Microencapsulation of bioactive compounds: developing customised and sustainable solutions

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ABSTRACT:

Microencapsulation is introduced as a key enabling technology to protect sensitive bioactive compounds, improve their stability and control their release in pharmaceutical, cosmetic, food and related products (Picot et al., 2015). The main objective of this project is to research and apply microencapsulation techniques to protect bioactive substances such as polyphenols and promote the development of personalised and sustainable products. In it, polyphenols from by-products of the wine industry (grape peels) were selected as target bio actives due to their antioxidant and health-promoting properties. Different extraction techniques were applied, followed by microencapsulation using ionic gelation and coacervation methods with natural polymers such as alginate, pectin and hydroxypropyl methylcellulose (HPMC). The resulting microcapsules were incorporated into pilot formulations of functional foods and cosmetic products. Experimental results demonstrate that the selection of encapsulation technique and material strongly influences capsule size, stability and suitability for final applications.

Keywords: Microencapsulation, polyphenols, circular economy

INTRODUCTION:

In recent years, the growing demand for healthier, safer and more sustainable products has driven innovation in food, cosmetic and pharmaceutical sectors. Bioactive compounds such as polyphenols are secondary metabolites present in natural foods that exert beneficial effects on health, such as antioxidant, anti-inflammatory, and anti-cancer properties. However, many of these compounds are highly sensitive to environmental factors such as temperature, oxygen, light, humidity or pH, leading to degradation, thereby reducing their effectiveness.

Microencapsulation emerged as an effective technological solution to overcome these limitations. This technique consists of enclosing active compounds within a protective matrix of micrometric size (1-1000 µm), creating a physical barrier between the bioactive compound and the surrounding environment. As a result, microencapsulation allows protection of sensitive compounds, improvement of physicochemical stability, masking of undesirable flavours or odours, separation of incompatible ingredients and controlled release of the active principle, enhancing bio accessibility and bioavailability.

Within the context of Vocational Education and Training (VET), microencapsulation offers an excellent opportunity to integrate applied research, multidisciplinary learning and sustainability principles.

The main objective of this project is to research and apply microencapsulation techniques to encapsulate polyphenols extracted from grape skins. Therefore, in addition to providing technical training to teachers and students in these emerging techniques, this project also adds value to agro-industrial waste by transforming it into natural bioactive compounds under a circular economy model.

RESULTS:

Polyphenols Identification and Quantification

According to the results obtained, green chemistry methods showed higher yields than traditional methods. As shown in figure 1, the most efficient method would be ultrasound extraction, followed by direct digestion extraction, while the Soxhlet extraction would be the lowest performing method.

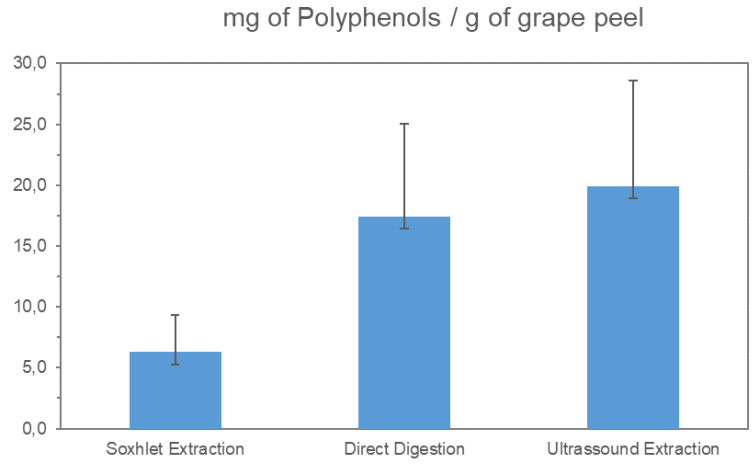

Figure 1 Comparative analysis of the different extraction methods used.

