

EVALUATION OF PHARMACOLOGICAL STABILITY OF PERILLA OIL AND PERILLA OIL CAPSULE

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ABSTRACT

Objective: The present study explained the effect of storage conditions on the stability (total phenolic content (TPC), acid and peroxide values, and antioxidant capacity) of free perilla oil (PO) and capsulated PO (POC).

Methods: PO from Mae Hong Son cultivar was used for making POC. The capsules were prepared by Nature Nutri Co., Ltd., Pathum Thani, Thailand. PO and POC samples were stored in different containers (plastic, glass, clear, and amber bottles) at various temperatures (4, 30, and 40°C) for 3 months. The physical changes (color, odor, sedimentation, and separations), TPC, and antioxidant capacity of the samples were assessed by organoleptic, Folin-Ciocalteu colorimetric, and 1,1-diphenyl-2-picrylhydrazyl and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid assay methods, respectively. The acid and peroxide values of the samples were also studied by the titration method.

Results: The color and odor of PO were not changed during storage, and also, there was no sedimentation and layer separation. The acid values were not significantly changed during the storage period, but the peroxide values were significantly increased in PO samples stored at 40°C while no changes were observed in POC. The TPC and antioxidant capacity of the samples were not influenced by any of the storage conditions.

Conclusion: The results suggested that POC was relatively more stable than free oil in terms of peroxidation.

Keywords: Antioxidant, *Perilla frutescens*, Perilla oil, Perilla oil capsule, Stability.

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INTRODUCTION

The essential oils (*Thymus* spp. and *Commelina nudiflora*) are commonly known for several bioactivities, majorly antioxidant properties [1,2]. Lamiaceae family members are known for fragrance and bioactive compounds. *Perilla frutescens* (L.) Britton is one of the important plants belongs to the mint family. *P. frutescens* is commonly used in Asian countries to improve the flavor and quality of the foods, and perilla seed oil can be used as cooking oil. Perilla is known for several pharmacological properties [3] and it has been used in traditional medicines. Perilla seed oil (PO) is one of the rich plant sources of polyunsaturated fatty acids (omega-3 and omega-6), phenolic compounds (catechin, apigenin, luteolin, ferulic, rosmarinic, and caffeic acids), and antioxidants. The active compounds of perilla (perillic acid, perillaldehyde, and perillyl alcohol) reported for anticancer, antioxidant, and antidepressant activities [4-6].

PO has been used as dietary supplements among the Asian peoples, especially popular in Thailand. In general, PO is marketed in the form of a capsule for the convenient consumption. A recent preprint reported that the alpha-linolenic acid content of PO obtained from the perilla seeds grown in higher altitude (Maehongson, Thailand) was relatively high than that of the other samples [7].

The unsaturated fatty acids in seed oil are easily susceptible to isomerization and oxidation during processing and storage, which ultimately affects the quality of the oil [8]. The optimization of suitable process and storage condition is necessary to maintain the quality of the oil. The spray-dried PO powder was stable when stored at a low temperature without light and air in terms of acid and peroxide values, and fatty acids composition [9].

PO food supplements are available mostly in the form of capsules. The stability of PO capsules is not yet clearly registered as a research

document. Thus, we, in the present manuscript, attempted to study the stability of PO capsules stored at different conditions.

MATERIALS AND METHODS

Materials

PO seed used in this study was derived from perilla seeds that planted in Mae Hong Son, Thailand. PO seed capsule (POC) was prepared at Nature Nutri Co., Ltd., Pathum Thani, Thailand, using ISO 9001:2008 certified equipment as per the regulations of the Food and Drug Administration.

Physical and chemical properties of perilla oil and perilla oil capsule

PO and POC were observed to notice the changes in color and odor by organoleptic techniques [10,11].

The acid and peroxide values of PO and POC were measured as described previously [3]. Briefly, 50 ml of 95% ethanol was mixed with 0.5 mL of 1% phenolphthalein solution, and the solution was neutralized with 2-3 drops of 0.1 M potassium hydroxide. Then, 2 g of PO or POC was mixed and titrated against 0.1 M potassium hydroxide by automatic titrator (Mettler Toledo, Titration Excellence T50, Switzerland). The acid value of the samples was calculated as follows:

$$\text{Acid value (AV)} (\text{mg KOH /g of oil}) = \frac{v (\text{ml}) \times 5.61}{\text{Mass of sample (g)}}$$

Where, v is the titration amount of 0.1 M potassium hydroxide for the sample in ml and 5.61 is a constant value (equivalence of mass of 0.1 M KOH).

