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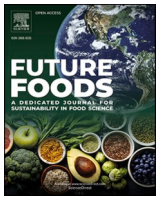

Optimisation of the carvacrol encapsulation method into PHBV nanoparticles

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ABSTRACT

The need of sustainable food packaging preserving food from degradation conducted to increase research on active packaging using essential oil, as carvacrol, for their antimicrobial and antioxidant properties. The encapsulation of this kind of volatile molecules is necessary and nanoencapsulation into biopolymers, as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) showed an increasing interest as a green solution, although this method again need to be improved. In this study, a full experimental design was developed to select the best method (nanoprecipitation and emulsification) and operating conditions (PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratios) to encapsulate carvacrol into PHBV. In this purpose, for each tested conditions, encapsulation efficiency (process efficiency, carvacrol recovery, PHBV recovery and loading capacity), as well as nanoparticles' morphology and size were estimated, and statistically analysed. Carvacrol recovery and loading capacity were significatively highest (61 % and 100 % respectively) using emuslification method, low surfactant concentration, high carvacrol/PHBV ratio (for loading capacity) and low PHBV molecular weight (for carvacrol recovery). To the contrary, PHBV recovery increased (93 %) using the nanoprecipitation method, a high surfactant concentration and a low carvacrol/PHBV ratio, while process efficiency increased (73 %) with a low carvacrol/PHBV ratio and a low aqueous/organic phase volume ratio. Moreover, small spherical-shaped and separated nanoparticles were obtained using emulsification method, high surfactant concentration but low carvacrol/PHBV ratio. Therefore, including all the aspects of carvacrol nanoencapsulation into PHBV (shape and encapsulation efficiency) using emulsification method, with a low level for all parameters except the surfactant concentration are the most suitable strategy.

1. Introduction

The increasing need for environmentally friendly and efficient food packaging solutions has prompted the development of encapsulation techniques that enhance the stability and effectiveness of bioactive compounds, including volatile essential oils and derivatives. These compounds, known for their inherent antimicrobial and antioxidant properties, have the potential to enhance the shelf life and safety of perishable products when utilized in food packaging (Varghese et al., 2020; Rzayeva et al., 2023). However, these substances often lose their efficacy during material processing due to their sensitivity to light, oxygen, and temperature, and they are easily lost from the material due to their volatility. This release is triggered by humidity or temperature exposure, which can hinder long-term storage and residual efficacy of active packaging materials (Mascheroni et al., 2011; Kurek et al., 2017). Encapsulation of the bioactive ingredients within a carrier or protective layer has addressed these issues related to processing and storage while also controlling the volatile bioactive compound release (Mascheroni et al., 2011; Nedovic et al., 2011; Rezaei et al., 2019).

When choosing a carrier for volatile bioactive encapsulation, several criteria must be satisfied. Two primary criteria are the encapsulation efficiency (EE, % w/w) and the loading capacity (LC, % w/w), which are crucial in determining the concentration of encapsulated material in the active-carrier complex and the amount of active loaded per unit weight of carrier, respectively. Additionally, other factors such as process efficiency or yield, morphology and dissociation of the complexes must also be considered. In literature, various carriers have been studied, with carbohydrate-based and protein-based biopolymers, lipids and clays being the most commonly used (Abdullayev and Lvov, 2011; Rehman et al., 2020; Alu'datt et al., 2022). But, other food-grade biopolymers (e.

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g., chitosan, starch, gum, etc.) or biopolymers (PLA, PHA, etc.) have been employed to encapsulate essential oils. These biopolymers possess advantageous physical and chemical properties, are safe for use, can naturally degrade, are bio-benign, and cheap. Therefore, the use of biopolymer, as poly(hydroxybutyrate-co-hydroxyvalerate), instead of synthetic one allow to minimize the use of fossil resources, but also the burden due to plastic accumulation, or post-usage waste management (Guillard et al., 2018). This makes them well-suited for enclosing essential oils and controlling their gradual release (Rehman et al., 2020), although sparsely studied.

Beyond encapsulation strategie, the incorporation of the active-carrier complex in the packaging material is of upmost importance. Basically, it could be added in the bulk of the polymer and consequently a homogeneous active concentration exists in the whole material, or it could be added on the surface of the packaging material in the form of a thin layer or coating, concentrating the whole quantity of active compound on the film surface (Rzayeva et al., 2023). This last configuration permits to better design the antimicrobial packaging by choosing the *just necessary* quantity of active compound to be added in contact with the food and optimal transport parameters for a given situation (Ben Arfa et al., 2007; Arfa et al., 2007; Guillard et al., 2009; Mascheroni et al., 2010). Using coating strategy necessitate to anticipate the formulation of the active layer, that must be compatible with the intended support. This will in turn affect the encapsulation strategy and thus the active-carrier formulation.

Recent concerns related to persisent plastic pollution and depletion of fossil ressources have promoted the emergence of bio-based and biodegradable biopolymers in food packaging application. One of this promising polymer family, cumulating the advantages to be bio-based (but non food based), bioprocessed and biodegradable in the most common conditions prevailing on earth is the PHAs (poly-hydroxyalkanoates) family (Rzayeva et al., 2023). Commercial PHAs are currently quasi-exclusively the copolymer polyhydroxy (butyrate-co-valerate), PHBV. PHBV could be shaped into rigid packaging (e.g., trays) using thermomechanical processes, and in their final shape, display good oxygen barrier properties that make them promising for fresh and other processed food packaging applications (Berthet et al., 2015; Angellier-Coussy et al., 2017; Bossu et al., 2020). To make PHAs based material active, active coatings using PHAs as encapsulant for bioactive compound would be an asset. Recently, the possibility to use PHBV to form nanoparticles encapsulating essential oils was explored (Shakeri et al., 2014; Freiberger et al., 2015; Corrado et al., 2022). The two main methods to encapsulate volatile components in PHAs polymers are nanoprecipitation and emulsification, both lay on the principle of precipitation of an organic phase (containing dissolved PHBV) into an aqueous one. They differ based on the mixing way of the immiscible solutions and aqueous solution content. The nanoprecipitation is a low energy consuming method, showing a good reproducibility and an easy scaling up, but is not adapted for hydrophilic compounds (Rivas et al., 2017; Lammari et al., 2020), while emulsification shows a good stability of the entrapped molecule, a high encapsulation efficiency and controlled release (Lu et al., 2016). However, literature is scarce, and research is still needed to clarify the process of PHBV nanoparticle formation using these two methods. The impact of some parameters during the nanoencapsulation procedure, as surfactant type and concentration, molecular weight of encapsulant, or aqueous/organic phase ratio were already explored (Leimann et al., 2013; Farrag et al., 2018; Senthilkumar et al., 2018; Hernández-Giottonini et al., 2020), but not in combination. For instance, Leimann et al. (2013) reported that the molecular weight of PHAs may influence the nanoparticle's shape and size distribution but in an extend that remain to be determined. Similarly, Farrag et al. (2018) showed that the type and concentration of the surfactant added in the aqueous phase, as well as the polymer concentration, impacted the shape and size distribution of PHBV particles. If ability of these two methodologies (nanoprecipitation and emulsification) to encapsulate bioactive into PHBV nanoparticles has already been evidenced, no complete study comparing the encapsulation efficiency and loading capacity of these two methods was never performed. Nowadays, the nanoencapsulation method still need to be improved (Yammine et al., 2024), by exploring in one study the impact of combined parameters. This will enable to propose an improved protocol for nanoencapsulation of essential oil into PHA, this encapsulation strategy being a high promising green solution for food preservation (Plati and Paraskevopoulou, 2022).

In this context, this research aims to identify the most effective encapsulation method for volatile active compounds, and more specifically carvacrol, in PHBV, by exploring the impact of different parameters (method, PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratios) on process efficiency, carvacrol recovery, PHBV recovery and loading capacity. To the best of our knowledge, it is the first time that a full experimental plan (including combined parameters) was proposed to select the best encapsulation strategies of essential oil in PHBV. It will enable to propose an improved green encapsulation strategy to increase the food preservation. The use of PHBV as carrier will make easier the further application of the active-carrier complex as coating on PHBV film support. The active compound chosen as model is carvacrol, abundant and naturally existing molecule in aromatic plants of the Labiatae family, including Origanum, *Satureja, Thymbra, Thymus,* and *Corydothymus.* Carvacrol is a monoterpenic phenol, with USA and Europe approval as a safe food additive in small quantities (threshold of 1800 ug/person/day) (Nostro and Papalia, 2012). It shows strong antimicrobial and antioxidant properties (Ben Arfa et al., 2006; Kurek et al., 2013; Requena et al., 2017; Altan et al., 2018). To find the optimal encapsulation strategy for carvacrol in PHBV, a full design plan is set up to explore the role of the method (nanoencapsulation versus nano-emulsification), the role of PHBV molecular weight, of polymer and carvacrol concentration onto the encapsulation efficiency (carvacrol and PHBV recovery, loading capacity, and processing efficiency) measured on obtained active-carrier complex. Structural observation of PHBV nanoparticles is also performed. All the results are discussed in the perspective to select the best compromise among all encapsulation strategies of carvacrol in PHBV for further application as active coating on PHBV film.

2. Material and methods

2.1. Materials

A commercial grade of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) under the references PHI002 (pellets) and PHI003 (powder) was purchased from NaturePlast (Ifs, France). PHI002 (pellet) is a formulated grade containing about 3 % of valerate and 1wt% of boron nitride (nucleating agent). PHI003 (powder) is a non-formulated grade containing about 3 % of valerate.

