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Effects of Centrifugation, Encapsulation Method and Different Coating Materials on the Total Antioxidant Activity of the Microcapsules of Powdered Cherry Laurels

B. Cilek Tatar, G. Sumnu, M. Oztop, E. Ayaz

***Abstract*—**Encapsulation protects sensitive food ingredients against heat, oxygen, moisture and pH until they are released to the system. It can mask the unwanted taste of nutrients that are added to the foods for fortification purposes. Cherry laurels (*Prunus laurocerasus*) contain phenolic compounds which decrease the proneness to several chronic diseases such as types of cancer and cardiovascular diseases. The objective of this research was to study the effects of centrifugation, different coating materials and homogenization methods on microencapsulation of powders obtained from cherry laurels. In this study, maltodextrin and mixture of maltodextrin:whey protein with a ratio of 1:3 (w/w) were chosen as coating materials. Total solid content of coating materials was kept constant as 10% (w/w). Capsules were obtained from powders of freeze-dried cherry laurels through encapsulation process by silent crusher homogenizer or microfluidization. Freeze-dried cherry laurels were core materials and core to coating ratio was chosen as 1:10 by weight. To homogenize the mixture, high speed homogenizer was used at 4000 rpm for 5 min. Then, silent crusher or microfluidizer was used to complete encapsulation process. The mixtures were treated either by silent crusher for 1 min at 75000 rpm or microfluidizer at 50 MPa for 3 passes. Freeze drying for 48 hours was applied to emulsions to obtain capsules in powder form. After these steps, dry capsules were grounded manually into a fine powder. The microcapsules were analyzed for total antioxidant activity with DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method. Prior to high speed homogenization, the samples were centrifuged (4000 rpm, 1 min). Centrifugation was found to have positive effect on total antioxidant activity of capsules. Microcapsules treated by microfluidizer were found to have higher total antioxidant activities than those treated by silent crusher. It was found that increasing whey protein concentration in coating material (using maltodextrin:whey protein 1:3 mixture) had positive effect on total antioxidant activity for both silent crusher and microfluidization methods. Therefore, capsules prepared by microfluidization of centrifuged mixtures can be selected as the best conditions for encapsulation of cherry laurel powder by considering their total antioxidant activity. In this study, it was shown that capsules prepared by these methods can be recommended to be incorporated into foods in order to enhance their functionality by increasing antioxidant activity.

***Keywords*—**Antioxidant activity, cherry laurel, microencapsulation, microfluidization

B. Cilek Tatar is with the Middle East Technical University, Ankara, 06800, Turkey. (phone: +90 5376195888; fax: +90 3121202756; e-mail: bcilek@metu.edu.tr).

G. Sumnu, M. Oztop and E. Ayaz are with the Middle East Technical University, Ankara, 06800, Turkey.

I. Introduction

MICROENCAPSULATION is commonly used in food industry especially for increasing the shelf life of foods containing phenolic compounds. Recently, microencapsulation of phenolic compounds from many different natural sources such as black currant, cactus pear and red wine have been studied [1]-[3].

Natural phenolic compounds are easily degradable because of their sensitivity to light, oxygen and heat. Besides, their unpleasant taste limits their usage in food industry. Encapsulation can be applied to improve their processing stability because coating materials act as a physical barrier to oxygen and mask unwanted flavor and color, Phenolic compounds can undergo degradation easily. Encapsulation or entrapment of phenolic compounds or phenolic compound containing fruits in a wall material increases the shelf life of foods. Moreover, encapsulation method can also be implemented to prevent unwanted taste and odor of phenolics and extend their shelf life and usage areas.

Different kinds of encapsulating materials have been used for microencapsulation, namely polysaccharides (starches, maltodextrins, gum arabic and corn syrups), lipids (mono- and di-glycerides) and proteins (casein, milk serum and gelatin) [4], [5]. Microencapsulation of phenolic compounds extracted from fruits like black carrot [6], bayberry [7], and cactus pear [1] has been studied. Maltodextrins of different dextrose equivalents are one of the most commonly used coating materials in food industry [1]. They have solubility in water, mild flavor and colorless solutions [1]. The reason of choosing maltodextrin is that they protect encapsulated material from oxidation [6], by forming amorphous glassy matrices during the encapsulation process [8]. Gum arabic is also preferred in microencapsulation. It is an encapsulating agent due to its low viscosity and good emulsifying capacity [9]. There is lack of study on microencapsulation of cherry laurels in the literature.

Cherry laurel (*Prunus laurocerasus* L.) fruit contains considerable amount of phenolic compounds and it is naturally grown in northern coastal area of Turkey. Because of its

pungent taste, cherry laurels are not widely used in food industry. It would be beneficial to develop an approach in the utilization of cherry laurel because they are rich in polyphenols and encapsulation is the best way to hide its taste and to increase the shelf life of polyphenols.

