Selection of stabilizers and processing aids to encapsulate bitter gourd extract in a stable double emulsion

Urmila Choudhary¹ and Latha Sabikhi²

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Abstract: The aim of the present investigation was to assess the effect of three formulation variables and their levels on the stability of bitter gourd encapsulated double emulsion using a 3³ full factorial design. The ingredients used were i) NaCl (3-5%, w/w), ii) polyglycerol polyricinoleate (2-4%, w/w) and iii) protein:polysaccharide complexes (sodium caseinate- β -pectin / WPC-80- β -pectin, 1.5-4.5%, w/w). The dependent variables were apparent viscosity, zeta potential, sedimentation stability and turbidity. The optimized formulation consisting of bitter gourd extract (55.2%, w/w), 3% NaCl, 4% PGPR and 4.5% sodium caseinate-pectin as complex had higher apparent viscosity (2060 ± 17.32 cP), zeta potential (-30.33 ± 0.18 mV), lower turbidity (1.18 ± 0.03 cm⁻¹), and was stable for 29 days at 37°C, when compared to other combinations ($p < 0.001$). The study resulted in an optimized emulsion containing bitter gourd extract as a potential delivery vehicle, to incorporate the antidiabetic herbal component in functional foods.

Keywords: Dairy protein-polysaccharide complex; NaCl; PGPR; Bitter gourd extract; Stability

Introduction

Over the past few decades, with increased awareness and interest among consumers in health-enhancing bioactive food components, people have shifted to better alternatives such as

functional foods over medicine. The quality and quantity of different dietary components are determining factors for overall health. Dietary habits and life style are associated with various metabolic diseases such as diabetes mellitus. Bitter gourd is fast gaining attention as a potential management for diabetes, hence increasing its use in foods and as a dietary supplement (Wu and Ng 2008; Habicht et al. 2011). Incorporating bitter gourd extract in foods could be a convenient way to combat diabetes mellitus. Utilization of bitter gourd extract needs a medium by which its sensorial aspects can be made acceptable or masked. Double emulsion is one such technique which is used to mask the unacceptable flavor and odor of plant extracts by compartmentalizing it in inner phase, which is then protected by layer of another immiscible liquid. $W_1/O/W_2$ emulsions can replace conventional O/W emulsions, not only by reducing the oil content, but also having similar sensorial perception in mouth (Jiménez-Colmenero 2013; Oppermann et al. 2016). In addition, by way of encapsulation, they can protect heat- and light-sensitive ingredients such as flavor compounds, vitamins and bioactive compounds and facilitate their controlled release within food (Pawlik and Norton 2014).

Instability is the key issue in double emulsion which limit their applicability as food matrices. The type and concentration of surfactants, electrolytes, the nature of encapsulants, ratio of dispersed phase to continuous phase, osmotic balance between inner and outer phase, various processing regimes such as formulation methods, shear applied, temperature of operation and conditions, final desired droplet size and storage conditions are the predominating factors which affect the stability of the double emulsion (Shima et al. 2004). Instability can be reduced by balancing the osmotic pressure between the two aqueous phases (Sameh et al. 2012) and by using protein-polysaccharide electrostatic complexes (Salminen and Weiss 2014) as secondary surfactant. Two different types of surfactants are usually required to stabilize a double emulsion ($W_1/O/W_2$) i.e. primary emulsion surfactant for the inner aqueous droplets and secondary emulsion surfactant for intermediate oil phase droplets. Polyglycerol polyricinoleate (PGPR) is used as an oil-soluble surfactant, which prevents coalescence of newly formed water droplets by the formation of tiny water droplets in the oil phase and facilitates droplet break-up by reducing the interfacial tension (Landfester

Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India

Urmila Choudhary (✉)

Dairy Technology Division, ICAR-National Dairy Research Institute
Karnal-132 001, Haryana, India
Email: chaudharyurmila89@gmail.com

2006; Bahtz et al. 2016). Salt (NaCl) is an electrolyte that generates the osmotic pressure to counterbalance the Laplace pressure and stabilizes the W/O emulsions against Ostwald ripening (diffusional ripening) (Landfester 2006; Capek 2010; Colman et al. 2014). Therefore, the addition of electrolytes along with appropriate hydrophobic emulsifiers and hydrophilic emulsifiers is essential to prepare coalescence-stable $W_1/O/W_2$ double emulsion.

Biopolymers which includes proteins and polysaccharides may improve the stability of double emulsions by increasing the viscosity of inner and outer water phase of the double emulsion (Surh et al. 2007). Thus, change of particle size is controlled through formation of interfacial layer surrounding droplets (Lutz et al. 2009b; Sameh et al. 2012) and controlling osmotic pressure (Pawlik et al. 2010). Proteins are less effective emulsifiers near their iso-electric pH, which limits their application in acidic foods. Hence, electrostatic protein-polysaccharide complexes gave better functional properties: emulsifying, stability, encapsulation efficiency, texture/rheology, surface hydrophobicity and mouth feel, than that of the proteins and polysaccharides alone even near the iso-electric pH of the protein (Li et al. 2012; Hernández-Marín et al. 2013; Klein et al. 2013; Salminen and Weiss 2014). However, the stability of the emulsions stabilized with protein-polysaccharide complexes is influenced by several factors such as protein-to-polysaccharide molar mass ratio, pH, ionic strength, temperature and processing methods of the complexes (Lutz et al. 2009a,b).