Next, the main polyphenols present in the extracts were identified using TLC (thin-layer chromatography) and UV spectroscopy. Two spots attributable to anthocyanins based on the literature (Santos et al., 2013) were observed in the TLC. Identification using UV-Vis spectroscopy showed a peak between 270 and 280 nm, possibly corresponding to resveratrol.

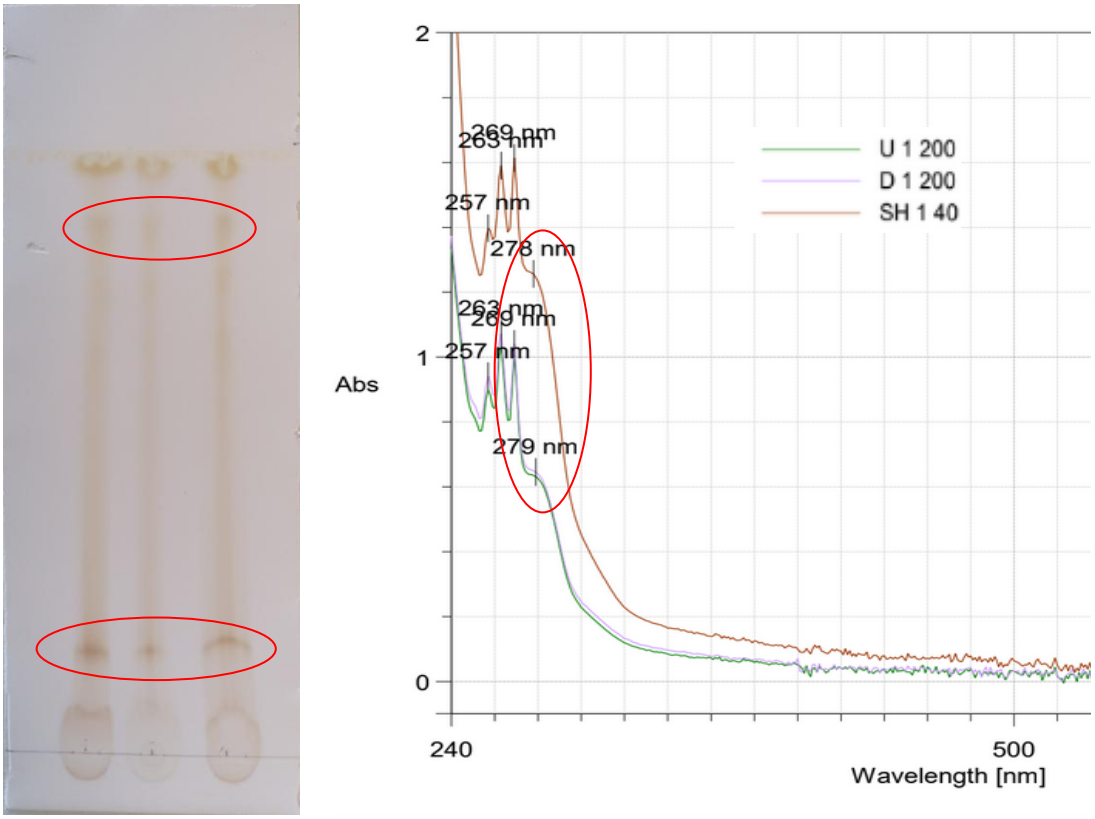

Figure 2 Main polyphenol presence. a) Result of the analysis by TLC. From left to right: digestion, Soxhlet extraction, and ultrasound. b) UV spectroscopy analysis.

Microencapsulation Techniques

Two main encapsulation strategies were assayed ionic gelation and coacervation. Microcapsules were successfully obtained using both encapsulation techniques. The results highlighted clear differences between the methods. Since the polyphenols were extracted using an ethanol-water mixture as a solvent, and due to the low solubility of the materials chosen for encapsulation (alginate and pectin) in this solvent-mixture, inverse ionic gelation was chosen as the optimal encapsulation technique.

