LWT - Food Science and Technology 136 (2021) 110297

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Development of kafirin-based nanocapsules by electrospraying for encapsulation of fish oil

Turgay Cetinkaya [a,b,c,1], Ana C. Mendes [c], Charlotte Jacobsen [b], Zafer Ceylan [d], Ioannis S. Chronakis [c], Scott R. Bean [e], Pedro J. García-Moreno [b,f,*]

[a] Department of Food Engineering, Graduate School of Science and Engineering and Technology, Istanbul Technical University, 34469, Istanbul, Turkey
[b] Research Group for Bioactives – Analysis and Application, National Food Institute, Technical University of Denmark, Kemitorvet, 2800, Kgs. Lyngby, Denmark
[c] Research Group for Nano-Bio Science, National Food Institute, Technical University of Denmark, 10, 2800, Kgs. Lyngby, Denmark
[d] Department of Seafood Processing Technology, Faculty of Fisheries, Van Yüzüncü Yıl University, 65080, Van, Turkey
[e] Grain Quality and Structure Research Unit, United States Department of Agriculture, Agricultural Research Service, 1515 College Avenue, Manhattan, KS, 66502, USA
[f] Department of Chemical Engineering, University of Granada, Avenida de la Fuente Nueva S/N, 18071, Granada, Spain

ARTICLE INFO

Keywords:
Kafirin
Sorghum
Omega-3
Nanocapsules
Electrospraying

ABSTRACT

Kafirin-based fish oil nanocapsules were successfully obtained by using electrospraying technique. A kafirin isolate containing mostly α-kafirins, but also γ- and β-kafirins, was used as shell material. Electrospraying solutions with 10 wt% kafirin and fish oil (20 wt% with respect to kafirin) led to the production of nanocapsules (552 and 861 nm) when electrosprayed at 20–25 kV voltage with flow rates ranging from 0.5 to 1 mL/h. Neither the flow rate nor the voltage had a significant effect ($p > 0.05$) on the average diameter of the kafirin electrosprayed capsules loaded with fish oil. The encapsulation efficiency of the nanocapsules was up to 94.0 ± 2.5%, indicating that most of the fish oil was encapsulated within the kafirin shell. These results suggest the feasibility of obtaining kafirin-based capsules for the encapsulation of fish oil by electrospraying. This will open up new possibilities for kafirin as hydrophobic shell material for the production of fish oil encapsulates, which can be applied to the fortification with ω−3 fatty acids of food matrices.

1. Introduction

Fish oil contains valuable nutrients for human diet such as long chain polyunsaturated fatty acids (EPA: C20:5 ω−3, DPA: C22:5 ω−3, and DHA, C22:6 ω−3). There are many known health benefits of intake of EPA and DHA (such as the limitation of cardiovascular and cancer diseases). Furthermore, DHA and EPA, that only to a limited extent are synthesized from α-linolenic (ALA, C18:3 ω-3) by humans, play a key role in the development of nervous and brain systems especially for infants (Dyall, 2015). Nonetheless, krill and fatty fish species, rich in ω-3 polyunsaturated fatty acids (PUFA) such as EPA and DHA, are consumed below the recommended intake in western countries (Calder, 2013; Innes & Calder, 2020). Thus, the incorporation of ω-3 PUFA in the human diet by producing fortified food with ω-3 has been considered by the food industry in the last decades (Arab-Tehrany et al., 2012; Valenzuela & Valenzuel, 2013). However, industry faces several challenges for the successful incorporation of ω-3 PUFA into food matrices. EPA and DHA are polyunsaturated fatty acids, containing a high number of bis-allylic hydrogens, which considerably increases their susceptibility to oxidation. Oxidation of EPA and DHA leads to the loss of their nutritional properties as well as to the quality deterioration of the fortified product (e.g. due to the development of several off-odor and off-taste compounds) (Jacobsen, 2015). Therefore, strategies to reduce lipid oxidation are needed when producing ω-3 PUFA enriched foods. In this regard, encapsulation of oil droplets within a shell material has been widely used (Aghbashlo, Mobli, Rafiee, & Madadlou, 2012). The shell material creates a physical barrier around the oil droplets, minimizing the contact of prooxidants (e.g. oxygen, trace of metals) and the oil, and thus increasing the oxidative stability of the oil (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007; García-Moreno et al., 2018).

The most commonly used technique to microencapsulate fish oil is spray-drying (Aghbashlo et al., 2012; Drusch et al., 2007). Nonetheless, an emergent encapsulation technology such as electrospraying presents

* Corresponding author. Department of Chemical Engineering, University of Granada, Avenida de la Fuente Nueva S/N, Granada 18071, Spain.
E-mail address: pjgarcia@ugr.es (P.J. García-Moreno).
[1] Present address: Food Processing Department, Armutlu Vocational School, Yalova University, 77500, Yalova, Turkey.

https://doi.org/10.1016/j.lwt.2020.110297
Received 13 July 2020; Received in revised form 20 August 2020; Accepted 23 September 2020
Available online 29 September 2020
0023-6438/© 2020 Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

several advantages compared to spray-drying: i) obtaining of capsules with a reduced size and high encapsulation efficiency, and ii) the possibility to dry/encapsulate at ambient temperature by applying a high-voltage electro-static field (Jacobsen, García-Moreno, Mendes, Mateiu, & Chronakis, 2018). The latter is of special importance for the encapsulation of thermolabile bioactives such as EPA and DHA (García-Moreno et al., 2017). In addition, recent engineering efforts are given to scale-up the electrospraying process, which may allow to reach productivity levels needed in the food industry (Busolo, Torres-Giner, Prieto, & Lagaron, 2019).

Encapsulation of fish oil by electrospraying requires the use of biopolymers solutions with low viscoelasticity. This implies a reduced number of polymer chain entanglements, allowing the charged jet ejected from the Taylor cone to break up in droplets. These charged droplets further disrupt in their travel to a grounded collector, facilitating solvent evaporation and the collection of dried capsules (García-Moreno et al., 2018). The biopolymers reported for the encapsulation of fish oil by electrospraying are mainly hydrophilic proteins (e.g whey protein) or hydrophilic carbohydrates (e.g. maltodextrin, glucose syrup, dextran) (García-Moreno et al., 2017, 2018; Prieto & Lagaron, 2020). Alternatively, zein, a hydrophobic corn protein, has been also reported for the encapsulation of fish oil (Busolo et al., 2019; Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010). It is worth noting the critical importance of the hydrophilic/lipophilic character of the shell material since it determines a potential disintegration of the capsules when incorporated into a food matrix. For instance, higher oxidative stability of zein electrosprayed capsules loaded with fish oil, when compared to glucose syrup electrosprayed capsules, has been reported both during storage and when incorporated into a water-based matrix such as low-fat mayonnaise (Hermund et al., 2019; Miguel et al., 2019). This was attributed to the fact that zein electrosprayed capsules remained intact when added to the food matrix, leading to the protection of the encapsulated oil; which was not totally the case for the glucose syrup capsules due to the hydrophilicity of the glucose syrup (Miguel et al., 2019). Nevertheless, despite of its oxygen-barrier and antioxidant properties, zein presents several corn impurities leading to undesired flavor, odor and color that hinder its utilization as shell material (Jacobsen et al., 2018).

