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Preparation and Characterization of Maltodextrin Microcapsules Containing Walnut Green Husk Extract

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***Abstract*—**In recent years, the field of natural antimicrobial and antioxidant compounds is one of the main research topics in the food industry. Application of agricultural residues is mainly cheap, and available resources are receiving increased attention. Walnut green husk is one of the agricultural residues that is considered as natural compounds with biological properties because of phenolic compounds. In this study, maltodextrin 10% was used for microencapsulation of walnut green husk extract. At first, the extract was examined to consider extraction yield, total phenolic compounds, and antioxidant activation. The results showed the extraction yield of 81.43%, total phenolic compounds of 3997 [mg GAE/100 g], antioxidant activity [DPPH] of 84.85% for walnut green husk extract. Antioxidant activity is about 75%-81% and by DPPH. At the next stage, microencapsulation was done by spry-drying method. The microencapsulation efficiency was 72%-79%. The results of SEM tests confirmed this microencapsulation process. In addition, microencapsulated and free extract was more effective on gram-positive bacteria's rather than the gram-negative ones. According to the study, walnut green husk can be used as a cheap antioxidant and antimicrobial compounds due to sufficient value of phenolic compounds.

***Keywords*—**Biopolymer, microencapsulation, Spray-drying, Walnut green husk

I. Introduction

OXIDATION reactions are a concern for the food industry; it caused great deterioration, thereby limited the shelf life of fresh and processed foodstuffs [1].

Synthetic antioxidants, such as BHT and BHA, and natural antioxidants such as tocopherol and ascorbate derivatives, are widely used in food processing because of their good protection from unsaturated fat and oils. However, BHA and BHT have recently been considered as unsafe for human health [1]. Thus, in recent years, many researches have been done to find effective non-toxic natural compounds, with antioxidative and antimicrobial activity [2], [3]. Several nuts such as walnuts and peanuts are dietary plants that have significant antioxidant and antimicrobial compounds [4], [5].

Juglans regia L. [Persian walnut] is a tree from Juglandaceae family. The walnut fruits are consumed extensively as a nutritious food, which are rich in unsaturated fatty acids. On the other hand, its leaves have been widely used in traditional medicine [6], [7]. In 2015, Iran Had produced 222 tons of walnut. About 64% of the net weight of wet walnut is attributed to its green husk that leads to accumulation of about 142.08 tons of walnut husk [8]. Also, it contains fruit related acids, minerals, Tanins, protein, oils, essential fatty acids and vitamin C [9]. Thirteen kind of phenolic compounds have been found in the green husk of walnut including: hydroxy cinnamic acid, hydroxy benzoic acid and flavonoids that made it as a rich source of natural antioxidant [10]. On the other hand, phenolic compounds, vitamins and many other trace nutrients are unstable in natural products [11]. Thus, these compounds should be stabilized in food products and preserved from incongruous environment. Encapsulation may be a suitable way to stabilize these compounds and control the release of them [12], [13]. Microencapsulation process consists of the formation of a wall or a husk around a compound and could be employed to protect sensitive substances against deteriorative reactions [14]. Encapsulation can prevent materials from decomposition (e.g. oxidation or hydrolysis). Therefore, this technique leads to stabilize food compounds, increase their bio-accessibility, and prevent formation of bad smell and flavor [15], [16]. Different carriers have various advantages and disadvantages including cost and encapsulation efficiency. Maltodextrin as a soluble modified starch has multifaceted functions and has been used alone or in combination with other materials in microencapsulation of aromatic additives, plant extracts, vitamins and carotenoids [17]. In this research, maltodextrin is used as a carrier for encapsulation of walnut green husk extracts [17].

