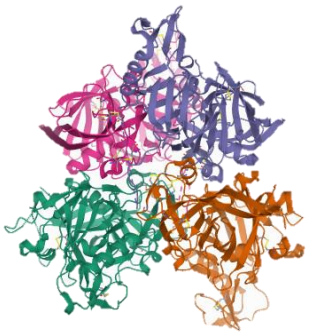
UNIVERSITAT ROVIRA i VIRGILI

SOY PROTEIN-BASED MATERIALS FOR ENCAPSULATION AND DELIVERY OF BIOACTIVE COMPOUNDS

Denisa Maria Ciuches

BACHELOR'S THESIS FOR THE DEGREE OF BIOTECHNOLOGY



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Jo, Denisa Maria Ciuches, amb DNI X8419498A, sóc coneixedora de la guia de prevenció del plagi a la URV Prevenció, detecció i tractament del plagi en la docència: guia per a estudiants (aprovada el juliol 2017) (http://www.urv.cat/ca/vidacampus/serveis/crai/que-us-oferim/formacio-competencies-nuclears/plagi/) i afirmo que aquest TFG no constitueix cap de les conductes considerades com a plagi per la URV

Tarragona, 05/06/2021

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Abstract

Soy protein isolate (SPI) is mainly a mixture of albumins and globulins. In aqueous environment, these exist majorly as globular molecules consisting of a hydrophilic shell and a hydrophobic kernel. Thus, the amino acid composition gives soy protein particles an advantage in encapsulation of highly hydrophobic drugs. In this bibliographic review, most recent research regarding soy protein-based materials for delivery of bioactive compounds, such as curcumin, is discussed. The results show great encapsulation efficiency, thermal and storage stability as well as resistance to acidic environments (such as gastric fluids) of encapsulated bioactive compounds with different soy protein blends and shapes. This flexibility regarding its formulations, allows the product to be used in several fields such as the biomedical, bioplastic and food industry. SP makes up a competitive product given that is low cost, biocompatible and animal-friendly.

Keywords: Soy protein, delivery system, encapsulation, bioactive compounds, hydrophobic drugs

1.Introduction

Nowadays, soya bean is on-demand worldwide. It's a source of several products such as soy oil, soy flour, soy protein isolate, fermentative derivates like tofu, tempeh and so on. Its popularity is due to its availability, nutritional value and low cost. The European Union and China are importer leaders of soy flour, being Brazil and United States of America (USA) leaders exporters. In 2018, the USA produced 123,66 thousand tones, followed by Brazil with 117,89 (Soybeans Product Trade, Exporters and Importers, OEC). Therefore, given the massive production of soya currently going on, there are a lot of protein sources available for

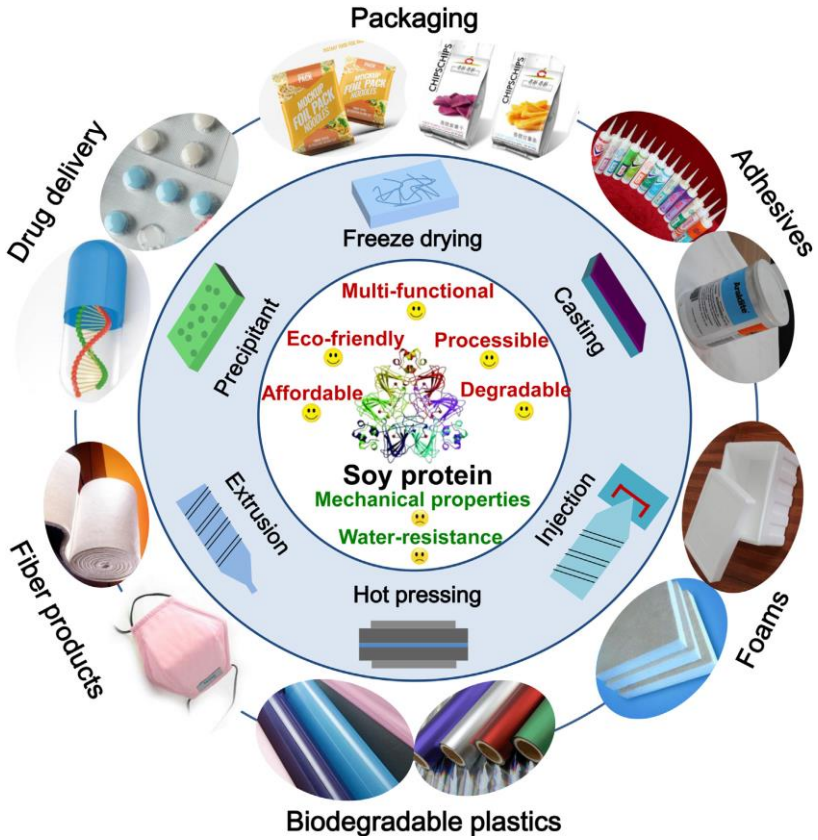

Figure 1 Applications of Soy protein in several fields. Figure adapted from Tian et al. 2018.

uncountable applications (Figure 1). Moreover, the environmental impact of animal products is by far higher than soy products (Figure 2). Soy protein is a suitable food for lactose-intolerant consumers, vegetarians and celiac patients, as it is free of cholesterol, gluten and lactose (Ashaolu, 2020).This is the reason why soy protein was chosen for this review instead of other animal origin proteins like whey protein.

The biocompatibility, low cost, animal-friendly and health effects of soy protein were the key-decisive characteristics (Table 1).

The soya bean (G*lycine max*) is a species of legume widely found in East Asia. It's edible beans have several uses that will be discussed among this review. Soy is a renewable

resource with favourable characteristics for use as a raw material due to its abundance, ease of processing, low cost and non-animal origin (Arias et al., 2021).

There are several commercial products derived from soybean. As a result from crushing the beans, soybean oil and meal are obtained. The soy flour is produced by grinding soybeans into fine powders with a 50% protein content. A higher concentration of protein is found in soy protein concentrate (SPC) which contains around 70% soy protein, and it comes from defatted soy flour lacking water-soluble carbohydrates. If high protein content is required, soy protein isolate (SPI) contains at least 90% of protein on a moisture-free basis.

The composition of soy protein extract is a mixture of albumins and globulins, both storage proteins. Mainly glycinin (40%) and β-conglycinin (30%) hence, these determine the nature of SP. On basis of sedimentation fractions, soy protein can be sorted into four main categories, 2S (S stands for Svedberg units), 7S, 11S, and 15S fractions. 7S (conglycinin) and 11S (glycinin) are the two major fraction, while 2S and 15S account for 8% and 5%, respectively. The isoelectric point of SPI is pH 4,5.

Glycinin is a hexamer composed of acidic and basic peptides which are bound with disulphide. All the essential aminoacids are present conferring good balance among polar, nonpolar and charged aminoacids. The polar functional groups, such as carboxyl, amine and hydroxyl groups that are capable of chemically reacting, making the manipulation of soy protein properties easier due to the fact that In aqueous environment,11s and 7s components exist majorly as globular molecules consisting of a hydrophilic shell and a hydrophobic kernel, together with a certain amount of small water-soluble aggregates (Teng et al., 2012).

β-conglycinin is a vicilin and is glycosylated. Vicilins are proteins able to inhibit yeast growth by supposedly binding to chitin-containing structures of yeast cells resulting into inhibition of H+ pumping and spore formation (Wong & Ng, 2011). Furthermore, previous studies have revealed that a certain percentage of β-conglycinin included in daily protein intake can promote cholesterol reduction (Ferreira et al., 2011). Since β-conglycinin peptides of enzyme-mediated production stimulate the growth of bifidobacterial, the protein may be beneficial to improve gastrointestinal health (Zuo et al., 2005). However, along with the benefits, it has been found β-conglycinin can result in food allergy reaction which may potentially harm human health (T. Wang et al., 2014). It has become a public problem all over the world, but our knowledge on it is still inadequate and there is a need of more data to asses the risk and define the mechanisms involved in the allergic process. Despite this, there are ingredient processing that reduce the allergy risk (T. Li et al., 2021; T. Wang et al., 2014).

Soy protein products can be produced in different forms as gels, films, nanofibers, microspheres and scaffolds for 3D bioprinting (Lin et al., 2016).These different forms will lead to different applications such as bioadhesives, bioplastics and drug delivery systems.

Membranes, fibers, microparticles/nanoparticles have been developed associating soy protein with other proteins polysaccharides, and synthetic materials (Ahn et al., 2018; Varshney et al., 2020a; Zhao et al., 2018). In other words, soy protein based materials show great flexibility regarding its formulation, combining it with different polymers and therefore, achieving different properties depending on the purpose of the application.

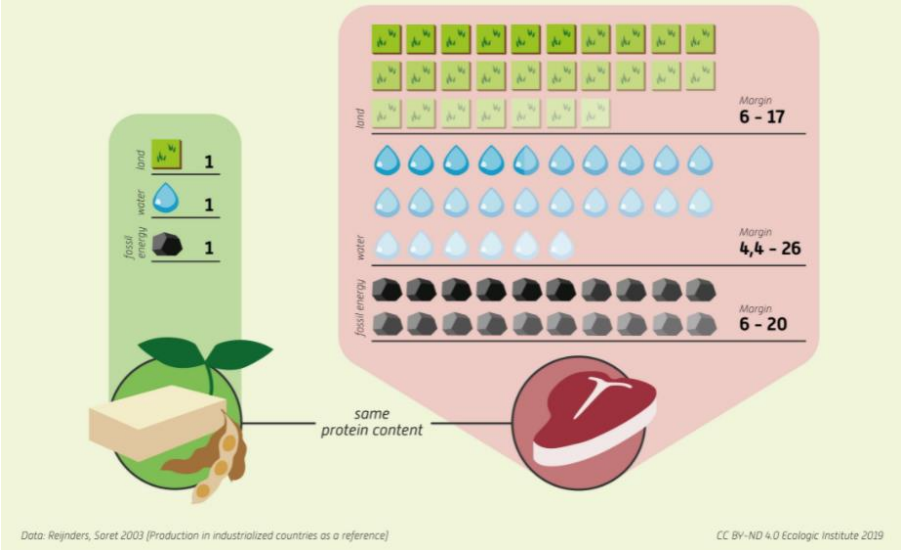

*Figure 2Meat and soy products comparison of environmental effect. With the same protein content, much more land,water and fossil energy is consumed for the production of meat than the production os soy-based vegetable products. Adapted from the Ecologic Institut.(*Meat and Soy Products: Comparison of Environmental Effects*, 2019)*

Furthermore, Soy protein concentrates (SPCs) and soy protein isolates (SPIs), provide revealing opportunities to develop improved packaging materials mainly in the food industry. The development of plastic replacement packages is beginning to become a reality in the food industry. The current trend is to functionalize (for antioxidant, antibacterial, antifungal properties) these packages and make them intelligent, and/or active. In order to achieve these properties two pathways are used: (i) design of controlled release packaging that incorporates natural or synthetic active molecules (Bi et al., 2021) or (ii) designing a package that changes along with the product status (pH, microorganisms, etc.) and informs the customer (Roy et al., 2021). Protein-based packages are a great alternative to conventional food packages. At this moment, they are more expensive alternative but are much more valuable and cost- efficient in terms of long-term properties such as biodegradability and eco-friendliness, vegetarianism, suitability, and consumer acceptance (Mihalca et al., 2021). Soy protein-based films have

some interesting properties of being biodegradable, biocompatible, and inexpensive. However, their weak mechanical property and high sensitivity to moisture are major obstacles to using this protein-based film for food packaging applications (Garrido et al., 2016). Besides this, there are different ways of overcoming this issue by adding plasticizers and other materials to soy proteins-based films.

