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Improving benzyl – isothiocyanate bioaccessibility in white mustard (*Sinapis alba*) sauce through spray – drying microencapsulation and Pickering emulsions

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ABSTRACT

This study compared the bioaccessibility and behaviour of different formulations of benzyl – isothiocyanate (benzyl – ITC) prepared using different approaches and the INFOGEST *in vitro* digestion protocol. The aim was to improve the low bioaccessibility of this bioactive compound caused by its lipophilic properties. Spray-dried microparticles were prepared using a matrix of either mannitol or maltodextrin, and compared with Pickering emulsions produced with cellulose nanofibres. The different systems were characterised and their ability to associate benzyl – ITC determined. The *in vitro* digestion characteristics provided by the different systems was evaluated. Microencapsulation of benzyl-ITC by spray-drying was not successful when mannitol was used as excipient, while maltodextrin resulted in a production yield around 70 % with an ITC association efficiency up to 75 %. Nevertheless, significant improvement of benzyl - ITC bioaccessibility in a mustard sauce was not achieved. In contrast, the formulation of benzyl – ITC in a Pickering emulsion prepared with cellulose nanofibres showed an association efficiency of around 100 % and high bioaccessibility with values up to 77 %. The chemical similarity between the mixed micelles formed for lipid absorption in the small intestine, and the structure of cellulose nanofibre emulsion could justify the observed improvement.

1. Introduction

Yellow or white mustard (*Sinapis alba*) is one of the main varieties of mustard, a cool season crop, widely spread into Asia, North Africa and Europe over thousands of years (Ekanayake et al., 2016). Although its cultivation is mainly for the production of seeds for seasoning, its green parts (shoots and leaves) have also proven to be a suitable source of inorganic micronutrients and bioactive compounds (Cámara-Martos et al., 2021; Martínez-Castro et al., 2023). One of these compounds is benzyl - isothiocyanate (benzyl–ITC), an organosulfur phytochemical obtained from glucotropaeolin upon the action of the enzyme myrosinase (Kassie et al., 2002). Benzyl-ITC has numerous properties as a functional ingredient including anti-cancer, antioxidant, antimicrobial, anti-inflammatory (Dinh et al., 2021; Hoch et al., 2024; Sun et al., 2021). However, it is a lipophilic molecule, thus poorly soluble in water (Dinh et al., 2021), which limits its use as a functional component in foods with high aqueous matrix.

Microencapsulation, where compounds of interest like micronutrients or bioactive molecules are surrounded by a coating or embedded in a matrix, can be an efficient alternative to circumvent the referred limitations. It permits the protection of the bioactive compound from undesirable reactions and interactions with other components of the food matrix, preventing its degradation and increasing its half-life (Sobel et al., 2023). Among the microencapsulation techniques, one of the most widely used, due to its low cost and effectiveness, is spray-drying. This methodology has been widely used to microencapsulate micronutrients (Carlos et al., 2018; Estevinho et al., 2016; Gonçalves et al., 2017; Grenha et al., 2023), producing microparticles upon drying

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of liquid droplets composed of bioactive compound and encapsulating material.

The formulation of emulsions may also be used as a means to protect molecules or provide their stabilisation. As a general concept, emulsions can be oil-in-water (O/W) or water-in-oil (W/O), the selection of the specific type depending on the nature of the molecule intended to be associated. Usually, that molecule is located in the inner phase in order to benefit of protection from the external environment. The stabilization of emulsions is usually mediated by molecular surfactants that are dissolved in one of the phases and decrease the interfacial tension. Pickering emulsions are a special type of emulsions in which the oil-water interface is stabilised by an inorganic element or a biopolymer, typically in solid form, which substitutes the surfactants (Meng et al., 2024; Saffarionpour & Diosady, 2022). In the food industry, they represent a promising alternative to synthetic emulsifiers with potential benefits such as reduced fat content, improved stability and increased consumer acceptance from a health point of view (Funami et al., 2025). These emulsions have recently been reported in the field of food technology to improve bioaccessibility of fat-soluble vitamins (Freitas Santos et al., 2021; Hao et al., 2024; Marisa Ribeiro et al., 2020; Ribeiro et al., 2022) and fat-soluble bioactive compounds such as curcumin (Song et al., 2023).

The use of the above methodologies for the formulation of benzyl – ITC is herein hypothesized to improve its bioaccessibility. The latter concept refers to the micronutrient or bioactive compound concentration that is solubilised in the intestinal lumen, therefore being available for absorption by enterocytes (Cámara et al., 2005), which is a first step for bioactivity and/or physiological effect. Bioaccessibility can be easily determined *in vitro*, simulating gastrointestinal digestion by reproducing the physiological conditions of the digestive tract (mouth, stomach and small intestine). In recent years, different working groups have come together with the aim of reaching a consensus on protocols to standardise results, yielding the INFOGEST static *in vitro* digestion model (Egger et al., 2016; Minekus et al., 2014).

