Kasetsart J. (Nat. Sci.) 45 : 521 - 529 (2011)

Characterization and Stabilization of the Extract from Mango Seed Kernel in a Cosmetic Emulsion

Pitchaon Maisuthisakul[1]* and Thepkunya Harnsilawat[2]

ABSTRACT

The aim of this study was to systematically investigate the antiradical capacity using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays, chelating activity and tyrosinase inhibitory activity (including solubility) of mango (*Mangifera indica* cultivar Chok-Anan) seed kernel extract compared to methyl gallate (MG). The mango seed kernel extract (MSKE) showed significantly ($P < 0.05$) higher antiradical capacity and tyrosinase inhibitory activity than that of MG. The extract showed higher solubility in glycerol than water. The emulsion-stabilizing properties of MSKE and MG preparations in cosmetic emulsion were investigated using apparent viscosity and total phenolic content. The phenolics affected emulsion stability under high temperature storage and a temperature cycling test. The apparent viscosity increased differently for the emulsion containing the MSKE and MG compared to the base lotion emulsion. The total phenolic content decreased during storage at higher temperature. The results showed that the interaction between the phenolics and the emulsifier, and the inclusion of other ingredients, in emulsion systems are important for cosmetic emulsion stabilization.

Keywords: mango, seed, antioxidant, tyrosinase, emulsion

INTRODUCTION

Mango (*Mangifera indica* L.) is a famous, commercial tropical fruit throughout the world, due to its excellent eating quality and nutritional composition. The increasing consumer demand for fresh and processed mango products is affected by the United States, European and Japanese market sizes. During processing of mango, by-products such as peel and kernel are generated. Kernel constitutes about 17–22% of the fruit (Soong and Barlow, 2004). The mango seed kernel was shown to be a good source of phenolic compounds (Arogba, 1997; Soong and Barlow, 2004) including microelements like selenium, copper and zinc (Schiber *et al.,* 2003). In addition, the mango seed kernel was shown to exhibit tyrosinase inhibitory activity (Maisuthisakul and Gordon, 2009). This information shows that the MSKE contains various compounds.

Many plant phenolic compounds are high value-adding chemicals and are used as ingredients in foods, cosmetics and pharmaceuticals. The potential use of phenolic compounds for the development of new skin care cosmetics has been emphasized (Kiken and Cohen, 2002). Phenolic compounds can be used as whitening, sunscreen and anti-wrinkle agents (González *et al.*, 2008).

1 Faculty of Science, University of the Thai Chamber of Commerce, Bangkok 10400, Thailand.

2 Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: pitchaon_mai@utcc.ac.th, pitchaon@yahoo.com

Received date : 15/06/10 Accepted date : 28/12/10

Melanin is the root cause for darkening of the skin. Its formation beneath the skin proceeds through a free-radical mechanism. UV-radiations facilitate this chain reaction and it could be disrupted by selective use of compounds, potent enough to inhibit this reaction (Choi *et al*., 2007). It is well documented that tyrosinase (E.C. 1.14.18.1) is an essential enzyme, which contributes towards pigment formation in a mammal's body as well as in plants, microorganisms and fungi (Nerya *et al*., 2003; Choi *et al*., 2007). The use of tyrosinase inhibitors is becoming increasingly important in the cosmetic industry due to their skin-whitening effects.

Emulsion stability is one of the most important factors governing the shelf life of products. Principally, emulsions are thermodynamically unstable systems and they tend to breakdown over time due to a number of different physicochemical mechanisms which may occur concurrently such as creaming, flocculation, coalescence, phase inversion and/or Ostwald ripening. Stability in cosmetic preparations must be considered. The present research involved the systematic study of the antioxidant properties and tyrosinase inhibitory activity of mango seed kernel including its stabilization in cosmetic emulsion.