The overall objective of the current investigation was to assess the effect of PGPR, the hydrophobic emulsifier as primary surfactant, NaCl as an electrolyte and dairy protein-polysaccharide complexes as secondary surfactant on stability of the bitter gourd extract encapsulated double emulsion ($W_1/O/W_2$). The formulated double emulsion was characterized for its physicochemical properties viz., apparent viscosity, zeta potential, sedimentation stability (by visual appearance) and turbidity.

Materials and Methods

Materials

PGPR Grinsted® PGPR-90, β -pectin [GENU® β -pectin with ~ 18% degree of acetylation and > 50% degree of esterification (DE)] were supplied free of cost by M/s DuPont Danisco India Pvt. Ltd. (Gurgaon, India) and CP Kelco, Huber India Company (Mumbai, India), respectively. Dairy proteins such as whey protein concentrate-80 (WPC-80) with 79.86% protein and 8.09% lactose and sodium caseinate (SC) with 88% protein were purchased from M/s Mahaan Proteins Ltd. (New Delhi, India). Aqueous extract of bitter gourd consisting 5.95% w/w total solids (TS) and 5% bioactive compound (Charantin) was purchased from M/s Ambe Phytoextracts Pvt. Ltd. (New Delhi, India). Rice bran oil, vinegar (4% acetic acid) and sodium chloride (NaCl)

were purchased from local market of Karnal (India). Reverse osmosis (RO) water was used as an aqueous phase.

Experimental factorial design

The double emulsion ($W_1/O/W_2$) composed varied concentrations of NaCl in inner aqueous phase (W_1) containing bitter gourd extract (55.2%), PGPR in middle oil phase (O) and dairy protein-polysaccharide (WPC-pectin, SC-pectin) complexes (WPC-P/SC-P, 1:2) in outer aqueous phase (W_2). All the variables are presented in percentage (%), w/w and their influence on the apparent viscosity, sedimentation stability (at 7 and 37°C) by visual observations, zeta potential and turbidity of the functional mayonnaise were evaluated. The experimental design consisted of 54 formulations (27 each for the two dairy protein-polysaccharide complexes) as shown in Table 1. A full factorial design approach was adopted to study the influence of the variables and their interaction on the properties of the double emulsion to optimize the double emulsion.

Preparation of dairy protein-polysaccharide complexes

The formulation of protein-polysaccharide complexes was done according to Salminen and Weiss (2014) with slight modifications. Dairy protein (WPC-80 and SC) and polysaccharide (β -Pectin-P) were used in the ratios 1:2 (Kumar et al. 2020). The protein and polysaccharide were weighed into separate beakers for solubilization with RO water according to Table 1. Protein solutions were prepared by magnetic stirring constantly at ambient temperature for 1-2 h, whereas polysaccharide solutions were prepared by magnetic stirring for 6-10 h at moderate speed to ensure complete dissolution. Protein and polysaccharide solutions were initially adjusted to pH 7.0 using 1.0 N NaOH before further mixing. Then, they were mixed together and pH was reduced up to 4.0 using 4% acetic acid (vinegar) and stored at ambient temperature for overnight.

Preparation of double emulsion ($W_1/O/W_2$)

Inner aqueous phase (W_1) was prepared with an aqueous soluble bitter gourd extract, (55.2%, w/w) and varying levels of NaCl (3.0-5.0%) in RO water. Middle oil phase (O) consisted of rice bran oil containing PGPR as hydrophobic emulsifier (2-4%, w/w). Outer aqueous phase (W_2) was added with either WPC-P or SC-P prepared as explained in the previous section. Both W_1 and O were pasteurized at 72°C for 15 sec, while outer aqueous phase heated at 85°C for 20 min to prepare biopolymer particles because of thermal denaturation and aggregation of the proteins followed by cooling to 4-7°C in ice water bath.

A primary water-in-oil (W_1/O) emulsion was premixed by mixing the inner aqueous phase (W_1) (30%, w/w) with the oil phase (O) (70%, w/w) at room temperature, using a magnetic stirrer at moderate speed for 5 min. The mixture was then homogenized using an Ultra-Turrax [IKA Ultra-Turrax T25 (IKA®) India Pvt.

Ltd., Bangalore, India] operating at 22,000 rpm for 5 min to form primary W_1/O emulsion. The primary (W_1/O) emulsion (30%, w/w) was gradually added to the outer aqueous phase (W_2) (70%, w/w) and mixed with magnetic stirring at moderate speed for 5 min. The pre-mix was finally homogenized using Ultra-Turrax at 15,000 rpm for 5 min to produce final double emulsion ($W_1/O/W_2$) (9:21:70:: $W_1/O/W_2$).

