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Effect of *Plectranthus amboinicus* Leaf Extract on the Quality Attributes of Microencapsulated Fish Oil Fortified Soup Powder

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Abstract

The effect of *Plectranthus amboinicus* leaf extract (PAE) (phenolic content of 2559.56 mg gallic acid equivalents/L) addition in a microencapsulated fish oil fortified soup powder was evaluated in the present study. Fortification of PAE significantly improved the color, rehydration and water activity of the soup powder. The oxidative indices unveiled the role of PEA in protecting the MFO fortified soup powder from oxidative deterioration during the storage period. No faecal coliforms, yeast and mould growth was observed in MFO and PAE fortified soup powder whereas mould growth was there in the control samples. The fortified soup powder has scored high in terms of aroma, taste, consistency and overall acceptability proving its sensory acceptance. The significant findings of the storage study clearly depicted the antioxidant, antibacterial, antifungal as well as flavour enhancing potential of PAE and hence it can be recommended as a potent natural preservative for fish oil incorporated food systems.

Keywords: *Plectranthus amboinicus*, microencapsulation, functional foods, rehydration, oxidative stability

Introduction

The last decade has witnessed a considerable change in the field of food production and consumers are now demanding foods that can provide not only nutritive values but also having positive physiological effects. This has led to the development of functional foods to fulfill the consumer's expectancy for products that are healthy and safe (Shah, 2007). Functional foods can be developed by incorporating ingredients which are having some health benefits such as omega-3 fatty acids, vitamins, minerals, phytochemicals, pigments etc. Presently, foods fortified with omega-3 fatty acids are having great demand because of the established fact that these are highly beneficial for the healthy functioning of the heart, brain, nervous system etc. (Ruxton et al., 2004; Fewtrell, 2006). This has fostered researchers around the globe to develop innovative functional products containing omega-3 fatty acids. Among the different dietary sources of long chain omega-3 fatty acids, fish oil remains as an excellent and economical option. Susceptibility to oxidation being a major problem, fish oil is often microencapsulated to retain its integrity and thereby easing its fortification.

Functional foods which are meant for longer shelf life is often added with synthetic preservatives owing to its low cost, broad range of activity and wide availability. However, the use of synthetic additives is debated as some of the long term studies indicated that the persistent use of such products can cause adverse health effects. Furthermore, consumers also started preferring minimally processed foods with negligible quantities of synthetic additives to avoid the health risks. This in turn has geared up the research for identifying preservatives from natural sources that are safe, effective without any side effects. As a result, many innovative functional foods with health promoting natural ingredients have been developed and commercialized by the food industries (Carocho et al., 2014; Caleja et al., 2015). Bacteriocins, essential oils or substances extracted from plants such as pure plant

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extracts and oils are some among the natural preservatives that are in use nowadays. Since many of these compounds are reported to have inherent antioxidant activity, they can be very well used as functional ingredients or even as natural preservative. *Plectranthus amboinicus* (Family - Lamiaceae, subfamily - Nepetoideae) commonly known as Indian borage is a succulent perennial herb known for its medicinal properties. It is reported to possess a wide range of bioactivities such as antioxidant, antibacterial (Praveena & Pradeep, 2012), antiepileptic (Buznego, 1999), antifungal, anti-inflammatory (Gurgel, 2009), antimutagenic, antitumourogenic and antigenotoxic effects (Annapurani, 1999). Damanik (2001) reported that the leaves of this plant are traditionally consumed by Batakneese women in North Sumatra Island Indonesia whilst nursing.

The juice obtained from the leaves has been reported to use in treating epilepsy and gastrointestinal disorders and as pain relieving agent in conjunctivitis. Furthermore, the fresh or dried leaves are used in many countries as a flavoring and seasoning agent in food preparations. Owing to the oregano-like flavor of leaves, it is often used to substitute oregano in meat preparations. In spite of all these potentialities, very few food products or drinks have been tried out of these leaves till date. Wadikar & Premavalli (2011) prepared an appetizer from the leaves of *P. amboinicus* and were found to cause reduction in leptin levels in experimental animal. They also reported that the sustained consumption of the beverage resulted in increased food intake and weight gain thereby indicating appetizing effect. Despite these well documented antioxidant, antibacterial and flavoring properties of *P. amboinicus*, its efficacy as a natural preservative in food systems has not been explored as of now. Hence, the present study has been designed to investigate the bio preservative effect of PAE in a functional freeze-dried shrimp soup powder.

