J. Life Science. (2000), 10(2), 27~32

JOURNAL OF
Life Science

Effects of *Chrysanthemum coronarium* L. on the Thermotropic Behavior of DPPC Liposomal Membrane

Ok-Jeong Noh, Sung-Bae Roh* and Song-Ja Bae†

Department of Food and Nutrition, Silla University
**Department of Food Science and Technology, Yangsan College*

Received: October 12, 2000

Abstract To understand the effects of the fraction from *Chrysanthemum coronarium* L. (CC), we prepared five different types of samples, denoted here as CCMM, CCMH, CCMEA, CCMB and CCMA. We studied the effects of these samples on the phase transition of liposomal membranes by high-sensitivity differential scanning calorimetry (nano-DSC). We used dipalmitoylphosphatidylcholine (DPPC) bilayers which make most stable liposomes among the other phosphatidylcholines. When the samples were added to the bilayers, the phase transition temperatures of DPPC liposomes incorporated with CCMH and CCMEA were decreased by 1.5 and 2℃, while the other three fractions showed less tendencies. The CCMH and CCMEA fractions markedly affected the thermotropic properties of DPPC liposomes, broadened and shifted the thermograms of DSC. It also significantly reduced the size of cooperative unit of the transition. In all cases, there was no change in enthalpy of transition within the concentration range of the CC fractions studied. We concluded that the incorporation of the CCMH and CCMEA into DPPC liposomes was preferentially located in the hydrophobic core of DPPC bilayers compared to the other three fractions CCMM, CCMB and CCMA. These results suggest that certain substances in CCMH and CCMEA fractions might have biologically significant effects on the fluidity of biological membrane.

Key words: *Chrysanthemum coronarium* L., DPPC, Liposomes, DSC, Membrane fluidity

Introduction

Chrysanthemum coronarium L. (CC), namely crown daisy, which is one of the *compositae* plants, has been widely cultivated in Korea as vegetable for years. It originated in the Mediterranean Sea, and came to Korea through China long time ago. This has a favorable scent to use as an appetizer for dishes, helps intestinal functions to use for constipation as well [1,2]. The composition compounds of CC consist of water (93.5g%), protein (2.5g%), lipid (0.4g%), carbohydrate (3.1g%), ash (4.9g%), calcium (74mg%), phosphorus (29mg%), iron (4.2mg%), vit.A (4950IU), thiamine (0.15mg%), riboflavin (0.30mg%) and vit.C (45mg%) [3]. Especially, it is rich in mineral and vitamin compared to other vegetables [1,3].

Recently, in Asian country, various researchers have studied for the screening of bioactive substances from natural products or food materials such as *Artemisia iwayomogi* (Mugwort) [4], *Allium cepa* L. (Onion) [5], *Ixeris sonchifolia* H. (Godulbaegi) [6] and *Angelica radix* (Danggui) [7]. These studies suggest that the natural products used in the experiments have similar effects like drug materials having therapeutic efficacy of cancer. There are several studies of *Chrysanthemum coronarium* L. (CC) [1,2,8-11], however, few is known about the interaction of CC with liposomal phospholipid bilayers on the thermotropic behavior for membrane fluidity. The fluidity of lipid bilayers of biological membrane has been known to play an important role in the physiological functions [12,13]. Model membrane systems composed of mainly phospholipids have been extensively used to investigate the interaction between drugs and toxicological agents in membrane lipids [14-17].

In this study, we analyzed the effects of CC fractions on the thermotropic properties and the fluidity of dipalmitoylphosphatidylcholine (DPPC) liposomal bilayers using high-sensitivity differential scanning calorimeter (nano-DSC). These five different fractions were prepared as shown in Scheme 1, in which they are denoted as CCMM (methanol), CCMH (n-hexane), CCMEA (ethylacetate), CCMB (n-butanol), and CCMA (aqueous) from the MeOH extract of *Chrysanthemum coronarium* L.. Multilamellar liposomes were prepared from DL- α -dipalmitoylphosphatidylcholine (DPPC) which makes most stable liposomes among the other phosphatidylcholines. The transition from gel to liquid-crystalline state

†Corresponding author
Phone: 82-51-309-5462, Fax: 82-51-309-5176
E-mail: sjbae@silla.ac.kr

was examined in the presence of various concentrations of CC fractions, and measured the fluidity of DPPC liposomal membrane by DSC.

