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IN VITRO ENCAPSULATION CHARACTERIZATION OF CLOVE OIL MICROENCAPSULATES

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Currently, spices have attained the potential to mitigate free radicals owing to the presence of numerous aromatic moieties. Due to their safe nature, these bioactive ingredients could be employed to replenish already existing artificial food additives to ensure healthy foods. Considering these facts, the present study was designed to probe the eugenol content present in the supercritical fluid extracted clove oil. Furthermore, it was encapsulated to avoid losses during thermal processing and to improve bioefficiency. In the current investigation, clove available in the Pakistani market was tested for compositional analyses that indicated higher content of nitrogen free extract, fiber and fat however, amongst minerals; calcium, sodium, magnesium and potassium were found in abundant proportions. Afterwards, the clove was subjected to supercritical fluid extractor at three different pressures; 1500, 3000 and 4500 psi. The clove oil carrying eugenol as the major fraction was optimally extracted at 4500 psi as analyzed by GC-MS. This clove oil was further coated via coating ingredients such as maltodextrin, gum arabic and their combination in 1:1, 1:2 and 2:1 ratio using freeze-drying technique. Characterization of all treatments were accomplished regarding multi-aspects including total polyphenols, ABTS assay and encapsulation efficiency that indicated best results in gum arabic based coatings with corresponding values as 47.68±1.91 mg GAE/g dry powder, 85.20±5.96% inhibition and 82.03±3.61, respectively. Conclusively it is depicted that microencapsulation is promising technique which ensued strong anti-oxidant potential of clove oil.
Keywords: Clove, super critical fluid extraction, GC-MS, freeze drying, anti-oxidant potential.

INTRODUCTION

Food safety becomes hot issue worldwide because everyone is more conscious about health status. In this regard, plant based food additives become idealistic choice of the food industries instead of artificial additives (Ameratunga *et al*., 2016). Spices and herbs are usually considered as a natural rich source of antioxidants that play an imperative role in chemoprevention of ailments especially resulting due to lipid peroxidation. They also possess the potential to be employed as an alternate to synthetic preservatives (Viuda-Martos *et al.,* 2011).

Antioxidants are vital fragment of processed foods. In past, mostly factitious antioxidants were employed to increase shelf life of food items but they have detrimental holdings on consumer's health. Now-a-days, food processors are utilizing antioxidants from natural vegetal matrixes. Vigorous antioxidant potential, negligible counteracts and mellow compatibility with foodstuff escalates the demand of natural antioxidants based products (David *et al.,* 2013; Gurbuz *et al.,* 2016; Ramadhan *et al*., 2017).

There are more than three thousand dietary sources of familiar antioxidants in the world which includes spices, herbs, foods, beverages and dietary supplements (Carlsen *et al.,* 2010). Amongst spices, clove (*Syzygium aromaticum;* Family: Myrtaceae) is an aromatic dried flower bud with dark brown color, distinctive fragrance and burning taste. Centuries ago, cloves were cultivated in Maluku spice island of Indonesia but now it has reached to all parts of the world (Kamatou *et al*., 2012). Normally, it is utilized for culinary purposes at domestic level, whereas employed as a flavoring agent or antimicrobial agent at industrial scale (Lekjing, 2016; Przygodzka *et al*., 2016). Considering nutritional worth, clove is a robust source of minerals; Ca, K, Mg, P, Se, Fe as well as Zn, vitamins; vitamin C, A, E, K as well as B and phytonutrients; eugenol, carotene-β, cryptoxanthin-β along with lutein-zeaxanthin (Ereifej *et al*., 2015). Furthermore, dried clove consists of nearly 15-20% of essential oil or 1 kg of dried clove buds could provide around 150 mL of clove oil (Bhowmik *et al.,* 2012). The chief lipophilic moiety in clove oil is eugenol (structure: L-hydroxy-2-methoxy-4-allylbenzene) that accounts up to 70-90% of the total essential oil (Gulcin *et al.,* 2012). This component plays important role as anti-oxidant.

Clove bioactive components can be extracted through various conventional extraction techniques such as soxhlet extractor, magnetic stirrer; maceration grinding and heat reflux (Yang *et al.,* 2008). Likewise, novel technique such as supercritical fluid extractor is an eco-friendly way of extraction and finest isolation mode to acquire lipophilic components from clove

hence considered as green technology (Yang *et al.,* 2011; Khaw *et al*., 2017). After extraction, most of the essential oils subsist in liquid form at room temperature. These oils are a fantabulous source of bioactive compounds but highly volatile hence easily get lost during processing or storage in response to high light intensity, temperature and oxygen. Such factors limit the usage of essential oil, but many techniques have developed to sort out this issue (Bergkvist, 2007; Ayala-Zavala *et al.,* 2008). Among these techniques, encapsulation is regarded as the most proficient and authentic method that involves blending and entrapment of lipophilic compounds in the excipient stuffs (Martin *et al*., 2010; Simon-Brown *et al.,* 2016). In past, many researches were executed regarding anti-oxidant capacity of oil. So present research is designed to evaluate the antioxidant potential of clove oil microencapsulates.

