CHAPTER 3

RESULTS AND DISCUSSION

1. Curcuminoids and volatile oil contents of turmeric and zedoary rhizomes at different growth stages.

The standard plots for quantitative determination of total curcuminoids of turmeric and zedoary rhizomes, obtained using a UV-spectrophotometric method are shown in Figures A-1 and A-2 (Appendix A), respectively. The curves show linearity in the concentration range from 0.81×10^{-3} to 3.30×10^{-3} mg/ml of curcumin, which is used as a standard to calculate the contents of total curcuminoids. Regression analysis gave a correlation coefficient (r^2) of 0.9994.

1.1 Curcuminoids and volatile oil contents of turmeric rhizome.

The curcuminoids and volatile oil contents of turmeric collected from Krasasin district, Songkhla province at various ages of growth (6, 9 and 12 months) are summarized in Table 3-1. The results suggested that the curcuminoids and volatile oil contents of 6-month-old turmeric are higher than those of 9- and 12-month-old turmeric from the same source. The decrease in levels of these constituents is only slight at 9- and 12-months, but this decrease in statistically significant ($P < 0.05$).

1.2 Curcuminoids and volatile oil contents of zedoary rhizome.

The curcuminoids and volatile oil contents of zedoary collected from Krasasin district, Songkhla province at various growth stages (6, 9 and 12 months) are shown in Table 3-2. It was found that the total curcuminoids and volatile oil of zedoary rhizome of 6-month-old plants were significantly higher than those of 9- and 12-month-old plants.

Table 3-1 Curcuminoids and volatile oil contents of turmeric rhizome at different growth stages.

Age of plant (months)	**Month of analysis**	**Curcuminoids content (% w/w) (Mean ± S.D.)**	**Volatile oil content (% v/w) (Mean ± S.D.)**
6	October	8.78 ± 0.29 a	9.50 ± 0.01 a
9	January	7.64 ± 0.03 b	8.33 ± 0.29 b
12	April	8.07 ± 0.07 b	8.50 ± 0.01 b
F-test		*	*
C.V. (%)		2.16	1.90

Mean ± S.D. (n = 3) followed by the different letters in the same column denote the significant differences, according to Duncan' s multiple range test.
* significant at $p < 0.05$.

Table 3-2 Curcuminoids and volatile oil contents of zedoary rhizome at different growth stages.

Age of plant (months)	Month of analysis	Curcuminoids content (% w/w) (Mean ± S.D.)	Volatile oil content (% v/w) (Mean ± S.D.)
6	June	1.81 ± 0.12 a	8.83 ± 0.28 a
9	September	1.46 ± 0.03 b	7.99 ± 0.01 b
12	December	1.50 ± 0.02 b	8.33 ± 0.29 b
F-test		**	*
C.V. (%)		4.50	2.80

Mean ± S.D. (n =3) followed by the different letters in the same column denote the significant differences, according to Duncan' s multiple range test.
*, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

Turmeric and zedoary have been known since antiquity for many medicinal properties and are indicated for stomachic, carminative, pharmaceutical aid (coloring agent) and astringent (Matsuda *et al.*, 2001b; Ministry of Public Health, 1998). Since the quality of turmeric and zedoary depends on the content of active constituents (curcuminoids and volatile oil), it is necessary to control the amount of these active constituents. The contents of total curcuminoids (calculated as curcumin) and volatile oil of turmeric have been specified as not less than 5.0 % w/w and 6.0 % v/w, respectively (Ministry of Public Health, 1998). The curcuminoids and volatile oil contents are influenced by many factors, such as the geographical and climatic conditions (Tewtrakul, 1993). In the present study,

active constituents of turmeric and zedoary rhizomes, curcuminoids and volatile oil, were determined at various growth stages (6-, 9- and 12-month-old) of these plants. Turmeric and zedoary rhizomes were cultivated in Krasasin district, Songkhla province in April (2002) and December (2001), respectively. In addition, observations on monthly mean rainfall and temperature in Songkhla province during field experiments of turmeric and zedoary were collected (Appendix A, Figures A-3 and A-4, respectively). Turmeric was cultivated from April 2002 to April 2003, during with the monthly mean rainfall and temperature were in the range of 0.9-12.7 mm and 27.4-29.7 °C, respectively. Zedoary was cultivated from December 2001 to December 2002, during which the monthly mean rainfall and temperature were in the range of 0-12.7 mm and 27.2-29.7 °C, respectively. Turmeric grows very well in rather hot climates with high humidity at night (Farnsworth and Bunapraphatsara, 1992). In the present study, both turmeric and zedoary at 6-month-old gave the highest contents of these active constituents. These results are similar to previous work of Chavalittumrong and Jirawattanapong (1992), which demonstrated that turmeric rhizomes from Prachuap Khiri Khan and Nakhon Pathom of 5-month-old gave the highest contents of curcuminoids and volatile oil. The present results indicated that the growth stages have an effect on the curcuminoids and volatile oil production in turmeric and zedoary rhizomes. From these results, 6-month-old turmeric and zedoary rhizomes, which gave both high curcuminoids and volatile oil contents might be promoted for harvesting. However, the lower production yield (kg/rai) of turmeric and zedoary at 6-month old should be evaluated and considered.

2. Curcuminoids, volatile oil and moisture contents of turmeric and zedoary rhizomes stored at room temperature at different storage periods.

Since the quality of turmeric and zedoary are based directly on the active constituents, it is necessary to control the amount of their active constituents. Turmeric and zedoary are exposed to a variety of conditions during processing, packaging and storage, such as exposure to air and light, that may be detrimental to the active constituents (Price and Buescher, 1996). Furthermore, an excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis (World Health Organization, 1998). Limits for moisture content should therefore be set for every given plant material. For turmeric, moisture content has been specified not less than 10.0 % v/w (Ministry of Public Health, 1998). In the present study, different containers (black polyethylene and paper bags) were used to store turmeric and zedoary rhizomes.

Curcuminoids, volatile oil and moisture contents of turmeric and zedoary rhizomes stored under different storage conditions at room temperature were investigated periodically (every three months) from January 2002 to March 2003 and March 2002 to March 2003, respectively. For determination of the transmittance of light through bags (black polyethylene and paper bags), pieces of black polyethylene and paper bags were attached to the sample holder of a UV-visible spectrophotometer. Transmittance of light through black polyethylene and paper bags were monitored spectrophotometrically between

200-800 nm. The results are shown in Figures 3-1 and 3-2, respectively. These results indicated that transmittance of light through black polyethylene bags was higher than that of the paper bags and the transmittance of light through black polyethylene bags also increased as the wavelength is increased from 200 to 800 nm.

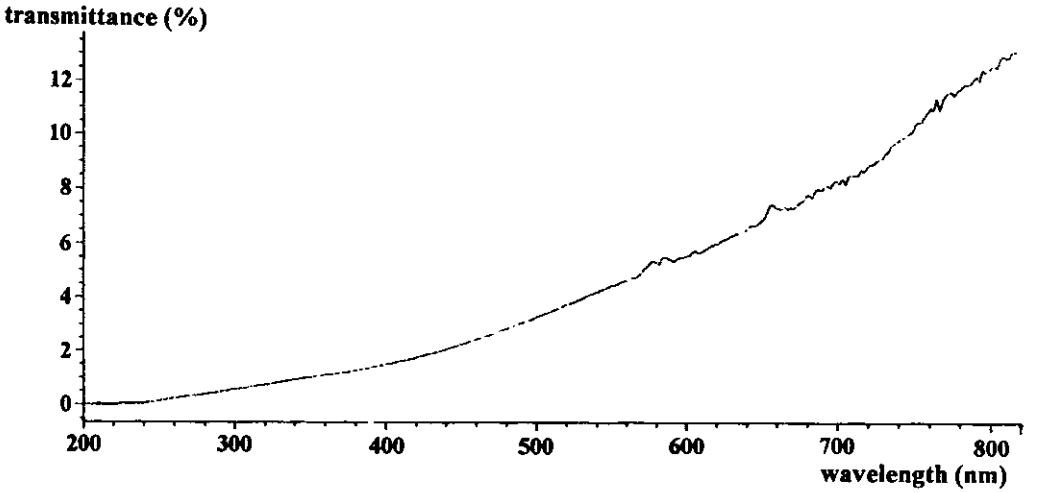


Figure 3-1 Transmittance of light through a black polyethylene bag.

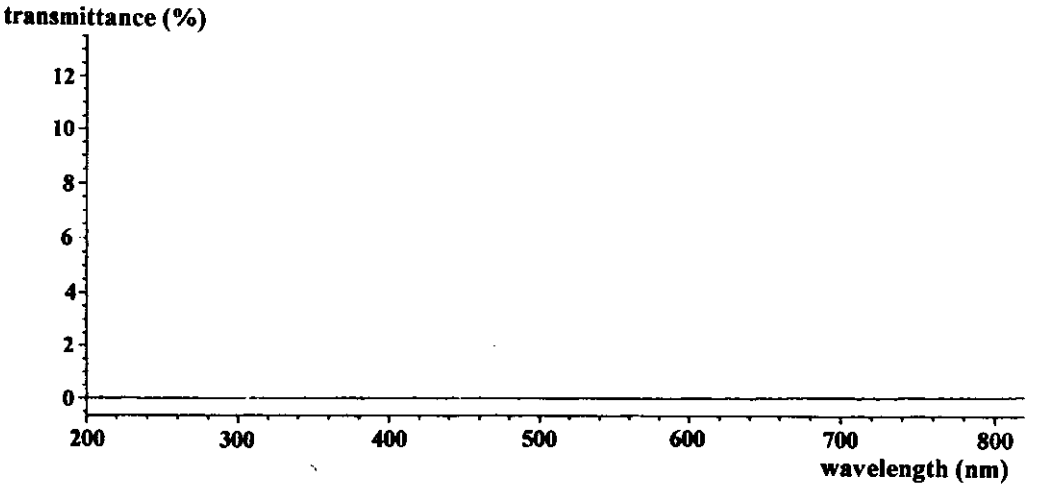


Figure 3-2 Transmittance of light through a paper bag.

2.1 Curcuminoids content of turmeric and zedoary rhizomes.

Curcuminoids content in the stored turmeric, zedoary (bulb) and zedoary (finger) rhizomes was determined as previously described in section 1. The standard plots are shown in Appendix A, Figures A-5, A-6 and A-7, respectively. Regression analysis gave correlation coefficients (r^2) of 0.9992, 0.9994 and 0.9995, respectively. Curcuminoids content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes are shown in Tables 3-3, 3-4 and 3-5, respectively.

Table 3-3 Curcuminoids content of turmeric rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	20.91 ± 0.30	20.91 ± 0.30	20.91 ± 0.30	20.91 ± 0.30
3	21.64 ± 0.22	21.19 ± 0.49	21.32 ± 1.06	21.23 ± 0.25
6	21.03 ± 0.45	20.78 ± 0.80	20.92 ± 0.55	20.22 ± 0.41
9	20.19 ± 0.60	20.02 ± 0.29	20.03 ± 0.69	19.50 ± 0.32
12	20.75 ± 0.09	20.15 ± 0.47	20.58 ± 0.55	20.21 ± 1.05
15	20.70 ± 0.18	20.13 ± 0.16	20. 38 ± 0.47	20.14 ± 0.11

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-4 Curcuminoids content of zedoary (bulb) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	1.38 ± 0.05	1.38 ± 0.05	1.38 ± 0.05	1.38 ± 0.05
3	1.22 ± 0.07	1.37 ± 0.11	1.17 ± 0.06	1.34 ± 0.03
6	1.13 ± 0.10	1.34 ± 0.06	1.07 ± 0.03	1.26 ± 0.12
9	1.10 ± 0.04	1.29 ± 0.04	1.07 ± 0.06	1.19 ± 0.10
12	1.09 ± 0.06	1.22 ± 0.06	1.02 ± 0.03	1.15 ± 0.01

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-5 Curcuminoids content of zedoary (finger) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	1.89 ± 0.04	1.89 ± 0.04	1.89 ± 0.04	1.89 ± 0.04
3	1.63 ± 0.12	1.81 ± 0.03	1.62 ± 0.05	1.81 ± 0.10
6	1.58 ± 0.05	1.74 ± 0.01	1.55 ± 0.03	1.68 ± 0.09
9	1.58 ± 0.00	1.67 ± 0.13	1.54 ± 0.01	1.59 ± 0.06
12	1.51 ± 0.00	1.57 ± 0.09	1.49 ± 0.02	1.56 ± 0.03

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

2.1.1 Comparison of curcuminoids content of turmeric rhizome among types of storage bags, forms of preparations and storage periods.

From the statistical values, no interactions were found between forms of preparations and types of storage bags, types of storage bags and storage periods, forms of preparations and storage periods ($p>0.05$). No interaction between forms of preparations, types of storage bags and storage periods was also found ($p>0.05$) (Appendix A, Table A-1).

