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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.231.238

Research Article
Functional Yoghurt Supplemented with Extract Orange Peel Encapsulated Using Coacervation Technique

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Abstract

Background and Objective: Orange peels (OP) as a fruit waste is a rich source of polyphenolic compounds (PC). In this research, the different concentrations of orange peel were extracted to obtain the highest PC concentration. **Materials and Methods:** The aqueous orange peel extracts (OPE) were encapsulated using coacervation method. Different ratios between wall materials (whey protein concentrate (WPC) and gum arabic ((GA) 3:1, 3:2 and 3:3) were investigated. The ratios between OPE and wall materials were 1:10 and 1:20. Encapsulated OPE was supplemented in yoghurt. The encapsulation efficiency (EE) was evaluated for capsules while phenolics content (PC), physiochemical and texture properties of yoghurt samples were evaluated during cold storage (fresh, 7 and 15 days). **Results:** The higher EE (95.4%) was observed when used WPC: GA at ratio 3:1 and OPE: wall materials at ratio 1:10. There aren't any significant influences on the physiochemical and texture properties of yoghurt samples. The organoleptic properties of supplemented yoghurt had gained acceptable flavor and satisfied scores from judging persons. **Conclusion:** Application of microcapsules as a carrier of orange peel extract in yoghurt (WPC: GA at ratio 3:1 and OPE: wall material at ratio 1:10) had the best potential to be successfully applied.

Key words: Microencapsulation, orange peel, coacervation methods, phenolic compounds, functional yoghurt and whey protein concentrate

Citation: Tamer Mohammed El-Messery, Marwa Mohamed El-Said, Nadia Mohamed Shahein, Hala Mohamed Fakhr El-Din and Atif Farrag, 2019. Functional yoghurt supplemented with extract orange peel encapsulated using coacervation technique. Pak. J. Biol. Sci., 22: 231-238.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yoghurt is one of the most popular fermented dairy products which are given its nutritional value and digestibility[1]. Past years have seen a global increase in yoghurt consumption due to its benefits including improved bowel function, enhanced immune system and reduced colon cancer[2]. Herbs, spices, fruits and vegetables have been used as supplements in the yoghurt industry as rich sources of antioxidants and phenolic compounds to improve antioxidant activity of yoghurt[3,4].

Annually produces tons of waste (including peel and segment membranes) during the processing of citrus and extracting juice at specialized factories, these wastes caused many problems of contamination of soil and environment in addition to pollution of the food industry. Sweet orange peel extract is known to have good radical antioxidative potential. Some researchers showed that orange peel contained phenolic compounds, flavonoids, carotenoids and anthocyanins, so, it can be used efficiently as medicines or as supplements[5].

Microencapsulation is a process in which bioactive materials are covered by coating material to maintain them, which are very small capsules. This technology was used in the food industry to cover oils, flavors acids, micro-organisms and vitamins to keep it from interaction with other compounds. The success of this technology depends on many factors such as; wall material, core material and the encapsulation technique[6,7]. Coacervation technique is one of the chemical encapsulation methods based on to form a liquid, neutral and polymer-rich phase within interacting cationic and anionic water-soluble polymers in water (ionic strength), pH, molecular weight, concentrations of the polymers and temperature[8]. This interaction forms insoluble complexes and produces phase separation[9]. The interactions between phenolic compounds and milk proteins were the major motivation behind this study and microencapsulation of phenolic compounds protect it from this interaction.

For that, this study aimed at the production of functional yoghurt using microparticles of orange peel extract containing high polyphenolic compounds by the complex coacervation method using whey protein concentrate and gum Arabic as wall materials.

