Preparation of Unsaturated Fatty Acids/Chitosan Microcapsules: Influence of Solvent

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Summary: In this work, the effect of solvent type on the characteristics of unsaturated fatty acids/chitosan microcapsules was evaluated. The unsaturated fatty acids were obtained from carp oil and chitosan was obtained from shrimp wastes. The emulsion containing microcapsules was obtained from an aqueous and an oil phases. In the oil phase, ethanol and hexane were evaluated as solvents. The microcapsules were characterized according to the encapsulation efficiency, peroxide values and scanning electron microscopy. The results revealed that the microcapsules with ethanol and hexane showed encapsulation efficiencies of 79.6 and 69.4%, respectively. The microcapsules with ethanol showed lower peroxide values, and these values were maintained during 30 days. Furthermore, these microcapsules were spherical and irregular, with mean diameter ranging from 0.5 to 2.5 μm. These results indicated that the ethanol was the more adequate solvent to obtain unsaturated fatty acids/chitosan microcapsules.

Keywords: carp oil; chitosan; ethanol; hexane; microcapsules

Introduction

The consume of nutritionally rich foods is a trend worldwide.[1] This new life model caused an increase in the search for foods with lower fatty content. However, fats are essential nutrients to the human health, being the more concentrated energy source, and diets with lower fatty content can provide inadequate contents of fat–soluble vitamins and essential fatty acids.[2] The recent changes in human diet and the appearance of diseases related with the low fatty acids consume, led to an interest for unsaturated fatty acids from various sources.[1] Fish oil is an alternative source to obtain essential fatty acids with therapeutic characteristics.[3]

The enrichment of foods with concentrated fish oil can increase the consume of essential fatty acids.[4] However, the production of enriched foods with essential fatty acids is difficult due to the high sensibility and oxidative degradation.[5] To avoid the oxidative degradation, it is necessary the use of special technologies, for example, encapsulation.[6] The encapsulation can protect these unsaturated fatty acids regarding to the oxidation and enzymatic reactions during the production process, increasing the shelf life and preventing losses in the metabolic value.[5]

The preparation of microcapsules by emulsion followed drying is one of the most common methods to the fatty acids encapsulation. The microcapsules are composed by a nucleus with unsaturated fatty acids, covered with a polymeric barrier. In this field, chitosan is a potential biomaterial to act as biopolymeric barrier. Chitosan is a cationic biopolymer derived from natural sources, and has been studied as a promising coating material due its adhesivity, biodegradability and film forming properties.[7–9]

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Apart from chitosan, solvents and emulsifying agents are necessary to obtain the unsaturated fatty acids microcapsules. The solvent type is one of the most important factors that influence the microcapsules characteristics.[5] Based on the above mentioned, this work aimed to verify the effect of solvent type (ethanol and hexane) on the characteristics of unsaturated fatty acids/chitosan microcapsules.

Material and Methods

Obtainment and Characterization of Unsaturated Fatty Acids

The unsaturated fatty acids (PUFAS and MUFAS) were obtained from carp oil (*Cyprinus carpio)*. The carp oil was extracted from carp viscera, which were provided from a commercial fish–processing plant located in Roca Sales–RS–Brazil. Firstly, 3.0 kg of carp viscera were cooked at 95°C for 30 min. The resultant material was pressed and centrifuged to obtain the crude oil.[3] After, the crude oil was refined by the steps of degumming, neutralization, washing, drying and bleaching.[10] Thus, the refined carp oil was obtained. The refined carp oil was submitted to the chemical hydrolysis with KOH in order to obtain free fatty acids (FFA). The FFA were submitted to the urea complexation, being the PUFAS and MUFAS, obtained in the non–urea complexing fraction.[11] The reaction yield (%R) was obtained gravimetrically,[12] according to the Eq. 1:

$$\%R = \frac{A_{FFA}}{A_{RO}} \times 100 \quad (1)$$

where, A_{FFA} is the amount of free fatty acids (g), A_{RO} is the amount of refined oil used in the reaction.[2]

