (ml) per 100 grams dried weight of stored sample (% v/w).

Statistical analysis

Data for curcuminoids, volatile oil and moisture contents from analyzed samples were subjected to analysis of variance (ANOVA) with Bonferroni adjustment and the mean comparison were performed using completely randomized factorial design. P-values <0.05 were considered as significant.

Results and Discussion

Moisture content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes among type of storage bags, form of preparations and storage times

Many compounds are moisture sensitive resulting in chemical and/or physical instability (Carstensen and Rhodes, 2000). In the present study, moisture contents of turmeric, zedoary (bulb) and zedoary (finger) rhizomes increased with increasing storage times. Significant differences were found ($p<0.05$) between moisture contents of powdered and sliced in all rhizomes during storage (Table 1). The data revealed that moisture content of powdered rhizomes was higher than that of sliced rhizomes; and was significantly different ($p<0.05$) after 3 months of storage. Statistical values indicated that moisture contents of all samples stored in paper bags were significantly higher than for samples stored in black polyethylene

Table 1. Moisture content of turmeric, zedoary (bulb) and zedoary (finger) rhizome

Storage time (months)	Moisture content (% v/w) (Mean ± S.D.*)											
	Turmeric				Zedoary (bulb)				Zedoary (finger)			
	Powdered /BPB	Sliced/ BPB	Powdered /PB	Sliced/ PB	Powdered/ BPB	Sliced/ BPB	Powdered /PB	Sliced/ PB	Powdered/ BPB	Sliced/ BPB	Powdered/ PB	Sliced/ PB
0	5.20±0.17	5.20±0.17	5.20±0.17	5.20±0.17	7.30±0.45	7.30±0.45	7.30±0.45	7.30±0.45	6.28±0.08	6.28±0.08	6.28±0.08	6.28±0.08
3	6.00±0.21	5.20±0.10	6.00±0.21	5.25±0.05	7.67±0.31	7.23±0.15	7.98±0.43	7.70±0.10	6.67±0.12	6.93±0.12	7.40±0.40	6.87±0.12
6	6.03±0.21	5.73±0.12	6.53±0.42	5.80±0.20	9.87±0.42	9.63±0.36	13.27±0.12	13.07±0.23	9.87±0.23	8.40±0.40	12.87±0.58	12.67±0.58
9	6.87±0.42	6.40±0.10	7.93±0.12	7.26±0.12	10.93±0.30	10.66±0.23	13.80±0.20	13.13±0.11	9.86±0.11	9.07±0.12	14.06±0.11	12.73±0.23
12	7.00±0.20	6.47±0.12	8.00±0.20	7.27±0.12	11.07±0.23	10.67±0.31	13.93±0.31	13.33±0.12	9.93±0.23	9.33±0.12	14.27±0.12	12.47±0.12
15	7.06±0.12	6.86±0.10	8.26±0.12	7.47±0.23	ND**	ND	ND	ND	ND	ND	ND	ND

***Mean ± S.D. from triplicate determinations, ** ND = Not determined, BPB = black polyethylene bag, PB = paper bag**

bags at the same storage time period. Moisture contents of turmeric, zedoary (bulb), and zedoary (finger) rhizomes in black polyethylene and paper bags were significantly different ($p<0.05$) after 9, 3 and 3 months storage respectively. Moisture contents of powdered turmeric rhizomes stored in black polyethylene and paper bags after 3 months were significantly higher ($p<0.05$), when compared to sliced rhizome stored in black polyethylene and paper bags.

Moisture contents of zedoary (bulb) powdered rhizome stored in black polyethylene and paper bags were significantly increased ($p< 0.05$) after 6 months storage. Similar effect was observed with sliced rhizome stored in black polyethylene and paper bags.

Zedoary (finger) rhizome in paper bags and black polyethylene bags showed significantly different ($p<0.05$) moisture content after 3 months of storage. Furthermore, moisture content of powdered zedoary (finger) rhizome in paper and black polyethylene bags significantly increased ($p<0.05$) after 3 and 6 months storage, respectively, whereas that of sliced rhizome stored in black polyethylene and paper bags significantly increased after 6 months.