Capsules can be formed by using either high energy treatment devices such as ultrasonicator or microfluidizer and high-speed homogenizator. The interest of microencapsulation of food components has been progressively increased due to physical, chemical and biological features of micro-sized materials. Ease of handling, change in flavor characteristics, enhanced stability and enhanced bioavailability are some of the advantages of micro-sized capsules [10]. Despite the fact that there are studies about the microencapsulation of natural antioxidants obtained from plants such as from blackberry [11], bayberry [7], olive pomace [12], cactus pear [1], sour cherry pomace [13], [14], and various medicinal plants; it is not very likely to come across with a comprehensive study about encapsulation of antioxidant containing cherry laurel fruit in literature.

The consumption of natural antioxidants has been increasing and this demand strengthens the importance of the research in this field. The aim of the present study was to investigate the possibility of preparation of the microcapsules of cherry laurel powder composed of polyphenolic compounds. Total antioxidant activities of encapsulated and uncoated cherry laurel powder were also investigated. In addition, the effects of different coating material types and encapsulation methods on capsules were studied. The effect of centrifugation on efficiency of capsules was also evaluated.

II. Material and Methods

A. Materials

Fresh cherry laurels (*Prunus laurocerasus* L.) were harvested from northern part of Turkey, mainly from Trabzon. Maltodextrin and whey protein supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) were used as coating materials. All other reagents used in this study (1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol (absolute), methanol G CHROMASOLV®, gallic acid, and acetic acid (100 %)) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and they are of analytical grade.

B. Core Material Preparation

The harvested cherry laurels were manually took out from stems and other foreign materials, which were then frozen at -18 °C in a deep freezer (D 8340 SM; Beko, Istanbul, Turkey) and then freeze dried (Christ, Alpha 1-2 LD plus, Osterode, Germany) at -52 °C for 48 hours at 0.075 mPa. Prior to encapsulation, dried content was ground into a fine powder using a mixer (Arzum, AR-151 Mulino Coffee Grinder, Turkey). Finally, freeze dried cherry laurels (FDCL) were obtained as core material.

C. Preparation of Coating Materials for the Microcapsules

Maltodextrin (MD) and whey protein isolate (WP) were used as coating materials. MD solutions were kept overnight at 27 °C (70 rpm) in a shaking water bath after dissolved in distilled water. WP is mixed with MD in a ratio of 1:3 with total solid content of 10%. The solutions were mixed using magnetic stirrer (MR 3001K, Heidolph Instruments GmbH & Co, Schwabach, Germany) at 1250 rpm. MD:WP mixture was prepared (90% distilled water) in a ratio of 1:3 (2.5 g MD with 7.5 g WP).

D. Preparation of the Microcapsules

Freeze dried cherry laurels and coating solution was mixed in order to obtain core to coating ratio of 1:10 by weight. Centrifugation at 4000 rpm for 1 min (Hettich Lab Technology, Mikro 200, Sigma Laborzentrifugen GmbH, Germany) was applied to the samples before high speed homogenization. Then, they were homogenized using a high-speed homogenizer at 4000 rpm for 5 min (IKA T25 digital Ultra-Turrax, Selangor, Malaysia). Later, in order to complete encapsulation process, silent crusher (Heidolph Instruments GmbH & Co.KG, Silent Crusher S, Germany) was used for 1 min at 75000 rpm or mixtures were treated by microfluidizer at 50 MPa for 3 passes (Nanodisperser, NLM 100, South Korea). Then, obtained emulsions were freeze dried for 48 hours. Finally, dried content was ground into a fine powder with the same method described in core material preparation. Each experiment was duplicated.

E. Total Antioxidant Activity

Total antioxidant activity of the capsules was determined as described in [13]. Total antioxidant activity was evaluated in accordance with the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by [15] with some modifications. Phenolic powder or microcapsules was accurately weighed as 100 mg and dissolved in 1 ml ethanol, acetic acid, and water mixture (50:8:42). This emulsion was agitated using Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min, and the liquid part of emulsion was drawn into syringe, then it was filtered with a filter of 0.45 µm. Then, 3.9 ml of 25 ppm DPPH radical solution (2.5 mg DPPH/ 100 ml MetOH) and 100 µl of methanol was added, and its absorption at 517 nm was measured (A1) by using UV/VIS spectrometer T 70, (PG Instruments LTD, UK) by using methanol as blank. Diluted samples of 100 µl were mixed with 3.9 ml DPPH radical solution and kept in the dark at room temperature. After 1 hour, the absorptions of samples were detected spectrometrically (A2). By using calibration curve, concentrations (C1 and C2) were found for A1 and A2, respectively. Then, the antioxidant activities were calculated (1):

$$TAA = \frac{(C1 - C2) * d * V}{m} * MW \quad (1)$$

where; d is for dilution factor, V is for volume of extract, m is for weight of dry sample and MW is for molecular weight of DPPH.

