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Investigation of anthocyanin extraction conditions from red grapes using microencapsulation method and its application in Red Velvet cake

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ABSTRACT

The effect of applying microencapsulated red grape peel extract with Persian gum and basil seed mucilage on the physicochemical properties (total phenolic content,DPPH radical scavenging activity, anthocyanin content, thermogravimetric analysis, and Fourier-transform infrared spectroscopy) was investigated with the aim of improving its stability and using it in red velvet cake. The results showed that the addition of microencapsulated red grape peel extract to red velvet cake led to an increase and maintenance of total phenolic content and antioxidant activity in the red velvet cake samples. Moreover, microencapsulation of the extract improved the stability of phenolic and antioxidant compounds in the microencapsulated extract compared to the free extract. The obtained results indicated that total phenolic content and DPPH radical scavenging percentage of the extract were significantly affected by microencapsulation (P≤0/05). By adding 6% microencapsulated extract to red velvet cake, the total phenolic compounds and DPPH radical scavenging percentage increase compared to the free extract, and up to the seventh day of storage, higher amounts of total phenolic compounds and DPPH radical scavenging percentage were observed in the red velvet cake containing microencapsulated extract than in the cake with free extract In addition, the microencapsulated samples with a ratio of 2:1 (2% Persian gum and 1% basil seed mucilage) significantly enhanced the stability of the red grape peel extract and showed higher efficiency in the stability tests against pH changes, environmental moisture, and solubility behavior compared to the control sample. In this study, sample No. 4, containing 2% Persian gum and 1% basil seed mucilage, was introduced as the superior treatment. This selection was made based on obtaining the highest scores in the conducted evaluations. The shelf life of red velvet cake in this treatment was confirmed for 7 days under refrigerated storage, without any considerable quality deterioration.

1-Introduction

Anthocyanins are natural, water-soluble pigments consisting of polyhydroxyl and methoxyl-2-phenylbenzopyrylium glycoside derivatives that are widely found in nature. Anthocyanins are an important and sought-after type of polyphenol in food coloring and exhibit a wide range of biological activities, including antibacterial, anti-inflammatory, antidiabetic, and anticancer effects [1,2]. Anthocyanins are a subgroup of phenolic compounds that fall within a larger class of plant secondary metabolites called flavonoids. Due to their special chemical structure, these compounds are able to play an effective antioxidant role by donating hydrogen atoms to free radicals and inhibiting the chain of oxidative reactions and prevent the formation of oxidation products [3]. Among the natural sources containing anthocyanins, red grape pomace, which is known as a by-product of grape processing industries, is considered a rich, accessible and economical source for extracting these compounds [4]. In addition to containing bioactive compounds, this industrial waste has a moisture content in the range of 50 to 72%, a lignin content between 16.8 and 24.2%, and a protein content of less than 4%. The majority of grapes produced are used to prepare grape juice, and as a result, the resulting pomace can be used as a raw material with high added value. The amount of anthocyanin present in plant tissues is affected by factors such as environmental conditions and harvest time. Also, the cell wall of grape pomace is mainly composed of pectic substances. This pulp contains a diverse range of polyphenols including anthocyanins, flavonols, phenolic acids, resveratrol, and citric acid [5]. Phenolic compounds in grapes play a role in the prevention of diseases such as cancer and atherosclerosis due to their antioxidant and functional properties [6]. Despite these advantages, the practical application of anthocyanins in food systems faces some limitations. The inherent instability of these compounds and their untargeted release reduce their biological efficacy [7,8]. Among the important challenges in the industrial use of anthocyanins are the loss of stability during processing and the difficulty of controlling their chemical reactions [9,10]. In order to overcome these problems, the use of encapsulation methods has been considered. This method allows for controlled release and targeted delivery of anthocyanins by creating a physical protective layer around the active compounds. [11,12] Various methods for microencapsulation of anthocyanins have been developed to overcome limitations such as stability, low oral bioavailability, and poor intestinal absorption, since anthocyanins are very sensitive to factors such as heat, light, and oxygen, especially in Products that require heat treatment have limited direct application. In this regard, spray drying technology is known as one of the most common and cost-effective methods for microencapsulation of hydrophilic and hydrophobic materials. This method is a fast, simple and scalable process in which a suspension or emulsion solution containing the active ingredient is sprayed in the form of fine droplets in a chamber at a high temperature (usually more than 100 ° C) and finally a dry powder is produced [13]. One of the most fundamental challenges in the microencapsulation process is the selection of a suitable wall material so that, in addition to having high stability, it has the ability to effectively protect the core compound against environmental factors such as heat, light, moisture and oxygen. Also, in food applications, these materials must be food grade in terms of safety and usability and approved by relevant standards [14]. Food gums or hydrophilic hydrocolloids show high microencapsulation ability. Among the gums that can be used in the food industry in the field of microencapsulation is gum zedo or almond mountain, which is the main part of the ingredients The chemical composition of this gum is composed of polysaccharides, along with terpenoid compounds.Proteins such as Bassorin and other compounds such as Glucuronic Acid and Glucoronique