The aim of the present work was to develop benzyl – ITC formulations that endow the necessary protection to the ITC, to foster the improvement of its bioaccessibility when incorporated in food matrices. Three excipients and two formulation approaches were studied: a) mannitol and maltodextrin as microparticle matrix materials in a process of spray-drying; b) crystalline nanocellulose as stabilizer in the production of Pickering emulsions. Spray-drying was selected because it is a cost-effective and well-established method in the food industry, with reported scalability for powder production (Har et al., 2018). Mannitol and maltodextrin were the chosen matrix materials in that case due to their natural origin and Generally Recognised as Safe (GRAS) status. In turn, Pickering emulsions with crystalline nanocellulose as stabiliser were selected due to environmental and economic interest of the latter, which production represents a breakthrough as technology for the valorization of agricultural by-products. The obtained formulation, either microparticles or Pickering emulsions, was incorporated into a mustard sauce in order to permit studying the benzyl – ITC bioaccessibility through the INFOGEST static *in vitro* digestion model. This work will contribute to providing strategies aimed at improving the bioaccessibility of this bioactive compound with a possible therapeutic role in human nutrition.

2. Materials and methods

2.1. Materials

All the reagents were of analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification system (Millipore, Madrid, Spain) was used throughout the experiments. For decontamination, glass laboratory materials were kept in a 50 % HNO_3 solution (v/v) for one day and then washed with deionized water and kept again in a 20 % HCl solution (v/v) for one additional day. Lastly, laboratory materials were washed with deionized water and dried.

Sodium bicarbonate (97 %) was obtained from Scharlau (Barcelona, Spain). Hydrochloric acid (35 %) was obtained from Panreac (Barcelona, Spain). Mannitol, maltodextrin (dextrose equivalent 4–7), calcium chloride, benzyl-ITC standard, Tween® 20, digestive enzymes (α – amylase from human saliva - A0521; pepsin from porcine gastric mucosa – P-7000); pancreatin from porcine pancreas – P-3292) and bile salts of porcine origin (B-8756), were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Working solutions of these enzymes were prepared immediately before use. Soybean oil was purchased from Guinama S.L.U. (Valencia, Spain).

Cellulose pulp (after subjecting the lignocellulose pulp to a bleaching treatment) was obtained from a soda pulping process of wheat straw as reported in previous works (Espinosa et al., 2017).

Nanofibres were prepared from bleached cellulose pulp obtaining cellulose nanofibres through a high-pressure homogenization process (HPH) on a PANDA GEA 2 K NIRO homogenizer following a pressure sequence of 4 passes at 300 bar, 3 passes at 600 bar, and 3 passes at 900 bar. Prior to the HPH treatment, cellulose pulps were subjected to a chemical or mechanical pretreatment to facilitate the nanofibrillation process (De Haro-Niza et al., 2022).

2.2. Benzyl – ITC formulation

Benzyl – ITC was microencapsulated using a process of spray-drying. Microparticles of two different compositions were prepared, corresponding to a matrix of mannitol or maltodextrin. In both formulations, a spraying solution of 40 mL was prepared with 0.5 mL of benzyl – ITC standard, 6 g of mannitol or maltodextrin and 0.5% of an emulsifying agent (Tween® 20).

The prepared dispersions were stirred for approximately 30 min and then spray-dried in a laboratory mini spray dryer (Buchi B-290 Mini Spray Dryer, Buchi Labortechnik AG, Switzerland) to obtain the microparticles. Stirring was maintained during the process to ensure homogeneity. The spray-dryer was operated in open-mode configuration, using compressed air as fluid. The operating parameters were set as follows: spray flow rate at 473 L/h, inlet temperatures of 105, 110, 120, 150 and 170 °C, aspirator at 100 %; feed rate of *ca.* 2 mL/min. The produced microparticles were stored in a desiccator until further use.

The production yield of the spray-drying process was calculated at the relationship between the resulting amount of microparticles and the total amount of solids initially present in the spraying dispersions.

The preparation of the Pickering emulsions was carried out by adapting the protocol proposed by Aw et al. (2023). Thus, 2 mL of benzyl – ITC standard were solubilized in 4 mL of soybean oil. In parallel, 12.94 g of cellulose nanofibre suspension (1.9316 %) were mixed with 100 mL of water to obtain a 0.25 % cellulose nanofibre suspension. Pickering emulsions were obtained by mixing 6 mL of the soybean oil containing benzyl - ITC and 24 mL of the cellulose nanofibre suspension. The mixture was homogenized in a high-shear homogenizer (IKA T18 digital Ultra Turrax) at 8000 rpm during 4 min to obtain the emulsion.

2.3. Morphological evaluation of microparticles

Scanning electron microscopy (SEM, Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M, Eindhoven, The Netherlands) was used to characterize the morphology and surface topography of microparticles. Microparticles obtained from spray-drying were fixed on a brass stub using double-sided adhesive tape and subsequently coated under vacuum by a thin layer of gold, before viewing.

2.4. Determination of particle size

The geometric size of microparticles was determined as the Feret's diameter based on the microphotographs obtained by SEM analysis, being estimated as the mean of 300 measurements ($n = 300$). The distribution of particle size was determined as D10, D50 and D90 (Grenha et al., 2023).

