Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Review
Volume 14, Issue 2, 2024, 45
https://doi.org/10.33263/BRIAC142.045

Porous Starch: Enzymatic Methods to Obtain it and Apply it as a Carrier Material in the Food Area

Heidi Maria Palma-Rodríguez[1], Apolonio Vargas-Torres [1], Román Leyva-López [1,*]

[1] Autonomous University of the State of Hidalgo, Institute of Agricultural Sciences (ICAp), State of Hidalgo, Mexico; palma.heidi@gmail.com (P.R.H.M); apolonio@uaeh.edu.mx (V.T.A); leyva.roma11@gmail.com (L.L.R);
* Correspondence: leyva.roma11@gmail.com; ro162750@uaeh.edu.mx (L.L.R);
Scopus Author ID 57209023984

Received: 1.04.2023; Accepted: 24.06.2023; Published: 4.02.2024

Abstract. Starch is a complex polymer produced by plants. It is formed by amylose and amylopectin, and it is present in some legumes, cereals, and fruits in a ripe state. Today, starch is mainly responsible for providing physicochemical, textural, and rheological properties to pharmaceutical, textile, and agricultural products and, most importantly, those from the food sector. Starch is used as a thickener, stabilizer, gelling, and loading agent. Nevertheless, native starch has inherent disadvantages, such as poor thermal resistance and weak mechanical strength. Diverse methodologies, such as physical, chemical, and enzymatic methods, have been used to redress these shortcomings. Porous starch obtained by enzymatic modification has gained interest due to improvements in thermal stability, protection, and controlled liberation of chemical compounds of interest in the food area. This review shows recent advances in porous starch obtained through enzymatic treatment or synergy with physical and/or chemical treatments. The treatments potentialize its use as an agent to absorb, encapsulate, and liberate different compounds such as probiotics, antioxidants, and essential oils in the food field.

Keywords: porous starch; encapsulation; antioxidants

1. Introduction

Starch is a renewable, affordable, and biodegradable biopolymer widely distributed in nature. It is formed by two polymers of glucose units: amylose, essentially linear, and amylopectin, highly ramified and of a higher molecular weight [1]. The shape and size of the granules differ depending on the source starch obtained from [2]. Starch granules have a semicrystalline structure. The crystalline part presents a high level of organization, and its main component is amylopectin, while the amorphous region has a lower organization formed by amylose. The latter is most vulnerable to exogenous factors like temperature, enzymes, and pH, among others [3-5], so it is the first to be hydrolyzed by enzymatic action. Jayakody and Hoover [6] stated that pores appear naturally on the surface of granules of some botanical species (e.g., corn, sorghum, and rice), a characteristic that favors enzymatic hydrolysis. Recent works have taken advantage of porous starches for different applications, such as the bioactive packaging of foods [7], a vehicle for bioactive compounds [8], adsorbent for natural drugs [9], and the formation of compounds with functional compounds [10]. These spaces (pores) favor the adsorption of chemical compounds and increase the volume, size, and specific surface of contact; as a result, there is a better adsorption yield [11]. In recent years, enzymatic methods have been widely used to obtain porous starches since they present a high substrate selectivity and specificity [12]. Even though enzymatic hydrolysis can produce a porous starch independently, its efficiency and desired characteristics can be improved in combination with physical and/or chemical methods [13]. When the starch is porous, the size of the chains of amylose and amylopectin can be reduced in the starch grain, affecting the viscosity of dispersions. However, the gastrointestinal tract's pH, temperature, and digestibility are improved [14]. This review aims to provide information on enzymatic methods and their combination with physical and/or chemical processes to obtain a porous starch and define its possible application as an encapsulating agent in the food sector.

2. Obtaining of porous starch

2.1. Enzymatic methods.

When selecting the method for enzymatic modification, there are important factors to consider, such as time, pH, enzyme, the mixture of enzymes to be used [15], and the source to obtain the starch. These physical and enzymatic conditions and the endemic properties of each starch source affect the morphology of the pores obtained in the hydrolyzed starch [16, 17], as reported in Table 1. The morphology of the obtained pores and the time of enzymatic erosion produce hydrolyzed starches that are widely used in the food sector. Amylolytic enzymes are widely employed in this type of hydrolysis [17], causing different formation patterns of pores. The commonly used ones are α-amylase, amyloglucosidase, branching enzyme, and cyclodextrin glycosyltransferase [7, 15, 18]. The size distribution, pore area, and water and oil adsorption capacity depend on the type of enzyme used in starch hydrolysis [19].

Table 1. Methodology of enzymatic treatment and morphological structure of porous starch produced.

Starch source	Enzymes	Experimental conditions	Characteristics of porous starch	Application	Ref
Purple sweet potato	α-amylase, glucoamylase	Temperature (35–60°C), pH (4.0–5.5), 10 min agitation. Enzyme: starch ratio (0.2:1.4)	Crater formation on the surface and extensive internal erosion. Larger specific surface area and improved adsorption capacity.	Significant improvement in oxidative stability of encapsulated olive oil.	[22]
Corn	α-amylase, glucoamylase	Starch suspension in acetate buffer 50mM (pH 4.02) (25% w/v), 30°C, hydrolysis time 16–20 h.	Superficial alterations and degradations (exocorrosion). The internal part is hydrolyzed by enzymes that penetrate small pores (endo-corrosion).	Mixed with Arabic gum, improved vitamin C stability protected during accelerated aging conditions and *in vitro* digestion.	[23]
Corn	Pancreatic α-amylase,	Starch suspension in phosphate buffer (5% w/v). Agitation 250 x g, 30 and	Increase in size and number of porous and interconnected structures.	Survival of probiotic *Lactobacillus plantarum* 299v microencapsulated	[24]