AcidGalacturonic Acid and Uronique Acid are found in gums by Gums are used at low concentrations due to their ability to produce high viscosity pro.Their special polysaccharide structure is very hydrophilic and can easily swell up to 200 times its volume.These materials do not have a freezing point and are insoluble in alcohol and other solvents. This The materials have wide application in industry due to their secondary physical properties [16, 15] of other compounds Natural substances of interest in microencapsulation include mucilages. These substances are products of plant metabolism.They are obtained and are very similar to gums in terms of structure and function. Mucilages are mainlyComplex polysaccharides are formed and are expected to break down into sugars and sugar acids upon hydrolysis.It is reported [17]. In basil seeds, upon exposure to an aqueous environment, the outer pericarp layer rapidly absorbs water and swells, creating a gel-like coating around the seed. This mucilage layer can be separated and, after drying, used as a natural polysaccharide in food product formulations. The swelling and gel-forming property of these seeds is due to the presence of polysaccharide compounds in their structure. Studies have shown that the polysaccharide extracted from basil seeds mainly consists of glucomannan (about 23%) with (2→1) cross-linkages and xylan (about 22.22%). In addition, a smaller amount of glucan (about 31.2%) has been reported in its structure. Also, the presence of highly branched arabinogalactans alongside glucomannan and xylan has been identified as part of the complex structure of this mucilage, which can play an effective role in its rheological and functional properties [18]. Chitgar et al. (2012) investigated the effect of temperature and solid content on the degradation kinetics of seedless barberry anthocyanins [19]. Abdel-Aal et al. (2018) microencapsulated concentrated (CE) and crude (RE) red cabbage (Brassica oleracea) anthocyanin extract to produce a natural food colorant using different concentrations of maltodextrin and gum arabic. The results showed that CE anthocyanin was 16 times higher than RE, and the formulation containing the highest amount of maltodextrin had the highest yield, with water activity in the range of 0.48 to 0.64 [20]. Tonon et al. (2008) microencapsulated the phenolic compounds of Northern berries with different wall materials, with the best results obtained using maltodextrin [21]. Mahdavi et al. (2016) used encapsulated barberry pigments in jelly powder as a substitute for synthetic color. The jelly containing 7% microencapsulated powder received a higher sensory score than the commercial jelly containing synthetic color [22]. Assous et al. (2017) stated that producing such products, in addition to the naturalness of their raw materials and high nutritional value due to high antioxidant activity, phenolic compounds, and anthocyanins, can be used as a completely natural pigment [23]. On the other hand, interest in developing food colors from natural sources as substitutes for synthetic colors has increased due to legal actions and consumer concerns. The aim of this research was, in the first stage, to optimize the extraction of anthocyanins from red grape skin using ethanol solvent. In the next stage, the extracted anthocyanins were microencapsulated using Persian gum and basil mucilage by spray dryer, and their physicochemical properties were investigated. Finally, the produced natural pigment powder was used in red velvet cake to evaluate its effect on the physicochemical properties and final quality of the product.

2- Materials and Methods

Red grape skin was prepared from the waste of a red grape juice production factory. It was then dried in an oven at 61°C (Behdad, Iran) and carefully ground using an electric grinder (Moulinex, Germany). To homogenize the particles, the powder was sieved through a 35-mesh sieve. The obtained powder was stored in dark glass containers in a freezer (-18°C) until the end of the experiments. Other required chemicals were purchased from Merck, Germany [24].

2-1. Preparation of Basil Mucilage and Persian Gum

First, basil seeds were manually cleaned. Then, to extract the hydrocolloid compounds, the seeds were placed under optimal conditions including a temperature of 37°C and a water-to-seed ratio of 50:1. Extraction was performed using a basket centrifuge (Hettich DMO412, Germany). The resulting extract was dried in a laboratory oven at 60±2°C. Afterwards, the dried material was ground and sieved using a mill (Artisan 5000) and a 19-mesh sieve. The final mucilage powder was stored in dry, cool conditions until use. The required Persian gum was also procured from a reputable herbal store in Tehran. This gum was ground using a mill (Bosch, model CNCM13ST1B) and then passed through a 100-mesh sieve to homogenize the particles [24].

2-2. Preparation of Extract Using Ultrasound Waves

To extract anthocyanins, 10 g of red grape pomace skin sample and 80 mL of solvent (a ratio of 1:8) were placed in a laboratory sonicator (model L20 - VCLEAN1). The solvent used consisted of ethanol and hydrochloric acid. The sonication process was performed at times of 15 and 25 minutes, and the ultrasound intensity was set to 100. The extracted extract was then filtered using Whatman No. 5 filter paper. In the final step, the extracted extract was concentrated using a rotary evaporator at a temperature of 19°C for 3 hours until reaching 49 °Brix [25].

2-3. Measurement of Total Phenolic Compound Content of the Extracted Extract

The total phenolic compound content of the produced extract was determined by the Folin-Ciocalteu method, and the results were expressed as mg of gallic acid per 199 mL of extracted extract. 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent. After 3 minutes, 2 mL of 7.5% sodium carbonate solution (75 g/L) was added, and the mixture was incubated at 27°C for 30 minutes. Then, the absorbance of the sample was read at 760 nm using a spectrophotometer. The total phenolic compound content was determined using equation (1)[26].

