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Research Article

Preparation of Prebiotic Pectin-Supplemented Vitamin C Microcapsules

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ABSTRACT

Microencapsulating vitamin C with dietary fibers and prebiotics can improve the storage, preservation, and marketing of vitamin C supplements. This research aimed to explore the feasibility of creating microcapsules using vitamin C, pectin, and alginate through a microencapsulation technique. Pectin was extracted from lemon peel using an acid treatment and then characterised. The morphology of the vitamin C-pectin-alginate microcapsules was examined by scanning electron microscopy. Time, temperature, and pH-dependent vitamin C release profiles of the vitamin C-pectin-alginate microcapsules were studied. The rate of release of vitamin C increased towards pH values close to 7.0, with a higher rate of 83.97% observed at pH 7.0. Additionally, temperature affected the release of vitamin C from the microcapsules, with approximately 47.2% release at body temperature (37°C) and a higher fluctuation in vitamin C release was observed at 20°C. This study revealed that pectin extracted from lemon peels can be used with alginate to encapsulate vitamin C.

Araştırma Makalesi

Prebiyotik Pektin Takviyeli C Vitamini Mikrokapsüllerinin Hazırlanması

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ÖZ

C vitamininin diyet lifleri ve prebiyotiklerle mikrokapsüllenmesi, C vitamini takviyelerinin depolanmasını, korunmasını ve pazarlanmasını iyileştirebilir. Bu çalışmanın amacı, bir mikrokapsülleme tekniği ile C vitamini, pektin ve aljinat kullanarak mikrokapsüller oluşturmanın fizibilitesini araştırmaktır. Pektin, asit muamelesi kullanılarak limon kabuğundan ekstrakte edildi ve karakterize edildi. C vitamini-pektin-aljinat mikrokapsüllerinin morfolojisi taramalı elektron mikroskobu ile incelenmiştir. C vitamini-pektin-aljinat mikrokapsüllerinin zaman, sıcaklık ve pH bağımlı C vitamini salım profilleri incelenmiştir. C vitamini salım hızı, pH 7.0'a yakın pH değerlerine doğru artarken, pH 7.0'da daha yüksek oranda %83.97 gözlendi. Ek olarak sıcaklık, vücut sıcaklığında (37°C) yaklaşık %47.2 salınım ile mikrokapsüllerden C vitamini salınımını etkiledi ve 20°C'de C vitamini salınımında daha yüksek bir dalgalanma gözlendi. Bu çalışma, limon kabuklarından ekstrakte edilen pektinin, C vitaminini kapsüllemek için aljinat ile birlikte kullanılabileceğini ortaya koydu.

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1. Introduction

Vitamin C is a water-soluble vitamin with antioxidant properties found in fruits and vegetables. It prevents cellular damage and lowers the risk of cancer by neutralizing reactive oxygen species that cause DNA mutations (Sdiri et al., 2020).

Vitamin C acts as a cofactor for collagen synthesis, balances hormones, and aids in cellular metabolism (Skrovankova et al., 2015). It can protect against oxidative damage by binding to metal ions or scavenging reactive oxygen species (Ngai et al., 2013; Yuswan et al., 2015). As a reducing agent, the ascorbic acid molecule is sensitive to oxidation. Its exposure to air, moisture, and heat causes it to oxidise, resulting in the loss of its bioactivity in its oxidised form (Zielinski et al., 2001; Shimoni, 2004; Blažević et al., 2020).

On average, women should consume 75mg and men 90mg of vitamin C daily (Blažević et al., 2020). To preserve the bioactivity of ascorbic acid and prevent oxidation, different encapsulation methods have been developed for its controlled release in pharmaceutical applications (Cho et al., 2003; Champagne and Fustier, 2007). For example, in one study, the stability of chitosan nanoparticles with L-ascorbic acid was examined during heat treatment in aqueous solutions (Jang and Lee, 2008). In another study, spray-dried microspheres with cross-linked chitosan and tripolyphosphate enabled the release of encapsulated ascorbic acid (Desai and Park, 2006).

Pectin (a.k.a. pectic polysaccharides) is a complex and heterogeneous polysaccharide found in plant cell walls, made of galacturonic acid and its methyl ester (Fishman et al., 1999). Pectin is found in dicotyledonous plants and plays a structural role. It's commonly associated with cellulose and hemicellulose and can be found in citrus fruits, apples, and sugar beets (Santos et al., 2020). Oranges and limes have pectins in their albedo (white part on the inside of the peel), mostly made up of anhydrogalacturonate. Homogalacturonans may also contain some xylose units. Rhamnogalacturonan is the second most common component, with side chains of arabinan, galactan, and arabinogalactan (Fishman et al., 2003).