For the PUFAS and MUFAS, the free fatty acids (FFA, Ca 5a–40) and peroxide values (PV, Cd 8–53) were determined according to the American Oil Chemists Society methodologies.[13] For fatty acids identification and quantification, chromatographic analysis was carried out. Fatty acid profiles were determined by preparation of methyl esters.[14] The fatty acid methyl esters (FAME) were identified by gas chromatography (GC) (Varian 3400 CX, Palo Alto, CA) equipped with an Agilent DB–23 (Santa Clara, CA) capillary column ($60\,m \times 0.25\,mm$, $0.25\,\mu m$ film thickness). FAME analysis was carried out in duplicate. The GC conditions were performed according to Crexi *et al.*[10]

Preparation and Characterization of Chitosan

Chitosan was obtained from shrimp (*Penaeus brasiliensis*) wastes. Firstly, 7.0 kg of shrimp wastes were submitted to the demineralization with 14 L of HCl solution $2.5\,g\,L^{-1}$ in stirred tank during 2 hours at $25 \pm 1°C$. Secondly, deproteinization step was carried out using 21 L of NaOH solution $5.0\,g\,L^{-1}$ (in stirred tank during 2 hours at room temperature). Then, deodorization step was realized with 35 L of NaClO solution $0.4\,g\,L^{-1}$ under agitation during 3 hours. After each above step, washings were done to remove the remaining solutions. Finally, chitin was dried, ground (Wiley Mill Standard, model 03, USA) and sieved until the discrete particle size ranging from 105 to 125 μm.[15] Chitosan paste was obtained by alkaline deacetylation of chitin (NaOH $421\,g\,L^{-1}$, at $130 \pm 1°C$ for 90 min), followed by purification according to Moura et al.[16] Chitosan paste was dried in spouted bed to obtain a chitosan powder.[17]

The average molecular weight of chitosan was determined by viscosimetric method. Reduced viscosity was determined by Huggins equation, and converted in molecular weight through Mark–Houwink–Sakurada equation.[18] Chitosan characteristic bands and deacetylation degree were determined by FT–IR analysis (Prestige 21, 210045, JPN), using diffuse reflectance in potassium bromide. Deacetylation degree was calculated according to 2:[19]

$$\%DD = 87.8 - [3(A_{C=O}/A_{-OH})] \quad (2)$$

where, %DD is deacetylation degree (%), $A_{C=O}$ is absorbance of C=O group, and A_{-OH} is absorbance of —OH group.

Preparation and Characterization of Unsaturated Fatty Acids/Chitosan Microcapsules

The suspensions containing microcapsules were prepared by the emulsion–solvent evaporation method.[5] Firstly, two phases were prepared: an aqueous phase (100 mL) and an oil phase (100 mL) (all dilutions were made with ultrapure water). In the aqueous phase, chitosan (2.0% m/v) was mixed with acetic acid (1.0%). The solution was stirred for 24 h at 25 ± 1°C, until the complete dissolution of chitosan. In the oil phase, the unsaturated fatty acids (1.0% m/v) and the surfactant agent (tween 80) (0.1% m/v) were dissolved in ethanol or hexane. The solution was stirred for 2 h at 25 ± 1°C. Finally, the aqueous and oil phases were homogenized at 20000 rpm for 5 min (Dremel, 1100–01, Brazil). Then, the emulsion containing microcapsules was lyophilized (Indrel, IULT 90–D, Brazil). The experiments were made in triplicate (n = 3) and the conditions were based in the literature.[1,2,4–9]

The encapsulation efficiency was obtained from the indirect method (2 mL of acetone were added into 1 mL of emulsion for precipitation. The samples were centrifuged at 14000 rpm and 3 °C for 30 min (Cientec, CT5000R, Brazil). The supernatant containing the non–encapsulated fatty acids was removed and dried at 36°C until solvent evaporation). In the lyophilized material the lipids were determined by Bligh Dyer.[3] The encapsulation efficiency (%EE) was calculated by the 3:

$$\%EE = \frac{UFA_{enc} - UFA_{ne}}{UFA_{enc}} \times 100 \qquad (3)$$

where, UFA_{enc} is the lipid fraction into the lyophilized microcapsules, and UFA_{ne} the supernatant fraction containing the non–encapsulated fatty acids.