The Pickering emulsion droplet size was determined by direct observation using an optical microscope (Olympus, EVIDENT) with a 40× objective lens connected to a digital camera to capture the images. Droplet size measurement was carried out using ImageJ software. The diameter of an average of 60 droplets was measured.

2.5. FTIR analysis

Fourier transform infrared spectroscopy (FTIR) analysis of microparticles was performed using a Spectrum Two™ instrument from Perkin Elmer (Massachussets, USA) equipped with an attenuated total reflectance (ATR) module. The spectra of each sample, from 4000 to 450 cm^{-1} wavenumbers, were recorded with a resolution of 4 cm^{-1} and 20 scans.

2.6. Thermogravimetric analysis (TGA) of microparticles

The thermal stability of microparticles was analyzed on a Mettler Toledo TGA/DSC 1 instrument from room temperature to 250 °C at a rate of 10 °C/min in a nitrogen stream flow rate of 50 mL/min.

2.7. Creaming index

The creaming index of the Pickering emulsions was determined as an indicator of the stability of the emulsion systems. This determination was carried out by measuring the height of the serum layer and the height of the entire emulsion with an electronic digital calliper Powerfix ® Profi+ (São Paulo, Brazil). The creaming index was calculated with the following equation:

$$Creaming\ index\ (\%) = \frac{Serum\ layer\ (mm)}{Total\ emulsion\ (mm)} x100$$

2.8. GC – MS benzyl – isothiocyanate analysis

The analyses were performed on a GC–MS system consisting of a Bruker GC Mod. 456 with a Bruker mass detector Mod Scion TQ. The chromatographic separation was achieved on a 5 % phenylsilicone column (30 m × 0.25 mm i.d., 0.25 µm film thikness). The GC conditions were as follows: column temperature began at 60 °C, was held for 1 min, then increased to 100 °C at a rate of 7 °C/min, then increased to 120 °C at a rate of 15 °C/min, then increased to 285 °C at a rate of 25 °C/min, was held for 2 min. The GC was operated in splitless mode whit a 1 mL/min continuous column flow rate using helium as a carrier gas. The sample injection volume was 1 µL and the injection temperature was 250 °C.

The MS was operated in Multiple Reaction Monitoring mode, using two MRM transitions ions for each compound. Transfer line GC–MS was maintained at 265 °C, and MS source temperature was 250 °C. Under these conditions, benzyl – ITC elutes at 6.6 min, approximately (Cámara-Martos et al., 2025).

2.9. ITC content in the formulations

The association efficiency (AE) and loading of benzyl - ITC in microparticles/emulsions was calculated by application of the following equation, using the methodology described above for the determination of benzyl - ITC:

AE (%) = (Real amount of Benzyl - ITC on microparticles or emulsions/Theoretical amount of Benzyl - ITC on microparticles or emulsions) x 100.

Loading (%) = (Real amount of ITC on microparticles/Weight of microparticles) × 100.

2.10. Static in vitro digestion model to estimate bioaccessibility

The conditions established in the INFOGEST digestion method (Egger et al., 2016) were used to estimate the benzyl – ITC bioaccessibility. Simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to Minekus et al. (2014).

For the digestion, briefly, 5 g of mustard sample (*Sinapis alba*) sauce was supplemented with benzyl – ITC standard free; maltodextrin – benzyl – ITC microparticles; mannitol – benzyl ITC microparticles; or benzyl ITC – cellulose Pickering emulsion at doses of 1, 3 and 8 mg of benzyl - ITC. The mustard sauce (supplemented or not) was mixed with 3.5 mL SSF, and manual shaking for 1 min. Then, 0.5 mL α – amylase solution (1500 U/mL to reach a final concentration of 75 U/mL oral digesta), 25 µL of 0.3 M $CaCl_2$ solution and 975 µL ultrapure water were added to obtain a final volume of 10 mL. The mixture was placed in a shaker bath for 2 min at 37 °C (oral phase). Each assay was performed three times.

To simulate gastric phase, 10 mL 0.1 M HCl was added to reduce the pH. Then, 7.5 mL SGF, 1.6 mL pepsin solution (to achieve 2000 U/mL in the final digestion mixture) and 5 µL 0.3 M $CaCl_2$ solution were added and mixed manually. The pH of the mixture was adjusted to 3 and ultrapure water was added up to a volume of 20 mL. Finally, the gastric mixture was incubated for 2 h at 37 °C in a shaking water bath.

To simulate intestinal phase, 11 mL SIF, 5 mL pancreatin solution (100 U/mL in the final mixture), 40 µL 0.3 M $CaCl_2$ solution, and 2.5 mL bile salts were mixed. The final mixture was stirred manually, adjusted to pH 7 with 1 M NaOH, and ultrapure water was added to a final volume of 40 mL. The intestinal mixture was incubated again for 2 h at 37 °C in a shaking water bath.