Pectin is a versatile substance extracted from fruit peels. It's used in food and pharmaceuticals as a gelling, thickening, and emulsifying agent. Thousands of tons of citrus fruit peels are processed annually to obtain pectin, which is essential for human health and nutrition. Pectic polysaccharides, found in dietary fibres, can help regulate lipid metabolism (Groudeva et al., 1997) and decrease glucose absorption in those with diabetes (Schwartz et al., 1988).

Pectic polysaccharides can stop pathogens from attaching to the intestinal mucosa. Moreover, they can be fermented by probiotic bacteria into short-chain fatty acids that can prevent colon necrosis (Wang and Friedman, 1998; Jun et al., 2006). Furthermore, certain types of pectin possess immunomodulatory properties that can impact the gastric mucosal immune system by activating Peyer's patch cells, leading to increased proliferation of lymphocytes and macrophages (Yamada and Kiyohara, 2007; Kratchanova et al., 2010). Earlier reports suggest that citrus pectins and their modified derivatives may have a preventative impact on the growth and spread of cancer (Ramachandran et al., 2011). Recent studies have indicated that pectins extracted from citrus fruits can be considered a viable prebiotic, supplying dietary fibre to probiotic bacteria in the gut microbiome (Ho et al., 2017; Islamova et al., 2017; Zhang et al., 2018).

Alginate is a naturally occurring polysaccharide commonly found in marine brown algae (Phaeophyceae). It is a structural component in soil bacteria, serving as a capsular polysaccharide (Robyt, 1998). Alginates are versatile additives used in food (E401), medicine, pharmaceutical, and textile industries for their ability to gel, thicken, stabilize, and retain water. Alginates undergo rapid crosslinking and sol/gel transition with Ca^{2+} ions, creating adjustable gels with temperature-insensitive properties in water-based solutions (Steinbüchel and Rhee, 2005; Donati et al., 2009). Additionally, alginate can retain fluids, making it useful for drug delivery, tissue engineering, gene delivery (Josef et al., 2010), and regenerative therapy (Smidsrød and Skja, 1990). Alginates are bioadhesive and can target mucosal tissues. They can also effectively encapsulate substances using microencapsulation, which involves coating particles with a polymer film (Bitton et al., 2006).

As discussed above, though there have been some formulations for vitamin C supplements in the literature, little attention has been paid to vitamin C-alginate-pectin microcapsules. Encapsulating vitamin C molecules in pectin and alginate may create a protective barrier against oxygen molecules that can cause the loss of biological activity of ascorbic acid. This can improve the storage, preservation, and marketability of vitamin C dietary supplements. Combining the pectin and vitamin C extracted from lemon peel can produce a prebiotic nutritional fibre source essential for the probiotic bacteria in the gut flora. This research explored the feasibility of producing microcapsules containing vitamin C using pectin isolated from lemon peels and alginate, a safe and edible polysaccharide known for its biocompatibility and mucoadhesive properties.

2. Experimental

2.1. Materials and method

2.1.1. Extracting pectin from lemon peels

A method reported in the literature was followed to extract pectin (Azad et al., 2014). Lemon peels (*Molla Mehmet*) were obtained from a local market and used for pectin extraction. To extract the lemon peel's albedo (white inner layer), a knife was used to remove it, followed by drying at room temperature (20°C). The dried samples were then placed in a citric acid solution (5.0 g in 100 mL of distilled water), and an HCl solution was added until the pH reached 1.0. The solution was stirred at 80°C for an hour before cooling to room temperature (20°C) and filtering through paper. The filtrate was cooled to +4°C and allowed to gel with added ethanol. 100 mL of 96% ethanol was added to the filtrate and kept at 4°C for 12 hours, allowing the pectin to precipitate. After the precipitated pectin was isolated by filtration, it was washed with ethanol. The gelled sample was dried in a Petri dish at room temperature (20°C). To reduce moisture content, the dried sample was kept in an oven at 60°C for an hour and then crushed into powder using a mortar.

The structural analysis of the pectin samples was done by FT-IR spectroscopy (Bruker Vertex 70 FT-IR spectrometer, 4000-400 cm^{-1}). The extraction yield, equivalent weight, moisture, ash, and methoxyl content of pectin were determined using a previously published method (Azad et al., 2014).