Inverse ionic gelation was carried out using alginate, pectin and alginate/pectin blends as encapsulating materials. Alginate-based capsules showed higher flexibility, whereas pectin and alginate/pectin capsules were more resistant, although their incorporation into aqueous formulations proved more challenging. In general, this method produced larger capsules than

coacervation method. The capsules size depends on encapsulation conditions thus various size influencing factors were analysed (Figure 3). Among these aspects were the type of propeller used and the dripping method employed, spraying or syringe. The best results were obtained using a 1x4 bladder stirrer together with a sprayer; these produced microcapsules so small that they can only be observed under a microscope.

Coacervation method was performed using hydroxypropyl methylcellulose (HPMC) as the encapsulating material. In addition, it was verified that the resulting microencapsulates withstand lyophilization, which favours their inclusion in solid formulations. This method allowed the formation of smaller microcapsules (figure 4), which were subsequently separated by centrifugation and washed with an acidic water solution before being lyophilized. Two different frozen strategies were followed: In the first, the samples were lyophilized directly, resulting in a "gummy" substance with high viscosity and low dissolution. In the second, the samples were pre-frozen in a conventional freezer. This second strategy resulted in a three-dimensional "polystyrene-like" structure with significantly faster redissolution times.

The characterization of the microencapsulates was carried out by microscopic observation, which allowed the evaluation of their general morphology and confirmed the formation of encapsulated structures. However, it was not possible to accurately determine the size of the microcapsules, as analysis tools with the necessary resolution or calibration were not available.

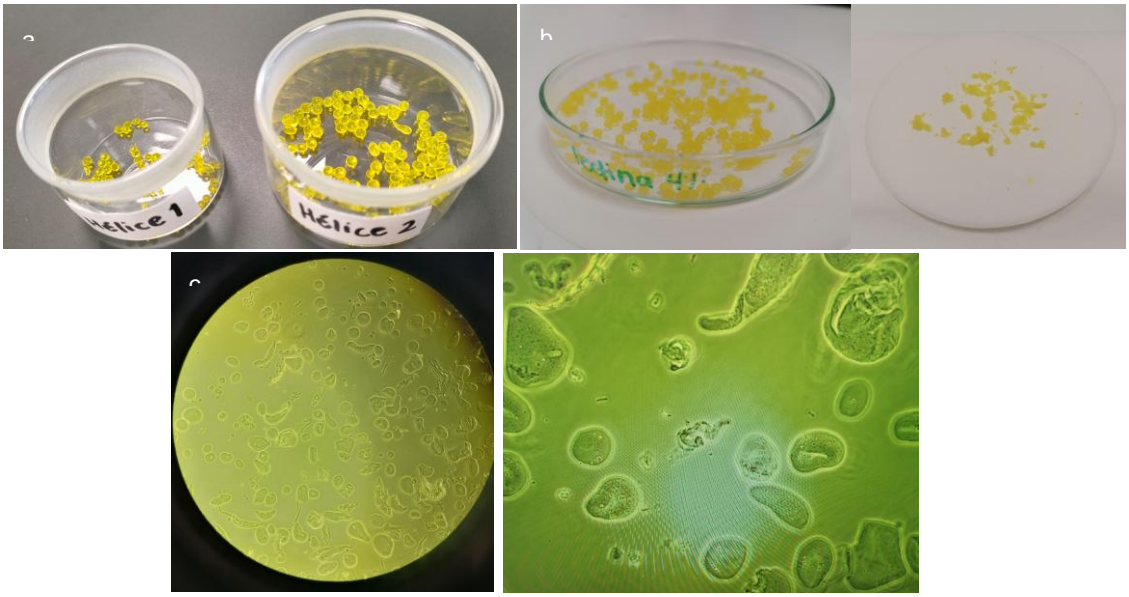

Figure 3 Dependence of capsule size on encapsulation conditions.