The oxidation of the encapsulated fatty acids was analyzed by the peroxide values.[3] Samples were analyzed at the end of lyophilization (zero time) and after 15 and 30 days of storage at 4°C. Furthermore, the unsaturated fatty acids/chitosan microcapsules were characterized by scanning electron microscopy (SEM) (Jeol, JSM–6060, Japan).

Results and Discussion

Characteristics of PUFAS and MUFAS

Table 1 shows the free fatty acids (FFA), peroxide value (PV) and reaction yield (%R) for the non–urea complexing fraction oil obtained from carp viscera. Table 2 shows the profile of the major fatty acids for non– urea complexing fraction.

From Table 2, it can be verified that C – 18:1 and C – 18:3 were the fatty acids with higher content in the non – urea complexing fraction, corresponding to approximately 41.3%. The fatty acids of series

Table 1.
Free fatty acids (FFA), peroxide value (PV) and reaction yield (%R) for the non – urea complexing fraction oil obtained of carp viscera.

Index	Value*
FFA (% oleic acid)	38.8 ± 0.8
PV (mEq. peroxide/kg)	3.1 ± 0.3
%R (%)	32.3 ± 1.5

Mean values ± standard error (n = 3).

Table 2.
Fatty acids profile for non – urea complexing fraction oil obtained of carp viscera.

Fatty acids	Non – urea complexing fraction (%)*
C 16:0	4.10 ± 0.03
C 16:1	9.40 ± 0.02
C 18:0	0.31 ± 0.01
C 18:1+ C 18:3 ω – 3	41.28 ± 0.04
C 18:2 ω – 6	15.21 ± 0.03
C 20:3 ω – 3	1.43 ± 0.01
C 20:4 ω – 6 (AA)	2.61 ± 0.01
C 20:5 ω – 3 (EPA)	4.57 ± 0.02
C 22:6 ω – 3 (DHA)	4.86 ± 0.01
Σ SFA (%)	8.81
Σ MUFA (%) + Σ PUFA (%)	85.24
Σ UFA (%)	5.95

Mean values ± standard error (n = 3). AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Σ SFA: sum of saturated; Σ MUFA: sum of monounsaturated; Σ PUFA: sum of polyunsaturated; Σ UFA: sum of unidentified fatty acids.

Table 3.
FT–IR bands and respective assignments for chitosan.

Bands (cm^{-1})	Assignments
3020	Broad band relative to the O—H and N—H stretchings.
1700	C=O vibration of amide band I
1550	C—N stretchings of amide
1400	C—O—H and H—C—H links
1075	C—N of amine links
1000	C—O links
680	Angular deformation of the N–H links

ω – 3 (C – 18:3, C – 20:3, C – 20:5, C – 22:6) accounted for approximately 52.1% of fatty acids, while, C – 18:2 was the highest of ω – 6 series (about 15%). These results were similar to Crexi et al.[10] and indicated that carp viscera refined oil can be considered a rich source of essential fatty acids.

Chitosan Characteristics

Chitosan powder presented average molecular weight of 154 ± 5 kDa and deacetylation degree (%DD) of 86 ± 1%. Chitosan characteristic bands were determined by FT–IR analysis and the results are shown in Table 3.

In Table 3, the chitosan characteristics bands can be observed. These bands confirm the presence of amino and hydroxyl groups on the chitosan structure. The chitosan amino and hydroxyl groups are the responsible for its unlimited application potential for use in a wide range of faculties, including oil encapsulation.[20]

Characteristics of Unsaturated Fatty Acids/Chitosan Microcapsules

The emulsions containing microcapsules were prepared, and, from the initial analyses, it was found that the solvents, ethanol and hexane with 15% of concentration were capable to form emulsions. The visual evaluation showed that the emulsions were stable during 24 h after homogenization. The textural characteristics of the lyophilized microcapsules are shown in Figure 1.

