Biopolymer Applications Journal (BAJ) ***Rebiha BELLACHE et al.*** ***Vol 2, N°1, 2023, pp.01-07***

e-ISSN : 2800-1729

Microencapsulation of olive oil by Poly-hydroxy-butyrate-co-valerate / Polyethylene glycol

Rebiha BELLACHE*, Dalila HAMMICHE, Amar BOUKERROU

Laboratoire des Matériaux Polymères Avancés, Département Génie des Procédés, Faculté de Technologie, Université de Bejaia, Algérie.

*Corresponding author email** *rebiha.bellache@univ-bejaia.dz*

Received: 04 January 2022; Accepted: 24 January 2022; Published: 26 January 2023

Abstract

Encapsulation is a technology widely used in the industrial field because it brings many advantages, especially in the pharmaceutical field. Among the existing microencapsulation techniques, microencapsulation by solvent evaporation in simple emulsion remains one of the most used for hydrophobic active ingredients because it does not require specific equipment and uses mild operating conditions (temperature and ambient pressure generally). Which was the subject of our present study which targets to encapsulation the olive oil by polyhydroxy (butyrate-co-valerate) PHBV using the method of evaporation of the organic solvent which is chloroform. The encapsulation yields obtained with respect to the different parameters are between 66 and 80 %, the best of which is obtained with the highest amount of active ingredient, which is 0.4 g/ml. Microscopy allowed us to observe the morphology of the powders obtained, where the latter are in the form of a powder (spherical) and other are aggregates. The macroscopic and microscopic analysis showed that the increase of the emulsion agitation speed, the amount of surfactant percentage and the nature of the latter, allow to modify the size of the microparticles, which has been proved, in agreement with the theory. The FTIR analysis showed the presence of olive oil in the PHBV microparticles, and highlighted the absence of chemical interactions between the polymers and olive oil. Thermal analysis by thermogravimetry confirmed the existence of olive oil in the PHBV microparticles by the presence of two losses of mass, first in the interval (200 to 250) which corresponds to the degradation of oil and another loss after this range of (250 to 400) attributed to the decomposition of the encapsulant (PHBV).

Keywords:*PHBV, PEG, olive oil, Microencapsulation, physico-chemical properties.*

I. Introduction

Polymers and copolymers of lactic and glycolic acids are the most commonly used to develop drug delivery systems due to their safe and authorized use applications in humans [1]. However, other biodegradable polymers have been studied to increase the number of biodegradable materials available for pharmaceutical and medical applications [2]. Poly-hydroxy-butyrate-co-valerateabbreviated usually as PHBV or PHBHV, is naturally occurring biodegradable polymer produced from a wide range of microorganisms [3]. Besides, it is non-toxic, 100% biodegradable, biocompatible with many types of cells, characterized by its high degree of crystallinity, and it is resistant to ultraviolet radiation and acceptable amounts of alcohols, fats, and oils [4] An alternative to giving protection and stability to active compounds is the encapsulation in biopolymers [5], the emulsion solvent evaporation has been the most frequently used microencapsulation method [6].

The oils encapsulation can be defined as a process in which the droplets of the bioactive oil are surrounded by a coating material, or embedded in a homogeneous or heterogeneous matrix, to give small capsules with many useful properties [7]. The oils encapsulation in a practical system is of potential interest and functional for pharmaceutical, food and cosmetic products[8].Other important application areas such as personal care, agricultural products, veterinary medicine, industrial chemicals, biotechnology,and biomedical engineering, are all in the biomedical engineering, are all within the range of interest. Because of the wide range of oils in nature, there is growing interest in the application of encapsulating such oils to fully exploit their fully exploit their beneficial benefits [9].

Krishnan et al.(2005) [10], encapsulated Cardamom oil which possesses potential antibacterial, antioxidant, anticancer, antiseptic, antispasmodic, digestive, diuretic and stimulant activities. The microencapsulation is prepared with a mixture of gum arabic, maltodextrin, and modified starch increased volatile stability.