Storage of powdered and sliced rhizomes in black polyethylene bags for 15 months did not have any adverse effect on curcuminoids content ($p>0.05$). Similarly, storage of powdered rhizome in paper bags for 15 months did not result in the change of curcuminoids ($p>0.05$) (Appendix A, Table A-2). These results are similar to the results obtained by other research groups; the color of powdered turmeric expressed as curcumin was found to be highly stable during storage at the high ambient temperatures (25-32 °C) for up to 12 months (Chatterjee *et al.*, 1998). The data obtained from this study suggested that polyethylene and paper bags can be used as protective containers, since curcuminoids content of turmeric rhizome stored for 15 months in these bags did not change. These results are in agreement with the 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 (Govindarajan, 1980). Water content did not affect the stability of curcuminoids pigments in turmeric oleoresin-microcrystalline cellulose model systems (Souza *et al.*, 1997). However, curcuminoids were reported to degrade

rapidly due to photooxidation on exposure to light, the rate of degradation being higher in acid brine than in methanol (Price and Buescher, 1996). Munnasiri *et al.* (1987) reported a slightly increase in color power of turmeric which was irradiated to 10 kGy and stored up to 8 months.

2.1.2 Comparison of curcuminoids content of zedoary (bulb) among forms of preparations, types of storage bags and storage periods.

The statistical values (Appendix A, Table A-3) showed that no interactions were found between forms of preparations and types of storage bags, and between types of storage bags and storage periods ($p>0.05$). No interaction between forms of preparations, types of storage bags and storage periods was also found ($p>0.05$), but different forms of preparations, storage bags and storage periods ($p<0.05$) affected curcuminoids content. The data indicated that curcuminoids content of zedoary (bulb) rhizome was also significantly correlated between forms of preparations and storage periods ($p<0.05$).

Curcuminoids content of sliced zedoary (bulb) were statistically ($p<0.05$) higher than those of powdered rhizome (Appendix A, Table A-4). Curcuminoids content was statistically significant different between sliced and powdered rhizome after 3 months storage (Appendix A, Table A-5). Curcuminoids content of zedoary (bulb) rhizome was also statistically different ($p<0.05$) between those stored in black polyethylene and paper bags. The data show that curcuminoids content of rhizome stored in black polyethylene bags was

higher than that stored in paper bags (Appendix A, Table A-6), and curcuminoids content of powdered zedoary (bulb) rhizome stored in black polyethylene and paper bags after 3 months was significantly decreased ($p<0.05$). Similarly, sliced rhizome stored in paper and black polyethylene bags revealed a significant decrease ($p<0.05$) in curcuminoids content 9 and 12 months after storage, respectively (Appendix A, Table A-7).

From these results, it is clear that curcuminoids of powdered zedoary (bulb) rhizome gradually decrease with increasing storage periods compared to sliced rhizome.

2.1.3 Comparison of curcuminoids content of zedoary (finger) rhizome among types of storage bags, forms of preparations and storage periods.

Statistical values showed that no interaction was found between forms of preparations and types of storage bags and between types of storage bags and storage periods ($p>0.05$) (Appendix A, Table A-8). No interaction between forms of preparations, types of storage bags and storage periods was also found ($p>0.05$), but interaction was found between forms of preparations and storage periods ($p<0.05$). The data indicated that curcuminoids content of zedoary (finger) rhizome was also significantly affected by forms of preparations and storage periods ($p<0.05$).

Curcuminoids content of sliced zedoary (finger) was statistically (p

<0.05) higher than that of powdered rhizome (Appendix A, Table A-9). Curcuminoids content of zedoary (finger) rhizomes prepared as slices and powders was significantly different ($p<0.05$) after 3 months storage (Appendix A, Table A-10). Table A-11 (Appendix A) shows that curcuminoids content of powdered zedoary (finger) rhizome, 3 months after storage in black polyethylene and paper bags significantly decreased ($p<0.05$). Similarly, both sliced rhizomes stored in black polyethylene and paper bags revealed a significant decrease ($p<0.05$) in curcuminoids content 6 months after storage.

As in the case of zedoary (bulb) rhizome, curcuminoids content of powdered zedoary (finger) rhizome decreased with increased of the storage periods, when compared to sliced rhizome. This may be due to the particle size of powdered is smaller than sliced rhizomes, resulting in higher surface area. Some factors such as air, heat and light could affect easily. Morover, the result from the moisture content study during the storage periods showed an increasing amount which was another factor that affect the rate of degradation (Tables 3-10 and 3-11 in section 2.3). The degradation rate of curcuminoids content of powdered zedoary (bulb) and zedoary (finger) rhizomes was higher compared to those of sliced rhizomes. This may be because of the differences in the moisture content in these samples, since an excess of water will encourage deterioration following hydrolysis of compounds (World Health Organization, 1998).

The complexity of curcuminoids degradation has been reported. Curcuminoids are sensitive to light and alkaline conditions (Souza *et al.*, 1997). They were more stable to photooxidation as dry powder than as alcoholic extracts

(Khurana and Ho, 1988). Photodecomposition of curcuminoids was found to be dependent on the type of solvents, i.e., they are 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; Tønnessen and Greenhill, 1992).

2.2 Volatile oil content of turmeric and zedoary rhizomes.

Volatile oil content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored under different conditions at room temperature expressed as the percentage of v/w are shown in Tables 3-6, 3-7 and 3-8.

Table 3-6 Volatile oil content of turmeric rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Volatile oil content (% v/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	13.83 ± 0.29	13.83 ± 0.29	13.83 ± 0.29	13.83 ± 0.29
3	12.38 ± 0.18	13.50 ± 0.01	12.33 ± 0.29	13.49 ± 0.01
6	12.32 ± 0.45	13.66 ± 0.29	11.50 ± 0.50	13.33 ± 0.29
9	11.66 ± 0.29	13.33 ± 0.28	11.00 ± 0.50	13.33 ± 0.28
12	11.49 ± 0.00	13.33 ± 0.29	11.16 ± 0.29	13.16 ± 0.28
15	10.50 ± 0.01	12.99 ± 0.00	10.00 ± 0.01	12.82 ± 0.30

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-7 Volatile oil content of zedoary (bulb) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Volatile oil content (% v/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	6.32 ± 0.29	6.32 ± 0.29	6.32 ± 0.29	6.32 ± 0.29
3	6.49 ± 0.01	6.50 ± 0.00	6.16 ± 0.29	6.16 ± 0.28
6	5.33 ± 0.29	6.33 ± 0.29	5.33 ± 0.29	6.33 ± 0.29
9	5.33 ± 0.29	6.33 ± 0.29	5.16 ± 0.29	6.16 ± 0.29
12	4.00 ± 0.00	5.83 ± 0.29	3.50 ± 0.00	5.50 ± 0.00

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-8 Volatile oil content of zedoary (finger) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Volatile oil content (% v/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	6.81 ± 0.27	6.81 ± 0.27	6.81 ± 0.27	6.81 ± 0.27
3	6.50 ± 0.01	7.00 ± 0.50	6.50 ± 0.00	6.83 ± 0.29
6	6.50 ± 0.00	7.00 ± 0.01	6.00 ± 0.00	6.66 ± 0.29
9	6.00 ± 0.00	7.00 ± 0.00	5.50 ± 0.00	6.66 ± 0.29
12	5.00 ± 0.00	6.50 ± 0.00	4.50 ± 0.00	6.33 ± 0.29

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

2.2.1 Comparison of the volatile oil content of turmeric rhizome among types of storage bags, forms of preparations and storage periods.

The statistical data of volatile oil content (Appendix A, Table A-12) revealed that no interaction was found between types of storage bags and storage periods ($p>0.05$). No interaction between forms of preparations, types of storage bags and storage periods was also found ($p>0.05$). However, forms of preparations and types of storage bags affected volatile oil content of turmeric rhizome ($p<0.05$). An interaction between forms of preparations and storage periods was found ($p<0.05$). The data indicated that volatile oil content of turmeric rhizome was significantly ($p<0.05$) affected by forms of preparations, types of storage bags and storage periods.

Volatile oil content was statistically different ($p<0.05$) between powdered and sliced turmeric rhizome during 15 months of storage (Appendix A, Table A-13). The data showed that volatile oil content of powdered rhizome was lower than those of sliced rhizome. Volatile oil content of rhizome stored in black polyethylene and paper bags was statistically different ($p<0.05$) (Appendix A, Table A-14). The data showed that volatile oil content of rhizome stored in black polyethylene bags was higher than those stored in paper bags. The results from data in Table A-15 (Appendix A) showed that volatile oil content of rhizome prepared as sliced and powdered was significantly different ($p<0.05$) after 3 months of storage. Volatile oil content obtained from powdered rhizome stored in black polyethylene bags was higher than those stored in paper bags ($p<0.05$), but

volatile oil content obtained from sliced rhizome stored in black polyethylene and paper bags was not statistically significant different ($p>0.05$) (Appendix A, Table A-16). The volatile oil content of powdered rhizome stored in black polyethylene and paper bags was significantly decreased after only 3 months ($p<0.05$), whereas sliced rhizome stored in black polyethylene and paper bags exhibited a significant decrease ($p<0.05$) only after 15 months storage (Appendix A, Table A-17).

2.2.2 Comparison of the volatile oil content of zedoary (bulb) rhizome among types of storage bags, forms of preparations and storage periods.

Analysis of variance (ANOVA) data (Appendix A, Table A-18) of volatile oil content revealed that no interaction was found between forms of preparations and types of storage bags and between types of storage bags and storage periods ($p>0.05$). No interaction between forms of preparations, types of storage bags and storage periods was also found ($p>0.05$). However, the statistical values showed that interaction between forms of preparations and storage periods affected volatile oil content of rhizome ($p<0.05$).

A significant difference was found ($p<0.05$) between volatile oil derived from powdered and sliced zedoary (bulb) (Appendix A, Table A-19). The data revealed that volatile oil content of powdered rhizome was lower than that of sliced rhizome. The results from ANOVA data (Appendix A, Table A-20) showed that volatile oil content of rhizome prepared as slices and powders was significantly different ($p<0.05$) 6 months after storage. Volatile oil content of

rhizome stored in black polyethylene bags was significantly higher than that stored in paper bags (Appendix A, Table A-21), and that of powdered rhizome stored in black polyethylene and paper bags significantly decreased ($p<0.05$) after 6 months storage. Conversely sliced rhizome stored in black polyethylene bags revealed a non-significant ($p>0.05$) decrease in volatile oil content over 12 months storage. However, volatile oil content of sliced rhizome stored in paper bags was significantly decreased after 12 months storage (Appendix A, Table A-22).

From these results, the data suggested that volatile oil content in zedoary (bulb) rhizome in black polyethylene bags can be better maintained by their storage rather than in paper bags. Moreover, the results indicated that rhizomes prepared as slices could better maintain volatile oil content, compared to powdered rhizome.

2.2.3 Comparison of volatile oil content of zedoary (finger) among types of storage bags, forms of preparations and storage periods.

ANOVA data (Appendix A, Table A-23) of volatile oil content revealed that no interaction was found between forms of preparations and types of storage bags, and between types of storage bags and storage periods ($p>0.05$). No interaction between forms of preparations, types of storage bags and storage periods was found ($p>0.05$). However, statistical values showed that forms of preparations and storage periods affected volatile oil content of zedoary (finger) rhizome ($p<0.05$), and the data indicated that volatile oil content of these rhizome

was significantly ($p<0.05$) affected by forms of preparations, types of storage bags and storage periods.

Significant differences were found ($p<0.05$) between volatile oil derived from powdered and sliced zedoary (finger) (Appendix A, Table A-24); the data revealed that volatile oil of sliced rhizome was higher than that of powdered rhizome. ANOVA data in Table A-25 (Appendix A) showed that volatile oil of rhizome prepared as slices and powders was significantly different ($p<0.05$) 6 months after storage. The statistical values indicated that volatile oil content of rhizome stored in black polyethylene bags was significantly higher than those stored in paper bags (Appendix A, Table A-26). Volatile oil content of powdered rhizome stored in black polyethylene and paper bags was significantly decreased ($p<0.05$) 9 and 6 months after storage, respectively. However, both sliced rhizome stored in black polyethylene and paper bags revealed a non-significant ($p>0.05$) decrease in volatile oil content within 12 months after storage (Appendix A, Table A-27).

Results from the present study showed that volatile oil content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes gradually decreased over 12-15 months storage. These results are in accordance with earlier reports (Chavalittumrong and Jirawattanapong, 1992). Volatile oil content was affected by forms of preparations and types of containers. The data suggested that volatile oil content obtained from these rhizomes stored in black polyethylene bags could be higher than those of the rhizome stored in paper bags. Moreover, preparations of turmeric and zedoary rhizomes as slices could maintain volatile oil

content better than in powdered rhizome. Volatile oil content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes prepared as powders was more unstable with increasing storage periods than sliced rhizome. This may be due to the particle size of powdered is smaller than sliced rhizomes. The higher surface area, the better expose to air, heat and light which are important factors to the content of volatile oil. The decreasing in volatile oil content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in paper bags was presumably due to moisture, heat and oxygen being able to diffuse through paper bags more easily than through black polyethylene bags. 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).

2.3 Moisture content of turmeric and zedoary rhizomes.

Moisture content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored under different conditions at room temperature expressed as the percentage of v/w is shown in Tables 3-9, 3-10 and 3-11, respectively.