Apparent viscosity

A controlled stress rheometer (RHEOPLUS/32 (M/s Anton Paar, GmbH, Ostfildern, Germany), using a cone and plate geometry (CP-75, D=0.149mm) was used for the measurement of apparent viscosity (cP) in the shear rate range $0.01-100\text{ s}^{-1}$ at 25°C (Lupi et al. 2011).

Zeta potential

The zeta potential of the double emulsions were measured using Zetasizer Nano-ZS90 (Malvern Instrument Ltd., Malvern, Worcestershire, UK) to determine the stability of the emulsion. The freshly prepared emulsions were diluted 100 times with RO water and the experiment was carried out at 25°C temperature. Zeta potential measurements were carried out in triplicate for each emulsion and the results were expressed in mV.

Turbidity

The turbidity of the double emulsions were determined in triplicates according to Pearce and Kinsella (1978). Aliquots (1 mL) of the emulsion were diluted serially with water and sodium dodecyl sulfate (SDS) solution to give final dilutions in the range 1/1000 to 1/5000 and an SDS concentration of 0.170%. The absorbance of the diluted emulsion was then determined in a 1-cm path length cuvette at a wavelength of 500 nm in a spectrophotometer (M/s Thermofisher Scientific Inc., Rochester, New York, USA). Identical cuvettes were used for all samples and rinsed with a jet of distilled water between determinations. Absorbance of triplicate aliquots of each emulsion was measured in each case.

$$T = \frac{2.303A}{l}$$

where A is the observed absorbance and l is the path length of the cuvette.

Table 1 3^3 full factorial designs to investigate the influence of variables on double emulsion

Variables (% w/w)	Levels		
	Low level (-1)	Medium level (0)	High level (+1)
NaCl	3	4	5
PGPR	2	3	4
Dairy protein-polysaccharide complexes (1:2)	1.5	3	4.5
WPC-P			
SC-P			

Sedimentation stability

The sedimentation stability (by visual appearance) of the double emulsion was determined in triplicates according to Sapei et al (2012). Freshly made double emulsions were poured into 40 mL glass vials (internal diameter = 21 mm; length = 90 cm; Corning India, Gurgaon, India) to a height of 4.6 cm and stored at 37°C . The emulsion was observed daily until the phase separation occurred.

Statistical data analysis

The data obtained were analyzed by two-way analysis of variance (ANOVA) using SPSS (IBM SPSS Statistics 21) software. Significant difference between variables and attributes (apparent viscosity, zeta potential, sedimentation stability and turbidity measurements) of the functional mayonnaise was determined at $p < 0.001$. Two-way ANOVA results table of 3^3 full factorial designs are shown in Table 2-5. All experimental attribute measurements were done in triplicate.

Results and Discussion

Effect of different variables on physicochemical properties of the double emulsion as presented below:

Apparent viscosity

The effect of NaCl (3-5%, w/w), PGPR (2-4%, w/w) and two different (WPC-P and SC-P) complexes on the apparent viscosity (at 100 s^{-1} shear rate) immediately after preparation of the double emulsion ($W_1/O/W_2$) shown in Fig. 1. The double emulsion stabilized with SC-P complex had a very highly significantly ($p < 0.001$) higher mean value of apparent viscosity than that with WPC-P complex (Table 2). It is clear from Fig. 1 (a,b) that the apparent viscosity increased ($p < 0.001$) with increase in concentration of both the complexes. Further, as PGPR and NaCl concentrations increased, apparent viscosity of emulsions stabilized with both complexes (WPC-P and SC-P) increased. The differences in the mean apparent viscosity values were very highly significantly affected by all the variables and their interactions (Table 2).

In the present study, the viscosity of the double emulsions was influenced mainly by relationships between the concentrations

of the protein-polysaccharide complexes and NaCl in the outer aqueous phase and inner aqueous phase, respectively, and the degree of mobility of the water in the external aqueous phase.

The effect of concentration of the protein-polysaccharide complexes on the apparent viscosity of the double emulsions ($W_1/O/W_2$) was the same as observed by Lutz et al. (2009a).

Table 2 Analysis of variance of apparent viscosity, zeta-potential and turbidity of double emulsion stabilized with WPC/SC-P complex at 37°C

