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MICROENCAPSULATION OF FLAXSEED OIL PRODUCED THROUGH THE COMBINATION OF DIFFERENT EMULSIFICATION METHODS AND DRYING TECHNOLOGIES

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1. INTRODUCTION AND OBJECTIVES

Flaxseed oil (FO) thrive of essential nutrients with an abundance of polyunsaturated fatty acids (PUFAs), particularly alpha-linolenic acid (ALA), an ω-3 fatty acid. PUFAs, like ω-3 and ω-6 fatty acids, are crucial for human health and can play vital roles in cell membrane development and serve in controlling inflammatory reactions, blood pressure, and preventing cardiovascular diseases. Additionally, ω-3 fatty acids offer benefits such as reduced risk of diabetes and certain cancers. In many food products, the addition of ω-3 fatty acids helps maintain a healthy balance between ω-3 and ω-6 fatty acids in the diet which is, unfortunately, in many modern diets, falling within the concerning range.

Due to this nutritional profile of flaxseed oil, many industries have aimed to incorporate it into various products leading to an increase demand in the food and biopharmaceutical industries. As an example, it was incorporated in formulations to prepare ice cream, soup powder, and bread, and it was also used to prepare formulations for the treatment and prevention of gastrointestinal disorders, cardiovascular disease, eczemas, hypertension, atherosclerosis, diabetes, and cancer.

In the other hand while FO offers all these benefits, unfortunately it is prone to oxidation which not only deteriorate its nutritional

value but also negatively impact the organoleptic properties of food products containing it. Therefore, microencapsulation of FO emerges as a critical step to ensure its stability within the food matrix.

Encapsulation is an evolving technology that aims to satisfy the demands of a stable product with high quality. It is used to protect food ingredients, to assure their quality and effectiveness and to control the release of property of active agents by coating small droplets of liquid or solid particles with a thin film of wall materials. Depending on the core material to be protected, wall materials can include a variety of polymers, carbohydrates, proteins, and waxes etc. Different techniques have been used for food-grade compounds encapsulation.

OBJECTIVES:

The objective of my PhD work is to dive into the details of various microencapsulation techniques for flaxseed oil (FO) and to offer promising formulations for the development of stable microcapsules systems that can protect FO from lipid deterioration. Optimizing microencapsulation techniques for FO, will pave the way for its wider application in the food and biopharmaceutical industries and will ultimately lead to an increase development of functional food products enriched with the health benefits of FO, while ensuring its stability.

The focus on membrane emulsification technique (ME) as an initial step for emulsion preparation can offer a cost-effective solution providing a foundation to achieve desired results in term of characteristics and properties of FO capsules.

In this regard, the following steps were set to accomplish:

- Preparing a base study through literature review and screening with preliminary and pilot studies for the selection of adequate wall materials and oil load in the case of ME and spray drying (SD).
- Optimizing the FO capsules obtained through ME and SD with different oil content and different composition of wall material such as, maltodextrin (MD), Gum arabic (GA), and modified starch (MS).
- Optimizing the FO capsules obtained through rotor stator homogenization (RSH) and SD with different oil content and different composition of wall material.
- Optimizing the FO capsules obtained through ME and freeze drying (FD) with different oil content and different composition of wall material.
- Evaluation and comparing of differently formed FO capsules from optimized formulation in the aim of studying their efficacity in offering the needed protection for FO. This evaluation consists of comparing the produced optimized emulsions stability and the droplet size and distribution and

studying the capsules characteristics by conducting an evaluation of particle size and distribution, oxidative stability, moisture content, and analysis that are in correlation with FO encapsulation efficiency and stability.

2. MATERIALS AND METHODS

2.1. Materials

The cold-pressed Flaxseed oil (FO) was purchased from a local shop in Hungary. Maltodextrin (Dextrose equivalent (DE)=19) (MD) was purchased from Buda Family Kft and Gum arabic (GA) was purchased from Bi-Bor Kft. High amylose maize modified starch (MS) and emulsifier soya lecithin were also used in emulsion formulation.

