

ENCAPSULATION EFFICIENCY AND RELEASE PROPERTIES OF LEMONGRASS OIL (*Cymbopogon citratus* (DC) Stapf.) WITH SOY PROTEIN ISOLATE

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Abstract

This study was aimed to investigate the encapsulation efficiency and release of lemongrass oil with soy protein isolate (SPI). Lemongrass oil was encapsulated by soy protein isolate shell at ratios 1:1, 2:1, and 1:2 by freeze-dried. Morphological properties, chemical reaction and thermal stabilities of lemongrass oil microcapsule were tested by Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and thermo gravimetric analysis (TGA). The morphologies of microcapsules lemongrass oil at ratio 2:1 and 1:2 appear the roughest surface and polygonal shape, while those based on microcapsule lemongrass oil at ratio 1:1 exhibit the smoothest surface, round shapes like SPI powder. The morphologies of microcapsules lemongrass oil at ratio 2:1 and 1:2 appear the roughest surface and polygonal shape, while those based on microcapsule lemongrass oil at ratio 1:1 exhibit the smoothest surface, round shapes like SPI powder, and all microcapsules lemongrass oil has a particle size larger than soy protein isolate powder. FTIR spectra of the all microcapsule lemongrass oil confirmed the presence of lemongrass oil. The TGA thermogram shown microcapsule lemongrass oil ratio at 1:1 exhibit two mass loss zones and losing the least weight. Microcapsule lemongrass oil can be applied in the agricultural industry such as add-in animal feed etc.

Keywords: Lemongrass oil, soy protein isolate, microcapsules, freeze-dried

Introduction

Lemongrass (*Cymbopogon citratus* stapf.) is a tropical and subtropical perennial plant which yields aromatic oil. Lemongrass oil can use in pharmaceutical, cosmetics, food and agriculture industry and has medicinal properties such as treating stomach pain and treating urinary tract diseases. The substance in lemongrass oil is citral group (65-80%) myrcene (12-20%) limonene citronell (citronellal) geraniol and linalool etc. (Guenther, 1950; Zaki *et al.*, 1975; Tyagi and Malik,

2010) and has anti-pathogenic activity (Pootong *et al.*, 2012) such as *E. coli*, *Streptococcus* spp., *Staphylococcus* spp. and inhibit fungal growth (Onawunmi *et al.*, 1984; Somda *et al.*, 2007; Irkin, and Korukluoglu, 2009). In addition, lemongrass oil has antioxidant property, inhibit lipid peroxidation (Cheel *et al.*, 2005). However, lemongrass oils can evaporate easily and quickly when exposed to air, heat and oxygen.

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Microcapsule techniques can reduce evaporation rate of essential oils and prevent the loss of some substances from chemical reaction. The microcapsule process is creating a layer between core and shell to avoid chemical reactions, maintain biological properties and the physicochemical properties of the core material. Normally, the microcapsule production process consists of 2 steps: emulsion and drying. The dry technique most common ones are encapsulation techniques, spray-drying and freeze-drying. There are reports freeze-dried blueberry powder has retention values of anthocyanins higher than spray-dried process (Wilkowska *et al.*, 2016). Microcapsules with emulsifier as oil in water are dried by spray drying or freeze drying. Both methods are commonly used to extend shelf life of essential oils (Lim *et al.* 2011).

Soy protein isolate has the properties, including gelling, emulsifying, fat-absorbing and water binding properties. (Nunes *et al.*, 2003; Caillard *et al.*, 2009). Kim and Morr (1996) study the encapsulation of orange oil using SPI which has better emulsifier than gum arabic (GA), whey protein isolate (WPI), and sodium caseinate (SC). Therefore, SPI is interesting to be applied in the production of microcapsules.

This study examined the potential of soy protein isolate (shell material) and lemongrass oil (core material) using freeze-dried techniques.

Materials and Methods

Materials

Lemongrass oil or LO (*Cymbopogon citratus* Stapf.) was purchased from Botanicesence Essential Oils store, Thailand. It has a specific gravity of 0.8921 and a refractive index of 1.4835. Extracted of essential oil was used as the core material. Soy protein isolate or SPI (minimum of 90.5% protein content) and Tween 80 (cationic surfactant); food-grade was purchased from Chemipan Corporation Co. Ltd. were used as wall materials and emulsifiers, respectively.