Other hydrophobic proteins such as gliadins (prolamine proteins from wheat) have been reported as shell material for the microencapsulation of fish oil via double emulsification and subsequent heat-polymerization (Liao, Luo, Zhao, & Wang, 2012). Gliadins particles have also been produced by electrospraying for the delivery and control release of an anticancer drug (e.g., cyclophosphamide) (Gulfam et al., 2012). The latter indicates that electrosprayed-gliadins capsules loaded with different core material (i.e., fish oil) could also be obtained. Nonetheless, gliadins cause an allergic response or a detrimental autoimmune reaction in celiac individuals, which limit their applications as food ingredients (De Mesa-Stonestreet, Alavi, & Bean, 2010). Therefore, there is a scientific and industrial interest in investigating the use of alternative hydrophobic proteins as biopolymers for the encapsulation of fish oil.

Kafirin, named as the class of cereal prolamin storage protein found in grain sorghum (Xiao, Wang, Perez Gonzalez, & Huang, 2016), may be an interesting alternative to zein as hydrophobic wall material, especially as some kafirin subclass have been reported to be more hydrophobic than the corresponding zein proteins (Belton, Delgadillo, Halford, & Shewry, 2006). Furthermore, and opposite to gliadins, kafarin is a safe ingredient to celiac patients (De Mesa-Stonestreet et al., 2010). Kafirin has been successfully utilized in several protein-based products (Taylor, Anyango, & Taylor, 2013). It has been reported that kafirin protein has adhesive (Li et al., 2011) and antimicrobial (Giteru et al., 2015) characteristics. Furthermore, compared to zein, kafirin has stronger gas (i.e. air) and water vapor barrier functions (Taylor, Taylor, Dutton, & De Kock, 2005). Kafirin protein can also be used in the food industry for production of plastic films (Wang et al., 2018) or application in food packaging materials (Dianda, Rouf, Bonilla, Hedrick, & Kokini, 2019). Olivera et al. (2019) evaluated LAPONITE® added kafirin nanocomposite films in terms of mechanical, surface and barrier properties in their study. They have claimed that kafirin protein could be utilized for coating, packaging, biomedical and other functional film applications. In another study, Huang et al. (2020) prepared irradiated kafirin-quercetin film with high physical properties to prolong the shelf life of cod (*Gadus morhua*) fillets. They found that kafirin-quercetin film could be used for protecting the quality of fresh fish products during cold storage. Kafirin has also been employed as shell material to obtain microcapsules by spray-drying or coacervation for the encapsulation of soybean oil (Bai et al., 2019) and tannins (Links, Taylor, Kruger, Naidoo, & Taylor, 2016), respectively. Nonetheless, to the best of the authors' knowledge, the development of encapsulates by electrospraying using kafirin as shell material remains to be investigated.

In this regard, this study aimed at investigating the development of kafirin-based capsules by electrospraying for the encapsulation of fish oil. First, the composition of the electrospraying solution (e.g. concentration of kafirin used) was optimized for obtaining the desired morphology (e.g. capsules). Secondly, the effect of the electrospraying processing conditions (i.e. voltage and flow-rate) on the morphology of kafirin-based encapsulates loaded with fish oil was evaluated. Finally, the encapsulation efficiency of the fish oil-loaded kafirin capsules obtained at the optimum processing conditions was assayed.

2. Material and method

2.1. Materials

Fish oil was obtained from Vesteraalens (Sortland, Norway) with a total EPA and DHA content of 20 wt%. The kafirin used in the present study was extracted from sorghum as described in section 2.2. The cereal source was a commercial white-tan sorghum hybrid F1000 obtained from NuLife Market (Scott City, Kansas, USA). The rest of reagents used were of analytical grade and were purchased from Sigma-Aldrich (Søborg, Denmark).

2.2. Kafirin isolation

Kafirin was isolated based on a modification of methods from Bean, Ioerger, Park, and Singh (2006) and Carter and Reck (1970), US Patent 3,535,305 as described in Lawton (2003) and Emmambux and Taylor (2003). The grain was decorticated to remove 20% of the outer layers by weight using a tangential abrasive dehulling device (Machine Works, Saskatoon, Canada) equipped with an 80-grit sandpaper disk. Decorticated grain was ground using a cyclone mill (cyclone sample mill, Udy Corporation, Fort Collins, Colorado, United States) equipped with a 0.5 mm screen. Kafirins were extracted from 300 g of decorticated sorghum flour (total of 8 batches; 2400 g flour) with 1050 mL of 70% ethanol containing 0.5% sodium metabisulphite for 1 h at 50 °C with occasional stirring. Sodium metabisulfite was used to break intra and intermolecular disulfide bonds, which enhanced the extraction yield. Cysteine and glutathione as potentially food safe reducing agents have also been tested, and have worked well, especially glutathione (Bean et al., 2006). Note that NaOH was not used in the extraction solvent to reduce any potential alkaline modification of the proteins during extraction. After extraction, sample was allowed to settle at room temperature for 1 h and then the supernatant was decanted and centrifuged at 10,000×*g* (5810 R, Eppendorf, Hauppauge, New York, United States) for 10 min to clarify. Supernatant was adjusted to a final volume of 30% ethanol and adjusted to pH 2.5 with 2M HCl and placed in a freezer (~-20 °C) overnight to precipitate kafirins. After storage in the freezer, the sample was again centrifuged as described above and the pellet was collected and lyophilized (FreeZone 6-plus, Labconco, Kansas City, Missouri, United States). After drying, isolated kafirin was defatted with hexane at a 1:2.5 ratio by stirring hexane and kafirin together for 1 h, then

allowing the mixture to settle and the hexane was carefully decanted. Defatting was repeated for a total of 3 times. After defatting, kafirin was allowed to dry at room temperature and then collected. Kafirin purity was determined by nitrogen combustion using a Leco FP-628 nitrogen determinator (Leco Corporation, St. Joseph, Michigan, United States) with a nitrogen to protein conversion factor of 6.25.