From SEM images, it can be observed, for both solvents, that the microcapsules were spherical and irregular, with mean diameter ranging from 0.5 to 2.5 μm. The microcapsules prepared with hexane (Figure 1b) were more irregular and agglomerated. This can be occurred because hexane is a non–polar solvent. So, when mixed with polar solvents (in aqueous phase), hexane caused repulsion of the microcapsules, leading to the agglomeration. On the other hand, ethanol (Figure 1a) caused a high dispersion, due its polar structure. Similar trend was found by Kolanowski et al.,[4] which obtained fish oil microcapsules with an irregular structure and mean diameter of 27 μm.

The microcapsules with ethanol and hexane showed encapsulation efficiency of 79.6 and 69.4%, respectively. These results

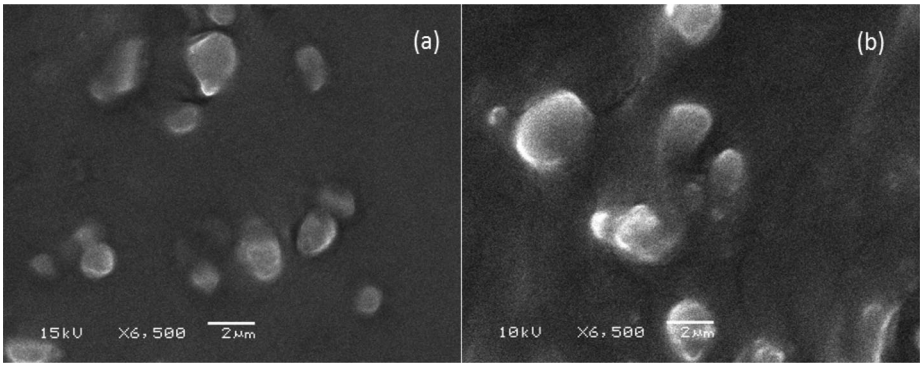

Figure 1.
SEM images of unsaturated fatty acids/chitosan microcapsules: (a) prepared with ethanol as solvent and (b) prepared with hexane as solvent.

Table 4.
Peroxide values (mEq. peroxide/kg) for the unsaturated fatty acids encapsulated with chitosan.

Solvent	Zero time*	15 days*	30 days*
Ethanol	3.77 ± 0.20^{a}	3.96 ± 0.18^{a}	3.95 ± 0.10^{a}
Hexane	5.76 ± 0.17^{b}	5.91 ± 0.13^{b}	5.99 ± 0.15^{b}

Mean values ± standard error (n = 3). Same letters in same line indicate $p > 0.05$.

coupled with SEM images indicated that ethanol was more adequate to improve the microcapsules stability. Yuen et al.,[21] in the encapsulation of pharmaceuticals with chitosan, obtained values of encapsulation efficiency ranging from 56.66 to 96,81%. Klaypradit and Huang,[22] studying the production of fish oil/chitosan microcapsules, found values from 79 and 83%. The peroxide values for the unsaturated fatty acids encapsulated with chitosan are shown in Table 4.

From the results presented in Table 4, it can be observed that all samples were within the legislation limit (mEq. peroxide/kg).[23] When ethanol was used, lower peroxide values were found, indicating that this solvent was more adequate to preserve the microcapsules in relation to the primary oxidation (Table 4). Furthermore, it was not found significant difference ($p > 0.05$) in the peroxide values during 30 days. This shows that the encapsulation with chitosan is a good way to avoid the primary oxidation of the unsaturated fatty acids during this time.

