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Scientific Research

Subject: Encapsulation of phenolic compounds of rhubarb's juice and their effects on the properties of pomegranate's juice

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ABSTRACT

The purpose of this research is to encapsulate the phenolic compounds of rhubarb stem's juice with chitosan and soy protein isolate and checking their effects on the properties of pomegranate juice. For this purpose, the phenolic compounds of rhubarb stem's juice were extracted by methanol and encapsulated with chitosan and soy protein isolate by nanoemulsion method, and finally the resulting microcapsules and nanocapsules were used to enrich pomegranate juice samples. The highest percentage of encapsulation efficiency of phenolic compounds was with soy protein isolate (45.26%). The results of SEM images showed that the particles were relatively spherical and with a relatively smooth surface, also chitosan nanocapsules with an average size of 281.4 nm and soy protein isolate microcapsules with an average size of 22.33 µm were formed. The results of pH analysis showed that the samples enriched with chitosan nanocapsules and soy protein isolate microcapsules showed more and less pH decrease than the control samples during the storage period. The results of DPPH radical scavenging activity showed that the enrichment of pomegranate juice with chitosan nanocapsules increased the antioxidant activity of the samples for up to 3 months of storage.

1- Introduction

Today's food industries are rapidly moving towards the formulation, production and promotion of health enhancing foods with increased nutritional value. Production of quality products, storage time and high nutritional value are the main concerns of producers. Formulation of fruit juice is one of the methods that can be used to improve the nutritional quality of fruit juice and improve the amount of ascorbic acid and minerals according to the type of fruit juice used. During the last decade, most of the research and studies related to the field of nutrition have focused on compounds of plant origin [1, 2 & 3].

Phenolic compounds are plant secondary metabolites and are classified into several groups including flavonoids and folic acids. These compounds are strong antioxidants and protect plants against oxidative stress. Phenolic compounds present in food products significantly affect their stability, sensory and nutritional characteristics and may prevent their spoilage by suppressing radical reactions responsible for lipid oxidation. However, many of these compounds have poor bioavailability and are sensitive to extreme environmental conditions such as pH, oxygen, light or enzymes [4, 5, 6 & 7].

One of the ways to protect active and sensitive components during the process is microencapsulation. Microencapsulation is a process in which bioactive or sensitive compounds are covered by wall materials and so called entrapment. Nanoemulsions are the most famous type of this method and are a suitable carrier for the delivery of lipophilic substances because they are easily prepared, have a very small size and increase their accessibility, biological impact and stability [8 & 9].

Wall materials play a key role in microencapsulation systems. The specific purpose of micoencapsulation and compatibility with the food matrix should be considered for the selection of wall materials. For example, the harsh conditions and higher sensitivity of the core material require a strong wall material such as one or two layers of material [10].

Chitosan is a deacetylated derivative of chitin and is the second most abundant copolymer on earth. Crustaceans, insects, molluscs and fungi are the main sources of chitosan. In industry, chitosan is made from the chitin exoskeleton of marine crustaceans such as crab, shrimp and krill, waste products originating from the fishing industry. Chitosan nanoparticles are known to facilitate the uptake of active molecules or compounds through cell membranes. The absorption-enhancing effect of chitosan nanoparticles improves the molecular bioavailability of the active substances in the nanoparticles [11, 12 & 13]. The effect of different combinations of microencapsulating agents on the physicochemical properties and stability of microcapsules loaded with plum's phenol was investigated by Li et al (2018) and The results showed that maltodextrin/chitosan microcapsules with high microencapsulation efficiency and smooth surface of particles had considerable protection against phenolic compounds and thus had better stability [14].

Among different natural polymers, vegetable proteins such as soy protein, zein and wheat gluten have attracted considerable attention in scientific research and industries due to their easy access and low cost. Soy protein isolate is generally used as a separate wall material, but it can also be mixed with polysaccharides. Combining proteins with carbohydrates as a carrier provides better protection, oxidative stability and better drying properties [15]. The structural, chemical and surface properties of proteins were investigated by Zhu et al (2021) and The results showed that microcapsules made of egg white protein and corn protein isolate reduced the destruction of enclosed vitamins by 20 and 40% after exposure to heat and ultraviolet radiation [16].

The rhubarb plant belongs to the Polygonaceae family, and various species of it are grown in countries such as Iran, India, China, and Turkey. A species of it called Rheum Ribes grows in Iran, mainly in the mountainous regions of provinces such as Khorasan, Kurdistan, West Azerbaijan, etc., and its stem is consumed as food. Valuable properties that can be attributed to rhubarb essential oil can also be attributed to vitamins E, B2, B1, A, C and K, respectively, which are abundantly found in rhubarb. Also, the polyphenolic compounds present in this plant provide antioxidant properties [17 & 18]. The determination of secondary metabolites including curcumin in rhubarb and its antioxidant and anticancer activity were investigated by Noori et al (2022) and The results showed high amounts of phenolic, flavonoid and total flavonol compounds in the extract [19]. In the research of Öztürk et al (2007) on the antioxidant activity of rhubarb stem and root extracts, antioxidant activity of 82.2 and 82% inhibition of chloroform and methanolic extracts was observed for the stem [20].

Pomegranate with the scientific name Punica granatum L. is one of the members of the pomegranate family, which researchers have identified as its origin in Iran and other neighboring countries. This fruit is famous for its nutritional and medicinal purposes in folk medicine. Currently, there is an increasing interest in pomegranate fruits in the scientific community due to its bioactive properties and potential for health promotion [21 & 22].

The consumption of pomegranate juice has increased significantly since the scientific literature mentioned its therapeutic benefits related to its antioxidant, antimicrobial, anti-cancer and anti-

inflammatory properties. However, most of the pomegranate juice in the market have important quality problems such as high turbidity, sediment and brown color, which prevent many consumers from consuming industrial pomegranate juice. Although thermal pasteurization is the most widely used preservation technology, it has adverse effects on the nutritional and sensory quality of fruit juices [23 & 24]. For example, in the research of Bhagat and Chakraborty (2022) the results showed that the sample pasteurized by thermal method (95°C for 3 minutes) showed a 30% and 37% reduction in phenolic compounds and antioxidant capacity, respectively, and only 34% of vitamin C in fruit juice [25].

2. Methods

2. 1 Chemicals and reagents

The materials used include rhubarb with the specifications listed in table 1, which was collected from the red mountains of Kashmer city, Khorasan Razavi province. Soy protein isolate (Shandong yuwang ecological food industry, China), DPPH (Sigma Aldrich, USA), chitosan medium with a molecular weight of 190-310 kDa, Folin Ciocaltio reagent, gallic acid and toin 80 (Merck, Germany), pomegranate juice (Negin Shahd Shahran Company, Iran), methanol 99%, ethanol 96%, glacial acetic acid, disodium hydrogen phosphate, citric acid and sodium carbonate (Dr. Mojallali Industrial Chemical Complex Co, Iran).

Table 1: Characteristics of rhubarb used in the current research [26]

Specifications	
Species	*R. khorasanicum*
Total phenol	2.916 $^{mg\ GA}/_{g\ DW}$
Antioxidant activity (DPPH)	15.51

2. 2 Collecting and preparing of rhubarbs

On April 13, 2022, rhubarbs were collected and the leaves and roots of rhubarbs were separated, washed with water and stored in the freezer. One day before mixing, the rhubarb was taken out of the freezer and placed in the refrigerator to defrost. A homemade blender (Shine Tech, Pars Khazar/Iran) was used to mix the rhubarbs.

2. 3 Extraction of phenolic compounds

In order to extract phenolic compounds, 10 ml of rhubarb's mix was blended with 100 ml of 70% methanol solution and stirred for 45 minutes on a stirrer (Heidolph/Germany) at room temperature. The methanol in the solution was removed using a rotary evaporator (Heidolph/Germany) at a temperature of 40°C and the rhubarb's juice was filtered and stored in a freezer [17].