Boron nitride (BN, 98 % purity), carvacrol (CA, 98 % purity), dichloromethane (DCM, ≥99.9 %), ethanol (EtOH, ≥99.8 % and 96 %), hexane (Hex, ≥95 %), and sodium dodecyl sulfate (SDS, ≥99 %) were supplied from Sigma Aldrich (France).

2.2. Preparation of PHBV polymer as encapsulant

2.2.1. Compounding and thermoforming

PHBV powder (PHI003) was mixed with 0.5 wt% boron nitride (BN) and dried at 60 °C for 48 h in a climatic chamber (Memmert, Germany) before using. The mixture was melt-blended using a corotating twin-screw microextruder (model "process 11″ Thermofisher) as described by Dedieu et al. (2022). The barrel temperature profile was set at 180 °C (from top to bottom), and the screw speed at 150 rpm. The residence time of the mixture inside the extruder was 1.5 min. Five processing cycles were successively performed in order to reduce the molecular weight of PHBV (Dedieu et al., 2022). After each cycle, the melt strain

was cooled down at room temperature, then pelletized (Pelletizer from Thermofisher, Germany) and dried under vacuum at 60 °C for 24 h.

The dried PHBV pellets (the produced PHI003 and the bought PHI002 dried 24 h at 60 °C) were thermocompressed using an hydraulic thermopress (CFM 20 T, Pinette Emidecau Industries, Chalon sur Saone cedex, France) at 178 °C. For that, around 6 g of pellets were positioned in a mould between two Teflon coated plates (Taconic, France) and were compressed using the following time/pressure conditions: 2 min at 5 bar, then 30 s at 25 bar, 30 s at 50 bar, 30 s at 75 bar, 30 s at 100 bar and 1 min and 30 s at 150 bar. Before demolding, the film was cooled down at room temperature (22–23 °C) posing a cold-water bath on the surface of the metal mould. The films have a size of 12 cm per 12 cm and a thickness around 300 um. They were stored in a desiccator containing silica gel at room temperature for a maximum two months before their use to encapsulate carvacrol.

2.2.2. Determination of molecular weight

The molecular weight was determined by the Toulouse White Biotechnology (TWB) as described by Perdrier et al. (2023). The PHBV samples were dissolved in 5 mL chloroform at room temperature for 3 h under magnetic agitation. To avoid the solvent evaporation, HeadSpace vials were used with PTFE caps. The obtained solution were adjusted to a concentration of approximately 2 $g.L^{-1}$ in a 10 mL graduated flask, then filtered through 0.45 µm PTFE membranes. Samples was injected in duplicate into a size exclusion chromatography associated with multi-angle light scattering (SEC-MALS) and refractive index detectors (both from Wyatt Technology Corp). The calibration of both detectors was made using a monodisperse 30 kDa polystyrene, prepared under the same conditions as the samples. The refractive index increment (dn/dc) in chloroform that was used for experiments was 0.16 $mL.g^{-1}$ for polystyrene (Bello et Guzman, 1966) and 0.0336 $mL.g^{-1}$ for P(3HB-co-3HV) (Kovalcik et al., 2020).

2.3. Encapsulation of carvacrol into PHBV nanoparticles

2.3.1. The encapsulation methods

The encapsulation of carvacrol was tested on the two types of PHBV (produced in 2.2): PHI002 and PHI003 (Parameter A) and with two different adapted methods: emulsification (Corrado et al., 2022) and nanoprecipitation (Shakeri et al., 2014) (Parameter B).

For nanoprecipitation, PHBV film was cut in small fragments (around 1 cm per 1 cm) and dissolved in dichloromethane solution at 5 mg/mL at 40 °C for 2 h under stirring (Organic phase). After cooling the organic phase at room temperature, 25 or 50 mg of carvacrol (Parameter C) was added in solution and stirred 1 h more at room temperature. In parallel, a SDS solution (2.2 mg/ml or 5 mg/ml: Parameter D) was prepared in a 30/70 water/ethanol solution and stirred 1 h at room temperature (Aqueous phase). The organic phase was added dropwise into the aqueous one, while homogenized using a shear mixer (L4RT High, Silverson, England) at 6000 rpm (15 min) in an ice bath. The aqueous/organic phase ratio tested was 6/1 and 9/1 (Parameter E). Then, the mixture were put at 40 °C under agitation to evaporate the dichloromethane, and centrifuge at 2795 g for 10 min at 20 °C using a Megafuge centrifuge (16R, ThermoScientific, Germany). The recovered nanoparticles were dried in a desiccator for 24 h, then stored in a closed container at refrigerated temperature conditions (-20 °C) until characterization.

For emulsification, the organic phase was prepared as described above for nanoprecipiation. Concerning the aqueous phase, the SDS was prepared into water (2.2 mg/ml or 5 mg/ml, parameter D) and stirred 1 h at room temperature. The organic phase was pouring into the aqueous one and homogenized 15 min at 6000 rpm using a shear mixer (L4RT, High, Silverson, England) in an ice bath. The aqueous/organic phase ratio tested was 2/1 and 4/1 (parameter E). Then, the dichloromethane was evaporated, mixture centrifuged and the nanoparticles dried, as described for nanoprecipitation.

For both method and all tested parameters, a control was also made using the same procedure without adding the carvacrol molecule.

2.3.2. Experimental design

An 2^5 factorial experimental design was applied to identify the impact of 5 different parameters: polymer's molecular weight (A), method (B), PHBV: carvacrol ratio (C), surfactant concentration (D), and Aqueous/Organic (A/O) phase ratio (E) on encapsulation efficiency of carvacrol into PHBV. The two levels tested for each parameter are described in Table 1 and the description of the 32 experiments are made in Supplementary table. The obtained carvacrol and PHBV recovery, loading capacity and the process recovery of every trial was studied for each experiment.

2.4. Nanoparticles characterization

2.4.1. Carvacrol extraction from PHBV nanoparticles

Considering hexane's affinity with carvacrol (Kurek et al., 2013) and the swelling effect of ethanol on PHBV, extraction of carvacrol from PHBV nanoparticles was done by stirring 10 mg of nanoparticles in 5 ml of hexane:ethanol (1:1) solution for 72 h at room temperature. Then, samples were filtered through 0.22 um (ait, France) and carvacrol was quantified by measuring absorbance at 275 nm (maximal absorbance) with a UV–visible spectrophotometer (Evolution 300 UV–Vis Spectroscopy, Thermo Scientific). The analysis was conducted in triplicate. The quantity of encapsulated carvacrol in the nanoparticles ($Carv_t$) (mg) was calculated using the following equation:

$$Carv = \frac{(Abs - \mathrm{Abs}_0) \times \mathrm{Dil} \times \mathrm{V}_{tot} \times \mathrm{W}_t}{k \times l \times \mathrm{V}_{te}} \quad (1)$$

where Abs is the sample absorbance at 275 nm; Abs_0 is the blank absorbance, corresponding to the negative control going through the same encapsulation and extraction protocol procedures than sample but without carvacrol; k is the extinction coefficient of carvacrol at 275 nm (15.1 mL mg^{-1} cm^{-1}), l is the width of spectrophotometer cell, Dil is the dilution factor used before measurement to specrophotometer if necessary, V_{tot} is the total volume of the assay (5 mL), W_t is the quantity of nanoparticles obtained after encapsulation (mg), and W_{te} is the quantity of nanoparticles used for carvacrol extraction.

2.4.2. Determination of efficiency of the encapsulation method

Process efficiency, carvacrol and PHBV recovery and loading capacity are determined as described by Anaya-Castro et al. (2017).

The process efficiency is defined as the quantity of obtained nanoparticles (mg) over the total quantity of initial material involved in the formulation and was calculated as follows:

$$PE = \frac{\mathrm{W_t}}{Carv_i + \mathrm{PHBV}_i} \times 100 \quad (2)$$

where $\mathrm{W_t}$ is the quantity of nanoparticles obtained after encapsulation

Table 1
The different factors and their levels used for the experimental design set for encapsulating carvacrol in PHBV nanoparticles.

Factors	Code	Level	
		Low (–)	High (+)
Molecular weight (Mw)	A	244 ± 14 kDa	351 ± 11 kDa
Method (Meth)	B	Nanoprecipitation (Np)	Emulsification (Em)
Carvacrol/PHBV ratio (Carv)	C	0.5/1	1/1
Surfactant concentration (SDS)	D	2.2 mg/mL	5 mg/mL
Aqueous/Organic phase ratio (Aq)	E	6/1 for Np; 2/1 for Em	9/1 for Np; 4/1 for Em

(mg), $Carv_i$ and $PHBV_i$ are the quantity of carvacrol and PHBV respectively, initially introduced in the preparation (mg).

The encapsulation efficiency (EE) or carvacrol recovery is defined as the quantity of encapsulated material (carvacrol) detected in the final formulation over the initial quantity used to make the formulation. Encapsulation efficiency (EE) was calculated using the following equation:

$$\boldsymbol{EE = carvacrol\ recovery = \frac{Carv_t}{Carv_i} \times 100} \quad (3)$$

where $Carv_t$ is the quantity of carvacrol extracted from samples (mg) and $Carv_i$ is the quantity of carvacrol initially introduced in the preparation (mg).

The PHBV recovery is defined as the quantity of encapsulant material (PHBV) in the final formulation over the initial quantity used to make the formulation. It was calculated using the following equation:

$$\boldsymbol{PHBV\ recovery\ = \frac{W_t - Carv_t}{PHBV_i} \times 100} \quad (4)$$

Where W_t is the quantity of nanoparticles obtained after encapsulation (mg), $Carv_t$ is the quantity of carvacrol extracted from samples (mg) and $PHBV_i$ is the quantity of PHBV initially introduced in the preparation (mg).