In this review, given that there are many possibilities to focus on, this time it will be focused on just one. Hence, the last developments regarding the encapsulation and delivery of bioactive compounds in biomedical field and food industry will be discussed. Although the food applications such as smart packaging will remain to be discussed for another time.

Table 1: Properties of proteins mentioned in this review.

Protein	Origin	Biodegradability	Solubility (in water)	Immunogenicity	Reference
Soy	Vegetable	High	High	Low	(Tansaz et al., 2018)
Collagen	Animal usually xenogeneic or allogenic	Low	High	Low	(D. Li et al., 2019)
Zein	Vegetable	High	Low	Medium	(F. Li et al., 2019)
Whey	Animal	High	High	Medium	(Bogahawaththa et al., 2018)
Casein	Animal	high	Low	Low	(Bhat et al., 2016)

1.1. Protein-based particles

One of the applications of nanoparticles is drug delivery systems. The aim is to release their load progressively via diffusion or degradation phenomena (or both), and the speed of these processes must be adequate to achieve therapeutic drug concentrations that remain below toxic levels. The choice of whether the drug is encapsulated inside the core of the nanomaterial, inside the shell (usually up to a few tens of nm thick), or even attached on the surface, depends on the desired application, the nature of the drug, the composition of the ENM, whether the drug can diffuse through the shell or not, and whether the core/shell has a different function than transporting and protecting the drug (Gubala et al., 2018).

Nanoparticles have a range of size that comes from 1 to 1000 nanometres. This size gives a high surface area to volume relationship, higher when compared to other conventional microparticles (Appendix 1). Microspheres can be made from several materials like metals, polysaccharides and proteins. Nonetheless, protein-based micro/nanospheres may confer better biocompatibility than others depending on the situation since their characteristics may be more suitable for specific issues. As a matter of fact, a combination of proteins, polysaccharides and metals is often practiced in order to achieve the best outcomes (Wu et al., 2020; Zhai et al., 2018). Nanospheres and microspheres are a polymer-drug combinations where the drug is homogenously dispersed in the polymeric matrix, and nanocapsules/microcapsules, have the drug particles or droplets entrapped in a polymeric membrane(Guterres et al., 2007).

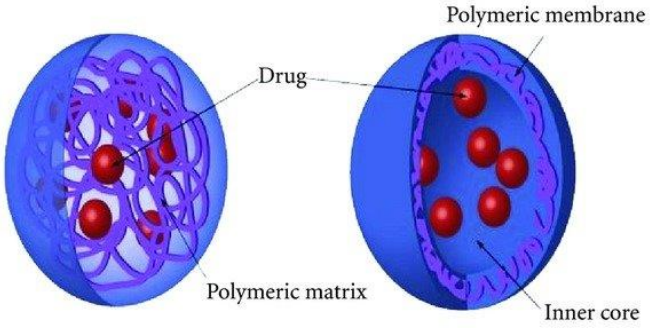

Figure 3 Difference between nanocapsule and nanosphere. Adapted from: Christoforidis, John & Chang, Susie & Jiang, Angela & Wang, Jillian & Cebulla, Colleen. (2012). Intravitreal Devices for the Treatment of Vitreous Inflammation. Mediators of inflammation. 2012. 126463. 10.1155/2012/126463.

The molecular properties of the therapeutic compound must be taken into account when fabricating the nanoparticle for the encapsulation of a specific compound, all the factors to be considered are detailed by (de Frates et al., 2018). Complex nanoparticles composed of polysaccharides and proteins are good carries for hydrophobic bioactive compounds due to the electrostatic interactions between them making them more stable and in consequence, stabilizing the encapsulated compound (S. Wang et al., 2020).

Depending on the application, nanoparticles can be embedded in hydrogels for their delivery(Ligorio et al., 2021). Nano particles for drug delivery have got more attention in the last decade given that their bigger surface area allows the reduction of the amount of drug needed to achieve the same effect, diminishing the collateral effects of higher dosage (Radacsi et al., 2012).

Soy protein nanoparticles are becoming more popular due to the high abundance and low cost of the protein, as well as its biodegradability and low immunogenicity. The amino acid composition gives soy protein nanoparticles an advantage in encapsulation of highly hydrophobic drugs (Anaya Castro et al., 2019; Goder et al., 2021; S. Wang et al., 2020) . Zein is also suitable for encapsulating hydrophobic compounds but it has low content in hydrophilic and charged amino acids resulting in a limited solubility in aqueous environment, unless a

considerable amount of polysaccharides or surfactants is added (table1). soy protein nanoparticles are soluble in aqueous environments which can be used in different oral drug delivery scenarios (Table 1).

1.1.2 Nanotoxicology

Besides the advantages that nanoparticles can provide there is also a dark side on it. In a cellular level, the nanosize of the particles increases the possibilities of cell entry. The uptake pathway varies depending on the size and the composition of the nanoparticle. The routes of entry are shown in figure 3. The harmful impact will depend on how the cells can process the

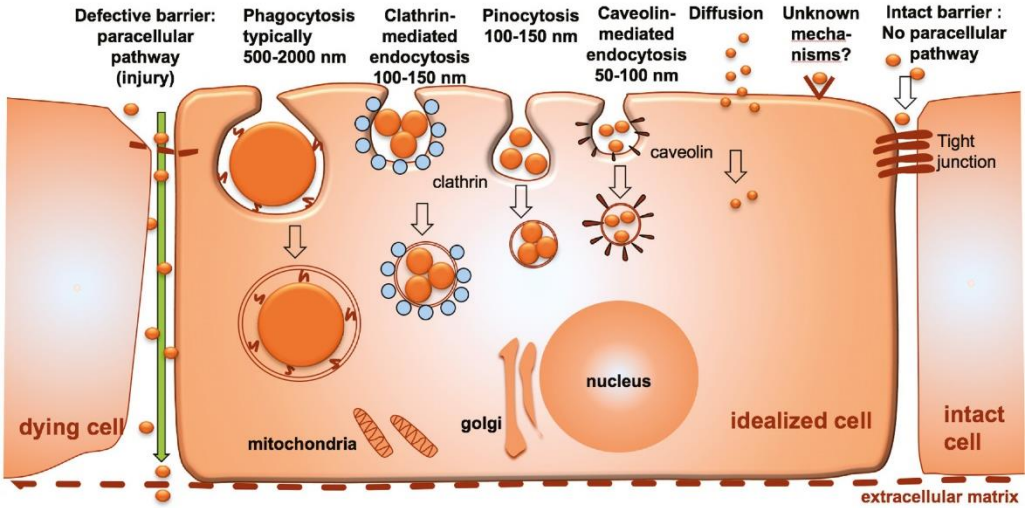

Figure 4 Uptake pathways for nanoparticles into cells and across cell barriers. From Gubala et al. 2018

nanoparticles. Large particles (about 500 nm) are taken up by phagocytosing cells, such as macrophages and hepatic Kupffer cells. Storage in these cells protects the organism from contact with engineered nanomaterials (ENM). However, if the phagocytosing cells are not able to degrade the ENM at a sufficient speed, then the cells will be overloaded. There is also a risk that ENM-loaded phagocytosing cells will send out signalling molecules that cause local inflammation. When ENM or their components are taken up by antigen presenting cells, a sensitizing immune reaction accompanied by intolerance may result. The small intestine, where the majority of orally taken drugs will be absorbed, constitutes a barrier between the intestinal lumen and the blood formed by densely connected epithelial cells covered by layers of mucus. Nanoparticles are, in general, too large to be absorbed by diffusion through the enterocyte membranes. Likewise, permeation through the intact paracellular pathway (between cells) is impossible for molecules or ions larger than approximately 1 nm. Additional possible uptake routes involve endocytosis through epithelial cells and passage via M-cells of the immune system, found in the gastrointestinal tract (X. Huang & Tang, 2021).

In this case SPI nanoparticles have been proved to be biocompatible (biocompatibility is referred to as the ability of a material to perform a desired function in a specific situation in body without eliciting toxic or deleterious impacts) with non-toxic effects on several kinds of cells. In this review we will find *in vivo* studies testing the cytocompatibility of soy protein based products. The cellular uptake routes that SPI nanoparticles could take would be clathrin-mediated transcytosis and macropinocytosis routes, only if the nanoparticles size is around 30nm (J. Zhang et al., 2015).

For the skin, it has been shown in many different studies and systems that almost none of a given ENM-dose applied to normal or UV-stressed skin reaches living cells in the deeper layers (Gimeno-Benito et al., 2021; Monteiro-Riviere et al., 2011).Thus, it is assumed that nanoobjects cannot reach the bloodstream via the skin barrier in any clinically relevant amounts unless they are specifically designed for this purpose(Gubala et al., 2018)

1.2. Hydrogels, nanofibers and skin substitutes

In this subtopic, the following concepts will be briefly defined given that these will often be mentioned in this review.

Hydrogels: Hydrogels are 3D networks consisting of physically or chemically crosslinked bonds of hydrophilic polymers in which water is the dispersion medium. Hydrogels are generally obtained by mixing two different polymers to achieve mixture with excellent characteristics compared to the pure polymers. Hydrogel-based wound dressings are one of the most promising materials in wound care, fulfilling important dressing requirements, including: (a) keeping the wound moist whilst absorbing extensive exudate, (b) adhesion-free coverage of sensitive underlying tissue, (c) pain reduction through cooling and (d) a potential tool for active intervention in the wound healing process(Zhai et al., 2018). Furthermore, hydrogels provide protection against denaturation of proteins in it.

Nanofibers: Ultra-fine fibers with diameters ranging from several micrometres down to few nanometres. These nano structures can be fabricated by several methods, electrospinning being the most used technique due to its simplicity, cost efficiency, and versatility (Appendix 2)(Ramos Carriles et al., 2021; Sapkota & Chou, 2020).

Skin substitutes: There are 3 major types of skin substitutes: dermal, epidermal, and dermal/epidermal, whose differences are discussed by Selvakumar et al. (Selvakumar et al., 2020). They mimic as much as possible the natural skin and its ECM, which is a non-cellular structure that regulates most of the cellular functions, whose main component is collagen III, produced by fibroblasts.(Selvakumar et al., 2020).

1.3. Protein based biomaterials for wound healing and tissue regeneration

Some wounds are hard to heal, and wound dressings enhance the healing process. It is important for these dressings to create a favourable environment for cells to proliferate and at the same time protect the wound from pathogens. For this purpose, compounds mimicking the properties of the natural extracellular matrix (ECM) that fasten the recovery of the wound site should be used. In Figure 5 below there is a schematic representation of the wound healing process to understand which processes are necessary to enhance.

