Current Research in Food Science 2 (2020) 77–83

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.editorialmanager.com/crfs/

Research Paper

Encapsulation of isoflavone with milk, maltodextrin and gum acacia improves its stability

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ARTICLE INFO

Keywords:
Isoflavone
Encapsulation
Gum acacia
Maltodextrin
Isoflavone degradation
Retention rate

ABSTRACT

This study was carried out for extraction of soy isoflavones and entrapment of the isoflavones so obtained into whole milk *via* encapsulation techniques. Three different solvent (ethanol, methanol and acetonitrile) were used for the extraction of isoflavone using three stage of extraction. The extracted isoflavone was encapsulated into 200 ml of whole milk by spray drying using different concentrations of gum acacia (4, 6 and 8% w/v) and 10% w/v maltodextrin DE 18. The ratio between cores to coating materials was 1:10. Though acetonitrile extracted higher amount of isoflavone, ethanol was selected for subsequent studies of extraction of isoflavone, as per the legislations regarding use of Food-grade solvents. There was no significant difference ($p > 0.5$) among all three samples 4% gum acacia+10% maltodextrin (A), 6% gum acacia+10% maltodextrin (B) and 8% gum acacia+10% maltodextrin (C) in terms of moisture content and hygroscopicity. However, insolubility index showed that sample A possessed a higher solubility index. Encapsulation techniques suggested that sample A showed higher encapsulation efficiency than others. Statistical analysis suggested that there was no significant difference among samples A, B and C during storage at 4°C for the time period (30 days) studied, in terms of isoflavone retention rate. However, samples stored at 25 and 37°C showed significant difference in the retention rate. Among all the three samples, sample B showed significantly lower isoflavone degradation rate of 3.80, 4.07 and 4.70 $\times 10^{-3}$/day at 4, 25 and 37°C, respectively. The highest amount of isoflavone degradation was observed at 37°C. Results indicate that isoflavone can be encapsulated using a combination of gum acacia either 4 or 6% w/v and 10% maltodextrin along with milk proteins at 4°C for longer shelf life.

1. Introduction

Soy isoflavones are one of the strongest candidates to be incorporated in the functional foods (Mazumder, 2016) due to their estrogenic and/or anti-estrogenic effects (Fatih, 2005) by sitting in and blocking receptor sites against estrogen α and β receptors (ERs) (Paterni et al., 2014). Isoflavone shows both estrogenic and antioxidant capacity. Research has revealed that soy isoflavones and their glycosides are strongly associated with lower incidence of cardiovascular diseases (Adlercreutz, 1990), hormone-dependent cancers (Yu et al., 1991), colon cancer (Rose et al., 1986), menopausal symptoms (Clarkson, 2000) and osteoporosis (Adlercreutz et al., 1992). Among isoflavones, genistein is a very powerful inhibitor of tyrosine (Akiyama et al., 1987). However, genistein could also act as an antioxidant in *in vivo* and *in vitro* (Rahman Mazumder and Hongsprabhas, 2016). The isoflavones are mainly genistein, daidzein, and glycitein, which belong to the phytoestrogens, found as four molecular species: a sugar free aglucone, a sugar-containing glucoside, and glucoside esters (malonyl-glucoside and acetyl-glucoside). The aglycone isoflavone are especially important, among others, due to their easily bioavailability to humans (Rahman Mazumder and Hongsprabhas, 2016; Monteiro et al., 2004). The health promoting effects of soy isoflavone has led to the development of isoflavone fortified functional foods. Isoflavone shows cholesterol lowering property in some soy foods (Anthony et al., 1996; Fukui et al., 2002). Soy protein reduces total cholesterol, low density lipoprotein cholesterol and triglyceride in humans (Mazumder and Begum, 2016) and inhibits atherosclerosis in animal (Arliss and Biermann, 2002). There is a strong correlation between high serum cholesterol and risk of coronary heart disease. Therefore, physical, chemical and biological methods were studied to reduce the cholesterol in dairy products (Kwak et al., 2001a,b; Ahn and

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https://doi.org/10.1016/j.crfs.2019.12.003

Kwak, 1999; Lee et al., 1999; Szejtli, 1988). Isoflaovne may be a useful functional ingredient added to milk and milk products.

Milk and milk products are one of the important food products in South Asia. Milk is an ideal food for both children and adults with abundance of calcium and vitamin D. Both help in building bones and help protecting against osteoporosis in later life. Dairy products can be fortified with non-dairy bioactives. The growth in functional food market indicates that milk-based beverages are ideal vehicles for newly discovered bioactive food ingredients. Several bioactive ingredients offer promising opportunities and challenges for development for functional dairy beverages (Sharma, 2005). Soy isoflavone, can be used as one of the functional ingredients added to milk products such as dried milk. Research on the incorporation of isoflavones into milk will not only help to produce functional foods, but also in the application of the latter. However, isoflavone cannot be added directly to the milk due to its bitter taste, beany flavor and brown color, therefore, encapsulation could be a better way to solve the problem.

