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**Sardinelle protein isolate as a novel material for oil microencapsulation:
Novel alternative for fish by-products valorisation**

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Abstract

The present study investigates the potential of sardinelle protein isolate (SrPI) combined to maltodextrin (MD), at different ratios ((1:0, 1:1, 1:2, 1:3 and 1:4, w/w), as wall matrix to stabilize and encapsulate corn oil (1:2, oil/ wall ratio). Emulsions were prepared by homogenization followed by sonication treatment and then dried by the spray-drying process. The obtained microcapsules were characterized regarding the encapsulation efficiency (EE), scanning electron microscopy (SEM), infrared spectroscopy (FTIR), and thermodynamic analyses (thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC)). Data revealed that the combination of SrPI and MD resulted in very high EE compared to SrPI used alone as wall material, and the EE increased with the amount of MD incorporated to the SrPI solution. SEM images showed the production of irregular and larger particles with the increase of MD concentration. Moreover, TGA showed that microparticles obtained by 1:4 w/w ratio (SrPI/MD) revealed the highest protection of corn oil. Thus, these findings revealed the effectiveness of SrPI and MD mixture to encapsulate and protect corn oil, which offered a promote application for food and pharmaceutical industries.

Keywords: Sardinelle protein isolate; Maltodextrin; Microencapsulation efficiency; Spray-drying process; Microstructure.

1. Introduction

The development of microcapsule technology attracts the attention of industry because it is one of the required technologies in which healthy principle ingredients are coated without reducing their bioavailability or functionality [1]. The microencapsulation presents various advantages such as protecting the active and sensitive ingredients, enhancing their stability and prolonging the period of storage and controlling their release [2,3], explaining their wide application in several areas like medicine, cosmetics, food, textile and advanced materials [4]. This protective process allows the entrapment of solid, liquid and gas materials inside tiny microcapsules with a micrometric diameter (from 1 to 1000 μm) [5]. Some of the relevant procedures are spray-drying [6,7], freeze drying [8], electrospinning [9], ionic gelation [10], coacervation [11-13], etc. Among the microencapsulation methods, spray drying is the most commonly used encapsulation technique in the food industry for encapsulation and stabilizing of various types of heat-sensitive compounds due to its simplicity, low cost and production of powders particles of good quality [14]. Different factors may affect the encapsulation efficiency during spray drying [15]. The choice of the coating materials, natural or synthetic, is one of the important factors impacting the encapsulation efficiency and the stability of the bioactive substance. In fact, the polymeric material is selected according to their nature, physiochemical and technofonctionnel properties [16]. Traditionally, gelatin, whey proteins, soy protein, maltodextrin, starches and gum Arabic are the most common protein and polysaccharide used as wall matrix for spray drying microencapsulation [17].

Proteins are excellent wall material for oil encapsulation due to their high emulsification properties, high water solubility and high film-forming properties [15]. Carbohydrates are generally used as a secondary wall material to improve the encapsulation efficiency and storage of encapsulated oil.

Maltodextrin (MD), a hydrolyzed starch, is commonly used as wall material in spray drying encapsulation of food ingredients due its low cost, high solubility, neutral aroma and taste, low viscosity and good oxidative protection of the core materials [18]. However, due to its low emulsification capacity MD is generally used as wall material in combination with other active biopolymers [19-22].

Due to their excellent physico-chemical and functional properties, marine proteins can be used as alternative to animal and vegetal proteins in spray-drying to encapsulate various types of bioactive compounds. The study of Shi, Beamer, Yang, Jaczynski [23] found that the krill protein isolated with pH-shifting process is a potential wall biopolymer for krill oil coating.

Therefore, this study evaluated the effects of combination of proteins isolated from sardinelle by-products and maltodextrin, at different ratios as wall materials, on the physicochemical characteristics, structural and thermal stability of a vegetable oil microparticles obtained by spray drying process.

2. Materials and methods

2.1. Materials

Maltodextrin (Dextrin equivalent 20) was purchased from Iobachimie (Pvt. Ltd., Mumbai, India). Corn oil was obtained from Nejma (OUED ELLIL, 2021, MANOUBA, Tunisie). Hexane was reagent grade and provided from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used were of reagent grade and purchased from Sigma–Aldrich (Oakville, ON, Canada).

2.2. Preparation of sardinelle protein isolate by pH shifting method

SrPI was obtained through the alkaline solubilization process as reported by Chaijan, Panpipat & Benjakul [24]. *Sardinella aurita* muscle (SrPI), fish mince was homogenized with cold water (4 °C) at a ratio of 1:9 (w/v). The homogenate was adjusted to pH 11.0 using 2 N NaOH solution, stirred gently for 60 min and then centrifuged at 9,500 g for 20 min at 4 °C. The soluble proteins in the supernatant were precipitated at their nominal isoelectric point 5.5 using 2 N HCl and then collected by centrifugation (9,500 g for 20 min). Finally, the obtained pellet was resuspended in distilled water, followed by pH adjustment to 7.0 using 2 N NaOH solution. SrPI was spray-dried using a Mini Spray Dryer B-290 apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland). The inlet air temperature was set at 180 °C, as it proved to be enough to achieve complete drying of the particles. Under these conditions, the outlet air temperature was 85 °C.