Starch source	Enzymes	Experimental conditions	Characteristics of porous starch	Application	Ref
	fungal α-amylase	120 min, 37°C.	Superficial pores and similar size among starches treated with amylases.	after treatments with acid, bile, and heat.	
Potato, corn, wheat, sweet potato	Glucosyl-transferase, branching enzyme	Glucosyltransferase: (35–55°C; pH: 3.0–7.0; 1–15 h). Branching enzyme: (40–70°C, pH 3.0–7.0, 2–16 h)	Increase in number of micropores (> 50 nm), volume, superficial area, and crystallinity. Adsorption was 2 times higher compared to the α-amylase and glucoamylase mix.	Significant increase in oil adsorption capacity, dyes (methylene blue, neutral red), and heavy metal ions (Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}).	[25]
Rice	α-amylase, glucoamylase	Starch in citrate buffer (25%) Ultrasound at 450 W, 25 min. α-amylase and glucoamylase, 14 h, 40ºC.	Formation of more uniform pores, some collapsed or merged.	Curcumin- encapsulated in combination with xanthan gum; showed controlled release *in vitro*.	[26]
Wheat	α-amylase	20% (w/w) starch in distilled water, pH 6.0. Ultrasound at 35 kHz; 240 W; for 20, 40, 60 min. Electric oven at 80°C for 12 h.	Combined treatments increased the size and number of pores. Sonication time and enzyme concentration were proportional to pore formation.	Excellent natural adsorbent with good water, oil, and methylene violet adsorption.	[27]
Canna edulis	thermostable α-amylase	Starch in phosphate buffer (0.1 M, pH 6.5) (25% w/v), α-amylase (100 U/g) at 60°C, 80 rpm, and 8 h.	Visible pores on the granule surface are proportional to enzyme concentration. A few big holes formed.	Non-conventional improvement in solubility and water and oil adsorption capacity.	[28]
Wheat	α-amylase, glucoamylase	15% starch (w/v) in 0.02 M sodium acetate buffer (pH 6.9), α-amylase (100 U/g), agitation at 45°C, 10 h.	The formation of big pores on the surface can result in granule breakage. Some small pores formed on the surface of the starch particles.	Innovative strategy to obtain a super absorbent material due to modification of internal and external structures.	[29]
	Sequential branching enzyme glucoamylase	The reaction stopped, pH was adjusted to 6.5 for sequential branching enzyme (100 U/g), and agitation was at 45°C for 10h	Many shallow pores on the starch particle surface.	Substantial increase in adsorption capacity of heavy metal ions (Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}) and water, oil, methylene blue, and neutral red.	
	α-amylase, sequential branching enzyme, glucoamylase	The reaction stopped, pH was adjusted to 5.5 for glucoamylase (1500 U/g), and agitation was at 48°C for 12 h.	Larger number of bigger, deeper holes on the surface.		

Papakhin *et al.* [20] concluded that corn starch-modified with α-amylase and amyloglucosidase shows physical, chemical, and rheological changes since water adsorption capacity is increased 1.6 fold and solubility 4 fold. Still, viscosity decreases 1.7-fold as compared to native starch. Based on the report by Dura *et al.* [12], this happens because the enzymes mainly hydrolyze the amorphous part, promoting a disintegration of the granules into hydrolyzed glucose and maltose fractions. Benavent-Gil and Rosell [21] reported that the use of amyloglucosidase in the hydrolysis of corn starch produces bigger pores (longer diameter). At the same time, cyclodextrin glycosyltransferase results in pores with a significantly smaller diameter. So, the number and the size of the pores formed can be modulated.

It is also possible to mix enzymes to improve enzymatic hydrolysis: cyclodextrin glycosyltransferase and the branching enzyme increase the superficial area of wheat, corn, potato, and sweet potato starches by 1216%, 1247%, 93%, and 410%, respectively, as compared to the native part [25]. Guo *et al.* [29] obtained granules of porous wheat starch through enzymatic combinations. The comparison of the hydrolysis treatments when using a complex of enzymes like α-amylase, sequential branching enzyme, and glucoamylase showed a pore volume of 0.005 cm3/g and a pore size of 36.35 nm. When an enzyme of the complex was eliminated, the volume and size of the pore in hydrolyzed wheat starch decreased. These same authors reported an adsorption efficiency of oil of 1472% in the enzymatic complex and a decrease by ~50% in oil adsorption when an enzyme of the complex in the starch hydrolysis was eliminated. These results confirm that combining enzymes efficiently prepares porous starches with higher adsorption capacities [21]. Studies reported that the pore diameter on the starch surface is positively correlated to the adsorption capacity of chemical compounds [9]. However, the authors also found that the internal channels of the starch granule contribute to increasing the transportation of the compounds by ~55% [30].

2.2. Synergistic methods.

2.2.1. Physical and enzymatic methods.

Physical treatments can cause partial alterations of the structure and surface, which translates into higher accessibility of the enzymes into amylose and amylopectin chains and a uniform pore distribution [24, 31].

Ultrasound has been used as an adjuvant in starch hydrolysis due to its capacity to get eroded granules and weaken their structure. Zhong *et al.* [32] improved kudzu root starch hydrolysis by combining ultrasound, α-amylase, and glucosyltransferase. The effects of the hydrolyzed starch were notoriously compared to those of native starch since crystallinity decreased by 48–38% but caused an increase in solubility from 11% to 85%. This behavior is due to lower amylose content and molecular weight and a higher branching degree [33]. Choi *et al.* [34] reported that a longer hydrolysis time by glucoamylase was proportional to increased size, depth, and micropores in corn starch. This pattern was more notorious in the sonication of starches, where this previous process may cause cracks or weaken the structure of the starch granule, promoting hydrolysis. Wang *et al.* [35] observed that after the ultrasound-glucoamylase treatment, the water adsorption capacity increased. This behavior happened since adsorption mainly started on the surface of potato starch to be absorbed by the larger contact area in the cavities through internal diffusion [36]. Majzoobi *et al*. [37] obtained microporous wheat starch. However, studies reported that excess treatment, such as sonication for 40 and 60 min, and using enzymes, almost destroyed the starch granules. Keeratiburana *et al*. [38] developed a new method, including ultrasound with a previous freezing-thawing treatment of starch granules in water followed by the combination of amyloglucosidase and/or maltogenic α-amylase. The freeze-thaw principle is based on the initial crystallization of water during the freezing process that results in physical stress on the granular matrix, inducing pore and crack formation [36]. Efficiency is promoted by the use of slow freezing rates and multiple cycles [39, 40]. This process has been used in wheat starch, causing a significant decrease in amylose content from 28.9 to 24.5 % due to leaching by the freezing pressure and resulting in slits on the granular surface [41].

Enzymatic hydrolysis assisted by microwaves is another efficient way to prepare porous starch. When starches are submitted to this process, heat is created inside the granules due to the quick alterations in the high-frequency electromagnetic field. This affects the granule surface and leads to structural damage in the shape of cracks, which favors enzymatic hydrolysis [42, 43]. Jiang *et al*. [44] obtained porous rice starch through enzymatic hydrolysis assisted by microwaves as an efficient preparation way. The water adsorption rate increased by 48% as compared to native starch. The pre-treatment with microwaves promoted the loss of crystallinity, leading to a quick hydrolysis by an enzymatic attack [45]. They modified corn starch for 2 min at 300 W, followed by hydrolysis with α-amylase [44].