2.2. Methods

2.2.1. Preparation of the emulsion through membrane emulsification

Emulsions were produced utilizing a continuous, crossflow specialized laboratory apparatus for ME, developed at the Department of Food process engineering within the Hungarian University of Agriculture and Life Sciences, Faculty of Food Science. This emulsification apparatus was used with a tubular ceramic membrane with 1,4 um pore size and an active membrane surface area of 50 cm^2. To enhance emulsion quality, a turbulence static promoter was integrated into the membrane module.

The wall material was dissolved in the continuous phase (water) and FO was pressed through the membrane pores with a pressure of 2 bars and dispersed into the continuous phase to create the emulsion.

Figure 1: Membrane emulsification apparatus

2.2.2. Preparation of the emulsion through the rotor-stator homogenization (RSH)

The homogenization process was carried out using a rotor-stator homogenizer (T 25 digital ULTRA-TURRAX, Hungary) equipped with a dispersing shaft. FO was added to the wall material solutions under high shear. The homogenization speed was set to 15,000 rpm for a duration of 5 minutes.

2.2.3. Spray drying

Microencapsulation was achieved using a laboratory-scale spray dryer (LabPlant SD-05, Hungary) equipped with a 0.5 mm diameter nozzle. During the SD process, Compressed air pressure was set to 3.6 bars to facilitate atomization. An inlet air temperature of 185 ± 5°C and an outlet air temperature of 105 ± 5°C were employed. An airflow rate of 74 m^3/h was maintained.

2.2.4. Freeze drying

This process was carried out using a freeze-dryer (ScanVac,

coolsafe, 110-4 apparatus, Labogene, Lillerod, Denmark). As a first step the emulsions were put to freeze for 24 hours at -40°C. Subsequently, a lyophilization process was employed in which the emulsions were maintained at a constant temperature of -109°C and a vacuum pressure of 12 Pa for a duration of 24 hours.

2.2.5. Experimental design.

To design the experiments the software Design-Expert 13.0.1.0. was used. Response surface methodology RSM was applied to investigate the effect of different formulation on the encapsulation efficiency. 3 factor – 3 level Box-Behnken experimental design was used. For the optimization of the encapsulation efficiency, three independent variables were used and were coded. These variables are the ratio MD/GA (X1) ranging from 0 to 1, the concentration of MS (X2) from 0 to 40% (w/w), and FO content (X3) from 10% to 40% (w/w).

2.2.6. Emulsion and microcapsules characterization

Emulsion stability was measured through determination of zeta potential and phase separation. Droplet size and size distribution were assessed, and morphological analysis was also carried out.

The evaluation of encapsulated flaxseed oil was conducted by examining the encapsulation efficiency, particle size and size distribution, microcapsules morphology and oxidative stability Flowability and cohesiveness powder wettability, and solubility were also determined.

3. RESULTS AND DISCUSSION

This work investigated the complex effect of wall material composition (Maltodextrin DE=19 (MD), Gum Arabic (GA), Modified starch (MS) and flaxseed oil load (FO)) on microencapsulation of flaxseed oil produced through the combination of different emulsification methods and drying technologies. Two different emulsification technologies were adopted; membrane emulsification and rotor stator homogenization, and two different drying methods were selected; spray drying and freeze drying.

After a pilot study and screening experiments to investigate the effect of varying variables on the encapsulation efficiency, emulsion stability and droplet and particle size and distribution of produced microcapsules through membrane emulsification and spray drying, it was noticed that the present of MD even though it has resulted in bigger droplet sizes, its capacity to improve encapsulation efficiency is notable. In this regard, there may be a trade-off between droplet size and encapsulation efficacy depending on the application, In the other hand, formulations which included a balanced mix of MD and GA, and MS, provided an effective combination. MD have enhanced the drying process by quickly forming a drying coat around the passing oil through the SD nozzle which led to efficient oil droplet coating and

reducing leakage. GA has contributed to increased encapsulation efficiency by its emulsifying and film-forming capabilities. Furthermore, MS, present in all formulations, have contributed to overall stability. Another important aspect that arose was the oil load as the observed tendencies indicated that a lower oil load may be beneficial for enhanced emulsion stability resulting in an effective encapsulation.