Materials and Methods

Indian white shrimp (*Fenneropenaeus indicus*), corn flour, fish oil (seven seas), skimmed milk powder (Nandini diary, Mysore), salt, onion, carrot, ginger, garlic, green chilly, coriander leaves, tomato were procured from local market (Mysore). *P. amboinicus* leaves were freshly collected from the Defence Food Research Laboratory (DFRL) campus, Mysore. All the ingredients used in the preparation of shrimp soup powder were of food grade and supreme quality.

Fresh *P. ambionicus* leaves collected from the DFRL, Mysore campus were thoroughly washed in distilled water and then grinded to fine paste. This was then filtered through a muslin cloth and the crude extract (PAE) thus obtained was kept in chilled condition (4°C) till its use. The total phenolics and DPPH radical scavenging activity of PAE was carried using the method of Singleton & Rossi (1965) and of Brand-Williams et al. (1995) respectively.

The fish oil emulsion was prepared using skimmed milk powder as wall material in the ratio of 1:2 (fish oil to skimmed milk powder) by subjecting to high speed homogenization at the rate of 10,000 rpm for 10 min. The emulsion prepared was then transferred to 25 ml graduated test tubes, sealed and observed for any separation. Emulsion stability was expressed as percentage of separation, which is the ratio of height of creamy layer to initial emulsion height. Emulsion microstructure was captured using an optical microscope (Leica ICC50 HD) equipped with microscope digital camera at an objective magnification of 40X. The emulsion microstructure was acquired using digital image processing software (image-pro plus^{Tm}, version 6). This formulation was made in bulk quantity and freeze dried in a pilot scale freeze dryer (Martin Christ GmbH & Co KG, Osterode, Germany) which was equipped with both freezing and drying facilities. Pre-freezing was done for 4 h at -40°C which was followed by contact drying at a temperature of 50°C under a chamber pressure of 100 - 300 Pa for 12 h. Once the process was finished, the samples were immediately transferred to a low humidity chamber and packed in paper foiled low density polyethylene (PFP) (45 gsm paper/ 20 μ aluminum foil/ 37.5 μ LDPE) packets for further studies. Encapsulation efficiency of the powder has been determined by following the method of Lekshmi et al. (2017).

Folch method (Folch et al., 1957) was followed for the extraction of lipid from the samples. Transesterification was performed according to the method of AOAC (2000). The fatty acid methyl esters were injected into a Varian gas chromatograph (Varian CP-3800, USA) equipped with a Capillary column (100 m × 0.25 mm ID; 0.25 μm film thickness) and a flame ionization detector. The identification of fatty acids separated were done by comparing the retention time with that of standard fatty acid methyl esters (Supelco 37 component FAME mix, Analytical standard, Sigma-Aldrich Co., St. Louis, MO, USA). Retention time and peak areas

of each fatty acid were recorded and expressed as percentage.

Commercially important and most commonly available species, 'Indian white shrimp' (*Fenneropenaeus indicus*) was used for soup preparation. Different ratios of fish oil powder (1 to 5%) and PAE extracts (1 to 10%) was tried for incorporation into the soup powder without affecting the sensory attributes. Among the different combinations tried, 3% fish oil powder and 4% leaf extract was selected based on the sensory preference. Four treatments each of 2 kg were prepared. They are: T1 (shrimp soup), T2 (shrimp soup+4% PAE), T3 (Shrimp soup+3% MFO), T4 (Shrimp soup+4% PAE+3% MFO). The following methodology was used for the preparation shrimp soup powder.

Shrimp soup was prepared by hot blanching the peeled and deveined shrimp for 5 min and then cooled before mixing with other ingredients. The ingredients for the base soup was standardized based on the sensory acceptance (Table 1). The ingredients were fried and mixed with the starch mix and MFO and PAE was fortified in the soup preparation accordingly. The mixture was then subjected to freeze drying for a period of 24 h. Freeze dried powder was immediately packed in the PFP pouches. Four different treatments were made and stored under two different conditions, *viz*., ambient room temperature and accelerated conditions. Storage analysis was carried out on 7 days' interval for a period of one month.

The nutritional profiling of the soup powder (moisture, protein, fat, and ash content) was measured as per the standard procedures (AOAC, 2003). The Muller & Tobin (1980) method was used to calculate the crude carbohydrate content (Eq. 2). The total caloric intake per 100 g of soup was also determined based on the percentage of proteins, carbohydrates and fat (Eq. 3).