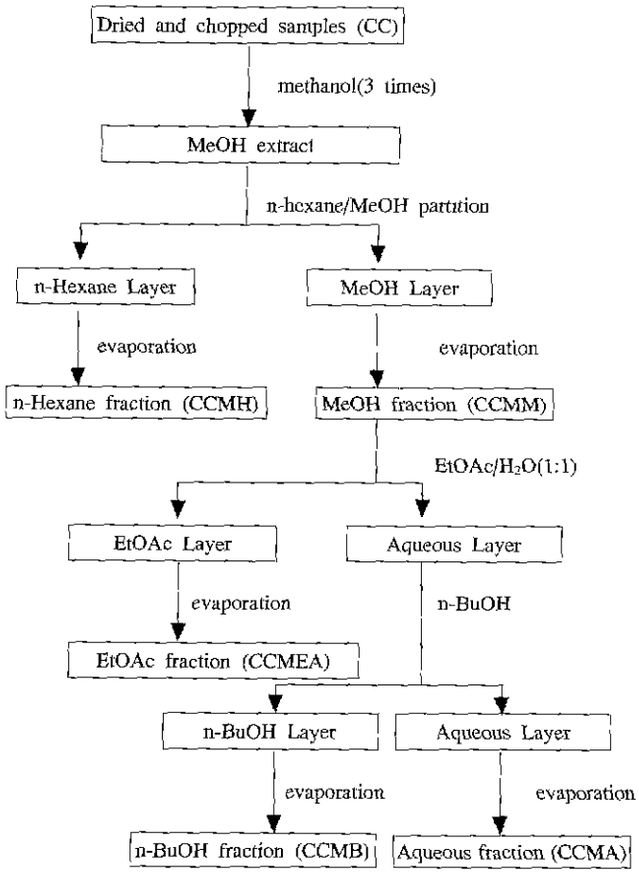
Materials and methods

Materials and reagents

Chrysanthemum coronarium L. (CC) was purchased from a local market in Pusan, Korea. DL- α -dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and was used without further purification. All other chemicals were of reagent grade.

Extraction and fractionation of CC

Dried and chopped *Chrysanthemum coronarium* L. (CC) was extracted three times with MeOH (1.5L). The methanol was removed by the evaporation under reduced pressure, and the resulting insoluble material was filtered off. The methanol extract was fractionated into n-hexane (CCMH), methanol (CCMM), ethylacetate (CCMEA), butanol (CCMB) and aqueous (CCMA) layers. Each partition layer was evaporated, and freeze-dried for the samples of this experiment (Scheme 1.).



Scheme 1. Fractionation procedure of *Chrysanthemum coronarium* L.

Preparation of multilamellar liposomes (MLVs)

Appropriate amounts of stock solutions of DPPC in methanol and the CC fractions in methanol/chloroform (1:1) were mixed to provide the desired concentration (mg ml^{-1}) for making multilamellar liposomes (MLVs). The organic solvents were then evaporated under a stream of dry N_2 to make a thin film of the lipid, and the last traces of solvents were completely removed by a further evaporation under high vacuum for 3 hours. The dried thin film was suspended in phosphate buffered saline (PBS) at pH 7.4 by mixing on a vortex mixer for 1 min, and then left in a thermobath at a temperature above their phase transition temperature for 2 min.

Differential scanning calorimetry (DSC)

The calorimetric measurements were performed using a CSC-5100 nano-differential scanning calorimeter. The lipid suspensions of all samples were heated from 5℃ to 60℃ at a scan rate of 0.25℃ min^{-1}. The final phospholipid concentration was 1 mg ml^{-1} Each sample was scanned three times to verify reproducibility.