MATERIALS AND METHODS

The current study was conducted at National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The details regarding the materials obtained and methods adopted for various analyses to meet the objectives of the study are summarized herein.

***Procurement and preparation of raw material*:** The clove (*Syzigum aromaticum* L.) samples were procured from local market and ground to fine powder for further analyses.

***Proximate composition*:** The clove powder was assessed for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract (NFE) as per their standard methods in AOAC (2006).

***Mineral profile*:** The clove samples were also subjected to mineral profiling by adopting the guidelines of AOAC (2006). Dried clove sample (0.5 g) was digested with HNO_3 and $HClO_4$ while heating on hot plate until sample becomes colorless and reduced to 1-2 mL in volume. Afterwards, it was diluted up to 100 mL using double distilled water. The minerals *i.e.* Na, K and Ca were estimated via Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) whilst P, Mg, Zn and Fe were determined using atomic absorption spectrophotometer (Varian AA240, Australia).

***Extraction of eugenol containing clove oil*:** Eugenol was extracted from clove buds via Supercritical Fluid Extractor SFT-150 system using 99.8% pure CO_2 at 50°C, using the method elucidated by Wenqiang *et al*. (2007). After placing the ground clove sample in extraction vessel; CO_2 was used at optimized pressure *i.e*. 1500, 3000 and 4500 psi, separately to enhance mass transfer as well as solvation ability of clove oil and T_1, T_2 and T_3 were renamed as T_{1500}, T_{3000} and T_{4500}, respectively.

***GC-MS analysis of eugenol*:** The clove oil obtained from supercritical fluid extraction (SFE) was analyzed using GC-MS (ISQ series, Thermo Fisher Scientific, USA) carrying fused silica column of 0.25 µm diameter. Helium was used as carrier gas at a flow rate of 1.15 mL/min. GC oven temperature was settled at 60°C for 5 min; slowly raised to 260°C @ 2°C/min and then kept at this constant temperature. 0.1 µL clove oil sample was injected at 250°C injection temperature. Carrier gas was transported at constant pressure *i.e.* 5 kg/cm^2. MS spectra were taken as E_1 ion source of 70 eV (Wenqiang *et al*., 2007).

Table 1. Treatments for supercritical CO_2 extraction of clove.

Treatments	Pressure (psi)
$T_{SFE1500}$	1500
$T_{SFE3000}$	3000
$T_{SFE4500}$	4500

SFE = Supercritical Fluid Extract

***Selection of best treatment*:** Based on GC-MS analysis, one best SFE treatment *i.e.* which shows highest amount of eugenol was selected for further microencapsulation.

***Microencapsulation of clove oil*:** The selected SFE treatment was encapsulated by devising emulsions using different combination *i.e.* maltodextrin: gum arabic (1:1, 1:2 and 2:1) as described in Table 3.2. There are five microencapsulated treatments *i.e.* M_1 **(**MD_{18}:GA_0), M_2 (MD_0:GA_{18}), M_3 (MD_9:GA_9), M_4 (MD_6:GA_{12}) and M_5 (MD_{12}:GA_6).

***Treatment plan for microencapsulation*:**

Treatments	Maltodextrin (g)	Gum Arabic (g)
M_1	18	-
M_2	-	18
M_3	9	9
M_4	6	12
M_5	12	6

Preparation of clove oil powder: Each emulsion was prepared by dissolving maltodextrin, gum arabic and clove oil in 100ml distilled water using magnetic stirrer at 10°C. All combination of emulsions was frozen for 24 hours and then lyophilized at -50°C and 1.09 Pa till all moisture was sublimated using Freeze Dryer-5 Model 75050, Labconco, USA. The lyophilized powder was stored in air tight zipper bags and kept in a desiccator at 20°C till further use (Hill *et al.,* 2013).