2. 4 Encapsulation of phenolic compounds

For encapsulating of phenolic compounds using chitosan, 0.5 g of chitosan was dissolved in 100 ml of 1% glacial acetic acid solution for 2 hours at 40°C and stirring speed of 750 rpm on a heater stirrer. 5 ml of phenolic extracted rhubarb juice was mixed with 200 µl of Tween 80. 50 ml of chitosan solution was stirred on a heater stirrer with a stirring speed of 1200 rpm and at the same time rhubarb's juice solution was added dropwise at a speed of 0.5 ml/min. The solution was stirred for another 45 minutes and then stored in the refrigerator. The encapsulation of phenolic compounds with soy protein isolate was according to the above method, with the difference that first the soy protein isolate was dissolved in a buffer solution with pH=7 (disodium hydrogen phosphate 0.2 M and citric acid 0.1 M) [27].

2. 5 Phenolic content and encapsulating efficiency

In order to measure the phenolic content of rhubarb juice, chitosan nanocapsules solution and soy protein isolate microcapsules solution, 1 ml of the sample solution was mixed with 5 ml of Folin–Ciocalteu reagent solution and allowed to rest in the dark for 3 minutes. Then 4 ml of sodium carbonate solution was added to the solution and the solution rested in the dark for 60 minutes. The absorbance of the solution was measured at a wavelength of 720 nm in a spectrophotometer (Unico/USA) [17]. The percentage of encapsulating efficiency of the samples was calculated with formula 1 [6]:

$$Encapsulation\ efficiency = \left(1 - {}^{F}/_{T}\right) \times 100 \quad (1)$$

In the above formula, T represents the total amount of phenolic compounds added during the encapsulating process and F represents the free phenolic compounds in the solution after the encapsulating process.

2. 6 Morphology

SEM (Zeiss sigma Sigma 300-HV/Germany) was used to examine the morphology of the particles in this research. To prepare the samples for SEM analyz, a drop of the sample solution was poured on an aluminum foil with an area of 1 square centimeter and dried in an oven (Memmert/Germany) at a temperature of 40 degrees Celsius. Before analyze, a coating of gold was placed on the samples with a desktop sputtering device (DSR-1/Iran) and then analyze was done [28].

2. 7 Sensory analysis

Pomegranate juice used in this research was prepared from Nagin Shahd Shahran Company of iran. In this research, sensory evaluation of traits was done based on a 5-point scale. The evaluation was done with the help of 24 evaluators (12 men and 12 women) who were randomly selected from among the students. Each evaluator was given one sample from each samples and the four factors of color, aroma, taste and overall acceptance were evaluated. A score scale was evaluated for all four factors from number 5 in the most favorable state to number 1 as unacceptable examples. A repetition of the samples enriched with chitosan nanocapsules, a repetition of the samples enriched with soy protein isolate microcapsules, which received the highest scores in the general acceptance section, and non-enriched pomegranate juice were selected as control samples for the next analysis.

Table 2: Pomegranate juice samples prepared for sensory analysis in the present study

Treatments	Compounds of constituent
C	Control (pure pomegranate juice)
CM1	3 mg of chitosan nanocapsules
CM2	4 mg of chitosan nanocapsules
CM3	5 mg of chitosan nanocapsules
CM4	50 mg of chitosan nanocapsules
CM5	94 mg of chitosan nanocapsules
SPM1	14 mg of soy protein microcapsules
SPM2	25 mg of soy protein microcapsules
SPM3	34 mg of soy protein microcapsules
SPM4	202 mg of soy protein microcapsules
SPM5	339 mg of soy protein microcapsules

2. 8 Storage of enriched pomegranate juices and related analysis

100 ml of pomegranate juice was added in glass flasks. The lids of the flasks were closed with aluminum foil. The flasks were heated in a bain-marie apparatus (Memmert/Germany) at 90°C for 15 minutes. Then the flasks were placed in another Bain-Marie device at 40°C for 30 minutes. The flasks were closed and stored in a refrigerator at 4°C [29]. The treatments were stored for 4 months in a refrigerator at a temperature of 4 degrees Celsius, and pH, colorimetric, viscosity and DPPH radical scavenging activity analysis were performed at the end of the first, second, third and fourth months of storage [30].

2. 8. 1 pH

The pH of the samples was evaluated using a pH meter (Jenway/England). In order to perform this evaluation, the desired sample was brought to a temperature of 20°C and poured into a beaker, and the electrode of the pH meter was placed inside the sample. The electrode was in contact with the sample for at least 60 seconds and then the desired number was measured.

2. 8. 2 Colorimetric

In order to measure the color of the samples, first, photos were taken of the samples with a digital camera in 3 repetitions. After that, L*, a* and b* were evaluated using ImageJ software. The L* indicates the brightness of the juice. In such a way that L*=0 indicates black color and L*=100 indicates light dispersion or full light. The a* indicates green and red color. So that its positive values indicate red color and its negative values indicate green color. b* indicates yellow and blue color. So that positive values show yellow color and negative values show blue color [31]. The color change of the samples was calculated by the method of Bursac Kovacevic et al (2016) using formula 2 [32].

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \quad (2)$$

2. 8. 3 Viscosity

To evaluate the viscosity of the samples, a rheometer (Anton-paar/Austria) was used and the shear rate was used in the range of 0.1 to 100 S^{-1} at a temperature of 25 degrees Celsius [33].

2. 8. 4 DPPH radical scavenging activity

To evaluate the percentage of DPPH radical scavenging activity, 2 ml of sample was combined with 2 ml of 0.2 mM DPPH ethanol solution. The

solution was allowed to rest in the dark for 30 minutes. The absorbance of the sample was measured at a wavelength of 517 nm with a spectrophotometer (Unico/USA). For the control sample, 2 ml of 70% ethanol solution was used instead of the sample. DPPH radical scavenging activity percentage of the samples was calculated using formula 3 [34]:

$$\%\ DPPH\ inhibition = \frac{(Acontrol - Asample)}{Acontrol} \times 100 \quad (3)$$

2. 9 Statistical Analysis

Statistical analysis of the data obtained from this research was done using SPSS and the difference between treatments was determined based on Duncan's method and at the 5% level. All tests except rheology test and SEM were done in 3 repetitions. Graphs were drawn using EXCLE 2019.

3. Results

3. 1 Phenolic content and encapsulating efficiency

According to the results, the phenolic content of rhubarb's juice was 44.63 ppm (equivalent to 0.044 milligrams of gallic acid per 1 gram of rhubarb stem), which results are shown in Figure 1. According to the results of Figure 1, the phenolic content of the solutions of chitosan nanocapsules and soy protein isolate microcapsules was less than the rhubarb's juice sample, which indicated the encapsulaiing of the phenolic compounds of rhubarb's juice by chitosan and soy protein isolate, and also the two solutions of chitosan nanocapsules and soy protein isolate microcapsules had a significant difference (P < 05) in phenolic content. According to the results of Table 3, encapsulation of phenolic compounds with soy protein isolate had a higher percentage of encapsulation efficiency (45.26%) than encapsulation with chitosan (38.28%). The encapsulation efficiency of microcapsules mainly depends on the physicochemical properties of wall materials, core materials and also on the interaction between wall and core materials. Previous studies have shown that the efficiency of encapsulation is greatly influenced by the molecular weight of chitosan, which in the present study chitosan used was of medium molecular weight (190-310 kilodaltons) [35]. Ran et al (2020) also in their study on the synergistic effect of antioxidant glutathione and edible phenolic acids and improving the protection of activity by overlapping in chitosan-coated liposomes, obtained a microencapsulation efficiency of over 50% for chitosan-coated microcapsules. brought and showed that after coating liposomes with chitosan, the microencapsulation efficiency increased from 54.64 to 61.32% [36]. In another study, Delfanian et al (2018) in their study on the effect of the main components of the emulsion on the physicochemical and functional properties of the water/oil/water nanoemulsion obtained a microencapsulation efficiency of over 90% for microcapsules coated with soy protein isolate and showed that microencapsulation with Nanoemulsion of soy protein isolate and basil seed gum shows a higher microencapsulation efficiency than whey protein isolate and basil seed gum and less than 100 Hi-Cap [37].