Results and discussion

To investigate the effects of *Chrysanthemum coronarium* L. (CC) on the thermotropic behavior of dipalmitoylphosphatidylcholine (DPPC) bilayers, we employed high-sensitivity differential scanning calorimetry (DSC) measurement. The DSC profiles for multilamellar vesicles (MLVs) of DPPC alone and in the presence of increasing concentrations of the CC fractions CCMH and CCMEA are shown in Fig. 1. In the absence of the samples, DPPC alone exhibited two endotherms upon heating, the gel to gel pretransition at 34.1℃ and the main gel to liquid-crystalline phase transition at 41.4℃, in agreement with previous data [15-18]. The transition peaks were broadened and drastically shifted to lower temperature with increasing concentrations of the samples CCMH and CCMEA. The incorporation of CCMH and CCMEA into DPPC liposomes led to a notable decrease in the transition temperature (T_m) of the bilayers, which was dependent on the concentrations of CCMH and CCMEA (Table 1.). The temperature of the main gel to liquid-crystalline phase transition decreased in proportion to each concentration of the samples. Addition of 0.2 mg ml^{-1} CCMH and CCMEA resulted in a decrease of the onset temperature of the main phase transition of about 1.5℃ and 2℃. The presence of these samples did not produce a significant effect on the enthalpy (ΔH_{cal}) change of the gel to liquid phase transition of DPPC. Differential calorimetric data can be used to obtain the van't Hoff enthalpy for the transition. From the fraction of the area under curves of the main transition thermograms of DPPC liposomes with CCMH, the reaction degrees versus temperature for the main transition of the DPPC bilayers are illustrated in

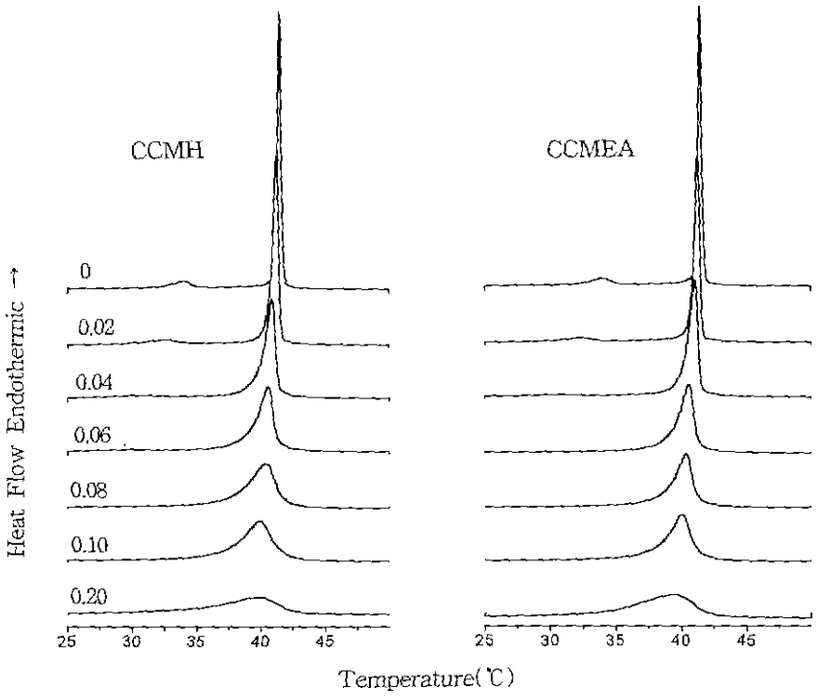


Fig. 1. The DSC thermograms of DPPC liposomes without and with various concentrations of CCMH and CCMEA. The concentration (mg ml^{-1}) of CC fractions in DPPC liposomes is expressed on the curves. * CCMH : Hexane fraction of CC, CCMEA : Ethylacetate fraction of CC.

Table 1. Thermodynamic parameters for DSC main transition curves of DPPC liposomes incorporated with CCMH and CCMEA

Conc. (mg ml^{-1})	CCMH			CCMEA		
	T_m (℃)	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}/\Delta H_{cal}$	T_m (℃)	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}/\Delta H_{cal}$
0	41.4±0.05	8.87±0.35	434	41.4±0.05	8.87±0.35	434
0.02	41.2±0.05	10.93±0.31	244	41.3±0.05	9.63±0.88	286
0.04	40.9±0.05	10.93±0.82	108	41.0±0.00	10.20±0.55	169
0.06	40.7±0.04	11.28±0.58	69	40.6±0.05	10.05±0.25	86
0.08	40.4±0.05	10.53±0.21	74	40.4±0.00	9.97±0.33	63
0.10	39.9±0.04	11.00±0.43	50	40.0±0.08	9.43±1.17	50
0.20	39.9±0.16	8.28±1.23	19	39.5±0.05	7.83±0.43	20

The transition temperatures (T_m) and the calorimetric enthalpies (ΔH_{cal}) were calculated by DSC, the temperature being scanned at 0.25℃ min^{-1}. All values are means±S.D. of at least three separate experiments.