Umesha et al.(2013) [11], encapsulated the garden cress oil (GCO) using different wall materials such as sodium caseinate (SACA), whey protein concentrate (WPC), blend of maltodextrin and gum arabica (MDGA) and skimmed milk powder (SKM) was examined using spray-drying method. In another work of Silva et al. (2014) [12], studied the influence of different combinations of wall materials

and homogenization pressure on the microencapsulation of green coffee oil by spray drying. The encapsulation of this oil facilitates its use in cosmetic powders and reduces the allergenic effects of cinnamic acid when applied directly to the skin.

Lim et al.(2012) [13], established the effects of different wall materials on the physicochemical properties and oxidative stability of spray-dried microencapsulated red fleshed pitaya (Hylocereuspolyrhizus) seed oil. This microencapsulation with sodium caseinate and whey protein has increased its stability to oxidation. The chitosan-carrageenan polyelectrolyte complex has been successfully used as an effective matrix to encapsulate neem seed oil by Devi et al.(2009) [14]. Kanakdande et al.(2007) [15], studied microencapsulation of cumin oleoresin by the mixture of gum arabic / maltodextrin / modified starch.

In recent years, olive oil has received great attention owing to its biological activities and sensory qualities. It has social and economical importance for the Mediterranean regions [16]. The importance of olive oil is mainly attributed to its high content of oleic acid and its richness in phenolic compounds, which act as natural antioxidants [17].

For this, in this work, we are interested in the encapsulation of olive oil by polyhydroxy (butyrate-co-valerate) or PHBV using the method of evaporation of the organic solvent which is chloroform, the choice fell on the olive oil as an active principle thanks to its important therapeutic properties, its availability and its low cost compared to other oils which present the same characteristics but with a high cost.

II. Materialsand Methods

II.1. Materials

PHBV copolymer used in this work was supplied by TianAn Biologic Materials Co. Ltd (China) with a molar ratio of 92:8 (HB: HV). It was commercialized in the form of pellets under the grade name ENMAT Y1000P.

The olive oil obtained by hot extraction (mountain of bejaia-Algeria).

The PEG used was grade 600 (Mw = 600 g/mol), which acted as a nonionic surfactant, and chloroform used in this study as a solvent was produced by Biochem, chemopharm, (Quebec), were supplied by Biochem Chemopharma (Montreal, Quebec).

II.2. Methods

Preparation of PHBV microcapsules containing olive oil (with different concentrations 0.15g/ml, 0.25 g/ml, 0.4g/ml**)**

The encapsulation technique by solvent evaporation was performed according to the following steps:

- Preparation of the organic phase (solution 1):

A dissolution of a desired amount of PHBV (1.2 g) in chloroform;add different concentrations (0.15g/ml, 0.25 g/ml, 0.4g/ml) of olive oil to this polymeric solution after cooling in 2 minutes.

- Preparation of the aqueous phase (solution 2):

A dissolution of a desired amount of PEG (1 %) in distilled water.

The introduction by drop, of the solution 1 to the solution 2 and leave under agitation;

Medium magnetic stirring is maintained overnight, at room temperature, so that the chloroform is completely removed.

The solution obtained is centrifuged at 6000 rpm for 3 minutes and recovery of the microparticles, then rinsed with distilled water 3 times; Put the microparticles in petri dishes and let dry in a desiccator.

III. Characterizations

- **Encapsulation yield (R%)**

In order to calculate the encapsulation yield we first measured the mass of the microcapsules recovered after centrifugation and is calculated with the following formula:

$$\mathbf{R\ (\%)} = \frac{\text{mass of recovered microcapsules}}{\text{mass of polymer} + \text{mass of olve oil}} * 100$$

This calculation of yield allowed us to deduce which is the best concentration of encapsulation of the active principle by the PHBV.

- **Macroscopic aspect**

The verification of the color and the homogeneity of the various concentrations were carried out by a simple visual analysis for the microparticles obtained.

