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Encapsulation of inulin loaded ovalbumin nanofibrils in toned milk to enhance the nutritional value

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Abstract

Self-assembled nanofibrils encapsulation was performed in the application of nutritional enhancement of toned milk. Inulin loaded nanofibrils (Self-assembled ovalbumin nanofibrils) were used for the encapsulation of toned milk. The physico-chemical parameters and nutritional value of inulin loaded ovalbumin nanofibrils were determined. The physic-chemical analysis of toned milk such as pH, titrable acidity, anti-oxidant activity, encapsulation efficiency and in-vitro release were calculated. The results show that sensory characteristics were not affected by encapsulation of nanofibrils on toned milk. The nutritional values of inulin loaded ovalbumin nanofibrils in toned milk was performed using Conventional Oven drain method (Moisture), Ignition method (Ash), gerber method (protein), kjeldahl method (fat), pearson's composition analysis (Carbohydrates), Titration method (Lactose), and HPLC method (Vitamin D). The result shows that the protein content is raised and also increased with other nutritional values.

Introduction

Inulin is composed of a linear chain of fructose units and ends with residues of glucose. This can improve the processing performance, nutritional value and texture of food. Also it functions as substitute of fat and it is also used to replace the flour in baked goods. It has low calories, high fiber content and has more health benefits (1). The fiber present in it is soluble in water and produces gelatinous substances in stomach which will increases fullness and reduces absorption of cholesterol while passing through the digestive tract. Good bacteria provides health benefits and inulin is used to stimulate these bacteria to grow. Inulin aids in increasing the number of good bacteria such as Bifidobacteria and Lactobacilli. These bacteria help in feed off unwanted pathogen (bad bacteria), stimulate our immune system and prevent infection (2).An emulsion gel that was induced by ultra sonification using inulin, rosemary essential oil and rice bran oil has improved greatly the efficiency of encapsulation and essential oils oxidative stability. Ovalbumin is one of the important protein components of egg white, which is of 54% to 58% of the total egg. It has the multifunctional properties and has the ability to form gel and foam upon heating (3). Ovalbumin has various biological activities and high nutritional value. Due to its functional properties it is considered as an ideal ingredient to encapsulate for the improvement of nutritional parameters in the food. The gel forming property on heating is one of the important functions of ovalbumin and can be used to determine the sensory attributes and texture (1).Therefore the combination of ovalbumin and inulin would enhance the stability and properties of food. Self-assembly is defined as the instinctive molecular arrangement of the disarranged units of molecules into orderly manner. As a result interactions among molecules will be enhanced. The self -assembly process is applicable both bulk materials and for 2D systems. Its salient feature is formation of ionic bonds between the molecules in organic as well inorganic compounds. Nano compounds with self-assembled structures will have excellent potential and can be used as simple and active materials. In the last few decades, boundless advances were made in self-assembly process (4).Ovalbumin was self-assembled into fibrillar nanostructures which lead to the formation of hydrogel (5). As it has high nutritional value, it acts as an immune booster. Toned milk is made from combination of cow and buffalo's milk and the concentration reached is 3% fat and 8.5% of non-fat solids of milk and this includes the proteins and milk sugars. Toned milk is great source of vitamins, proteins and minerals and it is a healthy choice for most of the people. The application of encapsulation of inulin in ovalbumin nanofibrils was done on adding them to toned milk and the fortification of milk was studied. The nanofibrils formed from self-assembly will be very strong and it will not break as fibrils (2).

Experimental methods

Preparation of ovalbumin nanofibrils and inulin loaded ovalbumin nanofibrils

2% (w/w) of ovalbumin i.e. 10 mg of ovalbumin was added to 0.5 ml of distilled water and pH was adjusted to 2 by adding 0.1 N HCl (4).Then the sample was centrifuged at 4000 RPM for 30 min and the residues were filtered. In eppendorf thermo mixer C – F 1.5, the filtered sample was shaked at 1000 RPM at a temperature of 80°C for 24hours. The resulting solution was used as control for characterization. To prepare inulin loaded ovalbumin nanofibrils, inulin was dissolved in double distilled water (2% w/w) and centrifuged at 4000 RPM for 30 minutes. The resulting solution was filtered. Then prepared ovalbumin solution was added to the inulin solution, heated to 80°C for 24hours and aforesaid procedure for loading of inulin as shown in figure 1.