2.3. Characterization of kafirin protein isolate by reversed-phase high-performance liquid chromatography (RR-HPLC)

Isolated kafirin was analyzed by RP-HPLC described by Bean, Ioerger, and Blackwell (2011). Kafirin isolates were dissolved (2 mg/mL) in 60% t-butanol (v/v) containing 0.5% sodium acetate (w/v) and 2% β-mercaptoethanol (v/v) and alkylated as described previously (Bean et al., 2011). For comparison, an analytical control extract of kafirin was prepared as described in Bean et al. (2011) and analyzed under the same conditions.

2.4. Preparation of electrospraying solutions

Kafirin-based electrospraying solution (KbES) was prepared by dissolving kafirin (5–15 wt%) in 85/15 (v/v) ethanol/water solvent. KbES was shaken 1000 rpm for 2 h at room temperature to allow complete solubilization.

For optimum KbE (10 wt% kafirin), fish oil (20 wt% with respect to kafirin) was dispersed in the KbE at 18,000 rpm using a POLYTRON® PT1200E (Kinematic Inc., New York, USA). The fish oil was slowly added to the KbE during the first minute while the total processing time was 4 min. After that, secondary homogenization, adopted from García-Moreno et al. (2020), was done to decrease droplet size using a sonicator Microson XL2000 equipped with a P1 probe (Misonix, Inc., New York, USA). Emulsion (10 wt% kafirin with respect to final emulsion) was homogenized at an amplitude of 75% (maximum amplitude of 180 μm), running 3 passes of 30 s with a break of 1 min between passes. During the sonication process, the emulsions were surrounded by iced water in order to limit the increase of temperature.

2.5. Electrospraying process

The electrospraying unit used consisted of a syringe pump (New Era Pump Systems, Inc., USA), a needle (Proto Advantage, Canada), high voltage power supply (Gamma High Voltage Research, USA) and stainless steel plate collector (see Fig. S1). The electrospraying solutions were electrosprayed at a flow rate ranging from 0.5 to 1 mL/h with an applied voltage varying from 20 to 25 kV. A 22 G needle was utilized and the distance between the collector and needle was arranged as 15 cm. The electrospraying process was conducted at 22 ± 2 °C and 30–40% relative humidity. Samples were stored at room temperature in a desiccator until further analysis.

2.6. Morphology

The morphology of fish oil-loaded electrosprayed capsules was investigated using a Scanning Electron Microscope (SEM) (Phenom Pro, Phenom-World B.V., Eindhoven, The Netherlands). After 10 min of electrospraying, a piece of approximately 0.5 × 0.5 cm electrosprayed aluminum foil, that was located on the collector and contained the sample, was placed on carbon tape and sputter coated with gold during 8 s at 40 mA by a Q150 Quorum Coater (Quorum Technologies Ltd, East Sussex, UK). The capsule diameter distribution was measured by using the open source processing program Image J (National Institutes of Mental Health, Bethesda, Maryland, USA). The capsules size distribution was obtained by measuring 30 random capsules.

2.7. Encapsulation efficiency

In order to reveal the encapsulation efficiency of fish oil-loaded kafirin nanocapsules, UV–vis spectrometry (Nanodrop OneC, Thermo Fisher Scientific, Roskilde, DK) was performed. The efficiency of the encapsulation process was determined as described by Miguel et al. (2019). Capsules (C) (25 mg) were placed on a filter paper and isooctane (IO) (C/IO: 5/1 w/v) was poured over the capsules. The absorbance of the solvent resulting after washing the capsules was measured at 284 nm. A calibration curve was prepared in the range of 0.1–1 mg/mL using fish oil as standard and isooctane as solvent. Measurements were carried out in triplicate. The encapsulation efficiency was calculated using Eq. (1), where A is the theoretical quantity of fish oil and B is the amount of oil present in the washing solvent.

$$\text{Encapsulation Efficiency } (\%) = \frac{A - B}{A} \cdot 100 \quad (1)$$

2.8. Statistical analysis

For data analysis the software Statgraphics Centurion XV (Statistical Graphics Corp., Rockville, MD, USA) was used. First, One-way ANOVA was performed to identify if there were differences between samples. Then, multiple sample comparison using Tukey's test allowed to identify means which were significantly different from each other. Differences between means were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Characterization of kafirin isolate

Isolated kafirins were analyzed by RP-HPLC and compared to a standard analytical extract of kafirins from the same flour used to prepare the isolates (Fig. 1). Overall the chromatogram of the kafirin isolate was similar to that of the kafirins extracted directly from flour on an analytical scale. Note that the kafirin isolate was at a much higher concentration due to being highly purified (~93% protein). As expected, the majority of the proteins in the isolate were the α-kafirins eluting ~8–14 min (Bean et al., 2011). The α-kafirins (22–26 kDa) are rich in

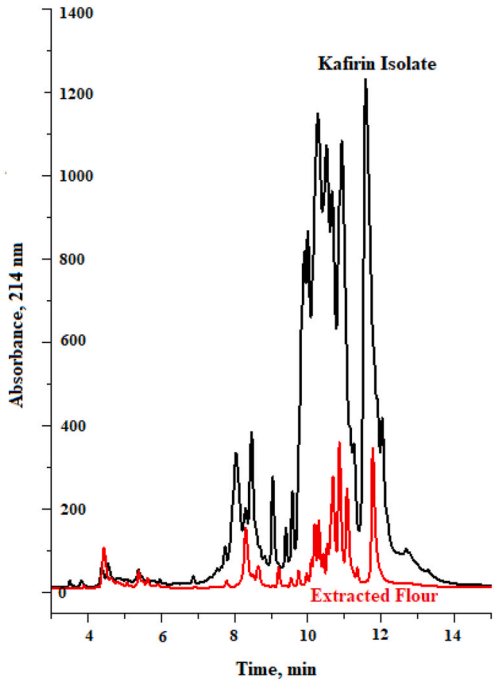


Fig. 1. RP-HPLC chromatograms of kafirin isolate and extracted sorghum flour.

nonpolar amino acids and form mainly intramolecular disulfide bonds (e.g. do not cross-link extensively) (De Mesa-Stonestreet et al., 2010). Peaks matching the elution times of γ- and β -kafirins (Cremer et al., 2014) were also present in the kafirin isolate chromatogram demonstrating all kafirin sub-classes were in the isolate as expected from past research on isolated kafirins (e.g. Emmambux and Taylor (2003)). β-and γ-kafirins are highly crosslinked due to the formation of intermolecular and intramolecular disulfide bonds (De Mesa-Stonestreet et al., 2010). There were minor differences apparent between the two protein samples in the ~7–10 min range with the kafirin isolate containing peaks not seen in the analytical kafirin extract. These were likely minor amounts of non-kafirin proteins present in the isolate due to the large-scale extraction procedure used.