Table 3-9 Moisture content of turmeric rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Moisture content (% v/w) (Mean ± S.D.*)			
	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
3	6.00 ± 0.21	5.20 ± 0.10	6.00 ± 0.21	5.25 ± 0.05
6	6.03 ± 0.21	5.73 ± 0.12	6.53 ± 0.42	5.80 ± 0.20
9	6.87 ± 0.42	6.40 ± 0.10	7.93 ± 0.12	7.26 ± 0.12
12	7.00 ± 0.20	6.47 ± 0.12	8.00 ± 0.20	7.27 ± 0.12
15	7.06 ± 0.12	6.86 ± 0.10	8.26 ± 0.12	7.47 ± 0.23

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-10 Moisture content of zedoary (bulb) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Moisture content (% v/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	7.30 ± 0.45	7.30 ± 0.45	7.30 ± 0.45	7.30 ± 0.45
3	7.67 ± 0.31	7.23 ± 0.15	7.98 ± 0.43	7.70 ± 0.10
6	9.87 ± 0.42	9.63 ± 0.36	13.27 ± 0.12	13.07 ± 0.23
9	10.93 ± 0.30	10.66 ± 0.23	13.80 ± 0.20	13.13 ± 0.11
12	11.07 ± 0.23	10.67 ± 0.31	13.93 ± 0.31	13.33 ± 0.12

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-11 Moisture content of zedoary (finger) rhizome stored in the forms of powders and slices in BPB and PB at room temperature.

Storage periods (months)	Moisture content (% v/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
0	6.28 ± 0.08	6.28 ± 0.08	6.28 ± 0.08	6.28 ± 0.08
3	6.67± 0.12	6.93 ± 0.12	7.40 ± 0.40	6.87 ± 0.12
6	9.87 ± 0.23	8.40 ± 0.40	12.87 ± 0.58	12.67 ± 0.58
9	9.86 ± 0.11	9.07 ± 0.12	14.06 ± 0.11	12.73 ± 0.23
12	9.93 ± 0.23	9.33 ± 0.12	14.27 ± 0.12	12.47 ± 0.12

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

2.3.1 Comparison of moisture content of turmeric rhizome among types of storage bags, forms of preparations and storage periods.

ANOVA data (Appendix A, Table A-28) of moisture content exhibited an interaction between forms of preparations and types of storage bags ($p < 0.05$). Interactions between types of storage bags and storage periods and between forms of preparations and storage periods were also found ($p<0.05$). However, statistical analyses showed no interaction between forms of preparations, types of storage bags and storage periods ($p>0.05$).

From Table A-29 (Appendix A), significant differences were found

($p<0.05$) between moisture content obtained from powdered and sliced turmeric rhizomes. The data revealed that moisture content of powdered rhizome was higher than that of sliced rhizome; the moisture content of powdered and sliced rhizomes was significantly different ($p<0.05$) after 3 months of storage (Appendix A, Table A-30). Statistical values revealed that moisture content of rhizome stored in paper bags was significantly higher than that stored in black polyethylene bags (Appendix A, Table A-31). Moisture content of powdered rhizome was higher than that of sliced rhizome stored in black polyethylene and paper bags ($p<0.05$) (Appendix A, Table A-32). Moisture content of rhizome stored in black polyethylene and paper bags was significantly different ($p<0.05$) after 9 months storage (Appendix A, Table A-33). Table A-34 (Appendix A) shows that moisture content of powdered rhizome stored in black polyethylene and paper bags after 3 months was significantly increased ($p<0.05$), when compared to sliced rhizome stored in black polyethylene and paper bags.

2.3.2 Comparison of moisture content of zedoary (bulb) among forms of preparations, types of storage bags and storage periods.

ANOVA data of moisture content exhibited an interaction between types of storage bags and storage periods ($p<0.05$) (Appendix A, Table A-35). However, no interactions between forms of preparations and types of storage bags and between forms of preparations and storage periods was found ($p>0.05$). In

addition, statistical analyses showed no interaction among forms of preparations, types of storage bags and storage periods ($p>0.05$).

Significant differences were found ($p<0.05$) between moisture content of powdered and sliced zedoary (bulb). The data revealed that moisture content of powdered rhizome was higher than that of sliced rhizome (Appendix A, Table A-36). Statistical values revealed that moisture content of rhizome stored in paper bags was significantly higher than that stored in black polyethylene bags ($p <0.05$) (Appendix A, Table A-37). Moisture content of rhizome stored in paper bags and black polyethylene bags was significantly different ($p<0.05$) after 3 months (Appendix A, Table A-38). Moisture content of powdered rhizome stored in black polyethylene and paper bags was significantly increased ($p<0.05$) after 6 months storage. Similar effects were observed with sliced rhizome stored in black polyethylene and paper bags (Appendix A, Table A-39).

2.3.3 Comparison of moisture content of zedoary (finger) among types of storage bags, forms of preparations and storage periods.

ANOVA data of moisture content exhibited an interaction between types of storage bags and storage periods, forms of preparations and storage periods, and interaction between forms of preparations, types of storage bags and storage periods was also revealed ($p<0.05$) (Appendix A, Table A-40). However there was no interaction between forms of preparations and types of storage bags

($p>0.05$). Results demonstrated that forms of preparations, types of storage bags and storage periods affected moisture content of zedoary (finger) ($p<0.05$).

Significant differences were found ($p<0.05$) in moisture content of powdered and sliced zedoary (finger) rhizome, and the data revealed that moisture content of powdered rhizome was higher than that of sliced rhizome (Appendix A, Table A-41). Moisture content of rhizome prepared as powders and slices was significantly different ($p<0.05$) after 6 months (Appendix A, Table A-42). Statistical analyses revealed that moisture content of rhizome stored in paper bags was significantly higher than that of rhizome in black polyethylene bags ($p<0.05$) (Appendix A, Table A-43). Moisture content of rhizome stored in paper bags and black polyethylene bags was significantly different ($p<0.05$) after 3 months (Appendix A, Table A-44). Moisture content of powdered rhizome stored 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 (Appendix A, Table A-45).

Many compounds are moisture sensitive resulting in chemical and/or physical instability (Carstensen and Rhodes, 2000). In the present study, moisture content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes increased with increasing storage periods. The data obtained from this study suggested that different containers and preparations affected the moisture content in these rhizomes. Moisture content in turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in paper bags was higher than in rhizomes stored in black polyethylene bags, and this may be due to high 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 storage periods.

3. GC-MS chromatograms of volatile oil obtained from turmeric and zedoary rhizomes during storage at room temperature.

Volatile oil of turmeric and zedoary rhizomes were obtained using a steam distillation method (see section 1.2.2), and their chemical constituents were determined by GC-MS.

3.1 GC-MS chromatograms of volatile oil obtained from turmeric rhizome.

The GC-MS chromatograms of volatile oil isolated from turmeric rhizome before storage (zero time) and after 6 and 12 months storage are presented in Figures 3-3, 3-4 and 3-5, respectively. The retention times and name of chemical constituents are listed in Table 3-12. The highest amount of components of volatile oil were sesquiterpenes. The major constituents of the volatile oil at rhizome before storage (zero time) and after 6 and 12 months storage are presented in Figures 3-3, 3-4 and 3-5, respectively. The retention times and name of chemical constituents are list in Table 3-12. The highest amount of components of

volatile oil were sesquiterpenes. The major constituents of the volatile oil at different storage periods are at the peak numbers 5, 6 and 7 (Figures 3-3, 3-4 and 3-5). These are bisaborane sesquiterpenes, and analysis of MS data show that these constituents were β-turmerone, ar-turmerone and α-turmerone, respectively. β-Turmerone, ar-turmerone and α-turmerone were found to be the major constituents in turmeric, which is in accordance with the earlier reports (Negi *et al.*, 1999; Govindarajan, 1980). The minor constituents were ar-curcumene, α-zingiberene, β-bisabolene and β-sesquiphyllandrene (peak numbers 1, 2, 3 and 4, respectively). The composition of volatile oil obtained in the present study is similar to that earlier reported (Singh *et al.*, 2002; Chatterjee *et al.*, 2000; He *et al.*, 1998). Six and 12 months after storage, the major component was β-turmerone (peak number 5 in Figures 3-4 and 3-5, respectively), whereas before storage (zero time) the major constituent was ar-turmerone (peak number 6 in figure 3-3).

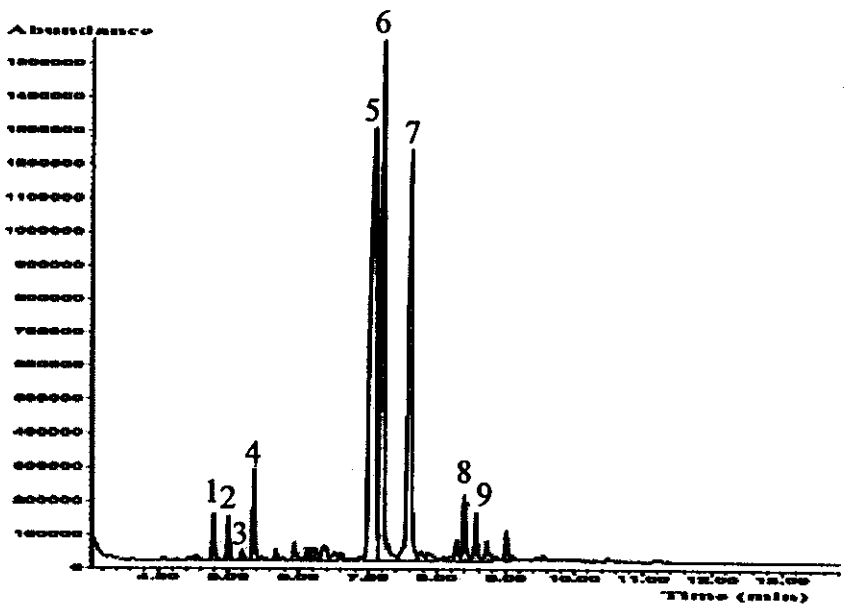


Figure 3-3 GC-MS chromatogram of volatile oil obtained from turmeric rhizome before storage (zero time).

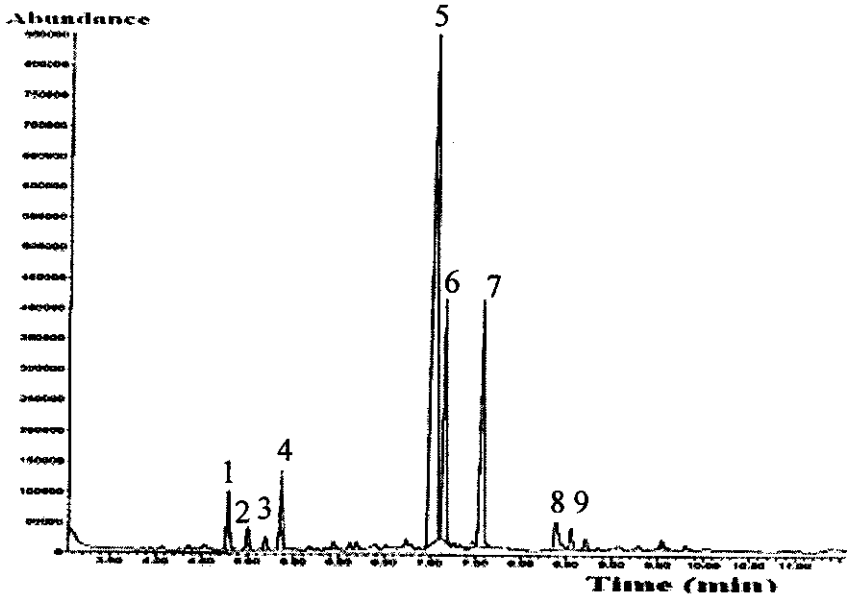


Figure 3-4 GC-MS chromatogram of volatile oil obtained from turmeric rhizome after 6 months storage.

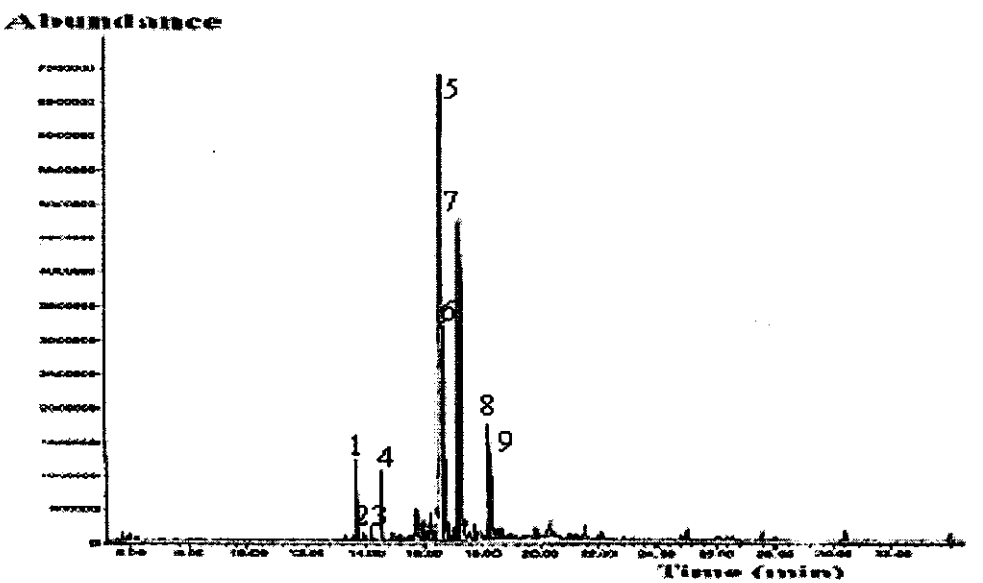


Figure 3-5 GC-MS chromatogram of volatile oil obtained from turmeric rhizome after 12 months storage.