Encapsulation is the process of entrapment or coating of active agents with another sub-substance (wall material/carrier material) (Faridi et al., 2015; Rajabi et al., 2015; Nedovic et al., 2015). Encapsulation improves the delivery of bioactive compounds and living cells into food (Nedovic et al., 2015). The main objective of encapsulation is to protect the active agents (core material) from adverse environmental conditions namely, undesirable effects of light, moisture and oxygen to increase the shelf life of the product and promoting controlled release of the encapsulate (Pourashouri et al., 2014a,b). Controlling the release of core materials and masking the undesired properties of core materials are very much necessary for the proper encapsulations (Dubey et al., 2009). Coating materials have significant role in stability of the encapsulation process. Different kinds of coating materials are used for encapsulation process such as polysaccharides, proteins and lipids (Mcnamee et al., 1998a,b). Coating materials for encapsulation must be food grade, biodegradable and able to form a barrier between the internal phase and its surroundings (Nedovic et al., 2015). Different types of wall materials have been used as encapsulating agents in spray drying, including, polysaccharides (starches, gum Arabic, corn syrups and maltodextrins), lipids (stearic acids, mono- and di-glycerides) and proteins (gelatin, casein, milk serum, soy and wheat) (Gibbs et al., 1999) for enzyme, flavor and iron microencapsulation in foods (Kwak et al., 2001a,b). Maltodextrin has low viscosity and good solubility even at high concentrations. As a result, maltodextrin could be used as coating materials. However, maltodextrins lack emulsifying and surfactant properties. Hence, maltodextrin is combined with other coating materials to form stable capsules (Rosenberg and Sheu, 1995). Amongst the coating agents, gum acacia is probably one of the most sought after. Gum Acacia is composed of branched arrangement of simple sugars like galactose, glucuronic acid, arabinose and rhamnose and small amount of covalently bonded protein which gives functional properties of gum acacia (Mcnamee et al., 1998a,b). Gum acacia has high water solubility and low viscosity than other gums (Madene et al., 2006). In addition to these, it can create a protective film around core materials and acts like emulsifier to prevent aggregation by forming a thick layer (Zuidam and Nedović, 2010). Currently no reports on the determination of the stability and efficiency of solvent extracted isoflavone microencapsulation using a combination of gum acacia and maltodextrin is available. Therefore, the objectives of this study were to examine the optimum conditions for solvent extracted isoflavone encapsulation and to examine the stability of encapsulation retaining isoflavone.

2. Materials and methods

2.1. Materials

Whole milk (4% protein and 4.5% fat) was purchased from the local market. Defatted soy flour (5.6% moisture, 75% protein); gum acacia (food grade) was supplied by Danisco India Pvt. Ltd.; ethanol, methanol, and acetonitrile (analytical grade, J.T. Baker, France), hexane (analytical grade; Mallinckrodt Chemicals; Saint Louis, USA), de-Ionized water (HPLC-grade) maltodextrin DE 18 were supplied by Danisco India Pvt. Ltd.

2.2. Extraction of isoflavone

Extraction of isoflavone was carried out using previously reported method by Greenfield et al. (2000) with modifications. Isoflavone was extracted from defatted soy flour (10 g) with 100 ml of solvent by magnetic stirring at 950 rpm for 30 min followed by sonication (OSCAR, Ultrasonic Microclean, India) for 60 min at 45°C. To find the best solvent in terms of isoflavone extraction, 96% ethanol, 96% methanol and 96% acetonitrile were used for extractions of isoflavone. Multistage (3 stage) extraction was used for extraction of isoflavone, i.e. isoflavone was extracted from 10 g defatted soy flour using 100 ml of solvent for 3 times. Multistage extraction is a process where extraction steps are repeated in order to increase the recovery of a product. This process is required when, due to a small partition coefficient, the recovery in a single extraction step is insufficient. The extract was centrifuged at 7600 g for 10 min at −15°C. The supernatant was evaporated using rotary evaporator (MKVI, Amketle Analysis, India). Nitrogen was passed through the isoflavone to remove the alcoholic flavor. The solvent extracted isoflavone was stored at 4°C till further processing and use.