MATERIALS AND METHODS

Materials

Three batches of sun dried seeds from ripened mango (*M. indica* cultivar Chok-Anan) were donated by Woraporn Co., Ltd. from March to June in 2007. The seed kernels (MSKs) were removed manually. The moisture content on a dry weight basis according to AOAC (1990) of MSK was 9.81 ± 0.34%. The dried material was kept in a freezer at -20 °C for no longer than 2 mth.

Folin Ciocalteu reagent, 1,1-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, and methyl gallate were purchased from Sigma Chemical Co., Ltd (Saint Louise, USA). The other chemicals and solvents used in this experiment were analytical grade purchased from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

MSKE preparation

Frozen kernel (80 g) was blended for 1 min with 95% ethanol and extracted according to Maisuthisakul and Gordon (2009). The dried extracts and reference samples (methyl gallate) were used to estimate the antioxidant properties using DPPH methods. The MSKEs were also used to evaluate the total phenolic content and chelating activity including tyrosinase inhibitory activity and solubility.

The free radical scavenging activity of samples was evaluated using the stable radical DPPH according to the method of Masuda *et al*. (1999) with modifications (Maisuthisakul *et al*., 2008).

The total phenolic content of samples was determined using the Folin-Ciocalteu's phenol reagent (modified from Kähkonen *et al*., 1999). The concentration of total phenolic compounds in all samples was expressed as milligrams of methyl gallate equivalent (MGE) per gram of dry weight of MSK using a linear equation.

The chelating activity of studied samples was estimated based on the decrease in the maximal absorbance of the iron (Fe^{2+})-ferrozine complex according to previously reported methods (Dinis *et al*., 1994).

The tyrosinase inhibitory activity of samples was determined using L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. All the samples were first dissolved in dimethyl sulfoxide (DMSO) and used for the experiment at 30 times dilution. The assay was performed as previously described (Fu *et al*., 2005).

The MSKE and MG were used to determine their solubility in oil, water and glycerol. Briefly, 1 g of each sample was mixed with 15 mL of solvent test and shaken gently at 25 °C for

3 h. The solution was then filtered through Whatman No. 1 filter paper to recover the remaining undissolved particles, and the residue was desiccated at 105 °C for 24 h and weighed. The solubility (S) was calculated using Equation 1:

$$S\ (\%) = [(Wo - Ws)/Wo] \times 100 \qquad (1)$$

where Wo = the initial weight of the sample expressed as dry matter and Ws = the weight of the undissolved desiccated sample residue. All tests were carried out in triplicate.

Preparation of cosmetic emulsion

1. The base emulsion obtained from the East Asiatic (Thailand) Public Company Limited (EAC) was an oil-in-water emulsion (formula 1). Three lotions was prepared respectively by melting the lipophilic phase consisting of Olivem 1000 (1.0%, 3.0% and 5.0%), Estrol 3609 (5.0%), Olivem 300 (3.0%) and olive oil (2.0%) in a water bath at 80 °C and separately mixing the hydrophilic compounds glycerine (3%), xanthan gum (0.2% and 0.4%) and water in a water bath at 60 °C. Subsequently, the water phase and lipophilic phase were gently poured and then homogenized by an Ystral mixer (Berlin, German) model X10/25 at 15000 rpm for 2 min and left to stand until the lotion reached room temperature; a volume of water equal to that of the evaporated water was added as compensation. The compositions of the prepared formulations are shown in Table 1. The appropriate base emulsions were selected from the viscosity and stability properties.

The apparent viscosity of the emulsion was determined at 25 °C at various shear rates (from 2 to 285 s^{-1}) using a Brookfield DV-II, LV viscometer (Brookfield Engineering Laboratories, USA), equipped with the LV spindle 2. The procedure for the measurement of apparent viscosity has been described by Mirhosseini and Tan (2009). The apparent viscosity range using the Brookfield programmable viscometer DV–II was measured in units of centipoints (*cPs*).

As a measure of stability with time (0, 1 and 6 h), the turbidity of the emulsion was monitored using a UV-Vis spectrophotometer model 1601 (Shimadzu, Japan) with a thermostatted cell holder set at 15 °C. The absorbance spectra between 200 and 800 nm were recorded, and even though the processes of sedimentation and coagulation could not be quantified separately, this gave a qualitative measure of the turbidity of the sample. However, water separation was not visually apparent in any system investigated.