Finally, aliquots of the digested sample were transferred to polypropylene centrifuge tubes (50 mL) and these were centrifuged for 30 min at 2000 rpm. The supernatant (soluble or bioaccessible fraction) and the solid residue (non – bioaccessible fraction) were collected in order to analyze the benzyl – ITC concentration. The ITCs analyses (Cámara-Martos et al., 2025) in the above samples were performed immediately after the gastrointestinal digestion process was completed.

2.11. Statistical analysis

The IBM SPSS 25 statistical software package was used for statistical analysis. The data were expressed as mean and standard deviation. Data were analyzed using ANOVA tests. Significant differences were considered when $p < 0.05$.

3. Results and discussion

3.1. Preparation and characterization of microparticles and emulsions

The formulation of molecules of interest, for food applications or others like pharmaceutical ones, is strongly affected by the particular features of the materials selected for the formulation process. The interaction established between the materials and the molecules being associated typically determines relevant properties of the final systems, including the solubility, swelling ability, efficiency of the association of the molecule and the proper release profile (Ribeiro et al., 2022). While these are transversal effects to consider, the particular methods of production and the specific systems produced also play relevant roles. Spray-drying is a process dependent on several pre-established parameters, which selection strongly impacts on the final properties of the obtained microparticles. One of the most impactful parameters is the inlet temperature, which is the temperature driving the drying process. High inlet temperatures increase the drying rate and decrease the moisture content of the microparticles (Gharsallaoui et al., 2007), resulting in smaller amount of microparticles deposited on the cyclonic separator and thus permitting a higher production yield (Estevinho et al., 2016).

As referred in the methodology, mannitol and maltodextrin were selected as microparticle matrix materials. Their ability to form microparticles containing ITC was rather different, as these were only formed when maltodextrin was used. Fig. 1 shows the morphological aspect of the dry powders that were obtained after the spray-drying process, where it can be seen that mannitol resulted in aggregates more than individual particles. In turn, maltodextrin was able to form microparticles, which present a convoluted shape.

A wide range of temperatures was tested in this work (between 105 and 170 °C). SEM visualization focused on powders with adequate results regarding other parameters under assessment, such as production yield and AE. All the mannitol microparticles that were viewed had a morphology corresponding to aggregates similar to those in Fig. 1, left image (data not shown). The literature provides countless reports on spray-drying of mannitol and our team has a long experience with this material. Most frequently the outcome corresponds to the production of spherical microparticles (Littringer et al., 2013; Grenha et al., 2023). Nevertheless, the presence of other substances in the spraying dispersions has been occasionally reported to induce alterations in the final topography, inclusive in some cases leading to a melted and aggregated aspect (Cunha et al., 2018), as observed in the present work. Therefore, the observed appearance is suggested to be justified by the interaction between the components. Indeed, the profile of mannitol crystallization was previously demonstrated to be affected by the presence of surfactants (Penha et al., 2021) which could justify the observations due to the presence of Tween® 20 in the formulation. Mannitol was further ineffective in the microencapsulation of benzyl – ITC, leading to production yields below 30 % and an AE of at most 21 % when an inlet temperature of 150 °C was used.

The use of maltodextrin as matrix material produced opposite results. Individual and defined microparticles were obtained (see Fig. 1) with a production yield around 70 %. Their size and parameters of size distribution were characterized and are shown in Table 1. Feret diameters of 4.5 and 5.7 μm were determined, along with D50 of 3.5 and 5.0 μm, respectively, for the particles produced at 150 and 120 °C, evidencing absence of significant differences. The sizes are considered adequate for a future incorporation in a food matrix such as the mustard sauce to be used in subsequent assays. Fig. 2 depicts the association efficiencies obtained at the different spray-drying temperatures tested. The microparticles produced at 120 °C registered an AE of 75 %, which resulted in a final loading of ITC of 8.2 %.

As already indicated, the differences in the results obtained for both matrix materials can be attributed to the intrinsic characteristics of each molecule. Mannitol is a glycoalcohol in the monosaccharide form, more

Table 1
Feret diameter and parameters of size distribution (D10, D50, D90) obtained for maltodextrin microparticles produced with different temperatures.

Microparticles	Feret diameter (μm)	D10 (μm)	D50 (μm)	D90 (μm)
Maltodextrin 120	5.7 ± 3.3	2.0	5.0	10.25
Maltodextrin 150	4.5 ± 3.1	1.4	3.5	8.8

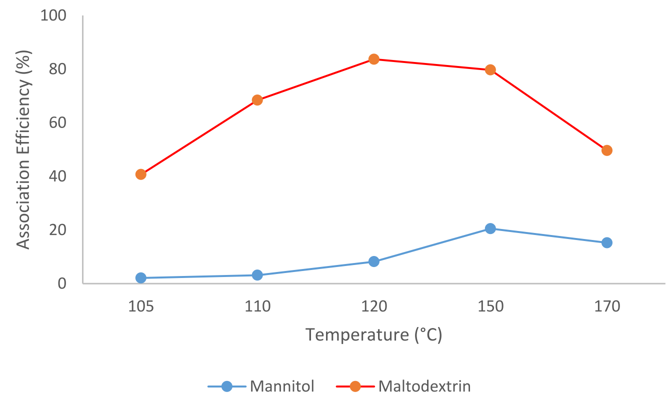

Fig. 2. Association Efficiency (%) for benzyl – ITC microencapsulates (mannitol and maltodextrin) at different inlet Temperatures (°C).