Formula (1): $y = 0.0012x - 0.0419$

In formula (1), x represents the concentration in µg/L, and y represents the absorbance percentage.

2-4. Evaluation of Antioxidant Activity of the Extracted Extract by the DPPH Radical Method

The antiradical property of the extracted extract was assessed based on the ability to donate hydrogen atoms or electrons in ethanol extracts or the decolorization of the purple solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol [27]. It was calculated using formula (2):

Formula (2): DPPH% = (A_control - A_sample / A_control) * 100

In formula (2), A_control is the absorbance of the control sample (DPPH radical solution without sample), and A_sample is the absorbance of the DPPH radical solution with the sample.

2-5. Measurement of Anthocyanins by the pH Differential Method

To investigate the effect of pH on the color efficiency of anthocyanins, 100 mg of red grape powder extract was added to 10 mL of aqueous solutions at pH = 1 and 4.5, and kept at room temperature for 30 minutes. Then, the absorption spectrum of the solution was measured at wavelengths of 520 and 700 nm using a spectrophotometer [28]. It was calculated using formula (3):

formula (3): A = (A_520nm - A_700nm)_pH1.0 - (A_520nm - A_700nm)_pH4.5

TAC = (A * MW * DF * 1000 * V) / (ε * L * M)

A = final absorbance, MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF = dilution factor, V = extraction volume (mL), ε = molar absorption coefficient of cyanidin-3-glucoside (29600 L/mol·cm), L = cell length (cm), M = weight of dry grape pomace skin (g)

2-6. Microencapsulation of Red Grape Skin Extract

For microencapsulation of the major bioactive compounds in the optimized extract from red grape skin, Persian gum and basil seed mucilage walls were selected in ratios of PG/BSG (0:1, 1:2, 2:1, 1:1 w/w) based on the results of other studies and preliminary experiments. Basil mucilage and Persian gum walls were added to the grape skin extract at a ratio of 3:1 and homogenized for 30 minutes at 1000 rpm using a homogenizer (APV Homogenisiers Albertslund 2000). A spray dryer (Buchi Laboratoriums-Technik, Switzerland Model B-19) manufactured in Korea was used to produce the powder, and the inlet temperature was set to 160°C [28].

2-7. Process Yield (Dry Weight Basis Efficiency) and Encapsulation Efficiency

Encapsulation yield refers to the total amount of encapsulated material (core material + wall) obtained relative to the initial raw materials. The process yield was obtained by calculating the ratio of the total powder after drying to the total weight of solids in the suspension before drying and is usually expressed as a percentage using formula (4), where Mp is the mass of the final encapsulated product and Mi is the mass of solids in the initial feed [29, 30].

Formula (4): EY(%) = (Mp / Mi) * 100

2-8. Morphology of Microcapsules

To accurately evaluate the morphology and surface characteristics of the produced capsules, a scanning electron microscope (SEM) model KYKY-EM3200 (KYKY, China) was used. The samples were first coated with a thin layer (a few nanometers) of a gold-palladium alloy using a coater. This sample preparation took about 3 minutes. After preparation, the samples were placed in the SEM device. In this method, by irradiating a high-energy electron beam onto the sample surface, backscattered and secondary electrons are received by the device's detector, and an image with 5000x magnification of the surface details of the capsules is recorded. These images provide valuable information regarding the shape, size, uniformity, and surface roughness of the capsules [31].

2-9. Thermogravimetric Analysis (TGA)

The thermal stability of the prepared samples was investigated using a thermogravimetric analyzer at a rate of 10°C/min in the temperature range of 25°C to 500°C [32].

2-10. Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared spectra of the samples were taken separately on an FTIR device (model Perkin Elmer, USA) equipped with OMNIC IR-FT software. FTIR spectroscopy was performed in the wavelength range (650-14000 cm^{-1}) [33].

2-11. Solubility

To determine the solubility percentage of the microencapsulated powder, 1 g of the powder was first added to 100 mL of distilled water. The resulting mixture was stirred for 5 minutes using a magnetic stirrer (Stuart, Scientific LTD, UK) at high speed to uniformly disperse the particles in the water. Then, the obtained suspension was centrifuged for 5 minutes at 3000 rpm (using the model mentioned in the main text). After centrifugation, 25 mL of the supernatant was carefully taken and transferred to a petri dish. This liquid was heated in a laboratory oven at 105°C for 5 hours until all the water evaporated. The weight remaining in the petri dish after drying indicated the amount

of dissolved substance. The final solubility percentage was determined by calculating the difference between the initial weight of the powder and the weight of the remaining material after water evaporation [34].

2-12. Moisture Content

The moisture content of the microencapsulated powder was calculated using the oven-drying method. 2 g of powder was dried in an oven at 110°C for 12 hours until a constant weight was reached, and the moisture content was calculated [35].