Table 3-12 Chemical constituents of turmeric rhizome after different storage periods as analysed by GC-MS.

Retention Time (min)	Chemical constituents		
	0 month	6 months	12 months
4.80	ar-Curcumene (1)	-	-
4.79	-	ar-Curcumene (1)	-
13.71	-	-	ar-Curcumene (1)
5.01	α-Zingiberene (2)	-	-
5.01	-	α-Zingiberene (2)	-
13.98	-	-	α-Zingiberene (2)
5.19	β-Bisabolene (3)	-	-
5.19	-	β-Bisabolene (3)	-
14.24	-	-	β-Bisabolene (3)
5.37	β-Sesquiphyllandrene (4)	-	-
5.37	-	β-Sesquiphyllandrene (4)	-
15.53	-	-	β-Sesquiphyllandrene (4)
7.09	β-Turmerone (5)	-	-
7.06	-	β-Turmerone (5)	-
16.65	-	-	β-Turmerone (5)
7.22	ar-Turmerone (6)	-	-
7.17	-	ar-Turmerone (6)	-
16.76	-	-	ar-Turmerone (6)
7.62	α-Turmerone (7)	-	-
7.58	-	α-Turmerone (7)	-
17.29	-	-	α-Turmerone (7)
8.40	β-Atlantone (8)	-	-
8.40	-	β-Atlantone (8)	-
18.28	-	-	β-Atlantone (8)
8.57	α-Atlantone (9)	-	-
8.57	-	α-Atlantone (9)	-
18.37	-	-	α-Atlantone (9)

Number in parenthesis is peak no.

3.2 GC-MS chromatograms of volatile oil obtained from zedoary (bulb) rhizome.

The GC-MS chromatograms of volatile oil isolated from zedoary (bulb) rhizome before storage (zero time) and after 6 and 12 months storage are presented in Figures 3-6, 3-7 and 3-8, respectively. The retention times and name of chemical constituents are listed in Table 3-13. The major constituents, namely, *p*-cymene, β-turmerone, ar-turmerone and α-turmerone, were present in all samples at different storage periods. Compounds, such as ar-curcumene and epicurzerenone were minor component in volatile oil obtained from zedoary (bulb) rhizome. Before storage (zero time), β-elemene and α-zingiberene were clearly detected in volatile oil (peak numbers 1 and 2, respectively in Figure 3-6), but these constituents were not detected in samples after 6 and 12 months storage. β-Bisabolene and germacrone were clearly visible in chromatograms of samples stored before zero time and after 6 months storage (peak numbers 4 and 2; and peak numbers 9 and 7 in Figures 3-6 and 3-7, respectively), but these constituents were not found in samples stored for 12 months. The highest amount of ar-turmerone was present in samples at zero time, and this constituent was affected after 6 and 12 months storage (peak number 7 in Figure 3-6, peak number 5 in Figure 3-7 and peak number 4 in Figure 3-8, respectively). The major constituent after 6 and 12 months storage was β-turmerone. These results were similar to those of volatile oil of turmeric rhizome.

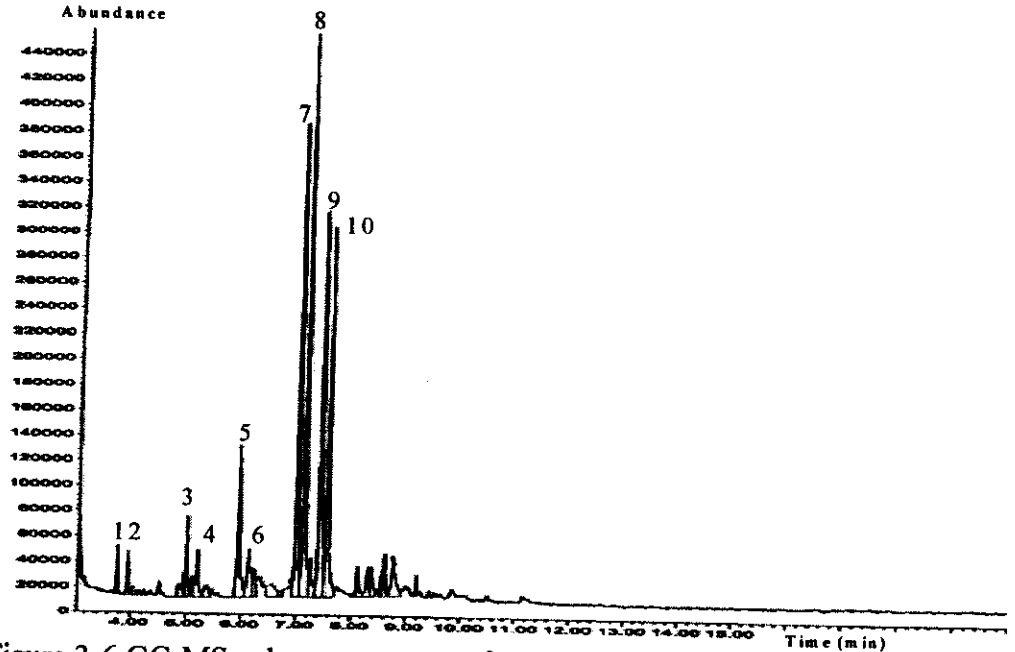


Figure 3-6 GC-MS chromatogram of volatile oil obtained from zedoary (bulb) rhizome before storage (zero time).

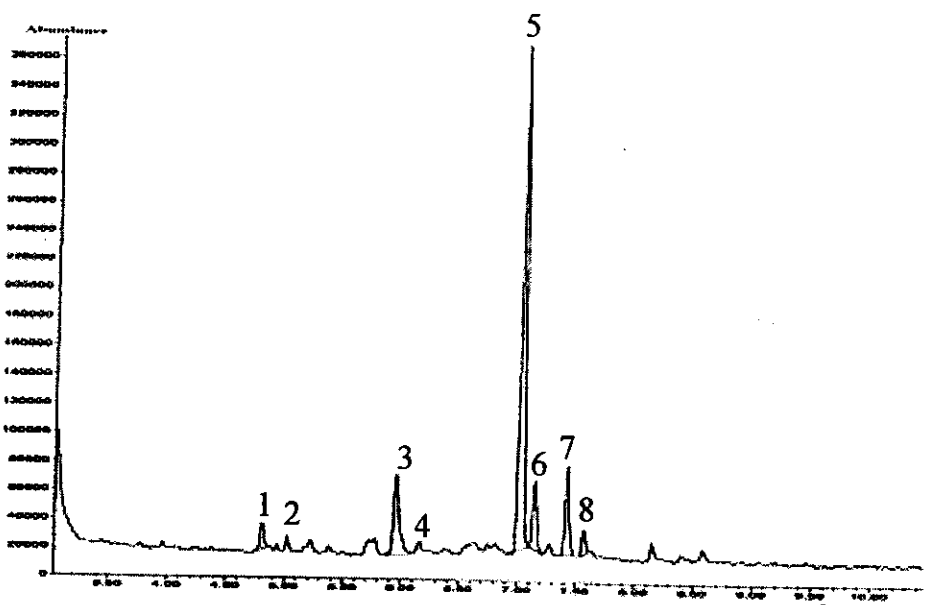


Figure 3-7 GC-MS chromatogram of volatile oil obtained from zedoary (bulb) rhizome after 6 months storage.

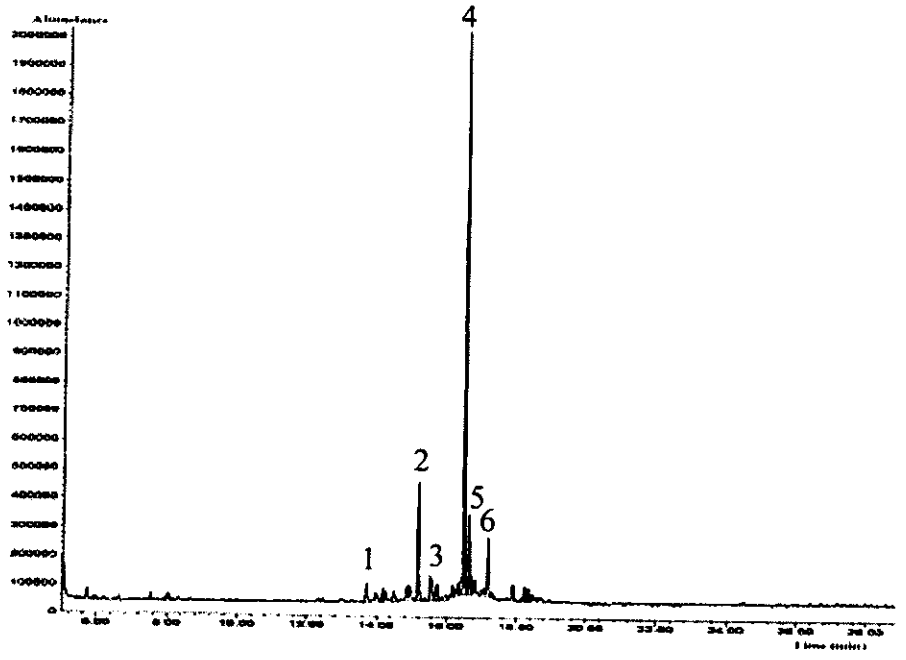


Figure 3-8 GC-MS chromatogram of volatile oil obtained from zedoary (bulb) rhizome after 12 months storage.

Table 3-13 Chemical constituents of zedoary (bulb) rhizome after different storage periods as analysed by GC-MS.

Retention Time (min)	Chemical constituents		
	0 months	6 months	12 months
3.78	β-Elemene (1)	-	-
3.97	α-Zingiberene (2)	-	-
4.82	ar-Curcumene (3)	-	-
4.82	-	ar-Curcumene (1)	-
13.70	-	-	ar-Curcumene (1)
5.23	β-Bisabolene (4)	-	-
5.23	-	β-Bisabolene (2)	-
5.97	*p*-Cymene (5)	-	-
5.96	-	*p*-Cymene (3)	-
15.20	-	-	*p*-Cymene (2)
6.16	Epicurzerenone (6)		-
6.16	-	Epicurzerenone (4)	-
15.56	-	-	Epicurzerenone (3)

Number in parenthesis is peak no.

Table 3-13 Chemical constituents of zedoary (bulb) rhizome after different storage periods as analysed by GC-MS (continued).

Retention Time (min)	Chemical constituents		
	0 months	6 months	12 months
7.04	β-Turmerone (7)	-	-
7.04	-	β-Turmerone (5)	-
16.51	-	-	β-Turmerone (4)
7.15	ar-Turmerone (8)		-
7.15	-	ar-Turmerone (6)	
16.67	-	-	ar-Turmerone (5)
7.43	Germacrone (9)	-	-
7.43	-	Germacrone (7)	-
7.57	α-Turmerone (10)	-	-
7.58	-	α-Turmerone (8)	-
17.22	-	-	α-Turmerone (6)

Number in parenthesis is peak no.

3.3 GC-MS chromatogram of volatile oil obtained from zedoary (finger) rhizome.

The GC-MS chromato grams of volatile oil isolated from zedoary (finger) rhizome before storage and 6 and 12 months after storage are presented in Figures 3-9, 3-10 and 3-11, respectively. The retention times and name of chemical constituents are listed in Table 3-14. Aromatic volatiles, such as ar-curcumene, zingiberene, β-bisabolene, β-sesquiphyllandrene, β-turmerone and ar-turmerone, were present in all samples at different storage periods. However, some compounds such as β-elemene, α-zingiberene, β-caryophyllene and γ-elemene were only detectable in samples at zero time and 6 months after storage. The MS data indicated that β-turmerone, ar-turmerone, and α-turmerone were

present as the major compounds, whereas only small amounts of ar-curcumene, zingiberene, β-bisabolene, β-sesquiphyllandrene and germacrene B were detected in volatile oil obtained from zedoary (finger) rhizome. The major compounds of volatile oil from samples stored for 6 and 12 months were β-turmerone, whereas samples at zero time were rich in ar-turmerone. These results were similar to those of volatile oil obtained from turmeric and zedoary (bulb) rhizomes. These results suggested that constituents in volatile oil obtained from turmeric and zedoary rhizomes, such as ar-turmerone decreased during storage of these rhizomes. It is possible that ar-turmerone is more sensitive to factors such as oxygen and moisture, than other constituents in volatile oil. The other factor that could have affect on the decreasing of ar-turmerone is the vapour pressure. ar-Turmerone has vapour pressure value less than that of β-turmerone and α-turmerone (vapour pressure values of ar-turmerone, β-turmerone and α-turmerone are 2.64×10^{-4}, 3.31×10^{-4} and 3.45×10^{-4} torr, respectively), thus ar-turmerone might be able to evaporate easier than β-turmerone and α-turmerone (Carolina *et al.*, 2003).