Attributes	Components	Type III Sum of Squares	Degree of freedom (Df)	Mean Square	F value
Apparent viscosity	NaCl	517864.778	2	258932.389	884.343***
	PGPR	10414484.11	2	5207242.056	17784.522***
	Complex	997813.556	1	997813.556	3407.876***
	Level of Complexes	34429487.44	2	17214743.72	58794.267***
	PGPR*NaCl	449568.556	4	112392.139	383.858***
	Level*PGPR	2713007.556	4	678251.889	2316.463***
	NaCl*Level	264671.556	4	66167.889	225.986***
	NaCl*Complex	7991.148	2	3995.574	13.646***
	Complex*Level	502617.815	2	251308.907	858.306***
	Complex*PGPR	207872.259	2	103936.130	354.978***
	Complex*NaCl*Level	193465.926	4	48366.481	165.188***
	Complex*NaCl*PGPR	99229.148	4	24807.287	84.725***
	Complex*Level*PGPR	127744.370	4	31936.093	109.073***
	NaCl*Level*PGPR	988721.333	8	123590.167	422.103***
	Complex*NaCl*Level*PGPR	109522.444	8	13690.306	46.757***
Zeta-potential	NaCl	295.678	2	147.839	91.085***
	PGPR	86.445	2	43.222	26.630***
	Complex	116.366	1	116.366	71.694***
	Level of Complexes	522.680	2	261.340	161.014***
	PGPR*NaCl	645.063	4	161.266	99.357***
	Level*PGPR	45.391	4	11.348	6.992***
	NaCl*Level	180.934	4	45.233	27.869***
	NaCl*Complex	37.710	2	18.855	11.617***
	Complex*Level	364.239	2	182.120	112.206***
	Complex*PGPR	0.711	2	0.356	0.219***
	Complex*NaCl*Level	68.927	4	17.232	10.617***
	Complex*NaCl*PGPR	285.325	4	71.331	43.948***
	Complex*Level*PGPR	85.474	4	21.369	13.165***
	NaCl*Level*PGPR	123.520	8	15.440	9.513***
	Complex*NaCl*Level*PGPR	146.819	8	18.352	11.307***
Turbidity	NaCl	40.174	2	20.087	1419.210***
	PGPR	193.374	2	96.687	6831.230***
	Complex	5.296	1	5.296	374.157***
	Level of Complexes	49.793	2	24.897	1759.026***
	PGPR*NaCl	104.094	4	26.024	1838.639***
	Level*PGPR	9.174	4	2.293	162.038***
	NaCl*Level	0.259	4	0.065	4.579***
	NaCl*Complex	54.875	2	27.438	1938.540***
	Complex*Level	0.696	2	0.348	24.589***
	Complex*PGPR	23.536	2	11.768	831.443***
	Complex*NaCl*Level	6.720	4	1.680	118.698***
	Complex*NaCl*PGPR	40.578	4	10.144	716.735***
	Complex*Level*PGPR	4.722	4	1.180	83.404***
	NaCl*Level*PGPR	13.021	8	1.628	114.992***
	Complex*NaCl*Level*PGPR	6.495	8	0.812	57.362***

***Significant at $p < 0.001$

Fig 1. Effect of NaCl, PGPR and dairy protein-polysaccharide complexes (a): WPC-P, (b): SC-P on apparent viscosity of double emulsion; n=3

Apparent viscosity of the primary emulsion (W_1/O) increased with increasing PGPR concentration (Choudhary et al. 2018). Hence, the increased apparent viscosity of the final double emulsion ($W_1/O/W_2$) may be due to increased resistance to shearing during secondary emulsification. Our results were in agreement with studies reported by Su et al. (2006) and Bahtz et al. (2016), who found that the viscosity of the W/O emulsion increased with increasing concentrations of PGPR. This may have led to a subsequent reduction in the rate of coalescence and Ostwald ripening of the water droplets by decreasing the droplet diameter. On the other hand, though with initial increase in NaCl concentration, the apparent viscosity increased, it reduced at higher NaCl concentration. This may be because NaCl plays an important role in the formation of double emulsion by matching the osmotic and laplace pressure between the two aqueous phases (W_1 and W_2) of the double emulsion. Presence of excess NaCl concentration in inner aqueous phase causes a migration of the water droplets from outer aqueous phase (W_2) to inner aqueous phase (W_1), leading to subsequent swelling and sometimes bursting of the W_1/O droplets in the presence of concentration gradient between the two aqueous phases (Lutz and Garti, 2006; Rosano et al. 1998).

Zeta potential

The effect of the variables on ζ -potential of the double emulsion is shown in Fig. 2 (a,b). The double emulsion stabilized with the WPC-P complex exhibited higher ζ -potential than their SC-P stabilized counterparts. The mean ζ -potential values increased with increase in concentration of SC-P complex (1.5-4.5%), whereas it was found decreased with increase in concentration of WPC-P complex. The ζ -potential values of both complexes (WPC-P and SC-P) stabilized double emulsion increased with increasing concentration of PGPR while it was found to decrease up to 4% NaCl before increasing at 5% NaCl concentration. All

three variables and their interactions showed a very highly significant ($p < 0.001$) effect on ζ -potential values of the double emulsion (Table 2).

According to Mirhosseini et al. (2008), an emulsion is considered as stable if the absolute zeta potential value is more than 25. Hence, ζ -potential is considered a key indicator for the stability of double emulsion (W/O/W) because the stability is affected by surface charge (ζ -potential) of the emulsion droplets. ζ -potential values below 25 indicated flocculation, while those above 25 mV indicated deflocculating emulsions (Mirhosseini et al. 2009). The negative surface charge of the double emulsion increased with increase in concentration of the complexes in the external aqueous phase (W_2) of the double emulsions ($W_1/O/W_2$). This effect can be attributed to the increase in electrostatic repulsion between the protein-polysaccharide complex molecules associated with their increased electrical charge, which prevented the aggregation and flocculation of the droplets. Our results are in good agreements with Guzey and McClements (2007), who reported that as the pectin concentration (0-0.05%, wt%) increased, the magnitude of zeta potential changed from positive to negative because the anionic pectin molecules adsorbed to the surface of cationic protein-coated droplets until the saturation of pectin concentration occurred. Further adsorption of the pectin may be prevented because the cationic binding sites get saturated with anionic groups of the pectin particles or due to the electrostatic repulsion between adsorbed and non-adsorbed pectin particles.