MATERIALS AND METHODS

This study was made in National Research Centre at last September, 2018. Egyptian fresh orange (*Citrus sinensis*) was purchased from the local market. Whey protein concentrate (WPC) which contain 80% proteins as supplier data, gum arabic (GA) and maltodextrin (MD) from Alfasol Co., Turkey. Sodium acetate and acetic acid glacial were purchased from Carl Roth GmbH and Co. KG (Karlsruhe, Germany). Folin-Ciocalteu reagent, Gallic acid and 1,1-diphenyl- -2-pycrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Low-heat skimmed milk powder (USA) which composition of 34% protein, 51% lactose, 1.2% fat, 8.2% ash and 4% moisture (Data are presented by supplier) was used. Starter strains of *Streptococcus thermophilus* and *Lactobacillus delburkii*s sp. *Bulgaricus* were obtained from stock cultures from the Dairy Microbiology Lab., National Research Centre, Dokki, Cairo, Egypt and propagated in sterilized reconstituted skimmed milk (10% w/v) before use.

Extraction of orange peel phenolics: The orange fruits were washed well using tap water. The peel is separated, cut into small pieces then dried at room temperature (22-25°C). The dried peels were grinded properly to obtain the powdered form. The obtained powder was stored at -18°C until use[10]. Briefly 5, 10, 15, 20 and 25 g of orange peel extracts powder (OPEP) were soaked separately in 100 mL of distilled water at room temperature for 24 h under stirring. The obtained extracts were filtered using Whatman filter paper No.1. The extracts were frozen at -18°C, then freeze dried using freeze dryer (LABCONCO, USA) at -52°C for 48 h at pressure below 0.1 mPa. The dried extract was manually ground to fine powder and kept at -18°C until encapsulated[11].

Determination of phenolics content (PC): The TPC was determined according to Jayaprakasha *et al.*[12] using Folin-Ciocalteu reagent. The results were expressed as milligrams Gallic acid equivalent per gram of dry weight.

HPLC analysis: High-performance liquid chromatography measurement (HPLC) analysis was carried out according to the method described earlier[13] using an Agilent 1260 series. The separation was carried out using a C18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of water: 0.02% tri-floro-acetic acid in acetonitrile (80:20) at a flow rate 1 mL min^{-1}. The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μL for each of the sample solutions. The column temperature was maintained at 35°C.

Determination of total antioxidant activity (AA): The antioxidant activity was determined by the ability of antioxidants to scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a free radical[14]. The antioxidant activity was calculated by using the following equation:

$$\text{Antioxidant activity (\%)} = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100$$

Microencapsulation of OPEP: Microcapsules of OPEP were prepared using whey protein concentrate (WPC) and gum Arabic (GA) as wall materials using coacervation method[15]. The WPC solution (3%, w/w) was obtained by swelling WPC in demonized water and heating up to 40°C until the appearance of a homogeneous solution. The GA (1%, w/w) was dissolved in demonized water. The wall materials were prepared from WPC and GA at ratios of 3:1, 3:2 and 3:3. The microcapsules were prepared by adding OPEP into the WPC solution (1:10 and 1:20), then diluted 3-4 times with demonized water (50°C). The GA solution was added drop wise into previous mixture (OPEP and WPC) and stirred at 800 rpm. The pH of this mixture was adjusted to 3.75 by adding citric acid (1%) drop wise in order to induce electrostatic interaction between WPC and GA. Microencapsulation procedure was carried out at 25°C followed by cooling to 5°C at a rate of 5°C h^{-1}. Finally, the microcapsules were dried using freeze dryer (LABCONCO, USA) at -52°C for 48 h at pressure below 0.1 mPa.

Microencapsulation efficiency: The encapsulation efficiency (EE) was determined by measuring the phenolic contents of sample before encapsulation (TPO) and the total phenolic contents of the supernatant after centrifugation (TPS)[16]. The EE (%) was calculated using the following Eq:

$$\text{EE (\%)} = \left(\frac{\text{TPO-TPS}}{\text{TPO}}\right) \times 100$$

Fourier transform infrared spectroscopy (FTIR): The function groups for microcapsules powder, whey protein concentrate and orange peel extract were checked by FTIR (JASCO FT/IR 6100 using KBr Wafer technique)[17] in the region of 400-4000 cm^{-1}. Each spectrum was obtained at a resolution of 1 cm^{-1}.