The loading capacity (LC) is defined as the quantity of encapsulated material (carvacrol) detected in the final formulation over the quantity of encapsulant or carrier (PHBV) present in the final formulation (active-to-carrier ratio). It was calculated according to the following equation:

$$\boldsymbol{LC\ = \frac{Carv_t}{W_t\ - Carv_t} \times 100} \quad (5)$$

Where, $Carv_t$ is the quantity of carvacrol extracted from samples (mg) and W_t is the quantity of nanoparticles obtained after encapsulation (mg).

Carvacrol recovery, PHBV recovey, and loading capacity were measured in triplicate, while process efficiency was measured one time.

2.4.3. Determination of nanoparticles morphology: scanning electron microscopy (SEM)

Small amount of the powder samples was deposited on carbon stickers on aluminum stubs. Samples were then metallized with a thin layer (4 nm) of Au/Pd sputter coating at room temperature (Quorum Sputter Coater SC7620). Morphology of nanoparticles were then observed using a benchtop Phenom Pro X scanning electron microscope (Phenom World, Eindhoven, The Netherlands) with a backscattered electron detector and an acceleration voltage of 10 kV. Size of the particles were measured using Fiji software ImageJ-win64 (Bethesda, USA) on 20 nanoparticles for each condition.

2.4.4. Statistical analysis

The responses considered in experimental design (carvacrol and PHBV recovery, and loading capacity) were statistically analyzed using an ANOVA test with a significance level of 5 % ($p < 0.05$).

The impact of each parameter (polymer's molecular weight (A), method (B), PHBV:CA ratio (C), surfactant concentration (D), and Aqueous/Organic (A/O) phase ratio (E)) was measured as the sum of each response multiply by the level of the parameter, as described by the following equation:

$$\text{coefficient of parameter } n\ = \sum_{i=1}^{i=32} Resp_i \times fl_i \quad (6)$$

With n being the observed parameter (A to E) or combined parameter (AB to DE, ABC to CDE, ABCD to BCDE, or ABCDE), i the sample run (1 to 32), $Resp_i$ being the value of the observed response (Carvacrol or PHBV recovery, loading capacity or process efficiency) for the run i, and fl_i being the parameter level (1 or −1). In case of combined parameters, the parameter levels of each parameter taken in account were multiplied between them to obtain the final fl_i. A Pareto test was made on the coefficient of parameters for each response (carvacrol and PHBV recovery, loading capacity and process efficiency).

Moreover, to determine the significance impact of each parameter, a Student test was done on each parameter coefficient, by comparing the coefficient parameter when parameter level was high to the coefficient parameter when parameter level was low, with a significance level of 5 % ($p < 0.05$).

3. Results and discussion

3.1. Impact of parameters on efficiency of the encapsulation

This study investigated parameters that affect process efficiency, carvacrol recovery, PHBV recovery and loading capacity to encapsulate carvacrol into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). For this purpose, an experimental plan with 5 parameters and 2 levels (36 experiments, Table 1 and supplementary table) was applied to select the best method (nanoprecipitation or emulsification) and operating conditions (PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratios).

3.1.1. Carvacrol recovery

3.1.1.1. Efficiency of the encapsulation method. Fig. 1 displayed the impact of the different parameters on carvacrol recovery. Results showed that, carvacrol recovery was ranged between 0.4 % and 6.5 % with the nanoprecipitation method, while it was ranged between 15.5 % and 61.0 % with the emulsification method, whatever the molecular weight of PHBV, the carvacrol/PHBV ratio, the surfactant concentration, and the aqueous/organic phase volume ratio. This observation was due to the difference of solvent used for aqueous phase: being water for emulsification and 30/70 water/ethanol solution for nanoprecipitation. The carvacrol having higher affinity with ethanol than water (Requena et al., 2017; Ramos et al., 2014), an unwanted release of carvacrol into the aqueous phase, especially during and after evaporation of dichloromethane, for the nanoprecipitation method should occur. The carvacrol recovery was higher when surfactant concentration was lower (46.5 % with emulsification method and low surfactant concentration vs 27.8 % with emulsification method and high surfactant concentration). This observation was all the more important since the molecular weight of PHBV was low (for emulsification method and low PHBV molecular weight: 51.9% vs 23.4 % with low and high surfactant concentration respectively, while for emulsification method and high PHBV molecular weight: 41.0% vs 32.1 % with low and high surfactant concentration respectively), proving a combined effect between molecular weight and surfactant concentration parameters. Similarly, but to a lesser extent, the carvacrol recovery was higher when the aqueous/organic phase volume ratio was lower (42.0 % with emulsification method and low aqueous/organic phase volume ratio vs 32.2 % with emulsification method and high aqueous/organic phase volume ratio). These two latest parameters (surfactant concentration and aqueous/organic phase volume ratio) had an importance in the carvacrol recovery, because a low SDS concentration and aqueous/organic phase volume ratio allow to increase the affinity of carvacrol with the organic phase, and consequently avoid a loss of the molecule in the water phase (Donsì et al., 2012). In more details, when the concentration of surfactant is higher than critical micelle concentration (CMC), the SDS molecules tend to self-assemble into micelles in the aqueouse phase (Ryu et al., 2018; Jacumazo et al., 2020). Consequently, after overpass the CMC, a part of carvacrol was probably encapsulated in the self-SDS micelles instead of PHBV, reducing the carvacrol recovery. In this present study, the SDS concentrations were 7.6 mM (2.2 mg/mL) and 17.4 mM (5 mg/mL), while Fuguet et al. (2005) estimated the CMC of SDS in water around 8

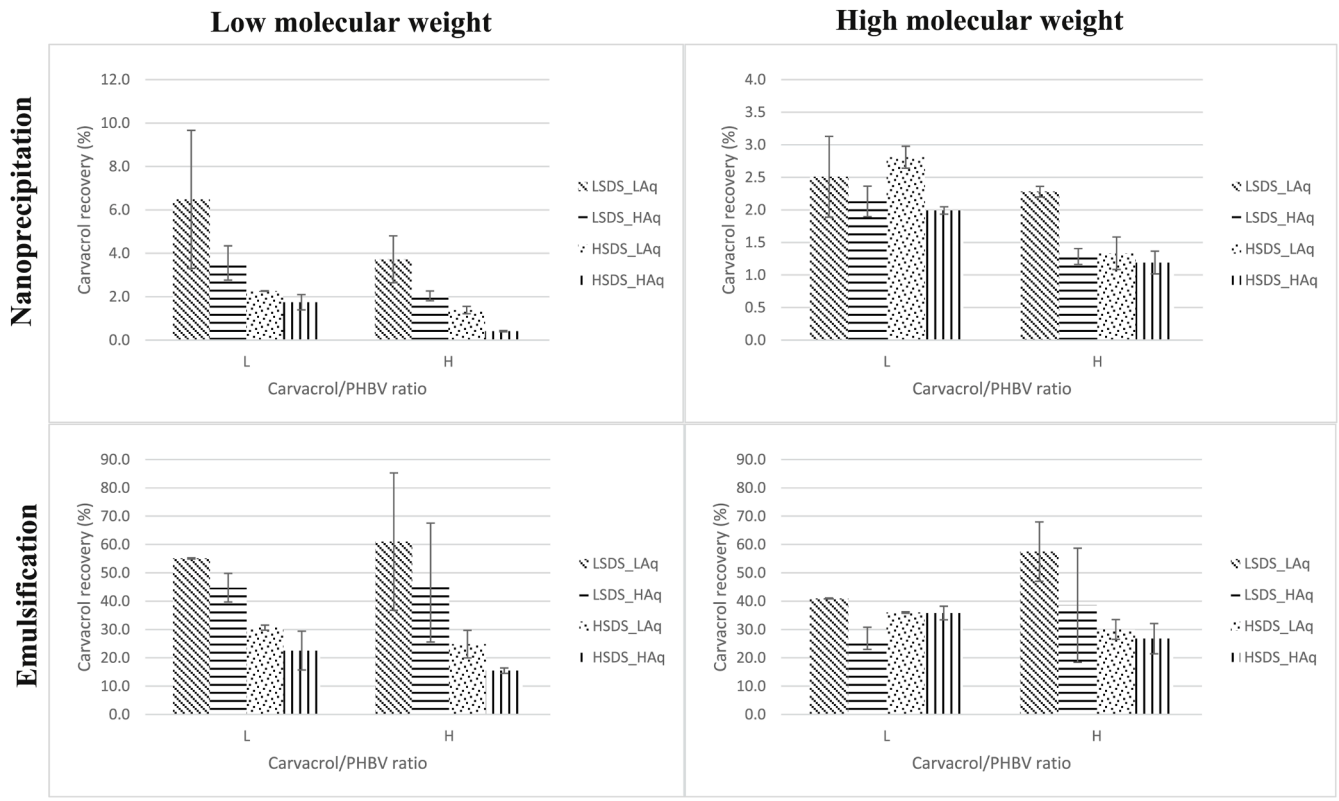

Fig. 1. Carvacrol recovery after encapsulation in PHBV nanoparticles depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio.

mM. Finally, the carvacrol recovery was not impacted by the carvacrol/PHBV ratio (36.6 % with emulsification method and low carvacrol/PHBV ratio vs 37.6 % with emulsification method and high carvacrol/PHBV ratio), neither PHBV molecular weight (37.7 % with emulsification method and low molecular weight vs 36.6 % with emulsification method and high molecular weight). The carvacrol recovery from this present study are in the same range than those found in literature (Shakeri et al., 2014; Marcet et al., 2018; Pignatello et al., 2019; Corrado et al., 2022). Indeed, Shakeri et al. (2014) obtained a carvacrol recovery of 21 % using an encapsulation procedure in PHB with a carvacrol/PHB ratio of 0.5/1, a surfactant concentration of 1 mg/mL and an aqueous/organic volume phase ratio of 4/1, while Corrado et al. (2022) observed a Mexican oregano oil recovery between 45 % and 70 % using an encapsulation procedure in PHB with a oil/PHB ratio of 0.5/1 or 1/1, a surfactant concentration of 2.2 mg/mL and an aqueous/organic volume phase ratio of 2/1.