Figure 3. Dependence of capsule size on encapsulation conditions. a) Two types of propellers were used: 1x4 bladder stirrer (helice 1) and straight stirrer (helice 2). b) The left picture shows the capsules size obtained using a syringe as a dripping method while capsules of the right picture were obtained by spraying. c) These pictures illustrate the capsules obtained with a 1x4 bladder stirrer together with a sprayer.

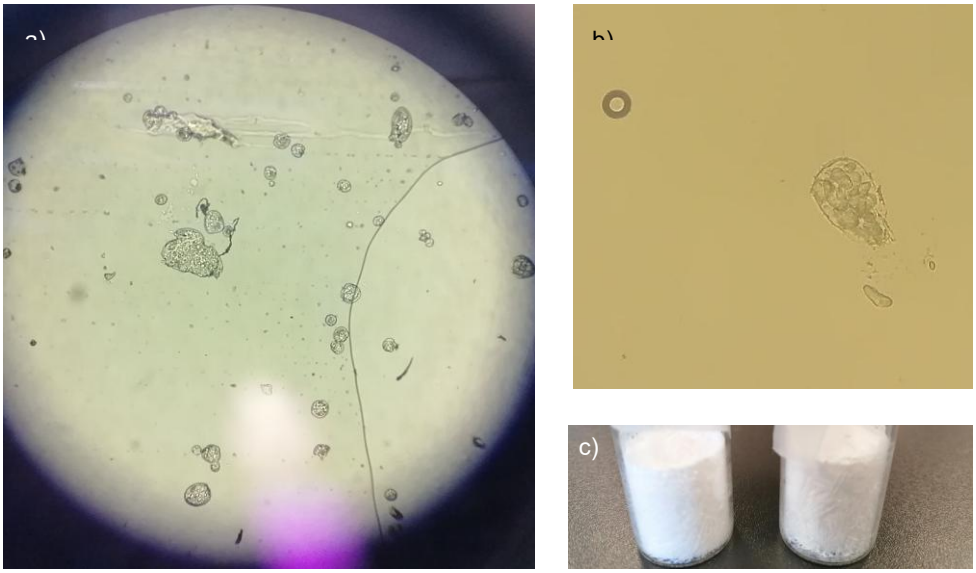

Figure 4 Microcapsules obtained by the coacervation method.

Figure 4. Microcapsules obtained by the coacervation method. a) Image obtained under an optical microscope of the microcapsules obtained. b) Magnified view of one of the microcapsules obtained. c) On the right is the freeze-dried sample, and on the left is the previously frozen sample.

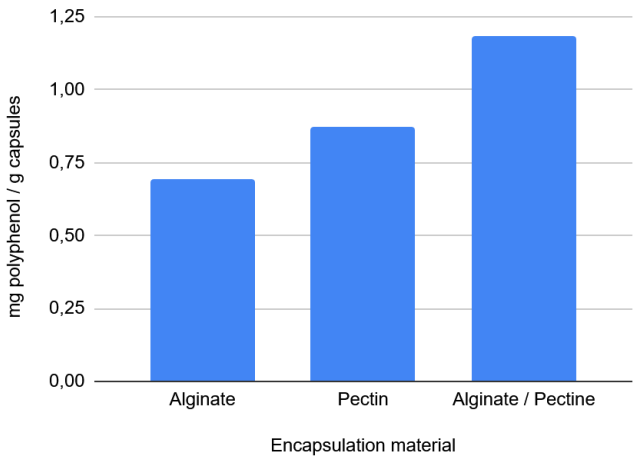

Figure 5 Evaluation of the encapsulation efficiency of the different coatings.

Possible Applications

Once the microcapsules had been obtained, their possible applications were studied. It was decided that the microcapsules obtained by inverse ionic gelation would be introduced into a dermo cosmetic formulation, while those obtained by the coacervation method were suitable for their inclusion in powders or solid capsules due to their small size and resistance to drying.