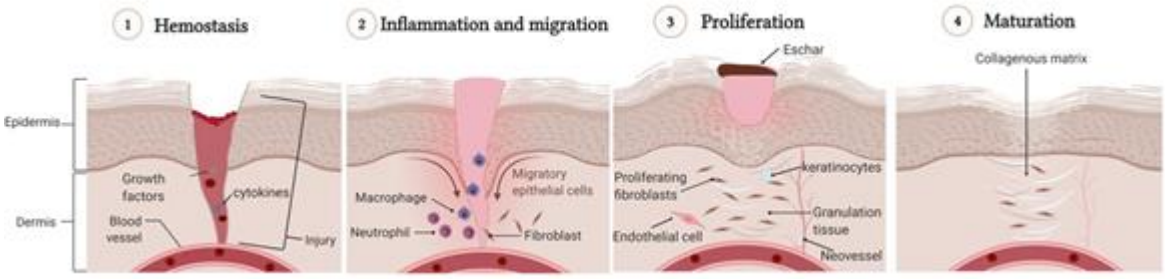

Figure 5. (1) Haemostasis: Blood coagulation by thrombocytes, which release cytokines and GFs that serve as pro-inflammatory signals. (2) Inflammation and migration: Start shortly after haemostasis; platelet secretions increase vasodilatation and recall immune cells (neutrophils and monocytes) to the wound bed; monocytes differentiate into macrophages, that release TGFβ and VEGF. Migration of cells occurs due to released GFs: EGF, FGF, and TGF-α recruit epithelial cells (keratinocytes); TGF-β, FGF, and PDGF affect the penetration of fibroblasts; Secreted VEGF and FGF induce the migration of vascular cells (endothelial). Note: The period of inflammation can be harmful for the tissue if it lasts longer than needed; (3) Proliferation: Angiogenesis/Revascularization by endothelial cells; reepithelialisation by epithelial cells; early ECM (collagen and fibronectin) formation by fibroblasts; granulation tissue formation to replace the clot; fibroblasts modulate into myofibroblasts, responsible for reducing the size of the wound size by contraction. (4) Maturation: Collagen deposit and scar formation; type III collagen replaced by type I; collagen fibres are rearranged, cross-linked, and aligned along tension lines; cells that are no longer needed are removed by apoptosis. Created with BioRender

The most abundant protein in the ECM is collagen. There are more than twenty different types of Collagens identified so far, and all display a triple-helical tertiary structure of polypeptide sequences. Collagen molecules aggregate to form fibrils with diameter in the range of 10–500 nm. This fibrous network facilitates cell migration to the wounded site, actively supporting tissue repair and increase epithelialization rate (Suarato et al., 2018). The fibroblasts are the cell producers of collagen and therefore, it is important to make sure that fibroblast can produce efficiently collagen in terms of wound healing. Additionally, growth factors are secreted along the process by many types of cells, and strategies of making hydrogels loaded with nanoparticles liberating growth factors are being developed (S. Zhang

et al., 2021). Another approach is using cells that will eventually secrete these growth factors (Skardal et al., 2017).

Collagen and gelatin have been widely in combination with polysaccharides like chitosan and alginate(S. Huang et al., 2021; Ort et al., 2021) but since these have either an animal-origin or an allogenic one, respectively, these are prone to cause an immune reaction(J. Luo et al., 2020).

On the other hand, polysaccharides also play a key role and have been used as previously said, as a good complement for many products in biomedicine and other areas(Dalmoro et al., 2012; Mallakpour et al., 2021a, 2021b). Alginate in particular is a polysaccharide (made up of the monosaccharides D-mannuronic and L-guluronic) extracted from the cellular wall of marine algae cells. It has a major use in the industry, it is mostly used in the food industry as a food additive and it is very utilized in odontology for prothesis. It's availability, biocompatibility and low cost make it a good option for many applications. Depending on the pH in which the alginate is found, it has a different structure(Efentakis & Buckton, 2002). In this sense, at neutral pH the alginate turns into a gel that helps hydration and release of the content, and at acidic pH 3 the alginate swells. In the pharmaceutical industry, alginates also serve to bind various substances together and make them visible as tablets for oral use (Fertah, 2017). Because of these properties, we can find alginate applications in countless different areas such as the pharmaceutical industry, the chemical industry, the textile industry, the freezing industry, the gastronomic industry, the immobilization of cells and enzymes, the controlled release of biomolecules, the industrial biomedical and its most recent use in 3D bioprinting within regenerative medicine (Hurtado et al., 2020).

In this matter, soy protein also has a role as they are proofed to promote collagen deposition and reepithelization in wounds (Santin & Ambrosio, 2008). Moreover, soy protein-based products bring no risk of transmissible diseases as in humas- and animal- derived products. Furthermore, SP based products degrade into natural component at a rate tuned with tissue regeneration, thus, avoiding the need for additional surgery(Varshney et al., 2020b). Its properties constitute a similarity to tissue constituents and a reduced susceptibility to thermal degradation, making soy protein a plant-derived bioactive macromolecule of interest in the biomedical field

2. Goals

Revision of last advances done regarding soy protein-based materials for encapsulation and release of bioactive compounds and define if it's the most suitable protein source for each of the applications discussed.

3.Methods

The National Centre of Biotechnology Information (NCBI) and Google scholar websites were mainly used for this bibliographic review.

The research started the 1st of April and the last time an article was looked up for the purpose of getting information for this Bachelor's thesis was 31st of May 2021.

Firstly, a general quest was done to see how many articles include 'soy' included in their title or abstract. NCBI advanced tool was used for this purpose. By selecting the Title/Abstract filter a search with 'soy' was done. The search resulted in 19.661 entries dating from year 1915 until now. Afterwards, "soy protein" was searched by the same method and 3.537 entries were shown. As it is still a large amount of articles, a time range from 2015 to 2021 was requested. As a result, 1.094 entries were obtained with this filter. The aim of this research is to search for the most advanced research on soy protein-based delivery systems so the following search was done: "soy protein"[Title/Abstract]) AND ((delivery[Title/Abstract]) OR (release[Title/Abstract])OR (encapsulation[Title/Abstract])) filter: 2018-2021. Consequently, 58 articles showed up.

This search was also done:

("soy protein"[Title/Abstract] AND ("delivery"[Title/Abstract] OR "release"[Title/Abstract]) AND (wound[Title/Abstract])) AND (2018:2021[pdat]).It got 4 articles as a results but these already appeared in the previous query.

The inclusion criteria used was that soy protein had to be one of the subjects of the experiment and not just mentioned through the paper. Some articles were discarded because they were similar to others that were recently done, so those recent ones were chosen over the older similar ones.

Since most of the results had to do with nanoparticles, a research on nanotoxicology was done as well using the same methodology. Researchers started publishing about Nanotoxicology in the year 2004 until now, making a total of 198 papers. In the last 4 years, 37 articles were found to have Nanotoxicology in their title. The two most recent articles were chosen regarding this matter. Same process was performed for additional research.

The three articles discussed in this review were selected because:

- Their approach is innovative
- All three are a soy protein-based delivery system applied in a different field (Food industry, drug delivery and wound healing)

4.Results

The results of the selected results from the bibliographical search are described in the following table (table 2).

Table 2: Selected results from researched bibliography

Soy protein-based release systems over the past 4 years			
Title	**Material added to SPI**	**Outcome**	**Reference**
Bupivacaine-eluting soy protein structures for controlled release and localized pain relief: An in vitro and *in vivo* study	Glyoxal glycerol	-Controlled drug release - Longer time without changing the dressing - Longer pain relief	(Goder et al., 2021)
Electrospun, sepiolite-loaded poly(vinyl alcohol)/soy protein isolate nanofibers: Preparation, characterization, and their drug release behaviour	Polyvinyl -alcohol	-SPI into PVA nanofiber increased mechanical strength up to 40% SPI content -Longer lasting effect	(Gutschmidt et al., 2021)
Design of biopolymer carriers enriched with natural emulsifiers for improved controlled release of thyme essential oil	Alginate Soy lechitin	-Stabilization of antibacterial thyme oil by encapsulation -Improved thyme oil bioavailability -Stabilized hydrogel carriers	(Volić et al., 2020)

Fabrication of soy protein isolate/cellulose nanocrystal composite nanoparticles for curcumin delivery	Cellulose nanocrystal	-Improved water solubility and chemical stability of curcumin -Controlled drug release in Simulated gastrointestinal conditions -Protection of drug under harsh conditions	(S. Wang et al., 2020)
Fabrication of Composite Hydrogels Based on Soy Protein Isolate and their Controlled Globular Protein Delivery	Polyacrylic	-High absorption of globular protein (BSA) -Sustained release of globular protein	(He et al., 2019)
Survival and Behaviour of Encapsulated Probiotics in Calcium-Alginate-Soy Protein Isolate-Based Hydrogel Beads in Pasteurized Mango Juice	Calcium Alginate	-Resistance to high temperaturas -Protection of probiotics -Stability under longer storage periods	(Praepanitchai et al., 2019)
Soy Protein-based composite hydrogels: Physico-chemical characterization and in vitro cytocompatibility	Alginate Bioactive glass	.-Support their attachment, growth of HUVEC and fibroblasts -Enhances spreading and metabolic activity of cells -Better colonization and growth of fibroblast	(Tansaz et al., 2018)
In vitro characterization of novel multidrug-	Glycerol & glyoxal	-Multiple drugs release in a desired manner for each drug individually.	(Matsliah et al., 2021)

eluting soy protein wound dressings		-Enhanced wound healing	
Spray-Dried Succinylated Soy Protein Microparticles for Oral Ibuprofen Delivery	Succinic acid	-High encapsulation efficiency for hydrophobic drugs -Rapid dissolutions at neutral pH and delayed delivery -Easy administration, via oral route	(Anaya Castro et al., 2019)
Soy Protein-Based Hydrogel under Microwave-Induced Grafting of Acrylic Acid and 4-(4-Hydroxyphenyl)butanoic Acid: A Potential Vehicle for Controlled Drug Delivery in Oral Cavity Bacterial Infections	acrylic acid-co-4-(4-hydroxyphenyl)butanoic acid	-Noncytotoxic, polycrystalline in nature with a network structure -Good porosity -Increased thermal stability - pH-responsive behaviour	(Mehra et al., 2020)

As commented by several researchers (Tang, 2021), there is a need for more invitro cell models to assess the effectivity and compatibility (on regards of health issue) of soy protein-based products and despite the *in vitro* studies found regarding this matter, there are not yet many studies *in vivo*.

An *in vivo* study with a SPI-based delivery system was done by. Goder et.al. They used soy protein isolate as the base material for bupivacaine-loaded hybrid wound dressing. This way the pain from wounds is treated in a non-systemic manner. The authors found that the drug release could be modified by regulating the degree of crosslinking or choosing the drug with different hydrophilicity or higher molecular weight. An *in vivo* rat model study was performed and the results showed a drug release and pain relief in burn wounds for several days without the need for dressing changes, which themselves cause a great deal of pain.

Soy protein-based nanofibers were also performed by Gutschmidt et al . Their study showed that SPI improved the mechanical strength of the mats making them longer-lasting. A summary of the outcomes of the selected studies is shown in table 1.

From the results shown in table 2, three of these articles will be discussed more in detail. The reason why these will be discussed deeper is because these three have quite different application as a delivery system and for no other reason than to comment real results and get a deeper view of the mechanism of these systems.