Total crude carbohydrate (%) = 100 – [Moisture (%) + crude protein (%) + crude lipid (%) Total ash (%)

(2)

Total energy value (Kcal/100g) = (Fat X 9) + (Protein X 4) + (Total carbohydrate X 4)

(3)

Color of the different treatments during the storage period was determined using Hunter colorimeter (Color Flex, CFLX-45-2, Hunter lab, Reston, VA, USA). Water activity of the treatments was determined by Labmaster-aw water activity meter (Novasina, Switzerland) at 25°C. Rehydration ratio was measured according to Krokida & Marinos-Kouris (2003) with suitable modifications. The peroxide and Free Fatty Acid value of the samples was estimated as per AOAC (2000). The secondary oxidation products were measured using Thiobarbituric acid reactive substance (TBARS) assay (Taraldgis, 1960).

The total plate count was carried out by pour plate technique according to the standard methods (APHA, 1992). Enumeration of coliforms and yeast and moulds was done by plating one ml of aliquot each in molten VRBA agar and molten PDA acidified with 10% tartaric acid respectively. The enumerated results were reported as $CFUg^{-1}$. The sensory characteristics of the soup powder were evaluated based on a 9-point hedonic scale by a panel of judges from DFRL Mysore, keeping nine for excellent and one for very poor as per the method of Murray et al. (2001).

Analysis of variance and Duncan's Multiple Range Test (DMRT) were used to analyse the results, using software SPSS version 16 (Chicago IL. USA) (IBM). The level of significance was set up at (pdH0.05). All the analysis was done in triplicates and results were expressed as mean±SD.

Results and Discussion

The total phenolic and flavonoid content of the plant extract general depicts the overall antioxidant activity (Xu, 2012). The PAE were found to have total phenolic content of 2559.56 mg gallic acid equivalents/L. Praveena & Pradeep (2012) have reported that the ethyl acetate and acetone extract of *P. ambionicus* showed higher phenolic content than the ethanol, methanol and hydroalcoholic extracts. Koolen et al. (2013) have reported that the phenolic compounds having the ability to donate electrons beyond their capacity and forming stable radical intermediates are strong active antioxidant metabolites from plants. The radical scavenging activity of the extracts was found to increase with the increase in concentration. The IC50 value which is the sample concentration needed to inhibit the radical scavenging activity by 50% was found to be 22.451 µl.

The ability of emulsion to resist changes in physicochemical properties over time is often

referred as the emulsion stability (McClements, 2007). Because of the surface-active and colloid stabilizing property of milk proteins, it has been used in the present study as an encapsulant and emulsifier. The emulsion stability, which was expressed as the percentage of separation was found to be 2.38%, which means about 97.62% remained as unseparated fraction. It was observed that the oil droplets were without any coalescence or aggregation and seemed to be separate from each other (Fig. 1). Lizarraga (2008) have reported that milk proteins have the ability to protect the emulsion from coalescence and further destabilization by virtue of its adsorption at the lipid-water interface and generation of electrostatic and steric repulsion. The absence of coalescence or aggregation can be attributed as probable reason for the higher emulsion stability.

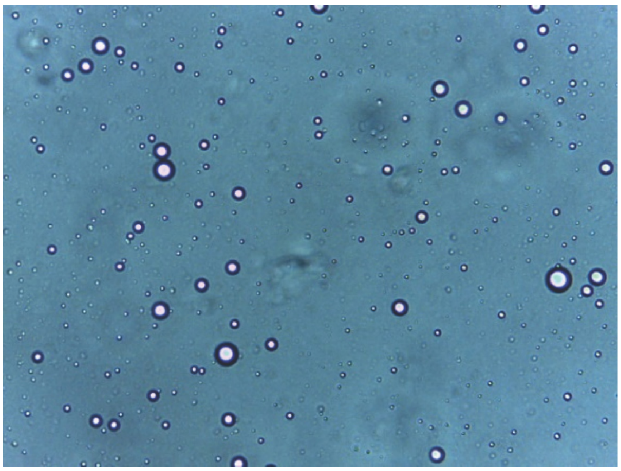

Fig. 1. Microstructure of fish oil emulsion stabilized with skimmed milk powder taken at a magnification of 40X