hydrophilic, while maltodextrin is a low molecular weight oligosaccharide with a high capacity to form gels. This gelling capacity has been shown in some polysaccharides to be the reason for retaining and enveloping compounds of nutritional interest in their matrix (Cámara-Martos, 2024). Moreover, considering the hydrophobicity of benzyl-ITC, its binding to maltodextrin is possibly more efficient than to mannitol due to the presence of several hydrophobic regions in the former that easily permit establishing hydrophobic interactions with benzyl-ITC (Li et al., 2020; Xiao et al., 2022).

On the other hand, Wu et al. (2014) have studied the stability and efficiency of encapsulation of other isothiocyanates, such as sulforaphane, by spray-drying. Among the different materials used (maltodextrin, k-carrageenan and β-cyclodextrin), maltodextrin showed the best results with association efficiency up to 39 %. The lower percentages referred in that study compared to those presented herein may be due to the higher inlet temperature used in the spray-drying process (190 °C). At such temperature, sulforaphane is easily decomposed (Chiang et al., 1998), which is why these authors recommend the use of lower inlet

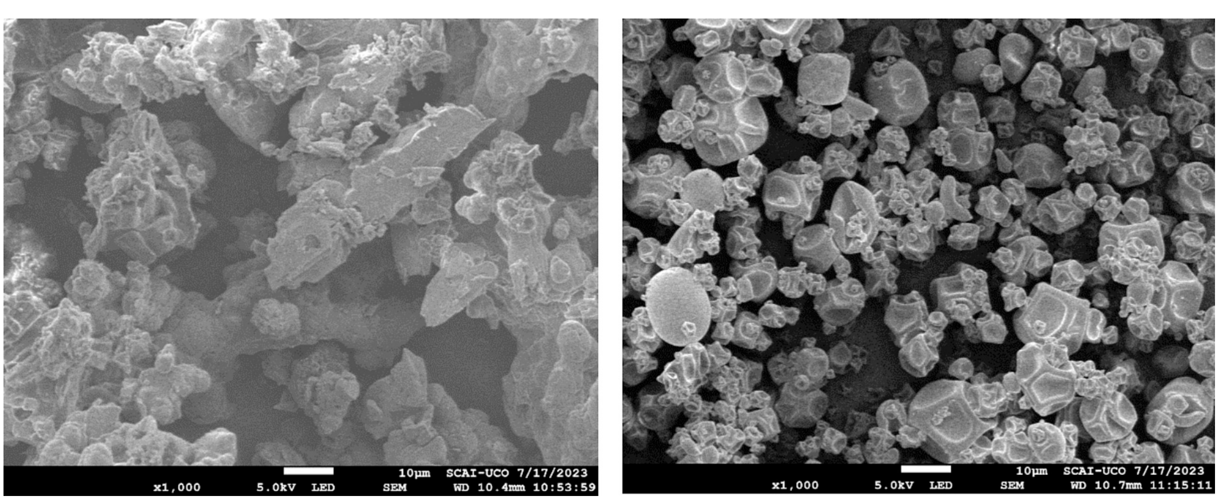

Fig. 1. morphological properties observed by scanning electron microscopy (SEM) of representative dry powders resulting from spray-drying of mannitol (left) and maltodextrin (right). Scale bar represents 10 μm.

temperatures. Also relevant is the fact that, when the dispersion to be spray-dried is an emulsion, which is occasionally proposed when hydrophobic molecules are to be encapsulated in a spray-drying process that uses hydrophilic matrix materials, the efficiency of microencapsulation is also improved by the addition of surfactants to the spraying dispersions. Emulsifiers not only stabilize emulsions during microencapsulation, but also prevent volatilization, thus improving ITC retention. Ko et al. (2012) have proved for allyl – ITC and Arabic gum an improvement in allyl - ITC retention from 39 % (without surfactant) to 83 % (with 0.3 % of Tween® 20). In the present work, preliminary studies showed that the best surfactant concentration was 0.5 %.

Regarding the Pickering emulsions, the content of benzyl – ITC was 8.75 ± 0.53 g/100 g which implies an AE ≥ 99 %. Such high values of benzyl - ITC AE were also reported in the literature for other formulation strategies. Uppal et al. (2017) reported inclusion ratios of benzyl - ITC in β-cyclodextrins of 95–96 % when sonication-assisted complexation was applied. In similar systems produced using the same methodology, Li, Liu, et al. (2015) reported an encapsulation efficiency of 86 % for benzyl - ITC extracted from papaya seeds. High shear homogenization technologies such as the one carried out in the present work increase the contact area promoting a higher degree of emulsification.

