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Optimization of chitosan for microencapsulation of flaxseed oil through spray drying

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Abstract: The study is aimed to characterize the wall material chitosan in combination with maltodextrin for microencapsulation of flaxseed oil to enhance the delivery of polyunsaturated fatty acids in functional foods. Four formulations of water-based oil emulsions containing flaxseed oil were prepared, homogenized, and analyzed in terms of stability and viscosity. Resulting emulsions were spray dried and further evaluated physicochemically. Results revealed that total moisture content of spray dried microcapsules was in range of 3.1 ± 0.01-3.32 ± 0.04, while highest microencapsulation efficiency found for spray dried powder was 88.8 ± 1.45. Peroxide and anisidine value revealed high oxidative stability of dried powders during storage at room temperature as compared to bulk FO. Among the four formulations, formulation 3 was found to be highly efficient with high encapsulation efficiency and lower PV (3.93 ± 0.13) and AV (2.93 ± 0.21) value. Fatty acid profile was stable after 30 days and slight change observed in composition. Formulations with different concentration of chitosan has significant ($p < 0.05$) impact on emulsion properties and oxidative stability values. It is concluded that spray dried flaxseed oil microcapsules prepared using chitosan as coating material enhance the stability and delivery of omega-3 fatty acids.

Keywords: Flaxseed, microencapsulation, chitosan, spray drying.

INTRODUCTION

There is always an increasing demand of functional foods to remain healthy. This motivates the researchers to test and explore various options. Polyunsaturated fatty acids (PUFA's) or Omega-3 are considered as essential fatty acids and there is substantial increase in the use of PUFA's in dietary supplements (Parikh *et al*., 2019). Flaxseed oil is a vital source to fulfill omega-3 fats requirement in human and valuable for maintaining normal functioning of body because of their anti-inflammatory, anti-arrhythmic properties. Flaxseed oil is major indirect source of omega-3 through metabolism of Alpha-Linolenic Acid (ALA) with a total 57% of fatty acids (Lemahieu *et al*., 2015). ALA is converted to Eicosapentaenoic acid (EPA) and Docosahexaenoic acids (DHA) in body. PUFA's are essential to control cardiovascular, inflammatory diseases, a vast variety of cancer and proper development of fetus (Balic *et al*., 2020).

PUFA's are highly unsaturated follows retrogression due to oxidation as a result primary and secondary oxidative products are generated. These products change the sensory qualities odor and color of oil, produce toxic compounds thus reduces its acceptability and shelf life (Pendule *et al*., 2021). Deterioration of oil can be prevented using microencapsulation thus increasing its shelf life (Goyal *et al*., 2015). Microencapsulation allowed the controlled release of active substances and their utilization in a protected way. Application of microencapsulation technique is studied in several industries especially those related to food as nutritive food requirements are high in market (Gowda *et al*., 2018).

Microencapsulation is done by using a variety of techniques including spray drying, extrusion, liposome entrapment, coacervation, molecular inclusion and co crystallization (Comunian and Favaro-Trindade, 2016). However, microencapsulation employing spray drying is commonly used in food related industry and wide application in terms of encapsulation of oils. Its mechanism consists of enveloping the core material like oil in a coating material followed by emulsion formation and their conversion into powder microcapsules as the passed into spray dryer (Kak *et al*., 2019). Furthermore, spray drying method is cheaper, easily handled and produces superior quality powder capsules (Rezvankhah *et al*., 2019).

Selection of wall material important factor that is associated with the formation of high-quality microcapsules (Encina *et al.,* 2016). Proteins, polysaccharides, lipids and gums are utilized as usual coating materials for the purpose of microencapsulation (Yakdhane *et al*., 2021). Chitosan is an amino polysaccharide derived from chitin through deacetylation process. Chitosan has variety of applications in food industry due to its antimicrobial, palatable nature, and

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antioxidant properties (Anandan *et al*., 2015). Maltodextrin is filler matrix that can hydrolyzes starch and considered as acceptable coating material in combination with other types of wall materials for microencapsulation of active substances (Can Karaca *et al*., 2013).