The encapsulation efficiency of MFO was found to be 73%. Similar results were reported by Aghbashlo (2013) while encapsulating fish oil with skimmed milk powder using spray-drying technique. The fatty acid profile of both the MFO and unencapsulated fish oil were analyzed with the help of a gas chromatography. It was clear from the analysis that the encapsulation by freeze drying didn't seem to affect the fish oil composition adversely. The fatty acid profile (Table 1) was mainly contributed by 18 fatty acids of which the predominant fraction was monounsaturated (42%), followed by poly unsaturated (25%) and saturated fatty acids (17-20%). The eicosapentanenoic acid (EPA) and docosahexaenoic acid (DHA) content was 8.71 and 11.56% in the unencapsulated oil and 8.43 and 10.75% in the encapsulated powder. The results showed a higher retention of the major fatty acids during encapsulation with freeze drying technique. This shows the potentiality of freeze drying technique to encapsulate fish oil efficiently without much loss in its integrity.

The yield of soup powder after freeze drying was 18.25%. Similar results of process yield were reported by Nguyen (2012) in the case of freeze dried desalted tusk fish. The low yield is because of the fact that the freeze drying process removes

Table 1. Fatty acid composition of the major fatty acids in unencapsulated and encapsulated fish oil

Designation	Fatty acid	% Fatty acid To Total Fatty acid Fish oil	encapsulated oil
C14:0	Myristic	4.08	4.76
C16:0	Palmitic	10.14	11.51
C16:1	Palmitoleic	7.49	7.85
C18:0	Stearic	2.14	2.54
C18:1	Oleic	15.79	16.14
C18:2	Linoleic	1.80	1.87
C18:3	Linolenic	2.83	2.54
C20:1	cis -11- Eicosenoic	9.58	8.97
C20:5	Eicosapentanoic	8.71	8.43
C22:1	Erucic	9.05	8.41
C22:6	Docosahexaenoic	11.56	10.75

Table 2. Proximate composition of different treatments

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
T1	3.41 ± 0.01^{c}	8.75 ± 0.00	12.28 ± 0.07^{a}	9.59 ± 0.01^{d}	65.97 ± 0.09^{c}
T2	3.41 ± 0.02^{c}	8.75 ± 0.00	12.34 ± 0.03^{a}	7.35 ± 0.01^{a}	68.14 ± 0.0.3^{d}
T3	2.70 ± 0.02^{a}	8.75 ± 0.00	15.31 ± 0.01^{b}	7.95 ± 0.00^{b}	65.30 ± 0.03^{b}
T4	3.09 ± 0.02^{b}	8.75 ± 0.00	15.48 ± 0.25^{b}	9.20 ± 0.01^{c}	63.48 ± 0.27^{a}

In the column, values (mean±SD) with different letters (a-d) were significantly different at $p<0.05$

about 97-98% of water content from the product. But on reconstitution the product was found to give the same freshness and aroma as that of the original preparation. The better reconstitution of the powder can be attributed to the drying technology adopted, freeze drying.

The moisture content of the treatments was in the range of 2.7-3.4%, with the lowest moisture content in T3, treatment with only MFO. In general, the lower moisture content in all the treatments can be related to the freeze-drying as about 97- 98% of water will be removed from the product during the drying process. The fat content was high in T3 and T4 as MFO powder has been incorporated at 3% in both these treatments. Ash content of the treatments remains almost the same with a value ranging from 7.35±0.01 to 9.59±0.01%. After freeze drying, starch content might have concentrated in the powder form than its liquid form and hence a higher value. The calorific value of treatments varied from 409.32 to 433.99 Kcal (Table 2). In general, it can be concluded that the fish oil fortified soup is a high energy drink with the richness of omega-3 fatty acids and phytochemicals.

Irrespective of the storage temperature, the lightness values (L*) showed an increasing trend during the storage in all treatments (Fig. 2A). The increase in L* values can be due to dehydration of water by sublimation during freeze-drying, resulting in increase in lightness. A similar trend of increasing lightness in desalted task fish after the process of freeze drying was reported by Nguyen (2012). Regardless of the treatments, a* (redness) and b* (yellowness) values of all the treatments decreased during the storage period (Fig. 2B, 2C). One probable reason for the decrease in a* value can be due to the degradation of carotenoid pigments of the tomato paste during the storage time. The water activity of all the treatments increased slightly during the storage period and there was a significant difference among all the treatments ($p<0.05$) (Table 3). The increase in water activity can be explained by the porous structure of freeze dried product and the unchanged structure of protein

Fig. 2. Color changes of treatments stored at different temperature conditions (A, B & C depicts the changes in L*, a*b* during the storage period respectively)