Methods

Preparation of Microcapsules

In this study, shell material emulsions were prepared from soy protein isolate (SPI). Shell material at 50 66.66 and 33.33 g, respectively dissolved in DI water at 400 ml. by mixed for 90 min and left overnight. In another vial, lemongrass oil at 50 33.33 and 66.66 g, respectively mixed with Tween 80 at 50 g by mixed for 30 min. After that, both substances are mixed at 1,200 rpm. for 30 min according to the method of Sittipummongkol *et al.* (2018) as shown in Figure 1. It is dried by freeze-

dried machine (FDB-5503, Operon Co., Ltd.) at -20°C for 3 days, pressure 40 Pa according to Fioramonti *et al.* (2017) as shown in Figure 2. The microcapsule lemongrass oil or MLO was divided into 3 ratios: 1:1 2:1 and 1:2.

Efficiency and Release of Microcapsules

Scanning Electron Microscopy (SEM)

Examination of the morphology of microcapsules lemongrass oil at a ratio of 1:1, 2:1 and 1:2 by scanning electron microscope; SEM. It studied at 200 and 500 magnification and the samples were mounted on the specimen holder with double-sided adhesive tape and coated with gold, then measure the size with ImageJ programs and calculate the results into an average in micrometer (µm.).

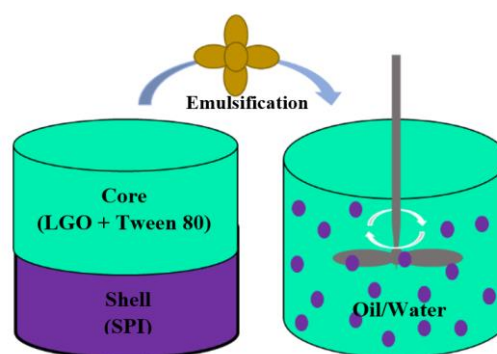


Figure 1. Emulsion production methods of microcapsules lemongrass oil

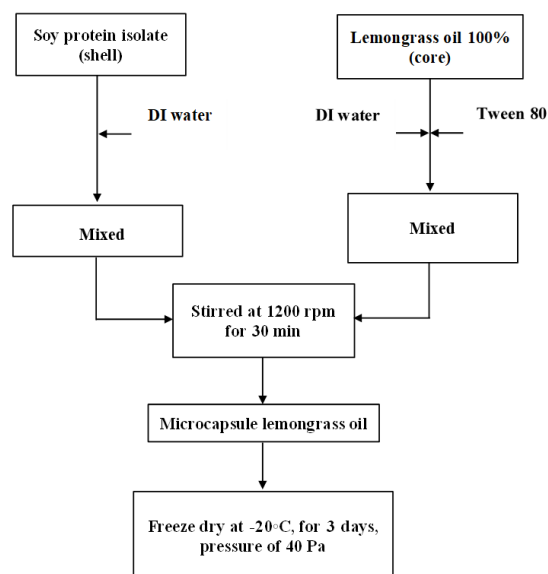


Figure 2. Production process of microcapsules lemongrass oil with freeze-dried

Fourier Transform Infrared Spectroscopy (FTIR)

Microcapsules lemongrass oil at a ratio of 1:1, 2:1 and 1:2 tested with FTIR measurements were using as a model Nicolet iS50, Thermo Fisher Scientific, USA. All FTIR absorption spectra were recorded in 400-4,000 cm^{-1} range at a resolution of $\pm 4 \text{ cm}^{-1}$, over 32 scans.

Thermogravimetric Analysis (TGA)

Test the thermal decomposition properties of microcapsules lemongrass oil at the ratio of 1:1 2:1 and 1:2 with the weight change analyzer using the thermal property model TGA/DSC3+/HT/1,600, METTLER TOLEDO, Switzerland studied at 30-700°C and the heat rate at 5°C/min (Sittipummongkol *et al.*, 2018).

Statistical Analysis

A study of three different microcapsules lemongrass oil ratio wave conducted in a randomized design. There is an evaluation of the size of the microcapsules at the ratio of SPI per LO at 1:1 2:1 and 1:2, respectively. The data obtained to analyze variance (ANOVA) and compare the difference between the mean by Duncan's New Multiple Range Test (DMRT) at 95% confidence level by SAS® University Edition.