Prichapan and Klinkesorn (2014) reported that zeta potential was higher in $W_1/O/W_2$ emulsions prepared with SC than those with WPC, and therefore, it had more electrostatic repulsive force between droplets, which prevents droplet flocculation and coalescence. In presence of high salt concentration, the magnitude of ζ -potential decreased may be due to the screening effect of electrically charged ions. This meant that the counter-

Fig 2. Effect of NaCl, PGPR and dairy protein-polysaccharide complexes (a): WPC-P (b): SC-P on zeta potential and (c): WPC-P (b): SC-P on turbidity of double emulsion; n=3

ions (Na^+) in inner aqueous phase accumulated loosely around the negatively charged $-\text{COO}^-$ groups on the protein-polysaccharide complex surface, due to the electrostatic interaction between the emulsion droplets and ion-binding effect. Low ionic (salt) concentration facilitated a strong electrostatic repulsion between the emulsion droplets, which prevents ions coming into close contact. However, once a critical ion (salt) concentration was reached, above this concentration electrostatic repulsion was no longer strong sufficient to overcome the attractive forces such as van der Waals and hydrophobic forces acting between the emulsion droplets, thus the emulsion droplets aggregate (McClements 2004). As a result of salt addition, the negative surface charge (ζ -potential) of the double emulsions shifted towards zero (slightly positive). This results supports

the finding of Liu et al. (2012) and Onsaard et al. (2014) that the magnitude of the electrical charge (ζ -potential) of emulsions prepared with SC-carboxymethylcellulose and WPC-maltodextrin/carrageenan was negative at all salt (NaCl) concentrations studied (0-500 mM), but the magnitude of surface charge decreased as well as the salt concentration increased due to reduction in the electrostatic repulsion between oil-water emulsion droplets. Lutz et al. (2009b) also found a lower ζ -potential value (-37 ± 3 mV) of double emulsions stabilized with most negatively charged WPI/U63 as compared to other pectin type WPI/C63 (-28 ± 3 mV). This negative surface charge prevented flocculation and coalescence by facilitating electrostatic repulsion between the emulsion droplets.

Turbidity

Fig 3. Effect of salt, PGPR and dairy protein-polysaccharide complexes (a): WPC-P (b): SC-P (37°C) (c): WPC-P (d): SC-P (7°C) on sedimentation stability of double emulsion; n=3

The effect of the variables on the turbidity of the double emulsion is shown in Fig. 2 (c,d). The average turbidity values were found lower in SC-P stabilized double emulsion than WPC-P stabilized samples and it increased with increasing concentration of both complexes (1.5-4.5%). The range of turbidity values with the minimum and maximum concentration of the complex were as follow: DE_{SC-P} ($2.95 \pm 0.22 \text{ cm}^{-1}$ to $5.04 \pm 0.43 \text{ cm}^{-1}$) < DE_{WPC-P} ($3.63 \pm 0.21 \text{ cm}^{-1}$ to $4.94 \pm 0.34 \text{ cm}^{-1}$). The mean turbidity values of both complexes (SC-P and WPC-P) stabilized double emulsion increased up to 3% PGPR, after that it decreased at 4% PGPR concentration. The range of turbidity values with the minimum and maximum concentration of NaCl are as follow: DE_{SC-P} ($3.12 \pm 0.30 \text{ cm}^{-1}$ to $4.04 \pm 0.27 \text{ cm}^{-1}$) < DE_{WPC-P} ($3.86 \pm 0.28 \text{ cm}^{-1}$ to $4.97 \pm 0.33 \text{ cm}^{-1}$). There was very high significant ($p < 0.001$) difference in the turbidity of double emulsions stabilized with each of the three variables i.e., NaCl, PGPR and complexes (WPC-P and SC-P). Their interaction also showed a very high significant ($p < 0.001$) effect on turbidity of the double emulsion (Table 2).

Several factors were reported to influence the emulsifying properties of protein-polysaccharide complexes such as protein-to-polysaccharide molar mass ratio, their total concentration, polymer charge density, pH, ionic strength, temperature and method of mixing (Benichou et al. 2002; Schmitt and Turgeon 2011). Ettelaie et al. (2005) reported that electrostatic protein-polysaccharide complexes onto the surface of protein-coated oil droplets led to increased emulsion stability by a thick secondary layer of charged polysaccharide. This could be due to the electrostatic and steric interaction between the protein and charged polysaccharide molecules. It has been reflected that the chemically modified proteins via electrostatic and steric complexation with polysaccharide could enhance emulsifying properties, especially below their isoelectric pH and at low ionic (salt) concentrations by maintaining molecular integrity and altered solubility (Surh et al. 2006; Lutz et al. 2009b). Emulsion activity (EA) and emulsion stability (ES) of double emulsion can be enhanced by covering the emulsion droplets by surfactants.