The prepared Pickering emulsions (Fig. 3) were highly stable with a creaming index of 96.81 ± 1.32 %. The creaming index was monitored for 10 days and was found to be stable, with no phase separation observed. The stability of the emulsions was corroborated by optical microscopy where it was observed that while cellulose nanofibre alone in emulsion formed a heterogeneous droplet pattern in size distribution (Fig. 3a), Pickering cellulose nanofibre – benzyl - ITC emulsions resulted in homogeneous droplet distribution (Fig. 3b) with an average diameter of 25.54 ± 10.34 μm. Thus, it has been demonstrated that cellulose nanofibres could be an effective material to successfully carry out the encapsulation of compounds of interest such as benzyl - ITC.

FTIR spectra of mannitol and maltodextrin microparticles (Fig. 4) showed that benzyl - ITC were efficiently microencapsulated when these materials were applied. Both FTIR spectra showed a wide band in the region of 3300 cm^{-1}, which corresponds to ν(O – H) stretch vibration; weak absorption bands due to ν(C – H) stretch vibration of between 3000 and 2850 cm^{1}; bands at 1024 cm^{-1}, 1079 cm^{-1}, and 1154 cm^{-1}, related to ν(C – O) stretch vibration and ν(C – OH) bending vibrations (Castro-Cabado et al., 2016).

Thermogravimetric analysis (TGA): Benzyl – ITC is a volatile compound and loses mass rapidly when subjected to temperatures above 50 °C, reaching a total decomposition before 175 °C in its free form (Li, Liu, et al., 2015). To improve the thermal resistance of benzyl – ITC is, it was microencapsulated by spray – drying using two different materials to compose the microparticles matrix, namely maldodextrin and mannitol. Normally, manitol shows a stable thermal behaviour up to the range of 250–340 °C, where it is totally degraded in a single stage (Li, Zhao, et al., 2015). In the case of maltodextrin a degradation temperature has been reported from 200 °C, where there are two stages of degradation, characteristic of carbohydrates, (i) a rapid degradation at 200–300 °C and (ii) a slower degradation step from 330 °C onwards (George et al., 2021). Fig. 5 shows the results of the TGA of the mannitol and maltodextrin based benzyl - ITC microparticles. The maltodextrin – benzyl - ITC sample showed a two-step weight loss, as expected due to the presence of maltodextrin, with some particularities. The first weight loss of 12.62 % occurred in the 50–193 °C range in a slow manner attributable to the decomposition of benzyl - ITC, while a more rapid and pronounced weight loss, attributable to maltodextrin, began at this temperature. In the case of mannitol – benzyl - ITC, a slow and prolonged one-step degradation typical of mannitol was observed, with no degradation events typical of benzyl - ITC. These results suggest that the coating used exert a protective effect on the ITC core. Similar results have been reported by other authors for the complexation of benzyl - ITC with β-cyclodextrins. Thus, it is demonstrated that the rapid thermal degradation of isothiocyanates can be circumvented by strategies that provide its microencapsulation or coating in any way (Li, Liu, et al., 2015; Uppal et al., 2017).

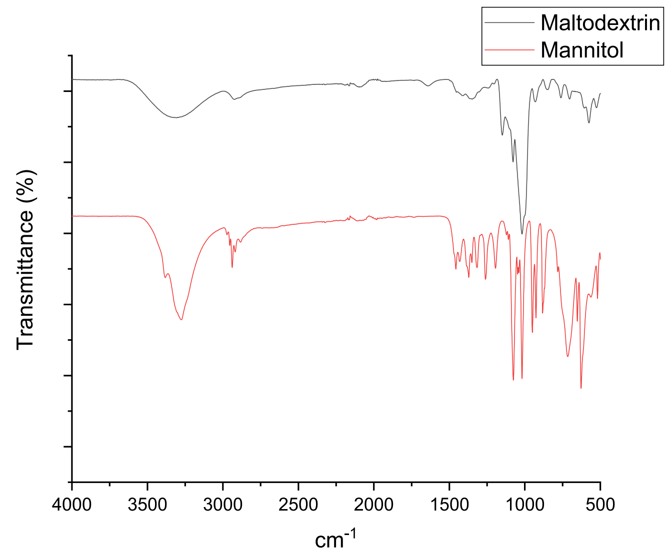

Fig. 4. FTIR spectra for microencapsulates (mannitol and maltodextrin).

3.2. Benzyl – ITC bioaccessibility of formulations

The content of benzyl – ITC analysed in the unsupplemented mustard sauce samples was negligible (data not shown). Both the bioaccessible and non-bioaccessible fractions obtained after *in vitro* gastrointestinal