It should be noticed that carvacrol recovery showed highly variable results (high error bars in Fig. 1) when surfactant concentration was low and carvacrol/PHBV ratio was high. It was probably due to the fact that this combination of low surfactant concentration and high carvacrol/PHBV ratio increased the viscosity of the solution, consequently decreasing its homogeneity. Moreover, it seems that parameters that increased the solution viscosity (low surfactant concentration coupled to low molecular weight, low aqueous/organic phase volume ratio) increased the carvacrol recovery, probably because the high viscosity reduced the carvacrol diffusivity inside the solution and its evaporation during the heating step at 40 °C (dichloromethane evaporation) (Asua, 2002; Freiberger et al., 2015).

3.1.1.2. Statistical analysis. These results were confirmed by the measurement of coefficient for each parameter (Fig. 5, A). The higher (in positive or negative) was the value of coefficient, the higher was the impact of parameter on carvacrol recovery. Between the five studied parameters, the method was the most impacting parameter on carvacrol recovery (parameter B: coefficient of +17.4 %, so in favor of emulsification method), following by the surfactant concentration (parameter D: coefficient of −5.0 %), the aqueous/organic phase volume ratio (parameter E: coefficient of −2.7 %), the molecular weight of PHBV (parameter A: coefficient of −0.5 %), and the carvacrol/PHBV ratio (parameter C: coefficient of −0.1 %). The statistical analysis revealed that only method and surfactant concentration parameters had a significant impact on carvacrol recovery. Similarly, Pareto test on coefficient of parameters indicated that method and surfactant concentration parameters represented 67.8 % and 19.5 % of the total impact respectively, so more than 80 % cumulatively (10.6 % for aqueous/organic volume phase ratio, 1.8 % for molecular weight and 0.3 % for carvacrol/PHBV ratio, supplementary figure). These two observations means that an appropriated setting for these two parameters should be sufficient to obtain a high carvacrol recovery. However, the statistical analysis also showed a significant combined effect between surfactant concentration and molecular weight parameters (parameters AD: coefficient of +2.7 %). Consequently, the aggregation of all these results allowed to recommend that parameters setup should be the following one: emulsification method, low surfactant concentration and low molecular weight (correlated parameter and coefficient A was negative), to obtain the better carvacrol recovery (between 44.8 % and 61.0 %, Fig. 1).

3.1.2. PHBV recovery

3.1.2.3. Efficiency of the encapsulation method. Fig. 2 displayed the impact of the different parameters (method, PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratio) on PHBV recovery. This material being expensive, it could be interesting to minimize its lost during the encapsulation process. Similarly to carvacrol recovery, the PHBV recovery was impacted by encapsulation method and surfactant concentration, but in an

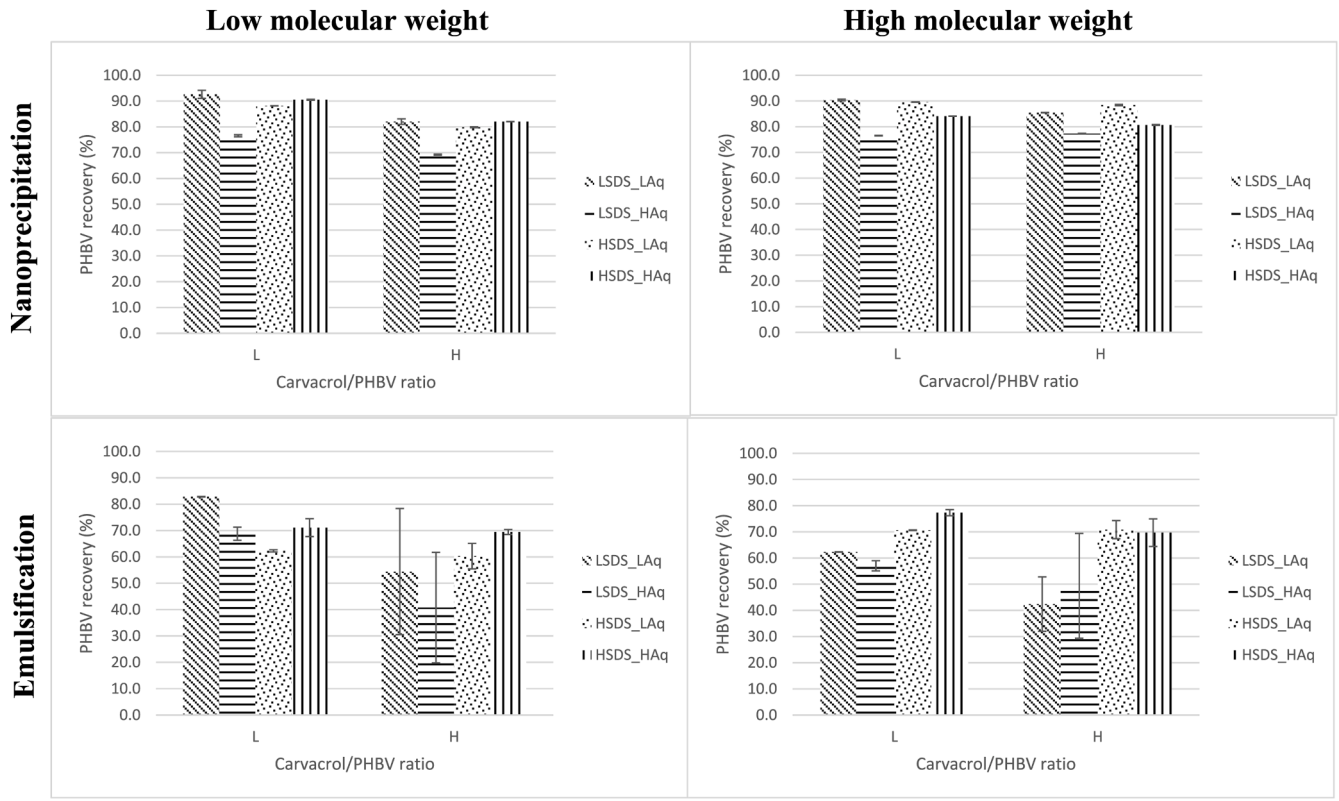

Fig. 2. PHBV recovery after encapsulation of carvacrol in PHBV nanoparticles depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio.

opposite way. Indeed, the PHBV recovery was higher with the nanoprecipitation method (83.4 %) than emulsification one (63.1 %), as it was higher with high surfactant concentration (77.2 %) than with low surfactant concentration (69.3 %). Moreover, the PHBV recovery increased when the carvacrol/PHBV ratio decreased (77.6 % with low carvacrol/PHBV ratio vs 68.7 % with high carvacrol/PHBV ratio). It should be noticed that the impact of surfactant concentration and carvacrol/PHBV ratio was amplified with the emulsification method, in comparison with the nanoprecipitation one (around 2 and 3 time more important for carvacrol/PHBV ratio and surfactant concentration respectively). In a less extent, the PHBV recovery was also positively impacted by a decrease of aqueous/organic volume phase ratio (75.2 % with low aqueous/organic volume phase ratio vs 71.3 % with high aqueous/organic volume phase ratio). Nevertheless, this trend was more pronounced with nanoprecipitation method (87.1% vs 79.6 %) than emulsification one (63.3% vs 63.0 %) and was dependent on surfactant concentration (an increase of aqueous/organic volume phase ratio induced a decrease of PHBV recovery at low surfactant concentration (74.1% vs 64.4 %) and an increase at high surfactant concentration (76.3% vs 78.1 %)). Finally, the PHBV recovery was not impacted by the molecular weight of PHBV (73.2 % with low molecular weight and 73.3 % with high molecular weight).

As for carvacrol recovery, the variability of the PHBV recovery results (error bars) was important when surfactant concentration and carvacrol/PHBV ratio were low and high respectively, exclusively for emulsification method. However, to the contrary of carvacrol recovery, it seems that the way that decreased the solution viscosity (high surfactant concentration, nanoprecipitation method) increased the PHBV recovery. This is probably because high viscosity hamper the sedimentation of molecules during centrifugation, letting part of them in the supernatant (Momen-Heravi et al., 2012).

3.1.2.4. Statistical analysis. The coefficient of parameters (Fig. 5, B) confirmed that the most impacted parameter on PHBV recovery was the method (parameter B: coefficient of −10.1 %, so in favor of nanoprecipitation method), following by the carvacrol/PHBV ratio (parameter C: coefficient of −4.3 %), the surfactant concentration (parameter D: coefficient of 4.0 %), the aqueous/organic phase volume ratio (parameter E: coefficient of −1.9 %), and the molecular weight of PHBV (parameter A: coefficient of 0.03 %). The statistical analysis revealed that encapsulation method, carvacrol/PHBV ratio, and surfactant concentration parameters had all a significant impact on PHBV recovery, while Pareto test indicated that the three of us had a cumulative impact higher than 80 % (49.7 %; 21.2 % and 19.4 % for method, carvacrol/PHBV ratio, and surfactant concentration respectively, supplementary figure). As, there was no significative combined effect between the parameters, the most appropriated setting parameters to obtain the better PHBV recovery was the nanoprecipitation method, a low carvacrol/PHBV ratio and a high surfactant concentration (between 84.1 % and 90.5 %).