2. The phenolic emulsion was made using the appropriate components from part 1.

Table 1 Formulation of base lotion emulsions.

	Net weight (g)		
	1	2	3
Oil phase			
Olive oil	2.00	2.00	2.00
Olivem 1000	5.00	1.00	3.00
Estol 3609	5.00	5.00	5.00
Olivem 300	3.00	3.00	3.00
Water phase			
Glycerine	3.00	3.00	3.00
Xanthan gum	0.20	0.40	0.40
Water	81.8	85.6	83.6

Note: 1 = Formula obtained from the East Asiatic (Thailand) Public Company Limited
2 = Modified formula number 1
3 = Modified formula number 2

above, by stirring the oil phase containing olive oil (2.00%), Olivem 1000 (1.00%), Estol 3609 (5.00%) and Olivem 300 (3.00%) at 80 °C until they were completely dissolved. In parallel, an aqueous phase consisting of glycerine (3.00%), xanthan gum (0.40%), the MSKE (0.10%) or MG (0.08%) and water was prepared. Both clear solutions were mixed and homogenized for 2 min, and then quenched to 25–30 °C. The pH of the lotion was 5.8 ± 0.6. At this pH, an active ingredient was not ionized (pKa_1 of methyl gallate = 8.03 and pKa_2 = 11.6) in the MSKE while Olivem 1000 retained its emulsifying properties.

Effect of thermal treatment on stability of phenolic lotion emulsion

An amount of 1 g of the MSKE, MG, base and phenolic lotion emulsions were stored in closed containers at 45 , 37 and 4 °C for 1, 3, 6, 24 and 48 h, respectively, in dark conditions. The pH of the lotion was 5.7 ± 0.9 throughout the experiment. Sampling emulsions were then frozen at -30 °C for 6 h before thawing in the refrigerator at 7 °C for 24 h. The samples were centrifuged at 15000× *g* for 30 min at 4 °C in a refrigerated centrifuge (Sorvall model RC5B, USA) to break the emulsion. The lower layer was removed from the centrifuge tube with a syringe. The aqueous phase was determined quantitatively for its total phenolic content as mentioned above.

Effect of temperature cycling tests on stabilization of phenolic lotion emulsion

To obtain more information about the emulsion structure and its dependency on temperature cycling, the emulsion underwent temperature cycles from 5 to 45 °C within 48 h for a period of 10 d. This type of test is frequently used in the pharmaceutical industry as an accelerated test in order to predict the long term stability and in particular the shelf life of the product under investigation. The pH of the lotion ranged from 5.0 to 5.8 during the experiment. The emulsions were sampled every 24 h to evaluate viscosity. Emulsions were broken for total phenolic content evaluation as mentioned above. The emulsions were also used to evaluate viscosity as mentioned above.

Statistical analysis

All tests were carried out in triplicate. The data were analyzed by the SPSS software program (SPSS Inc., Chicago, IL, USA). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values with the level of significance set at $P < 0.05$. Mean values and the pooled standard error of the mean (SEM) were then estimated.

RESULTS AND DISCUSSION

Characterization of mango seed kernel extract

Many researchers have reported on the correlation of free-radical-scavenging and tyrosinase-inhibition activities with the phenolic contents (Masuda *et al*., 1999; Kariotia *et al*., 2007; Maisuthisakul and Gordon, 2009). Tyrosinase is a copper-containing enzyme. Hence, some tyrosinase inhibitors act as metal chelators. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanization of human skin. Therefore, these agents have good commercial potential in both the food processing and cosmetic industries. The MSKE showed the strongest antioxidant activity and tyrosinase inhibitory activity (Table 2) compared with MG which is the main active ingredient of the extract (data not shown); hence it was used as the reference. The phenolic content of the ethanolic extract reached 285.70 ± 0.28 mg of MGE/g (dry basis).