The current study is therefore aimed to optimize the combination of maltodextrin and chitosan as wall materials and to prepare the flaxseed oil microcapsules employing different core to wall ratio of materials and evaluation of lipid oxidation and oxidative stability during storage period. Emulsions evaluated for viscosity, moisture content and water activity. Powdered microcapsules were analyzed through surface oil, encapsulation efficiency and water solubility. Lipid oxidation of powder microcapsules was also evaluated.

MATERIALS AND METHODS

Flaxseed oil was acquired from local market of district Okara, Pakistan. Maltodextrin and Chitosan were procured by radical scientific traders Lahore, Pakistan.

Emulsion preparation

Ratio of wall material chitosan, maltodextrin as filler and flaxseed oil as core material is given in table 1. Maltodextrin was dissolved in water prior to emulsion formation. Chitosan solution with different concentrations was prepared in 0.50ml/100ml of acetic acid solution. It was constantly stirred until complete mixing of contents. Wall material solutions (chitosan and maltodextrin) were retained on shaking water bath overnight in order to hydrate polymer molecules and to obtain dissolution of material. Core wall material with ratio 1:4 was added in formulations and subjected to laboratory homogenizer operated at 5000 rpm for 30 minutes to form emulsion.

Table 1: Different emulsion composition (F1-F4) to be fed for spray drying.

Formulation	F1	F2	F3	F4
Flaxseed oil (FO)g/100g	10	10	10	10
Maltodextrin (MD) g/100g	20	20	20	20
Chitosan (CS) g/100g	0.5	1	1.5	2

Emulsion Characterization

Emulsion stability

Method of Mehrad *et al*., (2015) was employed for evaluation of emulsion stability. Emulsion stability was calculated through the Height of serum (HS) layer was estimated and divided by Total height of emulsion (HE) and expressed as creaming index (Wang *et al*., 2017).

$$\text{Creaming index} = 100\left(\frac{HS}{HE}\right)$$

Emulsion viscosity

Carneiro *et al*. (2013) method was employed for measurement of emulsion viscosity with temperature set at 25^0C and emulsion viscosity was calculated according to empirical models between shear stress and shear rate. Measurements were recorded in triplicates.

Microencapsulation process

Emulsions after preparation were subjected to a laboratory scale spray dryer (Toption Lab Spray Dryer, Xi'an, China) machine with a nozzle diameter of 1.5mm, inlet temperature 180^0C, outlet temperature 80 ^{0}C and 6ml/min emulsion pumping rate. Emulsions wereconstantly stirred before being fed to spray dryer machine to avoid amalgamation of oil droplets. Powdered microcapsules were stored in airtight glass container soon after their formation at room temperature for further characterization. Experiments were executed in triplicates.

Microcapsule Characterization

Moisture Content

Moisture content was calculated as the weight difference before and after placing the sample in oven at 105^0C (Zhong *et al*., 2009).

Microcapsules solubility in water and Controlled release

Powder (0.1g) was added in a test tube containing 5ml of distilled water followed by centrifugation and incubated at 37^0C for 5 h. Resulting supernatant was used to check concentration of soluble protein employing biuret method (Mehrad *et al*., 2015).

Controlled release of active material from microcapsules was carried out according to method described by Pourashouri *et al.* (2014) and expressed as percentage of flaxseed oil released from dry capsules to their percentage in microencapsulated powder.

Total oil of microcapsule

In test tube 0.5g of microcapsules and 2ml of n-hexane were taken and vortexed for 2 min. Components were mixed with 25 ml of 3:1 ratio n-hexane and isopropanol solution. After centrifugation aqueous phase was re-extracted and filtered. It was then transferred to rotary evaporator for solvent evaporation at 70^0C. Extracted oil was measured gravimetrically (Baik *et al*., 2004).

