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MICROENCAPSULATION OF GLUCOSYL-HESPERIDIN IN ALGINATE/CHITOSAN HYDROGEL BEADS

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Summary

Glucosyl-hesperidin is a water soluble derivate of hesperidin. Both these derivates have many health-promoting properties such as antioxidant, anti-inflammatory and antimicrobial activities. However, the low water solubility of hesperidin disables its wide utilization in the food and pharmaceutical industries so glucosyl-hesperidin has an advantage concerning new product development. The aim of the study was to produce hydrogel beads filled with glucosyl-hesperidin by applying microencapsulation technique using vibration technology. Beads were fabricated under the same operating conditions of the encapsulator and obtained by dropping a mixture of glucosyl-hesperidin and alginate into different hardening solutions (calcium chloride or calcium chloride-chitosan) with different times of complexation (30 min or 90 min). The highest retention ability of glucosyl-hesperidin had chitosan-alginate beads, which were complexed for 30 min (590.93 mg/kg), while the lowest retention ability was observed for alginate beads with a complexation time of 30 min (409.94 mg/kg). Beads were stored for 7 days at ambient temperature and in the presence of light. The highest amount of glucosyl-hesperidin was detected in chitosan-alginate beads as after preparation. Results of this study give insight into glucosyl-hesperidin encapsulation into hydrogel beads and its behavior during storage.

Keywords: microencapsulation, glucosyl-hesperidin, beads, alginate, chitosan

Introduction

Hesperidin is a flavanone found in citrus fruits and is composed of aglycone (hesperetin) and a disaccharide unit (rutinose). Different health-promoting properties of this polyphenol were demonstrated such as anti-inflammatory (Corciovă et al., 2021), antioxidant and antibacterial activity (Balakrishnan et al., 2021), analgesic, antitoxic and metal-chelating properties, neuroprotective and cardioprotective effects, prevention of glucose homeostasis, bone resorption (Sa'Ayinzat et al., 2021) and kidney diseases (Li and Schluesener, 2015). It also controls blood pressure (Chen et al., 2018) and has a lipid-lowering effect (Xiong et al., 2019). However, its low solubility limits its wide utilization in the food or pharmaceutical industries. Hayashibara Co., Ltd. proposed a solution to this problem by improving the solubility of hesperidin using a commercial enzymatic process that involved attaching glucose to its structure. The molecule obtained was called glucosyl-hesperidin and had 100 000 times better solubility than hesperidin (197 g/100 mL). Safety studies were conducted and it was concluded that glucosyl-hesperidin is safe to use as a food ingredient (Matsumoto, 2019). Since polyphenols are very unstable, sensitive to heat, light and oxygen, it is necessary to protect them using various encapsulation techniques (Popović et al., 2019). Also, bioactive compounds are often microencapsulated and then added to various foods as functional ingredients which subsequently enrich the final product (de Moura, 2018). Microencapsulation is a process, in which the final products are particles ranging from 1 to 1000 μm. Produced microcapsules can have different structural forms and various natural and synthetic materials are used as wall material(s) (Whelehan and Marison, 2011). This process can improve the physical properties of bioactive compounds, ensure their targeted release and also prolong their shelf-life by preventing degradation reactions (Macías-Cortés et al., 2020). The microencapsulation techniques are categorized as chemical (polymerization reactions), mechanical (spray-drying, extrusion methods) and physicochemical processes (complex coacervation). Vibrating-jet (nozzle) method is a mechanical procedure based on generating droplets from a polymer that is extruded through a nozzle and broken after it passes through it at a certain flow rate. Prepared alginate particles are gelified into beads upon landing in a bath of calcium chloride. They can be used immediately, stored, or further processed (e.g. adding a new membrane). Size and other characteristics of the beads depend on the nozzle diameter, the flow rate of the laminar jet, the frequency at defined amplitude and the viscosity of the extruded liquid. This procedure allows the production of beads which will protect the encapsulant from external factors, enable its

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controlled release or improve its organoleptic properties (Whelehan and Marison, 2011). Different biopolymers such as proteins and polysaccharides are used as wall material(s) in microencapsulation processes (Capablanca et al., 2017). There is a growing interest in natural polymers such as alginate and chitosan since they are known for their low toxicity, safe usage, biodegradability and biocompatibility. Lower animals and humans can consume edible polymers without any harmful effects and thus the Food and Drug Administration has regarded edible polymers as safe (GRAS) (Ćorković et al., 2021). Encapsulation of different polyphenols in hydrogel beads has been studied in other studies as well (Stoica et al., 2013; Kim et al., 2016; Bušić et al., 2018; Maleki et al., 2020), however as far as we know glucosyl-hespeirdin was not studied. Considering all these aspects, the microencapsulation technique was applied for the production of alginate beads filled with glucosyl-hesperidin. In addition to alginate, the effect of chitosan and different times of complexation on the encapsulation of glucosyl-hesperidin in beads were investigated after preparation, as well as after 7 days of storage.