It is worth mentioning that the white color of kafirin isolate, that will result in white nanocapsules (Fig. S1), is an advantage when compared to yellow zein for production of encapsulates to be used to fortify food (Jacobsen et al., 2018). This will result in enriched food products without an undesired yellowish color.

3.2. Effect of kafirin concentration on morphology of electrosprayed capsules

Fig. 2 shows that capsules with diameter below 1 μm could be obtained when electrospraying kafirin (5, 10 and 15 wt%) dissolved in ethanol/water (85:15, v/v). Higher concentration (20 wt% kafirin) resulted in larger capsules (0.988 ± 0.288 μm) with fibers connecting the capsules (Fig. S2), which is in the same line as the results obtained by Xiao, Shi, et al. (2016). These authors reported the production of nanofibers when dissolving 20 wt% kafirin in acetic acid/dichloromethane mixture (4:1, v/v) at 0.6 mL/h flow rate and 17.5 kV.

High solids content, while keeping constant the ratio kafirin:fish oil and maintaining capsule morphology, is desired in the electrospraying solution. This allows increasing productivity of the electrospraying process. Nevertheless, high concentration of biopolymer results in larger capsules and it also hinders the dispersion of fish oil in the continuous phase during homogenization, which may negatively affect encapsulation efficiency. This was observed in one pre-trial when using 15 wt% kafirin when compared to 10 wt% kafirin (data not shown). Therefore, and due to the limited amount of kafirin that we had, we selected 10 wt % kafirin for evaluating the influence of the processing conditions.

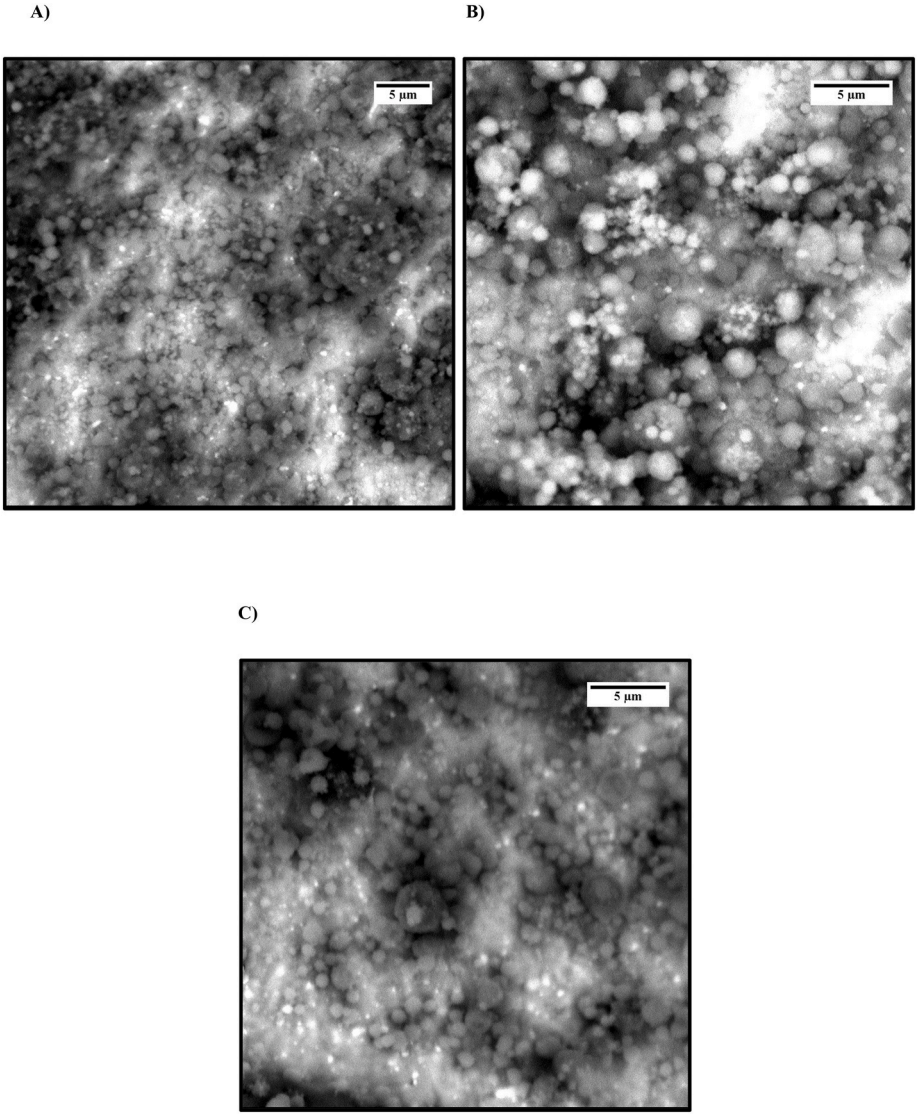


Fig. 2. Morphology of nanostructures obtained when eletrospraying kafirin solutions in ethanol/water (85:15, v/v): A) 5 wt% kafirin, B) 10 wt% kafirin, and C) 15 wt% kafirin. Electrospraying conditions: 20 kV and 0.5 mL/h.

3.3. Influence of processing conditions on the morphology of kafirin-based electrosprayed capsules loaded with fish oil

Electrospraying of KbE solutions (10 wt% kafirin) containing fish oil (20 wt% with respect to kafirin) were electrosprayed at different processing conditions to evaluate the effect of voltage and flow rate on morphology of the capsules. Four different flow rates (0.5, 0.6, 0.8, and 1 mL/h) were assayed at two different voltages (20 or 25 kV).

Fish oil-loaded kafirin-based nanocapsules with spherical shape were successfully produced at 20 and 25 kV, independently of the flow rate used (Figs. 3 and 4). From SEM images and using Image J, average diameter of the capsules was determined. It was expected that increasing flow rate would lead to larger capsules, due to the formation of larger droplets during electrospraying (Tapia-Hernández, Rodríguez-Félix, & Katouzian, 2017). However, ANOVA analysis of the data indicated that there were no significant differences in the diameter of electrosprayed kafirin capsules produced at 20 kV when increasing flow rate. Likewise, increasing flow rate did not significantly increase the diameter of the electrosprayed kafirin capsules when produced at 25 kV (Table 1). In addition, no significant differences in average diameter of the kafirin capsules were obtained when increasing voltage from 20 to 25 kV for any of the flow rates assayed (Table 1). Higher voltage would imply higher electrostatic forces that would cause droplets to repel between each other and break in smaller droplets in their way to the collector (Tapia-Hernández et al., 2017). Nevertheless, we only increased the voltage by 5 kV, which did not cause significant differences when electrospraying kafirin. Altogether, we obtained a polydisperse sample and consequently we did not observe a significant effect of the voltage and flow rate on the size of the electrosprayed caspules.