Table 3: Microencapsulation efficiency percentage

Wall material type	Microencapsulating efficiency
Chitosan	37.28%
Soy protein isolate	45.26%

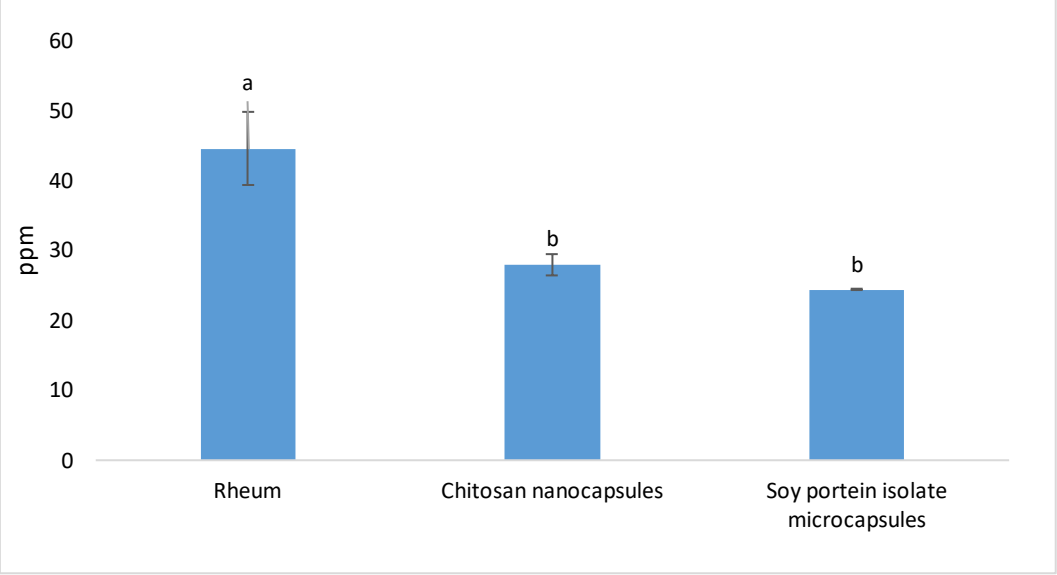


Figure 1: The phenolic content of the samples is equivalent to ppm of gallic acid in 1 ml of the sample

3. 2 Morphology

The results of examining the particle morphology of chitosan nanocapsules and soy

protein isolate microcapsules using SEM are shown in Figure 2. According to the pictures, the particles are relatively spherical and with a relatively smooth surface, chitosan nanocapsules with an average size of 281.4 nm and soy protein isolate microcapsules with an average size of 22.33 µm were formed. The phenomenon of agglomeration of chitosan nanocapsules particles is probably created during the drying process of solutions containing nanocapsules to prepare for SEM analyse. In the research of Gopalakrishnan et al (2014) on ellagic acid encapsulated with chitosan nanoparticles as an anti-hemorrhage agent, it was also observed that chitosan nanoparticles coated with ellagic acid appeared as clusters with an average particle size of about 80 nm. A slight increase in the size of nanoparticles compared to chitosan microcapsules without ellagic acid can be due to the incorporation of core materials between the polymer complex [38]. Regarding soy protein isolate microcapsules, a similar situation was observed during the research of Di Giorgio et al (2019) on the microencapsulating of fish oil in soy protein particles by emulsification and spray drying. In this study, the average apparent diameter of microcapsules for all samples was between 15 and 20 micrometers, and the particles showed a smooth spherical shape, which was characterized by surface depressions [39].

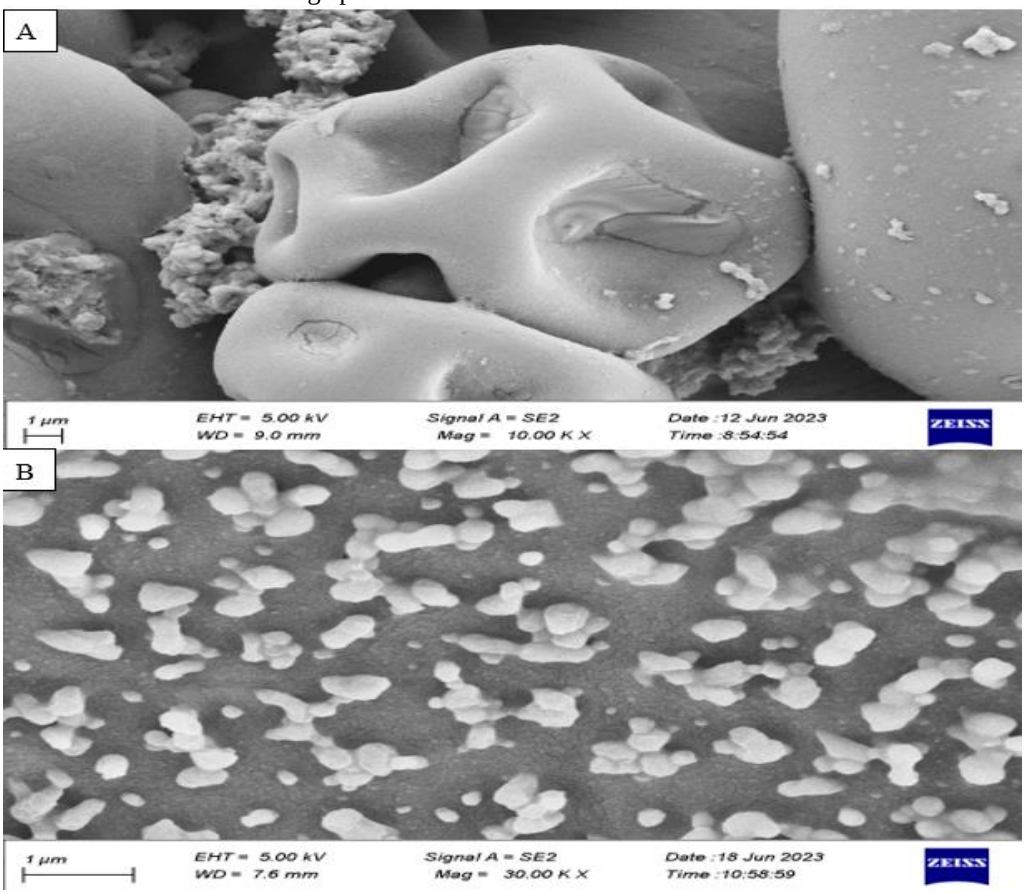


Figure 2: SEM images of (A) soy protein isolate microcapsule and (B) chitosan nanocapsule

3. 3 Sensory analysis

The sensory analysis of the pomegranate juice samples prepared by the participants in the hedonic test was done. In terms of color, taste and overall acceptance, samples containing 94 mg of chitosan nanocapsules and samples containing 339 mg of soy protein isolate microcapsules (CM5 and SPM5) received the lowest scores. Analysis of the aroma of the samples shown in Figure 3 showed that enrichment of pomegranate juice with chitosan nanocapsules and soy protein isolate microcapsules is not significantly different (P<05) from the control sample. According to Figure 4 in terms of color, enrichment of pomegranate juice with chitosan nanocapsules more than 50 mg of nanocapsules decreased the color score of pomegranate juice. In the case of enrichment with soy protein isolate, a decrease in score was observed in the enrichment of more than 202 mg of microcapsules which in any case can be due to the turbidity of the samples due to the contents of the capsules. According to Figure 5, in terms of taste, samples containing 50 and 94 mg of microcapsules (CM5) showed the lowest taste score in enrichment with chitosan nanocapsules. This can be due to the fact that chitosan has the ability to reduce the acidity of fruit juices and create a relatively bitter taste [42]. Regarding soy protein isolate microcapsules, the samples containing 14 and 25 mg of microcapsules (SPM1 and SPM2) did not show significant difference (P<05) with the

control sample. According to Figure 6, in terms of overall acceptance, samples containing 4 and 5 mg of chitosan nanocapsules (CM2 and CM3) did not show significant difference (P<05) with the control sample. In enrichment with soy protein isolate microcapsule, the sample containing 25 mg of microcapsule (SPM2) showed no significant difference (P<05) with the control sample. Similar to this, it was also observed in a similar study by Martín-Diana et al (2009) on chitosan-enriched orange juice to optimize shelf life. In this research, with the increase in chitosan concentration, a significant decrease in the overall acceptance of the samples was observed [40]. In another similar study, Potter et al (2007) on the characteristics of blueberry and soy beverages observed that beverages containing soy protein isolate had more blueberry flavor than other beverages and caused chalkiness. Also, in these samples, the acidity of the sample was significantly reduced during 4 weeks of storage [41]. According to the obtained results, CM3 and SPM3 samples were selected for further analysis.