Encapsulation efficiency (EE)

Encapsulation efficiency is quantity to encapsulated oil and non-encapsulated oil. It is considered an important factor regarding stability, storage, release and morphology of microcapsules for further processing and it was calculated by following equation (Gomes *et al.,* 2011).

EE(%) = (Total amount of encapsulated oil) - (non-encapsulated oil) × 100/Total oil content

Oxidative stability of microcapsules

Peroxide value (PV) was evaluated according to standard IDF (International Dairy Federation) protocol. Following equation was used to calculate the PV:

Peroxide value ($meqO_2$/Kg of oil) = $(A_S - A_B)m/55.84 \times m_o \times 2$ (Pedro *et al*., 2011)

Anisidine value determination

Anisidine value was determined according to British standard protocol (BS 684 1998) for the evaluation of secondary oxidation products (AOCS, 2004).Anisidine value was evaluated using formula:

$AV = 25 \times (1.2A_2 - A_1)$/sample weight (Tolouie *et al*., 2013).

Fatty acid profile

Fatty acid profile of FO was determined using AOAC protocol (2000). For the analysis of fatty acids methyl esters flame ionization detector along with Gas Chromatography (GC) was used (7890,B Agilent Technology). Fused silica capillary column with temperature 140-220°C was used in GS. Fatty acid methyl esters were identified on the basis of retention time compared with standard provided by GC-MS controlling computing system. Peaks of each fatty acid were analyzed for the calculation of absolute response.

STATISTICAL ANALYSIS

Statistical Analysis System (SAS) of 9.1version was used to obtain and compare the means. Data was analyzed by one way ANOVA (Analysis of Variance) to evaluate the mean data from different treatments. Duncan's Multiple Range Test (DMRT) was applied to compare the means at probability level of ($p \leq 0.05$).

RESULTS

Focus of present study was the microencapsulation of flaxseed oil by means of different combination of chitosan and their effect on physiochemical characteristics employing spray dried methods.

Table 2: Comparison of emulsion characteristics produced with different concentration of chitosan.

Formulation	% Separation	Viscosity (Mean±SD)
F1	-	5.5 ± 0.05^a
F2	2.4%	42.5 ± 0.08^b
F3	-	74.2 ± 0.17^c
F4	-	96.1 ± 1.66^d

Means in column with different letters are statistically significant at $p < 0.05$.

Emulsion stability

Stability of flaxseed emulsions prepared with different concentration of chitosan is presented in table 2. Emulsions developed with different formulations were seemed to be kinetically stable with no phase separation except was noted except F2. So, emulsion formed with chitosan were able to cover the oil droplets while preventing its creaming (Pedro *et al*., 2011). Creaming formation in emulsion with chitosan 1g/100g mixed with maltodextrin resulted in separation of phase and formation of semi-transparent serum in the lower layer. This creaming promoted droplets coalescence and accumulation of oil at surface. Results proposed that low concentration of chitosan is inadequate to enhance the viscosity of emulsion leading to droplet aggregation and instability of emulsion.

Emulsion viscosity

Viscosity determined for different combination of wall materials in emulsion is presented in table 2. Significant changes observed in viscosity due to changes in chitosan ratio. Highest viscosity was observed for emulsion 4 followed by 3 and 2. Chitosan is a thickening agent and responsible for high viscosity of emulsion as emulsion prepared with low concentration of chitosan showed less viscosity as compared to others. Moreover, different formulations of chitosan effect the viscosity significantly.

Moisture content

Moisture content was analyzed as described previously and results shown that average moisture content of microcapsules was 3.1% with minimum 3.11±0.01 for emulsion 1 and highest for emulsion 4 (3.3±0.04). High moisture content of emulsion 4 with chitosan 2.0g/100 and maltodextrin 20g/100g can be explained by operating conditions of spray dryer machine. Lower moisture content can be achieved by selecting high inlet temperature of drying chamber.