The biopolymers form a bulky and flexible film that may be strongly anchored on the O/W interface (Benichou et al. 2007; Lutz and Aserin 2007). Surh et al. (2006) associated the high viscosity of SC-emulsion with the addition of pectin at pH 3 and 4, due to high degree of droplet flocculation caused by charge neutralization and bridging flocculation of oppositely charged SC-(+ve)-stabilized emulsion droplets and pectin (-ve) molecules. Double emulsions ($W_1/O/W_2$) exhibited higher optical turbidity with increasing concentration of the dairy protein-polysaccharide complexes, probably at higher concentration of the complexes led to high viscosity of the emulsion by inducing strong electrostatic interaction. Lutz et al. (2009a,b) reported that WPI-modified pectin soluble complexes had net negative zeta-potential at pHd^o6.0 that enable the repulsive interaction (electrostatic and steric) between the droplet interface, which results in a stable emulsion. Su et al. (2008) reported that the ionic environment reduced the emulsifying ability of the hydrophobic emulsifier

(PGPR) from 4 % (w/w) PGPR in a buffered system to 2 % (w/w) PGPR in distilled water systems and with an encapsulation efficiency >90% of W/O/W emulsions prepared with SUPER GUMTM.

Sedimentation stability

The double emulsion samples were kept at $37\pm1^\circ\text{C}$ and $7\pm1^\circ\text{C}$ and observed daily for phase separation. It was observed that the sedimentation stability (by visual separation) is a function of storage temperature. Fig. 3 depicts the effect of variables such as NaCl, PGPR and complexes (WPC-P and SC-P) on sedimentation stability. The double emulsions prepared with SC-P were stable for almost one month (29 days) and more than one year (383 days) while WPC-P stabilized emulsions were stable for 23 days and 229 days at 37°C and 7°C , respectively. It was found that as concentration of both complexes increased, sedimentation

Table 3 Analysis of variance of sedimentation stability of double emulsion stabilized with WPC/SC-P complex

Temperature	Components	Type III Sum of Squares	Degree of freedom (Df)	Mean Square	F value
37°C	NaCl	27.938	2	13.969	11.203***
	PGPR	405.975	2	202.988	162.792***
	Complex	769.191	1	769.191	616.876***
	Level of Complexes	7665.198	2	3832.599	3073.668***
	PGPR*NaCl	25.988	4	6.497	5.210***
	Level*PGPR	118.840	4	29.710	23.827***
	NaCl*Level	267.210	4	66.802	53.574***
	NaCl*Complex	54.531	2	27.265	21.866***
	Complex*Level	606.309	2	303.154	243.124***
	Complex*PGPR	35.901	2	17.951	14.396***
	Complex*NaCl*Level	334.025	4	83.506	66.970***
	Complex*NaCl*PGPR	109.321	4	27.330	21.918***
	Complex*Level*PGPR	76.543	4	19.136	15.347***
	NaCl*Level*PGPR	560.420	8	70.052	56.181***
	Complex*NaCl*Level*PGPR	99.679	8	12.460	9.993***
7°C	NaCl	12291.012	2	6145.506	10479.705***
	PGPR	25711.272	2	12855.636	21922.242***
	Complex	279000.469	1	27900.469	47577.642***
	Level of Complexes	685042.123	2	342521.062	584088.547***
	PGPR*NaCl	24835.877	4	6208.969	10587.926***
	Level*PGPR	34540.210	4	8635.052	14725.037***
	NaCl*Level	44290.802	4	11072.701	18881.868***
	NaCl*Complex	23422.864	2	11711.432	19971.074***
	Complex*Level	66340.198	2	33170.099	56563.747***
	Complex*PGPR	13283.864	2	6641.932	11326.242***
	Complex*NaCl*Level	53243.025	4	13310.756	22698.342***
	Complex*NaCl*PGPR	30957.802	4	7739.451	13197.800***
	Complex*Level*PGPR	24984.358	4	6246.090	10651.226***
	NaCl*Level*PGPR	141898.642	8	17737.330	30246.816***
	Complex*NaCl*Level*PGPR	33340.420	8	4167.552	7106.774***

stability increased. The sedimentation stability was found to increase with increase in PGPR (2-4%) concentration in both SC-P and WPC-P complex stabilized double emulsion. It could be seen from Fig. 3(a,c) that sedimentation stability was highest at 3% NaCl, followed by 5% and 4% NaCl at 37°C and it was found to decrease with increase in salt concentration (3-5%) at 7°C. Whereas Fig. 3(b,d) shows that it was highest at 4% NaCl, followed by 3% and 5% NaCl at 37°C and, it increased with increase in NaCl concentration (3-5%) at 7°C.

It is evident from Table 3 that the sedimentation stability (by visual observation) of double emulsion was very highly significantly affected by the variables such as NaCl, PGPR, WPC-P and SC-P complexes and their interaction.