Materials and methods

Materials

The alginic acid sodium salt, with very low viscosity, was procured from Alfa Aesar (Kandel, Germany). Chitosan was obtained from Sigma-Aldrich (St. Louis, MO, USA) and calcium chloride and ascorbic acid from Gram-mol (Zagreb, Croatia). Glucosyl-hesperidin was the product of Hayashibara Co., Ltd. (Okayama, Japan). Methanol (HPLC grade) was purchased from J.T. Baker (Deventer, Netherlands) and orthophosphoric acid (HPLC grade, > 85%) from Fisher Scientific (Loughborough, UK). Hydrochloric acid (37%) and methanol were purchased from Carlo Erba Reagents (Sabadell, Spain).

Preparation of hydrogel beads

Beads were prepared using Encapsulator B-390 (BÜCHI Labortechnik AG, Flawil, Switzerland) with a 1000 µm vibrating nozzle. The device was operated under fixed conditions: pressure 500 mbar, frequency 200 Hz, electrode 1000 V and ambient temperature. As an encapsulation mixture glucosyl-hesperidin solution (1500 mg/L) with 3.75% alginate was used. Two types of hardening solutions were used, 10% $CaCl_2$ and 10% $CaCl_2$ with the addition of 1.25% chitosan and 2.5% ascorbic acid. The influence of different complexation times (30 and 90 min) was also investigated. One set of the beads was analyzed immediately after preparation and the other was stored for 7 days at ambient temperature and in the presence of light and then analyzed.

Extraction of glucosyl-hesperidin from hydrogel beads

Extraction of the beads was performed according to Kopjar et al. (2021). For the extraction of glucosyl-hesperidin, 1 g of hydrogel beads was weighed and 10 mL of acidified methanol (methanol:hydrochloric acid ratio was 99:1) was added. The mixture was left for 24 h and then filtered. Extracts were further analyzed.

Determination of glucosyl-hesperidin concentration in extracts using high-performance liquid chromatography (HPLC)

The concentration of glucosyl-hesperidin in extracts was determined using Agilent HPLC 1260 Infinity II system (Agilent Technology, Santa Clara, CA, USA) equipped with Poroshell 120 EC C-18 column (4.6 x 100 mm, 2.7 µm), quaternary pump, DAD detector and a vial sampler. As solvent A, 0.1% phosphoric acid solution was used and methanol as solvent B. Elution conditions were: 0 - 38 min from 3% to 65% B and 38 - 45 min 65% B. Injection volume was 5 µL. Prior to injection into the system, extracts were filtered using PTFE filters with a pore size of 0.2 µm. All chromatograms were recorded with DAD in the 190-600 nm range and visualization and peak integration were done at 280 nm. The calibration curve of the glucosyl-hesperidin standard was constructed in concentrations ranging from 100 to 800 mg/L and the linearity was confirmed by $r^2 = 0.9992$. Each extract was injected twice and concentrations were expressed as mg of glucosyl-hesperidin per kg of beads (mg/kg).

Statistical analysis

All results were expressed as the mean values ± standard deviation. Statistical analysis was performed using software STATISTICA 13.1 (StatSoft Inc., Tulsa, OK, USA). Analysis of the variance (ANOVA) and Fisher's least significant difference (LSD) with the significance defined at $p < 0.05$ were used for the data analysis.

Results and discussion

The aim of this study was preparation of hydrogel beads that could be efficient delivery systems of glucosyl-hesperidin. Two sets of hydrogel beads were prepared. The first one based on alginate ALG-30 and

ALG-90 (30 and 90 minutes of complexation, respectively). The second one on alginate and chitosan ALG/CHIT-30 and ALG/CHIT-90 (30 and 90 minutes of complexation, respectively). In Table 1, the concentration of glucosyl-hesperidin in hydrogel beads after preparation as well as after 7 days of storage are presented. In the beads analyzed after preparation, the concentration of glucosyl-hesperidin ranged from 409.94 mg/kg to 590.93 mg/kg with a statistical difference between all samples. Encapsulation of different polyphenols within alginate beads has been the subject of research in numerous studies. The addition of fillers (cocoa and carob powder) improved the encapsulation of dandelion polyphenols since a very porous alginate structure limits its performance as an encapsulation system for small molecules (Bušić et al., 2018). On the other side, molecules such as proanthocyanidins were encapsulated with higher encapsulation efficiencies because their diffusion is limited by their high molecular weights (Kim et al., 2016). Interactions between polyphenols and alginate occur due to hydrogen bonds, which are formed in a presence of hydroxyl groups of phenols and the carboxyl and hydroxyl groups of alginate (Plazinski and Plazinska, 2011).