SPI/CNC for curcumin delivery

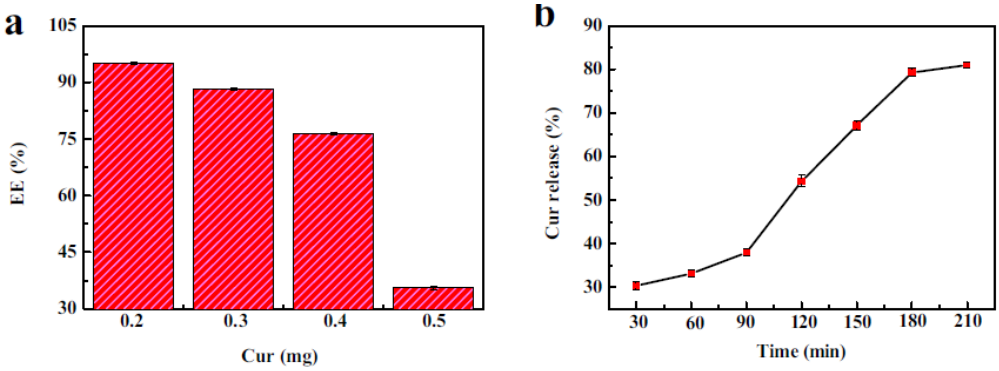

Figure 5.1 a)Effect of different Cur dosages on the encapsulation efficiency of Cur/SPI-CNC nanoparticles b) Release profile of Cur from Cur/SPI-CNC nanoparticles. Adapted from (S. Wang et al., 2020)

The authors of this article encapsulated curcumin in nano capsules made of cellullose nanocrystal and soy protein isolate. Curcumin (Cur) is known for its use in the food industry as a dyer, but it has been widely investigated for its anti-inflammatory, anticancer and antidiabetic effects (Franzone et al., 2021; Lai et al., 2021). However, Cur has low solubility in water, is unstable and has low bioavailability. The fabricated nanocapsules are meant to overcome these limitations. Cellullose nanocrystals were chosen for this experiment because

of their biocompatibility, low cost and hydroxil group in their surface area. This polysacharide and protein combination is proofed to stabilize hydrophobic compounds like curcumin in this case. Different ratios or CNC and SPI nanocapsules were fabricated and the results showed better ζ-potential at a ratio of 6:1 (SPI/CNC). The ζ-potential is key indicator of electrostatic repulsión, the higher potential, the higher repulsion and therefore better stability due to resistance to aggregation. The size on the nanocapsule 6:1 is close to 200 nm which was the lowest size of all ratios. As the nanoparticles will interact with several electrolytes during the delivery of Cur to the gastrointestinal tract, the ionic strength (NaCl) on the stability of SPI-CNC(6:1) was evaluated. It was concluded that higher salt concentration led to decrease of repulsion between nanoparticles and their size increased. At 5 to 25 mmol/L salt, the electrostatic repulsion was strong enough to resist accumulation of particles above that there is aggregation. Regarding the influence of pH on the nanoparticles, it remained stable in general. The effect of temperature was also evaluated and the results show negligible impact on the nanoparticles. The encapsulation efficiency, as shown in figure 5, decreased with increasing amounts of Cur, having 0,2 mg Cur a 95% encapsulation efficiency. The authors give Cur overloading as an explanation for the decreasing encapsulation phenomenon.

The simulated gastrointestinal digestion was made by putting the Cur loaded SPI/CNC nanoparticles dispersion into simulated gastric fluid (SGF) and simulated intestinal fuid (SIF) at 37 ºC and pH 2. Interestingly, the drug release in SGF was lower than in SIF, which shows a controlled drug release from Cur/SPI-CNC complex nanoparticles. The authors suggests that CNC are prone to aggregate under acidic conditions hence, creating a barrier against enzymatic degradation of SPI and Cur diffusion. When the conditions in the SIF switched, the CNC particles did degrade by action of the bile contents and peptides of SIF. Similar results where achieved with other vegetal protein encapsulating hydrophobic bioactive compounds (Y. Luo et al., 2012).

Conversely, a previous study (Peng et al., 2020) did curcumin nanoparticles(CNP) and coated them with several biopolymers, SPI was one of the biopolymers tested. The method for obtaining the nanoparticles was the pH shift process (Appendix 3) So, unlike Wang et al., these authors did not use SPI nanoparticles, but used Curcumin nanoparticles instead. As in the investigation with CNC, the authors of this article tested the stability and the EE of curcumin under different conditions. The results in comparison to the ones Wang et. Al obtained are shown in table 4 and Peng's article results are in table 3. In addition, Wang et. al did not test the ionic strength under $CaCl_2$ salt while Pen et al. tested both Na^+ and Ca^{2+} ions. There was a lack of storage stability evaluation on Wang et al. investigation.

Table 3: Peng's et al. Results. Arabic gum (GA); Soy protein Isolate (SPI); Whey protein isolate (WPI); Sodium Caseinate (SC)

Factor	**Biopolymer coating performance (higher→lower)**
Diameter Size	GA>SPI>WPI>SC
Encapsulation efficiency	SC>GA>SPI>WPI
Loading capacity	SC>WPI>SPI>GA
Thermal stability	SC>WPI=SPI>GA
Storage stability	SC>WPI>SPI>GA

Table 4: Peng's et a results and Wang's et al. results

Factor	CNP coated with SPI biopolymer	SPI-CNC nanocapsules
Diameter Size	156.2 ± 1.2 nm	197.7 ± 0.2 nm
Encapsulation efficiency	81,8%	95%
Loading capacity	2 mg Cur	0,2 mg Cur
Thermal stability	60ºC→ 9,9 % Cur did not remain in the SPI coating 90ºC→ 49,7% Cur did not remain in the SPI coating	Remaining Cur was not measured. Zeta potential remained generally stable in the range of 60 to 90ºC.
Ionic strengh	No size changes nor sedimentation observed with NaCl. $CaCl_2$	Increased size with increasing NaCl concentration. Precipitation observed at 40mM NaCl
Storage stability	4ºC→EE remained high up to 21 days. 25ºC→Dramatically decreased EE after 1st day of storage.	Not evaluated

Survival and Behaviour of Encapsulated Probiotics (Lactobacillus plantarum) in Calcium-Alginate-Soy Protein Isolate-Based Hydrogel Beads in Different Processing Conditions (pH and Temperature) and in Pasteurized Mango Juice

Innovative drinks are going out to the market with bioactive compounds as a new and healthy improvement. One of these improvements has been the addition of probiotics in fruit juices. The authors of this article aimed to encapsulate a probiotic Lactic acid bacteria (*L. plantarum* TISTR050) into calcium-alginate-soy protein isolated based hydrogel beads. Encapsulation of probiotics with alginate and other proteins like Whey protein showed good results an so SPI was never researched on this area before. Therefore, the authors consider that experimenting with SPI would be suitable for people allergic to dairy proteins.

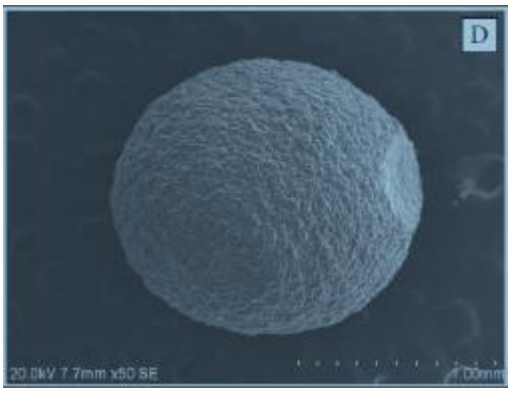

Figure 6 Scanning Electron Microscopy image (magnification 50x). SA-SPI(1:8) hydrogel beads containing Lactobacillus Plantarum. Adapted from (Praepanitchai et al., 2019)

Alginate properties are suitable for crosslinking with calcium and SPI. As it was seen previously that sodium alginate(SA) beads are suitable for encapsulating probiotics, the question in this paper is whether SPI has a synergetic effect that will enhance better protection of encapsulated cells against gastrointestinal conditions.

Different proportions of SPI and SA were investigated. All of them had a 90-92% encapsulation efficiency but only the one with 1:8 (SA/SPI) formulation achieved the highest survival rate under acidic solution. It is worth mentioning that all the formulations had a significative survival rate compared to free cells (which did not survive at all) and SA

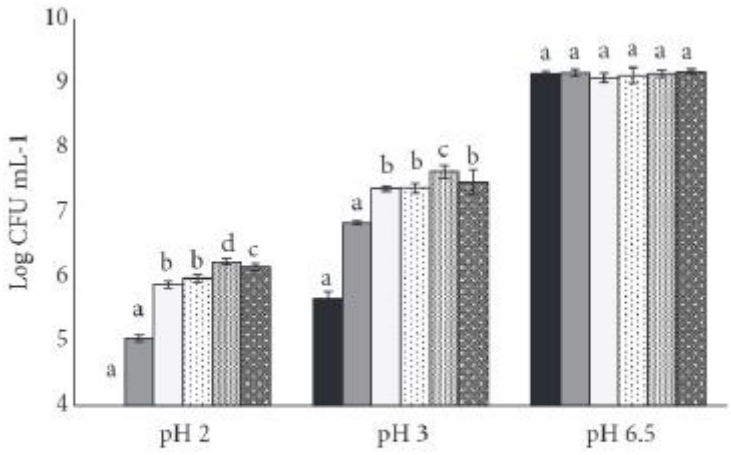

Figure 7 Survival of free cell and encapsulated probiotics in SA bead (bead A) and SA/SPI bead (B 1:2, C 1:4, D 1:8, E 1:12) under acidic conditions at 37ºC for 3 hours. Adapted from(Praepanitchai et al., 2019)

encapsulated ones. This results prove that SPI has a synergetic effect when compared to SA hydrogel beads, thus, validating the initial hypothesis.

In milk-yeast extract, encapsulated probiotics in SA hydrogel beads and free cell showed no changes regarding viability. In contrast, the viability of the encapsulated probiotics in the SA/SPI beads increased gradually from 3 to 6 h of incubation with increasing SA/SPI ratios to 1:8. This is due to the growth of the probiotics encapsulated in the SA/SPI beads; a milk–yeast extract medium, used as nutrient, penetrated the interface between the electrostatically charged SA and SPI complexion particles and the SA wavy matrix. Increasing the bile salt concentrations from0.5 to 1.0% (w/v), the survival of the free cells and the probiotic cells encapsulated in SA beads only decreased with increasing incubation periods. However, the survival of the probiotic in the SA/SPI beads remained nearly the same as that of the starting content (9 log CFU mL−1)after 6 h owing to protection from the electrostatically charged SA and SPI complexion in the hybrid beads. Hence, bile salt concentrations had no diminishing effects on the survival level of the probiotics encapsulated in the SA/SPI beads.

In order for probiotic cells to be effective and remain viable in food and beverage products, they must withstand the recommended pasteurization temperatures. Survival of probiotic cells in SA/SPI beads was therefore evaluated under the heat treatment at 50, 63, and 72ºC. All the free cells were killed at within a min. In contrast, addition of SPI in beads significantly improved the survival of the probiotic cells in SA/SPI beads.