Solubility is a useful indicator of phenolics performance in emulsion systems. Average solubilities (n=3) are shown in Table 3. The solubility of the MSKE was significantly different ($P < 0.05$) among the three solvents. The

MSKE was most soluble in glycerol then water and then oil, respectively. In addition, MG was more soluble in water than the others (Table 3). It can be assumed that the solubility of the MSKE was partly related to its polarity and that MG was more soluble in water which has high polarity. The polarity followed the order: water > glycerol > oil. These findings suggested that the MSKE and MG should dissolve in the water phase of lotion emulsion preparation.

Characterization of base lotion emulsion

The base lotion emulsion from the EAC formula was an oil-in-water (O/W) emulsion. Initially, the influence of emulsifier (Olivem 1000) and xanthan gum on the apparent viscosity of the base lotion emulsion formulations was investigated. Rheology measurement was selected for a preliminary evaluation of the stability of the lotion emulsion because it is a simple and effective means of comparing properties. None of the prepared lotion emulsions separated during storage for 60 d at 30 °C by visual inspection. Formula 2 which had a higher content of xanthan gum showed a higher viscosity value (Figure 1).

The turbidity experiments used the UV–vis spectrophotometer, scanning from 200 to 800 nm (Figure 2). The UV–vis absorbance of the emulsion formed over time decreased slowly as the emulsion destabilized (Knight and Caruana, 2007). Formula 2 was more stable than the others, so it was selected because its texture was good as well as the stability. Moreover, the water content of formula 2 was greater than the others which made it suitable for MG solubility.

Stabilization of phenolic lotion emulsion

Rheological measurements carried out in parallel with chemical studies represent a complete, rational and necessary approach to predict physical sample behavior during expected shelf life. The experiments showed that the phenolics of the lotion emulsions containing the MSKE were more stable to thermal treatments (37–45 °C) than those of MG (Figure 3). The higher the temperature storage, the lower the

Table 2 Chemical characterizations of MSKE and MG [1].

	Antiradical activity (A_{AR}, $1/EC_{50}$)	Chelating activity (CA_{50}, μg/mL)	Tyrosinase inhibitory activity ($1/AA_{50}$)	Total phenolic content (mg of MGE/ g)
MSKE	4.16^{c}±0.04	32.26^{a}±0.01	4.13^{b}±0.03	285.70±0.28
MG	4.01^{b}±0.01	32.12^{a}±0.15	4.01^{a}±0.03	ND

[1] Dry weight basis.

Means of three replications ± SD.

Different superscript letters in each column indicate a significant difference ($P < 0.05$) between conditions.

ND = not determined.

Table 3 Solubility of MSKE and MG in various solvents [1].

	Solubility (%)	
	MSKE	MG
Water	83.10^{b}±0.54	91.94^{c}±0.18
Oil	4.49^{a}±0.53	1.17^{a}±1.38
Glycerol	85.70^{c}±0.37	89.49^{b}±0.25

[1] Dry weight basis.

Means of three replications ± SD.

Different superscript letters in different columns indicate significant differences ($P < 0.05$) between conditions of MSKE and MG.

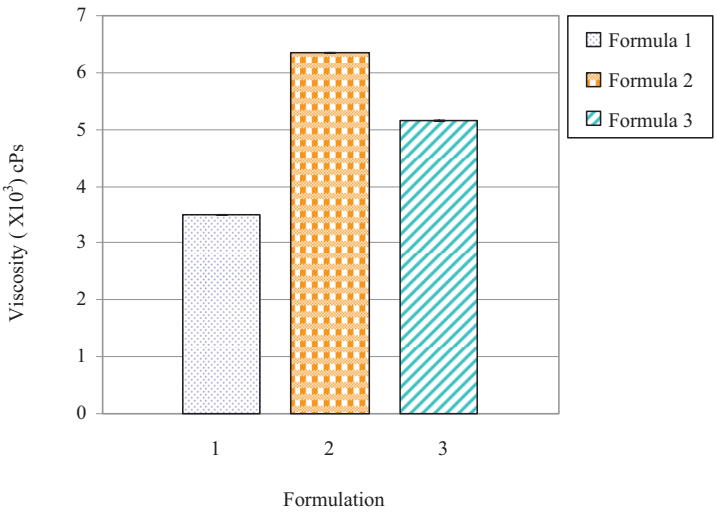

Figure 1 Viscosity of base lotion emulsions. Formulations are given in Table 1.