Table 1. Concentrations of glucosyl-hesperidin in produced hydrogel beads determined using HPLC method

Sample	Concentration after preparation (mg/kg)	Concentration after 7 days of storage (mg/kg)
ALG-30	409.94 ± 2.37^{a}	318.59 ± 6.32^{a}
ALG-90	512.20 ± 5.03^{c}	376.10 ± 0.73^{c}
ALG/CHIT-30	590.93 ± 2.86^{d}	456.23 ± 0.47^{d}
ALG/CHIT-90	425.61 ± 2.73^{b}	357.45 ± 0.13^{b}

ALG and ALG/CHIT: alginate or alginate/chitosan as wall material(s). 30 and 90: different times (minutes) of complexation. Within the column, means followed by superscript different letters are significantly different at $p \leq 0.05$ (ANOVA, Fisher's LSD).

For the beads with only alginate as wall material, it was observed that prolonged complexation had a positive effect on glucosyl-hesperidin concentration since it increased from 409.94 mg/kg to 512.20 mg/kg when the time of complexation was extended from 30 to 90 minutes. The opposite effect was observed for beads that had wall material composed of both alginate and chitosan, where longer complexation time caused a decrease of glucosyl-hesperidin concentration (from 590.93 mg/kg to 425.61 mg/kg). In this study, exposure time affected the concentration of encapsulated glucosyl-hesperidin and the same was observed in the Deladino et al. (2008) study. They concluded that for chitosan-coated beads, a shorter time of complexation was more favorable as opposed to alginate beads.

The addition of chitosan as a wall material caused a higher effect on glucosyl-hesperidin concentration than the extension of complexation time from 30 to 90 minutes (590.93 mg/kg for ALG/CHIT-30 and 512.20 mg/kg for ALG-90). The addition of other biopolymers such as chitosan, pectin, cellulose derivates improved the encapsulation of bioactives in different investigations as well (Bušić et al., 2018). Results in the present study were in accordance with Kim et al. (2016) who concluded that the addition of chitosan enhances encapsulation of polyphenols. The same was observed by Stoica et al. (2013). They observed that with the addition of chitosan, a denser membrane was formed. The reasons for such a phenomenon were hydrogen bonding and van der Waals forces between functional groups of chitosan and polyphenols (Spagna et al., 1996).

Encapsulation efficiencies (%) calculated from the concentration of the glucosyl-hesperidin in the encapsulation mixture and its concentration in beads are depicted in Fig. 1. It was observed that prolonged complexation (90 min) has a better effect on encapsulation efficiency of alginate beads than on alginate/chitosan beads (34.15% for ALG-90 and 28.37% for ALG/CHIT-90). The addition of chitosan caused an increase in encapsulation efficiency only when complexation time was 30 min (from 27.33% to 39.40% for ALG-30 and ALG/CHIT-30, respectively). Lavelli and Harsha (2018) concluded that encapsulation efficiency was affected by the loss of polyphenols from the droplet which occurs prior to gelling in the hardening solution. The diffusion rate of polyphenols through liquid-liquid diffusion is higher than solid-liquid diffusion which occurs after gelling. To obtain higher values of encapsulation efficiency, nozzles with smaller orifices than used in the present study could be a possible solution. It was reported that the encapsulation efficiency of bioactives was improved when smaller particles are produced (de Moura et al., 2018). Also, higher concentrations of alginate solution create a network with a ticker membrane that inhibits the loss of encapsulated material to the environment (Najafi-Soulari et al., 2016).