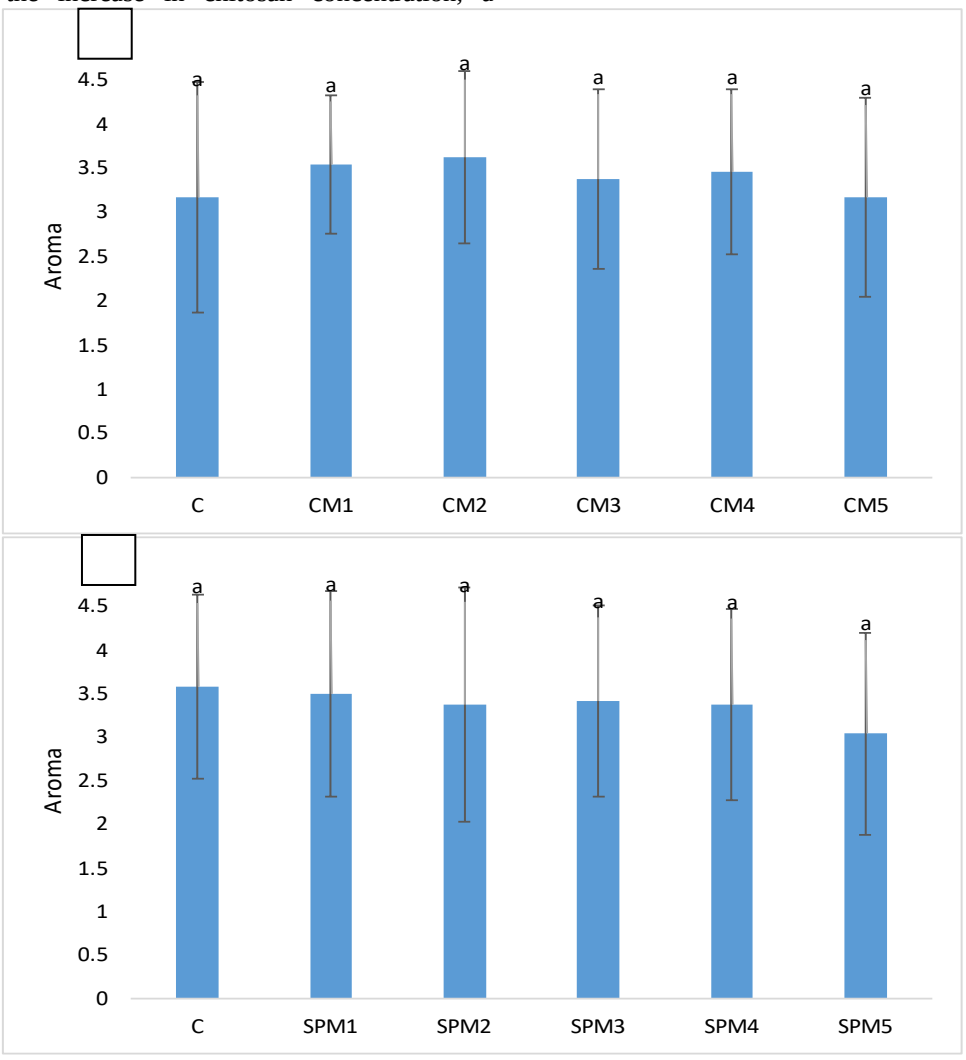


Figure 3: Aroma analysis of pomegranate juice samples enriched with (A) chitosan nanocapsules and (B) soy protein isolate microcapsules

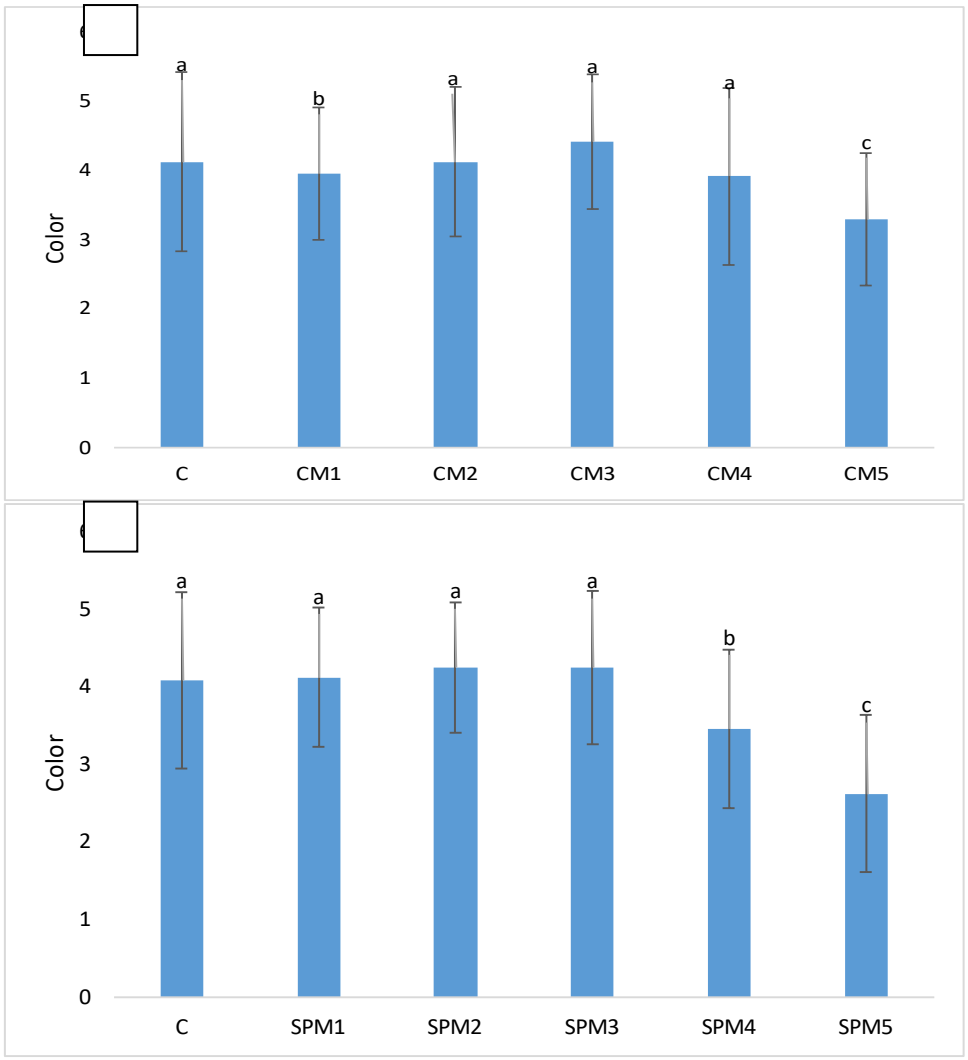
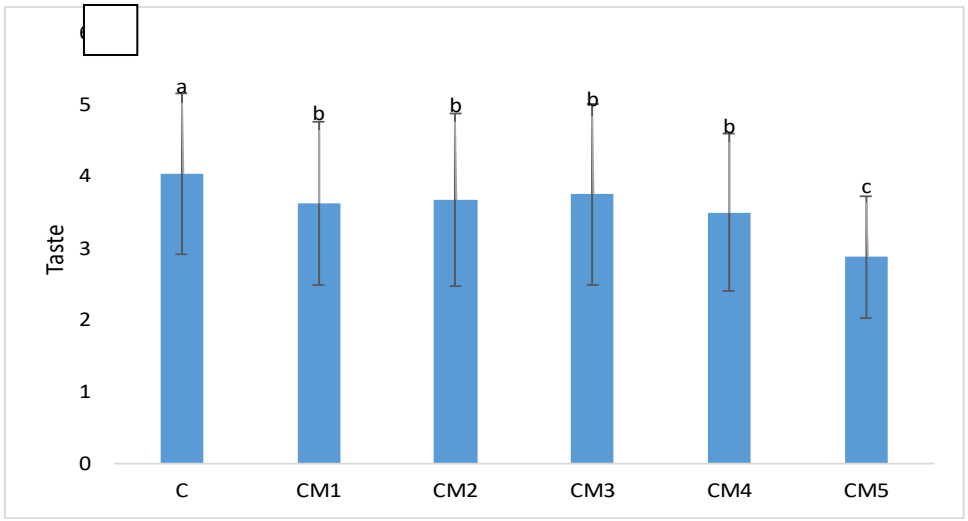


Figure 4: Color analysis of pomegranate juice samples enriched with (A) chitosan nanocapsules and (B) soy protein isolate microcapsules