The data obtained from this study suggested that different type of containers and sample preparations affected the moisture content in these stored rhizomes. Moisture contents in turmeric, zedoary (bulb and finger) rhizomes stored in paper bags were higher than in rhizomes stored in black polyethylene bags, and this may be due to the higher porosity of the paper bags. Powdered material clearly has a higher total surface area compared to sliced rhizome, and this probably accounts for the higher capacity to absorb water. The increase in moisture content might result in an increase in deterioration of active constituents, curcuminoids and volatile oil, in turmeric and zedoary rhizomes over the time of storage.

Effect of storage on curcuminoids content among type of storage bags, form of preparations and storage times

Storage of powdered and sliced turmeric

rhizomes both in black polyethylene and paper bags for 15 months (Figure 1) did not result in a decrease of curcuminoids content ($p>0.05$). These results are similar to those obtained by Chatterjee *et al.* (1998), where the color of powdered turmeric expressed as curcumin was found to be highly stable during storage at high ambient temperatures (25-32°C) for up to 12 months. The curcuminoids content of turmeric rhizome showed no significant decrease during 15 months storage, therefore, either polyethylene or paper bags could be used by manufacturers as protective containers. These results are in agreement with the previous observation that the color of turmeric was little affected by packaging or storage for up to 6 months, even under the drastic conditions of exposure to sunlight (Price and Buescher, 1996). However, curcuminoids were reported to degrade rapidly due to photo-oxidation on exposure to light, the rate of degradation being higher in acid brine than in methanol. The color of turmeric powder was slightly increased when irradiated to 10 kGy and the resultant powder could be stored for up to 8 months (Chosdu *et al.*, 1995; Price and Buescher, 1996; Chatterjee *et al.*, 1998).

Curcuminoids content of stored zedoary (bulb) and zedoary (finger) rhizome were statistically significantly different between sliced and powdered rhizome after storage (Figure 2). The data show that curcuminoids content of zedoary (finger) rhizome were higher than that of zedoary (bulb) rhizomes. The stability results indicated that curcuminoids content of sliced samples stored in either black polyethylene bags or paper bags was higher than that of the powdered samples stored in the same type of containers. Curcuminoids contents of sliced zedoary (bulb and finger) rhizomes stored in black polyethylene and paper bags showed no significant decrease during first 6 months of the storage, but significantly decreased ($p<0.05$) was observed after 6 months. Conversely, powdered zedoary (bulb and finger) rhizome stored in paper and black polyethylene bags revealed a rapid decrease ($p<0.05$) in curcuminoids content after the beginning of the storage during first 3 months and a slight decrease after that. No significant different of curcuminoids content during further storage from 3-12 months.

The degradation of curcuminoids of zedoary (bulb and finger) rhizomes could possibly be due to the increase in moisture over the time storage (Table 1). The degradation of curcuminoids content of powdered zedoary (bulb and finger) rhizomes was higher compared to those of sliced rhizomes. Even though black polyethylene bags could prevent moisture uptake by stored samples better than paper bag, they could not prevent degradation rate of curcuminoids of the samples with the same type of preparation. Therefore, zedoary samples should be prepared as slices in order to better maintenance of the curcuminoids content than as powdered form.

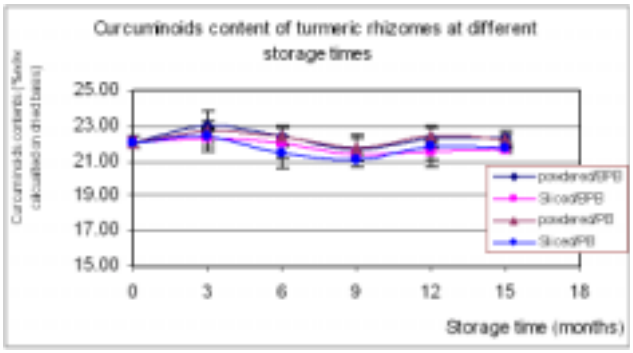

Figure 1. Curcuminoids content (%w/w) calculated on dried basis of turmeric rhizome at different storage times
BPB = black polyethylene bag, PB = paper bag