2-13. Production of Red Velvet Cake Containing Microencapsulated Red Grape Powder

In this research, red velvet cake was prepared using microencapsulated red grape skin powder as a natural pigment. The required materials are presented as percentages: Egg: 8%, Sugar: 18%, Flour: 30%, Cocoa powder: 0.5%, Powdered milk: 5%, Baking powder: 0.05%, Salt: 0.5%, Oil: 20%, White chocolate: 9%, Vanilla: 0.5%, Microencapsulated anthocyanin color: 6%. To prepare the cake, first sugar, oil, and eggs were mixed well in a mixer until uniform. Then cocoa powder, vanilla, and the microencapsulated color were added to this mixture. In the next stage, flour, baking powder, and salt were mixed in a separate container to achieve uniformity. Then the dry ingredients were gradually added to the cake batter and gently mixed. Baking was done at 175°C [36].

2-14. Measurement of Phenolic Compounds and Antioxidant Activity on Production Days, 3rd, and 7th

The total amount of phenolic compounds in the sponge cake was measured according to the method of Mau (2005) with minor modifications. After production, the cake was stored in a closed container in a refrigerator and monitored and tested until the seventh day. In this regard, the control sample (containing free extract) was also stored in a separate container in the refrigerator. Then, on the designated days, samples were taken and prepared for relevant tests. The free radical scavenging property of the extract from the cake was assessed based on the ability to donate hydrogen atoms or electrons in ethanolic extracts or the decolorization of the purple solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. To draw the standard curve, standard gallic acid dilutions of 100-700 mg/L and equation (5) were used [37].

Formula (5): $y = 0.0062x + 0.3007$

3- Results and Discussion

3-1. Investigation of the Efficiency of Anthocyanin Pigment Extraction from Red Grape Skin by Solvent and Ultrasound

Under optimal extraction conditions using ultrasound at 50°C with 96% ethanol solvent, a time of 25 minutes, and ultrasound intensity set to 100, the amount of anthocyanin was 129.635 mg/mL of extract, and phenolic compounds were 115.118 mg of gallic acid per 100 mL of extract. Zahed et al. (2022) in a study on the extraction of anthocyanins from pomegranate peel found that ethanol solvent had the highest extraction yield. According to their report, this yield was 1.445 ± 0.76%, consequently, the maximum total anthocyanin content was 3.146 ± 0.035 mg cyanidin-3-glucoside per gram of pomegranate peel powder, and the highest amount of phenolic compounds was 310.589 ± 4.246 mg of gallic acid per 100 g of extract [38]. Ansari and Hojjati (2017) stated that increasing temperature decreases the amount of anthocyanins, and increasing the solvent-to-solid ratio increases extraction and reduces extraction time [39]. Consequently, increasing the solvent-to-solid ratio increases the extraction efficiency because the concentration difference between the solid and liquid phases increases, facilitating the diffusion of bioactive compounds into the liquid phase. Also, increasing the solvent volume reduces the viscosity of the medium and accelerates

permeability, thus reducing the time required to achieve the desired extraction yield.

3-2. Investigation of the Antioxidant Activity of the Extract

The investigation of the antioxidant activity of the red grape skin extract showed a significant difference between the samples extracted with ethanol and acidic water. In other words, the highest antioxidant activity was related to sample No. 1 (extract extracted with 96% ethanol at 25 minutes), which had a significant difference from other samples extracted with acidic water ($P \leq 0.05$). The antioxidant power of the extracted extract was calculated as 70.35%. Escribano-Bailon et al. (2019) also acknowledged that there is a direct relationship between the extraction of phenolic compounds and antioxidant activity, which is due to the inherent reducing property of compounds with high reducing potential that allows them to transfer electrons to other molecules [40]. Therefore, the increase in antioxidant activity in the ethanolic extract can be attributed to the higher amount of phenolic compounds and their greater ability to neutralize active radicals. Hence, the more phenolic compounds in the extract, the greater the rate and extent of DPPH radical reduction. For this reason, the results of Bucić-Kojić et al. (2011) regarding the effect of longer extraction times on increasing antioxidant activity can be easily explained [41].

3-3. Phenolic Compound Content of the Extract

The results of the evaluation of phenolic compounds in the investigated samples shown in Table 1 indicated that the highest amount of phenolic compounds was observed in sample 1 (extract extracted with ethanol, 25 minutes) with 53.44 mg/mL and sample 2 (extracted with 96% ethanol at 15 minutes) with 45.64 mg/mL. There was a statistically significant difference compared to other samples extracted with acidic water ($P \leq 0.05$). Zahed et al. (2022) stated that the highest measured total phenolic compounds were recorded for the solvent (ethanol) extraction, equivalent to 310.589 ± 4.246 mg of gallic acid per 100 g of extract, which had higher DPPH radical scavenging activity than the anthocyanin extract extracted with (acidic) solvent [38]. In a study conducted by Bagheri et al. (2015), they also acknowledged that phenolic compounds in red grape juice were higher when extracted with 96% ethanol than with other solvents [42]. Consequently, the highest amounts of phenolic compounds were observed in ethanolic extracts (especially 96% ethanol) and at extraction times (15-25 minutes). These results are due to the polarity compatibility of ethanol with the structure of phenolic compounds, preventing the decomposition or oxidation of these compounds in a non-aqueous environment, and providing sufficient time for solvent penetration and release of compounds from the plant matrix. These findings are consistent with previous studies and emphasize the superiority of ethanol as an extraction solvent for phenolic compounds.