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II. Materials and Methods

Maltodextrins with Dextrose equivalent 18-33 [DE =18-22] from food chem Co. [Japan], methanol, ethanol and Brain Heart infusion culture provided from Merk Co. [Germany], and Folin, Acetic acid, Gallic Acid, 2-2, Diphenyl, 1-Pykryl Hydrazyl [DPPH], Acetate Buffer, Ferric chloride have been provided from Sigma [American Company]. *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 2592, *Bacillus cereus* PTCC 1154 have all been provided from the scientific and industrial center. Lyophilized vials which contain the above-mentioned bacteria are prepared under the sterile conditions.

A. Preparation of Green Walnut Husk Extract

The green walnut husk was dried for 73 hours in ambient

temperature and then milled and sifted. Extraction procedure was done according to the method of Pereira et al. [7]. 5 g of powdered green skin and 250 ml of 80 ºC distilled water was mixed for about 45 minutes and then filtered and freeze dried [4].

B. Preparation of Maltodextrin Microcapsules

First, 20 g maltodextrin was added to 100 ml of distilled water and then gently mixed at room temperature. Then, 1 to 2 g of dried extract was added to maltodextrin solution by magnetic stirrer for 30 minutes. Ultimately, the solution was fed to spray drier [17].

C. Determining Spray Drying Yield

The yield of spray drying process was obtained as the percentage of the microcapsule collected from spray drier over the total initial solid content of the feed solution.

D. Determination of Total Phenolic Compounds at the Surface of Microcapsules

100 mg of each microcapsule was added to 1 ml ethanol-methanol mixture [in ratio of 1:1]. Then, it was filtered with 0.45-micrometer filter paper. Ultimately, the amount of total phenolic compounds was determined by the FOLIN method [18].

E. Total Phenolic Content of Microcapsules

Folin-Ciocalteu method was used to determination of total phenolic content of the microcapsules with some modification. Distilled water containing 0.1% [w/v] Tween 80 was used to dissolve 25 mg of each sample and stirred overnight, then 0.1 ml solution extract, 7 ml Folin-Ciocalteu reagent and 7 ml distilled water were added. After 8 min, water and 1.5 ml 2% w/v sodium carbonate were added to obtain a final volume of 10 ml. Then, the solution was stayed for 2 h at room temperature, and the absorbance of it was measured at 765 nm. Gallic acid was used as the standard, and the results were expressed as mg of gallic-acid equivalents [GAE] per gram of microparticle [19].

F. Antioxidant Activity

The antioxidant activity of sample was determined based on scavenging properties of radical 2,2-diphenyl-1-picryhydrazyl [DPPH] that was expressed by decrease in absorbance at 517 nm because of reduction of DPPH radical in the presence of an antioxidant. Decrease in absorbance indicates an increase of the DPPH radical scavenging activity. 25 mg of each sample was dissolved in distilled water containing 0.1% [w/v] Tween 80 and stirred overnight and then was mixed with 3.9 ml of methanolic DPPH solution [0.1 mM] containing 0.1 ml of each extract. The absorbance decrease was determined after incubation for 60 min in darkness and ambient temperature at 517 nm. A DPPH radical solution merely was used as a control. The DPPH radical-scavenging activity [%] was measured using the following equation [19]:

$$DPPH\ svavening\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}}$$

G. Determining the Antimicrobial Activity of the Extract

Stock cultures of the selected bacterial strains such as *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922, *Staphylococcus aurous* ATCC 25923, and *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853 [Iranian Research Organization for Science and Technology [Tehran, Iran]] were grown in MHB at 30 °C for 24 h before the tests. The antimicrobial activity of microcapsules was assessed using the modified agar well diffusion method. First, 100 µl of culture containing 107–108 CFU/ml of the mentioned organisms was inoculated on the MHA plates. Three wells of 6 mm diameter were punched in each plate, and 50 mg lyophilized microcapsules were loaded in each well and then incubated at 30 °C for 24 h. The caliper was used to measure the diameter of inhibition zone. The average of diameter of six inhibition zones for each sample was reported as the "zone of inhibition" [19].