The samples (PO or POC) were mixed with 20 ml of a mixture containing acetic acid and chloroform (3:2 %v/v) in 100 ml beaker. Then, about 0.5 ml of saturated potassium iodide was added, and the flask was soaked in hot water until boiling point. The boiled mixture

was transferred to 250 mL flask that contains 20 ml of 5% potassium iodide solution and the beaker was rinsed twice with 15 and 10 ml of deionized water, respectively. The blank was prepared the same way without a sample. They were titrated with 0.002 N sodium thiosulfate using automatic titrator (Mettler Toledo, Titration Excellence T50, Switzerland). The peroxide value was calculated as follows:

$$\text{Peroxide value(PV)}(\text{mEq/kg of oil}) = \frac{A(\text{ml}) - B(\text{ml})}{\text{Mass of sample (g)}}$$

Where, A and B are the titration amount of 0.002 N sodium thiosulfate for sample and blank (in ml), respectively.

Determination of stability and shelf-life of PO and POC

The samples were stored in different containers such as transparent glass bottles, amber glass bottles, and plastic containers (Fig. 1). The samples were stored at 4, 30, and 40°C for 3 months. The changes in physical and chemical properties of PO and POC were assessed in terms of acid and peroxide values, total phenolic content (TPC), and antioxidant activity.

TPC and antioxidant activity

TPC of PO and POC was determined by Folin-Ciocalteu colorimetric method as detailed previously [12]. The changes in the antioxidant capacity of PO and POC stored at different temperature in different containers were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assays as described previously [13,14].

Statistical analysis

All the experiments were done in triplicates except fatty acid analysis, and the results were represented as mean \pm SD. Duncan's new multiple range test was performed to determine the significant differences, at the 95% confidential level ($p < 0.05$) using SPSS software version 17.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

PO derived from the perilla seeds cultivated in the high range region of Thailand (Mae Hong Son, Thailand) was used in this study. POC was made by Nature Nutri Co., Ltd., Pathum Thani, Thailand, as per ISO 9001:2008 standard. Both PO and POC were studied for their pharmacological stability for 3 months stored in different containers at various temperatures. The physical changes during storage were recorded by organoleptic techniques. There were no changes in color, and the odor of any of the samples. The formation of sedimentation and layer separation was also not observed in PO samples for 3 months (Table 1). The results suggested that storage at a wide range (4–40°C) in both plastic and glass amber bottles caused no any physical changes in PO samples.

The acid values of PO and POC stored in transparent and amber glass bottles at different temperature were not significantly affected up to 3 months. The initial acid value of PO and POC was 3.78 ± 0.09 and 3.79 ± 0.08 mg KOH/g of oil, respectively. After 3 months, the acid value of PO and POC stored in an amber glass bottle at 40°C was 3.87 ± 0.06 and 3.91 ± 0.07 mg KOH/g of oil, respectively (Table 2).



Fig. 1: The representative picture shows the perilla oil, stored in clear (a), and amber bottles (b), and oil capsules (c-g)

The initial peroxide value of PO and POC was 3.26 ± 0.04 and 3.10 ± 0.09 mEq/kg of oil, respectively. After 3 months, the peroxide value of PO stored in a transparent and amber glass bottle at 40°C was 5.75 ± 0.02 and 5.52 ± 0.13 mEq/kg of oil, respectively. The peroxide value of POC was not significantly affected after 3 months at 4 and 30°C , whereas storage at 40°C affected the peroxide value of POC after 2 months (Table 3). The results suggested that POC was relatively stable in terms of peroxide value compared to PO for 3 months at various temperatures. It was noted that the storage container greatly influences the stability of POC. Regarding peroxide values, POC stored in a plastic bottle was more stable compared to amber glass bottled samples (Table 3).