Table 3. Water activity of treatments stored at different temperature conditions

Treatment	0th Day	10th Day	20th Day	30th Day
T1 RT	0.105 ± 0.001^{b}	0.123 ± 0.001^{c}	0.134 ± 0.001^{c}	0.144 ± 0.002^{d}
T2 RT	0.083 ± 0.002^{a}	0.096 ± 0.001^{b}	0.121 ± 0.001^{b}	0.124 ± 0.002^{b}
T3 RT	0.081 ± 0.001^{a}	0.091 ± 0.001^{a}	0.113 ± 0.002^{a}	0.120 ± 0.001^{a}
T4 RT	0.124 ± 0.001^{c}	0.135 ± 0.002^{e}	0.159 ± 0.001^{g}	0.161 ± 0.001^{f}
T1 37	0.105 ± 0.001^{b}	0.129 ± 0.001^{d}	0.149 ± 0.001^{f}	0.156 ± 0.002^{e}
T2 37	0.083 ± 0.002^{a}	0.136 ± 0.001^{e}	0.146 ± 0.001^{e}	0.153 ± 0.002e
T3 37	0.081 ± 0.001^{a}	0.129 ± 0.001^{d}	0.133 ± 0.001^{c}	0.140 ± 0.001^{c}
T4 37	0.124 ± 0.001^{c}	0.143 ± 0.001^{f}	0.139 ± 0.001^{d}	0.166 ± 0.002^{g}

In the column, values (mean±SD) with different letters (a-g) were significantly different at ($p<0.05$)

after freeze-drying. Though the values differ among all treatments, the maximum value reached to only about 0.166. Foods having water activity in the range of (<0.3) are reported to have long shelf life. Hence, it was assumed that soup powders developed in the present study can have a longer shelf life.

A significant increase ($p<0.05$) in rehydration ratio was observed within the initial 2 minutes of rehydration and approached the equilibrium state after 5 min (Table 4). The increase in rehydration with in minimal time can be due to the unchanged nature of protein and porous structure of the sample after freeze drying. The rehydration was lowest in T3 and T4 (both with fish oil microcapsule addition) which can be due to its hydrophobicity of fish oil that affected the rehydration. Similar trend in rehydration was observed in case of freeze dried products such as desalted tusk fish (Nguyen, 2012), garlic (Sablani, 2007) and apple and potato (Karathanos, 1996). Abdel-Haleem (2014) reported a similar rehydration ratio in case of a dried vegetarian soup supplemented with legumes.

Table 4. Rehydration ratio (RR) of treatments at different time (2 and 5 min)

Treatments	RR
T1-2 min	5.806 ± 0.048^{c}
T2-2 min	5.602 ± 0.007^{b}
T3-2min	5.310 ± 0.010^{a}
T4-2 min	5.275 ± 0.001^{a}
T1-5 min	6.905 ± 0.005^{f}
T2-5 min	7.713 ± 0.006^{g}
T3-5 min	6.373 ± 0.046^{d}
T4-5 min	6.423 ± 0.015^{e}

The results showed that there is a significant difference in the oxidative stability among the treatments stored at different temperature ($p<0.05$). The treatments stored at 37±2°C showed significantly higher peroxide value than that at room temperature (Fig. 3A). Among the treatments, T3 and T1 showed higher peroxide values. The trend is expected only as both these treatments are prepared without any preservatives to retain the shelf stability of the product. The significant reduction in PV in treatments T2 and T4 may be because of the addition of PAE (4%). Though there is an increase in the PV values throughout the storage period of 30 days, the values were within the acceptable range of 20 mEq of O_2 kg^{-1} of oil as per the CODEX/FAO standards. Similar trends were observed in case of TBARS and FFA assays (Fig 3B, 3C). Kumaran & Karunakaran (2007) have studied the activity guided isolation of free radical scavenging components from Indian Borage and reported chlorogenic acid, caffeic acid and rosmarinic acid as the major scavenging compounds. They have also reported that rosmarinic acid being the major phenolic compound, is mainly responsible for the antioxidant activity of leaves. Similarly, Orabi et al. (2000) reported the presence of abietene diterpenoids and sesquiterpenes in *Plectranthus* spp. extracts. This underpins the potential of PAE as an antioxidant agent in food systems.