From the results, the volatile oil of zedoary (bulb) and zedoary (finger) rhizomes have a similar compositions, presenting some quanlitative differences. β-Caryophyllene, γ-elemene, zingiberene, β-bisabolene, β-sesquiphyllandrene and germacrene B were only detected in volatile oil of zedoary (finger) rhizome, whereas epicurzerenone was only detected in volatile oil of zedoary (bulb) rhizome. These results suggested that different parts of rhizomes produce different constituents of volatile oil. This could be due to different ages of rhizomes having an effect on the constituents of the rhizomes. The volatile oil from turmeric and

zedoary rhizomes had some qualitative differences. While β-sesquiphyllandrene, β-atlantone and α-atlantone were only detected in turmeric oil, some constituents such as β-elemene, epicurzerenone, germacrone were only identified in zedoary oil.

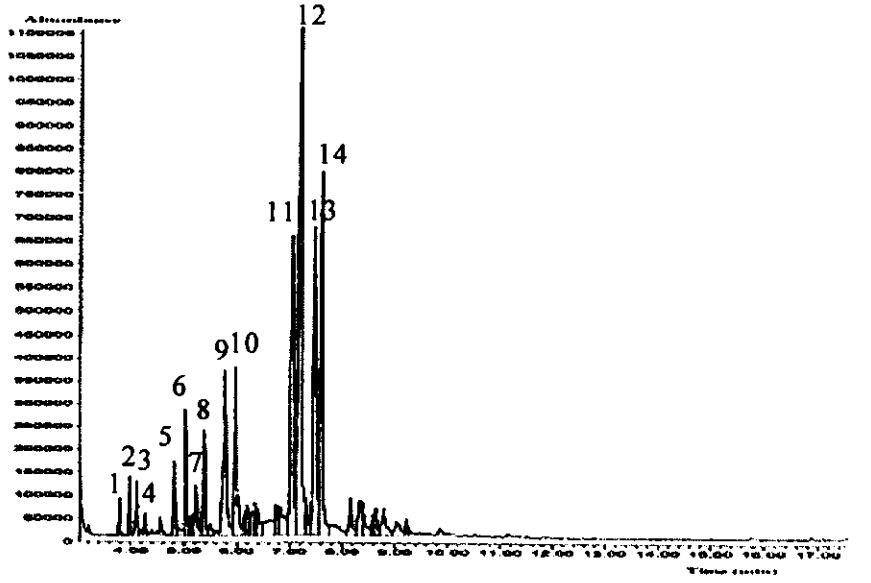


Figure 3-9 GC-MS chromatogram of volatile oil obtained from zedoary (finger) rhizome before storage (zero time).

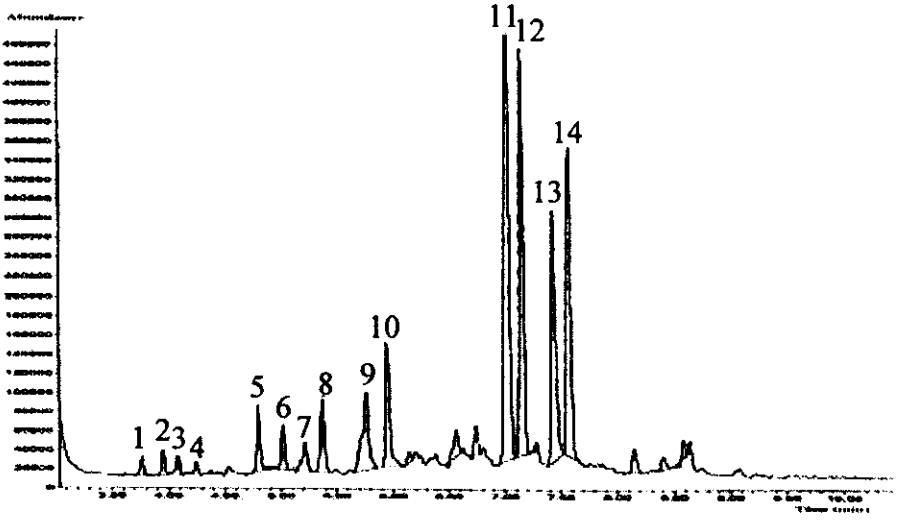


Figure 3-10 GC-MS chromatogram of volatile oil obtained from zedoary (finger) rhizome after 6 months storage.

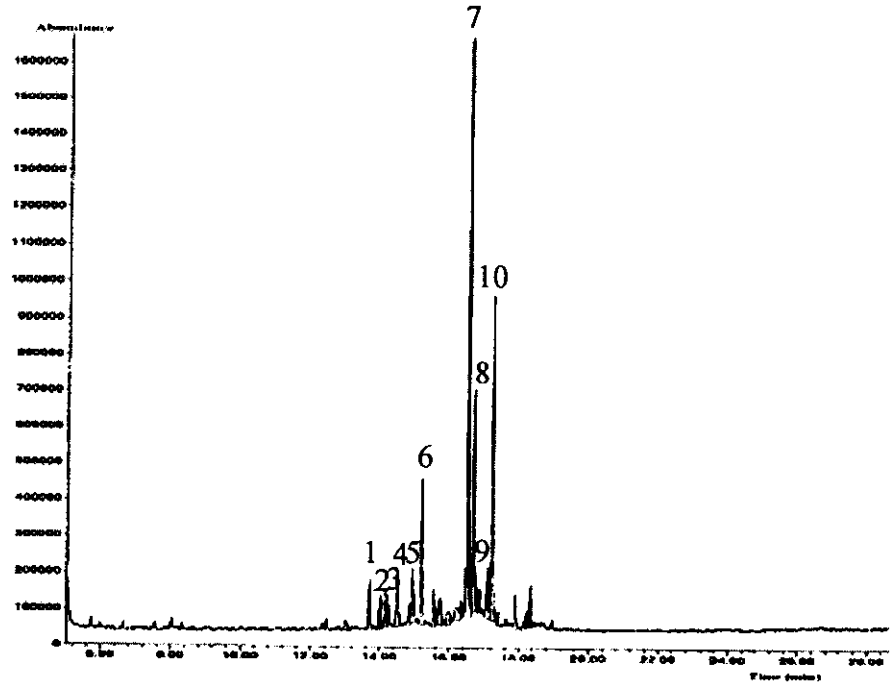


Figure 3-11 GC-MS chromatogram of volatile oil obtained from zedoary (finger) rhizome after 12 months storage.

Table 3-14 Chemical constituents of zedoary (finger) rhizome after different storage periods as analysed by GC-MS.

Retention Time (min)	Chemical constituents		
	0 months	6 months	12 months
3.79	β-Elemene (1)	-	-
3.77	-	β-Elemene (1)	-
3.97	α-Zingiberene (2)	-	-
3.98	-	α-Zingiberene (2)	-
4.09	β-Caryophyllene (3)	-	-
4.09	-	β-Caryophyllene (3)	-
4.27	γ-Elemene (4)	-	-
4.27	-	γ-Elemene (4)	-
4.83	ar-Curcumene (5)	-	-
4.81	-	ar-Curcumene (5)	-
13.71	-		ar-Curcumene (1)

Number in parenthesis is peak no.

Table 3-14 Chemical constituents of zedoary (finger) rhizome after different storage periods as analysed by GC-MS (continued).

Retention Time (min)	Chemical constituents		
	0 months	6 months	12 months
5.05	Zingiberene (6)	-	-
5.03	-	Zingiberene (6)	-
14.04			Zingiberene (2)
5.24	β-Bisabolene (7)	-	-
5.22	-	β-Bisabolene (7)	-
14.20	-	-	β-Bisabolene (3)
5.40	β-Sesquiphyllandrene (8)	-	-
5.38	-	β-Sesquiphyllandrene (8)	-
14.51			β-Sesquiphyllandrene (4)
5.80	Germacrene B (9)	-	-
5.78	-	Germacrene B (9)	-
14.95	-	-	Germacrene B (5)
5.99	*p*-Cymene (10)	-	-
5.97	-	*p*-Cymene (10)	-
15.21	-	-	*p*-Cymene (6)
7.06	β-Turmerone (11)	-	-
7.04	-	β-Turmerone (11)	-
16.51	-	-	β-Turmerone (7)
7.22	ar-Turmerone (12)		-
7.17	-	ar-Turmerone (12)	-
16.68	-	-	ar-Turmerone (8)
7.49	Germacrone (13)	-	-
7.45		Germacrone (13)	-
17.08	-	-	Germacrone (9)
7.62	α-Turmerone (14)	-	-
7.59	-	α-Turmerone (14)	-
17.22	-	-	α-Turmerone (10)

Number in parenthesis is peak no.

The major compounds so far identified in the volatile oil of turmeric and reported in literature include α-phellandrene, 1,8-cineol, zingiberene, ar-curcumene, turmerone, ar-turmerone, β-sesquiphellandrene, curlone and dehydrozingerone (Hiserodt *et al.*, 1996). Earlier studies (McCarron *et al.*, 1995; Vaisan *et al.*, 1989) on the volatile oil of turmeric have shown the presence of several monoterpenes, such as α-pinene, α-terpinene, camphene, limonene and γ-terpinene in the volatile oil of turmeric. In the present study, however the above monoterpene compounds were not detected. This could possibly be due to the variation in the type of raw materials and age of samples.

The major sesquiterpene compounds, including dehydrocurdione, furanodiene, germacrone, çurdione, neocurdione, curcumenol, isocurcumenol, aerugidiol, zedoarondiol, epicurzerenone and curzerene have been identified in the volatile oil of zedoary (Mau *et al.*, 2003; Yoshioka *et al.*, 1998). The gas chromatographic profiles and constituents of volatile oil obtained from zedoary (bulb) and zedoary (finger) rhizomes were similar to the finding of Mau *et al.* (2003) and Tang and Eisenbrand (1992) that the volatile oil of *Curcuma zedoaria* contained high ratios of sesquiterpene compounds in the identified compounds. From the results, the major constituents in zedoary (bulb) and zedoary (finger) rhizomes were ar-turmerone, β-turmerone. Ealier works Mau *et al.* (2003) and Yoshioka *et al* (1998), the major constituents in volatile oil of zedoary were dehydrocurdione and epicurzerenone, respectively. Difference with these major components compared to those previously reported in the literatures for the same aromatic plants collected in other geographic areas in the world could be attributed

to some factors, such as climatic, nature of soil, age of the tree, time of collection, mode of extraction, etc (Cimanga *et al.*, 2002; Rao *et al.*, 1996). The data suggested from the study, qualitative difference could be observed in GC-MS profiles of volatile oil obtained from turmeric and zedoary rhizomes during different storage periods. This could possibly be due to certain factors (e.g. air, moisture and heat), causing the degradation of constituents in volatile oil. Atmospheric oxygen, heat and light have been shown to change the chemical composition of volatile oil (Tisserand and Balaces, 1995).

4. Curcuminoids content of turmeric and zedoary rhizomes during accelerated stability studies.

The stability of curcuminoids in powdered and sliced turmeric and zedoary rhizomes stored in black polyethylene and paper bags was performed at 3 elevated temperatures; 45°, 55° and 70 °C (75 ± 5 % RH). Curcuminoids content were determined as previously described (section 1). The standard curve is shown in Figure A-8 (Appendix A). Regression analysis gave correlation coefficients (r^2) of 0.9995.

Curcuminoids content of powdered and sliced turmeric, zedoary (bulb) and zedoary (finger) rhizomes at different accelerated temperatures expressed as the percentage of w/w are shown in Tables 3-15, 3-16 and 3-17, respectively.

Curcuminoids content of powdered and sliced turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in black polyethylene and paper bags subjected to accelerated thermal stability testing during zero to 90 days storage were analyzed

by the Arrhenius law to predict half-life ($t_{1/2}$) and shelf-life ($t_{90\%}$). Plots of ln value of curcuminoids content (c) versus storage periods (t) of exposure at each temperature provided linear regression (t) (Appendix A, Figure A-9 to A-12, Figure A-13 to A-16 and Figure A-17 to Figure A-20, respectively).

Curcuminoids decomposed according to the first order kinetics for all treatments. Similar kinetic behavior was observed for the degradation of curcuminoids pigments in curcumin and turmeric oleoresin microcrystalline cellulose model systems during storage at 21 ± 1 °C (Souza *et al.*, 1997). First order reaction was also observed for the degradation of curcuminoids by light, solvent system and oxygen (Price and Buescher, 1996). The extrapolations of Arrhenius plots of curcuminoids content of powdered and sliced turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in black polyethylene and paper bags are shown in Appendix A, Figure A-21 to Figure A-24, Figure A-25 to Figure A-28 and Figure A-29 to Figure A-32, respectively.

Table 3-15 Curcuminoids content of turmeric rhizome at accelerated temperature.