Antioxidant assay:

***Total phenolic content*:** Total phenolic content (TPC) of microencapsulated clove oil was assessed using Folin-Ciocalteu method (Sultana *et al*., 2014). Purposely, clove microencapsulates were first dissolved in water then 50 µL of the resultant sample was added to a test tube carrying 750 µL of 20% Na_2HCO_3 & 250 µL of Folin-Ciocalteu's reagent and total volume was made up to 5 mL using distilled water. After two hours, absorbance of the sample was recorded at 765 nm via UV/Visible light Spectrophotometer (CECIL-CE7200) in comparison to control. TPC of the samples was expressed as

mg GAE/100g (mg gallic acid equivalent/100g).
TPC of each clove sample and control was calculated using following equation:

$$C=\frac{c\times V}{m}$$

C = TPC (mg GAE/g clove's encapsulates), c = Conc. of gallic acid (mg/mL), V = Volume of encapsulates in mL, m = Weight of clove encapsulates

***DPPH method*:** DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ability was assessed by using the protocol of Teixeira *et al*. (2014). Purposely, 2 mL of 0.2 mM/L fresh DPPH solution prepared using ethanol and was mixed with 20 mg of clove encapsulates. The sample and blank were placed in dark for 30 min followed by centrifugation. Reduction in absorbance was determined in these reaction mixtures using UV/Vis light spectrophotometer at 517 nm. The percent inhibition of the DPPH radical was determined by using the equation as below.

$$\%\ \text{Reduction of Absorbance} = [(\frac{A_a - A_b}{A_b})]\times 100$$

Where; A_a = Absorbance of sample, A_b = Absorbance of control

***Encapsulation efficiency (EE)*:** The encapsulation efficiency of the prepared encapsulates was analyzed by dividing TPC enclosed in microbeads (m_b) by the quantity of TPC of clove oil used to make microbeads (m_s) following the equation below:

$$EE = \frac{mb}{ms}\times 100$$

TPC in microbeads (m_b) was quantified by mixing them in $NaNO_3$ (2% w/v) in 1:5 ratio using vortex mixer at room temperature and Folin-Ciocalteu method was used to determine TPC by following protocol of Isailovic *et al*. (2012).

***Wettability*:** Wettability of clove encapsulates was analyzed by using protocol of Fernandes *et al*. (2014). Clove encapsulates (1 g) were scattered on 100 mL distilled water at 20°C. Time that powder took to sediment, sink and to get submersed or disappear from the surface of water was noted and used for comparing the extent of wettability of clove encapsulates.

***Solubility*:** Solubility of clove encapsulates was reckoned as per method proposed by Fernandes *et al*. (2014). Clove encapsulates (1 g) were mixed in 25 mL distilled water for 5 min using blender. Afterwards, the resultant solution was centrifuged at 760 rpm for 10 min. 20 mL aliquot of supernatant was poured in petri dish (pre-weighed) and dried overnight in oven at 105°C. The percent solubility was recorded as dried percent supernatant relative to amount of powder added initially (1 g).

***Water activity*:** The water activity of encapsulates was determined in triplicate using Hygropalm water activity meter; Rotronic a_w-Dio. The moisture content was determined immediately after freeze drying (Cortes-Rojas *et al*., 2014).

***Statistical analysis*:** All the analyses were repeated three times and data obtained for each parameter were statistically analyzed using statistical software package *i.e*. Costat-2003 (Co-Hort, v 6.1) under completely randomized design (CRD). Significance levels were incurred by applying one-way ANOVA for all parameters data interpretation (Mason *et al.*, 2003). The data was represented as Mean±S.D while graphs with standard error bar, graphs were constructed by using Microsoft Excel 2013.

RESULTS AND DISCUSSION

***Proximate composition of cloves*:** Proximate composition of clove (dry weight basis) indicated that moisture, protein, fat, fiber, ash and NFE were 7.01±0.33, 5.72±0.21, 14.63±0.53, 17.28±1.56, 4.96±0.10 and 50.4±1.76 g/100g D.W., respectively (Fig. 1).

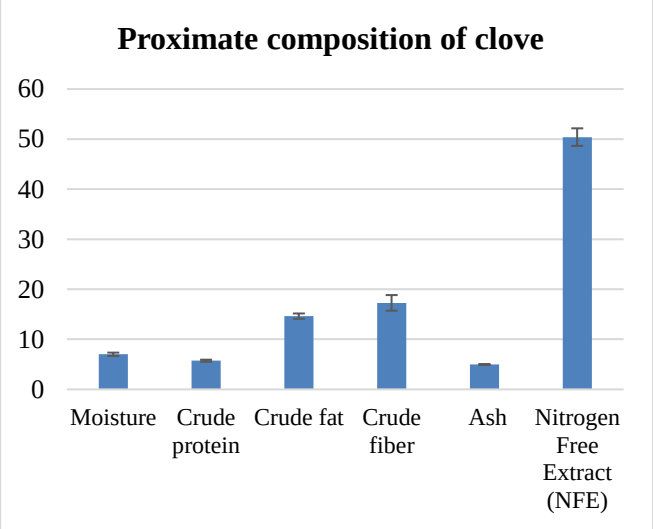

Figure 1. Proximate composition of clove (g/100g D.W.).