III. Results and Discussion

Antioxidant activity of freeze dried cherry laurels was determined as 3.02 ppm DPPH/g dry weight. As it can be observed on Table I, addition of WP to coating material had a

significant ($p < 0.05$) effect on antioxidant activity. The reason of this difference was using a more complex system when compared to MD. Core material was trapped inside the matrix and air contact was prevented [16].

TABLE I
TAA RESULTS OF CAPSULES PREPARED BY SILENT CRUSHER AND MICROFLUIDIZER HAVING DIFFERENT COATING MATERIALS (MD, MD AND WP ISOLATE IN A 1:3 RATIO 'MD:WP') WITH CENTRIFUGED (C) AND NON-CENTRIFUGED (NC) TECHNIQUES

	Silent Crusher		Microfluidizer	
	NC	C	NC	C
MD	0.64±0.02b*	1.04±0.07b	0.70±0.03b	1.24±0.10b
MD:WP	0.85±0.03a	1.36±0.06a	0.92±0.03a	1.49±0.05a

*Means followed by the different (a, b) letters within columns are significantly different at $p < 0.05$.

The related changes of antioxidant activities with centrifugation are shown in Table II. When the effect of centrifugation on antioxidant activity was examined, significant ($p > 0.05$) differences were observed between coating materials.

TABLE II
THE EFFECT OF CENTRIFUGATION ON THE TAA OF CAPSULES PREPARED BY SILENT CRUSHER (SC) HAVING DIFFERENT COATING MATERIALS (MD, MD AND WP ISOLATE IN A 1:3 RATIO 'MD:WP')

	MD	MD:WP
Non-centrifuged	0.64±0.02b*	0.85±0.03b
Centrifuged	1.04±0.07a	1.36±0.06a

*Means followed by the different (a, b) letters within columns are significantly different at $p < 0.05$.

As it can be observed in Tables II and III, centrifugation had a significant ($p < 0.05$) effect on total antioxidant activity (TAA). Centrifugation caused to have higher total antioxidant activity results. The reason of this difference is probably due to removing of non-phenolic impurities by centrifugation.

TABLE III
THE EFFECT OF CENTRIFUGATION ON THE TAA OF CAPSULES PREPARED BY MICROFLUIDIZER (MF) HAVING DIFFERENT COATING MATERIALS (MD, MD AND WP ISOLATE IN A 1:3 RATIO 'MD:WP')

	MD	MD:WP
Non-centrifuged	0.70±0.03b*	0.92±0.03b
Centrifuged	1.24±0.10a	1.49±0.05a

*Means followed by the different (a, b) letters within columns are significantly different at $p < 0.05$.

TABLE IV
THE EFFECT OF ENCAPSULATION METHOD ON THE TAA OF CENTRIFUGED CAPSULES PREPARED BY MICROFLUIDIZER AND SILENT CRUSHER HAVING DIFFERENT COATING MATERIALS (MD, MD AND WP ISOLATE IN A 1:3 RATIO 'MD:WP')

	MD	MD:WP
Microfluidizer	1.24±0.10a*	1.49±0.05a
Silent Crusher	1.04±0.07b	1.36±0.06b

*Means followed by the different (a, b) letters within columns are significantly different at $p < 0.05$.

Table IV clearly shows the effect of different encapsulation methods; namely, microfluidizer (MF) and silent crusher (SC) on total antioxidant activity.

According to results, it was shown that capsules prepared by MF had significantly higher total antioxidant activity compared to SC.

Consequently, the highest total antioxidant activity was found as 1.49 ppm DPPH / g dry weight for the capsule containing WP, which was centrifuged and prepared by MF.

IV. CONCLUSION

Natural phenolic compounds are easily degradable and their encapsulation can be applied to improve their processing stability. Because coating materials act as a physical barrier to oxygen and can mask unwanted flavor and color. Therefore, encapsulation of phenolic compounds or phenolic compound containing fruits in a wall material increases the shelf life of foods.

To conclude, freeze dried cherry laurels were successfully encapsulated by using microfluidization and coatings containing MD and WP. Microcapsules coated with MD:WP 1:3 with core to coating ratio of 1:10 and prepared by microfluidizer at 50 MPa had the highest total antioxidant activity.

Further research, however, would be beneficial before using microcapsules as food additives or nutraceuticals. Because it is crucial to know about storage stability, baking stability, sensory analysis and digestion of microcapsules before their industrial application.

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B. Cilek Tatar (BS'10 - MS'12 – PhD'cont) is research/teaching assistant at the Food Engineering Department of Middle East Technical University (METU), Turkey. She was born in Ankara, Turkey, in 1988. She received her Bachelor of Science and Master of Science degrees from METU Food Engineering Department in 2010 and 2012. While she was working as a project assistant for her graduate studies in the same department, she started working as a research/teaching assistant in 2011. As a Ph.D. student, she aims to advance her quantitative skills further, to deepen her understanding of various methods such as double emulsions and magnetic resonance imaging, and to widen her knowledge about their usage in food science. She is particularly interested in improving functionality of foods by increasing their bioactive components with the help of double emulsions.