Results and Discussion

Scanning Electron Microscopy of Lemongrass Oil with SPI

Morphology of microcapsules of lemongrass oil were analyzed by SEM (Figure 3). Microcapsules lemongrass oil at ratio 1:1 have a smooth surface and roughly spherical shape but microcapsules lemongrass oil ratio 2:1 and 1:2 has a rough surface and angular shape. Microcapsule lemongrass oil at ratio 2:1 and 1:2 have shell material appear the roughest surface and polygonal shape, while those based on microcapsule lemongrass oil at ratio 1:1 exhibit the smoothest surface and round shape like SPI powder. The freeze-dried methods make small particles, structure porous and rough surface that found similar reports encapsulated DHA algae oil by freeze-dried (Karthik and Anandharamakrishnan, 2013; Hundre *et al.*, 2015). In addition, the porosity on the surface of the microcapsules caused by contraction of uneven drying (Buma, 1971). The average sizes of SPI powder and microcapsules lemongrass oil at ratio 1:1, 2:1 and 1:2 particles were 21.96 ± 13.93 , 41.89 ± 20.74 , 94.58 ± 38.65 and $84.54 \pm 29.33 \mu\text{m}$, respectively (Table 1). All microcapsules lemongrass oil has a larger size than SPI powder. The greater

average diameter a result of reactions of core and shell, as oil is hydrophobic, while shell is hydrophilic due to the presence of polar protein/carbohydrate moieties (Sittipummongkol *et al.*, 2018). Moreover, the microcapsules at ratio 1:2 absorb too much oil cause high humidity, resulting in porous surface and larger size. The powders produced by freeze-drying had a resembled broken glass or flake-like structure. This structure could be due to the low temperature involved in the freeze-drying process which results in the lack of forces for breaking up the frozen liquid into droplets (Chen *et al.*, 2012). Wilkowska *et al.* (2016) explained that the freeze-dried powders showed much more satisfying retention values of anthocyanins when comparing the total amount of the received powder, for which it was about 1.5 times higher than for the spray-dried ones. Although the production of frozen microcapsules with porosity and uneven shape can help prevent oxidation of the core and the external environment (Silva *et al.*, 2016)

ATR-FTIR Spectroscopy of Lemongrass Oil with SPI

Analysis of the reaction between the core substance and the wrapper of microcapsules lemongrass oil by Fourier transform infrared (FTIR)

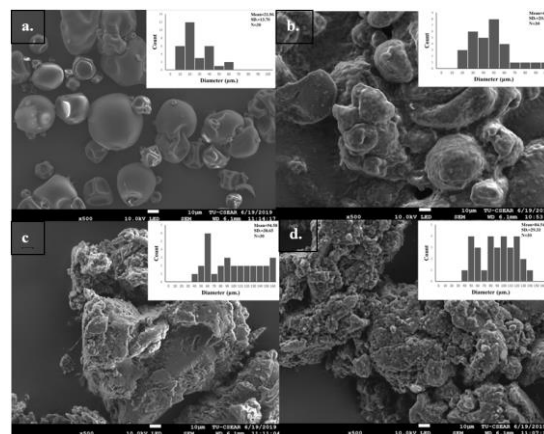


Figure 3. SEM micrographs of microcapsule lemongrass oil. (a) SPI; b MLO 1:1; c MLO 2:1; d MLO 1:2)

Table 1. Particle size of microcapsules lemongrass oil at ratio 1:1, 2:1 and 1:2.

Ratio (shell:core)	Size (μm)
SPI	21.96 ± 13.93^c
SPI:LO (1:1)	41.89 ± 20.74^b
SPI:LO (2:1)	94.58 ± 38.65^a
SPI:LO (1:2)	84.54 ± 29.33^a

Mean $\pm$ standard error; SPI: soy protein isolate, LO: lemongrass oil.

^{a,b,c} Values with different letters in the same column differ significantly ($p < 0.05$)

spectroscopy. Figure. 4 ATR-FTIR spectra of microcapsule lemongrass oil at ratio 1:1, 2:1 and 1:2. Comparison the spectra of each chemical composition and the corresponding microcapsule was made clear using lines. The ATR-FTIR spectra of lemongrass oil show characteristic peaks at 2,924 cm^{-1} ($\text{sp}^3\text{C-H}$ stretching), 1742 cm^{-1} (C=O stretching), 1,674 cm^{-1} (C=C stretching), 1,444 cm^{-1} (CH_2 bending), 1,377 cm^{-1} (CH_3 bending) and 1,154 cm^{-1} (C-H stretching) (Natrajan *et al.*, 2015). Peaks attributable to SPI appear at 3,273 cm^{-1} (N-H stretching), 2,930 cm^{-1} (C-H stretching), 1633 cm^{-1} (C=O), 1,516 cm^{-1} (N-H bending), 1447 cm^{-1} (CH_2 bending) and 1,393 cm^{-1} (CH_3 bending) (Hadzieva *et al.*, 2017). In addition, peaks attributable to all microcapsule lemongrass oil appear at 1,100 cm^{-1} (C-N stretching).