Besides the emergent technologies aforementioned, the external electric field is another adjuvant treatment for more efficient and homogeneous preparation of porous starch [46]. The technology is based on controlled permeability due to electric fields, generally in the range of 1–1000 V/cm [47], and arbitrary waves with or without heating effects. It has been reported that the enzymatic activity of α-amylase increases up to 41% through an electric field at an intensity of 1 V/cm and a frequency of 60 Hz [48]. Studies by Li *et al*. [49] reported that the hydrolysis rate in the first 40 min increases 4 times when the voltage changes from 2 to 20 V (50 Hz, room temperature), favoring the enzymatic activity in starch hydrolysis. A low-intensity electric field causes a light increase in enzymatic activity due to changes in the configuration, exposing the α-amylase active site. The opposite is observed in magnetic fields of high intensity, which destroy enzymes [50].

2.2.2. Chemical and enzymatic methods.

There are different chemical methods to improve the properties of porous starches since their applications may be restricted. As a result of the chemical modification, the structure of the starch granule is stabilized, and resistance to factors like pH, heat, and leaching improves [27]. The molecular weight of starch can be reduced through oxidation reactions and acid hydrolysis [51, 52]. On the other hand, it can increase through cross-linking, esterification, and etherification reactions [27, 53, 54].

Cross-linking consists of the insertion of intramolecular and intermolecular functional groups in random sites of amylose and amylopectin chains [55, 56].

Gao *et al.* [57] prepared cross-linked porous oxidized starch. The corn starch was hydrolyzed with α-amylase and glucoamylase, and the porous starch obtained was reacted with sodium hypochlorite to promote oxidation, followed by citric acid cross-linking. The starch granules suffered changes on the surface and severe deformations (erosion); the polygon shapes were completely lost, and even the surface of some granules started to collapse [58]. The starches obtained by an enzymatic method followed by a double chemical modification showed a significant increase (~100 fold) in the adsorption capacity of ammonium ions when compared to native starch. The carboxyl groups obtained by oxidation promoted adsorption through ionic exchange, while the intercrossing reaction stabilized the reaction sites, avoiding the release of ammonium ions already encapsulated.

Epichlorohydrin, followed by α-amylase, has been used in potato and taro starches as a cross-linking agent to improve mechanic resistance and form a porous starch. As the amount of epichlorohydrin grows, the structure becomes more complex due to the formation of crossed bonds; this characteristic improves the water adsorption capacity [59]. Sodium trimetaphosphate has been used to cross-link corn starch, followed by hydrolyzation with a mixture of α-amylase and glucoamylase [60]. Porous starch with 6% intercrossing showed an increase in the total pore area from 0.4 to 7 m2/g and a larger number of pores (from 7 to 62%) compared to native starch. The structure of the largest pores is formed due to the extensive enzymatic hydrolysis and the intercrossing that favors hydrolysis [61].

Wang *et al*. [27] obtained cross-linked porous starch from *Cyperus esculentus* through the enzymatic complex α-amylase: amyloglucosidase (1:3) and the insertion of sodium phytate. The results showed 3 times more oil adsorption capacity compared to native starch and better stability as well. The most formed pores resulted in more binding sites and a large specific surface [62]. The insertion of phosphoric acid groups promoted the formation of a more stable three-dimensional network due to intermolecular interaction with porous starch [63].

In another study, corn starch and calcium ions (Ca^{2+}) underwent oxidation and self-assembly [64]. It was observed that the depth of the micropores was proportional to the hydrolysis time with the glucoamylase and α-amylase mix. These modified starches showed longer slits and a highly rugged surface, attributed to the erosion of the surface after oxidation. The adsorption percentage increased from 56% in native starch to 161% in porous starch and 244% in porous starch with self-assembled calcium ions.

Additionally, etherification plays an important role when obtaining cationic starch. Chen *et al*. [54] carried out an enzymatic hydrolysis of corn starch with glucoamylase and α-amylase, followed by an assembly of quaternary ammonium groups through etherification. The results showed that the CH3-N+ groups were successfully inserted in the spine of the modified starch. They presented a water adsorption rate of 304% and increased swelling capacity (4 fold). The porous structure, increased specific surface formed by the enzymatic hydrolysis, and the etherification with quaternary ammonium groups considerably strengthened the adsorption.

3. Use of porous starches as a protector system of bioactive compounds

Porous starch has been used to encapsulate compounds of food interest, such as fatty acids, antioxidants, and even probiotics (Figure 1).