Because the microcapsules in aqueous bases tended to destabilise, for the dermo cosmetic formulation an oily formulation was chosen to ensure their integrity. An oil-based emulsion and serum containing microencapsulated grape extracts were prepared.

The obtained microcapsules were incorporated into pilot formulations including functional food products such as spirulina gummies with microencapsulated turmeric and kombucha capsules enriched with quinoa protein.

DISCUSSION:

The results obtained in this project confirm that microencapsulation is effective for protecting sensitive bioactive compounds, such as polyphenols. The use of grape peels from partner wineries demonstrated the potential to transform agro-industrial waste into high-value functional ingredients. This approach is in line with current sustainability challenges.

Regarding extraction methods, ultrasound-assisted extraction showed superior efficiency compared to conventional Soxhlet and direct digestion methods. The higher yields obtained under milder temperature and shorter extraction times highlight the advantages of green chemistry approaches, both in terms of energy consumption and preservation of thermolabile compounds.

The identification of anthocyanins by TLC and the detection of a UV absorption peak between 270–280 nm, potentially corresponding to resveratrol, confirm the presence of relevant bioactive compounds in the extracts.

Regarding encapsulation strategies, clear differences were observed between inverse ionic gelation and coacervation. The choice of inverse gelation was appropriate given the hydroalcoholic nature of the extract and the limited solubility of alginate and pectin in ethanol-water mixtures. This methodological adaptation demonstrates the importance of selecting encapsulation techniques according to the compatibility of the bioactive compounds and the coating materials.

Ionic gelation produced larger microcapsules compared to coacervation. The size of the capsules was strongly influenced by parameters such as the stirring system and the dripping method, reflecting the importance of conditions in controlling the size of the particles obtained. Encapsulation efficiency results indicate that the alginate–pectin hybrid matrix achieved higher polyphenol retention compared to single-polymer systems. This may be attributed to synergistic interactions between the polymers, leading to improved network density and reduced diffusion of the encapsulated compounds. These findings suggest that polymer blending may represent a promising strategy for optimising encapsulation performance.

The coacervation method, using HPMC as the coating material, produced smaller microcapsules that were resistant to lyophilization. Interestingly, direct lyophilization resulted in a viscous and poorly soluble material, while freezing prior to lyophilization generated a porous structure with better redissolution properties. This observation highlights the importance of post-processing in the final functionality/applicability of microcapsules.

Regarding potential applications, the different properties of the capsules obtained enabled them to be included in different formulations. The larger, more flexible capsules obtained through inverse ionic gelation were more suitable for oil-based dermo cosmetic systems, while the smaller, lyophilized coacervates showed greater potential for solid or powder formulations. This confirms that the selection of the encapsulation technique must be consistent with the desired end product.

Despite these promising results, the lack of advanced analytical equipment limited the accuracy of particle size measurements and detailed studies on release kinetics. In addition, long-term stability studies and *in vitro* bio accessibility tests would be necessary to evaluate the functional efficacy of encapsulated polyphenols.

Overall, the findings confirm that microencapsulation represents a versatile and scalable approach for protecting natural bio actives and facilitating their incorporation into customised and sustainable food and cosmetic products.

Beyond the technical outcomes, this project demonstrates the educational and innovation value of applied research within Vocational Education and Training. By integrating extraction, analytical chemistry, formulation science and sustainability principles, the project provided multidisciplinary learning opportunities while generating industry-oriented solutions. The valorisation of winery by-products further strengthens its contribution to circular economy strategies within the Basque region.

METHODS:

Extraction of Bioactive Compounds

Polyphenols were selected as bioactive compounds of interest for this project due to their antioxidant activity and relevance in food and cosmetic applications (Scalbert et al. 2005). The grape peels were obtained from the partner wineries Altún (D.O. Rioja) and HIKA (D.O. Getaria) after the trodden. Polyphenols were extracted following the methodology reported by Cruz et al., 2012, using conventional techniques, specifically Soxhlet extraction and direct digestion. In addition, a green chemistry approach based on ultrasound-assisted extraction (UAE) was applied according to the procedure described by Carrera et al. 2012. Prior to extraction, the grape peels were dried in an oven at a temperature of 60°C for 6 hours, so that the moisture content was less than 10%.