- **Microscopic aspect**

Optical microscopy allows to obtain information, certainly qualitative, but often essential to the general understanding of the systems.

- **Structural analysis:**

FTIR spectra of the different sampleswere recorded using Agilent Technologies Cary 630 FTIR in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.

- **Thermal analysis**

The thermal study of the samples was conducted using a LINSEIS STAPT 1600-type thermogravimetric apparatus in the range of temperature 20–700°C, and a heating rate of around 10°Cmin^{-1}.

IV. Results and discussion

IV.1. Encapsulation yield (R%)

From the calculation of the encapsulation yield for the three concentrations performed (see Table 1), it is deduced that the third concentration 0.4g/ml was the high concentration of the active ingredient (0.4 g/ml) has a better yield of 80% compared to the other concentrations (0.15 and 0.25 g/ml), This can be explained by the influence of the active ingredient on the encapsulation yield.And thus, increasingthe amount of olive oil is accompanied by an increase in encapsulation yield.

These results were confirmed by morphological analysis (macroscopic and microscopic aspects).

The HLB or Hydrophilic-Lipophilic Balanceis closely related to the structure of the surfactant molecule. Its values are determined according to an arbitrary scale ranging from 0 to 20, it is accepted that emulsifiers with an HLB value between 0 and 8 are lipophilic, those between 8 and 12 are said (intermediate) and those between 12 and 20 hydrophilic [18], and in our study, we used a surfactant

(PEG 600) with HLB = 13. Rabiskova et al. [19]found that the nature of the surfactant affects the oil uptake by the complex coacervate droplets as a function of the HLB value of the surfactant and the addition of surfactants with HLB values between 2 and 6 resulted in maximum uptake of the emulsions into the microcapsules. Although surfactants with HLB values outside this range improve the stability of the emulsions. It adversely modifies the surface properties of the oil droplets and thus prevents encapsulation.

IV.2. Macroscopic aspect

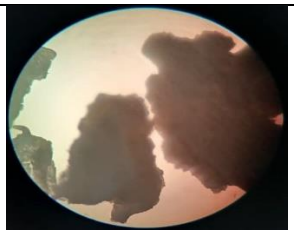
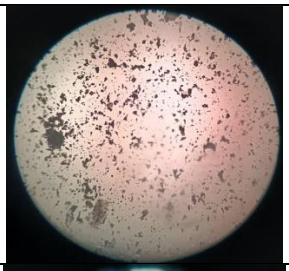
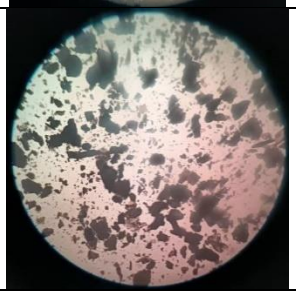
According to the table 2, the low quantity of olive oil used in our experimentation (0.15g/ml), which did not allow the formation of microparticles but rather a formation of aggregates. This result can also be explained by the low concentration of the surfactant which is insufficient to prevent the coalescence of the emulsion droplets. In this case, the microcapsules agglomerate and form aggregates. A similar finding was reported by Chacon et al.1996 [20], who explained the formation of polymer aggregates during the preparation of PLGA microcapsules containing cyclosporine, by the low concentration of the surfactant which was insufficient to stabilize the emulsion.

According to this, there is a proportional relationship between the concentration of the active ingredient and the size of the microcapsules, i.e. the higher the concentration of olive oil, the greater the increase in the size of the microparticles, which is confirmed by the appearance of the figures of the concentrations of 0.4 g/ml and 0.25 g/ml.

Table 1:The encapsulation yield according to the concentration of olive oil

Concentrations of olive oil (g/ml)	Encapsulation yield (R%)
0.15	66.51
0.25	79.25
0.4	80

Table 2: Macroscopic and Microscopic aspect of microcapsuls

Concentrations of olive oil (g/ml)	Macroscopic aspect	Observations	Microscopic aspect	Observations
0.15		The obtained microparticles are in the form of aggregates and of whitish color.		The presence of aggregates and therefore the absence of homogeneity.
0.25		The microparticles in the form of a fine powder and whitish color.		The presence of microparticles of homogeneous shape.
0.4		Microparticles in the form of a powder of medium granulometry and whitish color.		The presence of microparticles of non-homogeneous shape (large, small and medium).