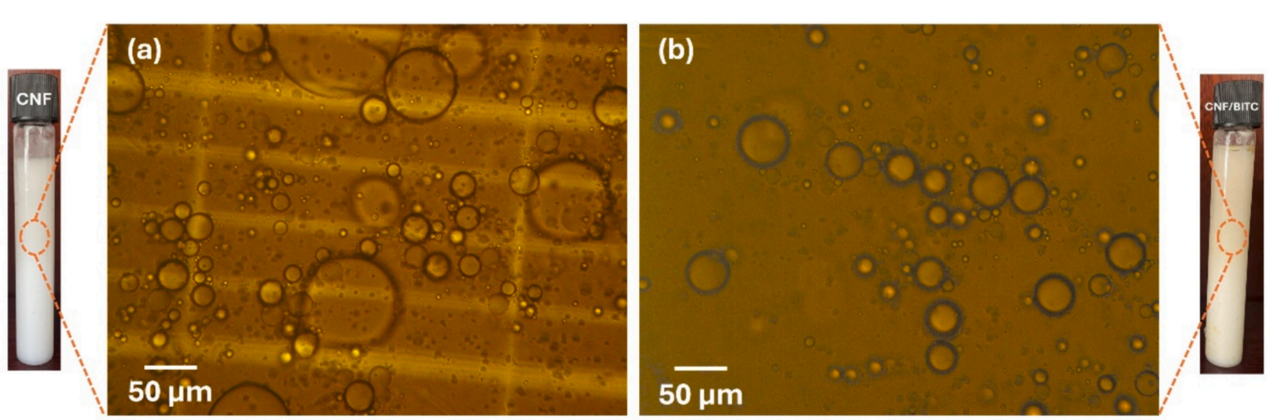

Fig. 3. Optical micrographs of (a) cellulose nanofibre (CNF) emulsion and (b) cellulose nanofiber – benzyl – ITC (CNF/BITC) Pickering emulsion.

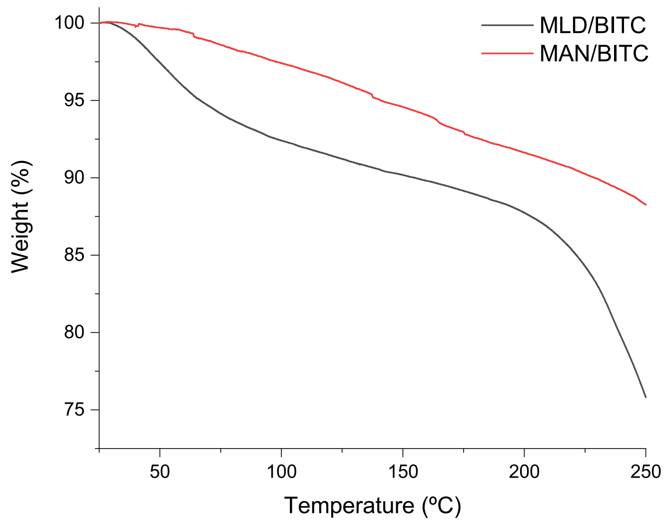

Fig. 5. FTGA curves from mannitol – benzyl – ITC (MAN/BITC) and maltodextrin – benzyl – ITC (MLD/BITC).

digestion showed benzyl – ITC values below the detection limit of the analytical method used (Cámara-Martos et al., 2025). The precursor glucosinolate of benzyl – ITC is glucotropaeolin. These results are in agreement with a previous study conducted by our research group (Martínez-Castro et al., 2023) which showed that whereas sinalbin and glucotropaeoline are the two major glucosinolates present in the green parts (stems and leaves) of white mustard *(Sinapis alba)*, the seeds of this cruciferous plant contain only sinalbin as the predominant glucosinolate.

The supplementation of mustard sauce with benzyl – ITC in the form of free ITC had a minimal effect on improving the bioaccessibility of this bioactive compound. The bioaccessibility values were below 10 % for the lowest supplementation dose (1 mg), dropping sharply to 5 % for the one of 3 mg, with no effect observed for the highest dose of 8 mg. The lipophilic properties and low water solubility of the benzyl - ITC molecule (Dinh et al., 2021; Tian et al., 2021) could be a contributing factor to these low bioaccessibility values. Another possible cause could be the high presence of anti-nutritional factors such as phytates, phenolic and hulls in mustard seeds, which limits their use as a dietary source of many nutrients (Sadeghi et al., 2006). In this regard, Martínez-Castro et al., 2023 observed that in the bioaccessible fraction of seeds from four cruciferous varieties (*Brassica carinata, Brassica rapa, Eruca vesicaria* and *Sinapis alba*), the bioaccessibility of most glucosinolates is below 1 %, implying that the bioaccessibility of derived ITCs is also very low. This suggests that strategies of benzyl - ITC formulation should be considered for supplementation.

However, the results of this work showed that supplementation with benzyl - ITC microparticles based on mannitol or maltodextrin did not significantly improve the bioaccessibility of this compound in mustard sauce (see Table 2). Most of the added ITC remains in the non-bioaccessible fraction, with only a percentage of around 10 % for the 1 and 3 mg doses remaining bioaccessible, and potentially absorbable by enterocytes in the small intestine. In contrast, the formulation of benzyl – ITC in the form of Pickering emulsion with cellulose nanofibre showed the highest bioaccessibility values ($p < 0.05$), ranging from 36 % to 77 %. For this approach, the highest content of supplemented benzyl – ITC after the gastrointestinal digestion process, was found in the bioaccessible fraction (0.77; 1.58; 2.87 mg for the three doses studied, respectively).