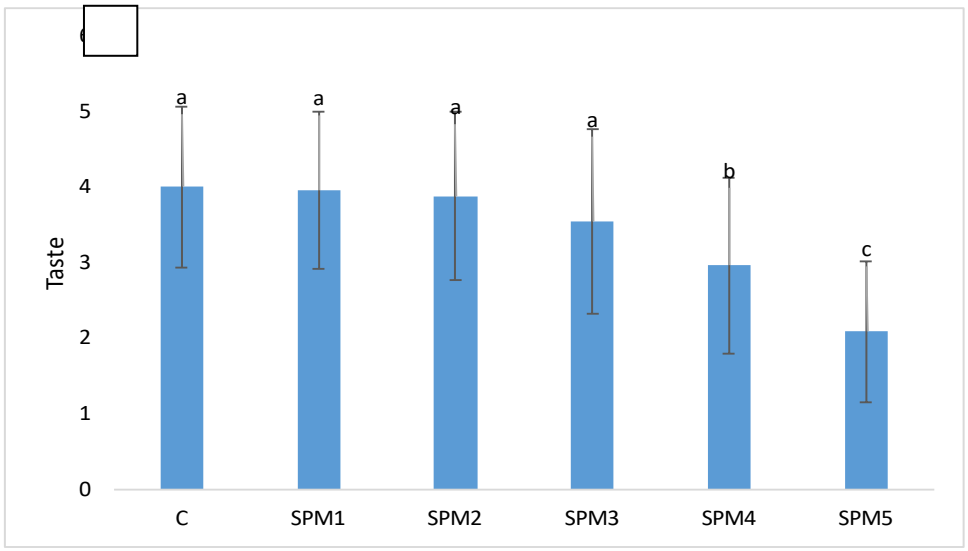


Figure 5: Taste evaluation of pomegranate juice samples enriched with (A) chitosan nanocapsules and (B) soy protein isolate microcapsules

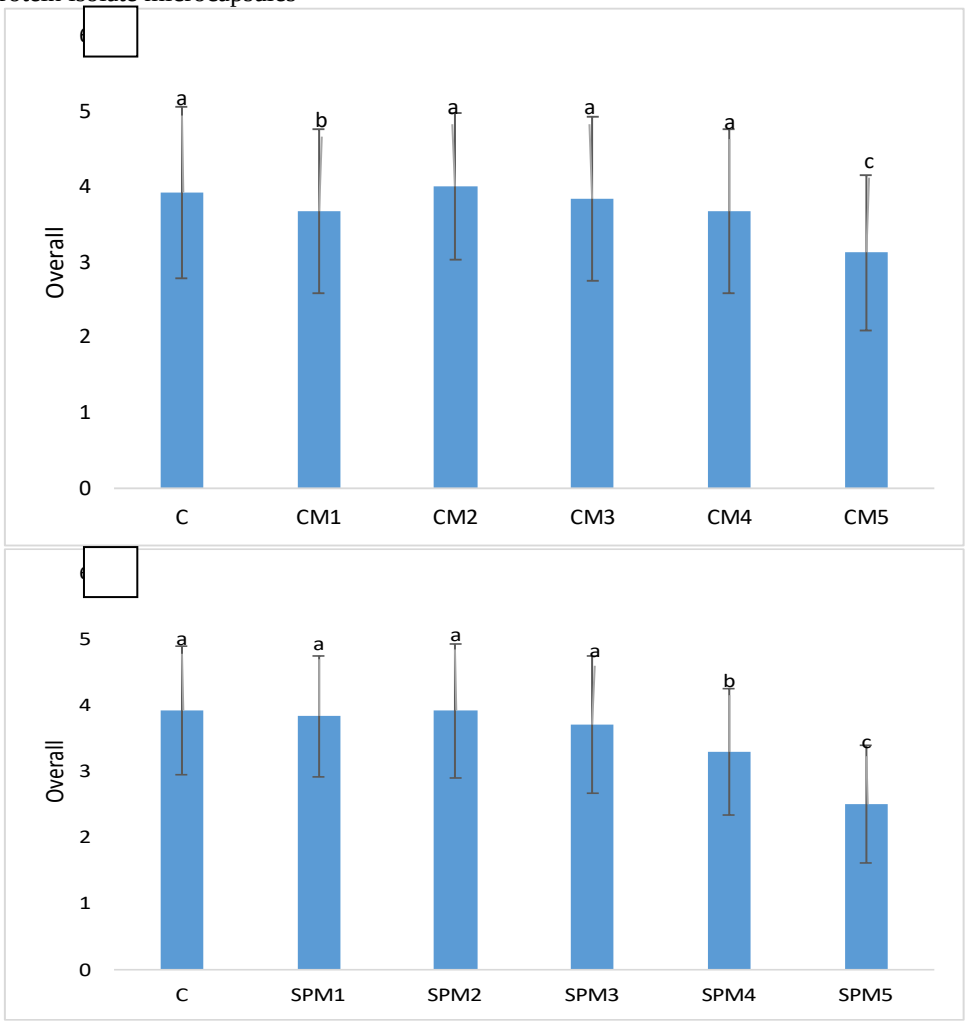


Figure 6: Overall of pomegranate juice samples enriched with (A) chitosan nanocapsules and (B) soy protein isolate microcapsules

3. 4 Analyzes during the storage period

3. 4. 1 pH

The results of the pH of the treatments during 4 months of storage in the refrigerator at 4°C are shown in Figure 7. According to the results of the control sample, pH increased in the second month of storage compared to the first month, and then pH decreased until the fourth month of storage. This can

be due to the increase in microbial growth in the samples and the subsequent increase in acidity and decrease in pH. In similar studies, Jafari et al (2021) investigated the kinetics of microbial inhibition of dimethyl bicarbonate and the qualitative characteristics of pomegranate juice in cold storage, and the research of Unluturk and Atilgan (2015) investigated the microbial safety and shelf life of freshly pressed white grape juice with UV- C was also observed similar to the same results [42 & 43]. The samples enriched with chitosan nanocapsules showed a greater decrease in pH during storage for 4 months. Probably, the use of citric acid and ascorbic acids in pasteurized fruit juice, as well as the use of glacial acetic acid in the preparation of chitosan nanocapsules, has reduced the pH in enriched pomegranate juice samples [44 & 45]. In the research of Bastos et al (2012) on the microencapsulation of cashew apple juice using a new commercial bovine whey protein isolate system and chitosan in spray drying, similar results were observed [44]. In the samples enriched with soy protein isolate microcapsules, a lower decrease in pH was observed than in other samples during the storage period. Probably, the use of buffer solution with pH = 7 to prepare soy protein isolate microcapsules has caused a lower pH decrease in the samples compared to other samples.