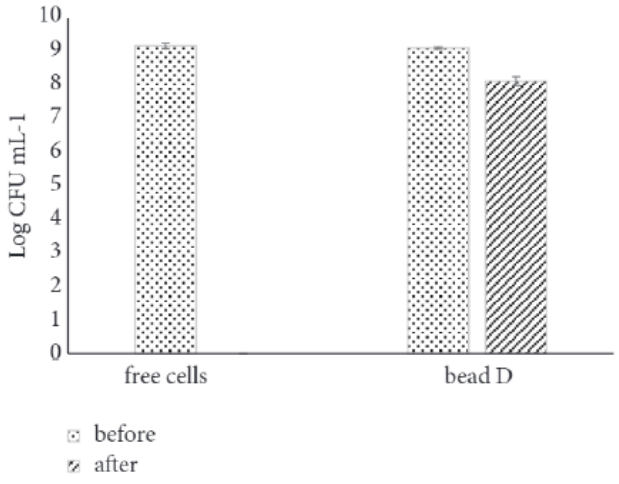

Figure 8 Survival level of the probiotics free cells and encapsulated in SA/SPI beads (bead D formulation) before and after pasteurization process.Adapted from(Praepanitchai et al., 2019)

The pH of mango juice with encapsulated bead remained constant at 4.70 during 35 days of storage. Encapsulated probiotics were found to be heat resistant during pasteurization process and thus, there were higher amount of viable cells in mango juice containing the probiotics loaded SA/SPI beads. The authors conclude that SPI is an alternative encapsulated material to create a barrier to the surrounding environment and it is suitable for the people who are allergic to dairy proteins.

Although the results done by the authors is satisfactory and suggest that SPI is able to encapsulate material and resist industrial processes arriving to the costumer with a minimum of viable probiotics, there is a lack of probiotic delivery study. This could be a future study to

do with formulation D hydrogel beads. This study would asses if the probiotics, once the nanocapsules resists the digestive process, are bioavailable and these are capable to adhere properly to the intestinal tract after all the industrial treatment these have been through.

A similar article (Mokhtari et al., 2019), encapsulated probiotics in Alginate beads in pasteurized grape juice. They also concluded that encapsulated probiotics are able to stay viable for longer storage periods.

In vitro characterization of novel multidrug-eluting soy protein wound dressings

In the current study, hybrid film structures were developed and studied as a novel wound dressing platform with controlled release of three bioactive agents. The dense top layer is designed to provide mechanical support, control the water vapor permeability and to elute the antibiotic drug cloxacillin and the analgesic drug bupivacaine to the wound site. The porous sub-layer is designed to absorb the wound exudates and release the haemostatic agent tranexamic acid for bleeding control.

Two film formulations were used in this study: highly crosslinked and highly plasticized. The films used for most tests were hybrid films and were composed of a top dense film and a bottom porous layer. Non-loaded films were compared to multidrug-loaded films containing bupivacaine and cloxacillin in the dense layer and tranexamic acid in the porous layer.

The cytotoxicity test was performed on human neonatal foreskin fibroblasts using an indirect method as described in ISO 10993:12 parts 5 and 12 for biological evaluation of medical

Figure 9 Soy protein-based wound dressings: (a) dense monolayer film; (b) hybrid film; (c) a hybrid film being bent to demonstrate its flexibility. Adapted from (Matsliah et al., 2021)

devices. Fibroblasts were exposed to 30% or 100% of the wound dressings' extract mediums and the viability of the cells was measured after 24 and 48 h. According to ISO 10993 part 5, at least 70% cell viability must be maintained for the device to be considered biocompatible. In their study, all samples maintained over 70% cell viability. The authors concluded that the films are biocompatible and safe for use as wound dressings, and therefore continued investigating them with the drugs, both in vitro and *in vivo*. Additionally, the analgesic and

antibiotic drugs which are more prone to induce cytotoxicity were tested separately in their preliminary study and did not induce toxic effects.

Overall, The formulation parameters, crosslinker and plasticizer concentrations, had almost no effect on the drug release profile. However, it appears that the drug-loaded highly plasticized films are best suitable for wound dressing applications, since they present the best mechanical properties for both handling and storage. The films released most of the analgesic drug (bupivacaine) within several hours, while only part of the antibiotic drug (cloxacillin) was released, probably due to formation of bonds between the drug molecules and the soy protein molecules. The haemostatic agent tranexamic acid was found to leave the films within several minutes, proving the efficiency of the strategy behind the choice of layers in which we incorporated each drug.

The authors arrive to the conclusion that their novel multidrug-loaded hybrid soy protein wound dressings demonstrated a biocompatible platform with the ability to release multiple drugs in a desired manner for each drug individually. They possess proper and adjustable mechanical and physical properties and their overall characteristics make them excellent candidates for life-saving wound healing applications.

5.Discussion

5.1. Improvement of SP-based products delivery properties by complexation with other compounds

The results show that SPI in combination with other compounds can improve its properties. Many authors from the selected articles (table 2) chose alginate to combine it with SPI. Alginate has been approved to be convenient as able to protect bioactive substances from degradation and from fast release in stomach conditions (due to shrinkage in acidic environment).On the other hand, there are some disadvantages usually correlated with alginate carrier such as severe leakage of entrapped molecules and low mechanical strength (Elnashar,Danial,& Awad, 2009).Therefore, alginate has been frequently blended with other polymers. Alginate-SPI blends have demonstrated better encapsulation efficiency than alginate alone and slower release of encapsulated compound (Volić et al., 2020). Moreover, it goes the other way around as well, soy protein isolate itself has a lower encapsulation efficiency than with Alginate (Chen et al., 2021). Volic et al (table 2) encapsulated thyme oil (known for its health benefits) in SPI-Alginate-Lecithin hydrogel microbeads and the results show an enhanced EE, thermal stability and mechanical strength. Soybean lecithin is a mixture of phospholipid derivatives, whereby phosphatidylcholine is the most prominent. Lecithin has high-affinity binding to the protein surface with specific hydrophobic groups,

whereas hydrophilic heads are oriented toward the water, thus promoting dispersion. lecithin may have another important role related to digestion of a thyme-oil delivery system. In specific, phosphatidylcholine in lecithin creates a mono layer in the GIT and enhances the diffusion of lipophilic compounds through the gastrointestinal mucosa(Volić et al., 2020). Their results

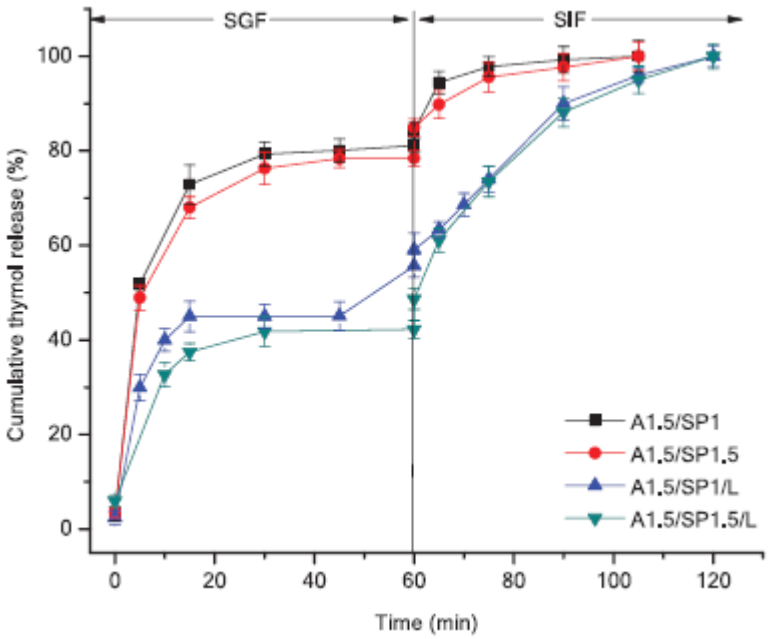

Figure 10 Cumulative release of thymol from SP-Alg and SP-Alg-Lecithin beads of different composition. Adapted from Volic et all 2020

show that SPI-Alg released 80% of thyme oil in Simulated gastric solution in the first hour of the test, while SPI-ALG-Lecithin released 60% of the oil (figure 10). Thus, Lecithin improves retention of the encapsulated compound under gastric solution.

Another approach would be succinylation, as Anaya Castro et.al did for encapsulating Ibuprofen. Succinylation is the addition of succinic acid to protein amino groups, resulting in the replacement of mostly positively charged lysine ε-amino groups with carboxyl groups, which are negatively charged (for a pH range of about 4 to 10). This modification leads to chain unfolding and a lower isoelectric point (pI), accompanied by a decrease in solubility below the pI and an increase in solubility above it. This chemical modification is used to enhance certain functional properties of proteins, such as solubility, emulsification, and foaming capacity. Succinylation is, thus, a possible method for modifying the pH sensitivity of soy protein and controlling the release of active ingredients in gastrointestinal conditions. Furthermore, succinylation is a soft modification, and the use of succinic anhydride is approved and is considered to be GRAS (generally recognised as safe) by the FDA (Food and Drug Administration)(Anaya Castro et al., 2019).

5.2. Discussion of reviewed results

For the wound healing process, the content of -soy protein isolate present certain advantages compared to polysaccharides like chitosan because the ECM is mainly formed by proteins more than polysaccharides which are also beneficials but not so efficient mimicking the ECM, Therefore polysaccharides like alginate and chitosan are preferably used as secondary support(Y. Luo et al., 2012; Su et al., 2021; Zhao et al., 2018). In comparison to other animal-origin proteins like collagen and gelatine, it has lower immunogenicity (Table 1). However, efforts are being done towards lowering the immunogenicity of collagen by finding other collagen sources (J. Luo et al., 2020; You et al., 2018). Therefore, taking into account that the final product would be applied on humans, a non-immunogenic collagen product would be better mimicking the ECM than soy protein that also has a quite good performance. Nevertheless, the cost of obtaining this improved collagen is higher than SPI which is cheaper. In terms of affordability and efficiency, soy protein-based products would be a good option.

On the results from the curcumin delivery, As Peng et al mentioned, curcumin stabilized by amphiphilic molecules, get a better storage stability. CNC have amphiphilic properties that stabilize better the curcumin and that's why on Wang's study the temperature and pH does not influence that much the stability of encapsulated Cur. If they had performed the storage stability test the results may have been satisfactory due to the CNC addition. Regarding the results of SPI coated CNP The relatively good stability of biopolymer-coated curcumin nanoparticles at high $CaCl_2$ levels(table 4) may be due to the strong adsorption of Ca^{2+} ions to the anionic droplet surfaces, thereby leading to charge reversal or strong hydration repulsion.

The storage of CNP coated with SPI biopolymer was not successful, the authors give the following probable reasons: (i) desorption of the biopolymers from the nanoparticle surfaces; (ii) dissolution of curcumin molecules from inside the nanoparticles, followed by their recrystallization; (iii) a reduction in the repulsive interactions between the biopolymer-coated nanoparticles. Given this possibilities, Wang's et al. approach could have possibly improved the storage period given that the CNC confer better protection against Cur diffusion, however, this remains on doubt since there is no research on that matter with SPI/CNC Cur loaded nanocapsules to date.