Yoghurt manufacture: Light yoghurt was made using skim milk powder (SMP) (12%) reconstituted in distilled water. Different ratios of encapsulated OPEP were added to obtain 300, 600 and 900 mg PC (T_1, T_2 and T_3, respectively) which equivalent the daily intake of PC requirement for human. All treatments subjected to heat at 85°C for 30 min and cooled directly to 45°C then inoculated with starter bacteria (*S. thermophiles* and *L. delburkii* ssp. *Bulgaricus* 3%). The previous treatments incubated at 42°C until the curd formed then stored in refrigerator at 5±2°C.

Physicochemical characteristics of yoghurt

pH and titratable acidity: The pH was measured by pH meter (JENWAY 3505) equipped with combined electrode. Titratable acidity of yoghurt was measured according to the AOAC[18] and the results were expressed as lactic acid (%).

Water holding capacity (WHC): Water holding capacity was determined according to Arslan and Ozel[19]. The WHC (%) calculated by using the following equation:

$$\text{EE} = \left(\frac{\text{NY-WE}}{\text{NY}}\right) \times 100$$

Where:
NY = Weight of native yoghurt
WE = Weight of whey expelled

Texture profile analysis (TPA): Texture profile analysis (TPA) was done for yoghurt samples using the double compression test (Multi test 1d Memes in, Food Technology Corporation, Slinfold, W. Sussex, UK). Experiments were carried out at room temperature by compression test that generate plot of force (N) versus time (s). A 25 mm diameter perplex conical shaped probe was used to perform the TPA analysis of samples in five different points on the sample surface. In the 1st stage, the samples were compressed by 30% of their original depth at a speed of 2 cm/min during the pretest compression and relaxation of the sample. From the force-time curve, the following parameters were determined according to the definition given by the International Dairy Federation (IDF)[20]:

$$\text{Hardness (N)} = \text{Maximum force of the 1st compression}$$

$$\text{Cohesiveness} = \frac{\text{Area under the 2nd compression}}{\text{Area under the 1st compression}}$$

$$\text{Adhesiveness (Ns)} = \text{Negative area in the curve (A3)}$$

$$\text{Springiness (mm)} = \frac{\text{Length 2nd compression}}{\text{Length 1st compression}}$$

$$\text{Gumminess (N) (g)} = \text{Hardness} \times \text{Cohesiveness}$$

$$\text{Chewiness (mJ) (g mm}^{-1}\text{)} = \text{Gumminess} \times \text{Springiness}$$

Sensory evaluation: The yoghurt samples were organoleptically evaluated by some panelists from the staff members of the Dairy Science Department, National Research Center, Egypt. Each yoghurt sample was evaluated and used a quality rating score card for evaluation of appearance, flavor and body/texture and color as described earlier[21].

Statistical analyses: The data obtained in this study were expressed as the mean of triplicate determinations. Statistical comparisons were made with Duncan's test which was analyzed with SPSS (SPSS for Windows, Version Rel. 15.0, 2006, SPSS Inc.,)[22]. The $p<0.05$ were considered to be significant.

RESULTS

Antioxidant activity (AA) and phenolics content (PC) of OPEP: The results in Table 1 showed the effect of OPEP concentrations on the AA and PC values. The mean values of AA and PC were increased by increasing the concentration of OPEP. The mean value of PC was 367.70 as equivalent mg Gallic acid/g at 5% of OPEP while this value increased to 683.25 as equivalent mg Gallic acid/g at 25% of OPEP. The mean value of AA was 53.41 at 5% OPEP and increased to 82.15% with increasing OPEP concentration at 25%.

Identification of phenolic compounds by HPLC: In Table 2, the identification and concentration of 12 phenolic compounds in OPEP have shown. From the result in Table 2, the major phenolic compound (PC) in orange peel was catechin (41.65 μg g^{-1}), while the lowest PC was Gallic acid (0.03) and cinnamic acid (0.11 μg g^{-1}).