3.1.3. Loading capacity

3.1.3.5. Efficiency of the encapsulation method. The loading capacity is represented as the quantity of carvacrol (encapsulated material) over the quantity of PHBV (encapsulant). Fig. 3 displayed the experimental impact of the different parameters (method, PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratio) on loading capacity. Firstly, the results showed that encapsulation method strongly impacted the loading capacity: values were ranged between 0.5 % and 4.5 % with nanoprecipitation method and between 15.9 % and 120.4 % with the emulsification method. Moreover, the loading capacity was higher with a higher carvacrol/PHBV ratio (26.2 % with emulsification method and low carvacrol/PHBV ratio vs 55.9 % with emulsification method and high carvacrol/PHBV ratio). As the loading capacity doubled when carvacrol quantity

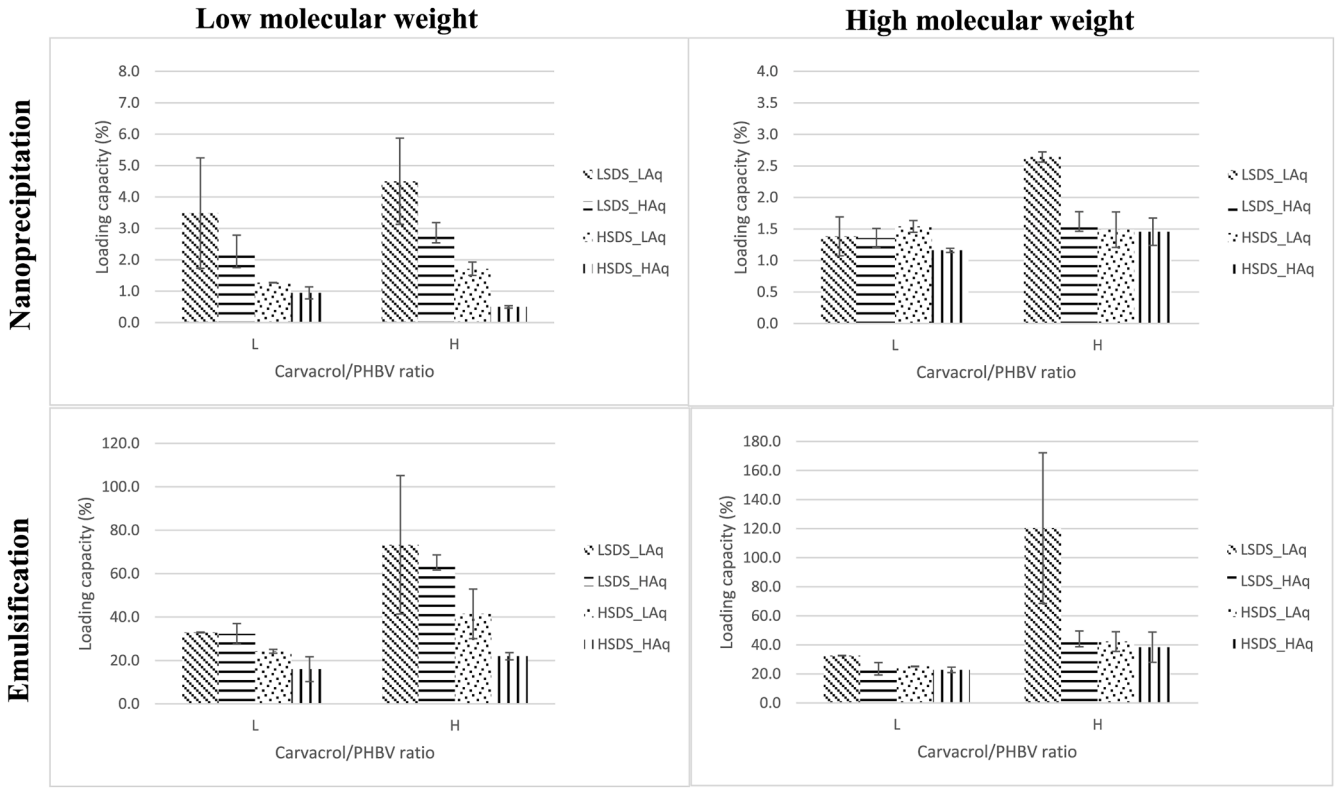

Fig. 3. Loading capacity after encapsulation of carvacrol in PHBV nanoparticles depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio.

was doubled, it could be supposed that the encapsulation saturation was not reached for these two carvacrol/PHBV ratio, as already observed by other authors testing the same oil/polymer ratio (Marcet et al., 2018; Corrado et al., 2022). The loading capacity increased when the surfactant concentration decreased (53.0 % for emulsification method and low surfactant concentration vs 29.0 % for emulsification method and high surfactant concentration). As for carvacrol and PHBV recovery, the loading capacity was also higher with a lower aqueous/organic volume phase ratio (49.0 % for emulsification method and low aqueous/organic volume phase ratio vs 33.0 % for emulsification method and high aqueous/organic volume phase ratio). However, the loading capacity was not strongly impacted by the molecular weight of the PHBV (38.4 % with emulsification method and low molecular weight vs 43.6 % with emulsification method and high molecular weight, that seems to be not significant regarding the error bars).

3.1.3.6. Statistical analysis. All these observations were confirmed by the coefficient of parameters (Fig. 5,C), that showed the method as the most impacted parameters on loading capacity (parameter B: coefficient of 19.6 %, so in favor of emulsification method), following by the carvacrol/PHBV ratio (parameter C: coefficient of 7.5 %), the surfactant concentration (parameter D: coefficient of −6.3 %), the aqueous/organic phase volume ratio (parameter E: coefficient of −4.2 %), and the molecular weight of PHBV (parameter A: coefficient of 1.2 %). Due to the way that loading capacity was calculated (Eq. (5)), when a parameter influenced the carvacrol and PHBV recovery in an opposite way, the impact of parameter on loading capacity was amplified. It was the case of encapsulation method, and surfactant concentration. The statistical analysis revealed that encapsulation method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic phase volume ratio had all a significant impact on loading capacity. However, Pareto test on coefficient of parameters indicated that the cumulative impact of encapsulation method, carvacrol/PHBV ratio and surfactant concentration parameters were sufficient to reach 80 % of the total impact (50.5 %; 19.4 % and 16.3 % for method, carvacrol/PHBV ratio, and surfactant concentration respectively, supplementary figure). The statistical analysis also showed a significant combined effect between numerous parameters, generally implying the encapsulation method and surfactant concentration parameters (BC, BD, ADE, BCD, ABDE, ACDE, ABCDE, Fig. 5C). Consequently, the most appropriated setting parameters to obtain the higher loading capacity was the emulsification method, a high carvacrol/PHBV ratio and a low surfactant concentration (between 44.1 % and 120.4 %), and eventually a low aqueous/organic phase volume ratio (between 73.3 % and 120.4 %). It should be noticed that these best solutions had a high variation (error bars, calculated in this case), due to the high variation of carvacrol recovery.

3.1.4. Process efficiency

3.1.4.7. Efficiency of the encapsulation method. The process efficiency was defined as the quantity of obtained nanoparticles over the total quantity of initial material introduced in the samples (Eq. (2)). Consequently, the parameters setup that could increase carvacrol and PHBV recovery should also increase the process efficiency. However, previous results showed that adjusted parameters that increased carvacrol recovery (emulsification method and high surfactant concentration) generated a decrease of PHBV recovery. Fig. 4 displayed the impact of the different parameters (method, PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratio) on process efficiency. Contrary to all other results, the most important parameter was not encapsulation method anymore, but the carvacrol/PHBV ratio, which decrease induced an increase of process recovery (44.6 % with high carvacrol/PHBV ratio, 58.3 % with low carvacrol/PHBV ratio and 92.9 % for the control without carvacrol). Due to the high volatility of the molecule, the loss of carvacrol during encapsulation process was higher than the loss of PHBV (carvacrol