Pectin yield: Pectin yield was calculated by averaging at least 3 replicates using Equation 1. The pectin samples were stored in glass bottles at 4°C.

$$\% \text{ Pectin Yield} = \left(\frac{\text{mass of pectin}}{\text{mass of albedo}}\right) \text{x } 100 \qquad \text{(Eq. 1)}$$

Moisture and ash content: To determine the moisture content of lemon peels dried at room temperature (20°C), the following steps were taken: 1.0 g of dried fruit peel (W_1) was placed in a weighted Petri dish (W), then put in a 100°C oven for 5 hours. After cooling it in a desiccator, the dish was weighed again (W_2). The per cent moisture content of lemon peels was calculated by Equation 2.

$$\% \text{ Moisture} = \left(\frac{W1 - W2}{W1 - W}\right) \text{x } 100 \qquad \text{(Eq. 2)}$$

W_1: mass of dried fruit peel, g
W_2: mass of Petri dish + sample, g
W: mass of Petri dish, g

To find out the ash content of the lemon peels (free from moisture), the sample was placed in a Petri dish and kept in a furnace at 600°C for 6 hours. After cooling to room temperature (20°C) in a desiccator, the dish's final weight (W3) was recorded. Using Equation 3, the percentage of ash content in the lemon peels was then calculated.

$$\% \text{ Ash} = \left(\frac{W3 - W1}{W2 - W1}\right) \text{x } 100 \qquad \text{(Eq. 3)}$$

W_3: final mass of Petri dish, g
W_1: mass of Petri dish, g
W_2: mass of Petri dish + sample amount, g

Equivalent weight: In a 250 mL flask, 0.5 g of pectin sample was taken and mixed with 5.0 mL of ethanol. Then, 1.0 g of sodium chloride and 100 mL of distilled water were added to the mixture. To prepare phenol red, 0.077 g of it was dissolved in 100 mL of water. Afterwards, 6 drops of the prepared phenol red were added to the solution and titrated against 0.1 M NaOH. At the titration point, a colour transformation close to purple was observed. This neutralised solution was stored for the determination of methoxyl content. The calculation of the equivalent mass was performed using Equation 4.

$$\% \text{ Equivalent weight } = \left(\frac{\text{Sample mass x 1000}}{\text{Alkaline mL x Alkaline molarity}}\right) \qquad \text{(Eq. 4)}$$

Methoxyl content: To determine the methoxyl content, the stored sample from the equivalent mass determination was taken, and 25 mL of sodium hydroxide (0.25 M) was added to the sample taken. The mixed solution was thoroughly mixed and incubated at room temperature (20°C) for 30 minutes. After 30 minutes, 25 mL of 0.25 M hydrochloric acid was added and titrated against 0.1 M NaOH. The methoxyl content was calculated according to Equation 5 below:

$$\% \text{ Methoxyl content } = \left(\frac{\text{Alkaline mL x Alkaline molarity x 31}}{\text{Sample mass}}\right) \qquad \text{(Eq. 5)}$$

2.1.2. Encapsulation of vitamin C

Sodium alginate (5.0 g) was stirred at 1000 rpm for 30 minutes at room temperature (20°C) to dissolve in distilled water (100 mL). After adding vitamin C (2.0 g) and pectin (0.5 g) to the mixture, it was mixed at 3500 rpm for 30 minutes to ensure homogeneity. Mixing was carried out in a closed vessel to reduce contact with air during mixing. Afterwards, the mixture was stirred at 750 rpm for 15 minutes to prevent bubble formation. The mixture was transferred to a burette, and a microcapsule was formed by dripping into a $CaCl_2$ solution (5.0 g in 100 mL of distilled water). The microcapsules in the calcium chloride solution were removed by filtration and washed with distilled water. It was stored in glass Petri dishes at +4°C without exposure to sunlight. The amount of ascorbic acid molecules encapsulated in the microcapsules was calculated spectrophotometrically by measuring their absorbance at 260 nm (using a calibration curve based on absorbance measurements).

To preserve the vitamin C microcapsules, they were dried at room temperature (20°C) in a dark environment and then stored in amber glass bottles at 4°C to avoid exposure to sunlight. The size, shape, and surface properties of the dried microcapsules were analysed using Scanning Electron Microscopy (SEM).

2.1.3. Release characteristics of vitamin C microcapsules

The release of ascorbic acid from vitamin C microcapsules was studied under the conditions listed below:

The release of ascorbic acid from vitamin C microcapsules was investigated under the following conditions.