The total plate count (TPC) of all the treatments slightly increased during the storage period. The bacterial counts were higher in treatments stored at 37°C than at room temperature as this temperature

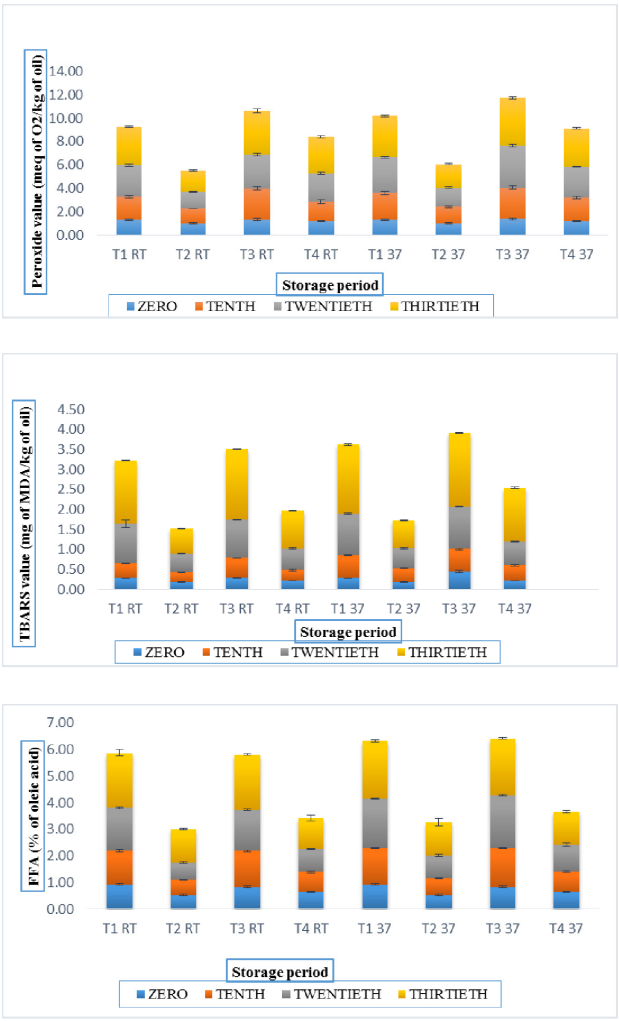

Fig. 3. Oxidative stability of treatments stored at different temperature conditions (A, B and C shows the changes in peroxide, TBARS & FFA values during the storage period respectively)

is considered ideal for the growth of all mesophiles. However, no faecal coliforms, yeasts and mould growth was observed during the entire storage period. Though there was an increasing trend in the bacterial growth during the storage period, the treatments T4 and T2 showed lower TPC values. The reason is that in both of these treatments PAE was added at the rate of 4%. The probable mechanism for the antibacterial activity of PAE can be the adsorption of polyphenolic compounds to bacterial membrane, thereby causing the membrane disruption, leakage of cellular contents and production of hydro peroxide from polyphenols (Ikigai et al., 1993; Otake, 1991; Akagawa, 2003). The antibacterial activity of the borage extracts was already established by Praveena & Bhatt (2012). The inhibitory effect of Indian borage leaves on both Gram positive and Gram negative organisms has been reported by Gurgel (2009) and Chandrappa (2010). Shubha (2015) have reported that the hot water extract of *P. amboinicus* leaves is having the potential to inhibit growth of pathogens such as *Escherichia coli* and *Salmonella typhimurium* and stimulates the growth of *Lactobacillus plantarum*. Murthy (2009) have reported the antifungal activity of Indian borage volatiles and suggested its use as botanical fungitoxicant. The aforesaid findings show the potentiality of Indian borage leaf extract as bio preservative because of its high antioxidant, antibacterial and antifungal activities.

There was significant difference in the sensory scores of different treatments irrespective of the storage temperature ($p<0.05$). The sensory scores have decreased significantly in all treatments at 30th day. In general, the treatments stored at 37°C received lower sensory score than that stored at room temperature (Table 5). The aroma, taste and the overall acceptability was low in control (T1) and in treatment without PAE (T3). The reason for this can be the accelerated oxidation of soup powders at 37°C as no preservatives is added to take care of the oxidation of the product. Among the different treatments, T4 received maximum sensory score proving its acceptability from a sensory point of view. This can be due to the addition of PAE which might have imparted flavoring effect as well as protective effect from oxidation. Wadikar & Premavalli (2013) has already reported the sensory acceptance of an Indian Borage based reconstitutable appetizer soup mix. Hence, it can be concluded that the sensory acceptance was higher when MFO was added along with PAE. This study clearly indicates the off flavor masking ability of PAE and hence can be exploited as a flavoring agent in products fortified with fish oil.