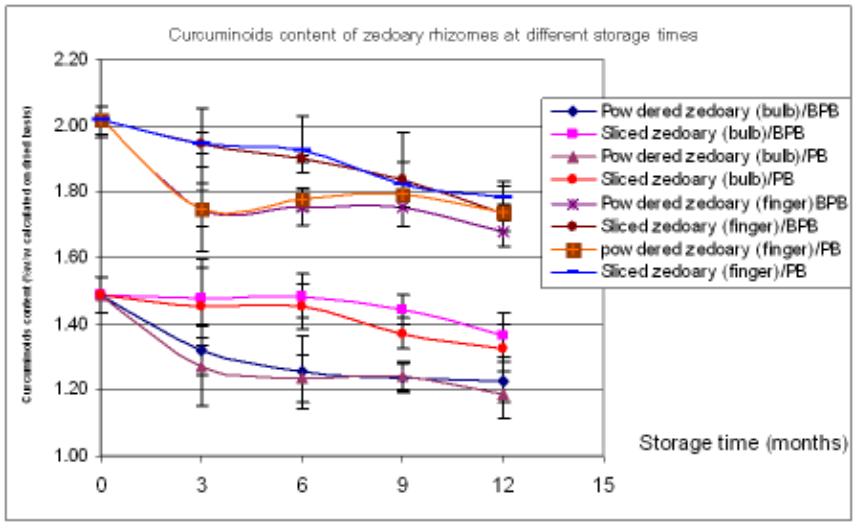

Figure 2. Curcuminoids content (%w/w) calculated on dried basis of zedoary (bulb and finger) rhizomes at different storage times
BPB = black polyethylene bag, PB = paper bag

The complexity of curcuminoids degradation has been previously reported. Curcuminoids are sensitive to light and alkaline condition (Price and Buescher, 1997). They are more stable to photo-oxidation as dried powder than as alcoholic extracts (Khurana and Ho, 1988). Photodecomposition of curcuminoids was found to be dependent on the type of solvent with it being more stable in methanol than in ethyl acetate, chloroform or acetonitrile (Price and Buescher, 1996). The main decomposition products of curcuminoids have previously been identified as feruloyl methane, ferulic acid, vanillin and *trans*-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal (Pfeiffer *et al.*, 2003).

Effect of storage on volatile oil content among type of storage bags, form of preparations and storage times

Turmeric rhizomes contain higher amount of volatile oil compared to bulb and finger rhizomes. However, the decreasing amount of volatile oil content in the stored samples (turmeric and zedoary rhizomes) displayed the same pattern during storage period (Figures 3-5). Volatile oil content of rhizomes during 12 or 15 months storage period was statistically different ($p<0.05$) between powdered and sliced preparations. The data shows that volatile oil content of powdered was lower than those for sliced rhizomes. The volatile oil contents of sliced rhizomes either stored in black polyethylene or paper bags showed no statistical difference ($p>0.05$) during 12 or 15 months storage period. The similar results were also observed with powdered rhizomes. Volatile oil content of turmeric rhizomes prepared as powders start decreasing after beginning of the storage, whereas decreasing of volatile oil contents of zedoary rhizomes were detected after 3 months of storage. Sliced zedoary (bulb and finger) and turmeric rhizomes exhibited a significant decrease ($p<0.05$) only after 9 and 12 months storage respectively.