Earlier, Sulieman *et al.* (2007) determined the compositional characteristics of dried clove buds; moisture 10±0.006%, protein 1.2±0.02%, fat 12.1±0.45%, fiber 20±0.1%, ash 5.2±0.01% and carbohydrates 51.5±0.02%. These values are in close harmony with the current study performed. Moreover, Shafique *et al.* (2010) measured the proximate composition of clove and the values of moisture 9.67±1.20%, protein 5.88±1.01%, fat 13.58±1.75% and ash 4.62±0.45% are close harmony with the present study results.

***Mineral profile of cloves*:** It is evident from Figure 4.2 that clove contains significant amount of minerals. The calcium content was maximum as 245.30±13.24 mg/100g D.W. followed by sodium (157.80±8.04 mg/100g D.W.), magnesium (111.30±6.56 mg/100g D.W.), potassium (100.80±5.64 mg/100g D.W.), phosphorous (83.80±4.44 mg/100g D.W.), iron (32.00±1.79 mg/100g D.W.) and zinc (0.50±0.02 mg/100g D.W.).

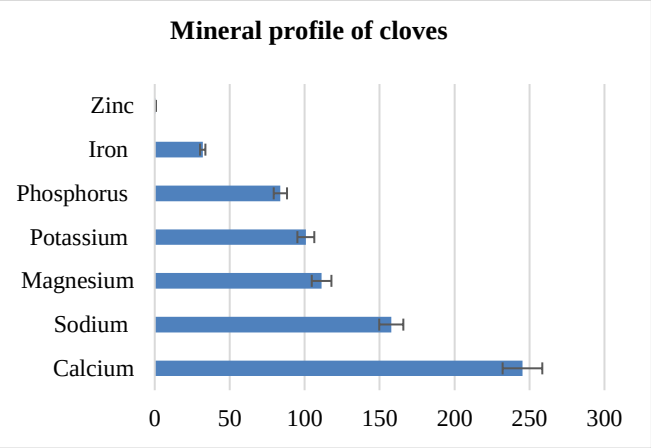

Figure 2. Mineral profile of cloves (mg/100g D.W.).

Adeyeye and Fagbohu (2005) measured the mineral profile of clove; calcium, sodium, magnesium, potassium, phosphorous, iron and zinc as 400, 60, 100, 150, 540, 100 and 10 mg/100g D.W. The sodium and magnesium, content was lower as compared to the current study though calcium, potassium, phosphorous, iron and zinc were higher as assessed in the present research.

***GC-MS quantification of eugenol in supercritical fluid extracted clove oil*:** Eugenol content was quantified via gas chromatographic mass spectroscopic technique in the supercritical fluid extract obtained at three different pressures. Eugenol content in $T_{SFE1500}$, $T_{SFE3000}$ and $T_{SFE4500}$ were 52.36 ± 1.83^{b}, 58.12 ± 3.77^{ab} and 77.93 ± 2.81^{a}%, respectively (Fig. 3).

In another study, Prado *et al.* (2011) extracted eugenol, eugenyl acetate, *β*-caryophyllene and *α*-humulene from clove using supercritical fluid extraction system. They retrieved eugenol as the main bioactive molecule, ranging from 71.12 to 72.74% at extraction time 70 and 130 min, correspondingly. These values are synchronized to the present research results.

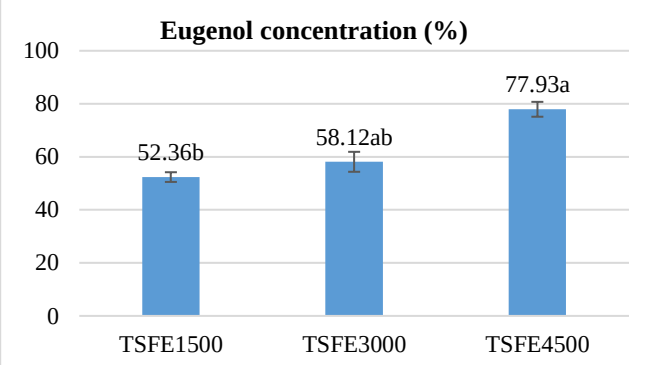

Figure 3. Quantification of eugenol concentration in different treatments.