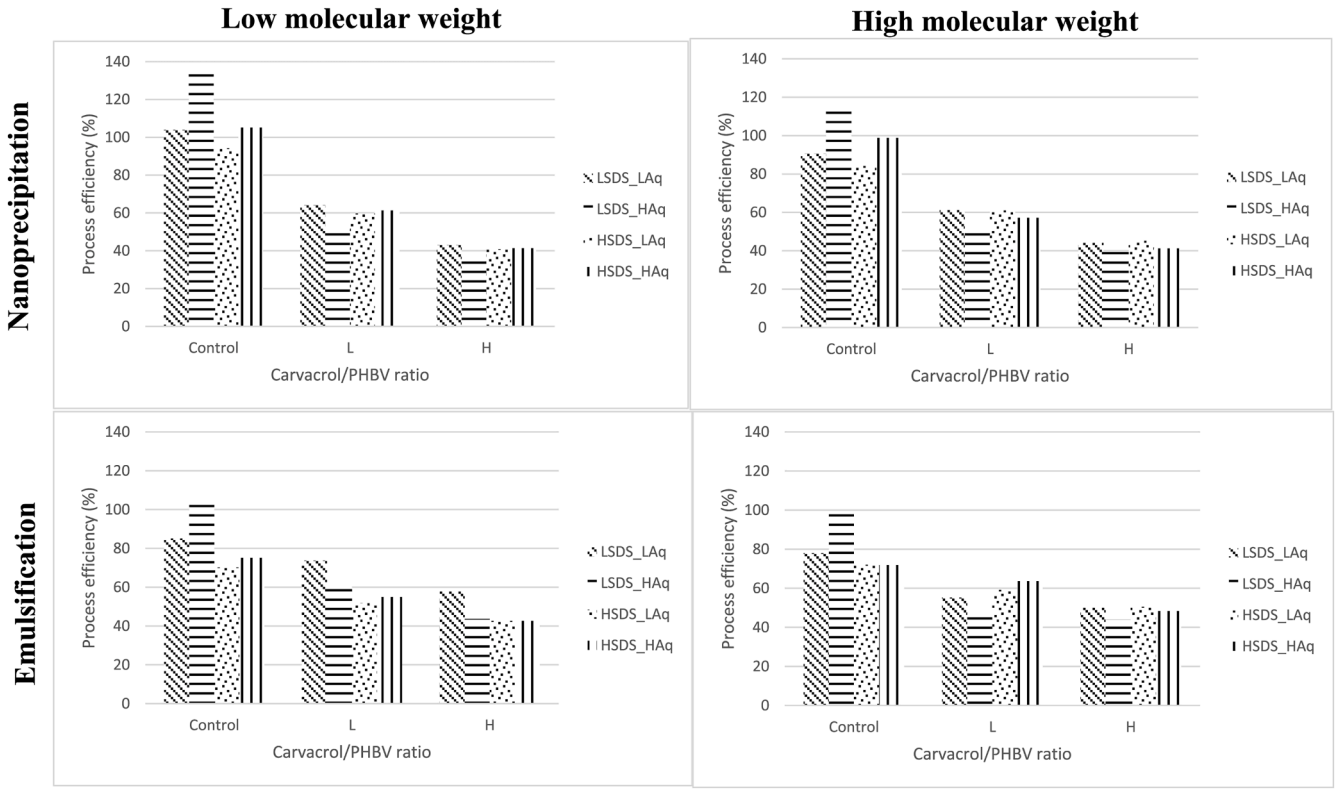

Fig. 4. Process efficiency after encapsulation of carvacrol in PHBV nanoparticles depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio.

recovery and PHBV recovery was between 0.41–61.0 % and 42.4–92.6 % respectively (Figs. 1 and 2), depending on parameters setup). Even if the carvacrol/PHBV ratio parameter did not affected the carvacrol recovery, the higher loss of carvacrol than PHBV during encapsulation process induced a decrease of process efficiency with an increase of carvacrol/PHBV ratio. Moreover, the negative impact of the carvacrol/PHBV ratio was amplified by the fact that this parameter also negatively impacted the PHBV recovery. The process efficiency increased when the aqueous/organic volume phase ratio decreased, but only in the case of a low surfactant concentration and the presence of carvacrol in the formulation (for low surfactant concentration: 56.1% vs 47.0 % with low and high aqueous/organic volume phase ratio respectively, and for high surfactant concentration: 51.4% vs 51.3 % with low and high aqueous/organic volume phase ratio respectively). In a less extent and when carvacrol was present in the formulation, the emulsification method tends to increase the process recovery in comparison with the nanoprecipitation method (53.2 % with emulsification method vs 50.1 % with nanoprecipitation method), and more specifically with low surfactant concentration and low molecular weight (59.1 % with emulsification method vs 53.1 % with nanoprecipitation method). Finally, the molecular weight and surfactant concentration did not impact the process efficiency (51.7% vs 51.5 % with low and high molecular weight respectively, and 51.6% vs 51.1 % with low and high surfactant concentration respectively).

It should be noticed that, on control samples, results were completely different than in presence of carvacrol: the process efficiency increased when the aqueous/organic volume phase ratio increased (84.8% vs 100.3 % with low and high aqueous/organic volume phase ratio respectively), with the nanoprecipitation method (82.1% vs 103.7 % with emulsification and nanoprecipitation method respectively), with low molecular weight (97.1% vs 88.8 % with low and high molecular weight respectively), and with low surfactant concentration (101.9% vs 83.9 % with low and high surfactant concentration respectively). These observations made sense: as parameters generally affected process efficiency in the same way than carvacrol recovery when carvacrol was present in the formulation (due to its high volatility), these parameters also generally affected the process efficiency in the same way than PHBV recovery when carvacrol was absent of the formulation (because in this case, process efficiency correspond to PHBV recovery). In some cases, the process efficiency of the control overpassed 100 %, that was probably due to a lack of solvent and surfactant evaporation, as already observed by other author with the encapsulation of roasted coffee in polylactic acid polymer (Freiberger et al., 2015). Moreover, the process efficiency obtained in this present study are in the same order than literature, as Shakeri et al. (2014) that get a 84 % process efficiency for encapsulation of carvacrol with PHB.

3.1.4.8. Statistical analysis. The coefficient of parameters (Fig. 5, D, calculated only with data from samples where carvacrol were present) confirmed that the most impacted parameter on process efficiency was the carvacrol/PHBV ratio (parameter C: coefficient of −6.8 %), following by the aqueous/organic phase volume ratio (parameter E: coefficient of −2.3 %), the method (parameter B: coefficient of 1.4 %, so in favor of emulsification method), the molecular weight of PHBV (parameter A: coefficient of −0.2 %), and the surfactant concentration (parameter D: coefficient of −0.1 %). Pareto test on coefficient of parameters indicated that carvacrol/PHBV ratio and aqueous/organic phase volume ratio represented 62.8 % and 21.1 % of the total impact respectively, so more than 80 % cumulatively (supplementary figure). Consequently, the most appropriated setting parameters to obtain the higher process efficiency was a low carvacrol/PHBV ratio and a low aqueous/organic phase volume ratio (between 51.9 % and 73.6 %), and preferentially with low surfactant concentration (correlated parameter with low aqueous/organic phase volume ratio) (between 55.2 % and 73.6 %).

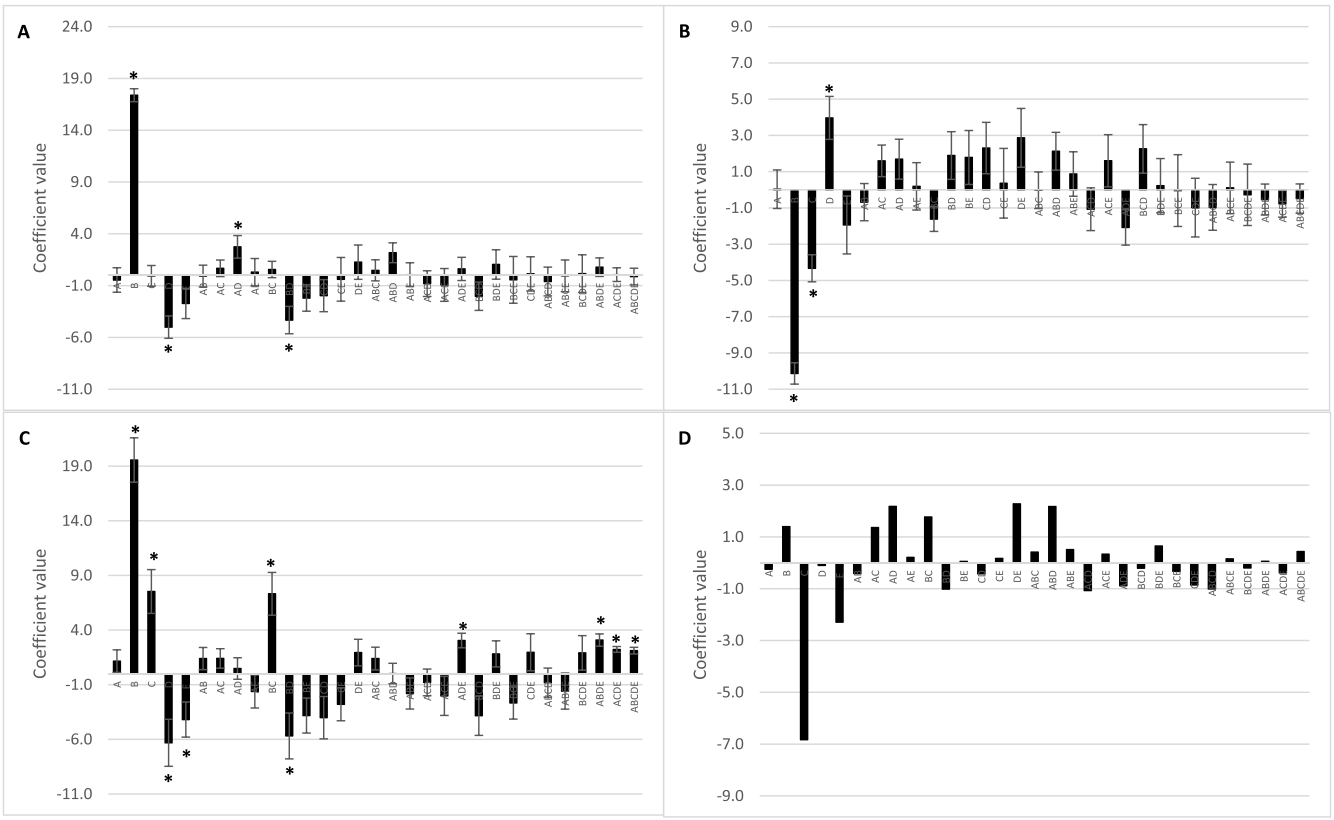

Fig. 5. Coefficient value of the different factors (A to E, corresponding to molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio) and combined factors (AB to ABCDE) on encapsulation efficiency: carvacrol recovery (A), PHBV recovery (B), loading capacity (C) and process efficiency (D). For (A), (B) and (C) graphics, stars indicated a significant impact of factor or combined factor (student test, $p = 0{,}05$).