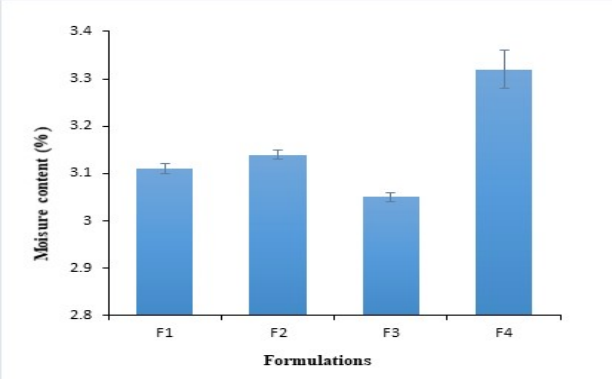

Fig. 1: Comparison of Moisture content of microcapsules in different formulations

Microcapsule solubility in water and controlled release

In current study release of core material was dependent on the wall material concentration. During first 2 hours of incubation flaxseed oil from microcapsules released quickly and then stabilized resulting in burst effect as

depicted in Fig. 2. Then there was steady phase being observed and highest flaxseed oil release was observed for third treatment (86%) after incubation. Other factors of microcapsules like porosity, thickness of shell, polarity and density strongly influenced release of core material. In current study complete release of flaxseed in not observed in any of the microcapsules with different concentration of chitosan and some oil remain trapped inside the matrix.

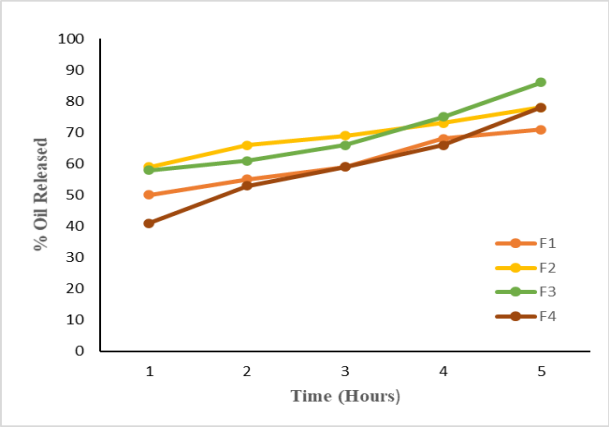

Fig. 2: Oil released (%) during incubation period at 37℃.

Total oil of microcapsule and encapsulation efficiency

For the evaluation of wall material encapsulation efficiency is considered as an important parameter. Highest encapsulation efficiency was observed among the treatment formulation 4 and formulation 1 has little lower encapsulation efficiency. It can be explained because of active material to coated material ratio.

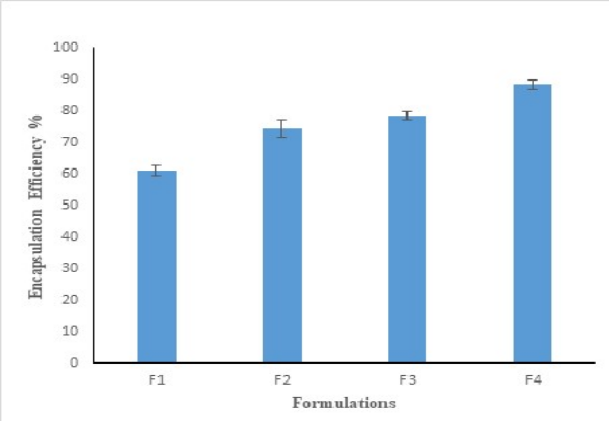

Fig. 3: Encapsulation efficiency of flaxseed oil microcapsules prepared with different Chitosan combinations.

Oxidative stability of microcapsules

Flaxseed oil contain high amount of PUFA's so highly prudent to oxidation by atmospheric oxygen. PV value is used to measure the formed primary products of oxidation like peroxides, dienes and free fatty acids. These primary products of oxidation do not produce bad odor and any color, but they have high toxicity, as a result fatty acid are unavailable. Microencapsulated flaxseed oil PV value was 1.51±0.03 and 1.68±0.08for formulation 1 and 4meqO_2/kg respectively at zero day of evaluation and bulk oil was 1.58±0.17 meq O_2/kg. There was not any significant difference observed in values. Flaxseed initial PV was 1.34 meq O_2/kg oil and increase in PV after microencapsulation can be explained because of increase in emulsion temperature due to homogenization and drying chamber conditions. Different combination of wall materials has strong impact on oxidative stability of microcapsules. All formulations prevented oxidation during storage and PV was in acceptable range (5.69-8.13 meqO_2/kg) as compared to bulk oil.