Moreover, Wenqiang *et al.* (2007) narrated that eugenol+eugenol acetate in clove as 58.8% (19.6 yield) using supercritical fluid extraction mode at 50°C, 10 MPa (1486 Psi) and 2 hours. This value achieved was almost like $T_{SFE3000}$ in the current study but more than $T_{SFE1500}$. They compared three conventional methods; steam distillation, hydrodistillation and soxhlet extraction systems with that of supercritical fluid extraction system. The maximum content of eugenol+eugenol acetate in clove was observed as 61.2% using steam distillation and minimum as 30.8 via soxhlet extraction system however, 50.3% by hydrodistillation process. One of their peers, Alma *et al.* (2007) explored eugenol, eugenyl acetate and β-caryophyllene via GC-MS as 87, 8.01 and 3.56%, respectively in clove essential oil extracted using steam distillation method.

Furthermore, Nassar *et al.* (2007) ascertained 71.56% concentration of eugenol using n-hexane extract of clove using GC-MS system. Later, Barakat (2014) reported 16

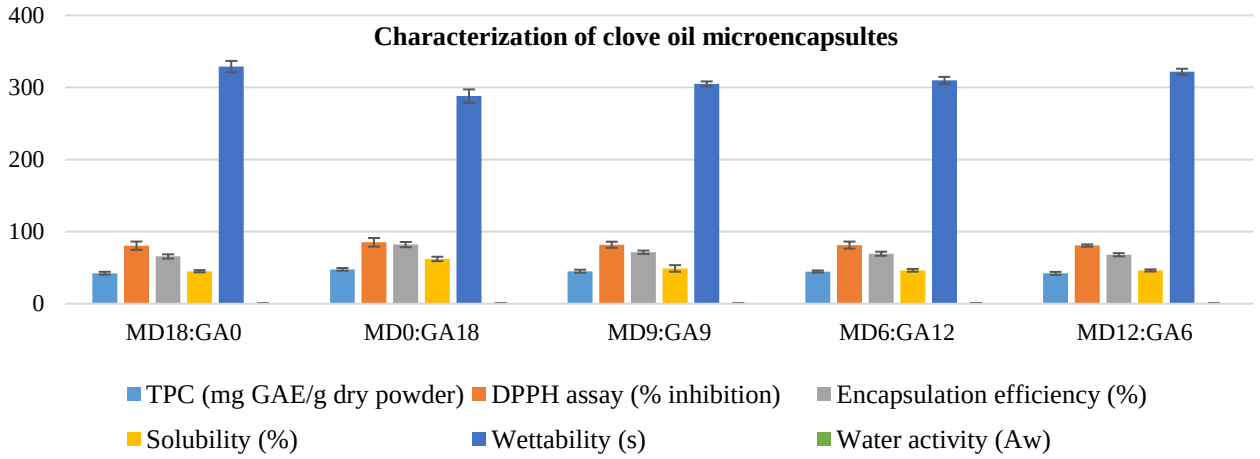

Figure 4. Characterization of clove oil microencapsulates.

components in the clove oil and measured eugenol as the major ingredient 80.19% obtained via GC-MS. Another researchers group quantified 88.58535% of eugenol in clove oil via GC-MS (Chaieb *et al*., 2007). In another study, Zengin and Baysal (2015) analyzed eugenol content as 75.20% in clove oil.

Characterization of microencapsulated clove oil powder:

***Antioxidant assays*:** The treatments exhibited significant differences with respect to TPC, EE, solubility and wettability, whereas non-significant variations were recorded in case of DPPH and water activity. Maximum total phenolic content was obtained in treatment M_2 as 47.68±1.91 mg GAE/g followed by M_3, M_4, M_1 and M_5 as 45.09±2.12, 44.68±1.52, 42.31±1.99 and 42.10±2.11 mg GAE/g, respectively (Fig. 4). The results of DPPH assay for M_1, M_2, M_3, M_4 and M_5 were 80.54±5.73, 85.20±5.96, 81.82±4.25, 81.37±4.88 and 80.69±1.61% inhibition, correspondingly.

Chatterjee and Bhattacharjee (2013) extracted clove oil using supercritical carbon dioxide and determined the total polyphenols as 287.76 mg GAE/g of dry clove bud and IC_{50} value for DPPH scavenging ability as 3.20 µg/mL. Afterwards, they encapsulated the extracted clove with gum arabic: maltodextrin using ratio as 1:2.4:4.8 via spray drying and measured the total polyphenols as 37.06 mg GAE/g of microencapsulated clove oil and IC_{50} value for DPPH scavenging ability as 208 µg/mL. The reason for reduction in phenolic content and associated antioxidant ability could be attributed to the existence of coating material. This study was in close harmony with the current investigation findings.