Encapsulation efficiency (EE): Encapsulation efficiency (EE %) was calculated using determined phenolics content between non-encapsulated of OPEP and encapsulated OPEP. The encapsulation efficiency will be higher when the amount of phenolic compounds on the surface of microcapsules is low. The highest EE (95.4%) ratio between OPEP to wall materials was 1:10 (WPC: GA 3:1), while OPEP: wall material was 1:20 (WPC: GA 3:3) showed the lowest EE (72.83%) (Fig. 1).

Fourier transforms infrared spectroscopy (FTIR): The results of FT-IR spectra of OPEP, GA, WPC and encapsulated OPEP are shown in Fig. 2. In the FT-IR spectrum, a broad band of approximately 3200-3500 cm^{-1} to relates OH vibrations. The N-H (amid-I) groups are characterized at 1640 and 1540 cm^{-1} there are signal was for CN (amide-II). In the FT-IR spectrum of OPEP characteristic bands of the C-O stretch alcohols and carboxylic acids functional groups can be observed at 1006 cm^{-1}, while vibrations C-C stretch (in-ring) aromatics group appear at 1400 cm^{-1}. The bands in the range of 3200-3500 cm^{-1} are characteristic of O-H stretch, H-bonded alcohols and phenols.

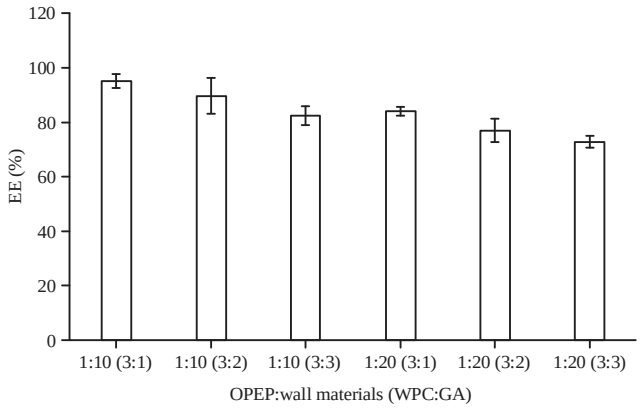

Fig. 1: EE (%) of encapsulated OPEP using different ratios of wall materials (WPC and GA)

Table 1: Antioxidant activity (AA) and phenolics content (PC) of OPEP at different concentrations

Concentration of OPEP (%)	AA (%)	PC (Equivalent mg gallic acid g^{-1})
5	53.41±0.01	367.70±0.01
10	74.61±0.01	568.55±0.02
15	87.23±0.01	622.70±0.02
20	80.44±0.05	678.30±0.01
25	82.15±0.02	683.25±0.01

Table 2: Phenolic compounds concentration (μg g^{-1}) of the OPEP

Phenolic compounds	Concentration (μg g^{-1})
Gallic acid	0.03
Catechin	41.65
Caffeic acid	3.43
Syringic acid	4.93
Rutin	20.15
Coumaric acid	1.23
Ferulic acid	13.06
Naringenin	10.28
Propyl gallate	6.26
Dihydroxyisoflavone	2.24
Quercetin	2.77
Cinnamic acid	0.11

Physicochemical characteristics and phenolics content of functional yoghurt

pH and acidity: In Table 3 showed the changes in pH and acidity values of yoghurt samples during storage at 4°C. The pH value of control sample was lower than yoghurt samples supplemented with encapsulated OPEP (T_1, T_2 and T_3), whereas at fresh or in the end of storage period. The mean value of acidity for control sample at fresh or in the end of storage