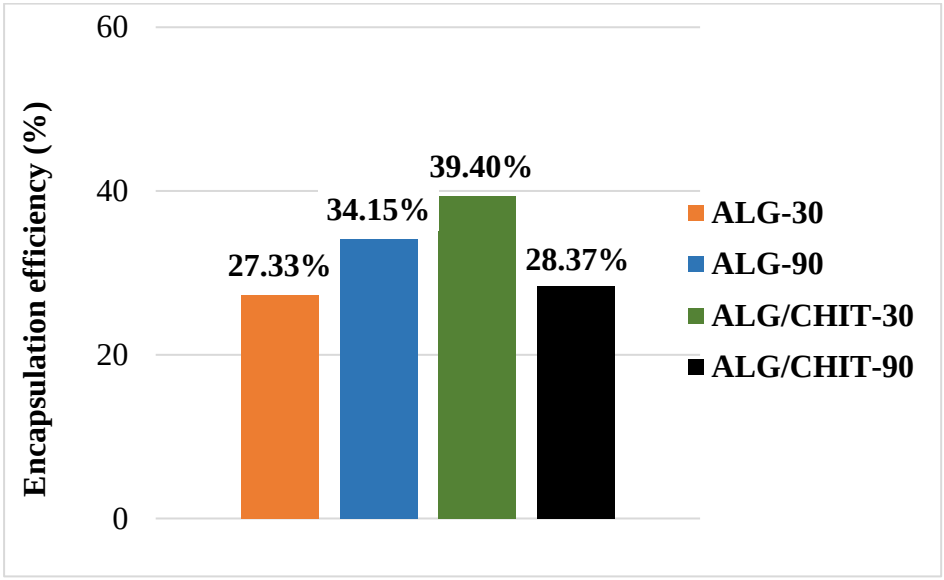

Fig. 1. Encapsulation efficiency of glucosyl-hesperidin in prepared alginate/chitosan beads (ALG and ALG/CHIT: alginate or alginate/chitosan as wall material(s). 30 and 90: different times (minutes) of complexation)

After storage, loss of glucosyl-hesperidin occurred. All of the stored samples had lower concentrations of glucosyl-hesperidin compared to fresh ones, ranging from 318.59 mg/kg to 456.23 mg/kg. The lowest concentration of glucosyl-hesperidin after storage was detected in the alginate beads complexed for 30 minutes, while the highest was determined in beads with chitosan complexed for 30 minutes. Calculating stability of glucosyl-hesperidin (Fig. 2) it was observed that the most stable beads were alginate/chitosan beads complexed for 90 minutes where the retention was 84%. The lowest retention 73.43% was calculated for alginate beads also complexed for 90 minutes, while the other two samples had retention around 77%. A storage study by Maleki et al. (2020) revealed that antioxidant properties, total phenolic compounds, hardness and thickness of alginate beads containing barberry extract decreased during the storage period, but higher alginate concentration showed the best results during the storage period.

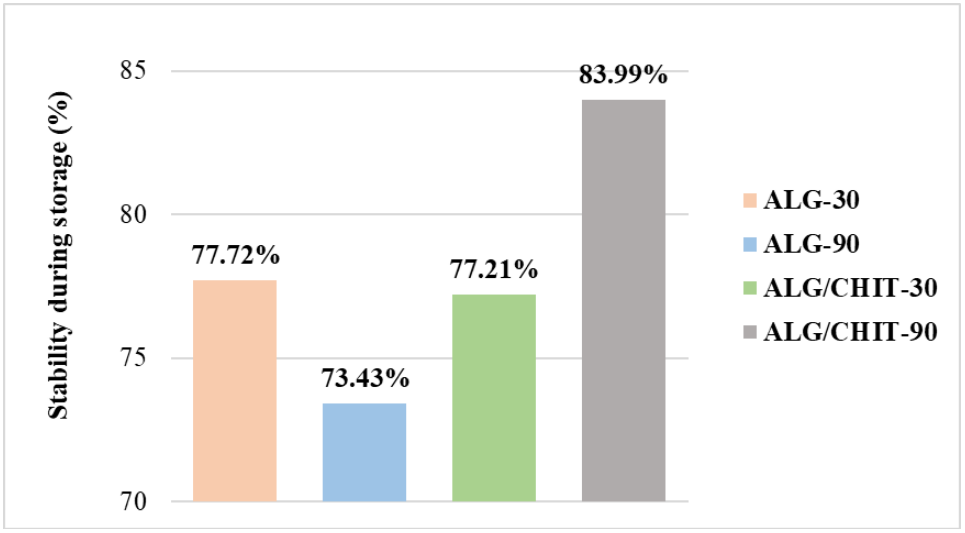

Fig. 2. Stability of glucosyl-hesperidin in alginate/chitosan beads during storage (ALG and ALG/CHIT: alginate or alginate/chitosan as wall material(s). 30 and 90: different times (minutes) of complexation)

Conclusions

The best retention ability of glucosyl-hesperidin had chitosan-alginate beads which were complexed for 30 min (590.93 mg/kg), while the lowest retention ability was observed for alginate beads with a complexation time of 30 min (409.94 mg/kg). From the presented results, it can be concluded that prolonged complexation had a positive effect on the concentration of glucosyl-hesperidin in hydrogel beads just in case when beads were prepared only from alginate. The addition of chitosan caused an increase in glucosyl-hesperidin concentration, but an extension of complexation from 30 to 90 minutes caused its decrease. Different interactions between used wall materials and glucosyl-hesperidin may occur such as hydrogen bonds and affect its encapsulation efficiency. For further investigations, the main challenge will be the application of vibration technology for the production of beads at an industrial scale since until now it was used mainly at a lab scale. Prepared beads could be used for encapsulation of different polyphenols and as antioxidant delivery systems that can be further added as ingredients of functional foods.

Acknowledgments

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