Following the pilot study the optimization of capsules obtained through three different technology combinations were studied. The first being membrane emulsification and spray drying (1), second being rotor stator homogenization and spray drying (2), and the third being membrane emulsification followed by freeze drying (3). RSM, statistical technique was used with Box-Behnken design to optimize these processes by evaluating the relationships between the multiple independent variables (Ratio of MD to GA, concentration of MS, and FO content) and the desired response variable. The response was expressed as encapsulation efficiency (EE).

The highest and the lowest encapsulation efficiency obtained after performing 15 runs for each of the combinations were, 92.05% and 62.95% for technology combination (1), 80.43% and 49.87% for technology combination (2), and 76.42% and 49.05% for technology combination (3).

Data was then analyzed using RSM software and a mathematical quadratic model that describes the relationship between the variables and the EE was fitted for each technology combination giving three actual equations.

The RSM analysis showed that all three independent variables and their interactions significantly affected the encapsulation efficiency. The optimal conditions for maximizing EE through ME and SD were found to be: 0.689 MD/GA, 19.835% MS and 30.148% FO. Under these conditions, the predicted encapsulation efficiency was predicted at 85.93%. The optimal conditions for maximizing EE through Rotor stator homogenization and SD were found to be 0.79 MD/GA, 20.23% MS and 24.62% FO. Under these conditions, the predicted encapsulation efficiency was 77.68 %, and finally, the optimal conditions for maximizing EE through ME and FD were found to be: 0 MD/GA, 26.26% MS and 25.13% FO. Under these conditions, the predicted encapsulation efficiency was 74.59 %.

Subsequently, the optimal composition from each encapsulation method was produced and analyzed through the study of the produced flaxseed oil in water emulsion and the study of flaxseed oil capsules after drying. The stability of the emulsions prepared using different encapsulation methods was assessed by measuring the zeta potential and phase separation. The results showed that all emulsions had moderate stability, with zeta potential values

between -29.5 mV and -41,3 mV. Emulsion droplet size was also analyzed giving the conclusion that ME samples resulted in larger droplets compared to the rotor-stator homogenization one due to its gentler pressure-driven process. Capsules produced through freeze drying, with no MD and more GA, had a smaller average droplet size compared to the one produced through membrane emulsification and spray drying despite using the same emulsification technique. This highlights the impact of wall material properties on droplet formation.

Capsules produced through membrane emulsification followed by drying displayed a narrower distribution of particle sizes compared to the one produced though rotor stator homogenization and drying. This suggests that ME might generate a more uniform distribution, potentially impacting encapsulation efficiency. Regarding oxidation examined microcapsules demonstrated significantly lower TBARS values compared to unencapsulated FO, indicating reduced lipid oxidation due to the protective barrier formed by the encapsulation materials. Microcapsules produced through membrane emulsification and spray drying exhibited a slightly lower TBARS value compared to rotor stator - spray dried microcapsules, suggesting potentially better protection achieved by ME. Additional observations were also done on encapsulated FO including moisture content, solubility, and bulk density.

4. CONCLUSION AND SUGGESTIONS

As a conclusion, this PhD research has made into light the effectiveness of encapsulation in protecting flaxseed oil against environmental factors and has successfully investigated the microencapsulation of flaxseed oil using various techniques and explored the impact of formulation variables on encapsulation efficiency. Response surface methodology (RSM) was successfully employed to optimize the microencapsulation process for three combinations: membrane emulsification (ME) and spray drying (SD), rotor-stator homogenization (RSH) and SD, and ME followed by freeze drying (FD), using maltodextrin, Gum arabic and modified starch as wall material.

Membrane emulsification, as an emulsification technology used prior to spray drying, appears to offer advantages in terms of emulsion stability, encapsulation efficiency, particle size distribution, oxidative stability, and powder flowability. Wall material composition plays a significant role in influencing these properties.

Further research can include the investigation of the effect of changing the apparatus and condition parameters on the encapsulation efficiency of flaxseed oil, or exploring alternative wall materials and emulsification and drying methods that could bring additional advantageous depending on the aimed capsules

characteristics. Further research could also explore the integration of the optimized microencapsulated flaxseed oil into functional food products, which can be conducted by studying the stability of flaxseed oil capsules within the food matrix, a sensory evaluation to assess consumer acceptance of the final product, and In vitro studies to simulate the digestive process and analyze the release profile of ω-3 fatty acids from the encapsulated flaxseed oil.