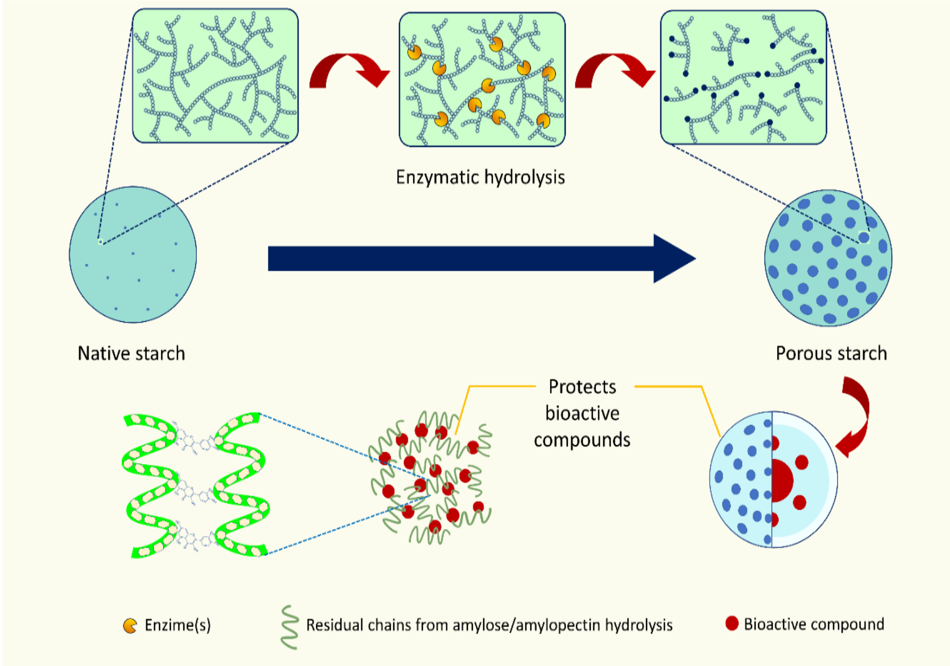

Figure 1. Representation of enzymatic hydrolysis to obtain porous starch and its application as a carrier material for compounds of interest in the food area. Adapted from "Hybrid spherical microparticles for *in vitro* tumor modeling", from BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Fatty acids are protected to reduce oxidation and, therefore, the unpleasant flavor of oxidized products. An alternative to solve this issue is encapsulation with porous starch [22, 65]. The encapsulation efficiency and oxidative stability of encapsulated oil are key parameters to evaluate the encapsulation efficiency [22, 66, 67]. According to Wang *et al.* [67], potato starch enzymatically modified with α-amylase and amyloglucosidase, followed by ultrasound treatments combined with freeze-thaw cycles, showed a better digestion resistance than native starch after encapsulating the soy oil. This is likely because the hydrolysis starch sites were blocked after oil adsorption, resulting in a more complete barrier than native starch. Belingheri *et al*. [66] obtained encapsulated sunflower oil with corn porous starch by spray drying, reducing the peroxide value of light exposure at any temperature. In turn, oxidation reactions were reduced when compared to non-encapsulated oil. In another study, Lei *et al*. [22] encapsulated olive oil with porous starch of purple sweet potato, previously hydrolyzed with α-amylase and glucoamylase. The microencapsulation protected the olive oil from environmental factors, and consequently, oxidation was reduced. The capsules showed low peroxide concentrations as compared to non-encapsulated olive oil. Similar results were presented by Piloni *et al*. [68]; they obtained porous microparticles with corn starch hydrolyzed with α-amylase and glucoamylase. The highest oxidative stability was achieved with a porous starch vacuum impregnated with 10–25% chia oil vs bulk oil. Encapsulation likely delayed the interaction with oxygen, showing that encapsulation with porous starches improves oil oxidation stability [22]. To preserve the antibacterial activity of orange oil, Qiu *et al*. [69] formed microcapsules using corn starch enzymatically hydrolyzed with α-amylase and glucoamylase as base to absorb the oils, while chitosan and sodium alginate were used as covering material. Orange oil was disseminated towards the wall material, and the low degradation of the coating material led to a controlled release [70].

Porous starches can be an innovative option to improve the protection of oils in the food industry.

Natural antioxidants potentially benefit human health. Their efficiency regularly depends on their bioactivity, stability, and bioavailability [71]. Still, they are vulnerable to heat, oxidant agents, and light, so they can lose their biological activity [72]. Therefore, porous starches have been used as protective materials. Ji *et al*. [51] modified corn starch through glucoamylase oxidation and hydrolysis, using trisodium phosphate for cross-linking. The obtained porous microgels exhibited a higher capacity to load anthocyanin, a controlled release, and improved compound stability under environmental conditions like humidity and light. Over 22% of the encapsulated anthocyanins (22.7%) are released from oxidated microgels vs 10.4 % released from porous microgels. In addition, the antioxidants taken orally usually have a low bioaccessibility [73] due to the high sensibility of the gastrointestinal tract [74]. According to Li *et al*. [75], corn starch enzymatically modified with α-amylase and amyloglucosidase, followed by an esterification with octenyl succinic anhydride, showed good bioaccessibility and emulsion capacity of β-carotene. Given this double function, this study opens a new road for the food industry since stable microcapsules are potential emulsion stabilizers in the processed foods industry. Another example of microcapsules using porous starch with double functionality was reported by Chen *et al.* [76]. They obtained microcapsules from porous wheat starch and esterified with caffeic acid to protect linoleic acid. The encapsulates presented excellent adsorption and antioxidant activity of up to 4 days of storage under environmental conditions, such as relative humidity and luminous intensity. The authors suggest that the stability of the encapsulated compound is due to the larger porous starch area. This increases the number of active sites for chemical interaction with caffeic acid, which chemically stabilizes the linoleic acid, thus extending its activity [77].

Probiotics are also of interest since they benefit human health only by passing through the gastrointestinal tract; their high viability is maintained until arrival in the intestine [78]. They play an important role in the maintenance of growth and balance of the gut flora. However, the conditions in the digestive system may negatively affect probiotics by reducing their activity [78]. Li *et al*. [24] encapsulated *Lactobacillus plantarum* in porous corn starch. The modified starches had significantly higher viable initial cells than the native starch. The modified starches had significantly more viable initial cells than the native starch. Unlike free cells, the microencapsulated probiotic bacteria showed a higher tolerance to acid. When used as protective materials, modified starches increased probiotic viability since this supported extreme conditions due to the exposition to acid, biliary salts, and conditions in the gastrointestinal tract. Benavent Gil *et al.* [79] obtained an encapsulation yield of 100% *Lactobacillus plantarum* using corn starch enzymatically hydrolyzed with α-amylase or glucoamylase, indicating better adsorption. The superficial wholes could facilitate bacterial trapping due to the expanded space that might be filled with bacteria [24].

Furthermore, the microencapsulation conferred stability to the cells, which survived even after 35 min at 55°C. The survival of the microorganisms largely depends on the concentration of porous starch [80]. However, even when the same starch concentration is used, the size and number of pores may significantly affect the microorganism's stability. So, the degree of hydrolysis of porous starches may be used to modulate the starch morphology and the effect on encapsulation and protection of microorganisms [79].

4. Conclusions and Future Perspectives

In recent years, the synergy of enzymatic hydrolysis and other methods, including emergent technologies, has been the most effective approach to preparing porous starches and offers a wider variety that will be useful for future applications. Compared to its native counterpart, porous starch shows improved stability, protection, and controlled release of compounds of interest in the food sector. It is a potential vehicle to carry and protect bioactive compounds.

In this sense, it is important to focus more studies on modifying starches, using a combination of techniques such as enzymatic, physical, and chemical, to obtain a synergistic effect to obtain porous starches. The physicochemical characterization of these porous starches contributes to understanding their use that would be given to them in the food and pharmaceutical sectors. So, a wider exploration of the properties of porous starch is needed. This review contributes to a better understanding of the behavior and properties of modified porous starches and their potential applications in different fields, such as the food and pharmaceutical industries.