2.3. Functional properties of SrPI

SrPI solubility was carried at a wide range of pH from 1.0 to 11.0 as described by Rawdkuen & Benjakul [25]. The emulsifying activity indice (EAI) of SrPI solution (10 mg/ml) was estimated at a wide range of pHs (1.0 to 11.0) as described by Pearce and Kinsella [26]. After incubation for 24 h at room temperature, the microscopic observation of all emulsions was conducted using a CX31-12C04 microscope (Motic 2048×1536 pixels, Olympus Co., Tokyo, Japan).

2.4. Preparation and characterization of emulsions

2.4.1. Emulsion preparation

SrPI powder was initially dispersed in distilled water at a concentration of 4%. The pH of the resulting solution was adjusted to 8.0 with 2 N NaOH solution and then stirred overnight (4 °C) to ensure complete hydration of the proteins. Afterward, MD was dissolved into the SrPI solution and the mixture was stirred for 1 h. The corn oil was added to SrPI/MD solution, at a ratio of oil to wall material of 1:2 (w/w), and then homogenized at 12 000 rpm for 5 min using Ultra-Turrax (IKA T18 basic, Wilmington, USA). The obtained emulsions were subsequently submitted to ultrasonication at 160 W of nominal power (Branson Digital Sonifier®, Model S-450D, Branson Ultrasonics Corporation, Dan-bury, USA) at a frequency of 20 kHz for 2 min. The height contact between the ultrasonic probe and the emulsions was standardized to 35 mm. Five emulsions were prepared with different SrPI/MD weight ratios of 1:0, 1:1, 1:2, 1:3 and 1:4 (w/w). pH of emulsions was adjusted to 8.0.

2.4.2. Emulsion stability evaluated by creaming index

The creaming index was determined to assess the stability of the emulsions as described by Surh, Decker, & McClements [27].

2.4.3. Z-potential and mean droplet size measurements

The oil droplet size and Z-potential were determined using a laser scattering size distribution analyzer Zetasizer Nano ZPS (Malvern Instruments, Ltd., Worcestershire, UK). Prior to the analysis, 20 µl of each emulsion were diluted in 180 ml of distilled water at 25 °C. Measurements were carried out in triplicate.

2.5. Microencapsulation by spray drying

Microencapsulation was manufactured by spray-drying technique. Briefly, emulsions were subsequently fed to a Mini Spray Dryer B-290 apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland). The inlet air temperature was set at 180 °C, as it proved to be enough to achieve complete drying of the particles. Under these conditions, the outlet air temperature was 90 °C. The atomizing air and aspiration rate flows were kept in 16% and 100%, respectively. The powders were collected, sealed in glass jar and then stored at 4 °C until further analysis.

2.6. Microcapsules analysis

2.6.1. Drying yield

Drying yield (%) was determined for each microcapsule and was expressed as the ratio of the mass of powder obtained at the spray-dryer output to the solid content in the initial feed solution.

2.6.2. Moisture content

The moisture content was measured gravimetrically after drying in a vacuum oven of the microcapsules at 110 °C for 24 h. MC was calculated using the difference in weight before and after drying.

2.6.3. Z-potential and particle size distribution

The Z-potential, mean particle size and particle size distribution (PSD) were determined for each sample using a laser scattering size distribution analyzer Zetasizer Nano ZPS (Malvern Instruments, Ltd., Worcestershire, UK) at 25 °C in triplicate.

2.6.4. Encapsulation efficiency

The determination of corn oil content in the microparticles was carried out as follows: a volume of 20 ml of hexane was added to 200 mg of powder by vigorous shaking at room temperature. The solvent was filtered through a Whatman filter paper n°1 and, then the filtrate was collected into a pre-weight glass tube. The solvent was evaporated and dried until a constant weight of glass tube. The surface oil was determined by mass difference between the initial clean glass tube and that containing the extracted oil.

The corn oil encapsulation efficiency (EE), which is the amount of oil encapsulated inside the encapsulation systems, was calculated as follows:

$$EE (\%) = \frac{MT - MS}{MT} \times 100$$

where MS is the amount (mg) of surface oil in microcapsules and MT is the total oil amount (mg) added to the emulsion. The oil content of each sample was carried out in triplicate.

2.6.5. Fourier-transform infrared (FTIR) analysis

FTIR spectra of SrPI, free corn oil and microcapsules were performed using a Perkin-Elmer spectrometer (Spectrum 65, France) equipped with an attenuated total reflectance accessory with a Zn Se crystal. A number of 32 scans were collected with 4 cm⁻¹ resolution in the wave length range 650-4500 cm⁻¹. Calibration was done using background spectrum recorded from the clean and empty cell at 25 °C. This analysis aimed to determine the modifications induced at the molecular scale between SrPI and its microcapsules. For FTIR data treatment, the Spectrum Suite ES software was used. Only the shifts were considered for specific chemical group interacting with the increasing content of microcapsules compared to sardinelle protein isolate.