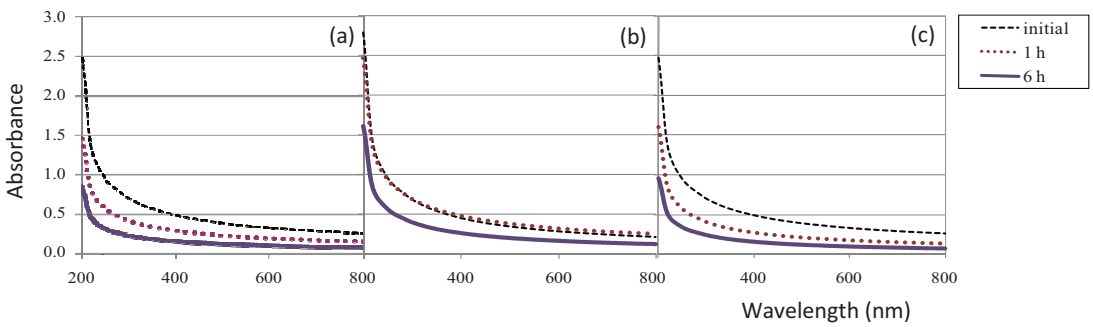

Figure 2 Emulsion stability of: (a) formula 1; (b) formula 2; and (c) formula 3 lotion emulsions.

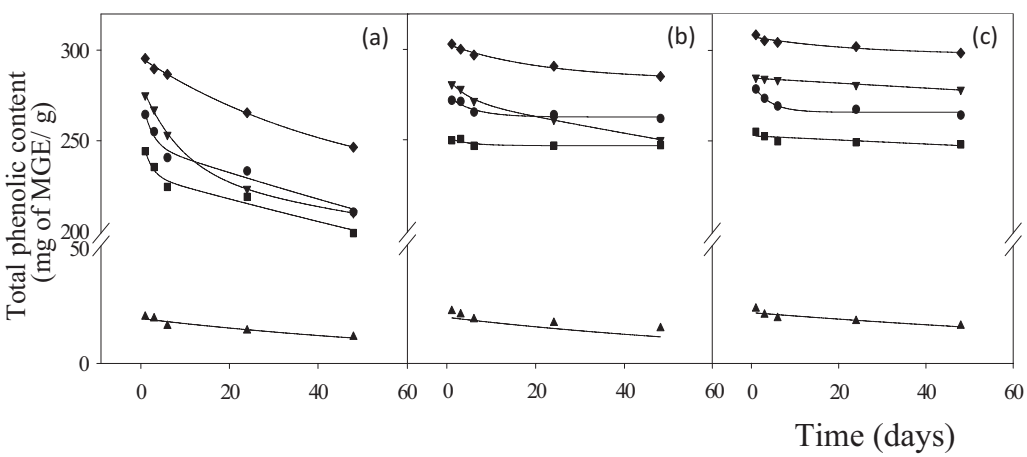

Figure 3 Influence of thermal treatment on the total phenolic content of several studied samples at: (a) 45 °C; (b) 37 °C; and (c) 4 °C. (▼ = MSKE; ■ = MG, ▲ = base formula; ◆ = MSKE formula and ● = MG formula).

stability of the phenolics in the lotion emulsion. The phenolics content of the base formula was similar at all storage temperatures (4–45 °C). The rheological parameters indicated that addition of the MSKE and MG to the basic formulation did not compromise its structure but altered some of the rheological parameters in the temperature cycling test (Figure 4). This observation was in line with other studies which reported that temperature cycling led to increase viscosity in lotions if the volume fraction was higher than 20%, which they attributed to partial coalescence (Tadros *et al.*, 2004). Furthermore, repeated cycling led to the formation of larger crystalline material at the droplet interface with the consequence that partial coalescence was supported (Boode *et al.,* 1991).