Table 1. Investigation of antioxidant and phenolic activity of the extracted extract

Variable	Code1	Code2	Code3	Code4	Code5	Code6
Antioxidant Activity	69.35±2.8^{a}	62.23±2.5^{b}	51.28±1.9^{e}	54.12±1.7^{c}	49.19±1.1^{f}	51.36±2.5^{d}
Phenolic activity)mg gallic acid/100ml(	53.44±2.8^{a}	45.64±1.7^{b}	41.45±1.4^{c}	37.26±1.2^{d}	36.61±1.3^{e}	29.98±1.20^{f}

Code (2): Extraction with ethanol at T=15min
Code (3): Aqueous acid solvent at T=15min
Code (4): Aqueous acid solvent at T=25min
Code (5): Acidic water solvent + ethanol extraction T=15 min
Code (6): Acidic water solvent + extraction with ethanol 96 T = 25min

3-4. Anthocyanin Content

The results of measuring the total anthocyanin content show that the highest amount of anthocyanin extracted from the extract was related to sample 1 (extraction with 96% ethanol, 25 minutes) at 40°C, which was calculated as 129.635 mg/mL of extract, and the solvent-to-solid ratio was predicted to be 1:8. There was a statistically significant difference with sample 6 (ethanol + acidic water solvent at 25 minutes), which was 71.68 mg/mL ($P \leq 0.05$). Malviya et al. (2014) found that the highest extraction yield of anthocyanins from pomegranate peel is achieved when using an ethanol/water solvent mixture at a volume ratio of 50:50. This yield was significantly higher than other solvents investigated [43]. Jackman et al. (1987) also confirmed that a high proportion of ethanol in the solvent has the greatest impact on the extraction yield of anthocyanins. Also, increasing the temperature to 55°C reduced the extraction yield. This phenomenon likely occurs because at higher temperatures, some volatile compounds may be released more rapidly from the extract, leading to a decrease in overall extraction yield [44]. The reason for these findings can be related to the chemical nature of anthocyanins and the type of solvent. Ethanol, due to its polarity compatibility with the structure of anthocyanins, proper penetration into plant tissue, and increasing the solubility of these compounds, plays an effective role in increasing extraction yield. In contrast, the use of acidic water or higher temperatures may reduce the stability and even degrade sensitive compounds like anthocyanins, consequently reducing the extraction yield. In general, the appropriate combination of ethanol, a suitable solvent-to-solid ratio, and temperature control are the main factors in achieving the highest anthocyanin extraction yield.

3-5. Measurement of Anthocyanins by the pH Differential Method

The results comparing the effect of pH on the extract, shown in Table 2, indicated that increasing pH caused a significant decrease in the stability of the samples compared to the control sample ($P \leq 0.05$). With increasing pH, the stability of the samples decreased significantly, such that the highest pH stability index was related to the extract at pH=1 at 520 and 700 nm ($P \leq 0.05$), and the lowest stability index was observed at pH=4.5. Farhadi Chitgar et al. (2013) in this study investigated the stability of extracts from three barberry species native to Iran at pH 1-7 for wider application in the food industry. The results of this study indicated that with increasing pH, the constant reaction rate increased in all three samples. Anthocyanins from all three species showed the highest stability at pH=1 [44]. Ansari and Hojjati (2018) also observed that by changing the pH of the environment, a reversible change in the structure of anthocyanins occurs; at pH=1, the colored flavylium cation form is dominant, and at pH=7, the colorless carbinol form is one of the dominant forms. In both, the highest absorption intensity at pH=1 is in the range of 500-530 nm, and no peak is observed at pH=7 [45]. The present findings are consistent with the results of Farhadi Chitgar et al. (2013); they reported that when investigating the stability of extracts from three barberry species native to Iran in the pH range of 1-7, with increasing pH, the constant reaction rate increased, and consequently, the stability of anthocyanins decreased. In that study as well,

the highest stability was observed at pH=1. Overall, the results of this study show that acidic conditions, especially pH=1, are the most suitable conditions for maintaining the stability and color characteristics of anthocyanin extracts, and increasing pH to the neutral range causes a significant decrease in their stability and absorption intensity due to the change in the chemical structure of these compounds.

Table 2. Measurement of anthocyanins by pH differential

wavelength	pH=1	pH=4/5
520nm	0.912[a]	**0.794[b]**
700nm	0.141[a]	**0.093** [b]