The initial TPC of PO and POC was 0.96 ± 0.09 and 0.94 ± 0.01 mg gallic acid equivalent (GAE) per g of oil, respectively. The TPC of PO stored in clear glass bottle at 4 , 30 , and 40°C was 0.89 ± 0.02 , 0.90 ± 0.01 , and 0.87 ± 0.01 mg GAE per g of oil, respectively. Likewise, the TPC of PO stored in an amber glass bottle at 4 , 30 , and 40°C was 0.90 ± 0.03 , 0.92 ± 0.01 , and 0.89 ± 0.01 mg GAE per g of oil, respectively. The TPC of

POC stored in an amber glass bottle at 4 , 30 , and 40°C was 0.90 ± 0.02 , 0.89 ± 0.06 , and 0.86 ± 0.04 mg GAE per g of oil, respectively. The TPC of POC stored in a plastic container at 4 , 30 , and 40°C was 0.90 ± 0.04 , 0.88 ± 0.03 , and 0.86 ± 0.05 mg GAE per g of oil, respectively (Table 4). The slight changes were observed in the TPC of PO and POC stored in different conditions. The results suggested that the storage container and temperature did not affect the quality of PO and POC for 3 months. The samples in clear glass bottle displayed significant changes even after 1 month of storage at 30 – 40°C , whereas samples stored in amber and plastic bottles were relatively stable (Table 4). The results suggested that light accelerates the degradation of PO during storage.

The antioxidant capacity of PO and POC was initially 0.30 ± 0.02 , and 0.29 ± 0.04 mg Trolox/g of oil in DPPH assay. After the 3 months of storage at different temperature and in various containers, the antioxidant capacity of PO and POC was not significantly changed in DPPH assay. After 3 months, antioxidant capacity of PO and POC was anything between 0.23 - 0.28 ± 0.01 - 0.03 mg Trolox/g of oil, which is like the initial value of the samples (Table 5).

Table 1: Physical characteristics of PO and POC stored at different storage conditions

Package (PO and POC)	Temperature ($^\circ\text{C}$)	Color	Odor	Sedimentation and separation
		Month 0-3	Month 0-3	Month 0-3
Plastic bottle	4	3	3	4
	30	3	3	4
	40	3	3	4
Amber glass bottle	4	3	3	4
	30	3	3	4
	40	3	3	4

Score: 1: Need improvisation, 2: Fair, 3: Good, 4: Excellent, PO: Perilla oil, POC: Perilla oil capsules

Table 2: The changes in the acid values of PO and POC stored in different containers at different temperature

Product	Packaging	Temperature storage ($^\circ\text{C}$)	Acid value (mg KOH/g of oil)			
			Month 0	Month 1	Month 2	Month 3
PO	Clear glass bottle	4	3.78 ± 0.09	3.88 ± 0.13	3.79 ± 0.04	3.57 ± 0.02
		30		3.91 ± 0.02	3.84 ± 0.14	3.96 ± 0.07
		40		3.77 ± 0.09	4.00 ± 0.06	3.95 ± 0.10
	Amber glass bottle	4		3.91 ± 0.13	3.69 ± 0.08	3.37 ± 0.05
		30		3.98 ± 0.04	3.77 ± 0.02	3.83 ± 0.05
		40		3.98 ± 0.10	3.94 ± 0.09	3.87 ± 0.06
PO capsule	Plastic bottle	4	3.79 ± 0.08	3.83 ± 0.04	3.07 ± 0.01	2.95 ± 0.08
		30		3.89 ± 0.01	3.75 ± 0.04	3.90 ± 0.02
		40		3.97 ± 0.06	3.86 ± 0.07	3.83 ± 0.04
	Amber glass bottle	4		3.89 ± 0.01	3.11 ± 0.09	3.06 ± 0.01
		30		3.84 ± 0.02	3.63 ± 0.11	3.61 ± 0.10
		40		3.95 ± 0.08	3.64 ± 0.03	3.91 ± 0.07

PO: Perilla oil, POC: Perilla oil capsules

Table 3: The changes in the peroxide values of PO and POC stored in different containers at different temperature

Product	Packaging	Temperature storage ($^\circ\text{C}$)	Peroxide value (mEq/kg of oil)			
			Month 0	Month 1	Month 2	Month 3
PO	Clear glass bottle	4	3.26 ± 0.04	2.93 ± 0.06	2.90 ± 0.02	3.41 ± 0.05
		30		3.45 ± 0.03	3.58 ± 0.06	3.48 ± 0.08
		40		$5.37\pm 0.06^{***}$	$5.35\pm 0.09^{***}$	$5.75\pm 0.02^{***}$
	Amber glass bottle	4		3.22 ± 0.12	3.34 ± 0.13	2.74 ± 0.02
		30		3.47 ± 0.14	3.48 ± 0.05	3.64 ± 0.14
		40		$5.41\pm 0.07^{***}$	$5.93\pm 0.06^{***}$	$5.52\pm 0.13^{**}$
POC	Plastic bottle	4	3.10 ± 0.09	2.92 ± 0.04	2.70 ± 0.04	2.70 ± 0.04
		30		3.82 ± 0.04	3.43 ± 0.01	3.43 ± 0.06
		40		3.2 ± 0.07	3.69 ± 0.07	3.69 ± 0.12
	Amber glass bottle	4		3.32 ± 0.03	2.86 ± 0.06	2.86 ± 0.09
		30		3.07 ± 0.05	3.06 ± 0.08	3.06 ± 0.01
		40		3.41 ± 0.08	$3.49\pm 0.06^*$	$3.49\pm 0.09^*$