2.3. Micro-encapsulations of solvent extracted isoflavone

Gum acacia (4, 6 and 8% w/v) suspension was prepared in distilled water at 60°C. Isoflavone was slowly added to the gum acacia suspension. The ratio between cores (isoflavone) to coating material (gum acacia) was 1:10. The suspension was mixed at 1100 rpm for 5 min with a mechanical stirrer. 200 ml of whole milk was added to the suspension and mixing continued with a mechanical stirrer (MKVI, Amketle Analysis, India). 10% (w/v) maltodextrin (DE 18) was added to the suspension as additional coating materials and mixed properly. The suspension was heated at 70°C for 2 min and cooled at room temperature. The particle size of suspension was reduced by passing through colloid mill (REMI motor, India) at speeds of 3000 rpm for 5 min. Optimization studies on encapsulation were carried out by varying the concentration of gum acacia (4%, 6% and 8% w/v), keeping constant maltodextrin concentration (10% w/v) and whole milk (200 ml). The samples were termed as A, B and C, respectively. The suspensions were spray dried using a laboratory spray dryer (Labultima, India) by a peristaltic pump and atomized into small droplets by passing through a nozzle (0.7 mm, 2′O′ rings) (4–5 bar air pressure) in a co-current air flow system. The liquid was fed at a flow rate of 20 ml/min and dried using inlet air temperature 175°C and outlet air temperature 80–85°C, with aspiration of 55 Nm^3/h by varying the feed rate in the range of 3–5 rpm. The powder was collected and sealed in multi layered laminate foil and stored at −20°C prior to for further analysis.

2.4. Physical properties of encapsulated powder

2.4.1. Determination of moisture content

Moisture was estimated by the method of AOAC (2000). Accordingly, 2 g of sample was weighed into pre-dried and tarred dish (3 dishes) and shaken until contents were evenly distributed. The samples were dried for 2 h at 130°C. The covers were placed on dishes and transferred to desiccator to cool. Weighed and calculated loss in weight as moisture.

$$\% \text{ Moisture} = \frac{\text{loss of moisture} \times 100}{\text{weight of sample}} \quad (1)$$

2.4.2. Determination of solubility

Solubility of the powder was determined according to the method of Sulieman et al. (2014). 5 g of the powders were mixed with 50 ml of distilled water at 25°C. The suspension was mixed at high speed for 2 min

by mechanical stirring. The suspension was allowed to settle for 15 min at room temperature and followed by stirring with glass rod for 1 min 25 ml of suspension was filled in a centrifuge tube with conically graduated bottom and centrifuged at 7600 g for 10 min at −15°C. After discarding the supernatant, the residue was washed with distilled water and stirred for 1 min. The content was again centrifuged at −15°C for 5 min and the sediment level noted. The sediment is termed as insolubility index and expressed in ml. The insolubility index should be below 0.2 ml in powder for good quality milk powder products.

Solubility of powder was determined based on time required to dissolve following the modified methods of Goula and Adamopoulos (2010). 2 g of powder was mixed with 50 ml of distilled water and agitated with magnetic stirrer at 900 rpm. The time required for the powder to complete dissolve was recorded by using a stop watch.

2.4.3. Hygroscopicity

Hygroscopicity was determined adopting the method of Sulieman et al. (2014). 2 g of powder was spread in a dried petri dish and kept in desiccator containing saturated sodium sulfate. Hygroscopicity was determined by the amount of moisture gained by the samples, every day till constancy. Hygroscopic moisture was expressed as g of moisture per 100 g dry solids (g/100 g).

$$\text{Hygroscopicity (g/100g)} = \frac{(Wf - Wi)\ \text{X}\ 100}{\left(Wi\ \text{X}\ \left(100 - \frac{\text{moisture}}{100}\right)\right.} \quad (2)$$

where, Wf = Final weight, Wi = Initial weight.

2.5. Color determination

Color of the isoflavone encapsulated spray dried powder was measured using a Minolta colorimeter (Chorma Meter, Cr-400/410, Japan). Results were analyzed according to the CIELB system with reference to the illuminant D65 and a visual angle of 10°. The parameters determine were L* (luminosity or brightness: L* = 0 black and L* = 100 white), a* (red-green component: a* = greenness and +a* = redness) and b* (yellow-blue component: b* = blueness and +b* = yellowness). All analyses were performed in triplicates.

Suspensions of 0.08% protein (w/v) was prepared in distilled water. The suspension was mixed at high speed (950 rpm) for 10 min using a magnetic starrier. It was then centrifuged at 8000 g for 10 min at a temperature −15°C. The supernatant was evaluated for no-enzymatic browning by measuring the absorbance (PerkinElmer, Lambda 25, UV/VIS spectrophotometer) at 420 nm and corrected for turbidity by subtracting with the absorbance at 620 nm using the method described by Pan and Melton (Pan and Melton, 2007).

2.6. Determination of percent transmittance (% T) of the encapsulated powder

Two (2) g of powder was mixed with 20 ml of distilled water and mixed at high speed for 10 min by mechanical stirring. The suspension was centrifuged at 7600 g for 5 min at −15°C. The supernatant was collected and the % Transmittance measured at 660 nm by spectrometer (PerkinElmer, Lambda 25, UV/VIS spectrophotometer).