3-6. Efficiency of Powder Preparation

According to the results shown in Table 3, considering the different input variables of the dryer and formulation, the highest extraction yield was related to sample number 4 (2% Zedo gum, 1% basil mucilage), which was calculated as a maximum of 19.81%, and a statistically significant difference was observed with other samples ($P \leq 0.05$). In fact, the higher percentage of Zedo gum compared to basil mucilage helps increase the extraction efficiency. The characteristics of wall materials can also affect the final performance of the device and increase the efficiency of the produced powder. Factors such as temperature, pressure, mixing time, and stirring can also affect the encapsulation efficiency and ultimately the yield [36, 37, 61]. Also, da Rosa et al. (2018) microencapsulated anthocyanin extract from blueberry using maltodextrin and corn starch with a wall material concentration of 18% and investigated its stability. The microencapsulation efficiency of the samples was between 74.4-85.22% [47]. Saberian et al. also investigated the thermal stabilization of saffron petal anthocyanin extract using microencapsulation and its application in a food model, and the efficiency of all walls was reported to be above 96%, with no significant difference between different wall materials (gum arabic, Persian gum, and maltodextrin) [48]. The difference in reported microencapsulation efficiency is related to the different content of core materials. In summary, the results indicate the optimality of the formulation containing Zedo gum and basil mucilage for achieving high efficiency in the dryer, and these findings can be interpreted considering the role of wall materials and process factors in previous microencapsulation studies. Overall, the combination of 2% Zedo gum and 1% basil mucilage was identified as the best combination, leading to the highest extraction efficiency. It was also found that the combination of wall materials and device operating conditions play a determining role in optimizing process yield.

3-7. Investigation of Microcapsule Morphology

The results of scanning electron microscopy (SEM) in Figure 1 showed that by increasing the percentage of basil mucilage relative to pure Zedo gum, the surface morphology changes from a spherical shape to an uneven shape with a low percentage of sphericity. In sample 3 (1% Zedo gum, 1% basil mucilage), the interactions between basil mucilage and Zedo gum caused a reduction in unevenness and a return to a flattened spherical shape. In sample 4 (2% Zedo gum, 1% basil mucilage), the surface morphology tends towards an uneven surface with a higher percentage of sphericity compared to sample 1 (2% basil mucilage, 1% Zedo gum). Gharanjig et al. (2020), in a study on microencapsulation of anthocyanins by complex coacervation using Zedo gum, cress seed gum, and gelatin type A and B as wall materials, prepared coacervates under optimal pH conditions and gum-to-gelatin ratio by freeze-drying. The morphology results showed that freeze-drying indicated that gelatin type A is a more suitable protein for coacervate

formation. The microencapsulated extract showed high thermal stability and less degradation [46]. Ahmad et al. (2018) encapsulated saffron anthocyanins using spray drying in beta-glucan and beta-cyclodextrin and investigated the release behavior of monomeric anthocyanins, antioxidants, and phenols. The structure of microcapsules was observed using SEM, and specific encapsulated particles in the cavities of the wall materials from SEM micrographs confirmed the presence of anthocyanin compounds in the microcapsules [49].

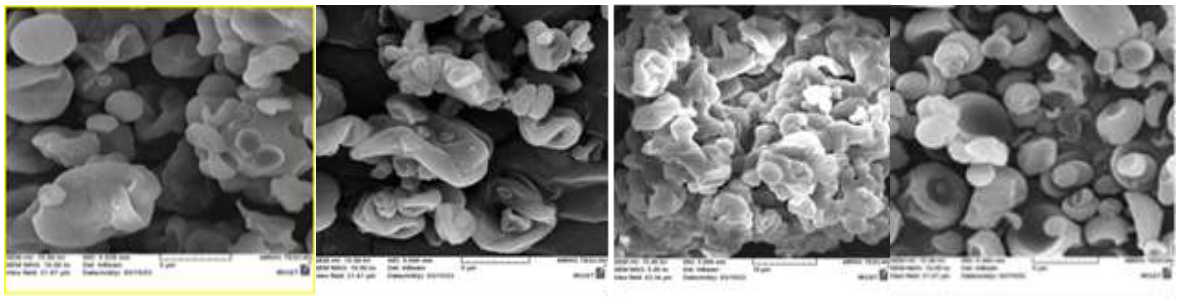

Fig 1 a: (1% gum zedo, 0% basil mucilage, 1% grape extract) b: (0% gum zedo, 1% basil mucilage, 1% grape extract) c: (1% gum zedo, 1% basil mucilage, 1% grape extract) d: (2% Zedo gum, 1% basil mucilage, 1% grape extract)

3-8. **Investigation of Thermogravimetric Analysis (TGA)**

The results of thermal analysis (TGA) in Figure 2 showed that thermal stability increased with the increase in the ratio of Zedo to basil. In the case of sample (4) (2% Zedo gum, 1% basil mucilage), electrostatic interactions between the functional groups of basil and Zedo gave more strength to the network structure, increasing thermal stability and consequently the stability of the compound. Whereas in sample (1) (1% Zedo gum, 2% basil mucilage), these electrostatic interactions were absent, and it had lower stability than the other three samples. Mahdieh Ansari et al. (2017), to increase the stability of the liquid color produced from red onion peel and red cabbage against environmental factors, performed microencapsulation by freeze-drying with two agents, gum arabic and maltodextrin. According to the results of TGA and DSC tests, gum arabic had greater resistance to heat, so that the onset degradation temperature of the encapsulated powder by this encapsulant increased from 63.82 to 250.87 for red onion peel and from 81.03 to 263.64°C for red cabbage [39]. Overall, increasing Zedo gum in the formulation improves the thermal stability and increases the resistance of the samples against thermal degradation. These findings are also consistent with the results of Mahdieh Ansari et al. (2017); in their study, gum arabic showed higher thermal resistance compared to maltodextrin and increased the onset degradation temperature of microencapsulated powders. Consequently, Zedo gum acts as a more effective encapsulant than basil mucilage, and using a higher ratio of it can improve the thermal stability and final quality of the microencapsulated compound.