Funding

This research received no external funding.

Acknowledgments

The authors acknowledge Biorender, a free online program for making scientific images.

Román Leyva-López is grateful to "Consejo Nacional de Humanidades, Ciencias y Tecnologías" (Conahcyt-Mexico) for the scholarship awarded to study the doctor degree in the Instituto de Ciencias Agropecuarias, (ICAp-UAEH). The authors acknowledge Biorender, free online program to make scientific images.

Conflicts of Interest

The authors declare that they have no competing interests.

References

1. Cao, P.;Wu, G.;Yao, Z.;Wang, Z.;Li, E.;Yu, S.;Liu, Q.;Gilbert, R.G.;Li, S. Effects of amylose and amylopectin molecular structures on starch electrospinning. *Carbohydrate Polymers*. **2022**, *296*, 119959, https://doi.org/10.1016/j.carbpol.2022.119959
2. Li, M.;Daygon, V.D.;Solah, V.;Dhital, S. Starch granule size: Does it matter? *Crit. Rev. Food Sci. Nutr.* **2021**, 1-21, 10.1080/10408398.2021.1992607
3. Kim, H.R.;Jeong, G.A.;Bae, J.E.;Hong, J.S.;Choi, H.D.;Lee, C.J. Impact of chemical modification by immersion with malic acid on the physicochemical properties and resistant starch formation in rice. *J. Food Sci.* **2022**, *87*, 1058-1068, 10.1111/1750-3841.16058

4. Pourmohammadi, K.;Abedi, E. Effect of alpha-amylase on the physicochemical and structural characteristics of corn starch before and after high pressure pre-treatment. *Journal of Food Science and Technology (Iran)*. **2022**, *19*, 293-302, 10.22034/FSCT.19.128.293
5. Xiang, G.;Li, J.;Han, W.;Yang, Y.;Lin, Q.;Yang, Y.;Liu, Q.;Guo, X.;Pan, Q., *et al.* The Influence of Temperature Changes on the Rice Starch Structure and Digestive Characteristics: One and Two-Step Annealing. *Foods*. **2022**, *11*, 10.3390/foods11223641
6. Jayakody, L.;Hoover, R. The effect of lintnerization on cereal starch granules. *Food Research International*. **2002**, *35*, 665-680, https://doi.org/10.1016/S0963-9969(01)00204-6
7. Miao, Z.;Zhang, Y.;Lu, P. Novel active starch films incorporating tea polyphenols-loaded porous starch as food packaging materials. *Int. J. Biol. Macromol.* **2021**, *192*, 1123-1133, https://doi.org/10.1016/j.ijbiomac.2021.09.214
8. Hu, D.;Zhang, Z.;Yuan, L.;Li, W.;Guo, Y.;Zhang, R.;Yang, X.;Peng, H. Load phycocyanin to achieve in vivo imaging of casein-porous starch microgels induced by ultra-high-pressure homogenization. *Int. J. Biol. Macromol.* **2021**, *193*, 127-136, https://doi.org/10.1016/j.ijbiomac.2021.10.127
9. Qian, J.;Yang, H.;Mo, C.;Chen, Y.;Zhao, C. Preparation of porous starch from native starch by using fungal amylase and evaluation of its adsorption property on natural pharmacodynamic compounds. *Polymers for Advanced Technologies*. **2022**, *33*, 2244-2253, https://doi.org/10.1002/pat.5675
10. Xu, T.;Li, X.;Ji, S.;Zhong, Y.;Simal-Gandara, J.;Capanoglu, E.;Xiao, J.;Lu, B. Starch modification with phenolics: methods, physicochemical property alteration, and mechanisms of glycaemic control. *Trends in Food Science & Technology*. **2021**, *111*, 12-26, https://doi.org/10.1016/j.tifs.2021.02.023
11. Davoudi, Z.;Azizi, M.H.;Barzegar, M. Porous corn starch obtained from combined cold plasma and enzymatic hydrolysis: Microstructure and physicochemical properties. *Int. J. Biol. Macromol.* **2022**, *223*, 790-797, https://doi.org/10.1016/j.ijbiomac.2022.11.058
12. Dura, A.;Błaszczak, W.;Rosell, C.M. Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers*. **2014**, *101*, 837-845, https://doi.org/10.1016/j.carbpol.2013.10.013
13. Veelaert, S. Powdered acid-loaded carrier material. Google Patents; 2014.
14. Xue, X.;Liang, Q.;Gao, Q.;Luo, Z. One-Step Synthesis of Cross-Linked Esterified Starch and Its Properties. Applied Sciences [Internet]. 2022; 12(8).
15. Jung, Y.S.;Lee, B.H.;Yoo, S.H. Physical structure and absorption properties of tailor-made porous starch granules produced by selected amylolytic enzymes. *PLoS One*. **2017**, *12*, 10.1371/journal.pone.0181372
16. Ahmad, I.;Xiong, Z.;Hanguo, X.;Lyu, F.;Aadil, R.M.;Khalid, N.;Walayat, N.;Taj, M.I.;Zhang, G., *et al.* Microstructural study of enzymatically and non-enzymatically hydrolyzed potato powder. *Journal of Food Processing and Preservation*. **2022**, *46*, e16998, https://doi.org/10.1111/jfpp.16998
17. Xie, Y.;Zhang, B.;Li, M.-N.;Chen, H.-Q. Effects of cross-linking with sodium trimetaphosphate on structural and adsorptive properties of porous wheat starches. *Food Chem.* **2019**, *289*, 187-194, https://doi.org/10.1016/j.foodchem.2019.03.023
18. Mohan, N.;Mellem, J.J. Structural and physicochemical characterization of porous starch prepared by enzymatic hydrolysis, solvent-exchange, and freeze–thaw cross-linking treatments. *Int. J. Food Sci. Technol.* **2022**, *57*, 2356-2364, https://doi.org/10.1111/ijfs.15590
19. Martinez-Alejo, J.M.;Benavent-Gil, Y.;Rosell, C.M.;Carvajal, T.;Martinez, M.M. Quantifying the surface properties of enzymatically-made porous starches by using a surface energy analyzer. *Carbohydrate Polymers*. **2018**, *200*, 543-551, https://doi.org/10.1016/j.carbpol.2018.08.035
20. Papakhin, A.A.;Kolpakova, V.V.;Borodina, Z.M.;Sardzhveladze, A.S.;Vasiliev, I.Y. Modified porous starch in development of biodegradable composite polymer materials. *Food Processing: Techniques and Technology*. **2020**, *50*, 549-558, 10.21603/2074-9414-2020-3-549-558
21. Benavent-Gil, Y.;Rosell, C.M. Comparison of porous starches obtained from different enzyme types and levels. *Carbohydrate Polymers*. **2017**, *157*, 533-540, https://doi.org/10.1016/j.carbpol.2016.10.047
22. Lei, M.;Jiang, F.-C.;Cai, J.;Hu, S.;Zhou, R.;Liu, G.;Wang, Y.-H.;Wang, H.-B.;He, J.-R., *et al.* Facile microencapsulation of olive oil in porous starch granules: Fabrication, characterization, and oxidative stability. *Int. J. Biol. Macromol.* **2018**, *111*, 755-761, https://doi.org/10.1016/j.ijbiomac.2018.01.051
23. Leyva-López, R.;Palma-Rodríguez, H.M.;López-Torres, A.;Capataz-Tafur, J.;Bello-Pérez, L.A.;Vargas-Torres, A. Use of enzymatically modified starch in the microencapsulation of ascorbic acid: Microcapsule characterization, release behavior and in vitro digestion. *Food Hydrocolloids*. **2019**, *96*, 259-266, https://doi.org/10.1016/j.foodhyd.2019.04.056
24. Li, H.;Thuy Ho, V.T.;Turner, M.S.;Dhital, S. Encapsulation of Lactobacillus plantarum in porous maize starch. *LWT*. **2016**, *74*, 542-549, https://doi.org/10.1016/j.lwt.2016.08.019