Fig. 2. Since the temperature was scanned at a constant rate, the slope of the curve of this plot could be used to calculate the van't Hoff enthalpy of the transition, ΔH_{vH} using the van't Hoff equation [19],

$$\Delta H_{vH} = 4RT_m^2(d\alpha/dT)_{Tm}$$

where α is the fraction of the lipid in the liquid-crystalline state, and T_m, the main transition temperature. The ratio of ΔH_{vH} to ΔH_{cal} is usually interpreted as the size of the cooperative unit of the transition (Scheme 2.) [20,21]. The ratios of ΔH_{vH} to ΔH_{cal} in the presence of various concentrations of CCMH and CCMEA into the DPPC liposomes were calculated and also included in Table 1. The penetration of various concentrations of CCMH and CCMEA into the DPPC bilayers reduced the ratios of ΔH_{vH} to ΔH_{cal} (cooperative unit), depicting a decrease of the cooperativity of the transition caused by two CC fractions CCMH and CCMEA. All these tendencies show that the CCMH and CCMEA are very effective in modifying the thermotropic behaviors and in fluidizing the lipid bilayers.

The DSC thermograms for the DPPC liposomal bilayers without and with various concentrations of the CC fractions CCMM, CCMB and CCMA are presented in Fig. 3 and the thermodynamic parameters in Table 2 (CCMA is not shown).

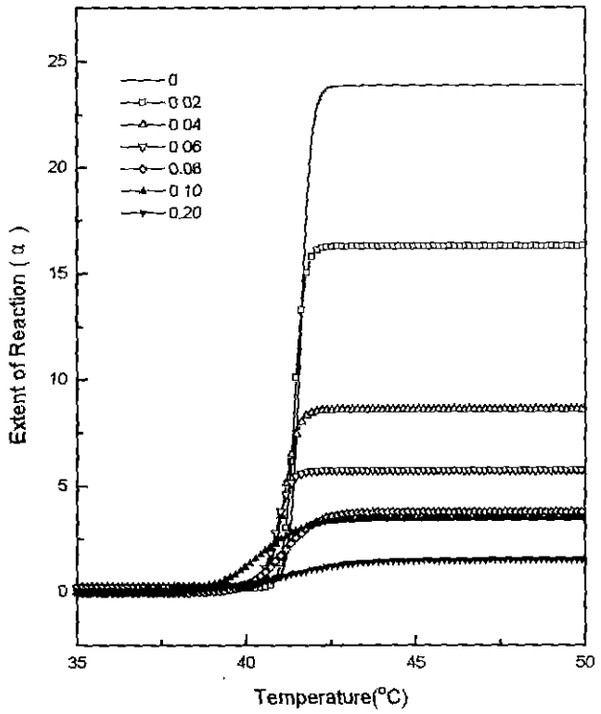
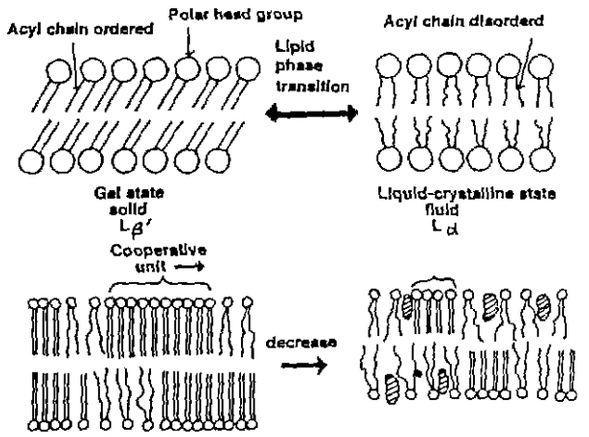


Fig. 2. Reaction extent (α) of the gel to the liquid-crystalline transition vs. the phase transition temperature (T_m) of DPPC liposomes without and with various concentrations (mg ml^{-1}) of CCMH.