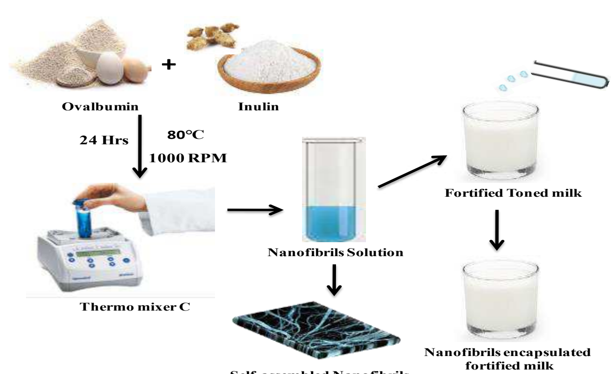

Figure 1. Schematic representation of encapsulation of nanofibrils

Encapsulation of nanofibrils on toned milk

0.5 ml of inulin loaded ovalbumin nanofibrils solution was added to 240 ml of toned milk. The resulting milk was pasteurized at 72℃ for 20 s, and then cooled to room temperature. The pH, titrable acidity, anti-oxidant activity, encapsulation efficiency, in-vitro release study, syneresis and sensory evaluation were measured for the fortified toned milk. The analysis was done for 7 hours of study period with 1 hour interval (6).

Analysis of nanofibrils encapsulated toned milk

Physio-chemical parameters were analysed. The pH values were measure using pH meter. Titrable acidity was done using potentiometric titrations. Total titrable acidity was calculated using equation 1. The antioxidant activity of encapsulated and unencapsulated toned milk was determined using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) by radical scavenging method. Encapsulated nanofibrils were dissolved in 3ml DPPH solution of different concentrations. Antioxidant activity of unencapsulated toned milk was also estimated for comparison. The samples prepared were incubated for 30 minutes under dark at room temperature. The absorbance of the sample was observed at 517nm and antioxidant activity was determined using equation 2 (7).The encapsulation efficiency was determined by adding 20 µl of inulin encapsulated oval albumin nanofibrils to 100 ml of 80 % ethanol and the solution was agitated for 10 minutes in thermo mixer C. The solution was then filtered using filter paper and the absorbance was measured at 306 nm under UV spectrophotometer (8). The encapsulation efficiency of the solutions was calculated using equation 3. Nutritional parameters such as protein, fat, carbohydrates, moisture, ash, energy and lactose for encapsulated and unencapsulated toned milk were also analysed and compared.

$$\text{Total Acidity (\%)} = \frac{\text{NaOH used (ml)} \times \text{milli equivalent factor}}{\text{Volume of sample}} \times 100 \qquad [1]$$

$$\text{DPPH Scavenging \%} = (A_c - A_s) \times 100 \qquad [2]$$

$$\text{Encapsulation efficiency(\%)} = \frac{\text{Amount of resveratrol in pellet } (R_T)}{\text{Total amount of resveratrol unentrapped } (R_F)} \times 100 \qquad [3]$$

Results and Discussion

Analysis of nanofibrils encapsulated toned milk

From figure 2a and 2b it is evident that there are no visible changes in the encapsulated toned milk, as it doesn’t affect the sensory characteristics of milk.

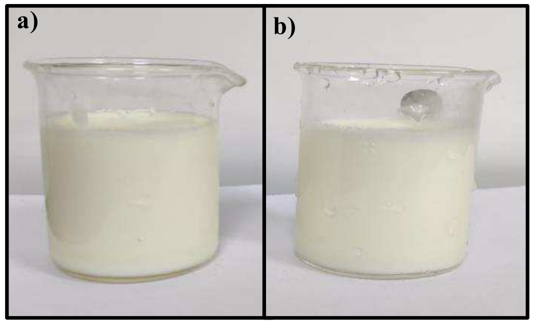

Figure 2. a) Encapsulated and b) unencapsulated toned milk

pH and titrable acidity

The pH for encapsulated and unencapsulated toned milk was found to have 6.7 and 6.8 respectively (table I). The percentage of titrable acidity was calculated for 5 hours duration with 1 hour time interval was shown in table I (9). The titrable acidity value for encapsulated and unencapsulated toned milk was found to be 0.0257 and 0.0193. There were no note worthy changes in both pH and titrable acidity for unencapsulated and encapsulated toned milk. This confirms that encapsulation doesn't affect the sensory characteristics of milk (10).