Parameters to be tested:
Duration: 0.25 – 0.50 – 1 – 2 – 4 – 8 – 16 – 24 hours (pH: 7.35, 37°C)
Temperature: 20 – 25 – 30 – 37°C (pH: 7.35, 1 hour)
pH: 2.0 – 3.0 – 4.0 – 5.0 – 6.0 – 7.0 – 8.0 (1 hour, 37°C)

In a typical experiment, 100 mg of vitamin C microcapsules were placed into 50 mL of distilled water and agitated at 50 rpm at a specified temperature, 2.0 mL of solution was taken at regular intervals. Its absorbance was measured at 260 nm in a UV-vis spectrophotometer, the amount of ascorbic acid in the solution was determined with the help of the calibration curve. Fresh ascorbic acid solutions were prepared and diluted to a certain extent for the calibration plot drawing. Dilute solutions of HCl and NaOH were used to adjust the pH.

3. Results and Discussion

3.1. Extraction of pectin from lemon peels

In the study, the pectin yield obtained from lemon albedo (the white part on the inside of the peel) was found to be 60.88%. The pectin content was higher than a report in the literature, which was around 13% (Azad et al., 2014). In our study, a specific type of lemon (*Molla Mehmet*) was chosen because of its thick skin and plentiful albedo. The fibrous inner portion of the fresh peel (albedo), which contains a high amount of pectin, was removed and used in the extraction. As a result, the extraction procedure followed in the study yielded a notably high pectin extraction yield.

Pectin is a significant ingredient in the food industry, and it is extracted from entire citrus peels for industrial purposes (Pereira et al., 2016; Adetunji et al., 2017). Therefore, in the study, pectin was also extracted from whole lemon peels (dried and pulverised) using the same extraction procedure employed for lemon albedo to investigate what the pectin content of the lemon peel would be. The pectin yield obtained from the whole lemon peel was 27.06%, lower than in the previous study (30-35%) (Da Silva and Rao, 2006).

3.2. Characterisation of pectin from lemon peels

The FT-IR spectrum of pectin obtained from lemon peels was obtained (Fig. 1) and compared with the commercial pectin spectrum (Baum et al., 2017; Şen et al., 2021). The spectrum has O-H stretching band at 3334 cm^{-1} and the C-H stretch of the alkyl groups (CH, CH_2, CH_3) of galacturonic acid at 2932 cm^{-1}. The bands around 1730 cm^{-1} and 1650-1510 cm^{-1} and at 1733-1619 cm^{-1} correspond to the C=O stretching vibration of esterified carbonyl groups and C=O of free carbonyl groups, respectively. As a result, the FT-IR spectrum analysis confirms that the obtained polysaccharides are pectin since the extracted lemon pectin sample spectrum has absorption bands compatible with the commercial pectin spectrum.

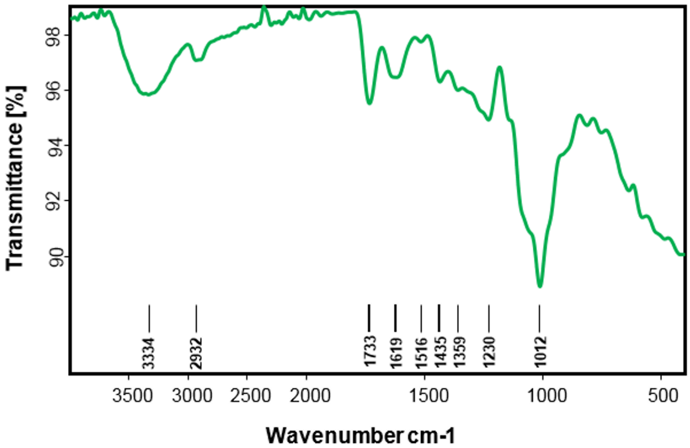

Fig. 1. FT-IR spectrum of pectin from lemon peel.

The moisture and the ash content of the pectin samples (previously dried at room temperature, 20°C) were found to be 10.13% and 0.43%, respectively, which was low and close to the figures (pectin: around 11% and ash: around 2-3%) reported in previous publications on pectin extraction from lemon, dragon fruit and sunflower head residues (Mohamadzadeh et al., 2010; Ismail et al., 2012; Azad et al., 2014). This low moisture content for pectin sources is desirable because excessive moisture levels in lemon peels can increase the proliferation of microorganisms and the secretion of pectinase enzymes, which can negatively impact the quality of the pectin (Maran et al., 2013). The lower ash content for pectin samples indicates their purity.