2.7. Thermogravimetric analysis

The thermal stability of the MD, SrPI and the microparticles was determined by thermogravimetric analysis using TGA Q500 High Resolution, TA Instruments (Corporation Shimadzu, Kyoto, Japan). The analysis was conducted under the following operating conditions: alumina pan; dynamic nitrogen atmosphere with heating rate: 20°C min⁻¹;

temperature range: 30-700 °C. The residual weight of samples (4 mg) was constantly measured with an accuracy of 0.01 mg.

2.8. X-ray diffraction analysis

The structural analysis of capsules was performed using XRD diffractometer D8 (Advance Bruker, Germany) used Cu-K α Ni-filter radiation ($k = 1.5406 \text{ \AA}$). The relative intensity was 171 recorded in the scattering range 2θ of 5–50 ° with a step size of 0.02 ° and a counting time of 5 172 s/step. The error of this measurement was $\pm 1^\circ$.

2.9. Microstructure analysis

Scanning electron microscopy (SEM) was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 2.0 kV and a working distance of 5 mm. Samples were sputter-coated with a gold-palladium mixture under vacuum prior to examination. Particle diameters were measured from the SEM micrographs in their original magnification using the Image J software.

2.10. Statistical analysis

All experiments and measurements were performed at least in triplicate. Data were expressed as mean value \pm SD (standard deviation) and analyzed using SPSS package ver. 17.0 for Windows, professional edition (SPSS Inc., Chicago, USA) using ANOVA analysis. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Functional properties of SrPI

SrPI was isolated using shifting pH technique. The content of proteins in SrPI powder was around 81 g/100 g (based on the dry weight matter).

Protein solubility is considered as a crucial parameter because it acts as a vital factor in their functional potential. Several factors, such as ion strength, temperature, pH and drying techniques could affect the proteins structural conformation and thereby their solubility behavior. The effect of different pH values on the solubility of SrPI was determined and reported in **Fig. 1a**. High solubility was recorded at extreme acidic and alkaline pH levels, suggesting the decrease of interactions between proteins and water molecules. However, the minimum solubility in water was observed at pH 4.0-5.0, which could be close to the isoelectric pH of SrPI. Indeed, at the isoelectric point, the net charge of the protein is equal to zero, which led to reduce the repulsion between protein molecules and promote their self- aggregation by hydrophobic forces, contributing to the precipitation of proteins and

decreasing their solubility in water. Similarly, in a study by Taktak et al. [28], it was reported high solubility of protein isolate from *Anguilla anguilla* at both acidic and alkaline pH values.

The effect of SrPI concentration on the EAI at pH 2.0 and 11.0 is presented in **Table 1**. At pH 11.0, the EAI of SrPI is significantly higher than that obtained at pH 2.0. Benelhadj, Gharsallaoui, Degraeve, Attia, & Ghorbel [29] explained this variation to the overall protein charge on the surface of proteins that is related to the balance between the dissociation of carboxylic and amine groups of protein molecules. In addition, as illustrated in **Table 1**, the EAI decreased with the increase of SrPI content. In fact, at low concentration, the adsorption of protein at the water-oil interface is controlled by the diffusion process. In contrast, higher concentrations of proteins limit protein-protein interactions involved in the formation of the interfacial membrane around droplets oil. Additionally, the microscopic images of the different emulsions are presented in **Fig. 1b**. Under the alkaline condition, lower droplet diameters were noted with the homogeneous distribution. In contrast, regarding SrPI concentration, images of emulsions elaborated at pH 2.0 presented polydisperse droplets along with a large diameter.

3.2. Physicochemical properties of the emulsions

The emulsion properties depend on various parameters such as the emulsification process and the type and concentration of the polymer. The present study assesses the effect of five different formulations consisting of either SrPI alone or SrPI/MD mixtures at four different ratios (1:1, 1:2, 1:3 and 1:4; w/w). Emulsions were obtained by homogenization followed by sonication. The creaming index, Z-potential, and droplet size values of the different emulsions are determined and presented in **Table 2**.

In term of stability, the creaming index of all emulsions was assessed after incubation during 24 h at 25 °C (**Table 2**). There was no phase separation detected for emulsions prepared using SrPI and MD ratios 1:2, 1:3 and 1:4. Hence, the MD incorporation seemed to be an efficient strategy for improving emulsion stability of sardinelle protein isolate.

Further, the Z-potential analysis was carried out to evaluate the surface charge and the stability of emulsion. Emulsions with a Z-potential over -30 mV are stable against flocculation, owing to mutual electrostatic repulsion [30]. Overall, negative Z-potential values, ranging from 19.10 to 24.7 mV were obtained for emulsions, indicating that emulsions exhibited good stability and no aggregation tendency (**Table 2**). The negative charge could be attributed to the anionic group (COO⁻) of protein. Moreover, emulsions prepared using SrPI/MD ratios over than 1:1 showed no flocculation over 30 days of storage period. A similar finding has been reported by Gould and wolf [31]. In addition, the results reveal that the surface charge of emulsions increased with the increase of MD content in SrPI/MD

complexes, leading to more stable droplets. Indeed, the Z-potential value (-24.7 mV) of emulsions prepared at SrPI/MD ratio of 1:4 was higher than that prepared at SrPI/MD ratio of 1:0 (-19.1 mV).