3.2. *Impact of parameters on nanoparticles' size and morphology*

Figs. 6 and 7 displayed the impact of the different parameters (method, PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratio) on the morphology and size of the nanoparticles thanks to SEM images. Controls were prepared, using the same procedure without the addition of the carvacrol molecule. The Fig. 6, A represented the best observation of nanoparticles (ideally spherical and no-agglomerated nanoparticles), that were obtained with emulsification method, for low molecular weight of polymer, low surfactant concentration and low Aqueous/Organic phase volume ratio, consequently all parameters that allowed to reduce the viscosity of solution. It should be noticed that a high surfactant concentration (with a remaining spherical shape in this case) and/or high Aqueous/Organic phase volume ratio and/or nanoprecipitation method tended to agglomerate the nanoparticles obtained in the control samples (data not shown). The propensity of nanoparticles to be deformed during the encapsulation process was probably due to the polymer flexible nature (Corrado et al., 2022).

With the presence of carvacrol, the results firstly showed that nanoprecipitation method led to nanoparticles agglomeration, regardless of the polymer molecular weight, the carvacrol/PHBV ratio, or the surfactant concentration (Fig. 6, B and C). However, it can be inferred that an increase in the Aqueous/Organic phase volume ratio had an impact on the size and shape of the particles, and more specifically, the particles became smaller (−43 nm, Fig. 7, A and B) and more irregular, even though they remained agglomerated (Fig. 6, C compared to B). Therefore, these results proved that the nanoprecipitation method was not adapted to the production of nanoparticles encapsulated carvacrol. Indeed, non-spherical and/or agglomerated nanoparticles might have complex diffusion pathways due to their irregular geometry, thus leading to a less controlled and predictable release profile (Mühlen et al., 1998; Freiberger et al., 2015).

The particles' size was higher with emulsification method (between 586 ± 125 and 881 ± 229 nm) than nanoprecipitation one (between 427 ± 118 and 585 ± 105 nm) (Fig. 7). The analysis of the shape and structure of PHBV nanoparticles using the emulsification method also showed a high impact of Aqueous/Organic phase volume ratio: as for nanoprecipitation method, the particles became more spherical and smaller (−10 nm, Fig. 7, C and D) with a high Aqueous/Organic phase volume ratio, regardless the other parameters (Fig. 6D–F vs G–I). The increase of aqueous phase in regards to organic one allow to generate a higher distance between the droplet of dispersed phase (organic phase) inside the continuous phase (aqueous), thus avoiding a coalescence phenomenon (Landfester, 2001; Song et al., 2006; Corrado et al., 2022). It seems that agglomeration was also more pronounced with higher molecular weight polymers (Fig. 6, E and H for high molecular weight in comparison with D and G for low molecular weight) as well as it increased particles' size (+78 nm, Fig. 7, C and D). More specifically, when polymer had a high molecular weight, the carvacrol/PHBV ratio and surfactant concentration did not affect the shape of the nanoparticles, that remained similarly agglomerated whatever the carvacrol/PHBV ratio and surfactant concentration (Fig. 6, E for low Aqueous/Organic phase or G for high Aqueous/Organic phase) (but affected the particles' size, Fig. 7, C and D). However, when polymer had a low molecular weight, these two parameters highly affected the agglomeration and shape of nanoparticles. Indeed, it was observed that a high concentration of surfactant and carvacrol/PHBV ratio caused irregular shapes and agglomeration of particles, especially at low Aqueous/Organic phase volume ratio and (Fig. 6, F, and particles' size of 690 ± 175 nm, Fig. 7, C). On the other hand, an increase of surfactant concentration and a decrease of carvacrol/PHBV ratio at low Aqueous/Organic phase volume ratio allowed to obtained separated and spherical nanoparticles with a particles' size of 610 ± 118 nm (Figs. 6, I,

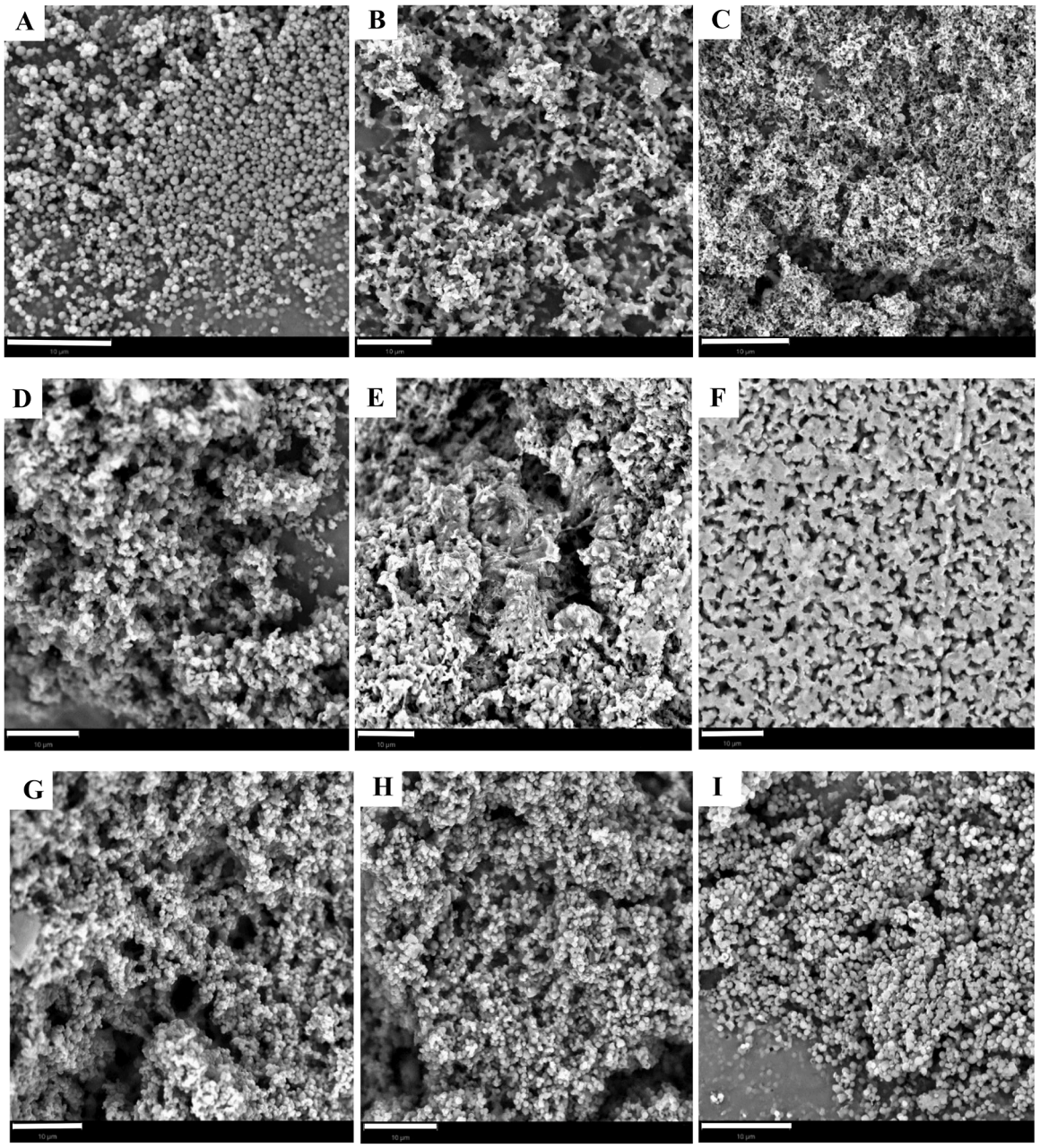

Fig. 6. SEM images of the nanoparticles obtained after encapsulation of carvacrol in PHBV depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic phase volume ratio. Bar scale represented 10 um.
(A) (control: low molecular weight, emulsification method, low surfactant concentration and low Aqueous/Organic phase volume ratio), (B) (low molecular weight, nanoprecipitation method, high carvacrol/PHBV ratio, low surfactant concentration and low Aqueous/Organic phase volume ratio), (C) (low molecular weight, nanoprecipitation method, low carvacrol/PHBV ratio, low surfactant concentration and high Aqueous/Organic phase volume ratio), (D) (low molecular weight, emulsification method, high carvacrol/PHBV ratio, low surfactant concentration and low Aqueous/Organic phase volume ratio), (E) (high molecular weight, emulsification method, high carvacrol/PHBV ratio, low surfactant concentration and low Aqueous/Organic phase volume ratio), (F) (low molecular weight, emulsification method, high carvacrol/PHBV ratio, high surfactant concentration and low Aqueous/Organic phase volume ratio), (G) (low molecular weight, emulsification method, low carvacrol/PHBV ratio, low surfactant concentration and high Aqueous/Organic phase volume ratio), (H)) (high molecular weight, emulsification method, low carvacrol/PHBV ratio, low surfactant concentration and high Aqueous/Organic phase volume ratio), (I)) (low molecular weight, emulsification method, low carvacrol/PHBV ratio, high surfactant concentration and high Aqueous/Organic phase volume ratio).