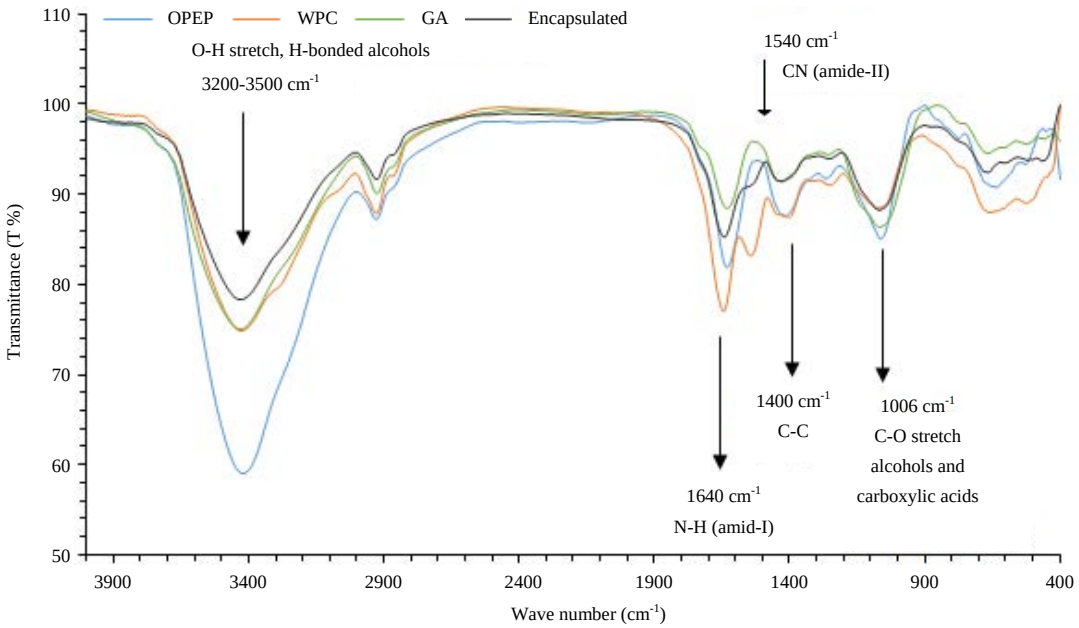

Fig. 2: FTIR spectra of OPEP, WPC, GA and encapsulated OPEP

Table 3: Physicochemical properties of functional yoghurt

	pH		Acidity (%)		WHC (%)	
Treatments	Fresh	15	Fresh	15	Fresh	15
Control	4.60±0.02^{b}	4.51±0.01^{c}	1.27±0.00^{a}	1.61±0.04^{a}	65.00±0.00^{c}	60.00±2.12^{c}
T_1	4.62±0.03^{ab}	4.53±0.01^{c}	1.25±0.00^{ab}	1.52±0.01^{b}	65.00±0.00^{b}	55.00±0.71^{bc}
T_2	4.64±0.01^{ab}	4.55±0.01^{b}	1.23±0.03^{bc}	1.34±0.01^{b}	55.50±2.12^{b}	50.00±0.00^{b}
T_3	4.65±0.01^{a}	4.59±0.01^{ab}	1.18±0.01^{c}	1.29±0.01^{b}	50.00±2.12^{a}	45.00±1.41^{a}

Data represent the average value±standard deviation of three replicates from each sample. The different letters in the columns (a-b) represent statistically significant differences ($p<0.05$). T_1: Yoghurt contains 300 mg PC, T_2: Yoghurt contains 600 mg PC, T_3: Yoghurt contains 900 mg PC

period was significantly ($p<0.05$) higher than T_1, T_2 and T_3, but the rate of increase was more pronounced in control sample.

Water holding capacity (WHC): Water holding capacity (WHC) is one of the most important parameters for yoghurt quality. Table 3 showed the changes in the WHC values of all yoghurt samples at fresh or at the end of storage period. Control sample exhibited the higher WHC value when fresh and after 15 days of storage compared to T_1, T_2 and T_3, while the mean value of WHC for T_1, T_2 and T_3 decreased markedly and this decrease parallel to increase encapsulated OPEP added.

Phenolics content: The mean values of PC of fresh yoghurt samples increased significantly by increasing addition of encapsulated OPEP ($T_3>T_2>T_1$) compare to control sample (Fig. 3). However, prolonged refrigerated storage, the mean values of PC for yoghurt samples supplemented with different concentrations of encapsulated OPEP increased and still high than the control sample.

Texture analysis: The results of the texture analysis performed on yoghurt samples presented in Table 4. The results revealed that hardness of yoghurt samples were not significantly affected with increasing the concentration of encapsulated OPEP, but during storage (15 days) the hardness was more stable than fresh time. Springiness, cohesiveness, chewiness and gumminess values for the treatments were relatively lower than in control, but during storage (15 days) the difference between data was highly significant with increasing of concentration of encapsulated OPEP.