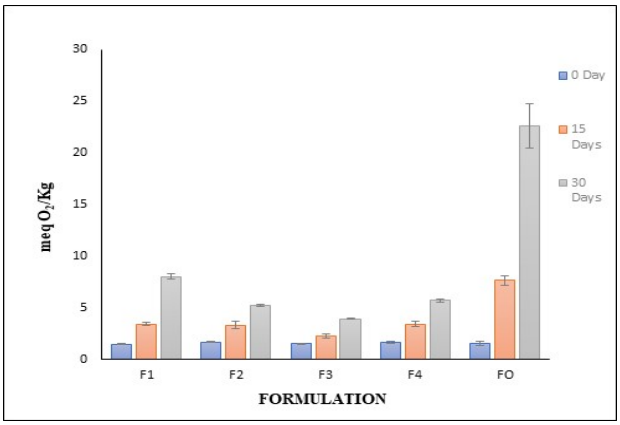

Fig. 4: Comparison of PV at different storage intervals with Bulk FO.

Anisidine value (AV)

Anisidine value is analyzed to check secondary products of oxidation like aldehydes, non-volatile carbonyl and ketones formed as a result of lipid degradation. In current study, AV analyzed on 1st day, 15 days and after a month of storage and found stable. This stability in AV before and after microencapsulation indicated low oxidation and good quality of oil in powders. AV recorded during storage period is given in table 3, according to which there is fast increase in AV of non-encapsulated oil when compared with encapsulated oil. Low AV was observed for formulation 3.

Fatty acid profile of FO and FOM

Fatty acid profile of FO was assessed and compared with FOM after 30 days. FO fatty acid component was linolenic acid representing 57.20±0.17g/100g of oil. Next in line were oleic acid (20.46±0.24g/100g, linoleic acid (14.30±0.14g/100g), palmitic acid (4.8±0.06g/100g), and stearic acid (3.10±0.02g/100g). Fatty acid profile show stability after 30 days when compared with FO. This indicates that microencapsulation is safe process that enhance the shelf life and stability of omega-3 fatty acids during storage.

Table 3: Assessment of p-anisidine value changes in different flaxseed oil microcapsules for 30 days storage period.

Time (Days) / Treatment Groups	0 days (Mean ± SD)	15 days (Mean ± SD)	30 Days (Mean ± SD)
Bulk oil	1.88 ± 0.26[c]	7.65 ± 0.73[a]	33.0 ± 5.45[a]
Formulation 1	1.81 ± 0.64[c]	4.39 ± 1.04[b]	5.91 ± 1.02[b]
Formulation 2	2.40 ± 0.36[a]	3.42 ± 0.33[c]	4.03 ± 0.09[b]
Formulation 3	1.24 ± 0.45[d]	1.88 ± 0.29[e]	2.93 ± 0.21[b]
Formulation 4	2.12 ± 0.08[b]	2.64 ± 0.29[d]	3.94 ± 0.08[b]

Rows having letters that are indifferent considered as non-significant at ($p \leq 0.05$)