Regarding the droplet size measurements, results reveal that the smallest particles size was observed in the emulsion containing SrPI only as wall material (3.79 μm). However, the size of emulsion oil droplet increased gradually with increasing SrPI/MD ratio, and the highest droplet size was observed with SrPI/MD ratio of 1:3 (8.68 μm). This could be explained by a reducing emulsifying capacity of formulations containing MD. Therefore, the SrPI/MD ratios had a significant influence on the oil droplet size.

The same trend was reported in the study of Bae and Lee [32] when the amount of whey protein isolate into MD decreases in emulsions. SrPI had an interesting emulsifying property, while MD is known by its lack of surface binding activities at oil/water interfaces. Thus, the increase in the MD concentration *vs* the amount of the SrPI in the formulations reduced the emulsifying ability of the wall material.

3.3. Characterization of the microcapsules

3.3.1. Moisture content, drying yield, zeta potential and particle size distribution

The moisture content is an important parameter, which gives an idea of the spray-dried microcapsules stability during storage. As seen in **Table 3**, the moisture content of the microcapsules ranged from 4.5% to 6%. The moisture content values are related to wall material composition [33]. In addition, the drying yield increased significantly from 40.33% to 83.3% with SrPI/MD ratio increase ($p < 0.05$). Thus, the mass loss for SrPI/MD 1:0 microcapsules could be explained by the adhesion of powder and oil to the cyclones of the apparatus, depending on their wall material composition [34, 35]. Additionally, the Z- potential values of all microcapsules were ranging from -18.54 mV to -25.59 mV (**Table 3**), and which are similar to surface charge of emulsion before drying process. The values decrease with increasing MD concentration in the SrPI/MD complexes.

In another aspect of the study, the particle size distribution (PSD) (**Fig. 2**) and the mean particle size (**Table 3**), as a function of SrPI/MD ratios, were determined. As mentioned in **Fig. 2**, PSD curves of all microcapsules show a distinct monomodal distribution. Furthermore, the microparticles prepared with only SrPI exhibited a thin peak shape, with diameters ranging from 2.15 to 3.77 μm , compared to those elaborated using SrPI/MD mixtures, which had broad peaks. In addition, results, illustrated in **Table 3**, reveal that the mean diameter of capsules increased with MD concentration increase. The particles size of microcapsules depends on the functional properties of wall polymers. Thus, the increase in the MD

concentration vs the amount of the SrPI in the formulations reduced the emulsifying ability of the wall material. Besides wall polymers characteristics, methods of nanoparticles preparation could affect particle sizes. In this context, Esfahani, Jafari, Jafarpour & Dehnad [36] reported that the homogenization speed is the main variable affecting particle size.

3.3.2. Encapsulation efficiency

The encapsulation efficiency of corn oil, selected as core material, was determined after spray drying. The EE values were ranged from 20.12 % to 77.52%, depending on the wall material composition (**Table 3**). Low EE (20.12%) was noted for SrPI using only as wall material, which may be explained by their small size. Interestingly, the addition of MD to the SrPI solution increased the EE of the microcapsules. The microcapsules prepared using SrPI/MD ratio of 1:4 displayed the highest oil EE (75.25%).

Several works also reported that microcapsules obtained by both homogenization and sonication processes had the highest encapsulation degree than those obtained by only homogenization [35, 37].

These findings suggested that oil entrapment was affected by the proportion of SrPI/MD ratio. Indeed, several works reported that the type and the composition of the wall material influences the emulsifying properties of the solution, and thereby, the encapsulating efficiency of the oil [23]. Gharsallaoui et al. [17] reported that the increase of total solids MD content in emulsions led to the reinforcement of the wall matrix structure during the spray drying step. Thus, despite its poor emulsifying ability, the MD may act as a plasticizer agent during the drying process. Similarly, Rosenberg, Rosenberg & Zhang [38] reported that microencapsulation of soy oil was influenced, to a certain degree, by the composition of the wall materials. Several other factors could affect encapsulation efficiency (EE) of microencapsulated oils such as properties of the core materials (concentration, volatility), the conditions of the spray drying process, particle size distribution, etc. [39]. High EE of orange peel oil in microcapsules based on pectin/whey protein as wall materials were also reported by Ghasemi, Jafari, Assadpour and Khomeiri [40]. Beside the type and composition of encapsulated materials, authors reported that pH values influenced the encapsulation efficiency. The EE of orange peel oil at pH 3.0, 6.0 and 9.0 were 88, 84 and 74%, respectively.

In summary, microcapsules prepared using SrPI/MD ratio of 1:4 had the largest droplet size and the highest encapsulated oil.