Accelerated temperatures/ Time	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
45°C/ (days)				
0	20.45 ± 0.28	20.14 ± 0.10	20.55 ± 0.07	20.14 ± 0.10
7	20.27 ± 0.15	20.13 ± 0.20	20.26 ± 0.14	20.00 ± 0.18
14	20.10 ± 0.36	20.10 ± 0.36	20.13 ± 0.33	19.98 ± 0.20
28	19.92 ± 0.28	19.82 ± 0.27	19.89 ± 0.29	19.87 ± 0.15
45	19.84 ± 0.29	19.63 ± 0.32	19.82 ± 0.35	19.77 ± 0.38
60	19.67 ± 0.22	19.53 ± 0.18	19.66 ± 0.24	19.62 ± 0.12
90	19.44 ± 0.13	19.43 ± 0.09	19.42 ± 0.07	19.27 ± 0.06
55°C/ (days)				
0	20.45 ± 0.28	20.14 ± 0.10	20.55 ± 0.07	20.14 ± 0.10
7	19.92 ± 0.31	19.94 ± 0.25	19.91 ± 0.23	19.90 ± 0.22
14	19.73 ± 0.18	19.74 ± 0.23	19.73 ± 0.14	19.70 ± 0.15
28	19.53 ± 0.25	19.52 + 0.27	19.51 ± 0.21	19.53 ± 0.42
45	19.38 ± 0.32	19.42 + 0.29	19.30 ± 0.05	19.39 ± 0.33
60	19.14 ± 0.10	19.22 ± 0.32	19.11 ± 0.19	19.17 ± 0.05
90	18.82 ± 0.14	18.88 ± 0.09	18.75 ± 0.14	18.77 ± 0.07
70°C/ (days)				
0	20.45 ± 0.28	20.14 ± 0.10	20.55 ± 0.07	20.14 ± 0.10
7	19.63 ± 0.16	19.76 ± 0.23	19.74 ± 0.25	19.59 ± 0.33
14	19.38 ± 0.13	19.29 ± 0.08	19.31 ± 0.06	19.26 ± 0.21
28	18.53 ± 0.06	18.54 ± 0.11	18.39 ± 0.13	18.72 ± 0.60
45	17.49 ± 0.04	17.16 ± 0.11	17.22 ± 0.14	17.15 ± 0.15
60	16.96 ± 0.09	16.66 ± 0.09	16.77 ± 0.25	16.57 ± 0.10
90	15.55 ± 0.07	15.39 ± 0.09	15.53 ± 0.17	15.36 ± 0.23

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-16 Curcuminoids content of zedoary (bulb) rhizome at accelerated temperatures.

Accelerated temperatures/ Time	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
45°C/ (days)				
0	1.10 ± 0.05	1.19 ± 0.04	1.09 ± 0.02	1.19 ± 0.04
7	1.06 ± 0.03	1.18 ± 0.02	1.02 ± 0.01	1.13 ± 0.04
14	1.06 ± 0.05	1.11 ± 0.03	1.01 ± 0.02	1.08 ± 0.03
28	1.04 ± 0.03	1.08 ± 0.06	0.98 ± 0.01	1.07 ± 0.06
45	0.91 ± 0.03	1.03 ± 0.02	0.89 ± 0.02	0.98 ± 0.01
60	0.90 ± 0.02	1.01 ± 0.01	0.87 ± 0.02	0.98 ± 0.01
90	0.84 ± 0.02	0.89 ± 0.02	0.79 ± 0.04	0.89 ± 0.02
55°C/ (days)				
0	1.10 ± 0.05	1.19 ± 0.04	1.09 + 0.02	1.19 ± 0.04
7	0.98 ± 0.00	1.06 ± 0.04	0.91 ± 0.05	1.07 ± 0.05
14	0.94 ± 0.05	1.02 + 0.02	0.89 ± 0.06	0.98 ± 0.02
28	0.89 ± 0.01	0.94 + 0.02	0.87 ± 0.02	0.93 ± 0.03
45	0.83 ± 0.03	0.91 + 0.03	0.78 ± 0.03	0.91 ± 0.06
60	0.82 ± 0.01	0.89 + 0.03	0.65 ± 0.03	0.88 ± 0.03
90	0.46 ± 0.03	0.49 ± 0.02	0.38 ± 0.00	0.49 ± 0.02
70°C/ (days)				
0	1.10 ± 0.05	1.19 ± 0.04	1.09 + 0.02	1.19 ± 0.04
7	0.83 ± 0.01	0.93 ± 0.03	0.78 ± 0.01	0.94 + 0.04
14	0.73 ± 0.00	0.78 ± 0.02	0.72 ± 0.01	0.75 ± 0.02
28	0.64 ± 0.03	0.67 ± 0.02	0.62 ± 0.02	0.65 ± 0.04
45	0.46 ± 0.04	0.51 ± 0.02	0.44 ± 0.01	0.51 ± 0.03
60	0.42 ± 0.01	0.50 ± 0.01	0.41 ± 0.03	0.50 ± 0.01
90	0.28 ± 0.01	0.30 ± 0.02	0.27 ± 0.03	0.30 ± 0.02

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

Table 3-17 Curcuminoids content of zedoary (finger) rhizome at accelerated temperatures.

Accelerated temperatures/ Time	Curcuminoids content (% w/w) (Mean ± S.D.*)			
	Powdered/BPB	Sliced/BPB	Powdered/PB	Sliced/PB
45°C/ (days)				
0	1.53 ± 0.05	1.57 ± 0.05	1.53 ± 0.03	1.54 ± 0.06
7	1.51 ± 0.02	1.55 ± 0.06	1.49 ± 0.03	1.53 ± 0.01
14	1.46 + 0.03	1.53 + 0.02	1.44 + 0.02	1.47 + 0.04
28	1.42 ± 0.05	1.42 ± 0.08	1.41 ± 0.07	1.46 ± 0.06
45	1.36 ± 0.03	1.42 ± 0.03	1.35 ± 0.05	1.40 ± 0.01
60	1.34 ± 0.03	1.37 ± 0.03	1.34 ± 0.02	1.36 ± 0.02
90	1.22 ± 0.01	1.27 ± 0.03	1.19 ± 0.02	1.25 ± 0.02
55°C/ (days)				
0	1.53 ± 0.05	1.57 ± 005	1.53 ± 0.03	1.54 ± 0.06
7	1.48 ± 0.03	1.51 ± 0.02	1.45 ± 0.02	1.51 ± 0.02
14	1.45 ± 0.02	1.48 ± 0.03	1.37 ± 0.03	1.42 ± 0.03
28	1.09 ± 0.03	1.09 ± 0.06	1.07 ± 0.01	1.03 ± 0.02
45	0.97 ± 0.03	1.03 ± 0.02	0.97 ± 0.03	1.02 ± 0.00
60	0.95 ± 0.02	1.01 ± 0.01	0.96 ± 0.02	1.01 ± 0.01
90	0.90 ± 0.02	0.94 ± 0.03	0.89 ± 0.02	0.90 ± 0.03
70°C/ (days)				
0	1.53 ± 0.05	1.57 ± 0.05	1.53 ± 0.03	1.54 ± 0.06
7	1.20 ± 0.02	1.32 ± 0.06	1.22 ± 0.01	1.31 ± 0.04
14	1.05 ± 0.03	1.20 ± 0.01	1.05 ± 0.05	1.13 ± 0.07
28	0.86 ± 0.02	0.87 ± 0.02	0.83 ± 0.02	0.86 ± 0.02
45	0.62 ± 0.02	0.66 ± 0.03	0.62 ± 0.03	0.63 ± 0.01
60	0.57 ± 0.03	0.62 ± 0.03	0.58 ± 0.02	0.59 ± 0.03
90	0.37 ± 0.03	0.46 ± 0.03	0.36 ± 0.03	0.43 ± 0.03

* Mean ± S.D. from triplicate determinations.
BPB = black polyethylene bag
PB = paper bag

4.1 Stability of curcuminoids content in turmeric rhizome.

Curcuminoids were fairly stable at 45 °C and 55 °C (mean content about 20.0 and 19.0 % w/w at zero and 90 days, respectively), and only decreased slightly (about 25 %) at 70 °C after 90 days (mean content about 20.0 and 15.5 % w/w at zero and 90 days, respectively).

The extrapolation of curcuminoids content of powdered and sliced rhizomes stored in black polyethylene and paper bags obtained from a relationship between ln value of rate constant (ln k) and reciprocal of thermodynamic temperature (T) led to the estimated k at room temperature (30 °C) of 1.27 $x10^{-4}$, 1.19 x 10^{-4}, 1.27 x 10^{-4} and 1.19 x 10^{-4} (Table 3-18), respectively.

Curcuminoids content of sliced rhizome stored in black polyethylene and paper bags was higher than that of the powdered rhizome and their predicted half-life ($t_{1/2}$) and shelf-life ($t_{90\%}$) were 16.2 and 2.5 years, respectively; and 16.1 and 2.5 years, respectively. The predicted half-life and shelf-life of powdered rhizome stored in black polyethylene and paper bags were 15.2 and 2.3 years; and 11.1 and 1.7 years (Table 3-18). From these results, it is clear that turmeric rhizome prepared as slices can be stored in black polyethylene and paper bags longer than that of the powdered rhizomes.

Table 3-18 Rate constant at 30 °C ($k_{30°C}$), half-life ($t_{1/2}$) and shelf-life ($t_{90\%}$) of turmeric rhizome.

Samples	$k_{30°C}$ (day^{-1})	$t_{1/2}$ (years)	$t_{90\%}$ (years)
Powdered/BPB	1.27×10^{-4}	15.2	2.3
Sliced/BPB	1.19×10^{-4}	16.2	2.5
Powdered/PB	1.27×10^{-4}	11.1	1.7
Sliced/PB	1.19×10^{-4}	16.1	2.5

BPB = black polyethylene bag
PB = paper bag

4.2 Stability of curcuminoids content in zedoary (bulb) and zedoary (finger) rhizomes.

4.2.1 Stability of curcuminoids content of zedoary (bulb) rhizome.

Compared to turmeric rhizome, zedoary (bulb) rhizome appears to lose an appreciable amount of its curcuminoids content when stored at all three temperatures (45°, 55° and 70 °C), with the highest loss at the highest temperature. At 45 °C, curcuminoids content was decreased slightly (about 18.0 %) at zero and 90 days (mean content about 1.1 and 0.9 % w/w, respectively). Curcuminoids content drastically decreased (about 55.0 %) at 55 °C after 90 days (mean content about 1.1 and 0.5 % w/w at zero and 90 days, respectively) and about 73.0 % at 70 °C after 90 days (mean content about 1.1 and 0.3 % w/w at zero and 90 days, respectively).

The extrapolation of curcuminoids content of powdered and sliced zedoary (bulb) rhizome stored in black polyethylene and paper bags obtained

from a relationship between ln value of rate constant (ln k) and reciprocal of thermodynamic temperature (T) led to the estimated k at 30 °C ($k_{30°C}$) of 1.35 $x10^{-3}$, 1.30 x 10^{-3}, 1.36 x 10^{-3} and 1.36 x 10^{-3} (Table 3-19), respectively. Half-life and shelf-life for the degradation of curcuminoids are summarized in Table 3-19. Predicted shelf-life of powdered and sliced rhizome stored in black polyethylene and paper bags were about 0.2 year. The results suggested that curcuminoids of sliced rhizome stored in black polyethylene bags had high stability over those stored in paper bags, and over those prepared as powders and stored in both polyethylene and paper bags. The data show that zedoary (bulb) rhizome prepared as slices and stored in black polyethylene bags tend to better preserve curcuminoids content.

Table 3-19 Rate constant at 30 °C ($k_{30°C}$), half-life ($t_{1/2}$) and shelf-life ($t_{90\%}$) of zedoary (bulb) rhizome.

Samples	$k_{30°C}$ (day^{-1})	$t_{1/2}$ (years)	$t_{90\%}$ (year)
Powdered/BPB	1.35 x 10^{-3}	1.4	0.2
Sliced/BPB	1.30 x 10^{-3}	1.5	0.2
Powdered/PB	1.36 x 10^{-3}	1.4	0.2
Sliced/PB	1.36 x 10^{-3}	1.4	0.2

BPB = black polyethylene bag
PB = paper bag

4.2.2 Stability of curcuminoids content of zedoary (finger) rhizome.

As in the case of zedoary (bulb) rhizome, curcuminoids in zedoary (finger) rhizome decreased with increasing temperature, with the highest loss at the highest temperature. At 45 °C, curcuminoids content was slightly decreased (about 20.0 %) at zero and 90 days (mean content about 1.5 and 1.2 % w/w, respectively). Curcuminoids content was rapidly decreased (about 40.0 %) at 55 °C after 90 days (mean content about 1.5 and 0.9 % w/w at zero and 90 days, respectively) and about 73.0 % at 70 °C after 90 days (mean content about 1.5 and 0.4 % w/w at zero and 90 days, respectively).

The extrapolation obtained from a relationship between ln value of rate constant (ln k) and reciprocal of thermodynamic temperature (T) led to the estimated k at 30 °C ($k_{30}{}^{\circ}C$) of 7.68×10^{-4}, 7.19×10^{-4}, 7.99×10^{-4} and 7.48×10^{-4}, respectively, for each of the four preparations (Table 3-20). Stability data revealed a predicted shelf-life of powdered and sliced zedoary (finger) rhizome stored in black polyethylene and paper bags of about 0.4 year. Curcuminoids content of sliced zedoary (finger) was more stable than of powdered rhizome stored in both black polyethylene and paper bags. The lowest curcuminoids content was found in the powdered rhizome. Therefore, it is clear that zedoary (finger) rhizome prepared as slices maintain curcuminoids content better than that of powdered rhizome. Furthermore, the result indicated that the types of containers during storage are an important factor for the quality for herbal materials. Curcuminoids and volatile oil contents of zedoary (finger) rhizome both in the forms of powdered

and sliced stored in black polyethylene bags could be better maintained than those stored in paper bags.

Table 3-20 Rate constant at 30 °C ($k_{30}°C$), half-life ($t_{1/2}$) and shelf-life ($t_{90\%}$) of zedoary (finger) rhizome.