Organoleptic properties: The results revealed that all yoghurt samples were accepted for all treatment although, fortification with encapsulated OPEP led to decrease in total scores, but still accepted and there isn't an unpleasant taste noticed. The results cleared that, it is possible to add encapsulated OPEP in the manufacturing of functional yoghurt up to equivalent 900 mg of PC (T_3) as shown in Table 5.

Table 4: Texture analysis of functional yoghurt supplemented with encapsulated OPEP during storage

Treatments	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N*mm)
Fresh					
Control	1.11±0.01[a]	0.66±0.01[b]	0.38±0.01[a]	0.41±0.01[a]	0.26±0.01[a]
T_1	1.11±0.01[a]	0.62±0.01[c]	0.36±0.00[a]	0.39±0.01[b]	0.25±0.01[a]
T_2	0.95±0.07[b]	0.60±0.01[d]	0.37±0.01[a]	0.36±0.01[c]	0.22±0.01[b]
T_3	0.95±0.07[b]	0.69±0.00[a]	0.38±0.01[a]	0.37±0.01[c]	0.26±0.01[a]
15 days					
Control	1.18±0.04[a]	0.73±0.01[a]	0.42±0.01[a]	0.51±0.01[a]	0.37±0.01[a]
T_1	1.18±0.04[a]	0.69±0.01[b]	0.44±0.01[a]	0.49±0.06[a]	0.36±0.01[a]
T_2	1.13±0.04[a]	0.58±0.01[c]	0.34±0.02[b]	0.35±0.01[b]	0.18±0.04[b]
T_3	1.08±0.11[a]	0.25±0.01[d]	0.30±0.02[b]	0.12±0.01[c]	0.13±0.01[b]

Data represent the average value±standard deviation of three replicates from each sample. The different letters in the columns (a-b) represent statistically significant differences ($p<0.05$). T_1: Yoghurt contains300 mg PC, T_2: Yoghurt contains 600 mg PC, T_3: Yoghurt contains900 mg PC

Table 5: Organoleptic properties of functional yoghurt supplemented different ratios of encapsulated OPEP

Indicator evaluation source	Control 95	T_1 93	T_2 91	T_3 90
Visual appearance	Not whey separation, no shrunken and surface is smooth	Not whey separation, no shrunken and surface is smooth	Not whey separation, no shrunken and surface is smooth	Not whey separation, no shrunken and surface is smooth
Flavor	Flavor is a clean acid and not undesirable flavors	Flavor is a clean acid, not undesirable flavors, not harsh and natural	Flavor is a clean acid, not undesirable flavors and the flavor was appeared and characteristic	Flavor is a clean acid, not undesirable flavors and The flavor was a highest appeared
Texture	Body is a smooth homogeneous texture like custard body	Encapsulated OPEP uniformly distributed throughout the product, flat and smooth surface	Encapsulated OPEP uniformly distributed throughout the product, flat and smooth surface	Encapsulated OPEP uniformly distributed throughout the product, flat and smooth surface
Color	Natural color and a bright-white	Off-white color	Creaming color was appeared	Creaming color was a highest appeared

T_1: Yoghurt contains 300 mg PC, T_2: Yoghurt contains 600 mg PC, T_3: Yoghurt contains 900 mg PC

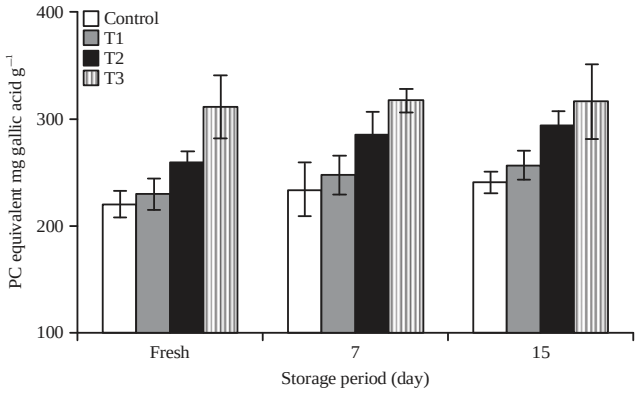

Fig. 3: PC of functional yoghurt supplemented with different ratios of encapsulated OPEP during cold storage