The equivalent weight value of extracted pectin depends on various factors, including the extraction method, the type of raw material used, the type of acid used for extraction, the extraction temperature, and the duration of extraction. The equivalent weight value of the extracted pectin was 6676.67 g. This high equivalent weight value indicates that the pectin samples were not undergone degradation during the extraction process (Ling et al., 2022). The methoxyl content of the pectin samples was found to be 4.96%, suggesting that the pectin extracted in the study could be classified as low-methoxyl pectin. Low-methoxyl pectins can form gels with low molecular mass sugars or divalent cations (Ismail et al., 2012). The methoxyl content is a crucial molecular index that determines pectin's ability to form a gel. This is because pectin's water solubility depends on the number and distribution of methoxyl groups and the degree of polymerisation (Peng et al., 2020).

3.3. Encapsulation of vitamin C with pectin and alginate

The optimum parameters of microencapsulation were determined as follows: The microcapsule solution; the amount of sodium alginate: 5.0 g, the amount of ascorbic acid: 2.0 g, and the amount of pectin: 0.5 g in 100 mL distilled water and the gelation solution; the amount of CaCl2: 5.0 g in 100 mL distilled water. The encapsulation of vitamin C was performed under optimum parameters. The SEM images of the microcapsules are presented in Fig. 2. In the SEM images, the diameters of the microcapsules were determined to be approximately 1.8 mm (the upper left image). In addition, $CaCl_2$ crystal formations were observed on the surfaces of the microcapsules (the lower right image).

The study examined the release of vitamin C from the microcapsules under varying conditions of time, temperature, and pH. The outcomes are presented in Table 1. When the vitamin C microcapsules were exposed to an aquatic environment at a pH of 7.30 for over 8 hours, swelling of the microcapsules occurred, as shown in Fig. 3.

Additionally, it was observed that the release of vitamin C fluctuated over an 8-hour period, possibly due to the oxidation of ascorbic acid molecules (Njus et al., 2020). The partial dissolution of the alginate matrix in the capsules could have caused these deviations. However, the vitamin C microcapsules prepared in the study could remain structurally stable for up to 8 hours at a pH close to physiological pH and could release vitamin C. The rate of vitamin C release in 8 hours reached 99%.

Table 1. Time, temperature, and pH dependant vitamin C (AA: ascorbic acid) release from the vitamin C-pectin-alginate microcapsules.

Time (h)	Amount of AA released (mg/mL)	Amount of AA released (%)	Temperature (°C)	Amount of AA released (mg/mL)	Amount of AA released (%)	pH	Amount of AA released (mg/mL)	Amount of AA released (%)
0.25	19.13	57.16	20	20.88	62.37	2.0	10.85	32.42
0.50	20.65	61.68	25	5.60	16.73	3.0	15.75	47.06
1	26.13	78.07	30	4.79	14.26	4.0	21.93	65.51
2	26.83	80.15	37	14.11	47.16	5.0	14.81	44.25
4	28.93	86.42				6.0	31.49	94.08
8	33.36	99.66				7.0	28.11	83.97
16	20.53	61.32				8.0	11.55	34.51
24	22.86	68.26						