H. Scanning Electron Microscopy

The size of microcapsules was determined with electron microscopy. Microparticles were sputtered with gold. SEM images were taken at different magnification at room temperature and examined using an acceleration voltage of 25 kV [19].

I. Statistical Analysis

Analysis of data was done using SPSS statistical software. In order to identify any significant differences between two treatments, t- test was applied at a level of 95% of significance. All of tests were performed in triplicate.

III. Results and Discussion

A. Preparation of Walnut Husk Extract

The total phenolic compounds of extract were reported in Table I that were in agreement with the other previous studies [20].

B. The Total Phenolic Content and the Level of Microcapsules

The extract of walnut husk extracts of was encapsulated in 10% maltodextrin.

TABLE I
TOTAL PHENOLIC CONTENT

Treatment	TPC	SPC
Maltodextrin10%- extract	50.67± 1.35	13.73± 1.55

C. Microencapsulation Efficiency and Yield

Table II showed the effect of using maltodextrin in coating formulation on the spray drier yields and encapsulation efficiency of microencapsulation process [17].

TABLE II
SPRAY DRYING YIELD AND MICROENCAPSULATION EFFICIENCY AND ANTIOXIDANT ACTIVITY

Treatments	Efficiency	Yield	DPPH
Maltodextrin 10 %	72.50±1.^{00a}	53.08± 1.85^{a}	75.17±1.42^{a}

D.Antioxidant Activity of Microencapsulated Walnut Green Husk Extracts

The walnut green husk extract is rich source of phenolic compounds that have many hydroxyl groups that can have antioxidant activity. The antioxidant activities of extracts of walnut green husk before and after encapsulation were showed in Table II. Both of two microcapsules had ability to cover the DPPH radical.

E. Antimicrobial Properties

The study of antimicrobial properties of walnut green husk extract before and after encapsulation showed more sensitivity of gram positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* than gram negative bacteria such as *E. coli* and *Salmonella typhimurium* [Table III].

TABLE III
ANTIMICROBIAL PROPERTIES

Treatments	Inhibition Zone			
	S. aureus	*B. cereus*	*E. coli*	*S. typhimorium*
Maltodextrine 10 %	9.17 ± 0.65^{Aa}	7.67 ± 0.86^{Aa}	-	-
Free extract	-	-	-	-

F. Scanning Electron Microscopy

The microcapsule micrograph shows spherical structure.

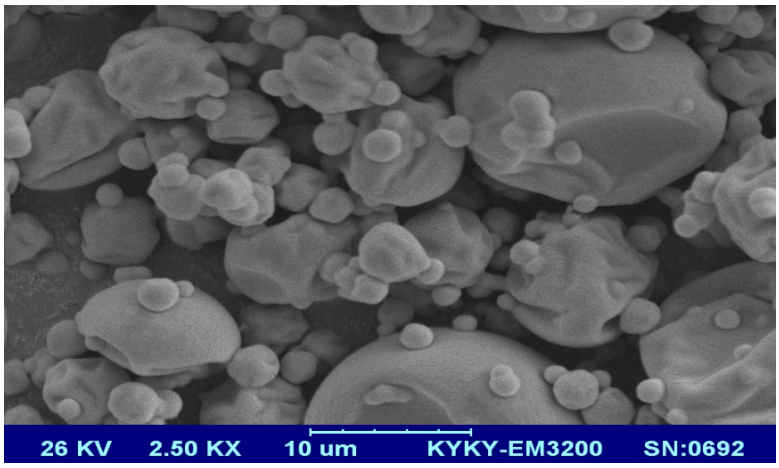

Fig. 1 Micrograph of microencapsulate maltodextrin-extract 10%

IV. CONCLUSION

This research showed the influence of the amount of maltodextrin and other polymers and the size of the particles on the microencapsulate performance. In the evaluation of antimicrobial activity of the extract, the gram-positive bacteria were more sensitive than the gram-negative bacteria.

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