Conclusion

In this work, unsaturated fatty acids/chitosan microcapsules were prepared and the effect of solvent type (ethanol or hexane) on the microcapsules characteristics was investigated. The emulsion containing microcapsules was formed using both solvents. The microcapsules with ethanol and hexane showed encapsulation efficiency of 79.6 and 69.4%, respectively. The microcapsules produced with ethanol presented lower peroxide values, indicating lower primary oxidation of the unsaturated fatty acids encapsulated with chitosan. These values were maintained during 30 days. In summary, ethanol was the more adequate solvent to obtain microcapsules with good textural characteristics, and the encapsulation with chitosan was a good alternative to avoid the primary oxidation of the unsaturated fatty acids.

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[1] R. B. Derner, S. Ohse, M. Villela, S. M. Carvalho, R. Fett, *Ciênc. Rural.* **2006**, *36*, 1959.
[2] P. Zimet, Y. D. Livney, *Food Hydrocolloid.* **2009**, *23*, 1120.
[3] V. T. Crexi, M. L. Monte, M. L. Monte, L. A. A. Pinto, *J. Am. Oil Chem. Soc.* **2012**, *89*, 329.
[4] W. Kolanowski, M. Ziolkowski, J. Weißbrodt, B. Kunz, G. Laufenberg, *Eur. Food Res. Technol.* **2006**, *222*, 336.
[5] N. T. Huynh, C. Passirani, P. Saulnier, J. P. Benoit, *Int. J. Pharm.* **2009**, *379*, 201.
[6] E. M. Kim, C. J. Steel, Y. K. Chang, *Braz. J. Food Technol.* **2005**, *8*, 268.
[7] P. Calvo, C. Remuñan-Lopez, J. L. Vila-Jato, M. J. Alonso, *Colloid. Polym. Sci.* **1997**, *27*, 46.
[8] S. A. Agnihotri, N. N. Mallikarjuna, T. M. Aminabhavi, *J. Control. Rel.* **2004**, *100*, 5.
[9] C. Prego, M. Garcia, D. Torres, M. J. Alonso, *J. Control. Rel.* **2005**, *101*, 151.
[10] V. T. Crexi, M. L. Monte, L. Souza-Soares, L. A. A. Pinto, *Food Chem.* **2010**, *119*, 945.
[11] R. M. Paim, M. L. Monte, J. Rizzi, L. A. A. Pinto, *Vetor.* **2012**, *22*, 18.
[12] AOAC. **1995**, Official Methods of Analysis. Ed. AOAC.
[13] AOCS. **1980**, American Oil Chemists Society. Ed. AOCS.
[14] L. D. A. A. Metcalfe, J. R. Schimitz, *Anal. Chem.* **1966**, *38*, 510.

[15] R. F. Weska, J. M. Moura, L. M. Batista, J. Rizzi, L. A. A. Pinto, *J. Food Eng.* **2007**, *80*, 749.
[16] C. M. Moura, J. M. Moura, N. M. Soares, L. A. A. Pinto, *Chem. Eng. Process.* **2011**, *50*, 351.
[17] G. L. Dotto, V. C. Souza, L. A. A. Pinto, *LWT Food Sci. Technol.* **2011**, *44*, 1786.
[18] H. Zhang, S. H. Neau, *Biomaterials.* **2001**, *22*, 1653.
[19] M. F. Cervera, J. Heinämäki, M. Räsänen, S. L. Maunu, M. Karjalainen, O. M. N. Costa, A. I. Colarte, J. Yliruusi, *Carbohydr. Polym.* **2004**, *58*, 401.
[20] K. V. Harish Prashanth, R. N. Tharanathan, *Trend. Food Sci. Technol.* **2007**, *18*, 117.
[21] C. W. M. Yuen, J. Yip, L. Liu, K. Cheuk, C. W. Kan, H. C. Cheung, S. Y. Cheng, *Carbohydr. Polym.* **2012**, *89*, 795.
[22] W. Klaypradit, Y. W. Huang, *LWT Food Sci. Technol.* **2008**, *41*, 1133.
[23] ANVISA. **1999**, Brazilian legislation, available in (http://portal.anvisa.gov.br).