2.7. Determination of degradation of isoflavone during storage period and kinetic study

Degradation of degradation of isoflavone during storage period and kinetic study were carried out according to the modified method of Seok et al. (2003). 1 mg of encapsulated isoflavone was added to 10 ml of 96% ethanol and stored at 4, 25 and 37°C for 30 days. The samples were sonicated at 45°C for 60 min and centrifuged at 9500 rpm for 10 min at −15°C. The supernatant was analyzed for the amount of isoflavone released from the microcapsules. The samples were analyzed at intervals of 10 days. Degradation rate constants (k) were obtained from the slope of a plot of the natural log of percentage retention of isoflavone measured by UV/VIS spectrophotometer *vs* time. For a first order reaction rate constant (k) was determine by the following equation:

$$\ln(C) = \ln C_0 - k(t) \quad (3)$$

where C_0 is the initial isoflavone content (day 0) after spray drying and C is the isoflavone content after t (time) of stability treatment at a given temperature.

2.8. Encapsulation efficiencies

The encapsulation efficiency was calculated by determining the total isoflavone (TI) and surface isoflavone (SI) after spray drying by using the modified method of Idham et al. (2012). For TI, 10 mg of powder was dissolved in 50 ml of 96% ethanol. The suspension was sonicated for 20 min and filtered through a glass filter. An aliquot from the filtrate was transferred to a 10 ml volumetric flask and made up to volume using potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate buffer at pH 4.5 (0.4 M), respectively. The absorbance was measured at 262 and 660 nm by UV-VIS spectrophotometer. Absorbance at 660 nm was measured for turbidity correction. Isoflavone was calculated as genistein according to the following equation:

$$\text{Isoflavone (mg/L)} = \frac{\Delta A}{\varepsilon} \times 1 \times \text{M} \times \text{D} \times 10^3 \quad (4)$$

where, ΔA = (Abs_{262} at pH 1.0- Abs_{660} at pH 1.0) - (Abs_{262} at pH 4.5- Abs_{660} at pH 4.5); E (molecular extinction coefficient) = 35,842 L/mol/cm for genistein at 262 nm and 96% ethanol; l = path length in cm; M (molecular weight) = 270.241 g/mol for genistein; D = dilution factor;

For SI, 10 mg of powder was mixed with 50 ml of 96% ethanol and vortex for 90 s. The suspension was centrifuged at 2600 g for 5 min at −15°C. The supernatant was separated and filter through 0.45 μm Millipore membrane. Encapsulation efficiencies were calculated shown in the following equation:

$$\text{Encapsulation efficiency} = \frac{\textit{Total isoflavone} - \textit{surface isoflavone}}{\textit{Total isoflavone}} \times 100$$

2.9. Sensory analysis

The consumer acceptability of isoflavone encapsulated milk powder was evaluated by a nine person testing panel. The panelists were semi-trained and semi-experienced in judging both dairy and soy products throughout the study. Sensory characteristics were studies for isoflavone encapsulated milk powder stored for 1, 4, 8, 12 and 16 days stored at 4°C. The panelists were asked to assign appropriate score to each product tested on 9-point hedonic scale for characteristics of color, flavor (beany flavor) and taste (bitterness) of samples (1 = none, 3 = slight, 5 = moderate, 7 = strong, and 9 = very strong).

3. Result and discussion

3.1. Effect of solvent on isoflavone extraction

Fig. 1 illustrates the effect of solvent on extraction of isoflavone. 70% isoflavone could be extracted using all three solvents. A single step extraction yielded only less than 50% of isoflavone. Increasing the stages of extraction increased the percent extraction above 70% for all three solvents. The result is in agreement with Achouri et al. (2005), who suggested that extraction of isoflavone increased by increasing the number of stages of extraction to four to five sequential extraction times.

Fig. 1 illustrates that acetonitrile extracted significantly ($p \leq 0.5$) higher amount of isoflavone than others. The results are in agreement

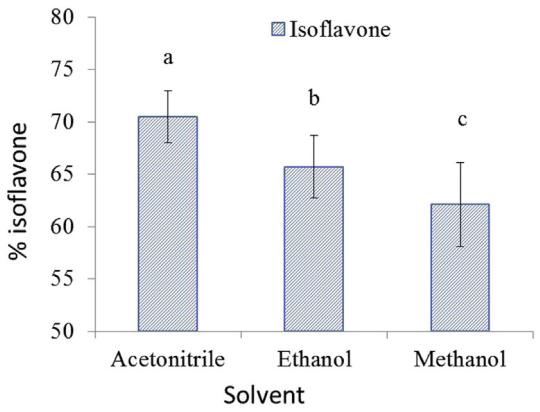

Fig. 1. Effect of solvent on extraction of isoflavone. Bar represents standard deviation.

with the reports by Murphy et al. (2002). In terms of extraction efficiency, the trend observed was acetonitrile > ethanol > methanol. Methanol was used as a solvent in the present study only for comparison purposes. Ethanol was selected for extraction of isoflavone, as per the legislations regarding use of Food-grade solvents.