Lutz et al. (2009a) observed no coalescence in double emulsions stabilized with soluble WPI/modified pectin complex for almost one month, because oil droplets were fully covered by external oil-water interfacial (O/W₂) film comprising of the WPI/modified pectin. The soluble complexes of WPI/pectin also reduced the interfacial tension on surface of the emulsion droplets. Further they reported that the level of creaming also depended on the NaCl concentration (1.0, 1.5 and 4.4%, w/w) in the inner aqueous phase. Salminen and Weiss (2014) studied the electrostatic adsorption of heat-treated biopolymer complexes [Whey protein isolate (WPI)-apple pectin at 85°C for 20 min] and stability of these complexes on emulsion interfaces. They found that the emulsions covered with the complexes showed good stability to salt (up to 200 mM) and heat (up to 90°C) by creating a thick polymer layer. Muschiolik et al. (2006) investigated the effect of addition of salt (NaCl) in inner aqueous phase of multiple emulsions prepared with 4% PGPR during storage. They found that presence of the salt in the inner aqueous phase (W₁) of multiple emulsions is essential to carry out coalescence free W/O emulsions prepared with PGPR, an addition of salt increased the encapsulation stability and oil droplet stability of multiple emulsions. Actually, salt is considered to be a co-stabilizer, a lipophobic that builds-up the osmotic pressure to counterbalance the Laplace pressure, and consequently stabilizes emulsions against diffusional degradation known as Ostwald ripening (Landfester 2006; Capek 2010; Colman et al. 2014). Bahtz et al. (2016) reported that PGPR, when used as an oil-soluble surfactant, was able to spontaneously form tiny water droplets in oil phase that cause structural changes in the oil layer. Further, they described that the overall water transport rate increases and water transport stagnates because of maximized structure formation at below and above a critical concentration of the oil-soluble surfactant, respectively.

Conclusions

An attempt was made to fabricate a stable water-in-oil-in-water (W₁/O/W₂) double emulsion encapsulating bitter gourd extract, using a two-step emulsification technique. The results of the

present study indicated that several factors such as NaCl, PGPR and protein-polysaccharide complexes (WPC-P and SC-P) and their interaction exhibited a synergistic effect on the stability of the fabricated double emulsion. The deciding attributes (turbidity, zeta-potential, apparent viscosity and sedimentation stability) were very highly dependent on the all the variables such as NaCl, PGPR and complex and their levels. SC-P stabilized double emulsions were found more stable than WPC-P stabilized samples.

References

- Bahtz J, Gunes DZ, Syrbe A, Mosca N, Fischer P, Windhab EJ (2016) Quantification of spontaneous W/O emulsification and its impact on the swelling kinetics of multiple W/O/W emulsions. *Langmuir* 32: 5787-5795
- Benichou A, Aserin A, Garti N (2002) Protein-polysaccharide interaction for stabilization of food emulsions. *J Disper Sci Tech* 23(1-3): 93-123
- Benichou A, Aserin A, Lutz R, Garti N (2007) Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocoll* 21: 379-91
- Capek I (2010) On inverse miniemulsion polymerization of conventional water-soluble monomers. *Adv Colloid Interface Sci* 156: 35
- Choudhary U, Sabikhi L, Abdul Hussain S, Khamrui K, Sharma V, Vij S (2018) Stabilizing the primary emulsion with hydrophobic emulsifiers and salt for encapsulating herbal extracts in a double emulsion. *J Food Process Preserv* 42:e13699 <https://doi.org/10.1111/jfpp.13699>
- Colmán MME, Chicoma DL, Giudici R, Araújo PHH, Sayer C (2014) Acrylamide inverse miniemulsion polymerization *in situ*, real-time monitoring using spectroscopy. *Braz J Chem Eng* 31(4):925
- Ettelaie R, Dickinson E, Murray BS (2005) Self-consistent-field studies of mediated steric interactions in mixed protein + polysaccharide solutions. *Food colloids: interaction, microstructure and processing* 74-84
- Guzey D, McClements DJ (2007) Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by β -lactoglobulin-pectin complexes. *J Agric Food Chem* 55: 475-485
- Habicht SD, Kind V, Rudloff S, Borsch C, Mueller AS, Pallauf J, Yang R, Krawinkel MB (2011) Quantification of antidiabetic extracts and compounds in bitter gourd varieties. *Food Chem* 126: 172-176
- Hernández-Marín NY, Lobato-Calleros C, Vernon-Carter EJ (2013) Stability and rheology of water-in-oil-in-water multiple emulsions made with protein-polysaccharide soluble complexes. *J Food Eng* 119: 181-187
- Jiménez-Colmenero F (2013) Potential applications of multiple emulsions in the development of healthy and functional foods. *Food Res Int* 52: 64-74
- Klein M, Aserin A, Ishai PB, Garti N (2013) Interactions between whey proteins isolate and gum Arabic. *Colloids Surf B* 79:377-383
- Kumar SM, Sabikhi L, Lamba H (2020) Emulsification properties of sodium caseinate-based conjugates with selected polysaccharides. *Int J Dairy Tech.*
- Landfester K (2006) Synthesis of colloidal particles in miniemulsions. *Annu Rev Mater Res* 36: 231
- Li B, Jiang Y, Liu F, Chai Z, Li Y, Li Y, Leng X (2012) Synergistic effects of whey protein-polysaccharide complexes on the controlled release of lipid-soluble and water-soluble vitamins in W₁/O/W₂ double emulsion systems. *Int J Food Sci Technol* 47: 248-254