Interestingly, the other biopolymers tested on Peng's investigation suggest that sodium Caseinate may be a better option for curcumin delivery although there was no evaluation of stability under gastrointestinal conditions. So far, CNC/SPI showed controlled delivery of Curcumin in SIF which demonstrated that this drug delivery system could work by oral delivering hydrophobic compounds, providing beneficial health effects to the host. Oral

administration of anti inflammatory drugs like ibuprofen, an essential oils like thyme oil, using SPI has been researched as well, as shown in the table of results (Anaya Castro et al., 2019). Although these results are promising, there is still need of more *in vivo* trials to asses tall the other factors interaction with the nanoparticles, such as whether is the intestinal microbiota somehow affected by these nanoparticles or if they interfere in the delivery process. There are also concerns about the nanotoxicology of CNC. Given that the human race cannot hydrolase the characteristic β-linkage in cellulose, the CNC can only be affected by the shaking movements and bile salts. In the article it is said that CNC are degraded in the SIF, but since there was no in vitro or *in vivo* test to be able to check the presence of cellulose in the cytoplasm, it cannot be confirmed that it has no cytotoxic effects. Despite studies proving that CNC are essentially biodegradable and show good biocompatibility such as hemocompatibility which suggest that the oral administration of such particles is potentially safe. there is still concern about its effects. In other words, (Pelegrini et al., 2019) CNCs are expected to trip through the gastrointestinal tract and end into stool. However, alimentary canal bioelectrolytes may influence the solubility and colloidal state of CNC particles which may in turn impact the interaction of CNCs with intestinal lumen components. For example, pancreatic secretion which is mildly alkaline may partially remove the sulphate groups from the surface of H2SO4-isolated CNCs, thereby diminishing charge repulsion among particles and eventually lead to particles aggregation. This would provide a longer residence time within the GIT and rises an opportune for CNCs interaction with gut microbiota(Koshani & Madadlou, 2018).

Regarding the encapsulated probiotics in fruit juice, SPI improved Calcium-alginate hydrogel properties by enhancing storage stability and thermal resistance. Other authors combined the Curcumin and probiotics delivery in one hydrogel (Su et al., 2021). It has been proved that Cur has a synergistic effect with probiotics. for the digestive health, which may manifest as the revivification of the gut flora and immunity enhancement (Peterson et al., 2018). The hydrogel they did contained a lactoglobulin from whey protein. Vegetable globulins could also be investigated for the same purpose, given the fact that these have also hydrophilic and amphiphilic properties. There was no study found to use SP globulins for the same purpose to date.

Overall, SPI improves the stability of different bioactive compounds achieving a balanced and controlled release in adverse conditions by modifying its formulation and improving its performance with other polymers in their hydrogel, nano/microparticles forms.

6.Conclusions

In conclusion, SP based products have proofed to be a good release system for bioactive compounds by improving their controlled release, encapsulation efficiency, storage time and stability. The studies in this review show no cytotoxic effects in *in vivo* studies and what's more important is that SPI has proved to be bioactive itself by promoting growth of different types of cells, collagen deposition and stimulating cells for production of new tissue which is remarkable in the biomedical field. Likewise, it is suitable for probiotics encapsulation, presenting resistance to acidic environments and enabling cell survival. Furthermore, Its vegetal origin makes is suitable for people allergic to dairy products.

Additional compounds like succinic acid, alginate ad cellulose reviewed in this thesis, can be utilized wisely for the purpose of improving stability and release of concrete bioactive compounds. The stability and performance of the delivery systems is likely to be highly product-dependent, since each product has a unique composition and processing/storage requirements, and so studies will need to be carried out on a case-by-case basis. Actually, the fact that they can be somehow "personalized" for each case is what makes SPI so advantageous. With this in mind, more studies *in vivo* are needed with the purpose of assuring the safeness of the product in each case. The reason for this is that although SP seems to be very safe, the other compound to which SP would be blended to, may not be so safe.

The performance of soy protein products may be better than other depending on the case, or not significantly better, as seen in the discussion of curcumin delivery, where maybe sodium caseinate could work better. Or as in the wound healing and tissue regeneration applications, where other animal origin like Collagen may suit better. However, its low cost and availability make a good quality and affordable product out of it, not only in the biomedicals industry, but also in the previously mentioned packaging, adhesive and food industry. Therefore, SP based products are a competitive product that can be available worldwide, thus, reaching countries with less resources and improving their life quality.

7.Autoevaluation

During my research I learned new concepts that I wouldn't have learned otherwise. More specifically, the methods for obtaining the different SP-based materials required some more chemistry and physical concepts that I hadn't done for a while, so it was refreshing for me to go over these concepts again and learn new ones. I acquired better organizational skills and learned how to manage my time as it was challenging for me to balance work with studies. Apart from that, I was also dealing with the fact that I was moving on my own to a new city (because of my internship) and had unexpected problems that eventually turned out well. I

believe this will help me cope with similar situations that I will probably have in the near future. To sum up, I'm satisfied with the final result although there is always room for some improvements.

8.References

Ahn, S., Chantre, C. O., Gannon, A. R., Lind, J. U., Campbell, P. H., Grevesse, T., O'Connor, B. B., & Parker, K. K. (2018). Soy Protein/Cellulose Nanofiber Scaffolds Mimicking Skin Extracellular Matrix for Enhanced Wound Healing. *Advanced Healthcare Materials*, *7*(9). https://doi.org/10.1002/adhm.201701175

Anaya Castro, M. A., Alric, I., Brouillet, F., Peydecastaing, J., Fullana, S. G., & Durrieu, V. (2019). Spray-Dried Succinylated Soy Protein Microparticles for Oral Ibuprofen Delivery. *AAPS PharmSciTech*, *20*(2). https://doi.org/10.1208/s12249-018-1250-6

Arias, A., González-García, S., Feijoo, G., & Moreira, M. T. (2021). Environmental benefits of soy-based bio-adhesives as an alternative to formaldehyde-based options. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-021-12766-4

Ashaolu, T. J. (2020). Health Applications of Soy Protein Hydrolysates. *International Journal of Peptide Research and Therapeutics*, *26*(3), 2333–2343. https://doi.org/10.1007/s10989-020-10018-6

Bhat, M. Y., Dar, T. A., & Singh, L. R. (2016). Casein Proteins: Structural and Functional Aspects. In *Milk Proteins - From Structure to Biological Properties and Health Aspects*. InTech. https://doi.org/10.5772/64187

Bi, F., Qin, Y., Chen, D., Kan, J., & Liu, J. (2021). Development of active packaging films based on chitosan and nano-encapsulated luteolin. *International Journal of Biological Macromolecules*, *182*, 545–553. https://doi.org/10.1016/j.ijbiomac.2021.04.063

Bogahawaththa, D., Ashraf, R., Chandrapala, J., Donkor, O., & Vasiljevic, T. (2018). In vitro immunogenicity of various native and thermally processed bovine milk proteins and their mixtures. *Journal of Dairy Science*, *101*(10), 8726–8736. https://doi.org/10.3168/jds.2018-14488

Chen, F. ping, Kong, N. qing, Wang, L., Luo, Z., Yin, J., & Chen, Y. (2021). Nanocomplexation between thymol and soy protein isolate and its improvements on stability and antibacterial properties of thymol. *Food Chemistry*, *334*. https://doi.org/10.1016/j.foodchem.2020.127594

Dalmoro, A., Barba, A. A., Lamberti, G., Grassi, M., & D'Amore, M. (2012). Pharmaceutical applications of biocompatible polymer blends containing sodium alginate. *Advances in Polymer Technology*, *31*(3), 219–230. https://doi.org/10.1002/adv.21276

de Frates, K., Markiewicz, T., Gallo, P., Rack, A., Weyhmiller, A., Jarmusik, B., & Hu, X. (2018). Protein polymer-based nanoparticles: Fabrication and medical applications. *International Journal of Molecular Sciences*, *19*(6), 1–20. https://doi.org/10.3390/ijms19061717

Efentakis, M., & Buckton, G. (2002). The effect of erosion and swelling on the dissolution of theophylline from low and high viscosity sodium alginate matrices. *Pharmaceutical Development and Technology*, *7*(1), 69–77. https://doi.org/10.1081/PDT-120002232

Ferreira, E. D. S., Silva, M. A., Demonte, A., & Neves, V. A. (2011). Soy β-conglycinin (7S globulin) reduces plasma and liver cholesterol in rats fed hypercholesterolemic diet. *Journal of Medicinal Food*, *14*(1–2), 94–100. https://doi.org/10.1089/jmf.2009.0204

Fertah, M. (2017). Isolation and Characterization of Alginate from Seaweed. In *Seaweed Polysaccharides: Isolation, Biological and Biomedical Applications* (pp. 11–26). Elsevier. https://doi.org/10.1016/B978-0-12-809816-5.00002-5

Franzone, F., Nebbioso, M., Pergolizzi, T., Attanasio, G., Musacchio, A., Greco, A., Limoli, P., Artico, M., Spandidos, D., Taurone, S., & Agostinelli, E. (2021). Anti-inflammatory role of curcumin in retinal disorders (Review). *Experimental and Therapeutic Medicine*, *22*(1), 790. https://doi.org/10.3892/etm.2021.10222

Garrido, T., Leceta, I., Cabezudo, S., Guerrero, P., & de la Caba, K. (2016). Tailoring soy protein film properties by selecting casting or compression as processing methods. *European Polymer Journal*, *85*, 499–507. https://doi.org/10.1016/j.eurpolymj.2016.11.007

Gimeno-Benito, I., Giusti, A., Dekkers, S., Haase, A., & Janer, G. (2021). A review to support the derivation of a worst-case dermal penetration value for nanoparticles. In *Regulatory Toxicology and Pharmacology* (Vol. 119, p. 104836). Academic Press Inc. https://doi.org/10.1016/j.yrtph.2020.104836

Goder, D., Giladi, S., Furer, A., & Zilberman, M. (2021). Bupivacaine-eluting soy protein structures for controlled release and localized pain relief: An in vitro and in vivo study. *Journal of Biomedical Materials Research - Part A*. https://doi.org/10.1002/jbm.a.37163

Gubala, V., Johnston, L. J., Krug, H. F., Moore, C. J., Ober, C. K., Schwenk, M., & Vert, M. (2018). Engineered nanomaterials and human health: Part 2. Applications and nanotoxicology (IUPAC Technical Report). In *Pure and Applied Chemistry* (Vol. 90, Issue 8, pp. 1325–1356). Walter de Gruyter GmbH. https://doi.org/10.1515/pac-2017-0102

Guterres, S. S., Alves, M. P., & Pohlmann, A. R. (2007). Polymeric Nanoparticles, Nanospheres and Nanocapsules, for Cutaneous Applications. *Drug Target Insights*, *2*, 117739280700200. https://doi.org/10.1177/117739280700200002

Gutschmidt, D., Hazra, R. S., Zhou, X., Xu, X., Sabzi, M., & Jiang, L. (2021). Electrospun, sepiolite-loaded poly(vinyl alcohol)/soy protein isolate nanofibers: Preparation, characterization, and their drug release behavior. *International Journal of Pharmaceutics*, *594*. https://doi.org/10.1016/j.ijpharm.2020.120172

He, N., Chen, X., Wang, L., Wen, J., Li, Y., Cao, Q., Liu, Z., & Li, B. (2019). Fabrication of Composite Hydrogels Based on Soy Protein Isolate and their Controlled Globular Protein Delivery. *Global Challenges*, *3*(9), 1900030. https://doi.org/10.1002/gch2.201900030

Huang, S., Yu, F., Cheng, Y., Li, Y., Chen, Y., Tang, J., Bei, Y., Tang, Q., Zhao, Y., Huang, Y., & Xiang, Q. (2021). Transforming Growth Factor-β3/Recombinant Human-like Collagen/Chitosan Freeze-Dried Sponge Primed With Human Periodontal Ligament Stem Cells Promotes Bone Regeneration in Calvarial Defect Rats. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.678322

Huang, X., & Tang, M. (2021). Review of gut nanotoxicology in mammals: Exposure, transformation, distribution and toxicity. In *Science of the Total Environment* (Vol. 773). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2021.145078