3.2. Physico-chemical properties of isoflavone encapsulated spray dried milk powder

Table 1 shows the different physical properties of isoflavone-encapsulated milk power. There was no significant difference among all the three samples in terms of moisture content and hygroscopicity. Moisture content of the isoflavone encapsulated spray dried milk powder ranged from 1.98 to 2.05%, as shown in Table 1. This moisture content value is typical for spray dried powders containing carbohydrates, such as honey powder with maltodextrin, as reported by Nurhadi et al. (2012) and Samborska & B Bieńkowska (2013). The critical moisture levels are around 5% for spray dried powders and 4% for roller powders, above those figures defects in the powder might be detected (Crossley, 1960). In our study, the moisture content is around 2.05% and microbial defects could be avoidable at this moisture content.

Insolubility index showed that sample A possessed a higher solubility index. However, the insolubility index for all three samples was on the lower side. The solubility index depends on the total solid content of suspensions, i.e. solid concentration ranges from 20 to 30% did not affect the insolubility index (Sulieman et al., 2014). The insolubility index must be below 0.2 ml in powder for better quality of milk powder (Sulieman et al., 2014). The microencapsulated milk powders obtained by spray drying were easily dispersible in water and completely soluble. Solubility time ranged from 40 to 58 s. The shortest time required in which microencapsulated milk powder was dissolved completely was achieved for sample A (40 s) and the longest time required for sample C (58 s). However, solubility of milk powder depends on the several factors such as atomization speed, heat treatment of milk, type of spray drying, and type of coating materials used (Sharma et al., 2012; Toikkanen et al., 2018; Lee et al., 2018). It is reported that maltodextrin showed significantly shorter time period than dextrin.

Table 1
Physical properties of isoflavone encapsulated milk powder.

Sample	Moisture content (%)	Insolubility index (ml)	Solubility time (sec)	Hygroscopicity (g/g solid)
A	2.01^{a} ±0.05	0.20^{a}±0.004	35^{a}±3.0	0.14^{a}±0.003
B	1.98^{a} ±0.07	0.35^{b} ± 0.005	42^{b} ± 2.0	0.17^{b} ± 0.002
C	2.05^{a} ±0.09	0.45^{c}±0.002	44^{b} ± 2.5	0.18^{c} ±0.005

*Superscripts with different letters in a column indicate significant differences with each other's.

Hygroscopicity of the isoflavone encapsulated milk powders ranges from 0.014 to 0.018 g/g solids (Table 1). Sample C is most susceptible to moisture absorption followed by sample B and A due to high gum acacia content in the sample C. Microencapsulation of isoflavone by spray drying in the milk with maltodextrin and gum acacia is less susceptible to hygroscopicity due to the low viscosity of maltodextrin at high concentrations and high solubility of gum acacia (Sansone et al., 2011). In our experiment, we found that sample A and B is less susceptible to moisture absorption, although all the conditions are same for all three samples except gum acacia. The result indicated that increasing the gum acacia content increased the moisture absorption due to high molecular content of gum acacia. This observation is in good agreement with the work of Suravanichnirachorn et al. (2018), who observed that Mao powders with 7% gum Arabic had higher moisture content than those with 7% maltodextrins.

3.3. Color determination

The luminosity (L*-value) of all the three samples did not show any significant ($p \geq 0.05$) differences (Fig. 2). Similarly, a*-value and b*-values for sample A, B and C are almost same and did not show any significant differences. As a result, it is necessary to determine the brown pigment formation by determining the optical density at 420 nm. The result suggested that combination of coating materials (gum acacia plus maltodextrin) did not have any effect on color formation during spray drying of milk.

There was no significant difference ($p > 0.5$) among all three samples in terms of color formation. Color formation in all three samples was higher as shown in Fig. 3. This might be due to the formation of turbidity in the suspension. The turbidity analysis suggested that sample C (8% gum acacia) shows higher turbidity than others. The result indicated that increasing the amount of gum acacia in the suspension increased the turbidity of the suspensions.