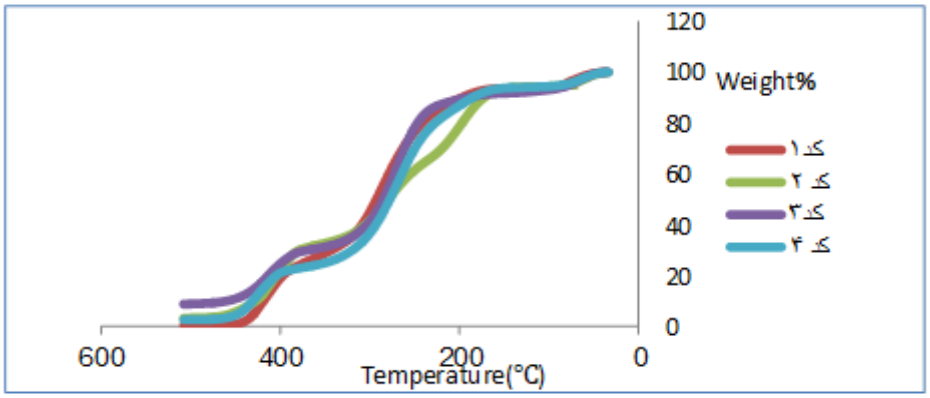

Fig 2 Thermal analysis of microencapsulated samples

acode1: (1% gum zedo, 0% basil mucilage, 1% grape extract) bcode2: (0% gum zedo, 1% basil mucilage, 1% grape extract)

ccode3: (1% gum zedo, 1% basil mucilage, 1% grape extract) dcode4: (2% Zedo gum, 1% basil mucilage, 1% grape extract)

3-9. Fourier-Transform Infrared Spectroscopy (FTIR)

Comparison of the FTIR analysis of the compounds in Figure 3 shows that with increasing percentage of Zedo gum, hydrogen interactions cause an increase in the peak in the 3100-3450 cm^{-1} region, and this broadening in this region confirms this electronic-electrostatic interaction. FTIR spectroscopy showed that the observed shift in the peak related to aromatic groups, between 1616 and 1641 cm^{-1}, indicates the interaction between basil and Zedo. Furthermore, the peaks related to the C-O bond, which appeared in the range of 1057 to 1100 cm^{-1}, confirm these interactions and prove the formation of a network between basil and Zedo. The shift of these peaks also justifies the color change. Mansour et al. (2020) conducted a study involving the microencapsulation of anthocyanin extract (AC) from red raspberry using freeze-drying including ultrasound, soy protein isolate (SPI), gum arabic (AG), and their combination [50]. Movahed Navi et al. (2015) stated that there is a mutual chemical interaction between wall materials and core materials. All microcapsules increased the thermal stability of AC in the temperature range of 80-114°C. Furthermore, the preservation of AC (up to 48%) during storage at 37°C for 60 days showed that the combined treatment of SPI and AG was the best [51].

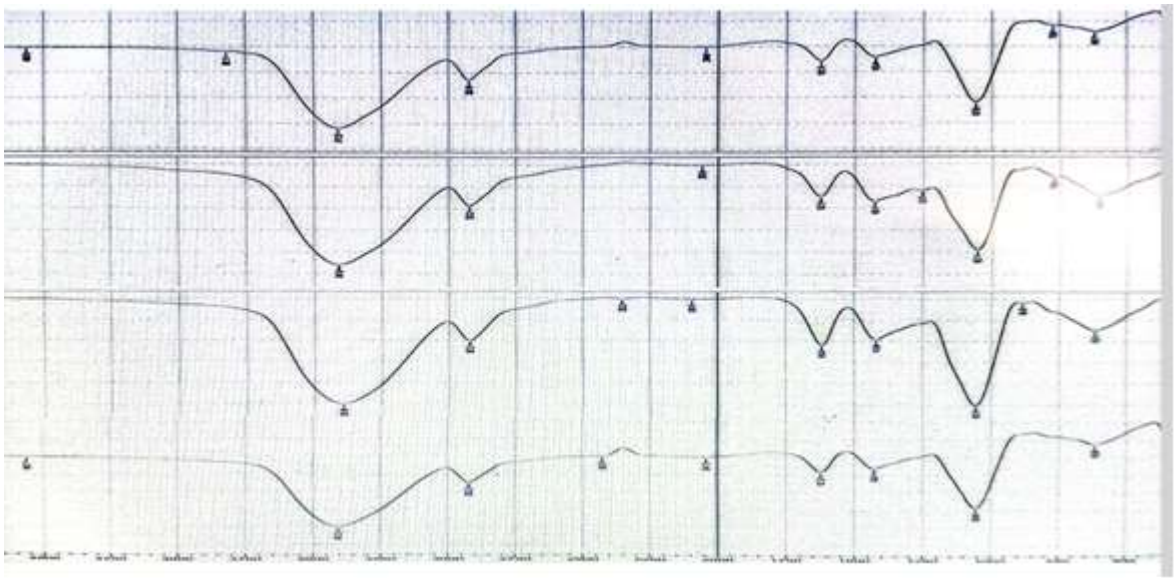

Fig 3 Infrared spectroscopy of microencapsulated samples

a: (1% gum zedo, 0% basil mucilage, 1% grape extract) b: (0% gum zedo, 1% basil mucilage, 1% grape extract)

c: (1% gum zedo, 1% basil mucilage, 1% grape extract) d:(2% Zedo gum, 1% basil mucilage, 1% grape extract