TABLE I. pH and titrable acidity of encapsulated and unencapsulated toned milk.

Physio-chemical parameters	Encapsulated toned milk	Unencapsulated toned milk
pH	6.7	6.8
Titrable Acidity	0.0257	0.0193

Anti-oxidant activity

The antioxidant activity of encapsulated fortified milk was done by DPPH radical scavenging method. The activity was performed to ovalbumin and inulin loaded ovalbumin encapsulated fortified milk. Both the milks showed scavenging activity. From figure 3, ovalbumin encapsulated fortified milk shows 10% of scavenging activity because of the presence of only protein that contains amino acids. Whereas the inulin loaded ovalbumin nanofibrils encapsulated fortified milk shows 30 to 50% due to the increased antioxidant property of inulin. The antioxidant activity of encapsulated fortified milk increases gradually by increasing the concentration of nanofibrils from 50 µl to 250 µl due to the scavenging capacity of the nanofibrils. This increased scavenging activity increases the quality and nutritional level of the fortified milk.

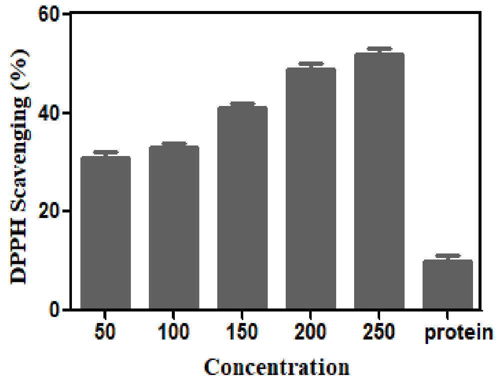

Figure 3. Anti-oxidant activity of encapsulated toned milk

Encapsulation efficiency

The concentration of ovalbumin was maintained constant (1.5%) and the concentration of inulin was varied as 0, 2, 2.5, and 3%. By increasing the concentration of the inulin the encapsulation efficiency will be reduced from 92% to 53% which proves that lower concentration of inulin results in higher encapsulation efficiency (2%) shown in table II.

TABLE II. Encapsulation efficiency.

Formulation	Ovalbumin Concentration (%W/W)	Inulin Concentration (%W/W)	Encapsulation efficiency (%)
Ovalbumin nanofibrils	1.5	0	0
Inulin loaded	1.5	2	92±0.52
Ovalbumin nanofibrils	1.5	2.5	79±0.63
	1.5	3	53±0.48

Analysis for nutritional parameters

From the nutritional analysis labeled in table III, encapsulated toned milk is increased with 78% of protein, 10% of fat, 11% of carbohydrates, and it decreased with 4% of moisture, and 3% of ash, whereas energy is increased to 24%and lactose is also increased to 15% compared to unencapsulated toned milk.

TABLE III. Nutritional values of encapsulated and unencapsulated toned milk.

Components	Encapsulated toned milk	Unencapsulated toned milk
Protein (%)	5.58	3.13
Fat (%)	3.42	3.1
Carbohydrates (%)	6.08	5.48
Moisture (%)	80.92	84.18
Ash on dry basis (%)	4.0	4.12
Energy (kcal/100g)	77.42	62.34
Lactose (%)	3.89	3.39

Conclusion

Self-assembly method was used for the formation of ovalbumin nanofibrils. The encapsulation efficiency, anti-oxidant, sensory characteristics such as pH, and titrable acidity of nanofibrils encapsulated fortified milk was studied. The encapsulation efficiency and in-vitro release study proves the successful encapsulation without altering the shelf stability of nanofibrils in the fortified milk. Successful encapsulation results increased in bioavailability of toned milk without any influence in sensory characteristics and quality, and this process maintain intact quality of the toned milk. The low calories with increased protein value have more acceptances in future and can be used for infant foods.

Acknowledgments

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