Scheme 2. Lipid phase transition from gel to liquid-crystalline state in lipid bilayer

Contrary to the systems CCMH and CCMEA in DPPC liposomes, smaller effects were observed for the fractions CCMM and CCMB in DPPC liposomes. The addition of 0.2 mg ml^{-1} CCMM and CCMB resulted in a decrease of the onset temperature of the main phase transition of 1℃ and 0.5℃, respectively. Obviously, CCMM and CCMB were less efficiently incorporated into DPPC liposomes than CCMH and CCMEA. This finding might be explained by the higher cohesion and thus lower flexibility of CCMM and CCMB in DPPC liposomes. However, DPPC liposomes with CCMA exhibited no significant effect on the phase behavior when CCMA was added to the phospholipid bilayers. The thermograms for DPPC liposomes alone and DPPC liposomal bilayers containing 0.02 to 0.2 mg ml^{-1} CCMA were almost undistinguishable. In addition to a variation of the CCMA concentration, DPPC liposomes described no disappearance of pretransition, and the gel to the liquid-crystalline phase transition temperature of the bilayers continued taking place at 41.4℃. The results show that the addition of CCMA causes no effective change in the thermotropic behavior of pure DPPC liposomal membranes.

All the figures and tables showed the overall effects of the incorporation of increasing amounts of the five fractions from CC on the thermotropic phase transition of DPPC liposomes. Among the five fractions of CC, CCMH and CCMEA have the remarkable effects on the fluidity of the lipid membranes. The penetration of various concentrations of CCMH and CCMEA into the lipid bilayers changed siginificantly their thermotropic phase behavior compared to that of CCMM, CCMB, and CCMA : it decreased the phase transition temperature of the lipid, broadened the thermograms, and dramatically reduced the ratios of ΔH_{vH} to ΔH_{cal} (cooperative unit) in proportion to its concentration. This means that probably the CCMH and CCMEA in DPPC liposomes are responsible for their preferential location in the hydrophobic core of the lipid bilayer, whereas the other fractions CCMM and CCMB preferentially reside at the lipid-water interphase. This localization of CC fractions in lipid bilayers may be important to determine for the permeability and fluidizing effects on biological membrane.

Conclusions

Effects of *Chrysanthemum coronarium* L. on the thermotropic behavior of DPPC liposomal membrane have been investigated by means of DSC. The profile of a DSC thermogram of a phospholipid phase transition is largely determined by the transition temperature (T_m), the enthalpy (ΔH_{cal}) change, and the ratio of $\Delta H_{vH}/\Delta H_{cal}$. Determining the temperatures of the transitions allows the construction of phase diagrams, which provide information regarding the equilibrium between different phases. The effects of CC fractions on the DPPC liposomes are through alteration of membrane physical properties. The results indicate that a liposomal encapsulation of *Chrysanthemum coronarium* L. depends on the kind of fractions at different solvents. The most drastic changes were observed for the incorporation of CCMH and CCMEA into DPPC liposomes. The CCMH and CCMEA are highly incorporated in DPPC liposomes, which might have a significant encapsulation efficiency of DPPC liposomes. On the other hand, a rather low incorporation in DPPC