Interestingly, all electrosprayed kafirin capsules loaded with 20 wt% fish oil produced in this study had an average diameter below 1 μm. This clearly shows the advantage of using electrospraying when compared to other encapsulation techniques for obtaining nanocapsules. For instance, Karim et al. (2017) used supercritical antisolvent process to obtain fish oil encapsulates with diameter of 58.35 μm when using hydroxypropyl methyl cellulose as a polymer. Similarly, spray-freeze-dried and spray-dried fish oil microcapsules having a diameter up to 50 μm were also reported in literature (Pang, Duan, Ren, & Liu, 2017). Recently, Rahmani-Manglano et al. (2020) encapsulated fish oil by spray-drying (15 wt% oil load), obtaining most of the capsules with a diameter below 30 μm. Nevertheless, there are only a few studies related to fish oil nanoencapsulation by using electrospraying process. Torres-Giner et al. (2010) produced zein-DHA nanocapsules (490 ± 200 nm) connected with fibrils under a flow-rate of 0.2 mL/h and 12 kV from 20 wt% zein solution in ethanol/water. Gómez-Mascaraque and López-Rubio (2016) encapsulated α–linolenic acid using gelatin, whey protein or soy protein isolate as shell materials by electrospraying, reporting capsule sizes up to 3, 1.5 and 1.25 μm, respectively. García--Moreno et al. (2018) reported the production of dextran or glucose syrup capsules loaded with 20 wt% fish oil using electrospraying assisted by pressurized gas. This type of electrospraying process allows for higher productivity when compared to normal electrospraying (e.g. by favoring solution atomization), which results in larger ejected droplets and thus larger dried capsules (Busolo et al., 2019). Accordingly, these authors

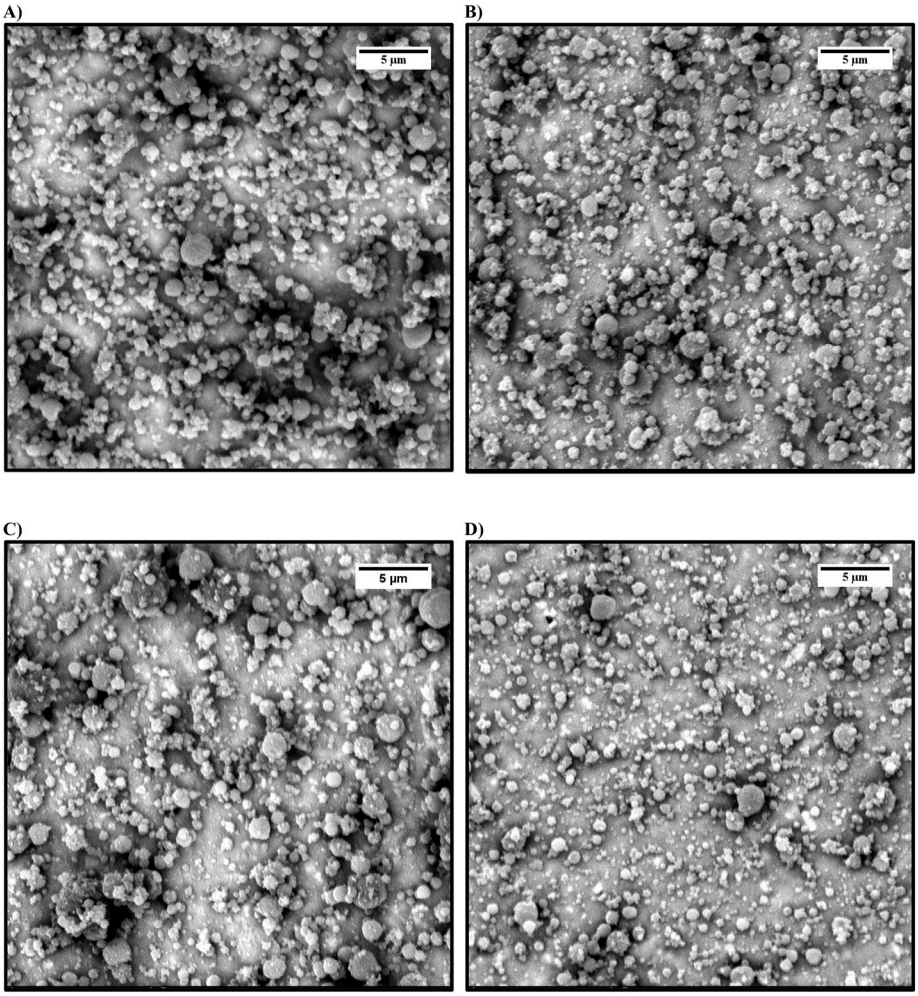


Fig. 3. Kafirin-based electrosprayed capsules loaded with 20% fish oil and produced at 20 kV and different flow-rates (mL/h): A) 0.5, B) 0.6, C) 0.8, and D) 1.