- Liu L, Zhao Q, Liu T, Kong J, Long Z, Zhao M (2012) Sodium caseinate/carboxymethylcellulose interactions at oil-water interface: relationship to emulsion stability. *Food Chem* 132: 1822-1829
- Lupi FR, Gabriele D, de Cindio B, Sánchez MC, Gallegos C (2011) A rheological analysis of structured water-in-olive oil emulsions. *J Food Eng* 107: 296-303
- Lutz R, Aserin A (2007) *Multiple Emulsions: Technology and Applications*, John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 85
- Lutz R, Aserin A, Wicker L, Garti N (2009a) Double emulsions stabilized by a charged complex of modified pectin and whey protein isolate. *Colloids Surf B* 72: 121-127
- Lutz R, Aserin A, Wicker L, Garti N (2009b) Release of electrolytes from W/O/W double emulsions stabilized by a soluble complex of modified pectin and whey protein isolate. *Colloids Surf B* 74: 178-185
- Lutz R, Garti N (2006) Double emulsions. In P. Somasundaran (Ed.), *Encyclopedia of surface and colloids science* (pp. 1816-1845). London: Taylor & Francis.
- McClements DJ (2004) Protein-stabilized emulsions. *Curr Opin Colloid Interface Sci* 9: 305-313
- Mirhosseini H, Tan CP, Hamid NSA, Yusof S (2008) Effect of Arabic gum, xanthan gum and orange oil contents on ζ -potential, conductivity, stability, size index and pH of orange beverage emulsion. *Colloids Surf A Physicochem Eng Asp* 315: 47-56
- Mirhosseini H, Tan CP, Taherian AR, Boo HC (2009) Modeling the physicochemical properties of orange beverage emulsion as function of main emulsion components using response surface methodology. *Carbohydr Polym* 75:512-520
- Muscholik G, Scherze I, Preissler P, Weiss J, Knoth A, Fechner A (2006) Multiple emulsions – preparation and stability. *IUFoST* 123-137 doi: 10.1051/IUFoST:20060043.
- Onsaard E, Putthanimon J, Singthong J, Thammarutwasik P (2014) Influence of maltodextrin and environmental stresses on stability of whey protein concentrate/ k-carrageenan stabilized sesame oil-in-water emulsions. *Revista de ATA* 20: 614-628
- Oppermann AKL, Piqueras-Fiszman B, de Graaf C, Scholten E, Stieger M (2016) Descriptive sensory profiling of double emulsions with gelled and non-gelled inner water phase. *Food Res Int* 85:215-23
- Pawlik AK, Cox PW, Norton IT (2010) Food grade duplex emulsions designed and stabilized with different osmotic pressure. *J Colloid Interface Sci* 352: 59-67
- Pawlik AK, Norton IT (2014) Bridging benchtop research and industrial processed foods: structuring of model food emulsions. *Food Struc* 1: 24-38
- Pearce KN, Kinsella JE (1978) Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique. *J Agric Food Chem* 26: 716-723
- Prichapan N, Klinkesorn U (2014) Effect of emulsifiers on properties and stability of water-in-rice bran oil-in-water (W/O/W) emulsions. 18th World Congress on Clinical Nutrition (WCCN)
- Rosano HL, Gandolfo FG, Hidrot JDP (1998) Stability of W₁/O/W₂ multiple emulsions influence of ripening and interfacial interactions. *Colloids Surf A Physicochem Eng Asp* 138: 109-121.
- Salminen H, Weiss J (2014) Electrostatic adsorption and stability of whey protein-pectin complexes on emulsion interfaces. *Food Hydrocoll* 35: 410-419
- Sameh H, Wafa E, Sihem B, Fernando LC (2012) Influence of diffusive transport on the structural evolution of W/O/W emulsions. *Langmuir* 28: 17597-17608
- Sapei L, Naqvi MA, Rousseau D (2012) Stability and release properties of double emulsions for food applications. *Food Hydrocoll* 27:316-323
- Schmitt C, Turgeon SL (2011) Protein/polysaccharide complexes and coacervates in food systems. *Adv Colloid Interface Sci* 167: 63-70
- Shima M, Tanaka M, Kimura Y, Adachi S, Matsuno R (2004) Hydrolysis of the oil phase of a W/O/W emulsion by pancreatic lipase. *J Controlled Release* 94: 53-61
- Su J, Flanagan J, Hemar Y, Singh H (2006) Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilization of water-oil-water emulsions. *Food Hydrocoll* 20: 261-268
- Su J, Flanagan J, Singh H (2008) Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUMTM). *Food Hydrocoll* 22: 112-120
- Surh J, Decker EA, McClements DJ (2006) Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocoll* 20: 607-618
- Surh J, Vladislavljević GT, Mun S, McClements DJ (2007) Preparation and characterization of water/oil and water/oil/water emulsions containing biopolymer-gelled water droplets. *J Agric Food Chem* 55: 175-84
- Wu S, Ng L (2008) Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. *LWT* 41: 323-330