Hurtado, A., Selgas, R., & Serrano Aroca, Á. (2020). El alginato y sus inmensas aplicaciones industriales. *Nereis. Interdisciplinary Ibero-American Journal of Methods, Modelling and Simulation.*, *12*, 137–149. https://doi.org/10.46583/nereis_2020.12.573

Koshani, R., & Madadlou, A. (2018). A viewpoint on the gastrointestinal fate of cellulose nanocrystals. In *Trends in Food Science and Technology* (Vol. 71, pp. 268–273). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2017.10.023

Lai, X., Geng, X., Li, M., Tang, M., Liu, Q., Yang, M., Shen, L., Zhu, Y., & Wang, S. (2021). Glutathione-responsive PLGA nanocomplex for dual delivery of doxorubicin and curcumin to overcome tumor multidrug resistance. *Nanomedicine*, nnm-2021-0100. https://doi.org/10.2217/nnm-2021-0100

Li, D., Gao, Y., Wang, Y., Yang, X., He, C., Zhu, M., Zhang, S., & Mo, X. (2019). Evaluation of biocompatibility and immunogenicity of micro/nanofiber materials based on tilapia skin collagen. *Journal of Biomaterials Applications*, *33*(8), 1118–1127. https://doi.org/10.1177/0885328218820180

Li, F., Chen, Y., Liu, S., Pan, X., Liu, Y., Zhao, H., Yin, X., Yu, C., Kong, W., & Zhang, Y. (2019). The effect of size, dose, and administration route on zein nanoparticle immunogenicity in BALB/c mice. *International Journal of Nanomedicine*, *14*, 9917–9928. https://doi.org/10.2147/IJN.S226466

Li, T., Bu, G., & Xi, G. (2021). Effects of heat treatment on the antigenicity, antigen epitopes, and structural properties of β-conglycinin. *Food Chemistry*, *346*, 128962. https://doi.org/10.1016/j.foodchem.2020.128962

Ligorio, C., O'Brien, M., Hodson, N. W., Mironov, A., Iliut, M., Miller, A. F., Vijayaraghavan, A., Hoyland, J. A., & Saiani, A. (2021). TGF-β3-loaded graphene oxide - self-assembling peptide hybrid hydrogels as functional 3D scaffolds for the regeneration of the nucleus pulposus. *Acta Biomaterialia*. https://doi.org/10.1016/j.actbio.2021.03.077

Lin, H. H., Hsieh, F. Y., Tseng, C. S., & Hsu, S. H. (2016). Preparation and characterization of a biodegradable polyurethane hydrogel and the hybrid gel with soy protein for 3D cell-laden bioprinting. *Journal of Materials Chemistry B*, *4*(41), 6694–6705. https://doi.org/10.1039/c6tb01501h

Luo, J., Yang, X., Cao, Y., Li, G., Meng, Y., & Li, C. (2020). Structural characterization and in vitro immunogenicity evaluation of amphibian-derived collagen type II from the cartilage of Chinese Giant Salamander (Andrias davidianus). *Journal of Biomaterials Science, Polymer Edition*, *31*(15), 1941–1960. https://doi.org/10.1080/09205063.2020.1786882

Luo, Y., Teng, Z., & Wang, Q. (2012). Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. *Journal of Agricultural and Food Chemistry*, *60*(3), 836–843. https://doi.org/10.1021/jf204194z

Mallakpour, S., Azadi, E., & Hussain, C. M. (2021a). Chitosan, alginate, hyaluronic acid, gums, and β-glucan as potent adjuvants and vaccine delivery systems for viral threats including SARS-CoV-2:

A review. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2021.05.155

Mallakpour, S., Azadi, E., & Hussain, C. M. (2021b). State-of-the-art of 3D printing technology of alginate-based hydrogels—An emerging technique for industrial applications. *Advances in Colloid and Interface Science*, *293*, 102436. https://doi.org/10.1016/j.cis.2021.102436

Matsliah, L., Goder, D., Giladi, S., & Zilberman, M. (2021). In vitro characterization of novel multidrug-eluting soy protein wound dressings. *Journal of Biomaterials Applications*, *35*(8), 978–993. https://doi.org/10.1177/0885328220975178

Meat and Soy Products: Comparison of environmental effects. (2019). https://www.ecologic.eu/16618

Mehra, S., Nisar, S., Chauhan, S., Singh, V., & Rattan, S. (2020). Soy Protein-Based Hydrogel under Microwave-Induced Grafting of Acrylic Acid and 4-(4-Hydroxyphenyl)butanoic Acid: A Potential Vehicle for Controlled Drug Delivery in Oral Cavity Bacterial Infections. *ACS Omega*, *5*(34), 21610–21622. https://doi.org/10.1021/acsomega.0c02287

Mihalca, V., Kerezsi, A. D., Weber, A., Gruber-traub, C., Schmucker, J., Vodnar, D. C., Dulf, F. V., Socaci, S. A., Fărcaș, A., Mureșan, C. I., Suharoschi, R., & Pop, O. L. (2021). Protein-based films and coatings for food industry applications. In *Polymers* (Vol. 13, Issue 5, pp. 1–24). MDPI AG. https://doi.org/10.3390/polym13050769

Mokhtari, S., Jafari, S. M., & Khomeiri, M. (2019). Survival of encapsulated probiotics in pasteurized grape juice and evaluation of their properties during storage. *Food Science and Technology International*, *25*(2), 120–129. https://doi.org/10.1177/1082013218801113

Monteiro-Riviere, N. A., Wiench, K., Landsiedel, R., Schulte, S., Inman, A. O., & Riviere, J. E. (2011). Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: An In vitro and in vivo study. *Toxicological Sciences*, *123*(1), 264–280. https://doi.org/10.1093/toxsci/kfr148

Ort, C., Chen, Y., Ghagre, A., Ehrlicher, A., & Moraes, C. (2021). Bioprintable, Stiffness-Tunable Collagen-Alginate Microgels for Increased Throughput 3D Cell Culture Studies. *ACS Biomaterials Science & Engineering*, acsbiomaterials.1c00129. https://doi.org/10.1021/acsbiomaterials.1c00129

Pelegrini, B. L., Ré, F., de Oliveira, M. M., Fernandes, T., de Oliveira, J. H., Oliveira Junior, A. G., Girotto, E. M., Nakamura, C. v., Sampaio, A. R., Valim, A., & de Souza Lima, M. M. (2019). Cellulose Nanocrystals as a Sustainable Raw Material: Cytotoxicity and Applications on Healthcare Technology. In *Macromolecular Materials and Engineering* (Vol. 304, Issue 8, p. 1900092). Wiley-VCH Verlag. https://doi.org/10.1002/mame.201900092

Peng, S., Zhou, L., Cai, Q., Zou, L., Liu, C., Liu, W., & McClements, D. J. (2020). Utilization of biopolymers to stabilize curcumin nanoparticles prepared by the pH-shift method: Caseinate, whey protein, soy protein and gum Arabic. *Food Hydrocolloids*, *107*, 105963. https://doi.org/10.1016/j.foodhyd.2020.105963

Peterson, C. T., Vaughn, A. R., Sharma, V., Chopra, D., Mills, P. J., Peterson, S. N., & Sivamani, R. K. (2018). Effects of Turmeric and Curcumin Dietary Supplementation on Human Gut Microbiota:

A Double-Blind, Randomized, Placebo-Controlled Pilot Study. *Journal of Evidence-Based Integrative Medicine*, *23*, 1–8. https://doi.org/10.1177/2515690X18790725

Praepanitchai, O. A., Noomhorm, A., Anal, A. K., & Potes, M. E. (2019). Survival and Behavior of Encapsulated Probiotics (Lactobacillus plantarum) in Calcium-Alginate-Soy Protein Isolate-Based Hydrogel Beads in Different Processing Conditions (pH and Temperature) and in Pasteurized Mango Juice. *BioMed Research International*, *2019*. https://doi.org/10.1155/2019/9768152

Radacsi, N., Ambrus, R., Szunyogh, T., Szabó-Révész, P., Stankiewicz, A., van der Heijden, A., & ter Horst, J. H. (2012). Electrospray crystallization for nanosized pharmaceuticals with improved properties. *Crystal Growth and Design*, *12*(7), 3514–3520. https://doi.org/10.1021/cg300285w

Ramos Carriles, Y., Suetel, M., Henze, S., Álvarez Brito, R., & Mueller, W.-D. (2021). Electrospun meshes of poly (n-butyl cyanoacrylate) and their potential applications for drug delivery and tissue engineering. *International Journal of Pharmaceutics*, 120735. https://doi.org/10.1016/j.ijpharm.2021.120735

Roy, S., Kim, H. J., & Rhim, J. W. (2021). Effect of blended colorants of anthocyanin and shikonin on carboxymethyl cellulose/agar-based smart packaging film. *International Journal of Biological Macromolecules*, *183*, 305–315. https://doi.org/10.1016/j.ijbiomac.2021.04.162

Santin, M., & Ambrosio, L. (2008). Soybean-based biomaterials: Preparation, properties and tissue regeneration potential. In *Expert Review of Medical Devices* (Vol. 5, Issue 3, pp. 349–358). Expert Rev Med Devices. https://doi.org/10.1586/17434440.5.3.349

Sapkota, S., & Chou, S.-F. (2020). Electrospun Chitosan-based Fibers for Wound Healing Applications. *Journal of Biomaterials*, *4*(2), 51–57. http://www.ncbi.nlm.nih.gov/pubmed/34056619

Selvakumar, G., Iyappan, K., & Suguna, L. (2020). *Biomaterials and Wound Healing: A Mini Review* (Vol. 1).

Skardal, A., Murphy, S. v., Crowell, K., Mack, D., Atala, A., & Soker, S. (2017). A tunable hydrogel system for long-term release of cell-secreted cytokines and bioprinted in situ wound cell delivery. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *105*(7), 1986–2000. https://doi.org/10.1002/jbm.b.33736

Soybeans (HS: 1201) Product Trade, Exporters and Importers | OEC - The Observatory of Economic Complexity. (n.d.). Retrieved May 19, 2021, from https://oec.world/en/profile/hs92/soybeans

Su, J., Cai, Y., Zhi, Z., Guo, Q., Mao, L., Gao, Y., Yuan, F., & van der Meeren, P. (2021). Assembly of propylene glycol alginate/β-lactoglobulin composite hydrogels induced by ethanol for co-delivery of probiotics and curcumin. *Carbohydrate Polymers*, *254*. https://doi.org/10.1016/j.carbpol.2020.117446

Suarato, G., Bertorelli, R., & Athanassiou, A. (2018). Borrowing from nature: Biopolymers and biocomposites as smart wound care materials. In *Frontiers in Bioengineering and Biotechnology* (Vol. 6, Issue OCT, p. 137). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2018.00137

Tang, C. H. (2021). Nano-architectural assembly of soy proteins: A promising strategy to fabricate nutraceutical nanovehicles. In *Advances in Colloid and Interface Science* (Vol. 291, p. 102402). Elsevier B.V. https://doi.org/10.1016/j.cis.2021.102402

Tansaz, S., Singh, R., Cicha, I., & Boccaccini, A. R. (2018). Soy Protein-based composite hydrogels: Physico-chemical characterization and in vitro cytocompatibility. *Polymers*, *10*(10). https://doi.org/10.3390/polym10101159