Table 4: Fatty acid profile of FO and FOM at different storage intervals*

Fatty acids %	FO (Mean±SD)	FOM after 30 days			
		Formulation 1 (Mean ± SD)	Formulation 2 (Mean ± SD)	Formulation 3 (Mean ± SD)	Formulation 4 (Mean ± SD)
Myristic acid (C14:0)	0.02±0.00	ND	ND	ND	ND
Palmitic acid (C16:0)	4.8±0.06	5.20±0.12	5.12±0.03	5.23±0.13	5.21±0.04
Stearic acid (C18:0)	3.10±0.02	3.92±0.23	3.96±0.08	3.98±0.05	3.80±0.06
Arachidic acid (C20:0)	0.03±0.01	0.31±0.14	0.39±0.16	0.32±0.09	0.23±0.12
Behenic acid (C22:0)	0.01±0.04	ND	ND	ND	ND
Oleic acid (C18:1)	20.46±0.24	19.10±0.05	19.46±0.07	19.40±0.03	19.27±0.02
Linoleic acid	14.30±0.14	16.24±0.19	16.35±0.16	16.29±0.12	15.12±0.09
Linolenic acid	57.20±0.17	54.21±0.34	54.65±0.12	54.25±0.33	55.10±0.18
Eicosapentaenoic acid - EPA (C20:5)	0.05±0.04	ND	ND	ND	ND
Docosahexaenoic acid - DHA (C22:6)	0.02±0.19	ND	ND	ND	ND
Tetracosaenoic acid	0.01±0.21	ND	ND	ND	ND

*FO represents flaxseed oil and FOM are flaxseed oil microcapsules.

DISCUSSION

Solubility of microcapsule in water reveals it functionality for core release. Microcapsule are designed in such a way to reduce their solubility in water. Food related industries are much concerned about control release of encapsulated product (Lakkis, 2007). Addition of solids in emulsion makes it more viscous (Karim *et al*., 2017). Humidity in drying chamber is main cause of moisture content in the formed microcapsules (Klinsorn *et al*., 2006). It is directly related to product stability as it effects the shelf life of products (Klaypradit and Huang, 2008).

Generally, there is phenomenon of fast release in first hour that is known as burst effect due to leakage of oil from cracks of microcapsules due to pepsin solution (Gan *et al*., 2008). Our results agree with Mehrad *et al*., (2015), they observed similar burst effect while working with fish oil microcapsules. Hydration of polymers increase their volume and straightening of polymer chain as they dissolved in aqueous media. So, flaxseed oil inside the matrix not linked with it and is released so contributed to burst effect.

Wall ratio directly related to encapsulation efficiency as less wall material is insufficient to encapsulate the core material and resulting in lower encapsulation efficiency (Gharsallaoui *et al*., 2007). Increase in content of wall material enhances the microencapsulation because of coating of each droplet. Highly stable emulsion lead to higher encapsulation efficiency as there is lesser amount of unencapsulated oil as stated by Barbosa *et al*. (2005). Flaxseed oil contact with oxygen resulted in accelerated oxidation process as explained by Heinzelmann and Franke (1999). According to them, this contact cannot be prevented thoroughly. Temperature of chamber accelerate the oxidation process in resulting powder. However, PV of free oil was increased significantly after 15 days and 30 days storage as compared of microencapsulated oil and proved by current study that fat deterioration process is much faster in bulk oil stored at room temperature. Oxidative stability of formulation prepared with lower amount of chitosan observed was poor. Encapsulation efficiency is a critical factor regarding high PV value. Microcapsules with low encapsulation efficiency have free oil on their surface that is susceptible to oxidation resulting in high PV value (Tonon *et al.,* 2012).

Kumar *et al*. (2017) reported that 1% of chitosan is insufficient to encapsulate the core material. AV was studied by different authors with reference to microcapsules and they documented different trends in results. This was probably due to type of wall materials, their concentration, type of oil and conditions of storage (Barroso *et al*., 2014).

CONCLUSION

In present study chitosan was used as wall material along with maltodextrin in different combinations and found that it has good emulsion formation property and high encapsulation efficiency due to formation of smooth microcapsules that retain the active material. At the end of flaxseed oil microcapsule storage period high PV and AV values were observed. Possible reason for low oxidative stability when storage period terminated is moisture and oxygen diffusion through chitosan in low concentration. Flaxseed oil powders with 1.5% chitosan showed long storage stability and can be used in value added products for fortification of Omega-3.

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