Thermal properties of lemongrass Oil with SPI

The TGA thermograms of microcapsules lemongrass oil (1:1, 2:1 and 1:2) are shown in Figure 5. SPI showed two-step degradation at a temperature 283.92 and 575.75°C, respectively that unlike those of the microcapsule lemongrass oil at ratio 1:1 and 1:2, which showed one-step degradation at a temperature 403.92 and 403.43°C, respectively (curves a and c in Figure. 5) but microcapsule lemongrass oil ratio 2:1 showed two-step degradation at a temperature 333.08 and 401.08°C.

SPI degradation occurs in two steps, beginning at 280°C followed by the final degradation at around 580°C. The weight of microcapsules decreases in the temperature range 200 to 600°C. Microcapsule lemongrass oil at ratio 1:1 exhibited around 78 and 20% weight loss at 360 and 560°C, respectively, and at ratio 2:1 exhibited around 47, 26, and 25% weight loss at 300, 400, and 560°C, respectively. Microcapsule lemongrass oil at ratio 1:2 exhibited around 82 and 17% weight loss at 360 and 560°C, respectively. The TGA thermogram of all microcapsule lemongrass oil exhibits weight loss zones which correspond to the degradation. The combination of substances is often associated with the optimum ratio of shell material (Rutz *et al.*, 2013).

Comparing the TGA thermograms of microcapsules lemongrass oil (1:1, 2:1 and 1:2) are show that microcapsules lemongrass oil at 1:1 have better thermal stability than the microcapsules of lemongrass oil at other ratios due to weight loss beginning at 360°C (78%) and 560°C (20%) compared microcapsules lemongrass oil at 2:1, resulting in weight loss at lower temperatures and microcapsules lemongrass oil at 1:2 lost more weight at 360°C.

Freeze drying using microcapsules lemongrass oil in the experiment can help to reduce thermal

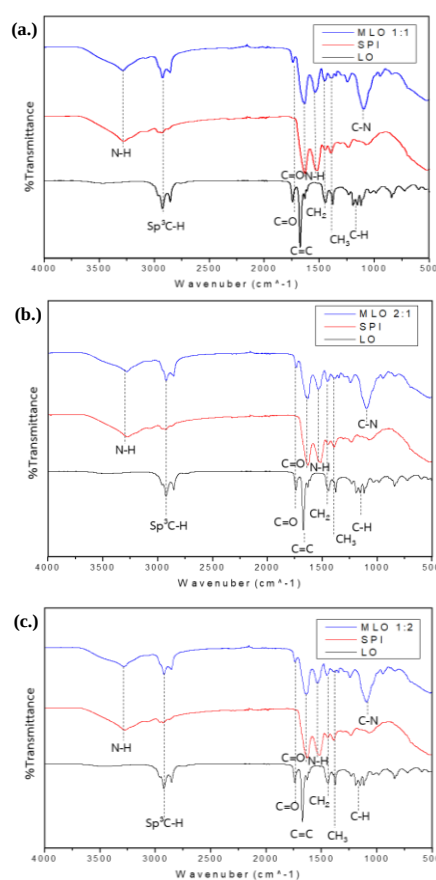


Figure 4. ATR-FTIR spectroscopy of microcapsule lemongrass oil. (a) MLO 1:1; b) MLO 2:1; c) MLO 1:2)

degradation when compared to the SPI produced from drying by incubators. SPI showed begins degradation at a temperature of 200°C, but microcapsules lemongrass oil begins to disintegrate at higher temperatures.