* $p < 0.05$, *** $p < 0.001$. PO: Perilla oil, POC: Perilla oil capsules

The antioxidant capacity of PO and POC was initially 0.94 ± 0.03 and 0.93 ± 0.03 mg Trolox/g of oil in ABTS assay. After the 3 months of storage at different temperature and in various containers, the antioxidant capacity of PO and POC was not significantly changed in ABTS assay. After 3 months, PO and POC antioxidant capacity was anything between $0.88 - 0.92 \pm 0.01 - 0.12$ mg Trolox/g of oil, which is like the initial value of the samples (Table 6).

In general, the antioxidant capacity of PO and POC was not affected greatly during storage, but samples stored at 40°C showed a significant level of reducing in antioxidant capacity after 3 months of storage. In some cases, like PO stored in clear glass bottle showed a significant level of quality reduction after 2 months. The results revealed that storage

temperature and container play a critical role in the stability of PO and POC. The amber bottle or plastic container, which protects the samples from light, are best for oil storage compared to transparent containers.

The degradation profile, regarding fatty acid composition, of naturally processed PO and refined PO during frying condition has been reported recently [15]. The essential oils are more sensitive to light, oxidation, and degradation. PO is also sensitive to physical harshnesses such as heat and light, which affect the quality and use in food and pharmacological industries. Recently, microencapsulated perilla essential oil has been proved for their ability to delay the fruit decay by acting against the microbial contaminants. Furthermore, the encapsulation process prevented the loss of essential oil by evaporation [16].

Table 4: The changes in TPC of PO and POC stored in different containers at different temperature

Product	Packaging	Temperature storage ($^\circ\text{C}$)	TPC (mg GAE/g of oil)			
			Month 0	Month 1	Month 2	Month 3
PO	Clear glass bottle	4	0.96 ± 0.09	0.95 ± 0.05	0.93 ± 0.04	0.89 ± 0.02
		30		$0.86 \pm 0.02^*$	$0.85 \pm 0.07^*$	0.90 ± 0.05
		40		$0.87 \pm 0.08^*$	0.88 ± 0.01	$0.87 \pm 0.01^*$
	Amber glass bottle	4	0.93 ± 0.03	0.90 ± 0.02	0.90 ± 0.03	
		30	0.90 ± 0.02	0.92 ± 0.04	0.92 ± 0.01	
		40	$0.89 \pm 0.08^*$	0.91 ± 0.07	$0.89 \pm 0.01^*$	
PO capsule	Plastic bottle	4	0.94 ± 0.01	0.93 ± 0.05	0.91 ± 0.01	0.90 ± 0.04
		30		0.94 ± 0.08	0.91 ± 0.01	0.88 ± 0.03
		40		$0.88 \pm 0.03^*$	0.88 ± 0.04	$0.86 \pm 0.05^*$
	Amber glass bottle	4	0.93 ± 0.01	0.90 ± 0.02	0.90 ± 0.02	
		30	0.92 ± 0.07	0.93 ± 0.06	0.89 ± 0.06	
		40	$0.89 \pm 0.07^*$	0.90 ± 0.04	$0.86 \pm 0.04^*$	

TPC: Total phenolic content, * $p < 0.05$, PO: Perilla oil, POC: Perilla oil capsules

Table 5: The changes in the antioxidant capacity (DPPH) of PO and POC stored in different containers at different temperature