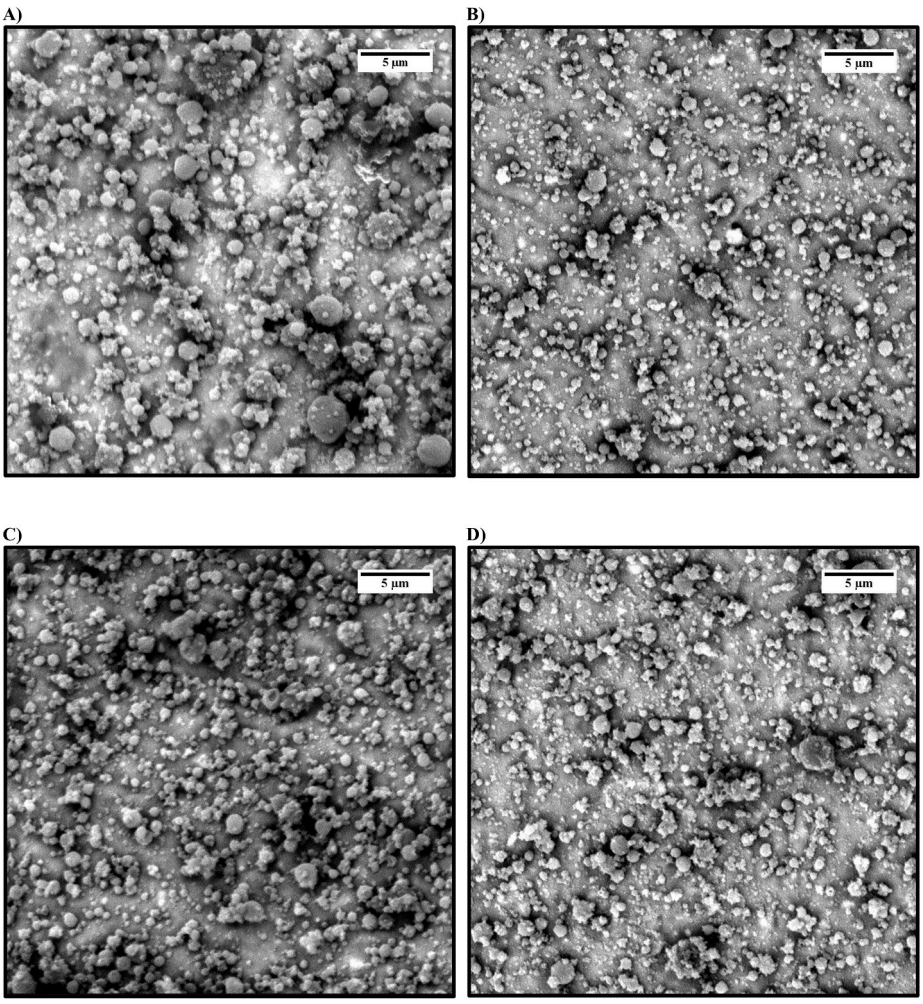


Fig. 4. Kafirin-based electrosprayed capsules loaded with 20% fish oil and produced at 25 kV and different flow-rates (mL/h): A) 0.5, B) 0.6, C) 0.8, and D) 1.

Table 1
Average diameter (nm) of kafirin-based electrosprayed capsules loaded with 20% fish oil.

Voltage, kV	Flow Rate, mL/h			
	0.5	0.6	0.8	1.0
20	741 ± 151^{aA}	623 ± 160^{aA}	663 ± 179^{aA}	604 ± 156^{aA}
25	861 ± 156^{aA}	552 ± 153^{aA}	667 ± 155^{aA}	594 ± 153^{aA}

Same lowercase letter show no significant differences ($p > 0.05$) between different flow rates at constant voltage.
Same uppercase letter show no significant differences ($p > 0.05$) between different voltages at constant flow rate.

found that up to 70% of the produced dextran or glucose syrup capsules were below 3 µm. Recently, Miguel et al. (2019), reported zein-based capsules loaded with 20 wt% fish oil with a diameter between 1 and 4 µm when produced with electrospraying assisted by pressurized gas.

The kafirin nanocapsules loaded with fish oil obtained in this study (with average diameter < 1 µm) present a high surface-to-volume ratio which is desired for a better release of the encapsulated bioactive compound. In addition, fortification of food with nanocapsules will reduce the impact on the properties of the enriched food matrix (e.g. texture). On the other hand, higher specific surface area also implies larger contact area between capsules and prooxidants (e.g. oxygen) which can be detrimental in terms of oxidative stability of the capsules (Jacobsen et al., 2018). This requires further research.

3.4. Encapsulation efficiency of kafirin-based nanocapsules loaded with fish oil

Although large fish oil droplets ($D_{4,3} = 2.585$ µm) were present in the KbE optimum solution (Fig. S3), encapsulation efficiency of fish oil within the kafirin matrix after electrospraying was 94.0 ± 2.5% (measured for nanocapsules produced at 25 kV and 0.8 mL/h) This value indicated that electrospraying was highly effective in order to retain most of the fish oil within the kafirin shell. Previous studies that used glucose syrup or dextran and zein as shell material revealed that EE values of nano-microcapsules were found as in the range of 78–92% and 83–84%, respectively (Busolo et al., 2019; García-Moreno et al., 2018; Miguel et al., 2019). In another study, Moomand and Lim (2014) produced 70% (w/w) ethanol-based 20% (w/w) zein nanofibers. EE value was found as 96.15 ± 0.23 and 91.19 ± 1.09% at 15% (w/w) and 30% (w/w) fish oil loading respectively (Moomand and Lim 2014). The achieved value (94.0 ± 2.5%) for 20% (w/w) fish oil loading in our study demonstrated that EE results were in coherence with their study, although we only used a kafirin concentration of 10 wt%. This finding denoted that, despite of the low emulsifying activity of the kafirin isolate used, the hydrophobicity of kafirin plays a critical role on encapsulating the hydrophobic bioactive (i.e. fish oil) in the core of the capsules. Indeed, kafirin needed to be defatted after extraction (i.e. to remove sorghum lipids), which indicated that kafirin exhibit a high affinity for lipids explaining the high encapsulation efficiency.

4. Conclusion

A kafirin isolate comprising mostly α-kafirins, but also γ- and β-kafirins, was successfully extracted from sorghum grains. Nano-processing applied at different electrospraying conditions (20–25 kV and 0.5–1 mL/h) revealed that, when kafirin isolate (10 wt%) was used as wall material, 20 wt% fish oil-loaded nanocapsules (552–861 nm) could be successfully produced. Interestingly, no significant differences in average diameter of the nanocapsules were observed when varying voltage or flow rate. The encapsulation efficiency of the nanocapsules was high 94.0 ± 2.5%, which denoted the impact of hydrophobic shell materials for retaining the hydrophobic bioactive encapsulated. Therefore, our results show the potential of kafirin as hydrophobic shell material for the encapsulation of fish oil by electrospraying, which will inspire further research on this topic.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Turgay Cetinkaya: Data curation, Investigation, Visualization, Writing - original draft, preparation. **Ana C. Mendes:** Conceptualization, Project administration, Supervision, Methodology, Writing - review & editing. **Charlotte Jacobsen:** Resources, Project administration, Supervision, Writing - review & editing. **Zafer Ceylan:** Formal analysis, Validation, Visualization, Writing - original draft, preparation. **Ioannis S. Chronakis:** Resources, Writing - review & editing. **Scott R. Bean:** Resources, Writing - review & editing. **Pedro J. García-Moreno:** Investigation, Conceptualization, Supervision, Methodology, Project administration, Writing - review & editing.

Declaration of competing interest

The authors report no conflicts of interest.

Acknowledgments

T. Cetinkaya acknowledges the Erasmus+Internship Programme for funding his research stay at the Technical University of Denmark. A.C.M. and I.S.C. acknowledge support from the Innovation Fund Denmark (PROBIO – project nr 7076-00053B).

Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product and use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable. USDA is an equal opportunity provider and employer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110297.