25. Guo, L.;Li, J.;Gui, Y.;Zhu, Y.;Yu, B.;Tan, C.;Fang, Y.;Cui, B. Porous starches modified with double enzymes: Structure and adsorption properties. *Int. J. Biol. Macromol.* **2020**, *164*, 1758-1765, https://doi.org/10.1016/j.ijbiomac.2020.07.323
26. Wu, L.;Tian, J.;Ye, X.;Fang, H.;Zhang, Z.;Xu, C.;Zhang, H. Encapsulation and Release of Curcumin with the Mixture of Porous Rice Starch and Xanthan Gum. *Starch - Stärke*. **2021**, *73*, 2000042, https://doi.org/10.1002/star.202000042
27. Wang, J.;Zhang, D.;Xiao, J.;Wu, X. Effects of Enzymatic Modification and Cross-Linking with Sodium Phytate on the Structure and Physicochemical Properties of Cyperus esculentus Starch. Foods [Internet]. 2022; 11(17).
28. Purwitasari, L.;Wulanjati, M.P.;Pranoto, Y.;Witasari, L.D. Characterization of porous starch from edible canna (Canna edulis Kerr.) produced by enzymatic hydrolysis using thermostable α-amylase. *Food Chemistry Advances*. **2023**, *2*, 100152, https://doi.org/10.1016/j.focha.2022.100152
29. Guo, L.;Yuan, Y.;Li, J.;Tan, C.;Janaswamy, S.;Lu, L.;Fang, Y.;Cui, B. Comparison of functional properties of porous starches produced with different enzyme combinations. *Int. J. Biol. Macromol.* **2021**, *174*, 110-119, https://doi.org/10.1016/j.ijbiomac.2021.01.165
30. Zhao, J.;Madson, M.A.;Whistler, R.L. Cavities in porous corn starch provide a large storage space. *Cereal Chem.* **1996**, *73*, 379-380,
31. Han, Z.;Zeng, X.A.;Fu, N.;Yu, S.J.;Chen, X.D.;Kennedy, J.F. Effects of pulsed electric field treatments on some properties of tapioca starch. *Carbohydrate Polymers*. **2012**, *89*, 1012-1017, https://doi.org/10.1016/j.carbpol.2012.02.053
32. Zhong, Y.;Xu, J.;Liu, X.;Ding, L.;Svensson, B.;Herburger, K.;Guo, K.;Pang, C.;Blennow, A. Recent advances in enzyme biotechnology on modifying gelatinized and granular starch. *Trends in Food Science & Technology*. **2022**, *123*, 343-354, https://doi.org/10.1016/j.tifs.2022.03.019
33. Sun, Q.;Gong, M.;Li, Y.;Xiong, L. Effect of dry heat treatment on the physicochemical properties and structure of proso millet flour and starch. *Carbohydrate Polymers*. **2014**, *110*, 128-134, https://doi.org/10.1016/j.carbpol.2014.03.090
34. Choi, E.H.;Lee, J.K. Effects of sonication on physicochemical properties and pore formation of maize starch. *Korean Journal of Food Science and Technology*. **2017**, *49*, 507-512, 10.9721/KJFST.2017.49.5.507
35. Wang, D.;Ma, X.;Yan, L.;Chantapakul, T.;Wang, W.;Ding, T.;Ye, X.;Liu, D. Ultrasound assisted enzymatic hydrolysis of starch catalyzed by glucoamylase: Investigation on starch properties and degradation kinetics. *Carbohydrate Polymers*. **2017**, *175*, 47-54, https://doi.org/10.1016/j.carbpol.2017.06.093
36. Charoenrein, S.;Preechathammawong, N. Effect of waxy rice flour and cassava starch on freeze–thaw stability of rice starch gels. *Carbohydrate Polymers*. **2012**, *90*, 1032-1037, https://doi.org/10.1016/j.carbpol.2012.06.038
37. Majzoobi, M.;Hedayati, S.;Farahnaky, A. Functional properties of microporous wheat starch produced by α-amylase and sonication. *Food Bioscience*. **2015**, *11*, 79-84, https://doi.org/10.1016/j.fbio.2015.05.001
38. Keeratiburana, T.;Hansen, A.R.;Soontaranon, S.;Tongta, S.;Blennow, A. Porous rice starch produced by combined ultrasound-assisted ice recrystallization and enzymatic hydrolysis. *Int. J. Biol. Macromol.* **2020**, *145*, 100-107, https://doi.org/10.1016/j.ijbiomac.2019.12.144
39. Tao, H.;Wang, P.;Ali, B.;Wu, F.;Jin, Z.;Xu, X. Structural and functional properties of wheat starch affected by multiple freezing/thawing cycles. *Starch - Stärke*. **2015**, *67*, 683-691, https://doi.org/10.1002/star.201500036
40. Yu, S.;Zhang, Y.;Li, H.;Wang, Y.;Gong, C.;Liu, X.;Zheng, X.;Kopparapu, N.K. Effect of freeze-thawing treatment on the microstructure and thermal properties of non-waxy corn starch granule. *Starch - Stärke*. **2015**, *67*, 989-1001, https://doi.org/10.1002/star.201500109
41. Zhao, A.-Q.;Yu, L.;Yang, M.;Wang, C.-J.;Wang, M.-M.;Bai, X. Effects of the combination of freeze-thawing and enzymatic hydrolysis on the microstructure and physicochemical properties of porous corn starch. *Food Hydrocolloids*. **2018**, *83*, 465-472, https://doi.org/10.1016/j.foodhyd.2018.04.041
42. Chen, X.;Ma, M.;Liu, X.;Xu, Z.;Zhang, C.;Sui, Z.;Corke, H. Microwave treatment alters the fine molecular structure of waxy hull-less barley starch. *Int. J. Biol. Macromol.* **2021**, *193*, 1086-1092, https://doi.org/10.1016/j.ijbiomac.2021.11.007
43. Yang, Q.;Qi, L.;Luo, Z.;Kong, X.;Xiao, Z.;Wang, P.;Peng, X. Effect of microwave irradiation on internal molecular structure and physical properties of waxy maize starch. *Food Hydrocolloids*. **2017**, *69*, 473-482, https://doi.org/10.1016/j.foodhyd.2017.03.011
44. Jiang, K.;Wang, W.;Ma, Q.;Wang, J.;Sun, J. Microwave-assisted enzymatic hydrolysis as a novel efficient way to prepare porous starch. *Carbohydrate Polymers*. **2023**, *301*, 120306, https://doi.org/10.1016/j.carbpol.2022.120306