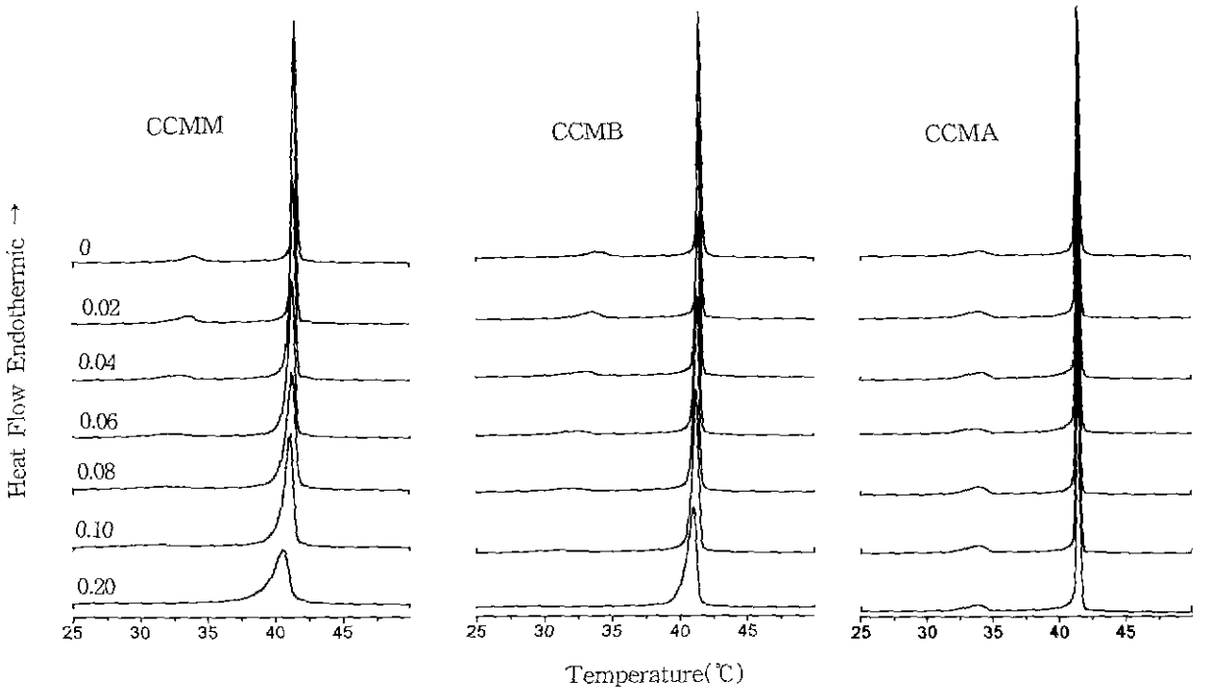


Fig. 3. The DSC thermograms of DPPC liposomes without and with various concentrations of CCMM, CCMB and CCMA. The concentration (mg ml^{-1}) of CC fractions in DPPC liposomes in expressed on the curves. * CCMM : Methanol fraction of CC, CCMB : Butanol fraction of CC, CCMA : Aqueous fraction of CC.

Table 2. Thermodynamic parameters for DSC main transition curves of DPPC liposomes incorporated with CCMM and CCMB

Conc. (mg ml^{-1})	CCMM			CCMB		
	T_m (℃)	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}/\Delta H_{cal}$	T_m (℃)	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}/\Delta H_{cal}$
0	41.4±0.05	8.87±0.35	434	41.4±0.05	8.87±0.35	434
0.02	41.4±0.00	11.73±1.16	429	41.4±0.00	10.80±0.43	714
0.04	41.3±0.00	11.43±0.79	309	41.4±0.00	10.47±1.28	443
0.06	41.2±0.05	11.90±0.50	178	41.3±0.05	11.8 ±0.37	333
0.08	41.2±0.00	11.33±0.17	187	41.3±0.00	11.73±0.33	301
0.10	41.1±0.00	11.80±0.33	173	41.2±0.00	11.97±0.56	249
0.20	40.6±0.12	11.70±0.92	53	41.0±0.05	11.27±0.12	167

The transition temperatures (T_m) and the calorimetric enthalpies (ΔH_{cal}) were calculated by DSC, the temperature being scanned at 0.25℃ min^{-1}. All values are means±S.D. of at least three separate experiments.

liposomes was observed for the fractions CCMM and CCMB. However, the CCMA had no effect on the thermotropic phase behavior of DPPC model membranes. These results might suggest that the incorporation of the CCMH and CCMEA into DPPC liposomes is preferentially located in the hydrophobic core of DPPC bilayers, enhancing the fluidity of the liposomal membranes.