Another scientists group, Ivanovica *et al.* (2013) quantified eugenol content, total polyphenol content (TPC), DPPH and FRAP scavenging abilities of clove oil extracted via supercritical fluid system as 64.1%, 530.56±0.09 mg GAE/g extract, 20.64±0.15 IC_{50} µg/mL and 1040.73±2.00 µM Fe^{2+}/g of extract, respectively. The TPC and FRAP trapping ability values in the study is far higher than attained in the present scrutiny. The difference might be due to the presence of entrapment material. On the other hand, eugenol concentration is comparable to the present study results.

Previously, Yadav and Bhatnagar (2007) expounded that clove possesses higher DPPH radical trapping ability *i.e.* 90% (IC_{50} value 10±1.1) followed by licorice, mace and cardamom. Additionally, they detected higher FRAP value as 0.532±0.009 µM trailed by mace, licorice and cardamom. In a research exploration, sunflower protein concentrate was employed to encapsulate clove essential oil that reported ABTS and FRAP assays as 1194.1 mg/g and 5733.3 mM/g, respectively (Salgado *et al*., 2013). Later, Sebaaly *et al*. (2016) ascribed the % DPPH scavenging potential of free eugenol, free clove essential oil, cyclodextrin encapsulated eugenol and cyclodextrin encapsulated clove oil as 88.16, 92.82, 92.31 and 91.32% that could be related to the present study outcomes. Additionally, Zengin and Baysal (2014) probed the total polyphenols in clove essential oil as 635.327±11.71 mg GAE/mL. However, the antioxidant assays based on FRAP and DPPH assays in clove essential oil were 4357.45±28.83 mM Trolox/mL and IC_{50} value 0.14±0.02 µL/mL, respectively.

***Encapsulation efficiency, solubility, wettability and water activity*:** Highest encapsulation efficiency was exhibited by M_2 (82.03±3.61%) while lowest was recorded in the treatment M_1 (65.57±2.95%). Likewise, the solubility ranged between 45.04±1.58 and 62.15±3.05%. The wettability results were obtained for M_1, M_2, M_3, M_4 and M_5 were 329±7.90, 288±9.33, 305±3.43, 310±4.72 and 322±3.99, respectively. The water activity of all the treatments varied from 0.304±0.03 to 0.331±0.04.

The encapsulation efficiency (EE) of phospholipid, cholesterol and clove oil (10:5:2.5) based liposomes was 78.4%, whereas phospholipid, cholesterol and eugenol (10:5:2.5) based liposomes possess EE as 86.6% (Sebaaly *et al*., 2015). One of the scientists group demonstrated EE of cardamom essential oil up to 92% by using gum arabic as wall material (Al-Ismail *et al*., 2015). In another study, gum arabic was used to encapsulate clove oil and the value of EE was noted upto 78% (Luo *et al*., 2014). Earlier, Carneiro *et al.* (2013) portrayed higher oil retention in coating materials carrying gum arabic however, the presence of maltodextrin resulted in poor oil stability characteristics as indicated in the current research. One of their peers, Chatterjee and Bhattacharjee (2013) encapsulated eugenol with gum arabic: maltodextrin in the ratio of 1:2.4:4.8 via spray drying and found the EE ranging from 60-65%. This study confirmed that the presence of maltodextrin reduced the EE in contrast to gum arabic as viewed in the investigation. This response could be related with that of the present study effects.

In a study, Cortes-Rojas *et al*. (2014) encapsulated clove extract using maltodextrin and combination of maltodextrin and gum arabic (1:1) and found that water solubility differed significantly. Higher water solubility was indicated by maltodextrin (70 g of powder/100 g of water) as compared to the combination of maltodextrin and gum arabic *i.e.* 45 g of powder/100 g of water, not supporting the present study results. In addition, they explicated inverse relationship between water activity and moisture content. The water activity and moisture content of maltodextrin coated clove extract were 0.329±0.004 and 4.06±0.149%, whereas 0.318±0.012 and 4.68±0.071 in maltodextrin and gum arabic coated clove extract. The findings regarding water activity of this study are in close harmony with the results of the present study. The above mentioned study also showed higher moisture content in case of gum arabic as compared to maltodextrin that means gum arabic possesses more hydrophilic groups hence could easily associate with water molecules *i.e.* expressed as wettability or rehydration potential. The coating materials carrying extra hydrophilic groups shorten the wettability time because of their ability to interact with water at a greater extent. Moreover, such coating

materials that have higher moisture content, allow more water to penetrate the pores (Fernandes *et al*., 2014a). Thus, it could be deduced from the previous studies that gum arabic possesses lesser wettability time owing to its higher moisture extent, justifying the current study finding related to wettability. In contrast to the current study results, the wettability time was lesser in maltodextrin and gum arabic combination coated rosemary oil (274 s) than that of gum arabic coated rosemary oil (301 s) owing to higher moisture content in combination (2.05%) as compared to gum arabic alone (1.64%). However, the similar study indicated higher solubility by gum arabic (46.57%) in contrast to gum arabic+maltodextrin (45.82%). Besides, they also observed higher oil retention capacity by gum arabic (56.83%) as compared to gum arabic+maltodextrin (45.45%). The higher solubility and oil retention in the study could be related to the instant study effects (Fernandes *et al*., 2014b).