3.3.2. Fourier Transform Infrared (FTIR) analysis

FTIR analysis was conducted to provide information about the interactions established between the SrPI and the MD, by the detection of changes in the intensity of IR absorption peaks of samples. The spectra of biopolymers applied in the microencapsulation are illustrated in **Fig. 3a**. The FTIR curve of SrPI powder presented the characteristic bands of proteins at 3292 cm^{-1} (Amide A, N-H or O-H), $2924\text{-}2835\text{ cm}^{-1}$ (amide B), 1641 cm^{-1} (Amide I, C=O and C-N stretching vibration), 1536 cm^{-1} (Amide II, C-N and N-H), and 1397 cm^{-1} (Amide III) [41]. Regarding charged microcapsules spectra, specific bands of oil are detected in all microcapsules with unchanged frame after the encapsulation process, demonstrated the presence of oil in the microcapsules. In the case of microcapsules prepared with MD and SrPI mixtures, two novel characteristic bands of MD are observed at 1024 cm^{-1} , assigned to C-O-C stretching group, and at 931 cm^{-1} , corresponding to the C-O stretching group for MD.

Furthermore, a reduction on the wavenumber of Amide I from 1641 cm^{-1} for SrPI to 1627 cm^{-1} for microcapsules prepared with both SrPI and MD was noted (Fig. 2b). This finding may be related to the formation of hydrogen interactions among SrPI and MD polymers. Otherwise, the shift of the Amide I intensity could be also attributed to the conversion of the primary amino groups to the secondary amine groups during the spray drying process.

3.4. Microcapsule morphology

SEM micrographs of the spray dried microparticles are shown in **Fig. 3b**. All microcapsules hold a spherical shape and an irregular surface, as a result of the spray drying process. In addition, no apparent cracks were detected in the external morphology, suggesting low gas permeability and thereby, high protection of the active ingredient against oxidation.

Similar morphological characteristics were found by Tonon, Grosso, & Hubinger [42] and Carneiro et al. [19]. Le et al. [20] also reported that microcapsules prepared using whey protein and MD displayed intact surface with no formation of cracks.

3.5. Thermogravimetric analysis

The thermogravimetric analysis permits to evaluate the stability of the encapsulate systems exposed to high-temperature treatment such as pasteurization, cooking, spray-drying. The decomposition temperature (Td) was determined using the DTG curves, which correspond to the maximum weight loss. As seen in **Fig. 4a**, the DTG profile of free oil revealed mass losses at the range of $400\text{-}440\text{ }^{\circ}\text{C}$, while, two degradation stages were distinguished in SrPI curve. The first one, around $100\text{ }^{\circ}\text{C}$, ascribed to the evaporation of water and the main one, which took place at a temperature range of $220\text{-}350\text{ }^{\circ}\text{C}$, characterizes the decomposition of myofibrillar proteins. Besides, the Td of MD powder occurred at $310\text{ }^{\circ}\text{C}$.

The DTG thermogram of microcapsules prepared using only SrPI (SrPI/MD, 1:0) showed decomposition peaks, at 310 and 390 °C, matching with SrPI and oil, respectively (**Fig. 4b**). Regarding DTG thermograms of microparticles prepared using SrPI/MD mixtures, results show a novel Td, around 200-220 °C, which could be attributed to the SrPI/MD complexes. A slight decrease in the Td of corn oil from 400 °C to 390 °C was observed in microparticles obtained using SrPI/MD ratios of 1:0 and 1:1. Nevertheless, the increase of the MD content leads to the preservation of Td of corn oil. At a temperature of 400 °C, distinct differences in weight loss between samples could be detected. The highest loss percentages of 50% were for the microcapsules prepared by only SrPI compared to system elaborated by SrPI/MD mixture (31-35%) and free corn oil (54%).

These findings indicate the importance of incorporation of MD to the SrPI as carrier agents in the spray drying of oil to increase the thermal stability of the powder.

3.6. X-ray diffraction analysis

X-ray diffraction analysis is widely applied to assess crystalline and amorphous structures. In fact, the crystalline solids provide sharp and well-defined peaks, while amorphous molecules present a large band [43].

As seen in **Fig. 5**, the diffractograms of various capsules and the biopolymers of the wall matrix showed broad peaks, indicating an amorphous structure of all samples. In addition, SrPI powder and microcapsules prepared using only SrPI present one peak at 2θ of 12.97°, typical diffraction peak of protein. Regarding the XRD patterns of the other microcapsules, the addition of MD to the wall matrix formulation occurred with the apparition of a novel diffraction peak at 2θ of 19.4°, which is assigned to the diffraction of X-ray by MD [22]. Furthermore, the incorporation of MD, at different concentrations to SrPI, generated also amorphous carrier state. A similar results and patterns are reported in the studies of Silva et al. [18] and Campelo et al. [22]. However, Zhou et al. [22] reported that microcapsules prepared using soy protein isolate and MD give sharp peak, due to the formation of crystalline state in the microcapsules. It is interesting to note that amorphous materials exhibit higher water evaporation ability during spray drying process. Therefore, their application, as carrier agent, is benefic and recommended in food dry process, to obtain high food powder properties and stability. Marabi et al. [45] reported that formulations prepared using amorphous structures present high solubility compared to the crystalline component, due to the low energy of bonds between molecules.

As consequently, the obtained data indicate that microcapsules prepared using maltodextrin and SrPI mixtures preserve amorphous character after spray drying process. This

structural property is extremely desirable in particles powders which improves their homogeneity, solubility and enhance their incorporation.