References

Aghbashlo, M., Mobli, H., Rafiee, S., & Madadlou, A. (2012). Energy and exergy analyses of the spray drying process of fish oil microencapsulation. *Biosystems Engineering, 111*(2), 229–241. https://doi.org/10.1016/j.biosystemseng.2011.12.001

Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. In *Trends in food science and technology* (Vol. 25, pp. 24–33). Elsevier. https://doi.org/10.1016/j.tifs.2011.12.002. Issue 1.

Bai, X., Li, C., Yu, L., Jiang, Y., Wang, M., Lang, S., et al. (2019). Development and characterization of soybean oil microcapsules employing kafirin and sodium caseinate as wall materials. *Lebensmittel-Wissenschaft & Technologie, 111*, 235–241. https://doi.org/10.1016/j.lwt.2019.05.032

Bean, S. R., Ioerger, B. P., & Blackwell, D. L. (2011). Separation of kafirins on surface porous reversed-phase high-performance liquid chromatography columns. *Journal of Agricultural and Food Chemistry, 59*(1), 85–91. https://doi.org/10.1021/jf1036195

Bean, S. R., Ioerger, B. P., Park, S. H., & Singh, H. (2006). Interaction between sorghum protein extraction and precipitation conditions on yield, purity, and composition of purified protein fractions. *Cereal Chemistry Journal, 83*(1), 99–107. https://doi.org/10.1094/CC-83-0099

Belton, P. S., Delgadillo, I., Halford, N. G., & Shewry, P. R. (2006). Kafirin structure and functionality. In *Journal of cereal science* (Vol. 44, pp. 272–286). Academic Press. https://doi.org/10.1016/j.jcs.2006.05.004. Issue 3.

Busolo, M. A., Torres-Giner, S., Prieto, C., & Lagaron, J. M. (2019). Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. *Innovative Food Science & Emerging Technologies, 51*, 12–19. https://doi.org/10.1016/j.ifset.2018.04.007

Calder, P. C. (2013). Nutritional benefits of omega-3 fatty acids. In *Food enrichment with omega-3 fatty acids* (pp. 3–26). Elsevier Inc. https://doi.org/10.1533/9780857098863.1.3.

Carter, R., & Reck, D. R. (1970). Low temperature solvent extraction process for producing high purity zein (Patent No. 3,535,305). In *Google patents (3,535,305)*. Washington, DC: U.S. Patent and Trademark Office. https://patents.google.com/patent/US3535305A/en.

Cremer, J. E., Bean, S. R., Tilley, M. M., Ioerger, B. P., Ohm, J. B., Kaufman, R. C., et al. (2014). Grain sorghum proteomics: Integrated approach toward characterization of endosperm storage proteins in kafirin allelic variants. *Journal of Agricultural and Food Chemistry, 62*(40), 9819–9831. https://doi.org/10.1021/jf5022847

De Mesa-Stonestreet, N. J., Alavi, S., & Bean, S. R. (2010). Sorghum proteins: The concentration, isolation, modification, and food applications of kafirins. *Journal of Food Science, 75*(5). https://doi.org/10.1111/j.1750-3841.2010.01623.x. no-no.

Dianda, N., Rouf, T. B., Bonilla, J. C., Hedrick, V., & Kokini, J. (2019). Effect of solvent polarity on the secondary structure, surface and mechanical properties of biodegradable kafirin films. *Journal of Cereal Science, 90*, 102856. https://doi.org/10.1016/j.jcs.2019.102856

Drusch, S., Serfert, Y., Scampicchio, M., Schmidt-Hansberg, B., & Schwarz, K. (2007). Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. *Journal of Agricultural and Food Chemistry, 55*(26), 11044–11051. https://doi.org/10.1021/jf072536a

Dyall, S. C. (2015). Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. In *Frontiers in aging neuroscience* (Vol. 7). https://doi.org/10.3389/fnagi.2015.00052. Issue APR.

Emmambux, N. M., & Taylor, J. R. (2003). Sorghum kafirin interaction with various phenolic compounds. *Journal of the Science of Food and Agriculture, 83*(5), 402–407. https://doi.org/10.1002/jsfa.1379

García-Moreno, P. J., Gregersen, S., Nedamani, E. R., Olsen, T. H., Marcatili, P., Overgaard, M. T., et al. (2020). Identification of emulsifier potato peptides by bioinformatics: Application to omega-3 delivery emulsions and release from potato industry side streams. *Scientific Reports, 10*(1), 1–22. https://doi.org/10.1038/s41598-019-57229-6

García-Moreno, P. J., Özdemir, N., Stephansen, K., Mateiu, R. V., Echegoyen, Y., Lagaron, J. M., et al. (2017). Development of carbohydrate-based nano-microstructures loaded with fish oil by using electrohydrodynamic processing. *Food Hydrocolloids, 69*, 273–285. https://doi.org/10.1016/j.foodhyd.2017.02.013

García-Moreno, P. J., Pelayo, A., Yu, S., Busolo, M., Lagaron, J. M., Chronakis, I. S., et al. (2018). Physicochemical characterization and oxidative stability of fish oil-loaded electrosprayed capsules: Combined use of whey protein and carbohydrates as wall materials. *Journal of Food Engineering, 231*, 42–53. https://doi.org/10.1016/j.jfoodeng.2018.03.005

Giteru, S. G., Coorey, R., Bertolatti, D., Watkin, E., Johnson, S., & Fang, Z. (2015). Physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2014.07.077

Gómez-Mascaraque, L. G., & López-Rubio, A. (2016). Protein-based emulsion electrosprayed micro- and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. *Journal of Colloid and Interface Science, 465*, 259–270. https://doi.org/10.1016/j.jcis.2015.11.061

Gulfam, M., Kim, J., Lee, J. M., Ku, B., Chung, B. H., & Chung, B. G. (2012). Anticancer drug-loaded gliadin nanoparticles induce apoptosis in breast cancer cells. *Langmuir, 28*(21), 8216–8223. https://doi:10.1021/la300691n.