IV.3. Microscopic aspect

In order to select the best concentration from the morphological point of view(see Table 2), we performed an optical analysis, according to our observation we noticed that the best concentration is 0.4 g/ml; this result was explained by the impacts of the different parameters of encapsulation process.

The stirring speed is the main parameter to control the size of the emulsion droplets. Enhancing the stirring speed generally results in the decrease of the average diameter of the microcapsules [21], [22].

According to the study by André-Abrant et al [22], the size of microcapsules decreases from 281µm to 91µm when the stirring speed is enhanced from 300 to 700 rpm. Similar

results were found by Valot et al [21] who showed that the size of microcapsules prepared with two polymers (Eudragit and EC), decreases when the stirring speed is enhanced.

The composition of the solvent is a factor influencing the final size of the microcapsules which has been proven by Kim et al [23] and Maia et al [2]. Have proven that the use of solvent with high solubility in water in combination

To confirm the presence of olive oil in PHBV microcapsulesand to study the interactions that may be established between PA and polymer, the FTIR spectra of virgin PHBV olive oil, and PHBV microcapsules containing olive oil were recorded between 400 and 4000 cm^{-1}. The FTIR spectrum of PHBV (see Figure 1) revealing the presence of several absorption bands, in particular [24] :

A rather narrow peak located at 3440 cm characteristic of the elongation vibration of the -OH bond.

A series of bands with peaks centered at 2965, 2940 and 2875 cm^{-1} ; attributed respectively to the vibrations of asymmetric elongation of the CH_3 group, symmetric elongation of the CH_2 group and symmetric elongation of CH_3.

A very intense band centered at 1750 cm^{-1} attributed to the elongation of the C=O carbonyl group of the esters.

A very broad band of peaks at 1445, 1390 and 1290 cm^{-1} corresponding respectively to the deformation vibrations of CH_3, to the elongation of the C=O group of the esters, to the vibration of the C-O-H bond.

A series of peaks 1000 and 800 cm^{-1} characteristic of the elongation vibrations of the C-C bonds.

Figure 2 presents the FTIR spectrum of olive oil reveals the presence of several absorption bands, which are cited in the following table 3[25]:

with low soluble solvents decreases the size of the obtained particles. For Kim et al. 2005[23] the average sizes of poly(ε-caprolactone) microspheres resulting from the use of dichloromethane are 73.5µm compared to those obtained with a mixture of dichloromethane and ethyl formate which are 56.6 µm.