5. NEW SCIENTIFIC RESULTS

[1] Statistical modeling of flaxseed oil encapsulation through membrane emulsification and spray drying technologies: By studying the impact of different formulations of carbohydrates namely Maltodextrin with 19 dextrose equivalent (MD), Gum arabic (GA), and High amylose maize modified starch (MS) in combination with varied amount of the different cold-pressed, filtered Flaxseed oil (FO) bioactive loads, on the Encapsulation efficiency (EE%) in case of a combined microcapsule production technology via membrane emulsification and spray drying, with the application of Response Surface Methodology RSM (Box-Behnken experimental design) as modeling tool, the encapsulation efficiency was determined with the following actual equation (R^2=0.999):

$$\mathrm{EE\%} = 63.01 + 58.35\frac{MD}{GA} + 0.76\mathrm{MS} + 0.14\mathrm{FO} - 0.16\frac{MD}{GA}\mathrm{MS} - 0.34\frac{MD}{GA}\mathrm{FO} - 32.60\left(\frac{MD}{GA}\right)^2 - 0.02\,MS^2 - 0.01FO^2$$

where MD/GA is the ratio between MD and GA ranging from 0 to 1, MS is the concentration of modified starch in wall material ranging from 0 to 40% (w/w), and FO is the Flaxseed load ranging from 10% to 40% (w/w).

[2] Statistical modeling of flaxseed oil encapsulation through rotor-stator homogenization and spray drying technologies: By studying the impact of different formulations of carbohydrates namely Maltodextrin with 19 dextrose equivalent (MD), Gum arabic (GA), and High amylose maize modified starch (MS) in combination with varied amount of the different cold-pressed, filtered Flaxseed oil (FO) bioactive loads, on the Encapsulation efficiency (EE%) in case of a combined microcapsule production technology via rotor stator homogenizer and spray drying, with the application of Response Surface Methodology RSM (Box-Behnken experimental design) as modeling tool, the encapsulation efficiency was determined with the following actual equation (R^2=0.998):

$$\mathrm{EE\%} = 46.75 + 41.05\frac{MD}{GA} + 0.78\mathrm{MS} + 1.34\mathrm{FO} - 0.08\frac{MD}{GA}\mathrm{MS} - 0.34\frac{MD}{GA}\mathrm{FO} - 19.98\left(\frac{MD}{GA}\right)^2 - 0.02\,MS^2 - 0.04FO^2$$

where MD/GA is the ratio between MD and GA ranging from 0 to 1, MS is the concentration of modified starch in wall material ranging from 0 to 40% (w/w), and FO is the flaxseed oil load ranging from 10% to 40% (w/w).

[3] Statistical modeling of flaxseed oil encapsulation through membrane emulsification and freeze drying technologies: By

studying the impact of different formulations of carbohydrates namely Maltodextrin with 19 dextrose equivalent (MD), Gum arabic (GA), and High amylose maize modified starch (MS) in combination with varied amount of the different cold-pressed, filtered Flaxseed oil (FO) bioactive loads, on the Encapsulation efficiency (EE%) in case of a combined microcapsule production technology via membrane emulsification and freeze drying, with the application of Response Surface Methodology RSM (Box-Behnken experimental design) as modeling tool, the encapsulation efficiency was determined with the following actual equation (R^2=0.998):

$$\mathrm{EE\%} = 66.10 - 0.39\frac{MD}{GA} + 0.17\mathrm{MS} + 1.18\mathrm{FO} + 0.05\frac{MD}{GA}\mathrm{MS} - 0.25\frac{MD}{GA}\mathrm{FO} - 0.01\,MS^2 - 0.04FO^2$$

where MD/GA is the ratio between MD and GA ranging from 0 to 1, MS is the concentration of modified starch in wall material ranging from 0 to 40% (w/w), and FO is the flaxseed load ranging from 10% to 40% (w/w).

[4] By setting the importance, the range, and the limit of our factors (maltodextrin (DE=19), Gum arabic, high amylose maize modified starch and cold-pressed, filtered flaxseed oil bioactive loads), I was able to determine an optimal combination to maximize the encapsulation efficiency while keeping a balanced oil load. The

optimal wall material carbohydrate and oil combinations for the three investigated complex technology mentioned in the table 1 below yielded good results in term of encapsulation efficiency.