Samples	$k_{30}°C$ (day^{-1})	$t_{1/2}$ (years)	$t_{90\%}$ (years)
Powdered/BPB	7.68×10^{-4}	2.5	0.4
Sliced/BPB	7.19×10^{-4}	2.7	0.4
Powdered/PB	7.99×10^{-4}	2.4	0.4
Sliced/PB	7.48×10^{-4}	2.6	0.4

BPB = black polyethylene bag
PB = paper bag

Results of the studies described in sections 4.1-4.2, suggest that curcuminoids content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes prepared as powders were more unstable with increasing storage periods and temperatures than sliced rhizome. This may be due to the particle size of powdered is smaller than sliced rhizomes, having higher surface area, some factors such as air, heat and light could affect easily. Thus, it is expected that these rhizomes prepared as slices could maintain curcuminoids content for longer than powdered rhizome.

The predicted shelf-life of curcuminoids of turmeric and zedoary rhizomes agree well with real time stability data of curcuminoids content gathered under storage of these rhizomes at room temperature (e.g. predicted shelf-life of

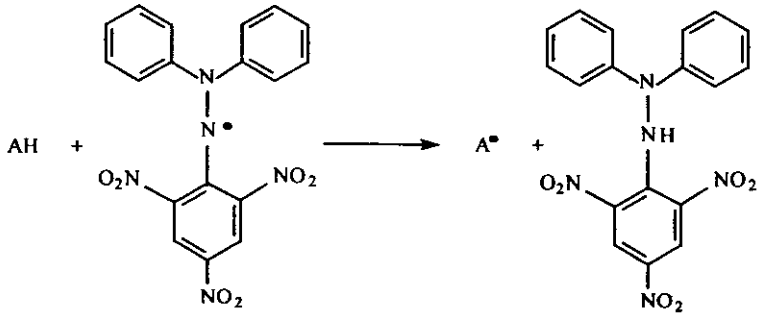
powdered zedoary (bulb) rhizome stored in black polyethylene bags was about 0.2 year and curcuminoids content obtained from this sample was decreased (about 12 %) after 3 months (0.25 year) during storage at room temperature.

5. Antioxidant activity of turmeric and zedoary rhizomes at different storage periods.

Free radical generation occurs normally in the human body, and rates of free radical generation probably increase in most disease states. Furthermore, since oxygen radical reactions and membrane damage play a key role in aging, it is not surprising that diet supplementation with antioxidants such as vitamin C and E or components of antioxidant enzymes: superoxide dismutase (Mn, Cu, Zn) or glutathione peroxidase (Se) are believed to increase life span and protect against artherosclerosis, age-related immune decline and liver-mitochondrial damage (Miquel *et al.*, 2002; Evans and Halliwell, 2001). The development of new assays applicable to humans should allow rapid evaluation of the role of free radicals in disease pathology and provide a logical basis for the therapeutic use of antioxidants (Aruoma, 1994). Antioxidants are elements of a collection of processes that retard *in vivo* free radical oxidation. The term antioxidation includes all of the processes that slow down or stop free radical oxidation. Antioxidation processes include 1) scavenging radicals to prevent their propagation, 2) enzymatic hydrolysis of ester bonds to remove peroxidized fatty acids from lipid, 3) sequestration of transition metal ions and 4) enzyme-catalyzed reduction of peroxides (Thomas, 2000). Antioxidant defences in the human body consist of low

molecular mass antioxidants such as vitamins C and E and enzymes, e.g. superoxide dismutase and these are summarized in Figure 3-12 (Evans and Halliwell, 2001).

Proton radical-scavenging is known to be one of the mechanisms for antioxidant action. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), commonly employed for screening plant extracts, is one of the many compounds that possess a proton free radical and shows a characteristic absorption at 520 nm (Kang *et al.*, 1997; Soares *et al.*, 1997; Mathiesen *et al.*, 1995; Yamasaki *et al.*, 1994). The absorbance of DPPH decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule. The main reaction is as shown below:



DPPH DPPH-H

The more rapidly the absorbance decreases, the more potent the antiradical activity of the compound in term of hydrogen donating ability (Bandoniene *et al.*, 2002).

To determine radical scavenging activity, the ethanolic extracts of powdered turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in black

polyethylene bags before (zero time) and after 6 and 12 months storage were tested. Radical scavenging properties of ethanolic extracts were evaluated against the DPPH radical. BHT, a well known compound as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat, 1997), was used as a reference compound.

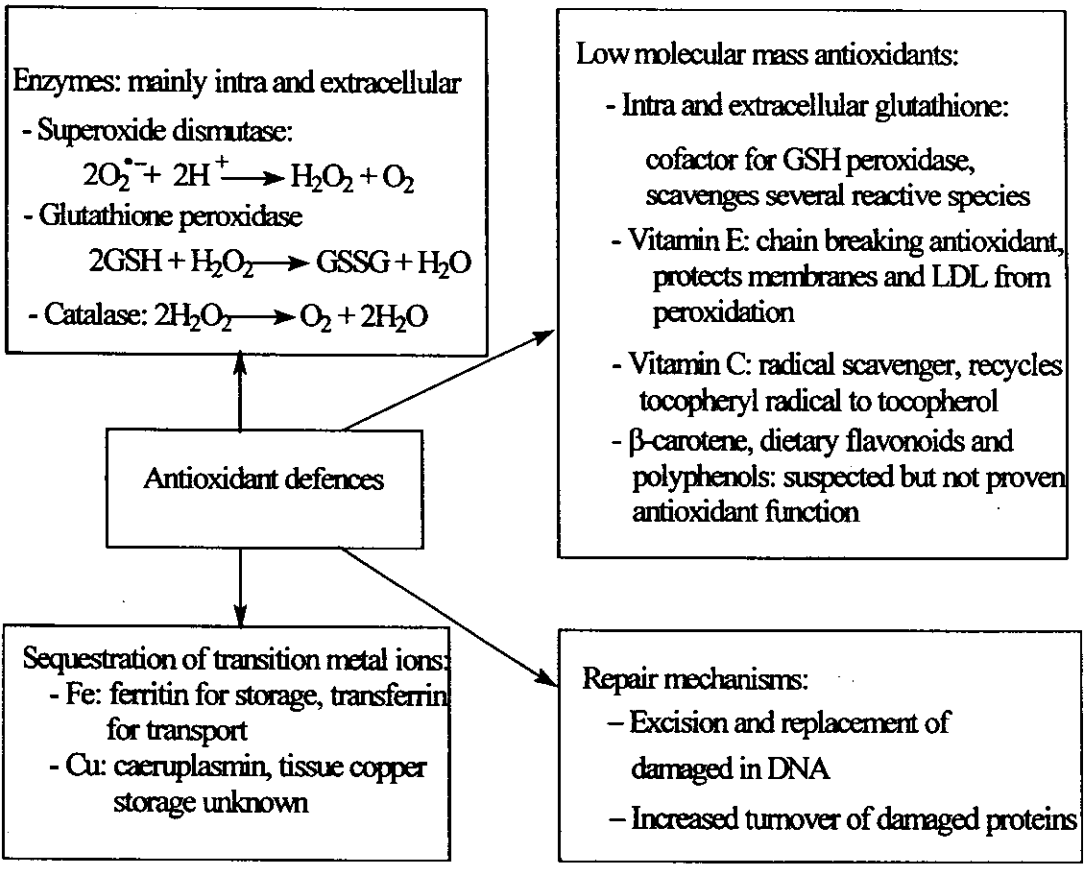


Figure 3-12 Summary of antioxidant defences in human body (Evans and Halliwell, 2001).

5.1 Radical scavenging activity of turmeric, zedoary (bulb) and zedoary (finger) rhizomes extracts at different storage periods.

The scavenging effects of turmeric, zedoary (bulb) and zedoary (finger) extracts before (zero time) and after 6 and 12 months storage on the DPPH radical are presented in Figures 3-13, 3-14 and 3-15, respectively. The scavenging effect of turmeric and zedoary extracts on the DPPH radical increased with increasing amount of extracts. The effect of antioxidants on DPPH radical scavenging is

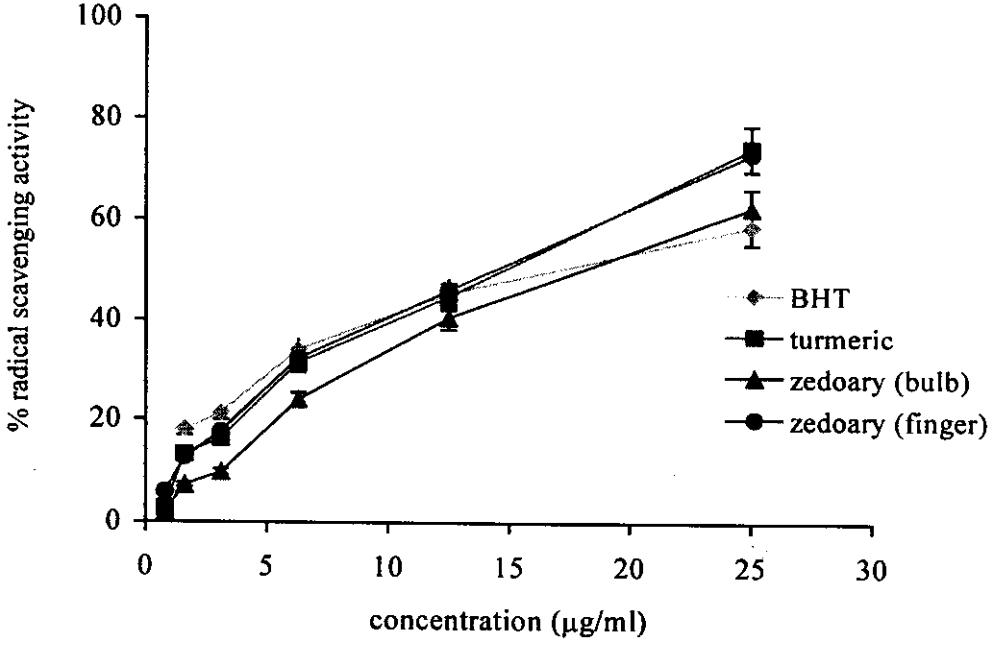


Figure 3-13 Radical scavenging effect of ethanolic extracts from turmeric and zedoary rhizomes before storage (zero time) on DPPH radical.

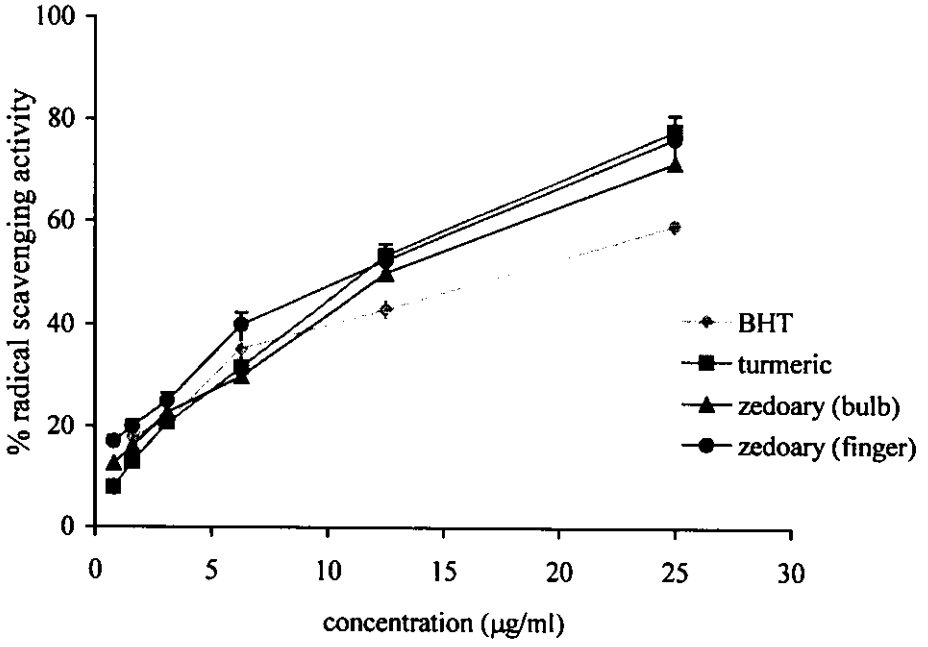


Figure 3-14 Radical scavenging effect of ethanolic extracts from turmeric and zedoary rhizomes after 6 months storage on DPPH radical.