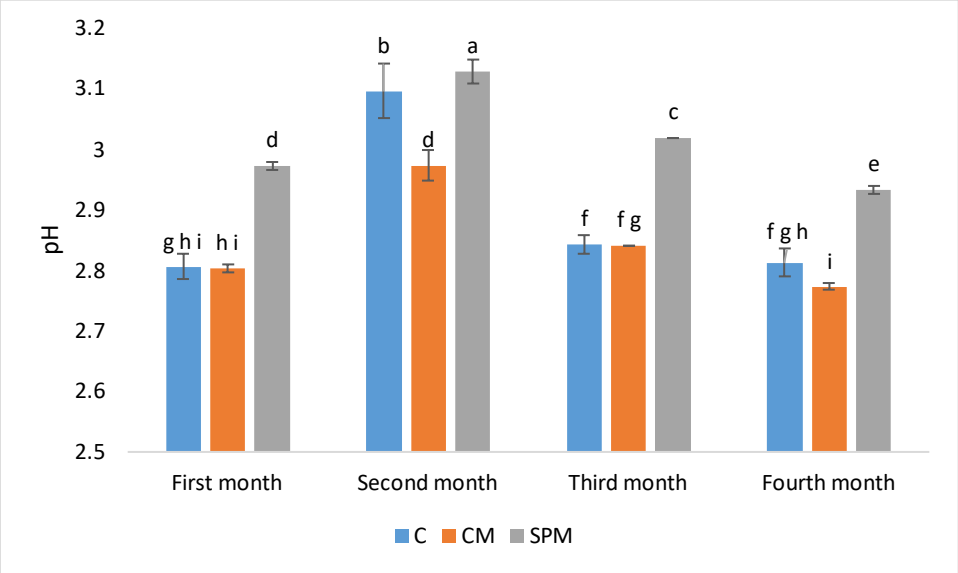
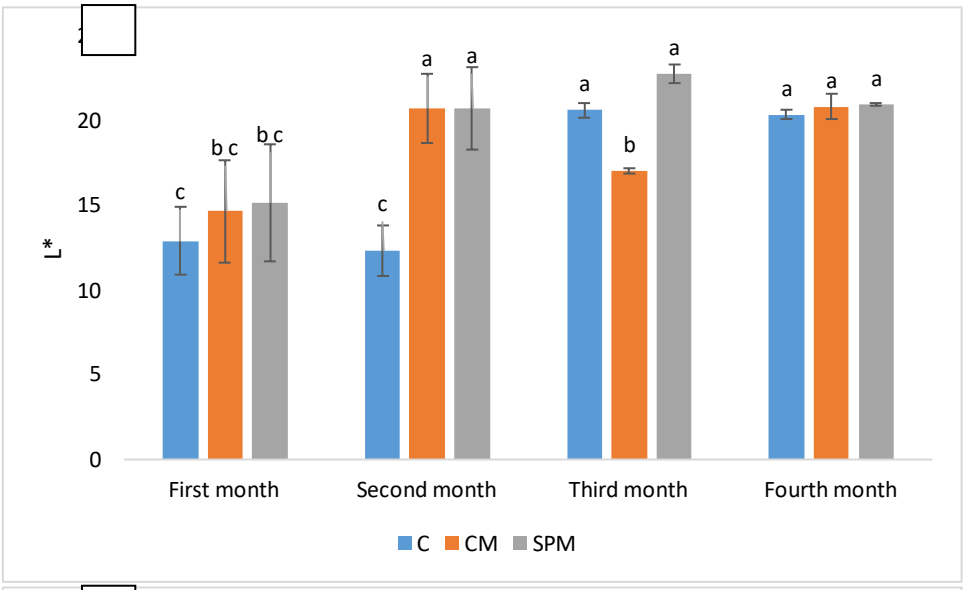


Figure 7: pH analysis of pomegranate juice samples during 4 months of storage in the refrigerator at a temperature of 4°C

3. 4. 2 Colorimetric

According to the results in figure 8, 9, 10 and 11 no significant difference (P<05) was observed in the L* until the second month, but it increased in the third month. Also, the same thing happened with the a* and the redness of the control pomegranate juice samples increased from the third month. Regarding the b* in the samples until the second month, the yellowness of the samples decreased, but from the third month the yellowness of the samples increased. In a similar study by Beaulieu et al (2020) on the changes in pomegranate juice of the pilot plant without concentrate, the same results were observed [35]. The samples enriched with chitosan nanocapsules had a higher L* than the control sample until the second month and after that it did not show a significant difference (P<05) until the fourth month. A similar situation was observed in the case of the a*. In the case of b*, there was an increasing trend until the second month, it decreased in the third month and increased again until the end of the fourth month. Regarding the ΔE, the samples showed a sharp increase in the second month of storage and did not show a significant difference (P < 05) in the rest of the months of storage. In a similar study by Celli et al (2018) on annatto enclosed in casein and chitosan complexes and its effect on the color quality of whey after acid coagulation of milk, it was also observed that after adding chitosan to the samples, the index a* and b* increased found, but the L* decreased [46]. Samples enriched with soy protein isolate microcapsule L* increased more than the control sample until the second month and then did not show a significant difference (P<05) until the end of the fourth month. Similar to this situation was also observed in the case of a*, with the difference that it showed a decreasing trend in the fourth month. In the case of the b*, the process of changing the results was similar to the L*. Regarding the ΔE, the samples showed the strongest increase in the second month of storage and did not show a significant difference (P<05) in the rest of the months of storage. In a similar study by Ziobro et al (2013) on the supplementation of gluten-free bread with non-gluten proteins and the effect on the rheological properties of the dough and the characteristics of the bread, it was also observed that in the samples containing soy protein, the a* and b* increased compared to the control sample. But it had no significant effect on L* [47].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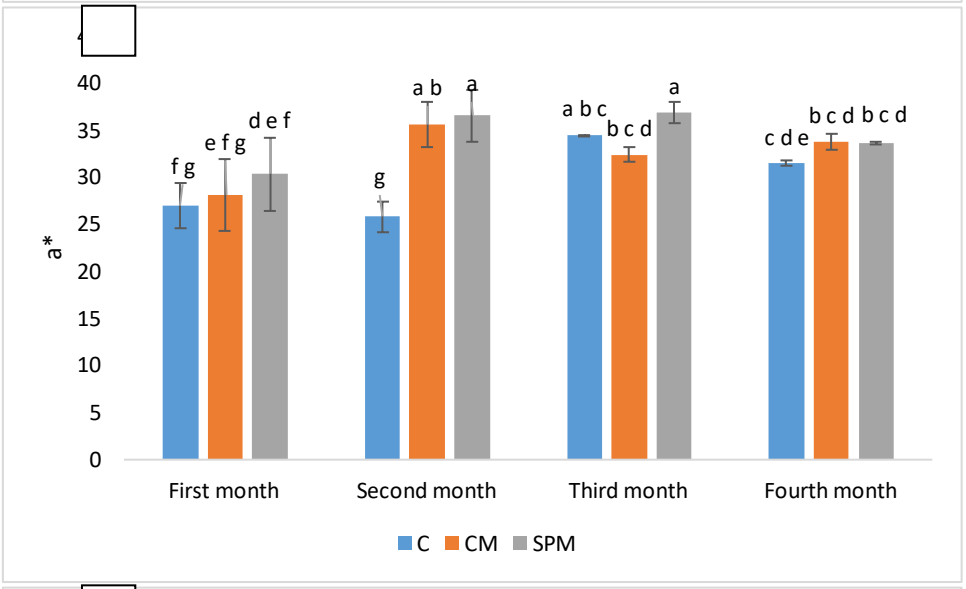
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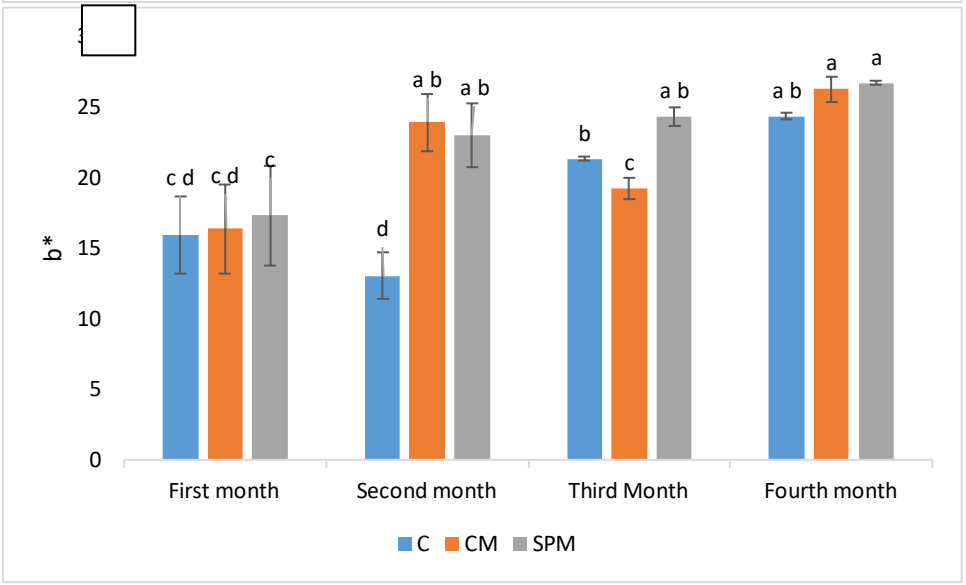
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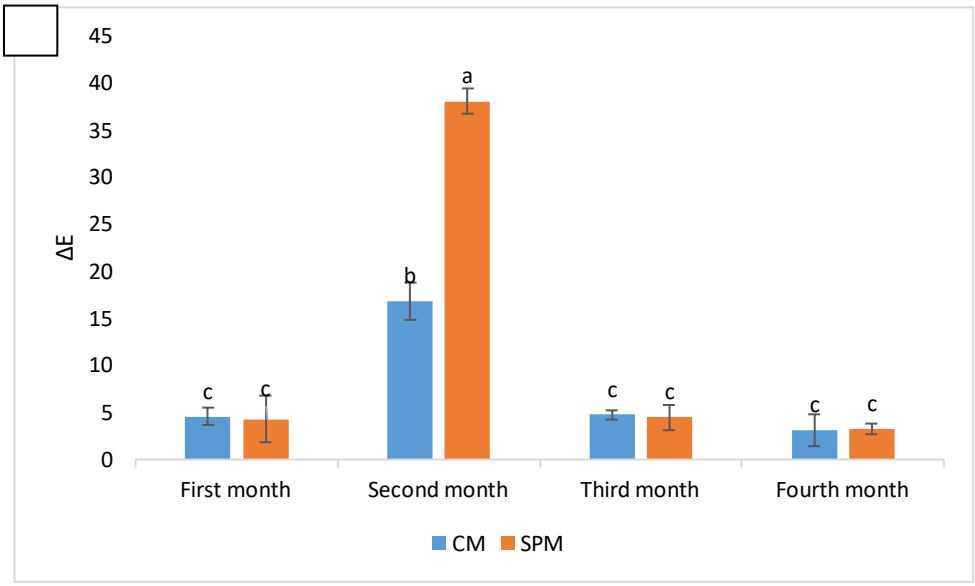
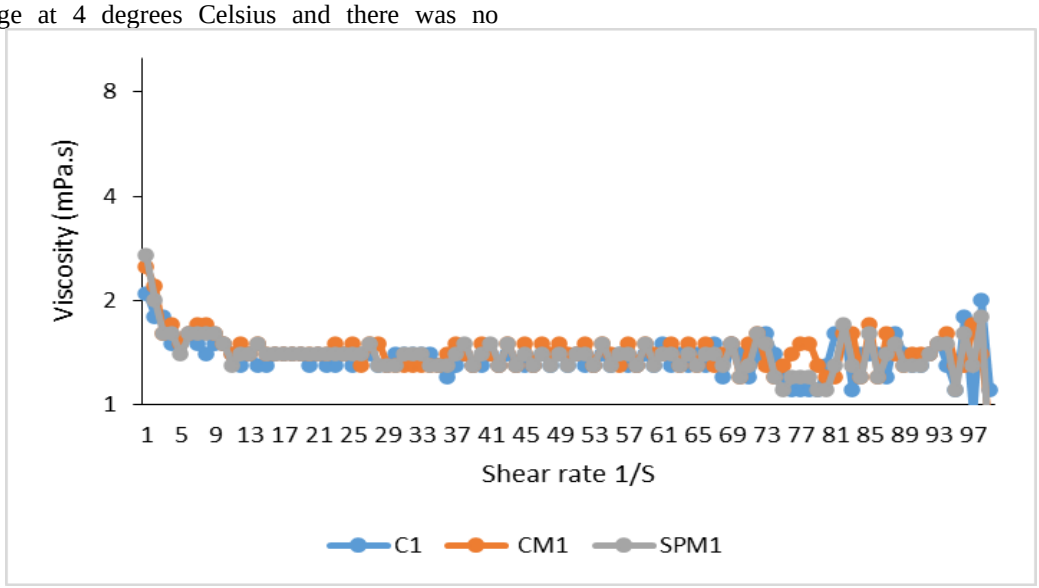