For conventional methods, hydroalcoholic mixtures of ethanol and water (1:1, v/v) were employed as a solvent. The extractions were carried out for 4–6 hours at 40–50 °C. In contrast, the extraction of phenolic compounds by means of ultrasound was performed using also a water–ethanol mixture (50:50) but 15 s on 10 s off cycles conducted for 15 minutes at 30–40 °C. All the extractions were carried out with a solute-solvent ratio (w/v) of 1:10.

Identification and Quantification

Total polyphenol content was determined using the Folin–Ciocalteu method as described by García et *al.*, (s.f.). To identify the anthocyanins, present in each extract, the obtained fractions were analysed by thin layer chromatography as described by Santos et al., 2013. For this purpose, 10 µL of each sample was spotted in a Silica Gel 60F254 plate. The used mobile phase was composed of ethyl acetate, glacial acetic acid, formic acid and distilled water (100:11:11:26). The presence of resveratrol was analysed by UV-VIS absorbance scan from 250 - 700 nm at room temperature using a Jasco V-630 UV-Vis double-beam spectrophotometer with a slit width of 2nm. These analyses ensured the presence and concentration of target compounds prior to encapsulation.

Microencapsulation Techniques

Two main encapsulation strategies were applied (Mosquera-Vivas et al., 2024).

Inverse Ionic Gelation

Microspheres containing polyphenol were prepared using pectin alone, alginate alone and pectin with sodium alginate together. Briefly 1 mL of grape peels extract was diluted in 9 mL of an ethanol-water (50:50) mixture. The resulting solution was filtered by gravity prior to the addition of calcium chloride at final concentration of 1% within. The encapsulation process was carried out using alternative polymer matrices (2% alginate solution, 4% pectin solution and a hybrid mixture of 2% alginate and 4% pectin) to compare structural outcomes. These polymers were added to the polyphenol-mixture solution under continuous agitation using different stirring conditions. Dispersing tools and dropwise systems were tested to study the differences in the size of the resulting capsules. The resulting microcapsules were separated by gravity filtration. The capsules obtained were stored for subsequent characterization in an opaque container in the refrigerator.

Coacervation

For the preparation of polyphenol dispersion 1 ml of extracted polyphenols was added to 49 mL of an ethanol-water mixture (50:50 v/v) under constant stirring. Then, HPMC was added little by little to the dispersion to reach a final concentration of 3% (w/v). The mixture was maintained at 40°C to ensure complete polymer dissolution. Coacervation was induced through controlled addition of approximately 10 mL of a 20% (w/w) sodium sulphate solution. The saline solution was added dropwise using a Pasteur pipette over a period of 20 minutes. To prevent aggregation, a progressive stirring protocol from 400 rpm to 600 rpm was implemented using a homogenizer ONILAB®OS40-Pro. To ensure structural integrity the microcapsules were stabilized by constant stirring at 600 rpm for 30 minutes to allow the coacervate to fully coat the polyphenols. In order to solidify the HPMC film, the solution was then acidified to pH 4.0–4.5 using 0.5 M acetic acid. The suspension was centrifuged at 1500 rpm for 5 minutes at 4°C. The supernatant was discarded, and the pellets were washed with acidified distilled water (pH 4.5) to maintain the gel

structure. The final product was dried by lyophilisation under specific conditions (48 h, 0.01 mBar). For this purpose, the samples were frozen in two different ways: in a conventional freezer prior to lyophilisation or directly in the lyophiliser.

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FIGURES:

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DATA AVAILABILITY STATEMENT:

The datasets generated and analysed during the current project are available from the corresponding author on reasonable request.

COMPETING INTERESTS STATEMENTS:

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