45. Zhu, J.;Li, L.;Zhang, S.;Li, X.;Zhang, B. Multi-scale structural changes of starch-based material during microwave and conventional heating. *Int. J. Biol. Macromol.* **2016**, *92*, 270-277, https://doi.org/10.1016/j.ijbiomac.2016.07.030
46. Zhu, F. Modifications of starch by electric field based techniques. *Trends in Food Science & Technology*. **2018**, *75*, 158-169, https://doi.org/10.1016/j.tifs.2018.03.011
47. Pereira, R.N.;Vicente, A.A.;Teixeira, J.A. Chapter 17: Food Structure Development/Production Through Flexible Processes: The Use of Electric Fields to Enable Food Manufacturing. Food Chemistry, Function and Analysis. 2020-January2020. p. 422-438.
48. Samaranayake, C.P.;Sastry, S.K. In-situ activity of α-amylase in the presence of controlled-frequency moderate electric fields. *LWT*. **2018**, *90*, 448-454, https://doi.org/10.1016/j.lwt.2017.12.053
49. Li, D.;Tao, Y.;Shi, Y.;Wu, Z.;Xu, E.;Cui, B.;Han, Y. Preparation of porous starch by α-amylase-catalyzed hydrolysis under a moderate electric field. *LWT*. **2021**, *137*, 110449, https://doi.org/10.1016/j.lwt.2020.110449
50. Tian, M.L.;Fang, T.;Du, M.Y.;Zhang, F.S. Effects of Pulsed Electric Field (PEF) Treatment on Enhancing Activity and Conformation of α-Amylase. *Protein Journal*. **2016**, *35*, 154-162, 10.1007/s10930-016-9649-y
51. Ji, Y. Synthesis of porous starch microgels for the encapsulation, delivery and stabilization of anthocyanins. *Journal of Food Engineering*. **2021**, *302*, 110552, https://doi.org/10.1016/j.jfoodeng.2021.110552
52. Zhang, H.;Hou, H.;Liu, P.;Wang, W.;Dong, H. Effects of acid hydrolysis on the physicochemical properties of pea starch and its film forming capacity. *Food Hydrocolloids*. **2019**, *87*, 173-179, https://doi.org/10.1016/j.foodhyd.2018.08.009
53. Chang, P.R.;Qian, D.;Anderson, D.P.;Ma, X. Preparation and properties of the succinic ester of porous starch. *Carbohydrate Polymers*. **2012**, *88*, 604-608, https://doi.org/10.1016/j.carbpol.2012.01.001
54. Chen, L.;Chen, X.L.;Zhou, C.H.;Yang, H.M.;Ji, S.F.;Tong, D.S.;Zhong, Z.K.;Yu, W.H.;Chu, M.Q. Environmental-friendly montmorillonite-biochar composites: Facile production and tunable adsorption-release of ammonium and phosphate. *Journal of Cleaner Production*. **2017**, *156*, 648-659, https://doi.org/10.1016/j.jclepro.2017.04.050
55. Han, X.;Wen, H.;Luo, Y.;Yang, J.;Xiao, W.;Xie, J. Effects of chitosan modification, cross-linking, and oxidation on the structure, thermal stability, and adsorption properties of porous maize starch. *Food Hydrocolloids*. **2022**, *124*, 107288, https://doi.org/10.1016/j.foodhyd.2021.107288
56. Zhou, D.;Li, D.;Liu, M.;Zhong, X.;Wei, H.;Wang, Z.;Jiang, Q.;Jin , F.;Cui, D. Experimental Parameters Affecting Cross-Linking Density and Free-Thaw Stability of Cross-Linked Porous Starch. *ES Food & Agroforestry*. **2021**, *5*, 20-28, 10.30919/esfaf493
57. Gao, J.;Zhang, L.;Gao, Q.;Wu, L.;Zhang, E. A study on physicochemical properties and adsorption performance of modified porous maize starch. *Materials Research Express*. **2018**, *5*, 125303, 10.1088/2053-1591/aae0b4
58. Spier, F.;Zavareze, E.d.R.;Marques e Silva, R.;Elias, M.C.;Dias, A.R.G. Effect of alkali and oxidative treatments on the physicochemical, pasting, thermal and morphological properties of corn starch. *J. Sci. Food Agric.* **2013**, *93*, 2331-2337, https://doi.org/10.1002/jsfa.6049
59. Zhu, J.;Sun, W.;Meng, Z.;Zhu, X.;Gan, H.;Gu, R.;Wu, Z.;Dou, G. Preparation and characterization of a new type of porous starch microspheres (PSM) and effect of physicochemical properties on water uptake rate. *Int. J. Biol. Macromol.* **2018**, *116*, 707-714, https://doi.org/10.1016/j.ijbiomac.2018.05.059
60. Gao, F.;Li, D.;Bi, C.-h.;Mao, Z.-h.;Adhikari, B. Preparation and characterization of starch cross-linked with sodium trimetaphosphate and hydrolyzed by enzymes. *Carbohydrate Polymers*. **2014**, *103*, 310-318, https://doi.org/10.1016/j.carbpol.2013.12.028
61. Chen, G.;Zhang, B. Hydrolysis of granular corn starch with controlled pore size. *Journal of Cereal Science*. **2012**, *56*, 316-320, https://doi.org/10.1016/j.jcs.2012.01.011
62. Kaur, L.;Singh, J.;Singh, N. Effect of cross-linking on some properties of potato (Solanum tuberosum L.) starches. *J. Sci. Food Agric.* **2006**, *86*, 1945-1954, https://doi.org/10.1002/jsfa.2568
63. Chen, B.;Dang, L.;Zhang, X.;Fang, W.;Hou, M.;Liu, T.;Wang, Z. Physicochemical properties and micro-structural characteristics in starch from kudzu root as affected by cross-linking. *Food Chem.* **2017**, *219*, 93-101, https://doi.org/10.1016/j.foodchem.2016.09.128
64. Chen, F.;Cao, X.;Chen, X.;Wei, J.;Liu, C. Calcium-modified microporous starch with potent hemostatic efficiency and excellent degradability for hemorrhage control. *Journal of Materials Chemistry B*. **2015**, *3*, 4017-4026, 10.1039/C5TB00250H
65. Yildiz, G.;Ding, J.;Gaur, S.;Andrade, J.;Engeseth, N.E.;Feng, H. Microencapsulation of docosahexaenoic acid (DHA) with four wall materials including pea protein-modified starch complex. *Int. J. Biol. Macromol.* **2018**, *114*, 935-941, https://doi.org/10.1016/j.ijbiomac.2018.03.175