Conclusions

Soy protein isolate in this study has the benefit of reducing the evaporation of lemongrass oil as core material by freeze-drying. Morphological investigations indicated that microcapsules lemongrass oil at ratio 1:1 have a smooth surface but ratio 2:1 and 1:2 has a rough surface, angular shape, and all microcapsules lemongrass oil has a particle size larger than soy protein isolate powder. FTIR spectra of the all microcapsule lemongrass oil confirmed the presence of both soy protein isolate and lemongrass oil. The TGA thermogram shown microcapsule lemongrass oil ratio at 1:1 exhibit two mass loss zones and losing the least weight which corresponds to the start of degradation. The results

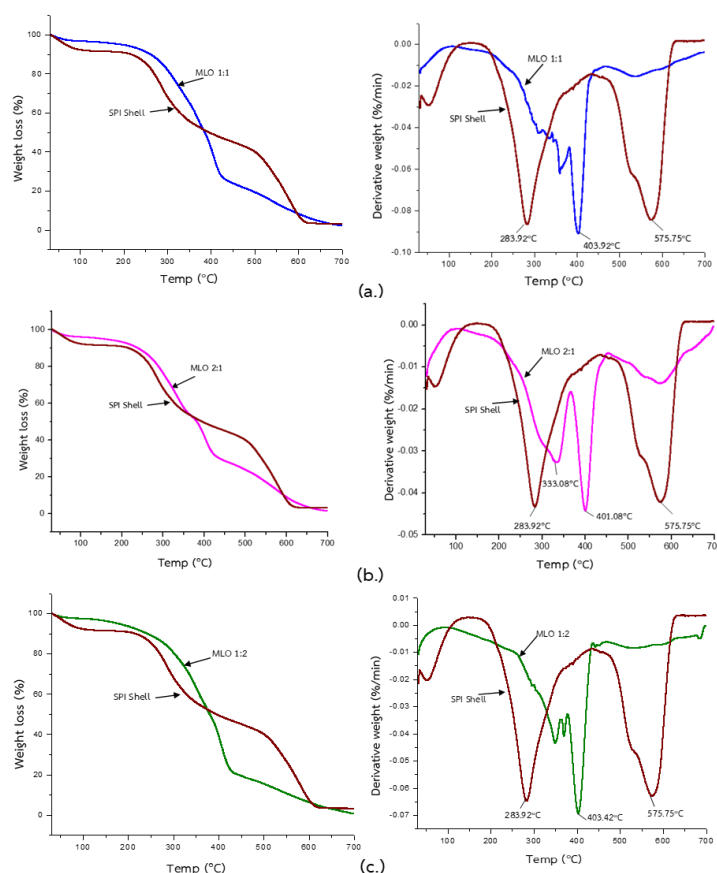


Figure. 5 The TGA thermograms of microcapsule lemongrass oil. (a) MLO 1:1; b) MLO 2:1; c) MLO 1:2

of this study of efficacy and release may be applied in the agricultural industry such as add-in animal feed etc.

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References

- Buma, T.J. (1971). Free fat in spray-dried whole milk. 8. The relation between free-fat content and particle porosity of spray-dried whole milk. *Nederlands melk-en zuiveltijdschrift*. Neth. Milk Dairy J., 25:123.
- Caillard, R., Remondetto, G.E., and Subirade, M. (2009). Physicochemical properties and microstructure of soy protein hydrogels co-induced by Maillard type cross-linking and salts. *Food Res. Int.*, 42(1):98-106.
- Cheel, J., Theoduloz, C., Rodrigues, J., and Schmida-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citrates* (DC) Stapf.). *J. Agric. Food Chem.*, 53:2,511-2,517.
- Chen, C., Chi, Y.J., and Xu, W. (2012). Comparisons on the functional properties and antioxidant activity of spray-dried and freeze-dried egg white protein hydrolysate. *Food Bioproc. Tech.*, 5:2,342-2,352.
- Fioramonti, A.S., Rubiolo, C.A., and Santiago, G.L. (2017). Characterisation of freeze-dried flaxseed oil microcapsules obtained by multilayer emulsions. *Powder Technol.*, 319:238-244.
- Guenther, E. (1950). *The essential oil*. Vol. IV. Florida, USA, REK Publishing Company, p. 602-615.
- Hadzieva, J., Mladenovska, K., Crcarevska, M.S., Dodov, M.G., Dimchevska, S., Geškovski, N., Grozdanov, A., Popovski, E., Petruševski, G., Chachorovska, M., Ivanovska, T.P., Petruševska-Tozi, L., Ugarkovic, S., and Goracinova, K. (2017). Lactobacillus casei encapsulated in soy protein isolate and alginate microparticles prepared by spray drying. *Food Tech. Biotech.*, 55(2):173-186. <https://doi.org/10.17113/ftb.55.02.17.4991>
- Hundre, S.Y., Karthik, P., and Anandharamakrishnan, C. (2015). Effect of whey protein isolate and β -cyclodextrin wall