4. Conclusion

The study described a spray-drying microencapsulation using a combination of SrPI and MD as carrier coatings for enhancing corn oil stability. Indeed, physicochemical characterization showed that the increase of MD content in the SrPI/MD complexes resulted in the increase of the mean diameters of emulsion and oil stability (DTG). Further it was found that the EE was highly improved when mixtures of SrPI with MD were used, while SrPI used alone as wall material resulted in low EE. In addition, after spray drying process, sphere-shaped microcapsules were found. The efficacy of SrPI/MD blend to entrap and protect oil was confirmed through FTIR, XRD and DTG analyses. The appropriate SrPI/MD ratio was 1:4 under which the EE was around 77.52%.

According to these results, a combination of SrPI and MD as wall materials allowed the preparation of microcapsules with desired properties. Further, the obtained results indicated the high emulsifying properties and the potential of SrPI (obtained from sardinelle by-products) as an alternative and highly microencapsulating agent in food and related applications.

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Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Author statement

Rim Nasri: Conceptualization, Methodology, Writing- Original draft preparation. **Wafa Taktak:** Investigation. **Marwa Hamdi:** Formal Analysis. **Nadia Ben Amor:** Formal Analysis, **Ahlem Kabadou:** Supervision. **Suming Li:** Supervision. **Moncef Nasri:** Supervision, Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Figures caption:

Fig. 1.(a) Effect of pH on SrPI solubility, (b) Optical microscopic observation of the SrPI emulsions at different pH values after 24 h (magnitude 400 x).

Fig. 2. Particle size distribution of microcapsules produced with different ratios of SrPI/MD as wall material. (SrPI: Sardinelle protein isolate: MD: Maltodextrin).

Fig. 3. FTIR profiles (a) and SEM images (b) of microcapsules produced with different ratios of SrPI/MD as wall material. (SrPI: Sardinelle protein isolate: MD: Maltodextrin).

Fig. 4.ATG and DTG thermograms of MD powder, SrPI powder, free corn oil (a) and microcapsules produced with different ratios of SrPI/MD as wall material (b). (SrPI: Sardinelle protein isolate: MD: Maltodextrin).

Fig. 5. XRD spectra of SrPI powder and microcapsules produced with different ratios of SrPI/MD as wall material. (SrPI: Sardinelle protein isolate: MD: Maltodextrin).

Table1: Emulsifying activity (EAI) indices of SrPI at pH 2.0 and 11.0.

	pH 2.0	pH 11.0
Concentration (%)	EAI (m2/g)	EAI (m2/g)
0.5	67.52 ± 1.01 ^b	84.80 ± 1.55 ^d
1	28.50 ± 0.76 ^c	40.50 ± 0.32 ^c
2	21.24 ± 1.51 ^b	25.24 ± 0.26 ^b
4	7.42 ± 0.39 ^a	12.88 ± 0.38 ^a

Results are the means of three determinations ± standard deviation. Different letters in the same column indicate a significant difference (p <0.05). EAI: Emulsifying Activity Index; SrPI: Sardinelle protein isolate.

Table 2: Z-potential value, mean particle size, creaming index of emulsions produced with different ratios of SrPI/MD as wall material measured 24 h after emulsion formation and storage at 25 °C.

SrPI/M D ratios	Z- potential (mV)	Mean particle size (µm)	Creaming index (%)
1:0	-19.10 ± 0.023 ^a	3.79	81.5± 2.5 ^a
1:1	-20.70 ± 0.001 ^b	5.00	97.65±1.21 ^b
1:2	-21.00± 0.019 ^c	6.63	100±0.00 ^c
1:3	-21.30± 0.035 ^c	8.68	100±0.00 ^c
1:4	-24.7± 0.005 ^d	7.97	100±0.00 ^c

Results are the means of three determinations ± standard deviation. Different letters indicate a significant difference between samples at $p < 0.05$. SrPI: Sardinelle protein isolate. MD: maltodextrin.

Table 3: Drying yield, moisture content, encapsulation efficiency (EE), z-potential and mean particle size values of microcapsules produced using SrPI/MD mixture.

SrPI/ MD ratio	Drying yield* (%)	Moisture content**(%)	EE*** (%)	Z- potential (mV)	Mean particle size (μm)
1:0	43.33 \pm 1.48 ^a	6.04 \pm 0.4 ^a	20.12 \pm 3.89 ^a	-18.54 \pm 0.025 ^a	2.32
1:1	54.65 \pm 2.08 ^b	5.75 \pm 0.1 ^a	60.85 \pm 0.48 ^b	-22.53 \pm 0.011 ^b	6.62
1:2	66.7 \pm 2.00 ^c	5.0 \pm 0.15 ^c	60.56 \pm 0.60 ^b	-22.59 \pm 0.062 ^b	5.22
1:3	75 \pm 2.30 ^d	4.5 \pm 0.05 ^d	64.00 \pm 3.44 ^c	-24.5 \pm 0.025 ^c	6.56
1:4	83.3 \pm 1.58 ^e	4.5 \pm 0.16 ^e	77.52 \pm 2.00 ^d	-25.59 \pm 0.17 ^d	6.14

Results are the means of three determinations \pm standard deviation. Different letters indicate a significant difference between samples at $p < 0.05$. SrPI: Sardinelle protein isolate. MD: maltodextrin.