66. Belingheri, C.;Ferrillo, A.;Vittadini, E. Porous starch for flavor delivery in a tomato-based food application. *LWT - Food Science and Technology*. **2015**, *60*, 593-597, https://doi.org/10.1016/j.lwt.2014.09.047
67. Wang, S.;Hu, X.;Wang, Z.;Bao, Q.;Zhou, B.;Li, T.;Li, S. Preparation and characterization of highly lipophilic modified potato starch by ultrasound and freeze-thaw treatments. *Ultrason. Sonochem.* **2020**, *64*, 105054, https://doi.org/10.1016/j.ultsonch.2020.105054
68. Piloni, R.V.;Bordón, M.G.;Barrera, G.N.;Martínez, M.L.;Ribotta, P.D. Porous Microparticles of Corn Starch as Bio-Carriers for Chia Oil. Foods [Internet]. 2022; 11(24).
69. Qiu, L.;Ma, H.;Luo, Q.;Bai, C.;Xiong, G.;Jin, S.;Wang, J.;Zu, X.;Li, H., *et al.* Preparation, Characterization, and Application of Modified Starch/Chitosan/Sweet Orange Oil Microcapsules. Foods [Internet]. 2022; 11(15).
70. Ozogul, Y.;Kuley Boğa, E.;Akyol, I.;Durmus, M.;Ucar, Y.;Regenstein, J.M.;Köşker, A.R. Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. *Food Bioscience*. **2020**, *36*, 100635, https://doi.org/10.1016/j.fbio.2020.100635
71. Fang, Z.;Bhandari, B. Encapsulation of polyphenols – a review. *Trends in Food Science & Technology*. **2010**, *21*, 510-523, https://doi.org/10.1016/j.tifs.2010.08.003
72. Mehran, M.;Masoum, S.;Memarzadeh, M. Improvement of thermal stability and antioxidant activity of anthocyanins of Echium amoenum petal using maltodextrin/modified starch combination as wall material. *Int. J. Biol. Macromol.* **2020**, *148*, 768-776, https://doi.org/10.1016/j.ijbiomac.2020.01.197
73. Flores, F.P.;Kong, F. In Vitro Release Kinetics of Microencapsulated Materials and the Effect of the Food Matrix. *Annu. Rev. Food Sci. Technol.* **2017**, *8*, 237-259, 10.1146/annurev-food-030216-025720
74. Annunziata, G.;Arnone, A.;Ciampaglia, R.;Tenore, G.C.;Novellino, E. Fermentation of Foods and Beverages as a Tool for Increasing Availability of Bioactive Compounds. Focus on Short-Chain Fatty Acids. Foods [Internet]. 2020; 9(8).
75. Li, H.;Ma, Y.;Yu, L.;Xue, H.;Wang, Y.;Chen, J.;Zhang, S. Construction of octenyl succinic anhydride modified porous starch for improving bioaccessibility of β-carotene in emulsions. *RSC Advances*. **2020**, *10*, 8480-8489, 10.1039/C9RA10079B
76. Chen, Z.;Ma, Y.;Gou, L.;Zhang, S.;Wang, Z. Construction of caffeic acid modified porous starch as the dual-functional microcapsule for encapsulation and antioxidant property. *Int. J. Biol. Macromol.* **2023**, *228*, 358-365, https://doi.org/10.1016/j.ijbiomac.2022.12.189
77. Liu, J.;Wang, X.;Bai, R.;Zhang, N.;Kan, J.;Jin, C. Synthesis, characterization, and antioxidant activity of caffeic-acid-grafted corn starch. *Starch - Stärke*. **2018**, *70*, 1700141, https://doi.org/10.1002/star.201700141
78. Ashwar, B.A.;Gani, A.;Gani, A.;Shah, A.;Masoodi, F.A. Production of RS4 from rice starch and its utilization as an encapsulating agent for targeted delivery of probiotics. *Food Chem.* **2018**, *239*, 287-294, https://doi.org/10.1016/j.foodchem.2017.06.110
79. Benavent-Gil, Y.;Rodrigo, D.;Rosell, C.M. Thermal stabilization of probiotics by adsorption onto porous starches. *Carbohydrate Polymers*. **2018**, *197*, 558-564, https://doi.org/10.1016/j.carbpol.2018.06.044
80. Xing, Y.;Xu, Q.;Ma, Y.;Che, Z.;Cai, Y.;Jiang, L. Effect of porous starch concentrations on the microbiological characteristics of microencapsulated Lactobacillus acidophilus. *Food Funct.* **2014**, *5*, 972-983, 10.1039/C3FO60438A