IV.4. Structural analysis

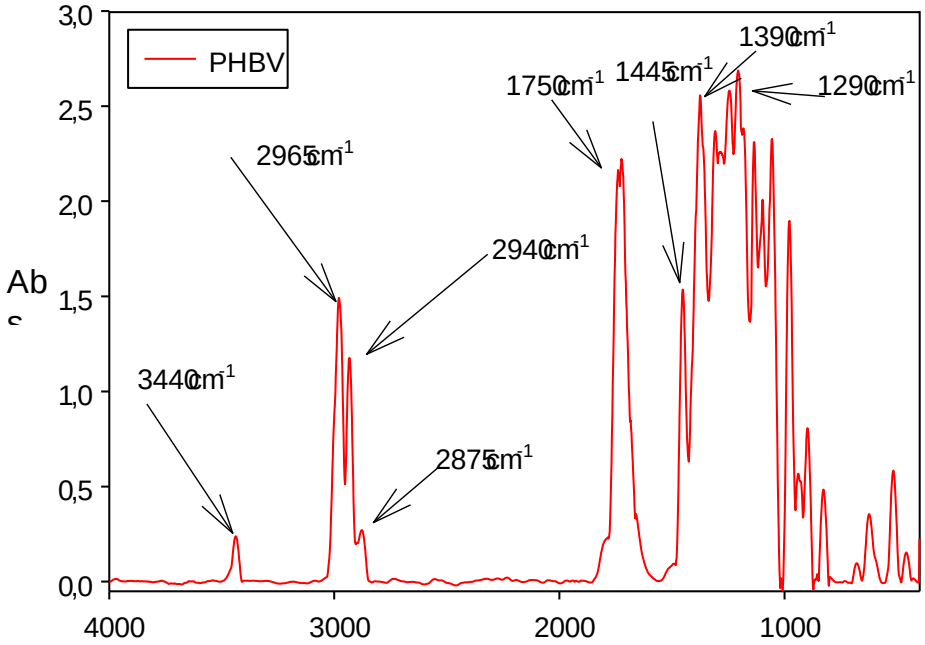


Figure 1: FTIR of PHBV

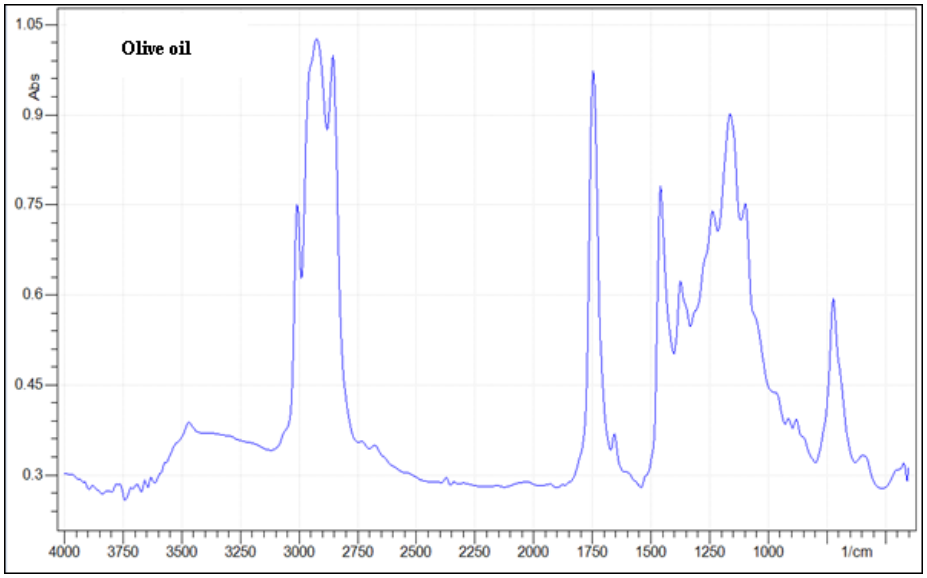


Figure 2: FTIR of Olive oil

Table 3: FTIR of Olive oil

Band (cm^{-1})	**Functional group**	**Intensity**
3464	(C=O)	Weak peak
3005	(=C-H) cis	Medium
2922	(C-H) CH_3	Very strong
2852	(C-H) CH_2	Very strong
1742	(C=O) ester	Very strong
1652	(C=C)	Weak
1459	(C-H) CH_2	Medium
1238 and 1162	(C-O)	Medium
961	(C-O)	Weak
898 and 839	–HC-CH–	Weak