The phenolic content of the base formula was steady (Figure 5), while the phenolics of the MSKE and MG formulas reduced toward the end

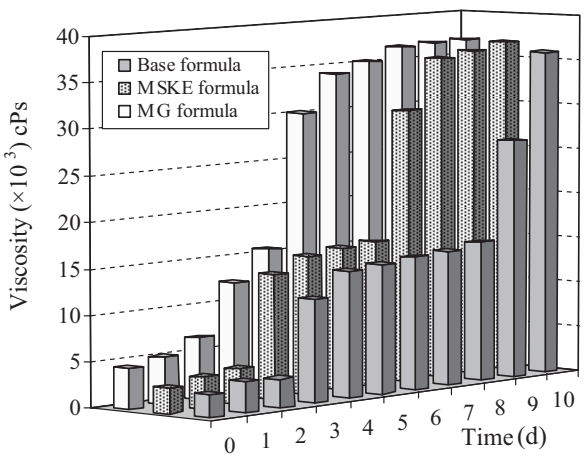

Figure 4 Influence of temperature cycling conditions on the viscosity of base lotion emulsion, lotion emulsion with MSKE and lotion with MG.

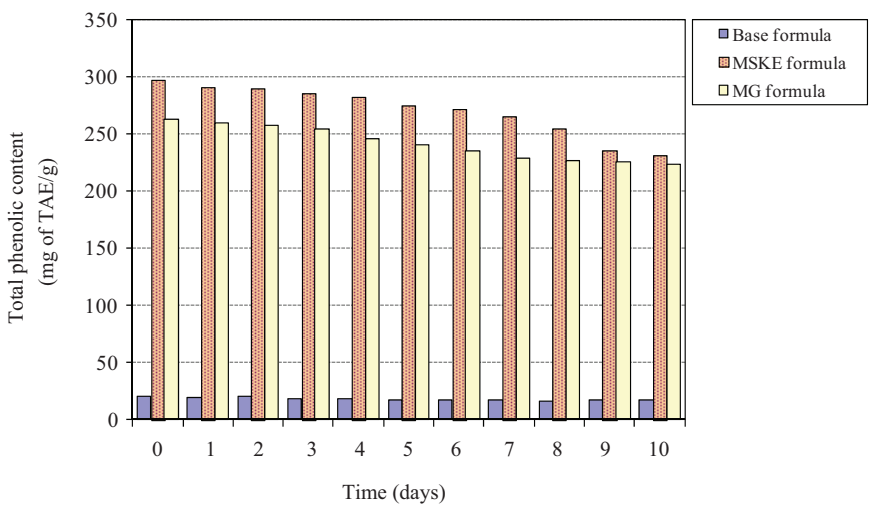

Figure 5 Influence of the temperature cycling conditions on the phenolic content of base lotion emulsion, lotion emulsion with MSKE and lotion with MG.

of the thermal cycling test. Typically, emulsifier facilitates the formation of an emulsion and improves the stability by reducing the oil–water interfacial tensions and by forming a protective layer around the fat droplets to prevent them from aggregating. However, a phenolic compound can interact with the emulsifier in the emulsion. The emulsifier's structural properties and its ability to form hydrogen bonds or hydrophobic interactions with the phenolics will affect the functional properties of the emulsifier (Stöckmann *et al*., 2000). The destabilized viscosity followed the order: MG formula > MSKE formula > base formula. From the results, the polarity of MG was greater than for the MSKE. These finding from the present study suggested that the polarity of the different phenolic compounds and emulsifiers affect the interaction between the emulsifier and phenolic compounds which will also affect the stability of the emulsion systems.