References

1. Sung-Hee Jung. 1984. Sterol Compositions in *Chrysanthemum coronarium*. *Thesis of master degree*, Korea University.
2. Gi-Wook Hwang. 1998. Effect of *Chrysanthemum coronarium* on the biological activity in streptozotocin-induced rat. *Thesis of master degree*, Kyungsung University.
3. Tanaka, A., Tadano, T., Yamada, M. 1973. Adaptability to calcium. *Nippon Dojo-Jiryogaku Zasshi*, **44**(9), 334-9.
4. Sang-Myung Nam, Jong-Goon Kim, Sung-Shi Ham, Soo-Jin Kim, Myung-Eun Chung and Cha-Kwon Chung. 1999. Effects of *Artemisia iwayomogi* extracts on antioxidant enzymes in rats administered benzo(α)pyrene. *J. Korean Soc. Food Sci. Nutr.*, **28**(1), 199-204.
5. Hee-Jin Kwak, Young-Joo Kwon, Pill-Ho Jeong, Joong-Ho Kwon and Hyun-Ku Kim. 2000. Physiological activity and antioxidative effect of methanol extract from onion(*Allium cepa* L.). *J. Korean Soc. Food Sci. Nutr.*, **29**(2), 349-355.
6. Song-Ja Bae, Nam-Hong Kim, Bae-Jin Ha, Bok-Mi Jung and Sung-Bae Roh. 1997. Effects of *Godulbaegi* leaf extracts on CCl_4-induced hepatotoxicity in rats. *J. Korean Soc. Food Sci. Nutr.* **26**(1), 137-143.
7. Eun-Joo Han, Sung-Bae Roh and Song-Ja Bae. 2000. Effects

of quinone reductase induction and cytotoxicity of the *Angelica Radix* extracts. *J. Korean Soc. Food Sci. Nutr.* **29**(1), 147-152.

8. Sanz, J.F., Falco, E. and Marco, J.A. 1990. New acetylenes from *Chrysanthemum Coronarium* L. *Justus Liebigs Ann. Chem.*, 303.
9. Tom, A. and Cornelius, S. 1960. Fluorescent petal constituents of *Chrysanthemum Coronarium* L. *Biochem. Biophys. Acta.*, **90**, 63.
10. Dae-Jin Kim, Sue-Hyeun Yoon, Young-Sue Cho and Mee-Ae Choi. 1999. The changes in structural carbohydrate on crown daisy and butterbur. *Korean J. Life Science*, **9**(2), 497-503.
11. Su-Mi Kim, Seung-Hee Ryu, Hui-Don Choi, Sung-Su Kim, Jeong-Hwan Kim and Jong-Sang Kim. 1998. Screening for Korean vegetables with anticarcinogenic enzyme inducing activity using cell culture system. *J. Food Sci. Nutr.*, **3**(3), 277-281.
12. Houslay, M.D. and Stanley, K.K. 1982. Dynamics of Biological Membrane, John Wiley and Son, N.Y.
13. Aloia,R.C. 1983. Membrane Fluidity in Biology, Academic Press, N.Y.
14. Song Ja Bae, Suk Kyu Han, Kwang Sik Im and Nam Hong Kim. 1988. A scanning calorimetric study of the effect of clover saponin on liposomal phospholipid membrane. *Arch. Pharm. Res.* **11**(3), 181-184.
15. Maria Rosa Moya-Quiles, Encarnaciòn Munoz-Delgado, Cecilio J. Vidal. 1996. The pyrethroid insecticide deltamethrin modifies the thermotropic properties and lipid packing order of model membranes. *Chemistry and Physics of Lipids*, **83**, 61-69.
16. Krzysztof Wòjtowicz, Wieslaw I. Gruszecki, Magdalena Walicka, Joanna Barwicz. 1998. Effect of amphotericin B on dipalmitoylphosphatidylcholine membranes : calorimetry, ultrasound absorption and monolayer technique studies. *Biochimica et Biophysica Acta*, **1373**, 220-226.
17. Corinna Bernsdorff, Regina Reszka, Roland Winter. 1999. Interaction of the anticancer agent Taxol™(paclitaxel) with phospholipid bilayers. *J. Biomed Mater Res*, **46**,141-149.
18. Alicia Grau, Juan C. Gòmez. Fernàndez, Francoise Peypoux, Antonio Oritz. 1999. A study on the interactions of surfactin with phospholipid vesicles. *Biochimica et Biophysica Acta*, **1418**, 307-319.
19. Fendler, J. H. 1983. Membrane Mimetic Chemistry, Wiley Interscience Publication, N.Y.
20. Castuma, C. E. and Brenner, R. R. 1983. Effects of fatty acid deficiency on microsomal membrane fluidity and cooperativity of the UDP-glucuronyl transferase. *Biochimica et Biophysica Acta*, **722**, 9.
21. Hinz, H. J. and Sturtevant, J. M. 1983. Calorimetric studies of dilute aqueous suspension of bilayers formed by synthetic L-α-lecithin. *J. Biol. Chem*, **19**, 6071.