- systems on stability of microencapsulated vanillin by spray-freeze drying method. *Food Chem.*, 174:16-24.
- Irkin, R. and Korukluoglu, M. (2009). Effectiveness of *Cymbopogon citratus* L. essential oil to inhibit the growth of some filamentous fungi and yeasts. *J. Med. Food*, 12(1):193-197.
- Karthik, P. and Anandharamakrishnan, C. (2013). Microencapsulation of docosahexaenoic acid by spray-freeze-drying method and comparison of its stability with spray-drying and freeze-drying methods. *Food Bioproc. Tech.*, 6:2,780-2,790.
- Kim, Y.D., and Morr, C. (1996). Microencapsulation properties of gum Arabic and several food proteins: Spray-dried orange oil emulsion particles. *J. Agric. Food Chem.*, 44:1,314-1,320.
- Lim, H.K., Tan, C.P., Bakar, J., and Ng, S.P. (2011). Effects of different wall materials on the physicochemical properties and oxidative stability of spray-dried microencapsulated red-fleshed pitaya (*Hylocereus polyrhizus*) seed oil. *Food Bioproc. Tech.*, 5(4):1,220-1,227.
- Natrajan, D., Srinivasan, S., Sundar, K., and Ravindran, A. (2015). Formulation of essential oil-loaded chitosan-alginate nanocapsules. *J. Food Drug. Anal.*, 23(3):560-568.
- Nunes, M., Batista, P., Raymundo, A., Alves, M., and Sousa, I. (2003). Vegetable proteins and milk puddings. *Colloids Surf. B*, 31:21-29.
- Onawunmi, G.O., Yisak, W.A.B., and Ogunlana, E.O. (1984). Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf. *J. Ethnopharmacol.*, 12:279-286.
- Pootong, A., Sam-angsri, J., Thongmalila, N., Khantisitiporn, O. and Cowawintaweewat, S. (2012). The effect of lemongrass volatile oil on pathogenic candida albicans. *TJST*, 20:293-301.
- Rutz, J.K., Zambiasi, R.C., Borges, C.D., Krumreich, F.D., Da Luz, S.R., Hartwig, N., and Da Rosa, C.G. (2013). Microencapsulation of purple Brazilian cherry juice in xanthan, tara gums and xanthan-tara hydrogel matrixes. *Carbohydr. Polym.*, 98(2):1,256-1,265.
- Silva, E.K., Azevedo, V.M., Cunha, R.L., Hubinger, M.D., and Meireles, M.A.A. (2016). Ultrasound-assisted encapsulation of annatto seed oil: Whey protein isolate versus modified starch. *Food Hydrocoll.*, 56:71-83.
- Sittipummongkol, K., Chuysinuan, P., Techasakul, S., Pisitsak, P., and Pechyen, C. (2018). Core shell microcapsules of neem seed oil extract containing azadirachtin and biodegradable polymers and their release characteristics. *Polymer Bulletin*. Springer-Verlag GmbH Germany, part of Springer Nature 2018, 16p.
- Somda, I, Leth, V., and Sereme, P. (2007). Antifungal effect of *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Azadirachta indica* oil extracts on sorghum seed-borne fungi. *Asian J. Plant Sci.*, 6(8):1,182-1,189.
- Tyagi, K.A. and Malik A. (2010). In situ SEM, TEM and AFM studies of the antimicrobial activity of lemon grass oil in liquid and vapour phase against *Candida albicans*. *Micron*, 41(7):797-805.
- Wilkowska, A., Ambroziak, W., Czyżowska, A., and Adamiec, J. (2016). Effect of microencapsulation by spray-drying and freeze-drying technique on the antioxidant properties of blueberry (*Vaccinium myrtillus*) juice polyphenolic compounds. *Polish J. Food Nutr. Sci.*, 66(1):11-16.
- Zaki, A.S.M., Foda, H.Y., Mostafa M.M., and Abd Allah M.A. (1975). Identification of the volatile constituents of the Egyptian lemongrass oil. Part II. Thin Layer Chromatography. *Mol. Nutr. Food Res.*, 19(3):201-205.