* g recovered powder.100 g dry solid⁻¹.

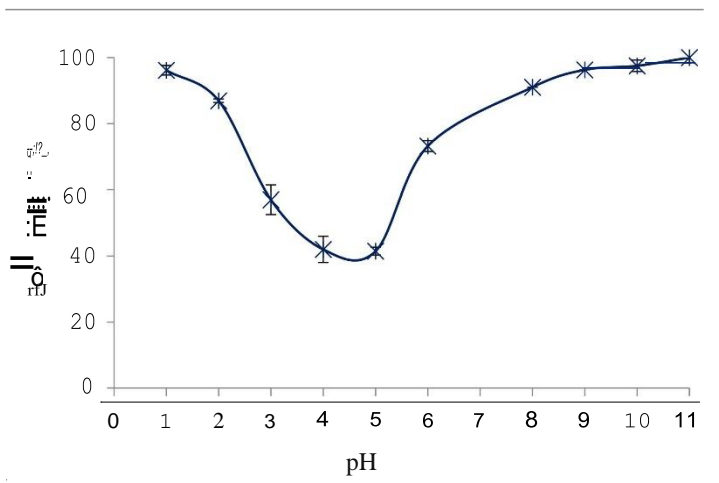
**g water.100 g dry solid⁻¹.

*** g corn oil.100 g microcapsules⁻¹.

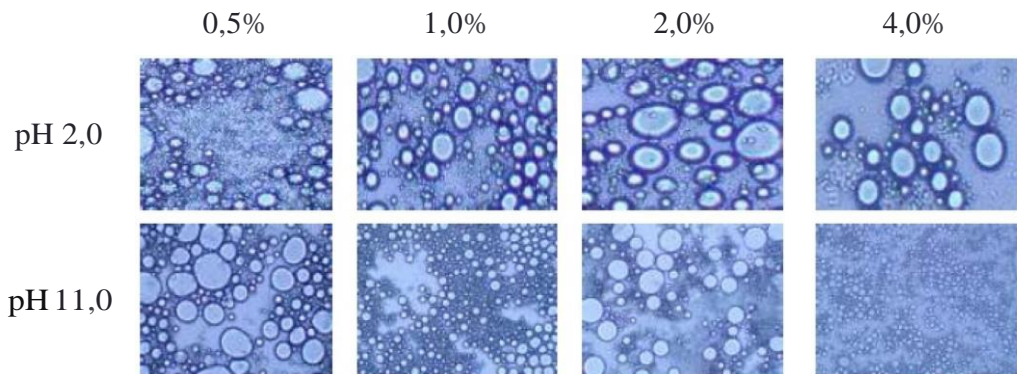
Highlights:

1. The potential of protein isolate from sardinelle by-products (SrPI) combined to maltodextrin (MD) to encapsulate corn oil was investigated.
2. Microencapsulation yield and encapsulation efficiency (EE) of oil were determined.
3. Highest EE and thermal stability was obtained with particles prepared using SrPI/MD ration of 1:4.
4. Incorporation of MD to the SrPI solution enhanced the protection and the encapsulation of corn oil.

a)



b)



Figur e 1

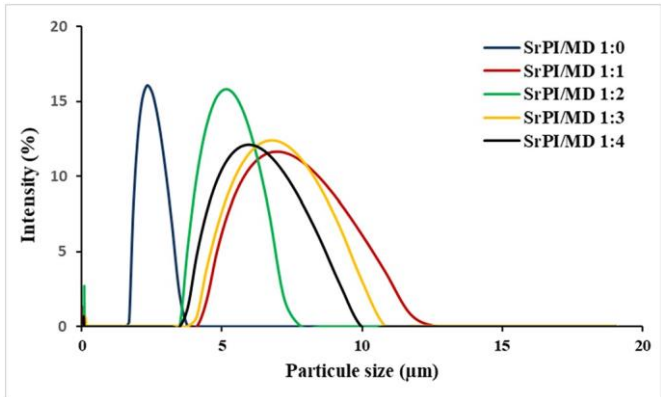
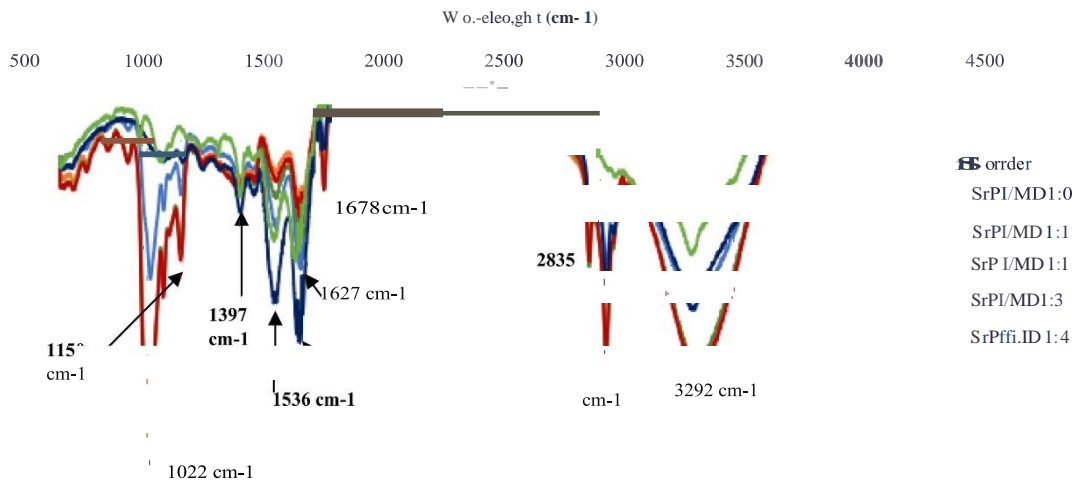