The growing increase in demand for functional foods of natural origin has fostered the screening of several plant based compounds for its bioactivity potential. In the present study, the inclusion effect of *P. amboinicus* leaf extract on the quality attributes of a functional food was evaluated. It was found that the incorporation of PAE improved the overall physico-chemical and microbial properties in freeze dried shrimp soup powder. Moreover, the sensory quality of the product was not adversely affected with the PAE addition. Instead it improved the sensory acceptance than the control samples proving its potentiality as a natural additive in food

systems. Based on the significant findings of the present study, it can be concluded that PAE holds promising potential as a functional food ingredient because of its antioxidant, antibacterial, antifungal and flavoring properties. However, this is a short term study and its results cannot be generalized. Hence, further research need to be carried out on these lines by isolating the individual molecules responsible for its bioactivity, to elucidate their activities using in-vitro and in-vivo assays and to establish its quantitative limits in foods.

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References

Abdel-Haleem, A. M. and Omran, A. A. (2014) Preparation of dried vegetarian soup supplemented with some legumes. Food Nutr. Sci. 5: 2274

Aghbashlo, M., Mobli, H., Madadlou, A. and Rafiee, S. (2013) Fish oil microencapsulation as influenced by spray dryer operational variables. Int. J. Food Sci. Technol. 48: 1707-1713

Akagawa, M., Shigemitsu, T. and Suyama, K. (2003) Production of hydrogen peroxide by polyphenols and polyphenol-rich beverages under quasi-physiological conditions. Biosci. Biotechnol. Biochem. 67: 2632-2640

Annapurani, S. and Priya, R. (1999) Antimutagenic, antitumourogenic and antigenotoxic effects of polyphenol extracts of selected medicinal plants. Indian J. Nutr. Diet. 36: 431-435

AOAC (2000) Official Methods of Analysis of AOAC International 17th edn., Washington D.C. USA, 2000; 4: 969.3 and 991.39

AOAC (2003) Official Methods of Analysis of AOAC International, 17th edn., revision 2, Vol II (Horwitz, W., Ed). Association of Official Analytical Chemists (AOAC) International, Gaithersburg, MD

APHA (1992) Standard Methods for Examination of Water and Wastewater. 18th edn., American Public Health Association. Washington DC

Brand-Williams, W., Cuvelier, M. E. and Berset, C.L.W.T. (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30

Buznego, M.T. and Perez-Saad, H. (1999) Antiepileptic effect of Plectranthus amboinicus (Lour.) Spreng. (french marjoram). Rev Neurol. 29: 388-389

Caleja, C., Barros, L., Antonio, A.L., Ciric, A., Sokovic, M., Oliveira, M.B.P., Santos-Buelga, C. and Ferreira, I.C. (2015). Foeniculum vulgare Mill. As natural conservation enhancer and health promoter by incorporation in cottage cheese. J. Funct. Foods. 12: 428-438

Carocho, M., Barreiro, M.F., Morales, P. and Ferreira, I.C. (2014) Adding molecules to food, pros and cons: A review on synthetic and natural food additives. Compr. Rev. Food Sci. Food Saf. 13: 377-399

Chandrappa, M.S., Harsha, R., Dinesha, R. and Gowda, T.S.S. (2010) Antibacterial activity of Coleus aromaticus leaves. Int. J. Pharm Pharm. Sci. 2: 63-66

Damanik, R., Damanik, N., Daulay, Z., Saragih, S., Premier, R., Wattanapenpaiboon, N. and Wahlqvist, M.L. (2001) Consumption of bangun-bangun leaves (Coleus amboinicus Lour) to increase breast milk production among Bataknese women in North Sumatera Island, Indonesia. Asia Pac. J. Clin. Nutr. 10: 267-274

Fewtrell, M. (2006) Long-chain polyunsaturated fatty acids in early life: effects on multiple health outcomes. In Primary Prevention by Nutrition Intervention in Infancy and Childhood. 57: 203-221, Karger Publishers

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol .Chem. 226: 497-509. PMID: 13428781

Gurgel, A.P.A.D., da Silva, J.G., Grangeiro, A.R.S., Oliveira, D.C., Lima, C.M., da Silva, A.C., Oliveira, R.A. and Souza, I.A. (2009) In vivo study of the anti-inflammatory and antitumor activities of leaves from Plectranthus amboinicus (Lour.) Spreng (Lamiaceae). J. Ethnopharmacol. 125: 61-363

Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T. (1993) Bactericidal catechins damage the lipid bilayer. Biochim. Biophys. Acta. 1147: 132-136

Karathanos, V. T., Anglea, S. A. and Karel, M. (1996) Structural collapse of plant materials during freeze-drying. J Therm Anal Calorim. 47: 1451-1461

Koolen, H.H., da Silva, F.M., Gozzo, F.C., de Souza, A.Q. and de Souza, A.D. (2013). Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (Mauritia flexuosa L. f.) by UPLC–ESI-MS/MS. Food Res. Int. 51: 467-473

Krokida, M. K. and Marinos-Kouris, D. (2003) Rehydration kinetics of dehydrated products. J Food Eng. 57: 1-7

Kumaran, A. and Karunakaran, R. J. (2007) Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of Coleus aromaticus. Food Chem. 100: 356-361

Lekshmi, R. G. K., Chatterjee, N. S., Tejpal, C. S., Vishnu, K. V., Anas, K. K., Asha, K. K., Anandan, R. and Mathew, S. (2017) Evaluation of chitosan as a wall material for microencapsulation of squalene by spray drying: Characterization and oxidative stability studies, Int. J. Biol. Macromol. 104: 1986-1995

Lizarraga, M.S., Pan, L.G., Anon, M.C. and Santiago, L.G. (2008) Stability of concentrated emulsions measured by optical and rheological methods. Effect of processing conditions—I. Whey protein concentrate. 2 Food Hydrocoll. 2: 868-878

Mcclements, D.J. (2007) Critical review of techniques and methodologies for characterization of emulsion stability. Crit. Rev. Food Sci. Nutr. 47: 611-649

Muller, H.G. and Tobin, G. (1980) Nutrition and food processing London: Croom Helm Ltd.

Murray, J.M., Delahunty, C.M. and Baxter, I.A. (2001). Descriptive sensory analysis: past, present and future. Food Res Int. 34: 461-471.

Murthy, P.S., Ramalakshmi, K. and Srinivas, P. (2009) Fungitoxic activity of Indian borage (Plectranthus amboinicus) volatiles. Food Chem. 114: 1014-1018

Nguyen, M.V., Jonsson, A. and Arason, S. (2012) Effect of Freeze Drying on Quality of Desalted Tusk Fish. As. J. Food Ag-Ind. 5: 388-394

Orabi, K. Y., Mossa, J. S., Muhammed, I., Alloush, M. H., Galal, A. M., El-Feraly, F. S. and McPhail, A. T. (2000) New eudesmane sesquiterpenes from Plectranthus cylindraceus. J. Nat. Prod. 63: 1665-1668

Otake, S., Makimura, M., Kuroki, T., Nishihara, Y. and Hirasawa, M. (1991) Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res. 25: 438-443

Praveena, B. and Pradeep S, N. (2012) Antioxidant and antibacterial activities in the leaf extracts of Indian borage (Plectranthus amboinicus). Food Nutr. Sci. 3:146-152 Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A. and Millington, K.J. (2004) The health benefits of omega 3 polyunsaturated fatty acids: a review of the evidence. J. Hum. Nutr. Diet. 17: 449-459

Sablani, S.S., Rahman, M.S., Al-Kuseibi, M.K., Al-Habsi, N.A., Al-Belushi, R.H., Al-Marhubi, I. and Al-Amri, I.S. (2007) Influence of shelf temperature on pore formation in garlic during freeze-drying. J. Food Eng. 80: 68-79

Shah, N. P. (2007) Functional cultures and health benefits. Int. Dairy J. 17: 1262-1277

Shubha, J.R. and Bhatt, P. (2015) Plectranthus amboinicus leaves stimulate growth of probiotic L. plantarum: Evidence for ethnobotanical use in diarrhea. J. Ethnopharmacol. 166: 220-227

Singleton, V. L. and Rossi, J. A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158

Tarladgis, B. M., Watts, M. T., Younathan and Duggan, L. R. (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44-48

Wadikar, D. D. and Premavalli, K. S. (2013) Development of a hot water reconstitutable appetizer soup mix from Coleus aromaticus using response surface methodology. Int. Food Res. J. 20: 3041-3046

Wadikar, D. D. and Premavalli, K. S. (2011) Effect of appetizer administration on plasma leptin level in human volunteers. Int. J. Food Sci. Nutr. 62: 148-151