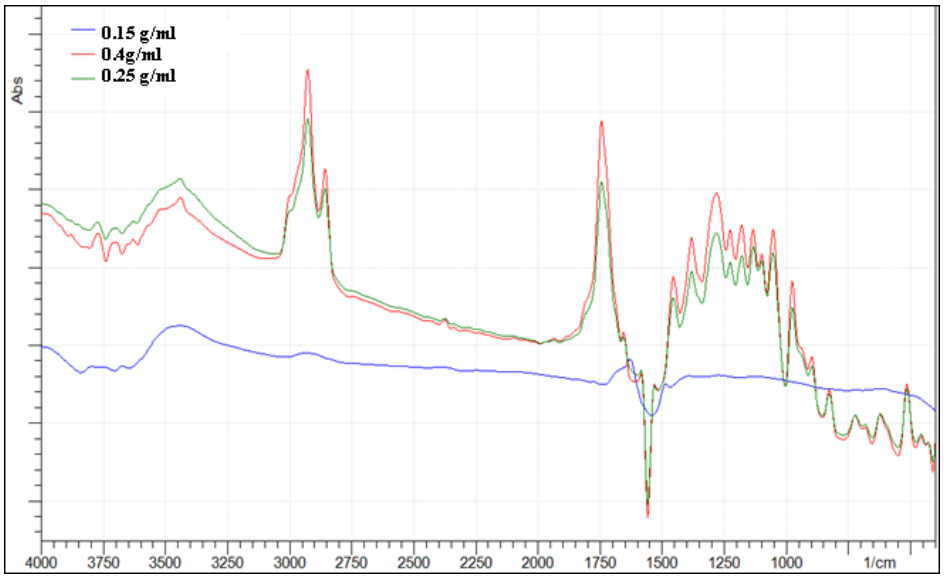


Figure 3: FTIR of PHBV/olive oil microcapsules in different concentrations

From the FTIR spectrum of PHBVmicroparticles containing olive oil(Figure 3):

- A broad band located in the 3464 area characteristic of the olive oil ester groupC=O.

- A band located in the area of the peaks centered around 3000 to 2875 cm-1; of the CH_2 group that occurred in PHBV and the CH band in olive oil
- A new peak at 2338, which signifies the formation of C≡C triple bonds correspond to the aromatic ring of the active ingredient.
- An intense peak located at 1725 cm^{-1} features the C=O band, which presented in PHBV and in olive oil and thus the presence of the latter in the PHBV matrix.

We also notice that the intensity of concentration 0.4g/ml is stronger than concentration 0.25g/mland to this effect; we can say that the encapsulation of olive oil with concentration 0.4g/mlis slightly better than that of concentration 0.25g/ml.

It is also observed that all the bands of PHBV appear in the spectrum of microparticles without any change in wave number, this indicates that the integrity of the active ingredient is preserved after the encapsulation process. This leads us to assume that the active ingredient remains stable and that there are no chemical interactions between the active ingredient and the polymer studied.

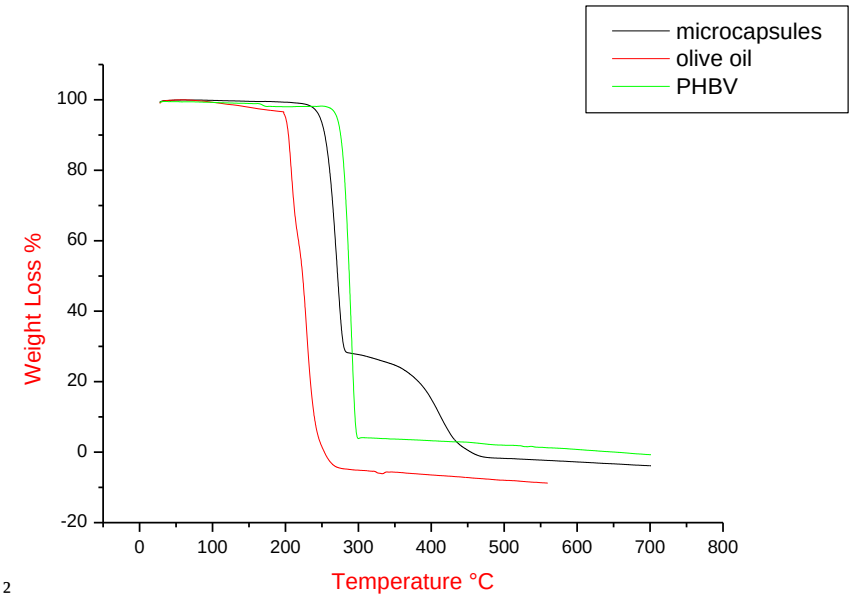
IV.5. Thermal analysis

According to the figure 4 and table4, we observe successively:

A first loss of mass from 80°C to about 100°C, which is perhaps attributed to the evaporation of water, then directly another loss at about 200°C to 250°C, which is probably related to the degradation of olive oil or the active principle used.