CONCLUSION

The extract of mango seed kernel exhibited the highest degree of free-radical-scavenging and tyrosinase-inhibition activities compared with methyl gallate. Moreover, the addition of phenolic compounds from the mango seed kernel and methyl gallate in emulsion affected the stability of the cosmetic emulsion systems. It was shown that the higher the temperature storage, the lower the stability of the phenolic compounds found in the cosmetic emulsions. These results suggested that the interaction between phenolic compounds and the emulsifier in cosmetic emulsion formulations does affect the stability of the emulsion system.

ACKNOWLEDGEMENTS

This research was supported by a grant from University of the Thai Chamber of Commerce (UTCC).

LITERATURE CITED

AOAC. 1990. **Official Methods of Analysis.** 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Arogba, S.S. 1997. Physical, chemical and functional properties of Nigerian mango (*Mangifera indica*) kernel and its processed flour. **J. Sci. Food Agric.** 73: 321–328.

Boode, K., C. Bisperink and P. Walstra. 1991. Destabilization of O/W emulsions containing fat crystals by temperature cycling. **Colloids Surf. A.** 61: 55–74.

Choi, S.W., S. Lee, E.O. Kim, J.H. Oh, K.S. Yoon, N. Parris and R.A. Moreau. 2007. Antioxidant and antimelanogenic activities of polyamine conjugates from corn bran and related hydroxycinnamic acids. **J. Agric. Food Chem.** 55: 3920–3925.

Dinis, T.C.P., V.M.C. Madeira and L.M. Almeida. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. **Arc. Biochem. Biophys**. 315: 161–169.

Fu, B., H. Li, X. Wang, F.S.C. Lee and S. Cui. 2005. Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. **J. Agric. Food Chem.** 53: 7408–7414.

González, S., M. Fernández-Lorente and Y. Gilaberte-Calzada. 2008. The latest on skin photoprotection. **Clin. Dermatol.** 26(6): 614–626.

Kähkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds. **J. Agric. Food Chem.** 47: 3954–3962.

Kariotia, A., A. Protopappaa, N. Megoulasb and H. Skaltsaa. 2007. Identification of tyrosinase inhibitors from *Marrubium velutinum* and *Marrubium cylleneum*. **Bioorg. Med. Chem.**

15: 2708–2714.

Kiken, D.A. and D.E. Cohen. 2002. Contact dermatitis to botanical extracts. **Am. J. Cont. Derma.** 13: 148–152.

Knight, M.K. and D.J. Caruana. 2007. Formation and characteristion of surfactantless oil-in-water and water-in-oil emulsions. **Colloids Surf. A.** 301: 352–360.

Maisuthisakul, P., S. Pasuk and P. Ritthiruangdej. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. **J. Food Comp. Anal.** 21: 229–240.

Maisuthisakul, P. and M.H. Gordon. 2009. Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. **Food Chem.** 117: 332–341.

Masuda, T., S. Yonemori, Y. Oyama,Y. Takeda, T. Tanaka and T. Andoh. 1999. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. **J. Agric. Food Chem.** 47: 1749–1754.

Mirhosseini, H. and C.P. Tan. 2009. Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure. **Food Chem.** 115: 324–333.

Nerya, O., J. Vaya, R. Musa, S. Izrael, R. Ben-arie and S. Tamir. 2003. Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. **J. Agric. Food Chem.** 51: 1201–1207.

Schiber, A., N. Berardini and R. Carle. 2003. Identification of flavonol and xanthol glycosides from mango peels by HPLC. **J. Agric. Food Chem.** 51: 5006–5011.

Soong, Y. and P.J. Barlow. 2004. Antioxidant activity and phenolic content of selected fruit seeds. **Food Chem.** 88: 411–417.

Stöckmann, H., K. Schwarz and T. Huynh-Ba. 2000. The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. **J. Am. Oil Chem. Soc.** 77: 535–542.

Tadros, T., P. Izquierdo, J. Esquena and C. Solans. 2004. Formation and stability of nano-emulsions. **Adv. Colloid Interface Sci.** 108: 303–318.