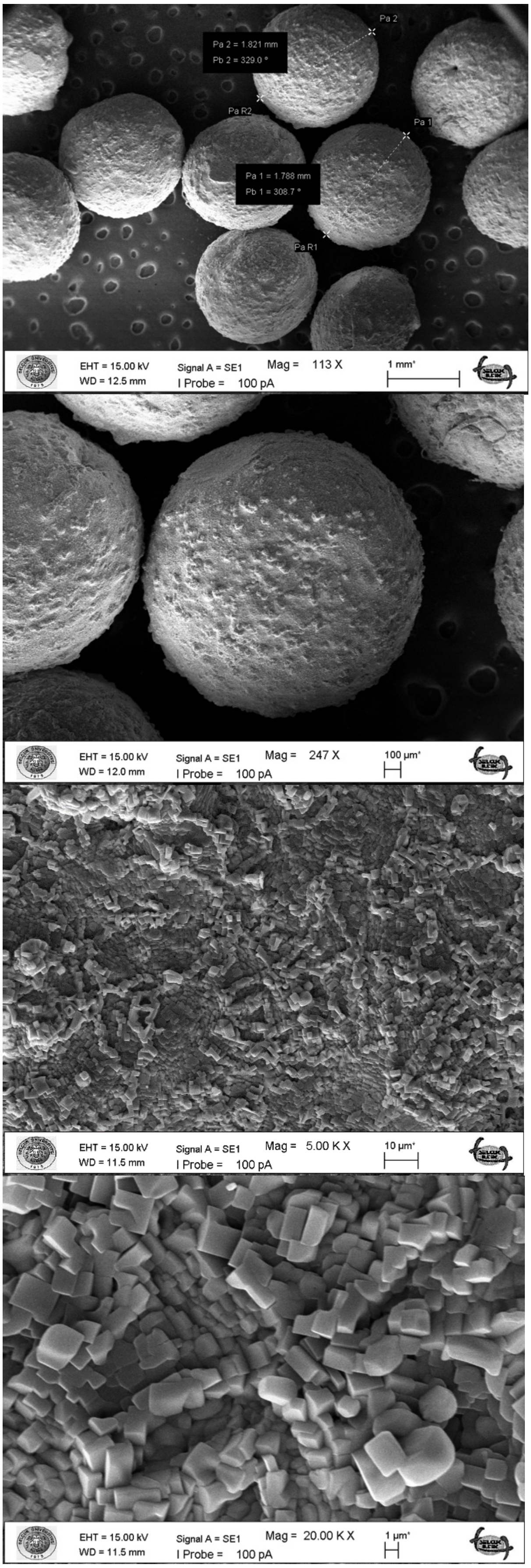

Fig.2. SEM images of vitamin C-pectin-alginate microcapsules at different magnifications.

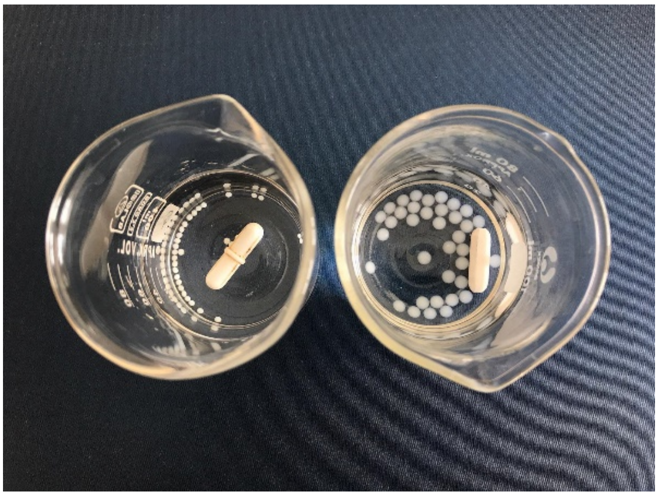

Fig.3. Swelling was observed when the vitamin C microcapsules were kept at pH: 7.35 in an aquatic environment for 8 hours (in the right beaker).

The study showed that the microcapsules used for vitamin C encapsulation are suitable for absorption in the small intestine. The release rate of vitamin C increased towards pH values close to 7.0, with a higher rate of 83.97% observed at pH 7.0. This is significant as vitamin C absorption takes place in the small intestine (Said, 2011). Additionally, temperature affected the release of vitamin C from the microcapsules, with approximately 47.2% release at body temperature (37°C) and a higher fluctuation in vitamin C release was observed at 20°C.

Vitamin C promotes the growth and repair of skin and connective tissues (Li et al., 2018). In a previous study, a pectin/modified alginate buccal patch was proposed as a drug delivery device for treating oral cavity diseases (Özkahraman et al., 2023). To improve its mucoadhesive properties, alginate was modified with acrylic acid and thiolated with cysteine. The study examined the impact of vitamin C on the healing process of the buccal adhesive patch formulation. The results showed that vitamin C promoted fibroblast proliferation, migration, and collagen synthesis, which had a positive effect on the wound healing process, particularly during the epithelialisation phase.

4. Conclusions

This study revealed that pectin extracted from lemon peels can be used with alginate to encapsulate vitamin C. The research study demonstrated that the microcapsules utilized for vitamin C encapsulation are well-suited for absorption in the small intestine. The release rate of vitamin C increased as the pH values approached 7.0, with an 83.97% higher rate being observed at pH 7.0. The temperature had an impact on the release of vitamin C from the microcapsules, with about 47.2% of the release taking place at the average body temperature (37°C). There was a greater fluctuation in the release of vitamin C at 20°C. The findings demonstrated that the effects of temperature and time on microcapsule stability and vitamin C release should be addressed in further studies. This study is a preliminary study, the encapsulation of vitamin C with vegetable, animal, and bacterial polymeric carbohydrates should be investigated in future studies.

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