The second loss of mass is located from 300°C, it corresponds to the degradation of the PHBV biopolymer.

This result confirms the presence of olive oil in the PHBV microcapsules, which has been verified by other previous analysis[26].



2

Figure 4 : Thermograms of PHBV, olive oil and microcapsules

Table 4: The onset temperature and Tmax of PHBV, Olive oil and PHBV/ Olive oil microcapsules

Simples	Ti (°C)	Tmax (°C)
PHBV	262	290
PHBV/ Olive oil microcapsules	239	300
Olive oil	196	253

V. Conclusions

The work presented is part of a research program that has set as an objective to prepare microparticles by the technique of emulsion evaporation of the solvent from biodegradable and biocompatible polymeric matrices. The active ingredient chosen for this study is olive oil, the polymers used are the PHBV as a base matrix and PEG as active tension.

- The encapsulation yields obtained with respect to the different parameters are between 66 and 80 %, the best of which is obtained with the highest amount of active ingredient which is 0.4 g/ml.
- The microscopy allowed us to observe the morphology of the powders obtained, where the latter are in the form of a powder (spherical) and other are aggregates.
- The FTIR analysis showed the presence of olive oil in the PHBV microparticles, and highlighted the absence of chemical interactions between the polymers and olive oil.

- Thermal analysis by thermogravimetry confirmed the existence of olive oil in the PHBV microparticles by the presence of two losses of mass, first in the interval (200 to 250) which corresponds to the degradation of oil and another loss after this range of (250a 400) attributed to the decomposition of the encapsulant (PHBV).

Acknowledgements

The authors would like to thank the students "OUHNIA Thinhineneand IBELHOULEN Kahina" in polymer engineering of process engineering department, university of Bejaia.

Disclosure of interest: The authors report no conflict of interest.

References

[1] D. Chulia, M. Deleuil, and Y. Pourcelot. Powder Technology and Pharmaceutical Processes. Elsevier Science B V Amsterdam, 557, 1994.

[2] J.L. Maia, M.H.A. Santana, M.I. Ré. The effect of some processing conditions on the characteristics of biodegradable microspheres obtained by an emulsion solvent evaporation process. Braz. J. Chem. Eng., 21, 01–12, 2004.

[3] A. A. Chowdhury. Poly-3-hydroxybuttersure abbauende Bakterien und Exoenzym. Arch. For Mikrobiol, 47,(2). 167-200, 1963.

[4] R. Bellache, D. Hammiche, A. Bettache, et A. Boukerrou. Enzymatic degradation of prickly pear seed (PPS)/Polyhydroxy(butyrate-co-valerate) (PHBV) biocomposite. Materials Today Proceedings, 53, 113-116, 2022.

[5] E. Franceschi. Precipitation of β-carotene and PHBV and co-precipitation from SEDS technique using supercritical CO_2 . J. Supercrit. Fluids, 47 (2), 259-269, 2008.

[6] E. T. Baran, N. Özer and V. Hasirci. Poly(hydroxybutyrate-co-hydroxyvalerate) nanocapsules as enzyme carriers for cancer therapy: an in vitro study. J. Microencapsul., 19 (3), 363-376, 2002.

[7] L. Sagalowicz andM. E. Leser. Delivery systems for liquid food products. Current Opinion in Colloid &Interface Sciences, 15, 61–72, 2010.

[8] L. Sanguansri, L. Day, Z . Shen, P. Fagan, R.Weerakkody,L. J. Cheng. Encapsulation of mixtures of tuna oil, tributyrin and resveratrol in a spraydried powder formulation. Food & Function, 4, 1794–1802,2013.