3.4. Degradation of isoflavone and kinetic study

Isoflavone encapsulated milk powder was examined for its ability to retain isoflavone in three different storage temperatures as shown in Fig. 4. For all three samples, the percentage loss of isoflavone lost (%) increased with the length of storage periods. After 10 days of storage, the loss (%) of isoflavone in sample A was only 5.0, 7.5 and 8.5% at 4, 25 and 37°C, respectively and it's significantly different with each other. Nonetheless 6.0, 8.0 and 9.5% isoflavone degradation (%) was observed for sample B and 7.0, 10.0 and 12.5% isoflavone degradation (%) for sample C at 4, 25 and 37°C, respectively, during 10 days of storage. The results are in agreement with those by Seok et al. (2003), who observed that the rate of water soluble isoflavone lost (%) increased with the length of storage period. While the loss of water soluble isoflavone (%) was 17, 18, and 20% at 4, 20 and 30°C, respectively after 5 days of storage, the same after 12 days increased to 18, 19 and 25% respectively at the temperatures studied.

After 30 days of storage period, the % losses of isoflavone for all three samples were around 1.5 times higher than that at 10 days of storage. Statistical analysis suggested that there was no significant difference among sample A, B and C during storage at 4°C, for the entire period studied, but significantly differed after 30 days of storage at 25 and 37°C temperature. The highest isoflavone lost (16%) was found for sample C. Isoflavone release was significantly higher at 37°C during 30 days of storage than 4 and 25°C. This might be due to the change in the lipid crystal structure, reducing the stability at 37°C (Jackson and Lee, 1991). There was no significant difference among samples stored at 4°C indicating that microcapsules can maintain water activity (a_w) less than 0.3 for better stability of isoflavone and inhibit chemical reactions (Mazumder, 2016; Davies et al., 1998). Although all the factors are same for all three samples, sample C shows highest isoflavone degradation at 25 and 37°C temperature after 10 and 30 days of storage. This might be

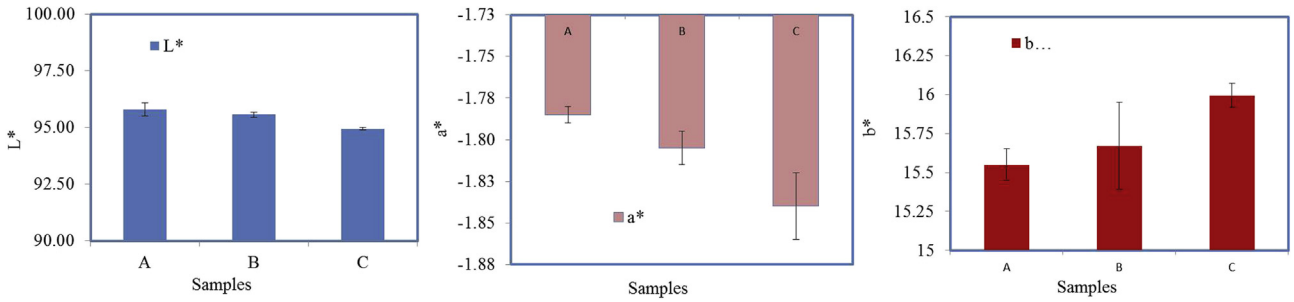

Fig. 2. Effect of isoflavone on L*-value, a*-value and b*-value. Bars represent standard deviation. A = 4% gum acacia+10% maltodextrin; B = 6% gum acacia+10% maltodextrin; C = 8% gum acacia+10% maltodextrin.

due to high gum acacia content in the sample C which tends to accumulate and sediment in the suspension before spray drying. As a result, high concentration of gum acacia cannot form strong film surrounding isoflavone resulting in the loss of isoflavone from the encapsulation.

Food quality loss can be described by zero order or first order reaction (Pua et al., 2008). Linear relationship between ln (C_t/C_0) and storage period with regression co-efficient >0.90 for micro-encapsulation of isoflavone in three different concentrations (4, 6 and 8% w/v) of gum acacia +10% maltodextrin (w/v) in whole milk at 4, 25 and 37°C. Results suggested that isoflavone degradation during storage period shows first order reaction for encapsulated samples. This result is in agreement with Idham et al. (2012), Wang and Xu (2007), Kirca and Cemeroglu (2003), Ochoa et al. (1999) and Ersus and Yurdagel (2007); they found that anthocyanins retention in encapsulated in different juice at different wall materials also showed first order reaction at different storage temperature.

Fig. 5 illustrates that degradation rate constant (k) of isoflavone at different storage temperature. Increase in storage temperature lead to an increase in the rate constant (k) for all three samples. However, at 4°C there is no significant ($p > 0.5$) difference among all three samples in terms of rate constant (k). Among all three samples, sample B shows significantly lower isoflavone degradation rate at all three storage temperature. A high encapsulation efficiency and low degradation rate indicates the stability and pronounced shelf life of isoflavone in milk powder.