These results suggest that volatile oil content in stored rhizome was affected by the form of preparation. The sliced preparations of turmeric and zedoary rhizomes could maintain volatile oil content better than powdered rhizome and can be stored in either black polyethylene bags or in paper bags. These results are in accordance with earlier reports (Chavalittumrong and Jirawattanapong, 1992). The decrease in volatile oil content of powdered turmeric, zedoary (bulb) and zedoary

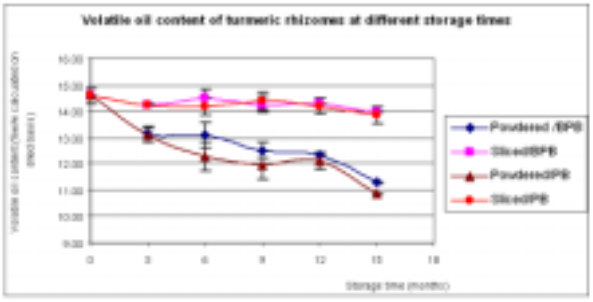

Figure 3. Volatile oil content (%w/w) calculated on dried basis of turmeric rhizome at different storage times
BPB = black polyethylene bag, PB = paper bag

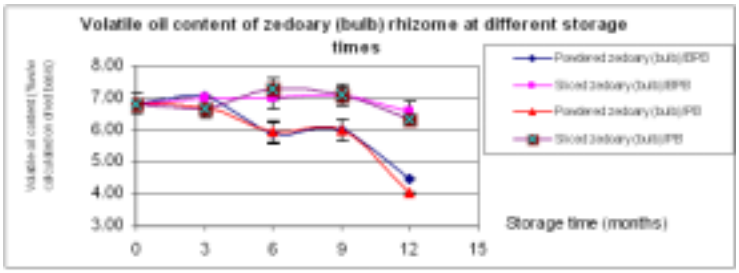

Figure 4. Volatile oil content (%w/w) calculated on dried basis of zedoary (bulb) rhizome at different storage times
BPB = black polyethylene bag, PB = paper bag

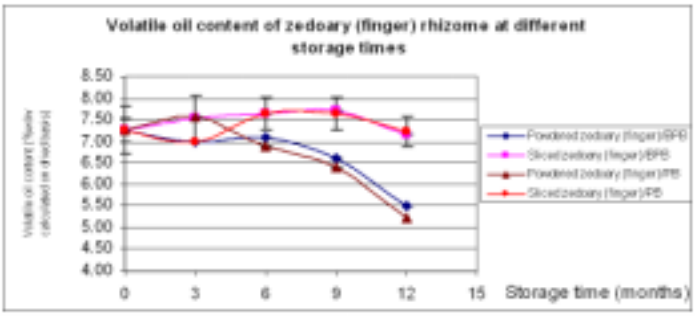

Figure 5. Volatile oil content (%w/w) calculated on dried basis of zedoary (finger) rhizome at different storage times
BPB = black polyethylene bag, PB = paper bag

(finger) rhizomes was probably due to higher surface area that allows moisture, heat and oxygen being able to be captured more easily than the sliced forms. Atmospheric oxygen can change the chemical composition of volatile oil, and this decomposition is speeded up by both heat and light (Tisserand and Balaces, 1995). The result from moisture content determination demonstrated that black polyethylene bags could prevent moisture uptake by stored samples better than paper bag,

but black polyethylene bags could not prevent decreasing volatile oil content of the powdered samples. Therefore, turmeric and zedoary samples should be prepared as slices in order to better maintenance of the volatile oil content than as powdered form.

Conclusions

Type of storage containers did not have significant affect on prevention the lost of active ingredients (curcuminoids and volatile oil) of turmeric and zedoary (finger and bulb) rhizomes, even though black polyethylene bag could protect moisture uptake by stored samples better than paper bag. Turmeric rhizome can be prepared as slices or powders and can be kept in either black polyethylene or paper bags. However, zedoary (finger and bulb) rhizomes should be prepared as sliced form to better maintain total curcuminoids and volatile oil contents and should be stored in black polyethylene bag to prevent moisture uptake by samples.

The results obtained from this study provide useful information to the industrial production scientists, traditional healers and consumers to reach decisions with regard to storing, or prescribing, stored turmeric and zedoary rhizomes. Further studies are undergoing investigation such as determination the effect of storage conditions on the content of individual curcuminoids (curcumin, demethoxycurcumin and *bis*-demethoxycurcumin) of turmeric and zedoary rhizomes and related biological activities for example antibacterial and antioxidant during storage period.

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