Figure 8: Examination of (A) L* (B) a* (C) b* (D) ΔE of pomegranate juice samples during 4 months of storage in the refrigerator at 4°C

3. 4. 3 Viscosity

The results of the viscosity of pomegranate juice samples are shown in Figure 9. According to the results, all the samples were part of Newtonian fluids and the enrichment of pomegranate juice samples with chitosan nanocapsules or soy protein isolate microcapsules had no effect on the viscosity of pomegranate juice samples. According to the results of Figure 9 (A), the viscosities of control samples, samples enriched with chitosan nanocapsules and soy protein isolate microcapsules were 1.37, 1.43 and 1.39 mPa.s in the first month of storage at 4 degrees Celsius and there was no significant difference (P<05). Also, keeping control and enriched samples for 4 months at 4°C did not have a significant effect (P<05) on the viscosity of the samples. In the research of Magerramov et al (2007) on the effect of temperature, concentration and pressure on the viscosity of pomegranate and pear juice concentrate, as well as the research of Hosseini et al (2021) on the clarification of pomegranate juice in a packed bioreactor by pectinase enzymes fixed on the bead Glass activated with polyaldehyde polysaccharides, similar results were observed [48 & 49].



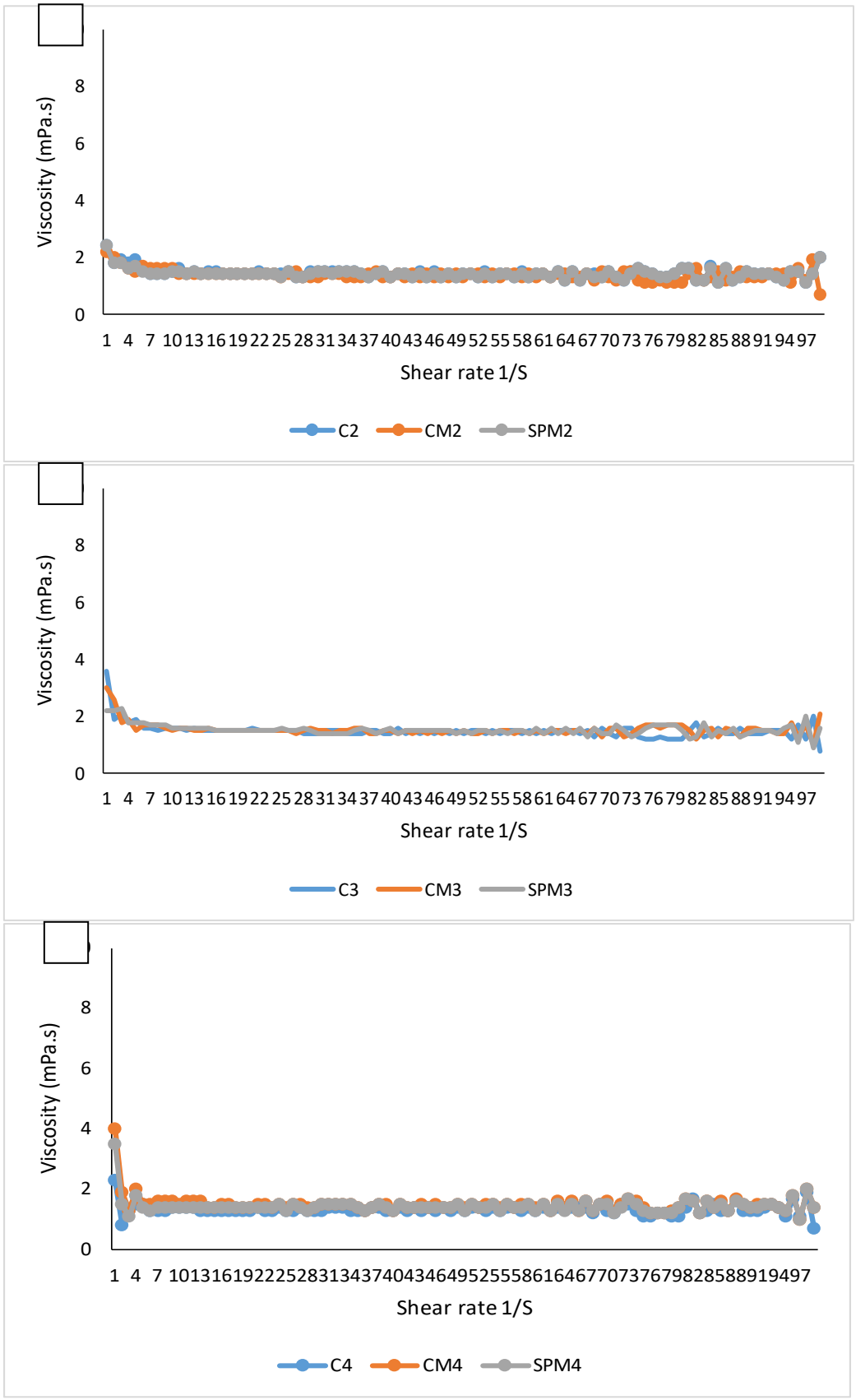


Figure 9: Checking the viscosity of pomegranate juice samples after (A) 1 month of storage, (B) 2 months of storage, (C) 3 months of storage and (D) 4 months of storage in a refrigerator at 4°C