Teng, Z., Luo, Y., & Wang, Q. (2012). Nanoparticles synthesized from soy protein: Preparation, characterization, and application for nutraceutical encapsulation. *Journal of Agricultural and Food Chemistry*, *60*(10), 2712–2720. https://doi.org/10.1021/jf205238x

Varshney, N., Sahi, A. K., Poddar, S., & Mahto, S. K. (2020a). Soy protein isolate supplemented silk fibroin nanofibers for skin tissue regeneration: Fabrication and characterization. *International Journal of Biological Macromolecules*, *160*, 112–127. https://doi.org/10.1016/j.ijbiomac.2020.05.090

Varshney, N., Sahi, A. K., Poddar, S., & Mahto, S. K. (2020b). Soy protein isolate supplemented silk fibroin nanofibers for skin tissue regeneration: Fabrication and characterization. *International Journal of Biological Macromolecules*, *160*, 112–127. https://doi.org/10.1016/j.ijbiomac.2020.05.090

Volić, M. M., Obradović, N. S., Djordjević, V. B., Luković, N. D., Knežević-Jugović, Z. D., & Bugarski, B. M. (2020). Design of biopolymer carriers enriched with natural emulsifiers for improved controlled release of thyme essential oil. *Journal of Food Science*, *85*(11), 3833–3842. https://doi.org/10.1111/1750-3841.15499

Wang, S., Lu, Y., Ouyang, X. kun, & Ling, J. (2020). Fabrication of soy protein isolate/cellulose nanocrystal composite nanoparticles for curcumin delivery. *International Journal of Biological Macromolecules*, *165*(Pt A), 1468–1474. https://doi.org/10.1016/j.ijbiomac.2020.10.046

Wang, T., Qin, G. X., Sun, Z. W., & Zhao, Y. (2014). Advances of Research on Glycinin and β-Conglycinin: A Review of Two Major Soybean Allergenic Proteins. *Critical Reviews in Food Science and Nutrition*, *54*(7), 850–862. https://doi.org/10.1080/10408398.2011.613534

Wong, J. H., & Ng, T. B. (2011). Plant Biochemistry: Antifungal Proteins Protecting Plants from Fungal Pathogens. In *Comprehensive Biotechnology, Second Edition* (Vol. 4, pp. 745–756). Elsevier Inc. https://doi.org/10.1016/B978-0-08-088504-9.00013-1

Wu, P., Zhao, Y., Chen, F., Xiao, A., Du, Q., Dong, Q., Ke, M., Liang, X., Zhou, Q., & Chen, Y. (2020). Conductive Hydroxyethyl Cellulose/Soy Protein Isolate/Polyaniline Conduits for Enhancing Peripheral Nerve Regeneration via Electrical Stimulation. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.00709

You, L., Weikang, X., Lifeng, Y., Changyan, L., Yongliang, L., Xiaohui, W., & Bin, X. (2018). In vivo immunogenicity of bovine bone removed by a novel decellularization protocol based on supercritical carbon dioxide. *Artificial Cells, Nanomedicine and Biotechnology*, *46*(sup2), 334–344. https://doi.org/10.1080/21691401.2018.1457044

Zhai, M., Xu, Y., Zhou, B., & Jing, W. (2018). Keratin-chitosan/n-ZnO nanocomposite hydrogel for antimicrobial treatment of burn wound healing: Characterization and biomedical application. *Journal of Photochemistry and Photobiology B: Biology*, *180*, 253–258. https://doi.org/10.1016/j.jphotobiol.2018.02.018

Zhang, J., Field, C. J., Vine, D., & Chen, L. (2015). Intestinal uptake and transport of vitamin B12-loaded soy protein nanoparticles. *Pharmaceutical Research*, *32*(4), 1288–1303. https://doi.org/10.1007/s11095-014-1533-x

Zhang, S., Vaida, J., Parenti, J., Lindsey, B. A., Xing, M., & Li, B. (2021). Programmed Multidrug Delivery Based on Bio-Inspired Capsule-Integrated Nanocoatings for Infected Bone Defect Treatment. *ACS Applied Materials and Interfaces*, *13*(10). https://doi.org/10.1021/acsami.0c20332

Zhao, Y., Wang, Z., Zhang, Q., Chen, F., Yue, Z., Zhang, T., Deng, H., Huselstein, C., Anderson, D. P., Chang, P. R., Li, Y., & Chen, Y. (2018). Accelerated skin wound healing by soy protein isolate–modified hydroxypropyl chitosan composite films. *International Journal of Biological Macromolecules*, *118*(Pt A), 1293–1302. https://doi.org/10.1016/j.ijbiomac.2018.06.195

Zuo, W. Y., Chen, W. H., & Zou, S. X. (2005). Separation of growth-stimulating peptides for bifidobacterium from soybean conglycinin. *World Journal of Gastroenterology*, *11*(37), 5801–5806. https://doi.org/10.3748/wjg.v11.i37.5801

9.Appendix

Appendix 1: Analogy to illustrate the surface-to-volume ratio of engineered nanomaterials.

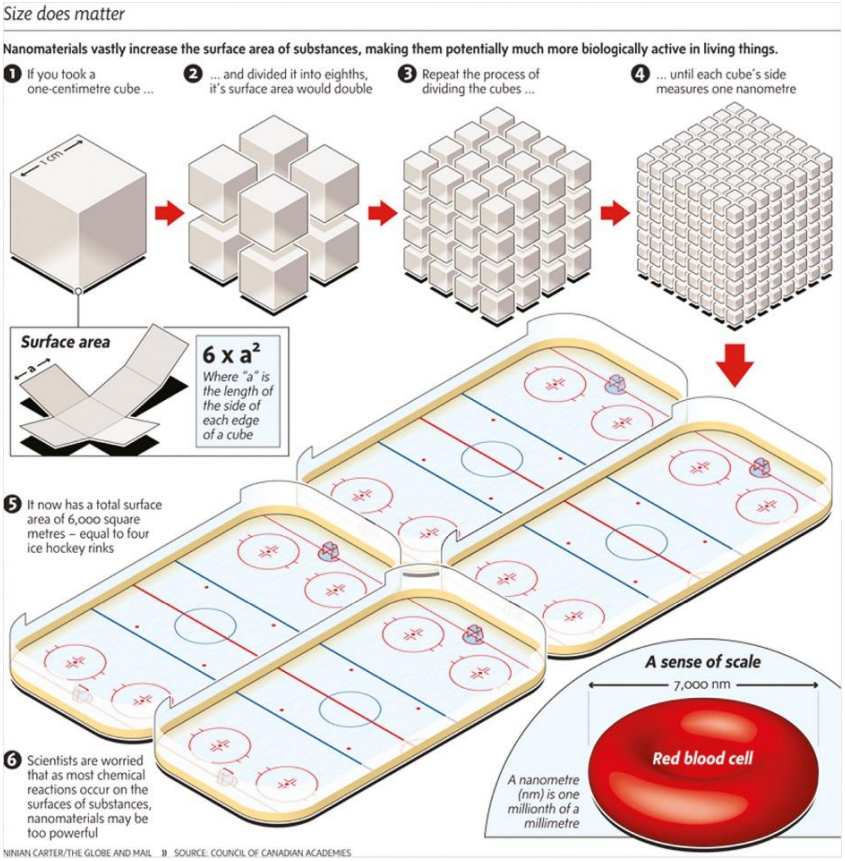

Figure a1: If the particle (e.g. cube) in the figure has a size a=1 cm, the surface area of that particle is 6 cm2. By decreasing its size to a=10 nm, while keeping the same overall volume and mass, the surface area now grows to 600 m2. By reducing each particle further to a=1 nm, the surface area is proportional to the surface of four ice-hockey rinks. Source: Council of Canadian Academies.

Appendix 2: Electrospinning

Electrospinning is a fiber production method which uses electric force to draw charged threads of polymer solutions or polymer melts up to fiber diameters in the order of some hundred nanometers

The process is the following: When a sufficiently high voltage is applied to a liquid droplet, the body of the liquid becomes charged, and electrostatic repulsion counteracts the surface tension and the droplet is stretched; at a critical point a stream of liquid erupts from the surface. This point of eruption is known as the Taylor cone. If the molecular cohesion of the liquid is

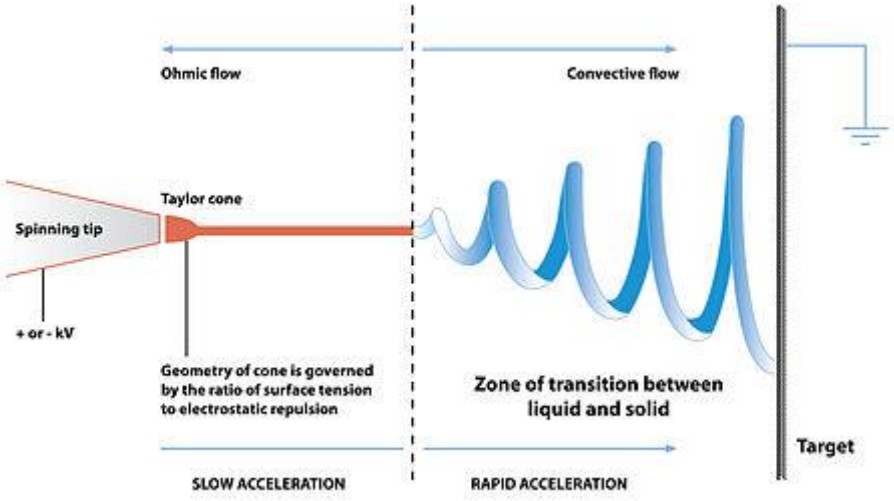

Figure a2: Diagram showing fibre formation by electrospinning. Source The New Zealand Institute for Plant and Food Research Ltd.

sufficiently high, stream breakup does not occur (if it does, droplets are electrosprayed) and a charged liquid jet is formed.

As the jet dries in flight, the mode of current flow changes from ohmic to convective as the charge migrates to the surface of the fiber. The jet is then elongated by a whipping process caused by electrostatic repulsion initiated at small bends in the fiber, until it is finally deposited on the grounded collector The elongation and thinning of the fiber resulting from this bending instability leads to the formation of uniform fibers with nanometer-scale diameters.

Appendix 3:pH shift process

In pH shift method, proteins will be exposed to high and low pH conditions that cause their solubility thus separating them from storage and membrane lipids.

The pH-shift method utilizes the fact that the solubility of curcumin within aqueous solutions is strongly dependent on pH. Below pH 8, the curcumin molecule is non-polar and non-charged so that it has an extremely low water-solubility. Conversely, above pH 8, the pH exceeds the pKa values of the hydroxide groups on the curcumin molecule, which causes them to lose a proton. As a result, the negative charge on the curcumin molecule progressively increases as the pH of the solution increases, which leads to an increase in its water-solubility. This phenomenon can be utilized to create curcumin nanoparticles using a simple experimental approach. Initially, the curcumin is dissolved within a basic solution (pH > 8) and then the system is acidified. As a result, the curcumin molecules become strongly hydrophobic and their water-solubility decreases. In pure water, this sudden change in curcumin water-solubility

leads to the spontaneous formation of curcumin nanocrystals. Conversely, in the presence of colloidal particles that have hydrophobic interiors (such as micelles, vesicles, or lipid droplets), the curcumin molecules move into these hydrophobic do- mains such as to reduce their contact with water.