[9] R. Abdallaoui. La microencapsulation des huiles : meilleure approche pour la valorisation des produits alimentaires, doctorat thesis maroc, 2018.

[10] S. Krishnan, R. Bhosale, R.S. Singhal. Microencapsulation of cardamom oleoresin: evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. Carbohydrate Polymers 61:95–102, 2005.

[11] S.S. Umesha, B. Monahar, K.A. Naidu. Microencapsulation of alpha-linolenic acid-rich garden cress seed oil: physical characteristics and oxidative stability. Eur J Lipid Sci Technol, 115;1474–82, 2013.

[12] V.M. Silva, G.S. Vieira, M.D. Hubinger. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Research International, 61;132–43, 2014.

[13] H.K. Lim, C.P. Tan, J. Bakar, S.P. Ng. Effects of different wall materials on the physicochemical properties and oxidative stability of spray-dried microencapsulated redfleshed pitaya (Hylocereus polyrhizus) seed oil. Food Bioprocess Technol 5:1220–7, 2012.

[14] N. Devi, T.K.Maji. A novel microencapsulation of neem (Azadirachta indica A. Juss.) seed oil (NSO) in polyelectrolyte complex of kappa-carrageenan and chitosan. J Appl Polym Sci 113:1576–83, 2009.

[15] D. Kanakdande,R. Bhosale, R. S. Singhal. Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrin and modified starch. Carbohydrate Polymers 67:536–41,2007.

[16] M. De Luca, W. Terouzi, G. Loele, F. Kzaiber, A. Oussama, F. Oliverio, R. Tauler, G. Ragno, Derivative FTIR spectroscopy for cluster analysis and classification of morocco olive oils, Food Chemical. 124; 1113–1118, 2011.

[17] Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura- Carretero, A., Fernández-Gutiérrez, A. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules, 12, 1679–1719, 2007.

[18] A. Harlay, A . Huard, L. Ridoux, V. Rolland. Guide du préparateur en pharmacie .Edition Masson, gondé sur noireau, 791, 2004.

[19] M. Rabiskova and J. Valaskova. The influence of HLB on the encapsulation of oils by complex coacervation. J. Microencapsul, 15, 747, 1998.

[20] M. Chacon, L. Berges, J. Molpeceres, M.R. Aberturas, M.Guzman. Optimized preparation of poly D, L (lactic-glycolic) microspheres and nanoparticles for oral administration. Int. J. Pharm, 141, 81-91, 1996.

[21] P. Valot, M. Baba, J. M. Nedelec, N. Sintes-Zydowicz. Effects of process parameters on the properties of biocompatible Ibuprofen-loaded microcapsules. Int. J. Pharm, 369, 53–63, 2009.

[22] A. André-Abrant, J.L. Taverdet, J. Jay. Microencapsulation par évaporation de solvant. Eur. Polym. J., 37, 955-963, 2001.

[23] B.K. Kim, S.J. Hwang, J.B. Park, H.J. Park. Characteristics of felodipine-located poly(epsilon–caprolactone) microspheres. J. Microencapsul, 22, 193–203, 2005.

[24] R. Bellache, D. Hammiche, A. Boukerrou. Physico-chemical characterization of hydrolytic Degradation of Prickly Pear Seed enhanced Poly-Hydroxy (Butyrate-co-Valerate) biocomposite. Macromolecular Symposia., 404, 2100371, 2022.

[25] R. Bellache, D. Hammiche, A. BoukerroU. Elaboration of ointments based on vegetable plants “Calendula arvensis” and “Dandelion”. Biopolymer Applications Journal, 1(2), 01-07, 2022.

[26] R. Bellache, D. Hammiche, A. Boukerrou, B. S. Kaith. Prickly pear seed oil (PPSO) encapsulated by biodegradable polymer Poly-hydroxy-butyrate-co-valerate (PHBV). https://doi.org/10.1016/j.matpr.2022.11.441. p.791.