4. 4. 3 Antioxidant activity

According to the results in Figure 10, the control samples showed a decrease in antioxidant activity in the second month of storage, and in the

third month of storage, their antioxidant activity increased, and until the fourth month of storage, there was a significant difference. (P<05) was not observed in DPPH radical scavenging activity. In similar studies, Varela-Santos et al (2012) on the effect of high hydrostatic pressure (HHP) processing on the physicochemical properties, bioactive compounds and shelf life of pomegranate juice in the case of control samples during 30 days of storage in a refrigerator at 4°C. Grad and the research of Bertolini et al (2020) on the optimization of the CO2 supercritical pasteurization process to maintain the high nutritional value of pomegranate juice in the case of control samples during 28 days of storage in the refrigerator at a temperature of 4 degrees Celsius, similar results were observed [50 & 51]. In the case of pomegranate juice enriched with chitosan nanocapsules, samples in the first month of storage showed more antioxidant activity than control samples and samples enriched with soy protein isolate microcapsules, but in the second month of storage antioxidant activity decreased. In the third month of storage, the antioxidant activity reached its highest level compared to all samples, and at the end of the fourth month of storage, a decreasing trend was observed. This may be due to the relatively good encapsulation efficiency percentage (37.28%) of phenolic compounds with chitosan. In a similar study by Liu et al (2021) on the isolation and microencapsulation of antibacterial compounds from wood vinegar, the release of phenolic compounds encapsulated in chitosan and alginate showed a strong increase before the 10th day of storage, and then until the 25th day of storage. The release increased gradually [52]. In the case of pomegranate juice enriched with soy protein isolate microcapsules, at the end of the first month of storage, the antioxidant activity of the samples did not show a significant difference (P<05) with the control samples. The trend of changes in the antioxidant activity of the samples until the end of the second month of storage was decreasing and at the end of the third month of storage it was increasing, and they showed a lower antioxidant activity than the control sample, which can be due to the greater ability of soy protein isolate to encapsulate phenolic compounds. At the end of the fourth month of storage, however, the antioxidant activity of the samples was higher than the control and chitosan microcapsule-enriched samples. This may be due to the higher percentage of encapsulating efficiency (45.26%) of phenolic compounds with soy protein isolate. In a similar study by Li et al (2015) on the microencapsulating of tomato oleoresin using soy protein isolate and Arasia gum as emulsifier and coating material, it was also observed that after 30 days of storage with a relative humidity of 33%, the shelf life of lycopene microcapsules from 82 decreased to 78.83% [53].

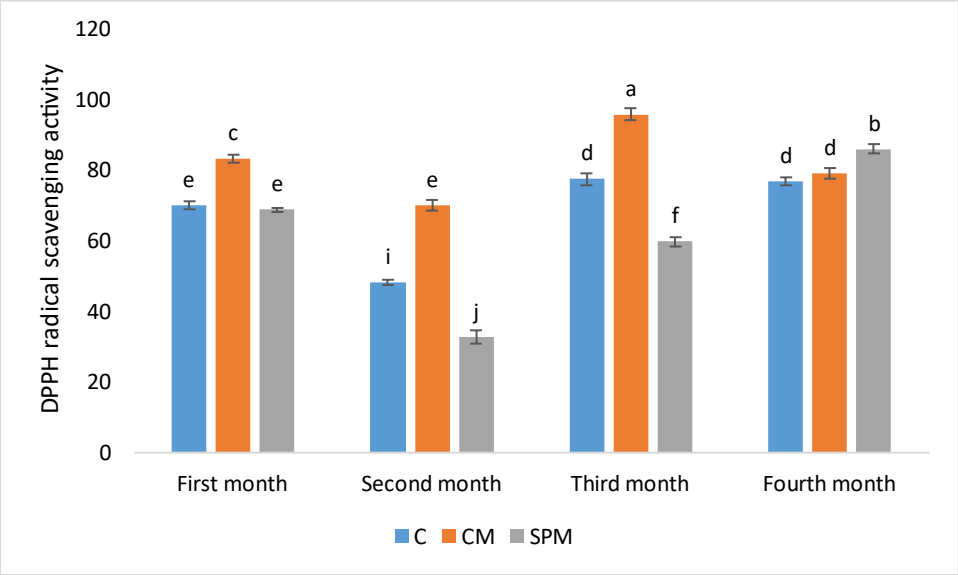


Figure 10: DPPH radical scavenging activity of pomegranate juice samples during 4 months of storage in the refrigerator at 4°C

4. Conclusion

In general, the current research includes the encapsulation of phenolic compounds of rhubarb's juice with chitosan and soy protein isolate and the investigation of encapsulation efficiency and particle morphology and the enrichment of pomegranate juice with different percentages of microcapsules and the evaluation of its sensory properties in order to choose the optimal enrichment percentage and then The characteristics of enriched pomegranate juice were during the storage period. The results of Folin- Ciocalteu test showed a higher percentage of encapsulating efficiency for encapsulating of phenolic compounds with soy protein isolate (45.26%). SEM images showed that the particles are relatively spherical and with a relatively smooth surface. Sensory evaluation results showed that mainly the samples containing

less than 5 mg of nanocapsules for chitosan nanocapsules and less than 34 mg of microcapsules for soy protein isolate microcapsules had a significant difference (05 /<P) did not affect the scores of color, taste and overall acceptance of the samples with the control samples, but increasing the percentage of enrichment decreased the scores. The samples enriched with chitosan nanocapsules showed a greater decrease in pH and the samples enriched with soy protein isolate microcapsules showed a lower pH decrease than the control samples during the storage period. The L* increased only at the end of the first month of storage under the influence of enrichment compared to the control samples, and in the rest of the months, it did not show a significant difference (P<05) with the control sample. Regarding a* and b*, enriched samples of pomegranate juice showed an increase during the storage period compared to the control sample. Enrichment of pomegranate juice with chitosan nanocapsules up to 3 months of storage increased the antioxidant activity of the samples, while enrichment with soy protein isolate microcapsules increased the antioxidant activity only at the end of the fourth month. According to the data of the present research, the enrichment of pomegranate juice using chitosan nanocapsules due to the appropriate morphology, lack of effect especially on the viscosity and sensory evaluation results, increasing the antioxidant activity of the samples and also the fact that chitosan is used in various industries is currently Now, it can be a promising and economical solution for food storage.

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درون پوشانی ترکیبات فنولی آب ریواس و تاثیر آن ها بر خواص آب انار

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چکیده

هدف از این پژوهش درون پوشانی ترکیبات فنولی آب ساقه ریواس با کیتوزان و ایزوله پروتئین سویا و تاثیر آن ها بر خواص آب انار است. به این منظور ترکیبات فنولی آب ساقه ریواس توسط متانول استخراج و با روش نانوامولسیون با کیتوزان و ایزوله پروتئین سویا درون پوشانی شد و در نهایت از میکروکپسول ها و نانوکپسول های حاصل برای غنی سازی نمونه های آب انار استفاده شد. بیشترین درصد بازده درون پوشانی ترکیبات فنولی با ایزوله پروتئین سویا (۴۵/۲۶ درصد) بود. نتایج تصاویر SEM نشان داد ذرات به صورت نسبتا کروی و با سطح نسبتا صافی تشکیل شده اند، همچنین نانوکپسول های کیتوزان با اندازه متوسط ۲۸۱/۴ نانومتر و میکروکپسول های ایزوله پروتئین سویا با اندازه متوسط ۲۲/۳۳ میکرومتر تشکیل شده بودند. نتایج بررسی pH نشان داد نمونه های غنی شده با نانوکپسول های کیتوزان و میکروکپسول های ایزوله پروتئین سویا به ترتیب کاهش pH بیشتر و کمتری نسبت به نمونه های شاهد در طی دوره نگهداری نشان دادند. بررسی مهار رادیکال DPPH نشان داد غنی سازی آب انار با میکروکپسول کیتوزان تا ۳ ماه نگهداری باعث افزایش فعالیت آنتی اکسیدانی نمونه ها شد.