DISCUSSION

Orange peel contained phenolic compounds, flavonoids, carotenoids and anthocyanins as a good radical antioxidative potential so, it can be used efficiently as medicine or as supplement[5]. The antioxidants of OPEP were measured by their ability to donate hydrogen for the scavenging the DPPH free-radical[23] it was about 85%. The phenolics content for OPEP was measured as equivalent mg Gallic acid/g and it was the highest at concentration of 25% of OPEP. The water extract of orange peel had a potential antioxidants activity due to the extracted water-soluble phenolic compounds and this agreed with the present results[24].

Microencapsulation is a process in which bioactive materials are covered by coating material to maintaining them, which are very small capsules and used in the current study to overcome the problem of fortification of dairy products with bioactive compounds which may interact with milk proteins, thus reducing the nutritional value of these compounds. In the present study, the encapsulation efficiency was increased by increasing WPC in the wall materials composition, this is due to the effect of WPC as emulsifier and stabilizer on the encapsulation technique. The capsules contained OPEP: wall materials (1:20) are expected to have high encapsulation efficiency, because it has more coating material relative to an OPEP (core material)[15].

The interaction between the function groups of WPC, GA and OPEP capsules was studied using FTIR. It is showed from FT-IR spectrum corresponding to OPEP formulation that the intensity of some peaks was higher or lower compared to those obtained individually by OPEP and WPC, this type of interaction take place between the carboxyl groups of OP and the amino groups of WPC in the encapsulated OPEP[25,26].

Yoghurt is one of the most popular fermented dairy products. In the present study, the pH for supplemented yoghurt showed that the addition of encapsulated OPEP slightly reduced the activity of the starter bacteria, WHC for yoghurt indicated that encapsulated OPEP weak the protein network of yoghurt which results is more serum to be released[27]. Texture of yoghurt effected by storage, this may be attributed to the moisture content of fresh samples have a higher concentration which weakens the protein network resulting in a less firmness[28].

The difference in PC values of yoghurt samples at fresh or after 15 days from cold storage could be explained by the activity of yoghurt bacteria which do degradation of milk proteins and resulting some of the degradation products capable to react with Folin-Ciocalteu reagent[29]. Also, *Lactobacilli* generally were more proteolytically active than the streptococci during milk fermentation and storage[30]. In addition, the release of phenolic amino acids during degradation of milk proteins itself and non-phenolic compounds like proteins and sugars could effect on total phenolic evaluation[31]. Finally, the physiochemical and texture properties of yoghurt samples supplemented by encapsulated OPEP were not any significant influences. Organoleptic properties of functional yoghurt had gained acceptable flavor and satisfied scores from judging persons.

CONCLUSION

Orange peel extract contain polyphenols can be encapsulated by different ratios WPC and GA using the coacervation complex method, the highest encapsulation efficiency was observed when used WPC:GA at ratio 3:1 and OPEP:wall materials at ratio 1:10. The chemical capsulation was done within the interaction between function groups of phenolic compound at OPEP and function groups for WPC and GA it was observed by FTIR. The application of microparticles in yoghurt, the highest encapsulation efficiency had the best potential to be successfully applied. Therefore, WPC and GA microparticles had the best potential to be successfully applied in the food industry, particularly in yogurt preparations.

SIGNIFICANCE STATEMENT

This study discovered the production of functional yoghurt supplemented with encapsulated orange peel extract phenolics using coacervation technique that can be beneficial for overcoming the problem of fortification of dairy products with bioactive compounds which may interact with milk proteins, thus reducing the nutritional value of these compounds.

ACKNOWLEDGMENT

This work was funded by Project No.11040104, National Research Centre. A lot of thanks to all staff members of this project.

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