Table 1: Composition of optimized formulation for flaxseed oil microencapsulation

	Optimized formulations of wall material and flaxseed oil		
	Membrane emulsification – Spray drying	Rotor stator homogenization – Spray drying	Membrane emulsification – Freeze drying
Ratio Maltodextrin/Gum arabic	0.69	0.79	0
Modified starch %	19.84	20.23	26.26
Flaxseed oil %	30.15	24.62	25.13
Encapsulation efficiency%	87.93	77.68	74.59

[5] Comparison between cross flow Membrane emulsification (ME) and Rotor stator homogenization (RSH) as emulsification techniques for producing flaxseed oil microcapsules of optimized formulations: I found that the RSH (15000 rpm for 5min) produced smaller droplet sizes but exhibited less homogeneous distribution than membrane emulsification (1.4 um pore size, pressure 2 bar). Spray-dried microcapsules showed superior encapsulation

efficiency for capsules achieved by cross flow ME and offered better oxidation protection than RSH for the resultant microcapsules. Results are mentioned in the table 2.

Table 2: Comparison between optimized flaxseed oil microcapsule obtained through membrane emulsification and rotor stator homogenization.

	ME	RSH
$D_{[4,3]}$ Emulsion (μm)	26.73 ± 0.04	13.62 ± 0.08
Span (emulsion)	0.53 ± 0.07	1.3 ± 0.02
EE (%)	85.93	77.68
Oxidative stability (MDA mmol/kg of FO)	0.6 ± 0.03	0.82 ± 0.02

[6] Comparison between emulsions and microcapsules properties of optimized formulations produced through membrane emulsification (ME) intended for spray drying (SD) and freeze drying (FD): I found that, for emulsion intended for FD (Temperature -109°C, vacuum pressure 12Pa) excluding maltodextrin and using only Gum arabic alongside high amylose maize modified starch resulted in smaller droplet size and enhanced stability. Furthermore, SD (nozzle diameter 0,5mm, inlet temperature of 185 ± 5°C and outlet temperature of 105 ± 5°C) offered a more homogeneous distribution and lower moisture content compared to FD. Additionally, SD microcapsules exhibited increased solubility and higher encapsulation efficiency (EE%) compared to FD, thereby establishing ME and SD as the optimal

combination for achieving high EE% with moderate flaxseed oil load. Results are mentioned in the table 3.

Table 3: Comparison between optimized flaxseed oil microcapsule obtained through membrane emulsification followed by spray drying and freeze drying

	Membrane emulsification – Spray drying	Membrane emulsification – Freeze drying
$D_{[4,3]}$ (μm) (droplets)	26.73 ± 0.04	23.59 ± 0.04
Span (capsules)	2.29 ± 0.18	2.95 ± 0.26
Separation (%)	10 ± 0.5	7 ± 0.5
Moisture content (%)	1.21 ± 0.9	2.1 ± 0.15
Solubility (%)	71.20 ± 0.75	57.99 ± 1.04

Articles in journals with impact factor and scopus cited	
Year	Publication
2021	**Yakdhane, A**., Labidi, S., Chaabane, D., Tolnay, A., Nath, A., Koris, A., & Vatai, G. (2021). Microencapsulation of Flaxseed Oil—State of Art. Processes, 9(2), 295. Doi:10.3390/pr9020295, IP = 3.5, Scimago rank Q2
2024	**Yakdhane, A**., Chaabane, D., Yakdhane, E., Molnár, M.A., Nath, A., & Koris, A., (2024) Microencapsulation of Flaxseed oil Produced by Sequential Rotor stator homogenization and Spray drying: Optimization of Emulsion composition; Progress in Agricultural Engineering Sciences; Manuscript number: 122, Doi: 10.1556/446.2024.00122, Scimago rank Q2
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2020	Nath, A., **Yakdhane, A**., Bánvölgyi, S., Koris, A., (2020), Encapsulation of probiotics and their release in food matrix; Ifjú Tehetségek Találkozója SZIEntific Meeting of Young Researches; ISBN: 978-963-269-937-0, P284-287.
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