Figure 2

a)



b)

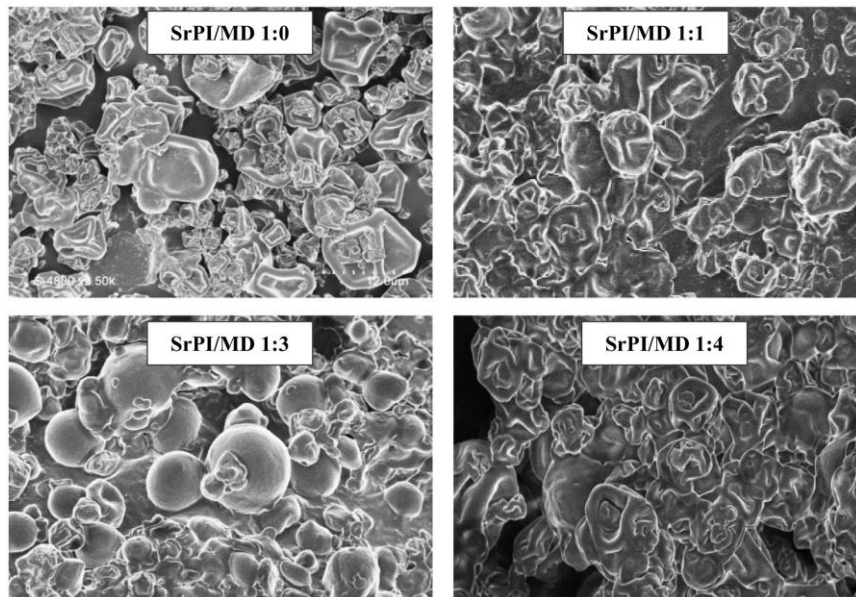


Figure 3

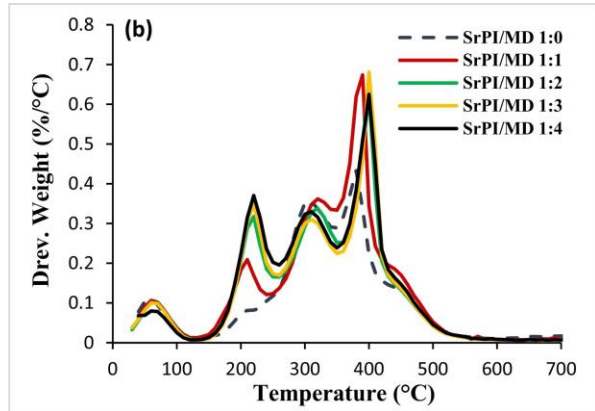
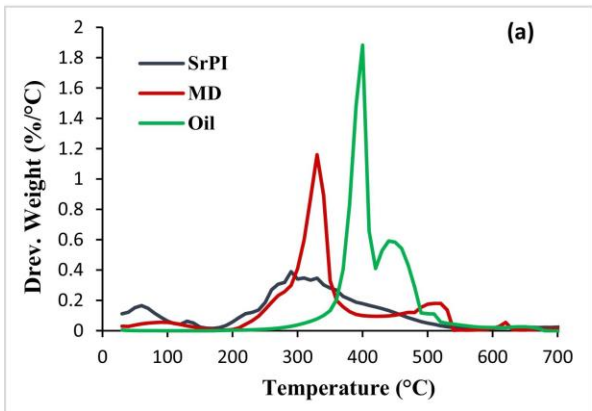


Figure 4

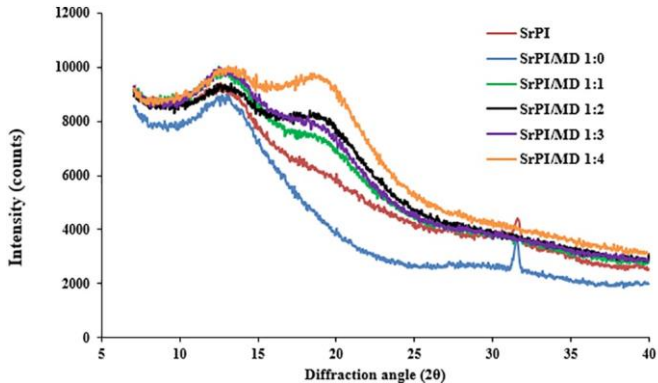


Figure 5