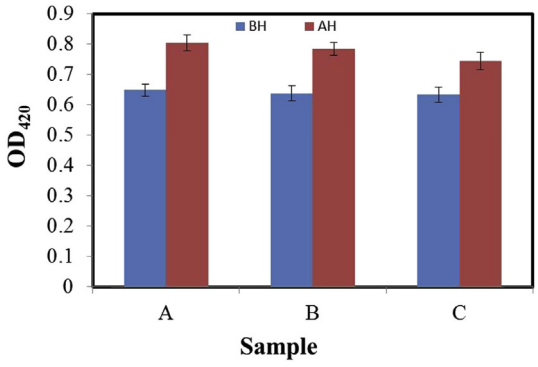

Fig. 3. Effect of isoflavone on the color formation. A = 4% gum acacia+10% maltodextrin; B = 6% gum acacia+10% maltodextrin; C = 8% gum acacia+10% maltodextrin. BH = before heating; AH = after heating. Bar represents standard deviation.

3.5. Encapsulation efficiency

Fig. 6 illustrates the encapsulation efficiencies of the three samples analyzed. The result mainly focuses on the effect of amount of coating materials on the microencapsulation. The result indicated that microencapsulation efficiency was affected by the amount of coating materials. While earlier report by Niamnuy et al. (2019), have highlighted the effectiveness of maltodextrins over gum acacia in terms of encapsulation efficiency, this work is an attempt to determine the efficiency of this combination, combined with the milk proteins on the encapsulation efficiency of isoflavones. Earlier reports have mentioned an Encapsulation efficiency of 80.59 and 69.63% for maltodextrin and gum Arabic, respectively. However, it is noteworthy that in this study, the encapsulation efficiency of all the samples were not less than 92%. Statistical analysis suggests that sample A and B did not significantly ($P > 0.5$) differ

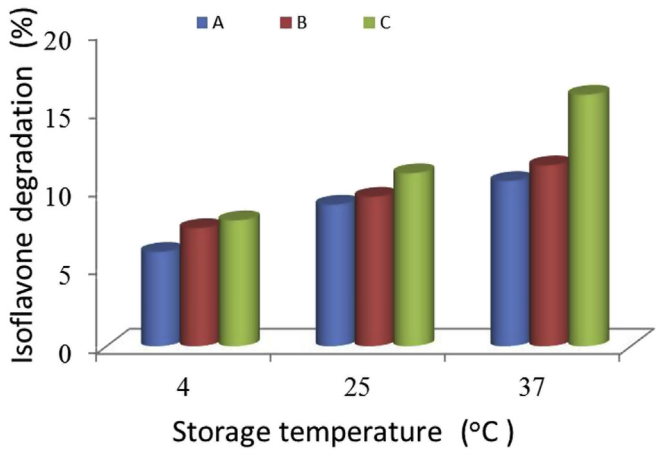

Fig. 4. Effect of storage temperature on isoflavone degradation at different temperature. A = 4% gum acacia+10% maltodextrin; B = 6% gum acacia+10% maltodextrin; C = 8% gum acacia+10% maltodextrin.

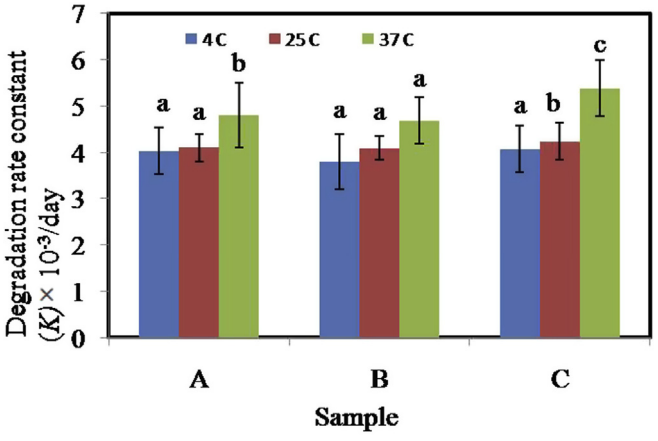

Fig. 5. Degradation rate constant (k) for isoflavone. A = 4% gum acacia+10% maltodextrin; B = 6% gum acacia+10% maltodextrin; C = 8% gum acacia+10% maltodextrin. 4 C = 4°C, 25 = 25°C, 37 = 37°C. Bar represents standard deviation.

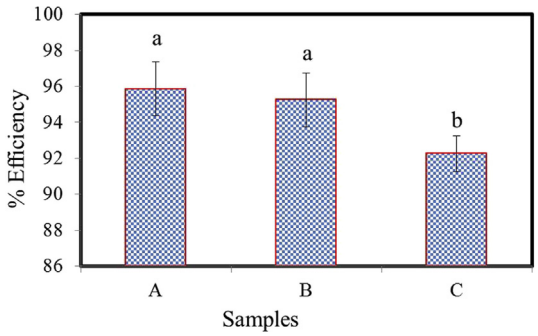

Fig. 6. Effect of different concentration of gum acacia on encapsulation efficiency. A = 4% gum acacia+10% maltodextrin; B = 6% gum acacia+10% maltodextrin; C = 8% gum acacia+10% maltodextrin. Bar represents standard deviation.

with each other in terms of encapsulation efficiency. It shows that core to coating ratio (1:10), 4 or 6% gum acacia with 10% maltodextrin is the optimal choice for microencapsulation of isoflavone in the milk powder. This might be due to synergy exhibited between the maltodextrins, gum Arabic with the milk proteins, ascribed to the ionic interactions between the negatively charged Arabic and maltodextrins with the proteins in milk (Eraso and Herrera, 2014).