Product	Packaging	Temperature storage ($^\circ\text{C}$)	DPPH (mg Trolox/g of oil)			
			Month 0	Month 1	Month 2	Month 3
PO	Clear glass bottle	4	0.30 ± 0.02	0.30 ± 0.01	0.27 ± 0.02	0.27 ± 0.01
		30		0.27 ± 0.01	0.26 ± 0.02	0.26 ± 0.01
		40		0.28 ± 0.03	$0.25 \pm 0.01^*$	$0.23 \pm 0.02^{**}$
	Amber glass bottle	4	0.29 ± 0.02	0.27 ± 0.03	0.25 ± 0.03	
		30	0.28 ± 0.02	0.28 ± 0.02	0.24 ± 0.03	
		40	0.26 ± 0.01	0.26 ± 0.03	$0.24 \pm 0.03^{**}$	
PO capsule	Plastic bottle	4	0.29 ± 0.04	0.28 ± 0.02	0.27 ± 0.01	0.25 ± 0.01
		30		0.27 ± 0.02	0.25 ± 0.01	0.25 ± 0.01
		40		0.27 ± 0.01	0.25 ± 0.03	$0.23 \pm 0.03^{**}$
	Amber glass bottle	4	0.29 ± 0.03	0.28 ± 0.03	0.26 ± 0.01	
		30	0.27 ± 0.03	0.27 ± 0.02	0.24 ± 0.03	
		40	0.26 ± 0.02	0.27 ± 0.03	$0.25 \pm 0.01^{**}$	

** $p < 0.01$, * $p < 0.05$, PO: Perilla oil, POC: Perilla oil capsules

Table 6: The changes in the antioxidant capacity (ABTS) of PO and PO capsule stored in different containers at different temperature

Product	Packaging	Temperature storage ($^\circ\text{C}$)	ABTS (mg Trolox/g of oil)			
			Month 0	Month 1	Month 2	Month 3
PO	Clear glass bottle	4	0.94 ± 0.03	0.93 ± 0.04	0.93 ± 0.04	0.91 ± 0.08
		30		0.93 ± 0.01	0.89 ± 0.01	0.89 ± 0.04
		40		0.91 ± 0.10	0.90 ± 0.05	$0.87 \pm 0.04^*$
	Amber glass bottle	4	0.94 ± 0.07	0.92 ± 0.07	0.91 ± 0.06	
		30	0.92 ± 0.04	0.92 ± 0.04	0.88 ± 0.04	
		40	0.92 ± 0.02	0.91 ± 0.02	$0.89 \pm 0.08^*$	
PO capsule	Plastic bottle	4	0.93 ± 0.03	0.93 ± 0.07	0.93 ± 0.06	0.92 ± 0.11
		30		0.91 ± 0.09	0.90 ± 0.05	0.89 ± 0.02
		40		0.91 ± 0.10	0.92 ± 0.10	$0.89 \pm 0.04^*$
	Amber glass bottle	4	0.92 ± 0.02	0.93 ± 0.13	0.92 ± 0.06	
		30	0.90 ± 0.05	0.90 ± 0.03	0.91 ± 0.01	
		40	0.91 ± 0.02	0.91 ± 0.01	$0.90 \pm 0.12^*$	

* $p < 0.05$, PO: Perilla oil, POC: Perilla oil capsules

The pre-treated (microwave, moist heat, dry heat, and sonication) perilla seed was subjected to oil extraction by solvent extraction method. PO was studied for their quality by assessing the tocopherol content, and fatty acid composition. The results revealed that the pre-treatments did not affect the quality of the PO. The α -glucosidase, α -amylase, and protein glycation inhibition property of PO were also not affected by the pre-treatment [3].

Another study explained the stability of the spray-dried PO. The powdered oils were generally used in several pharmacological and cosmetic products to nourish the product with natural fatty acids, which are one of the major bioactive ingredients in the product. The spray-dried PO was relatively stable compared to the oil for 3 months, if stored in dark and dry low temperature, regarding fatty acid composition, and peroxide value [9].

The results of the current study suggested that POC was more stable when compared to free PO samples stored at various temperatures for 3 months in different containers. As per our search, there was no report on the stability of PO obtained from Mae Hong Son cultivars. The present study primarily reported the stability of PO in the capsule, which aids to improve the quality and processing conditions of essential oils that are generally used as medicine or food supplements.

CONCLUSION

The stability of PO and POC was assessed primarily. The storage conditions and storage containers have an influence on the stability of any products, especially oils. The pharmacologically important PO in capsule form was more stable when compared to free PO for 3 months, even they were stored at 40°C. The present study concluded that POC can retain their pharmacological activities (measured by antioxidant assays, and acid and peroxide values) for 3 months when stored in plastic or glass containers at 4–40°C. Further, long-term stability evaluations are required to determine the life span of free PO and POC.

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AUTHORS' CONTRIBUTIONS

CC involved in the study design, experiments, review, and finalization of the manuscript. BSS and PK contributed to data analysis, manuscript preparation, and critical revision of the manuscript. NM, SS, and SP responsible for wet lab experiments and data collection. All the authors agree with the content of the manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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