The absorption of fat and lipophilic compounds in the small intestine occurs through mixed micelles, where bile salts play a major role as natural biosurfactants (Bauer et al., 2005; Griffin & Cunnane, 2009). These mixed micelles are spherical associations of amphipathic molecules (with hydrophobic and hydrophilic regions) similar to the Pickering emulsion that contains cellulose nanofibre with benzyl - ITC. This similarity enhances the bioaccessibility of ITC. In addition, a previous study (Dong et al., 2023) has pointed out that Pickering emulsions can promote the degree of lipolysis, improving the bioaccessibility of lypophilic compounds being emulsified. On the other hand, it can be observed that higher supplementation doses result in lower bioaccessibility. This is consistent with the fact that a high dose of benzyl-ITC may prevent its complete incorporation into the mixed micelles during the digestive process, thereby decreasing its bioaccessibility.

Our results are in agreement with previous studies showing that benzyl – ITC bioaccessibility is enhanced by using soybean protein isolate nanoemulsions (Tian et al., 2021). Similarly, cellulose nanofibre as an encapsulating agent has also been seen to increase the lipophilic compounds bioaccessibility such as melatonin (Li et al., 2019); coenzyme Q10 (Li et al., 2022); or vitamin D (Ahmad Wsoo et al., 2021).

Finally, the amount of benzyl-ITC present in both the bioaccessible and non-bioaccessible fractions after the gastrointestinal digestion process is lower than the supplemented dose, especially for the 3 and 8 mg doses. This suggests a degradation of this bioactive compound during digestion. Indeed, ITCs are highly reactive molecules, due to their electrophilic carbon (Andernach et al., 2024) and they can react with strong nucleophiles, such as thiol groups (Kawakishi & Kaneko, 1985; Kühn, Kupke, et al., 2018) or amine groups (Hanschen et al., 2012; Kühn, Kupke, et al., 2018; Kühn, von Oesen, et al., 2018). Other studies have indicated that ITCs are unstable molecules with a very short half-life (Andernach et al., 2024). However, despite this result, the high content of benzyl – ITC present in the bioaccessible fraction after formulation as an emulsion with cellulose nanofibres supports its use.

4. Conclusions

This study compared the morphology, efficiency and *in vitro*

Table 2
Benzyl - ITC bioaccessibility of microencapsulates (mannitol and maltodextrin) and emulsion (cellulose nanofiber) in the food matrix at different doses (mean ± standard deviation) (n = 3).

	Dose 1 mg			Dose 3 mg			Dose 8 mg		
	Bioaccessible (mg)	Non – bioaccessible (mg)	Bioaccessibility (%)	Bioaccessible (mg)	Non – bioaccessible (mg)	Bioaccessibility (%)	Bioaccessible (mg)	Non – bioaccessible (mg)	Bioaccessibility (%)
Free	0.080 ± 0.035[a]	0.916 ± 0.113[b]	8	0.139 ± 0.051[a]	2.946 ± 0.145[c]	5	0.169 ± 0.048[a]	3.981 ± 0.183[b]	2
Mannitol	0.100 ± 0.052[a]	0.743 ± 0.078[b]	10	0.340 ± 0.070[a]	2.213 ± 0.130[c]	11	0.281 ± 0.051[a]	3.516 ± 0.347[b]	4
Maltodextrin	0.117 ± 0.067[a]	0.795 ± 0.032[b]	12	0.212 ± 0.146[a]	1.692 ± 0.128[b]	7	0.294 ± 0.066[a]	3.622 ± 0.272[b]	4
Cellulose nanofibre	0.768 ± 0.116[b]	0.102 ± 0.043[a]	77	1.580 ± 0.218[b]	0.211 ± 0.002[a]	53	2.867 ± 0.189[b]	0.728 ± 0.232[a]	36

Within each column, different lowercase letters represent significantly different values at $p < 0.05$ according to the analysis of variance (ANOVA) and Scheffe tests.

digestion characteristics of benzyl – ITC microencapsulates or Pickering emulsions prepared with three different natural ingredients such as mannitol, maltodextrin and cellulose nanofibre. The aim was to improve the low bioaccessibility of this bioactive compound as a consequence of the lipophilic properties of its molecule. Microencapsulation of benzyl-ITC with mannitol by spray-drying was clearly ineffective while maltodextrin resulted in a production yield of around 70 % with an association efficiency of 75 %. Nevertheless, neither compound significantly improved benzyl - ITC bioaccessibility in a mustard sauce. In contrast, the preparation of benzyl – ITC in the form of an emulsion with cellulose nanofibre showed a high bioaccessibility with values of up to 77 %. The chemical similarities between the way lipids are absorbed in the small intestine as mixed micelles, and the cellulose nanofibre emulsion structure could be the cause of this improvement.

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CRediT authorship contribution statement

E. Rincón: Writing – original draft, Methodology, Investigation, Conceptualization. **A. Grenha:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J. Pinto da Silva:** Methodology, Investigation. **E. Espinosa:** Methodology, Investigation, Conceptualization. **F. Lafont-Déniz:** Formal analysis. **M.P. Almeida:** Investigation. **F. Cámara-Martos:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, 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.

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