Decreasing the amount of coating materials (gum acacia) from 8 to 4% resulted in increasing the efficiency of microencapsulation of isoflavone with maltodextrin (Fig. 3). The result is an agreement with Akdeniz et al. (2017), who found that encapsulation efficiency of onion skin phenolic compounds increased with decreasing the gum arabic concentration. However, combination of coating materials (gum acacia + maltodextrin) resulted in the highest encapsulation efficiency for isoflavone microencapsulation as reported by Akdeniz et al. (2017), Madene et al. (2006) and Mcnamee et al. (1998a,b). Gum acacia is an excellent film forming agent due to its complex structure containing L-arabinose, D-galactose, L-rhamnose and D-glucuronic acid (Akdeniz et al., 2017), thus better entrapping the encapsulated molecules. In addition, phenolic compounds and flavonols including isoflavone may form complexation with polysaccharides, however, depends on the solubility, molecular size, shape of polyphenols and mobility (Shahidi and Naczk, 2004). In this study, 200 ml of whole milk was added to the gum acacia, maltodextrin and isoflavone mixtures prior to colloidal mill processing, milk protein also form a coating on the isoflavone particles. As protein has the ability to form a coating *via* steric mechanism (McClements, 2004). However, encapsulation efficiency in Sample C is lower than sample A and B, although sample C contained higher amount of gum acacia. This might be due to the accumulation and sedimentation of gum acacia in the suspension before spray drying. Nevertheless, success of encapsulation relies on the achieving high retention of core materials and minimum core materials on the surface of powder particles. Several factors could effect the encapsulation efficiency includes, chemical properties of both coating and core materials, emulsion characters and drying parameter (especially conditions of the spray drying such as inlet and outlet temperatures, feed flow rate, air flow and humidity, powder particle size, etc.).

Table 2
Sensory scores of different isoflavone encapsulated milk powder for 16 days of storage in 4°C temperature.

Sensory description	Treatments	Storage period (days)				
		1	4	8	12	16
Beany flavor	Control (whole milk powder)	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Isoflavone encapsulated milk powder	1.15^{a}	1.25^{a}	1.55^{a}	1.80^{b}	2.15^{b}
Bitterness	Control (whole milk powder)	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Isoflavone encapsulated milk powder	1.10^{a}	1.21^{a}	1.45^{b}	1.77^{b}	2.0^{b}
Color	Control (whole milk powder)	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Isoflavone encapsulated milk powder	1.0^{a}	1.10^{a}	1.25^{a}	1.30^{b}	1.45^{b}

Superscripts with different letters in a column indicate significant differences with each other's.

3.6. Sensory analysis

Sensory evaluation of isoflavone encapsulated milk powder was observed during 14 days of storage at 4°C as shown in Table 2. Consumer preference for beany flavor suggested that isoflavone encapsulated milk powder showed significant difference compared with control at 12 days of storage. However, the value did not reach 3 (slight beany flavor), means the sample yet to reach beany flavor until 16 days of storage (Table 2). For bitterness, isoflavone encapsulated milk powder did not show significant difference until 8 days of storage, bitterness increased slowly during the storage period (16 days). Similar trend was also observed for color, isoflavone encapsulated milk powder little yellow color during storage period. The study suggested that isoflavone encapsulated milk powder was almost not affected with sensory attribute. Seok et al. (2003) also found the similar result in their study of microencapsulation of water soluble isoflavone.

Conclusion

Encapsulation of isoflavone with polysaccharides followed by appropriate processing may enhance the stability of isoflavone for efficient utilization in food systems. Microencapsulation of isoflavone with a combination of gum acacia and maltodextrin had the highest encapsulation efficiencies. The kinetics of study of isoflavone stability result supported that 10% maltodextrin and 6% gum acacia as wall material gave the longest shelf life. Hence, this study signifies that encapsulation process could stabilize and extend the shelf life of isoflavone. It was deemed necessary to perform further studies on release of encapsulated isoflavone in *in vivo* and animal model systems.

Author contribution

Ranganathan T.V.: Conceptualization, Methodology, Visualization, Supervision, Writing – review & editing, Anisur Mazumder.: Data curation, Writing- original draft preparation, Investigation, Validation.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors gratefully acknowledge Indian Science and Research Fellowship for research grants.

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