Hermund, D., Jacobsen, C., Chronakis, I. S., Pelayo, A., Yu, S., Busolo, M., et al. (2019). Stabilization of fish oil-loaded electrosprayed capsules with seaweed and commercial natural antioxidants: Effect on the oxidative stability of capsule-enriched mayonnaise. *European Journal of Lipid Science and Technology, 121*(4), 1800396. https://doi.org/10.1002/ejlt.201800396

Huang, T., Lin, J., Fang, Z., Yu, W., Li, Z., Xu, D., et al. (2020). Preparation and characterization of irradiated kafirin-quercetin film for packaging cod (Gadus morhua) during cold storage at 4 °C. *Food and Bioprocess Technology, 13*(3), 522–532. https://doi.org/10.1007/s11947-020-02409-w

Innes, J. K., & Calder, P. C. (2020). Marine omega-3 (N-3) fatty acids for cardiovascular health: An update for 2020. *International Journal of Molecular Sciences, 21*(4), 1362. https://doi.org/10.3390/ijms21041362

Jacobsen, C. (2015). Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. *European Journal of Lipid Science and Technology, 117*(11), 1853–1866. https://doi.org/10.1002/ejlt.201500137

Jacobsen, C., García-Moreno, P. J., Mendes, A. C., Mateiu, R. V., & Chronakis, I. S. (2018). Use of electrohydrodynamic processing for encapsulation of sensitive bioactive compounds and applications in food. *Annual Review Food Science and Technology, 9*(1), 525–549. https://doi.org/10.1146/annurev-food-030117-012348

Karim, F. T., Ghafoor, K., Ferdosh, S., Al-Juhaimi, F., Ali, E., Yunus, K. B., et al. (2017). Microencapsulation of fish oil using supercritical antisolvent process. *Journal of Food and Drug Analysis, 25*(3), 654–666. https://doi.org/10.1016/j.jfda.2016.11.017

Lawton, J. (2003). New uses of corn proteins. *International Starch Technology Conference, 39*. https://www.ars.usda.gov/research/publications/publication/?seqNo115=150405.

Liao, L., Luo, Y., Zhao, M., & Wang, Q. (2012). Preparation and characterization of succinic acid deamidated wheat gluten microspheres for encapsulation of fish oil. *Colloids and Surfaces B: Biointerfaces, 92*, 305–314. https://doi:10.1016/j.colsurfb.2011.12.003.

Links, M. R., Taylor, J., Kruger, M. C., Naidoo, V., & Taylor, J. R. N. (2016). Kafirin microparticle encapsulated sorghum condensed tannins exhibit potential as an anti-hyperglycaemic agent in a small animal model. *Journal of Functional Foods, 20*, 394–399. https://doi.org/10.1016/j.jff.2015.11.015

Li, N., Wang, Y., Tilley, M., Bean, S. R., Wu, X., Sun, X. S., et al. (2011). Adhesive performance of sorghum protein extracted from sorghum DDGS and flour. *Journal of Polymers and the Environment, 19*(3), 755–765. https://doi.org/10.1007/s10924-011-0305-5

Miguel, G. A., Jacobsen, C., Prieto, C., Kempen, P. J., Lagaron, J. M., Chronakis, I. S., et al. (2019). Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil. *Journal of Food Engineering, 263*, 348–358. https://doi.org/10.1016/j.jfoodeng.2019.07.019

Moomand, K., & Lim, L. T. (2014). Oxidative stability of encapsulated fish oil in electrospun zein fibres. *Food Research International, 62*, 523–532. https://doi.org/10.1016/j.foodres.2014.03.054

Olivera, N., Rouf, T. B., Bonilla, J. C., Carriazo, J. G., Dianda, N., & Kokini, J. L. (2019). Effect of LAPONITE® addition on the mechanical, barrier and surface properties of novel biodegradable kafirin nanocomposite films. *Journal of Food Engineering, 245*, 24–32. https://doi.org/10.1016/j.jfoodeng.2018.10.002

Pang, Y., Duan, X., Ren, G., & Liu, W. (2017). Comparative study on different drying methods of fish oil microcapsules. *Journal of Food Quality*. https://doi.org/10.1155/2017/1612708

Prieto, C., & Lagaron, J. M. (2020). Nanodroplets of docosahexaenoic acid-enriched algae oil encapsulated within microparticles of hydrocolloids by emulsion electrospraying assisted by pressurized gas. *Nanomaterials, 10*(2), 270. https://doi.org/10.3390/nano10020270

Rahmani-Manglano, N. E., González-Sánchez, I., García-Moreno, P. J., Espejo-Carpio, F. J., Jacobsen, C., & Guadix, E. M. (2020). Development of fish oil-loaded microcapsules containing whey protein hydrolysate as film-forming material for fortification of low-fat mayonnaise. *Foods, 9*(5), 545. https://doi.org/10.3390/foods9050545

Tapia-Hernández, J. A., Rodríguez-Félix, F., & Katouzian, I. (2017). Nanocapsule formation by electrospraying. In *Nanoencapsulation Technologies for the food and nutraceutical industries* (pp. 320–345). Elsevier. https://doi.org/10.1016/B978-0-12-809436-5.00009-4.

Taylor, J., Anyango, J. O., & Taylor, J. R. N. (2013). Developments in the science of zein, kafirin, and gluten protein bioplastic materials. *Cereal Chemistry Journal, 90*(4), 344–357. https://doi.org/10.1094/CCHEM-12-12-0165-IA

Taylor, J., Taylor, J. R. N., Dutton, M. F., & De Kock, S. (2005). Identification of kafirin film casting solvents. *Food Chemistry, 90*(3), 401–408. https://doi.org/10.1016/j.foodchem.2004.03.055

Torres-Giner, S., Martinez-Abad, A., Ocio, M. J., & Lagaron, J. M. (2010). Stabilization of a nutraceutical omega-3 fatty acid by encapsulation in ultrathin electrosprayed zein prolamine. *Journal of Food Science, 75*(6), N69–N79. https://doi.org/10.1111/j.1750-3841.2010.01678.x

Valenzuela, A., & Valenzuel, R. (2013). Omega-3 docosahexaenoic acid (DHA) and mood disorders: Why and how to provide supplementation?. In *Mood disorders*. InTech. https://doi.org/10.5772/53322.

Wang, W., Xiao, J., Chen, X., Luo, M., Liu, H., & Shao, P. (2018). Fabrication and characterization of multilayered kafirin/gelatin film with one-way water barrier property. *Food Hydrocolloids, 81*, 159–168. https://doi.org/10.1016/j.foodhyd.2018.02.044

Xiao, J., Shi, C., Zheng, H., Shi, Z., Jiang, D., Li, Y., et al. (2016b). Kafirin protein based electrospun fibers with tunable mechanical property, wettability, and release profile. *Journal of Agricultural and Food Chemistry, 64*(16), 3226–3233. https://doi.org/10.1021/acs.jafc.6b00388

Xiao, J., Wang, X., Perez Gonzalez, A. J., & Huang, Q. (2016). Kafirin nanoparticles-stabilized Pickering emulsions: Microstructure and rheological behavior. *Food Hydrocolloids, 54*, 30–39. https://doi.org/10.1016/j.foodhyd.2015.09.008