***Conclusion*:** Encapsulates of *Syzigum aromaticum* L. have promising antioxidant potential in terms of TPC and DPPH. Moreover, it can be used as replacer of counterfeit additives in processed food items. Further research should be accomplished in order to find out health boosting capacity of these encapsulates.

REFERENCES

Al-Ismail, K.M., G. Mehyar, H.S. Al-Khatib and M. Al-Dabbas. 2014. Effect of microencapsulation of cardamom's essential oil in gum arabic and whey protein isolate using spray drying on its stability during storage. Qual. Assur. Saf. Crops 7:613-620.

Alma, M.H., M. Ertas, S. Nitz and H. Kollmannsberger. 2007. Chemical composition and content of essential oil from the bud of cultivated Turkish clove (*Syzygium aromaticum* L.). Biol. Res. 2:265-269.

Ameratunga, R., C. Crooks, G. Simmons and S.T. Woon. 2014. Health risks and adverse reactions to functional foods. Crit. Rev. Food Sci. Nutr. 56:318-325.

AOAC. 2006. Official Methods of Analysis of Association of Official Analytical Chemists International, 18th Ed. AOAC Press, Arlington, VA, USA.

Ayala-Zavala, J.F., L.D. Toro-Sanchez, E. Alvarez- Parrilla and G.A. Gonzalez-Aguilar. 2008. High relative humidity in-package of fresh-cut fruits and vegetables: Advantage or disadvantage considering microbiological problems and antimicrobial delivering systems. J. Food Sci. 73:41-47.

Barakat, H. 2014. Composition, antioxidant, antibacterial activities and mode of action of clove (*Syzygium aromaticum* L.) buds essential oil. Br. J. Appl. Sci. Technol. 4:1934-1939.

Carneiro, H.C., R.V. Tonon, C.R. Grosso and M.D. Hubinger. 2013. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. J. Food Eng. 115:443-451.

Chaieb, K., H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf. 2007. The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (*Syzigium aromaticum* L. Myrtaceae): A short review. Phytother. Res. 21:501-506.

Chatterjee, D. and P. Bhattacharjee. 2013. Comparative evaluation of the antioxidant efficacy of encapsulated and un-encapsulated eugenol-rich clove extracts in soybean oil: shelf-life and frying stability of soybean oil. J. Food Eng. 117:545-550.

Cortes-Rojas, D.F., C.R.F. Souza and W.P. Oliveira. 2014. Encapsulation of eugenol rich clove extract in solid lipid carriers. J. Food Eng. 127:34-42.

David, J.R.D., L.R. Steenson and P.M. Davidson. 2013. Expectations and applications of natural antimicrobials to foods: a guidance document for users, suppliers, research and development, and regulatory agencies. Food Prot. Trends 33:238-247.

Fernandes, R.V.D.B., S.V. Borges and D.A. Botrel. 2014. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohydr. Polym. 101:524-532.

Fernandes, R.V.D.B., S.V. Borges, D.A. Botrel and C.R.D. Oliveira. 2014. Physical and chemical properties of encapsulated rosemary essential oil by spray drying using whey protein–inulin blends as carriers. Int. J. Food Sci. Technol. 49:1522-1529.

Gurbuz, U., H.A. Kahraman, A.E. Telli and D.B. Kulcu. 2016. The effect of eugenol on survival of *Listeria monocytogenes* inoculated inegöl meatball. Kafkas. Univ. Vet. Fak. 22:49-55.

Hill, L.E., C. Gomes and T.M. Taylor. 2013. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT- Food Sci. Technol. 51:86-99.

Ivanovic, J., S. Dimitrijevic-Brankovic, D. Misic, M. Ristic and I. Zizovic. 2013. Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds. J. Func. Foods 5:416-423.

Kamatou, G.P., I. Vermaak and A.M. Viljoen. 2012. Eugenol-from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. Molecules 17:6953-6981.

Khaw, K.Y., M.O. Parat, P.N. Shaw and J.R. Falconer. 2017. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. Molecules 22:1-22.