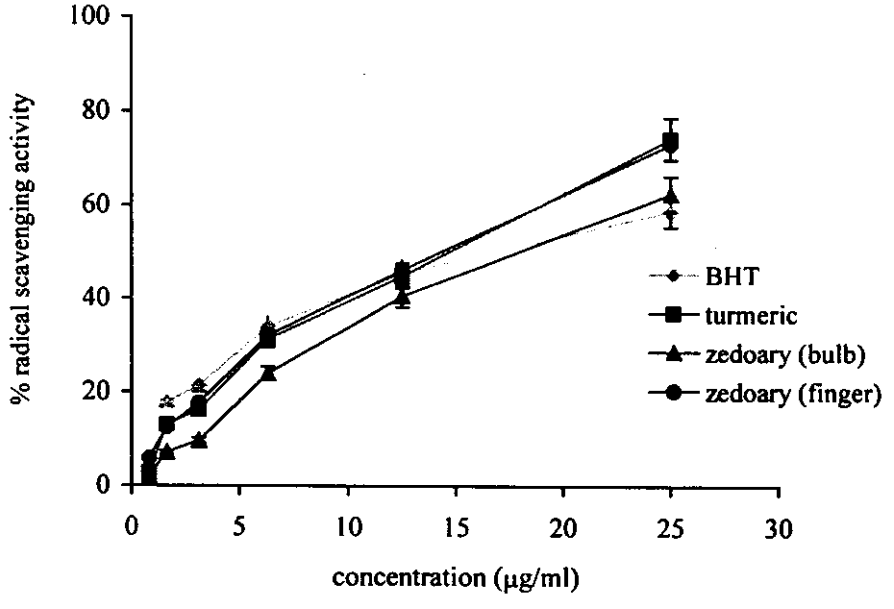


Figure 3-15 Radical scavenging effect of ethanolic extracts from turmeric and zedoary rhizomes after 12 months storage on DPPH radical.

thought to be due to their hydrogen donating ability (Brain-Williams *et al.*, 1995). Yen and Duh (1994) reported that the DPPH radical scavenging effect of phenolic compounds is due to their hydrogen-donating ability. Furthermore, phenolic extracts of plant have been shown to neutralize free radicals in various models (Lissi *et al.*, 1999; Wettasingha and Shahidi, 1999). The relative activity of different polyphenolic compounds was related to the number and location of hydroxyl groups (Lin *et al.*, 1996). These findings indicated that the general structure of curcumin-related phenols (curcuminoids), conjugated dienes with an adjacent phenolic group have the general property to act as potent antioxidant compounds, not only in food systems but also in biological systems (Anto *et al.*, 1996; Masuda *et al.*, 1993).

5.2 Concentration of turmeric, zedoary (bulb) and zedoary (finger) rhizomes extracts at different storage periods required to scavenge 50 % (IC_{50}) DPPH free radical.

The antioxidant activities of ethanolic extracts of turmeric and zedoary rhizomes, evaluated as IC_{50} values, are shown in Table 3-21. The IC_{50} values of the extract of turmeric stored at zero time, after 6 and 12 months were 10.7, 11.6 and 13.9 μg/ml, respectively, whereas IC_{50} values of BHT at zero time and after 6 and 12 months were 17.4, 17.9 and 17.7 μg/ml, respectively. The differences in IC_{50} of turmeric extract were observed between samples stored for different storage periods ($p<0.05$). The data revealed that turmeric extract obtained from rhizome before storage (zero time) had significantly higher (lower IC_{50}) antioxidant activity

than extracts from rhizome after 6 and 12 months storage. For the zedoary (bulb) extract, differences in IC_{50} were also observed among different storage periods ($p < 0.05$). IC_{50} of zedoary (bulb) extract before storage and after 6 and 12 months storage was 12.3, 12.7 and 15.2 μg/ml, respectively. IC_{50} of zedoary (bulb) extract at 12 months storage was significantly higher (lower activity) than extract before storage and after 6 and 12 months storage. For the zedoary (finger), IC_{50} values of zero time and after 6 and 12 months storage of this extract were 10.4, 11.2 and 13.4 μg/ml, respectively. A significant increase ($p<0.05$) in IC_{50} of zedoary (finger) extract was found at 12 months storage.

Table 3-21 Antioxidant activity of ethanolic extracts of turmeric and zedoary rhizomes at different storage periods.

Storage periods	**IC_{50} (μg/ml) (mean ± S.D.)**			
(months)	**Turmeric**	**Zedoary (bulb)**	**Zedoary (finger)**	**BHT**
0	10.7 ± 0.2 c	12.3 ± 0.2 b	10.4 ± 0.2 b	17.4 ± 0.8
6	11.6 ± 0.2 b	12.7 ± 0.3 b	11.2 ± 0.5 b	17.9 ± 1.1
12	13.9 ± 1.1 a	15.2 ± 0.1 a	13.4 ± 0.6 a	17.7 ± 1.5
F-test	*	*	*	NS
% C.V.	1.49	1.60	4.10	6.55

Mean ± S.D. (n = 3) followed by the different letters in the same column denote the significant differences, according to Duncan' s multiple range test.
* significant at $p < 0.05$.

The curcuminoids isolated from turmeric are good antioxidants (Das and Das, 2002; Bonte, *et al.*, 1997; Ruby *et al.*, 1995). Curcumin and its derivative are found to be potent antioxidants (Jovanovic *et al.*, 2001; Masuda *et al.*, 2001; Guha

et al., 1997; Masuda *et al.*, 1993). The quality of natural extracts and their antioxidative performances depends not only the quality of the original plants, the geographic origin, climatic condition, harvesting date and storage, but also environmental and technological factors (Cuvelier *et al.*, 1996)

From the results, increased in storage periods (0-12 months) showed differences in IC_{50} of turmeric and zedoary extracts (higher IC_{50} values on longer storage), and this might be presumed to be due to the decreasing content of antioxidative compounds in the extracts. Mau *et al.* (2003) reported that the volatile oil of zedoary was moderate to good in antioxidant activities by different methods, excellent in scavenging effect on DPPH radical, but low in chelating effect on ferrous ion. The decline in antioxidant activity may be due to the decrease in volatile oil content of turmeric, zedoary (bulb) and zedoary (finger) rhizomes at different storage periods (Appendix A, Tables A-17, A-22 and A-27, respectively). Furthermore, the decrease in curcuminoid content of zedoary (bulb) and zedoary (finger) rhizome (Appendix A, Tables A-7 and A-11, respectively) may also affect the increase in IC_{50} of these extracts. It is proposed that the decrease in antioxidant activities of turmeric, zedoary (bulb) and zedoary (finger) extracts is probably due to the increase in moisture content of these rhizomes at different storage periods (Appendix A, Tables A-34, A-39 and A-44, respectively), because an excess of water will encourage deterioration following hydrolysis of compounds, such as volatile oil in these rhizomes (World Health Organization, 1998).

The extracts of these turmeric and zedoary rhizomes at different storage periods have similar free radical scavenging activity (DPPH) in comparison to well-known dietary antioxidants, namely green tea, black tea, ascorbic acid (vitamin C) and Trolox ® (a water-soluble analogue of vitamin E) (Table 3-22). Tea, vitamin C and vitamin E are important dietary antioxidants (Wiseman *et al.*, 1997; Sies and Stahl, 1995). Tea, especially green tea, has been studied extensively for its antioxidant activity in relation to cancer (Katiyar and Mukhtar, 1997) and cardiovascular disease (Tijburg *et al.*, 1997). Tea contains tannin, with most of its antioxidant activity attributed to catechins (Nanjo *et al.*, 1996). Rather than being a single chemical, tea has the combined activity of flavonoids, most being catechins, theaflavins and flavonols (Wiseman *et al.*, 1997), that can lead to enhanced activity. Indeed, all of turmeric and zedoary extracts at different storage periods (IC_{50} about 10-15 μg/ml) performed better than carrot (IC_{50} about 832 μg/ml) which contains carotenes, and garlic extracts (IC_{50} about 833 μg/ml) against free radicals.

Interestingly, these results from this study suggested that the increase in storage periods of turmeric and zedoary rhizomes might tend to result in a decrease in antioxidant activity. However, after 12 months storage of these rhizomes, their extracts have similar free radical scavenging activity in comparison to well-known antioxidant agents (e.g. green tea, vitamin C and vitamin E).

Table 3-22 Antioxidant activity of common vegetables and standards as assessed with the DPPH assay.

Crude extract	IC_{50} (μg/ml) (mean ± S.E.*)
Green tea	6.8 ± 0.1
Black tea	15.2 ± 1.0
Carrots	831.5 ± 163.9
Garlic	833.3 ± 97.0
Ascorbic acid	5.2 ± 0.2
Trolox®, [a]	9.0 ± 2.1

* Mean ± S.E. (n = 3).
[a] Trolox ® (a water soluble analogue of α-tocopherol or vitamin E).
From: McCune and Johns, 2002.

6. Antibacterial activity of turmeric and zedoary rhizomes at different storage periods.

Bacteria, namely *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* are common microbes found in most natural environments including soil, water, plants and animal tissues. They have been known to act as a primary invader or secondary infectious agent in a number of diseases and have been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). Effects of the ethanolic extracts of powdered turmeric, zedoary (bulb) and zedoary (finger) rhizomes from black polyethylene bags before storage (zero time) and after 6 and 12 months storage on the growth of different bacteria (*S. aureus* ATCC 25923, *B. subtilis* and *E. coli* ATCC 25922) were determined. Tetracycline and DMSO were used as reference compounds.

6.1 Effect of turmeric, zedoary (bulb) and zedoary (finger) extracts at different storage periods on the growth of bacteria as determined by the disc-diffusion method.

As determined from the disc-diffusion assay, the antibacterial activities of turmeric rhizome extract at different storage periods on the growth of gram-positive (*S. aureus* ATCC 25923 and *B. subtilis*) and gram-negative (*E. coli* ATCC 25922) bacteria are shown in Appendix A, Tables A-46, A-47 and A-48, respectively. The results show that these extracts of all different storage periods were active against gram-positive bacteria, except *E. coli* ATCC 25922 was not sensitive to the extracts of all concentrations (1.3 – 10.0 mg/disc). Clear zone of inhibition of the extracts on *S. aureus* ATCC 25923 and *B. subtilis* were in the range of 6.00 - 10.00 mm and 6.00 - 9.83 mm, respectively, whereas those of tetracycline on *S. aureus* ATCC 25923, *B. subtilis* and *E. coli* ATCC 25922 were 26.50, 19.83 and 22.83 mm, respectively. This means that at the concentrations studied, the extracts of turmeric, zedoary (bulb) and zedoary (finger) rhizomes were less active as antibacterial agents than tetracycline.

6.2 Minimum inhibition concentration (MIC) of turmeric, zedoary (bulb) and zedoary (finger) extracts at different storage periods on the growth of bacteria as determined by the agar-dilution method.

MIC of turmeric, zedoary (bulb) and zedoary (finger) extracts before storage (zero time) and after 6 and 12 months storage are demonstrated in Table 3-23. MIC was reported as the lowest concentration of the compound capable for

inhibiting the complete growth of the bacterium tested (Jayaprakasha *et al.*, 2003). From this study, the results show that all ethanolic extracts at different storage periods were found to have effective antibacterial action against gram-positive bacteria. Moreover, MIC of all extracts on two strains of the gram-positive bacteria was considerably lower than those for gram-negative bacteria. None of the ethanolic extracts completely inhibited the growth of *E. coli.* These observations are likely to be the results of the differences in cell wall structure between gram-positive and gram-negative, with the gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Palombo and Semple, 2001).

The MIC of all extracts after 12 months storage on *S. aureus* ATCC 25923 was higher than zero time and after 6 months storage. Similarly, for *B. subtilis* the MIC of all extracts after 12 months storage was higher than those before storage and after 6 months storage. Thus, the antibacterial activity of turmeric, zedoary (bulb) and zedoary (finger) seems to decrease with increased storage periods. These might be presumed to be due to the different lower content of active compounds in the extracts. Antibacterial activities of turmeric and zedoary rhizomes previously have been reported (Araujo and Leon, 2001; Syu *et al.*, 1998; Lutomski *et al.*, 1974). The active compound of turmeric for the inhibition of bacteria was related to the presence of volatile oil (Martins *et al.*, 2001; Negi *et al.*, 1999; Ammon and Wahl, 1991).

According to stability of volatile oil in turmeric and zedoary rhizomes during storage at room temperature studies, volatile oil obtained from powdered

turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored in black polyethylene bags gradually decreased during 12-15 months of storage (Appendix A, Tables A-17, A-22 and A-27, respectively). This may result in the loss of antibacterial activity of turmeric, zedoary (bulb) and zedoary (finger) extracts after 12 months of storage. Moreover, the increase in MIC values of the extracts was presumed to be due to the increase in moisture content of turmeric, zedoary (bulb) and zedoary (finger) rhizome at different storage periods (Appendix A, Tables A-34, A-39 and A-44, respectively) because an excess of water will encourage deterioration following hydrolysis of compounds, such as volatile oil in these rhizomes (World Health Organization, 1998).

Hence, the results suggested that antibacterial activity of turmeric, zedoary (bulb) and zedory (finger) was dependent on the storage periods, with a decrease in antibacterial activity with an increase in storage periods.

Table 3-23 Minimum inhibition concentration (MIC) of the extracts of turmeric, zedoary (bulb) and zedoary (finger) rhizomes stored at different periods on the growth of bacteria.

Storage times (months)	Minimum inhibition concentration (MIC) (μg/ml)								
	Staphylococcus aureus ATCC 25923			*Bacillus subtilis*			*Escherichia coli* ATCC 25922		
	turmeric	zedoary (bulb)	zedoary (finger)	turmeric	zedoary (bulb)	zedoary (finger)	turmeric	zedoary (bulb)	zedoary (finger)
0	156.3	625.0	312.5	156.3	312.5	312.5	> 5000	> 5000	> 5000
6	156.3	625.0	312.5	156.3	312.5	312.5	> 5000	> 5000	> 5000
12	312.5	1250.0	625.0	312.5	625.0	625.0	> 5000	> 5000	> 5000
tetracycline	0.5			2.0			1.0		