7, C), whose aspects were more close to the control one (Fig. 6, A). Consequently, the most appropriated setting parameters to obtain the more spherical, small and separated nanoparticles were the emulsification method, a low molecular weight of polymer, low carvacrol/PHBV ratio and Aqueous/Organic phase volume ratio and high surfactant concentration.

Observations of polymer nanoparticles on SEM by other authors in literature also showed a variation of particles shape, size and agglomeration depending on parameters used to produce the nanoparticles (Leimann et al., 2013; Shakeri et al., 2014; Freiberger et al., 2015; Farrag et al., 2018; Granata et al., 2018; Pignatello et al., 2019; Samrot et al., 2021; Corrado et al., 2022). For example, Leimann et al. (2013) showed that an increase of molecular weight of PHBV induced an increase of nanoparticles size (Mw PHBV=44.350 g mol^{-1}, nanoparticles size=133 nm vs Mw PHBV=369.9 g mol^{-1}, nanoparticles size=300 nm), while Nachiyar et al. (2015), Senthilkumar et al. (2017) showed that the

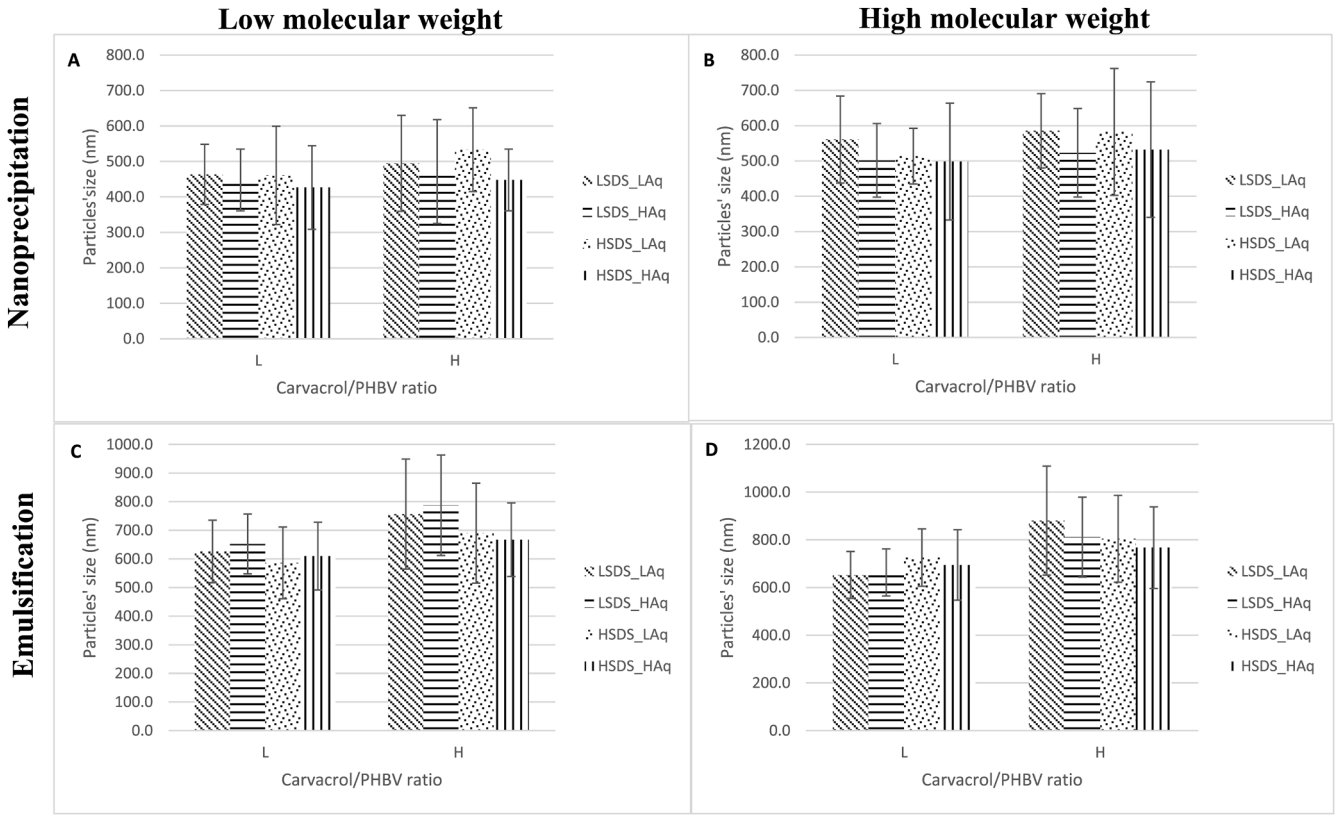

Fig. 7. Size of the nanoparticles obtained with SEM images after encapsulation of carvacrol in PHBV nanoparticles depending on different parameters: molecular weight of PHBV, method, carvacrol/PHBV ratio, surfactant concentration and aqueous/organic ratio.

presence of active molecules in the formulation (curcumin and levofloxaxin) also induced an increase of the nanoparticles size (based on PHA polymer), and Corrado et al. (2022) showed that an increase of Aqueous/Organic phase volume ratio allowed to avoid a nanoparticles agglomeration (PHA). Finally Farrag et al. (2018) showed that emulsification method induced an increase of nanoparticles size, but more separated than those obtained with nanoprecipitation method, but also that an increase of SDS concentration stabilized the emulsification and avoided the aggregation of nanoparticles.

4. Conclusion and recommendations

This present study investigated the impact of different parameters on encapsulation efficiency of carvacrol into PHBV nanoparticles; estimated through carvacrol and PHBV recovery, loading capacity, process efficiency, as well as the morphology of nanoparticles. More specifically, an experimental plan with 5 parameters and 2 levels per parameter was applied to select the best method (nanoprecipitation or emulsification) and operating conditions (PHBV molecular weight, surfactant concentration, carvacrol/PHBV ratio and Aqueous/Organic phase volume ratios).

The results showed that: (1) carvacrol recovery could be increased, using the emulsification method, low surfactant concentration and low molecular weight and, preferentially low Aqueous/Organic phase volume ratios; (2) PHBV recovery could be increased, using the nanoprecipitation method, low carvacrol/PHBV ratio, high surfactant concentration, and preferentially low Aqueous/Organic phase volume ratios; (3) loading capacity could be increased, using the emulsification method, high carvacrol/PHBV ratio, low surfactant concentration, and low Aqueous/Organic phase volume ratios; (4) process efficiency could be increased, using the emulsification method, low carvacrol/PHBV ratio, low aqueous/organic phase volume ratio, and preferentially low surfactant concentration; and (5) the morphology and size of nanoparticles could be improved (small, spherical shape and separated particles), using the emulsification method, low molecular weight of polymer, low carvacrol/PHBV ratio, low Aqueous/Organic phase volume ratio and high surfactant concentration, as reported in Table 2.

Therefore, to optimize the carvacrol encapsulation in PHBV nanoparticles (so carvacrol and PHBV recovery, loading capacity, process efficiency, and the morphology of nanoparticles), it is recommended to use a polymer with low molecular weight, a low Aqueous/Organic phase volume ratio (a consensus is reached for these two parameters), the emulsification method (because no carvacrol was entrapped with nanoprecipitation method), and a low carvacrol/PHBV ratio (the loading capacity was dependent of carvacrol quantity in any case). However, the better choice of the surfactant concentration will depend on the way that the molecule will be introduced in the packaging. Indeed, a low surfactant concentration allowed to obtain high carvacrol recovery (55.1 %), but the nanoparticles were agglomerated, which make it non-homogeneous, less processable for coating application, and will probably decrease the release rate during storage. A high surfactant concentration conducted to lower carvacrol recovery (30.1 %), but the nanoparticles were spherical and separated, which make it more homogenous and suitable for coating application and, as a consequence is a preferable strategy. Although these findings help to increase the encapsulation efficiency of carvacrol by proposing appropriate parameters setting for encapsulation protocol, some improvements could again be obtained: for example the process efficiency could probably be increased by screening the most appropriate centrifugation parameters; or the carvacrol recovery could also probably be increased by finding more adapted materials to avoid lost during the protocol. Moreover, in the future, the active film production and release of carvacrol through storage should be studied to confirm if this encapsulation strategy could be as good as the conventional one (encapsulation through beta-cyclodextrin).

Table 2
The optimum level of the different protocol parameters tested on cavracrol encapsulation in PHBV to optimize the criteria observed (encapsulation efficiency and morphology of nanoparticles) (n.*i* = no impact).

	Carvacrol recovery	PHBV recovery	Loading capacity	Process recovery	Shape and morphology
Molecular weight (Mw) (A)	–	n.i	n.i	n.i	–
Method (Meth) (B)	+	–	+	+	+
Carvacrol/PHBV ratio (Carv) (C)	n.i	–	+	–	–
Surfactant concentration (SDS) (D)	–	+	–	n.i	+
Aqueous/Organic phase ratio (Aq) (E)	n.i	n.i	–	–	–

Ethical statement

This work does not involve trials on any human or animals.

CRediT authorship contribution statement

Aynura Rzayeva: Writing – original draft, Methodology, Investigation, Conceptualization. **Valérie Guillard:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Lucie Bonny:** Methodology, Investigation, Conceptualization. **Nathalie Gontard:** Writing – review & editing, Supervision, Conceptualization. **Fanny Coffigniez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgments

AR, VG, NG and FC conceived and designed the experiments. AR and LB performed the experiments. AR, VG, LB, NG and FC analysed the data and wrote the paper. VG and FC supervised the work. The project was conducted thanks to the financial support provided by the Ministry of Science and Education of the Republic of Azerbaijan.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100466.

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