Lekjing, S. 2016. A chitosan-based coating with or without clove oil extends the shelf life of cooked pork sausages in refrigerated storage. Meat Sci. 111:192-197.

Luo, Y., Y. Zhang, K. Pan, F. Critzer, P.M. Davidson and Q. Zhong. 2014. Self-emulsification of alkaline-dissolved clove bud oil by whey protein, gum arabic, lecithin, and their combinations. J. Agric. Food Chem. 62:4417-4424.

Martin, A., S. Varona, A. Navarrete and M.J. Cocero. 2010. Encapsulation and co-precipitation processes with supercritical fluids: Applications with essential oils. Open Chem. Eng. 4:31-41.

Mason, R.L., R.F. Gunst and J.L. Hess. 2003. Statistical design and analysis of experiments. John Wiley & Sons, Inc., Hoboken, N.J. USA.

Nassar, M.I., A.H. Gaara, A.H. El-Ghorab, A. Farrag, H. Shen, E. Huq and T.J. Mabry. 2007. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. Rev. Latinoam. Quím. 3:47-57.

Przygodzka, K., H. Zielinski, Z. Ciesarova, K. Kukurova and G. Lamparski. 2016. Effect of selected spices on chemical and sensory markers in fortified rye-buckwheat cakes. Food Sci. Nutr. 4:651-660.

Ramadhan, A.H., W.M. Pembe, K.A. Omar, W. Xia and Y. Xu. 2017. Characterization of antioxidant activity of peptide fractions from Chinese giant salamander (*Andriaus davidianus*) protein hydrolysate. J. Glob. Innov. Agric. Soc. Sci. 5:14-19.

Salgado, P.R., M.E. Lopez-Caballero, M.C. Gomez-Guillen, A.N. Mauri and M. Pilar Montero. 2013. Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. Food Hydrocoll. 33:74-84.

Sebaaly, C., A. Jraij, H. Fessi, C. Charcosset and H. Greige-Gerges. 2015. Preparation and characterization of clove essential oil-loaded liposomes. Food Chem. 178:52-62.

Sebaaly, C., C. Charcosset, S. Stainmesse, H. Fessi and H. Greige-Gerges. 2016. Clove essential oil-in-cyclodextrin-in-liposomes in the aqueous and lyophilized states: From laboratory to large scale using a membrane contactor. Carbohydr. Polym. 138:75-85.

Shafique, M., S.J. Khan, Z. Nasreen and N.H. Khan. 2010. Appraisal of nutritional status and antimicrobial activity of clove, kalonzi, cinnamon, black pepper and sweet basil. PharmacologyOnLine 2:591-599.

Simon-Brown, K., K.M. Solval, A. Chotiko, L. Alfaro, V. Reyes, C. Liu, B. Dzandu, E. Kyereh, A.G. Barnaby and I. Thompson. 2016. Microencapsulation of ginger (*Zingiber officinale*) extract by spray drying technology. LWT-Food Sci. Technol. 70:119-125.

Sulieman, A.M.E., I. Boshra and E. El-Khalifa. 2007. Nutritive value of clove (*Syzygium aromaticum*) and detection of antimicrobial effect of its bud oil. Res. J. Microbiol. 2:266-271.

Sultana, B., F. Anwar, M. Mushtaq, M. Aslam and S. Ijaz. 2014. *In vitro* antimutagenic, antioxidant activities and total phenolics of clove (*Syzygium aromaticum L.*) seed extracts. Pak. J. Pharm. Sci. 27:893-899.

Teixeiraa, B., A. Marquesa, C. Piresa, C. Ramosa, I. Batistaa, J. A. Saraivab and M.L. Nunes. 2014. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties. LWT- Food Sci. Technol. 59:533-539.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez-Alvarez. 2011. Spices as functional foods. Crit. Rev. Food Sci. Nutr. 5:13-28.

Wenqiang, G., S. Li, R. Yan, S. Tang and C. Quan. 2007. Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food Chem. 10:1158-1564.

Yadav, A.S. and D. Bhatnagar. 2007. Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. Biofactors 31:219-227.

Yang, B., M. Zhao, J. Shi, N. Yang and Y. Jiang. 2008. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from Longan fruit pericarp. Food Chem. 106:685-690.

Yang, M., S.I. Koo, W.O. Song and O.K. Chun. 2011. Food matrix affecting anthocyanin bioavailability: review. Curr. Med. Chem. 18:291-300.

Zengin, H. and A.H. Baysal. 2015. Antioxidant and antimicrobial activities of thyme and clove essential oils and application in minced beef. J. Food Process Preserv. 39:1261-1271.