3-10. Moisture Content

According to the data in Table 3, two samples of microencapsulated red grape skin extract powder were compared in terms of moisture content. The highest moisture content was related to sample 3 (1% Zedo gum, 2% basil mucilage), which was calculated and had a statistically significant difference ($P \leq 0.05$). Kouroshian et al. (2015) found that basil samples with a higher mucilage ratio should retain more moisture. This is due to the heteropolysaccharide nature of basil mucilage, which has a branched structure and hydrophilic groups. These hydrophilic groups can bond with water molecules and prevent their evaporation and exit [52]. According to the results of Yu and Huang (2009), increasing the temperature decreased the moisture percentage of the samples because this factor facilitates water removal by increasing the rate of moisture transfer from the particles [53]. Athanasia et al. (2004) observed in their research that both drying air temperature and high pressure are effective in accelerating the drying process and reducing moisture. Their results also showed that increasing the inlet temperature to the dryer leads to a reduction in moisture. This phenomenon occurs because the greater the temperature difference between the drying environment and the particles, the greater the heat transfer to the particles, and consequently, the faster the moisture removal from the particles [54]. The results show that increasing the drying temperature reduces the moisture content in the produced powders. Also, the moisture content in samples with a higher ratio of basil mucilage is higher than in other samples because basil mucilage is a heteropolysaccharide complex with a branched structure that has hydrophilic groups, thus bonding with water molecules and preventing their exit.

3-11. Solubility

According to the data in Table 3, the highest solubility was observed in sample 4 (2% Zedo gum, 1% basil mucilage), and there was a statistically significant difference with sample 1 (1% Zedo gum, 2% basil mucilage) ($P \leq 0.05$).

Hesarinejad et al. (2021) stated that for most powdered foods, the goal is rapid and complete dissolution in water, wetting (submerging), and dissolving without lump formation. Therefore, it can be observed from the results that there is a positive relationship between solubility and the moisture content of the powders [55]. Fazaeli et al. (2012) investigated the effect of spray drying conditions and the use of encapsulants on the physical properties of blackberry extract powder. The results showed that a mixture of 2% maltodextrin (DE=6) and 6% gum arabic had the highest solubility (86%) [56]. Consequently, Zedo gum plays an effective role in improving the solubility of microencapsulated powder, and this property is desirable from a practical perspective because for powdered foods, the goal is rapid and complete dissolution in water without lump formation. Zedo gum plays an important role in increasing the solubility of the microencapsulated powder, and this desirable property is also positively correlated with the moisture content of the powder.

Table 3. Moisture, solubility, and extraction efficiency of microencapsulated powder

Variable	Code1	Code2	Code3	Code4
Solubility(%)	40.91 ± 0.01^{b}	39.67 ± 0.01^{d}	40.67 ± 0.01^{c}	43.33 ± 0.03^{a}
humidity(%)	3.35 ± 0.01^{c}	8.06 ± 0.00^{b}	8.19 ± 0.00^{d}	1.61 ± 0.00^{a}
Extraction efficiency(%)	14.59 ± 0.01^{b}	8.15 ± 0.01^{c}	5.23 ± 0.00^{d}	21.32 ± 0.01^{a}

3-12. Anthocyanin Content

The results of the evaluation of the total anthocyanin content in the 2 tested samples showed that the highest amount of anthocyanin in the microencapsulated sample was observed in sample 4 with a ratio (2% Zedo gum, 1% basil mucilage) at 122.117 mg/mL, which had a statistically significant difference ($P \leq 0.05$) with microencapsulated sample number 1 (1% Zedo gum, 2% basil mucilage) at 79.84 mg/mL. Shahidi et al. (2014), during a study on optimizing the spray drying of pomegranate juice, stated that at low amounts of maltodextrin, increasing the temperature causes a greater reduction in the total anthocyanin content because maltodextrin cannot effectively encapsulate anthocyanins, which in turn causes a rapid decrease in total anthocyanin content [57]. Tonon et al. (2010) investigated the degradation of anthocyanins during the storage of encapsulated powder produced from Açaí with different coating materials and concluded that the degradation reaction of anthocyanins follows a first-order equation. Also, increasing temperature had a negative effect on the anthocyanin content of the resulting powder, but the type of encapsulant did not have a significant effect on the degradation or preservation of anthocyanins, and the anthocyanin in the non-encapsulated powder was degraded more severely [58]. Overall, the findings show that the type and ratio of coating materials in the microencapsulation process play an important role in preserving anthocyanins, and the composition of sample 4 with a ratio (2% Zedo gum, 1% basil mucilage) was able to create greater stability and preservation of anthocyanins.

3-13. Investigation of Total Phenolic Compound Content of the Cake

The highest and lowest phenolic compound content were observed in the cake sample containing 6% microencapsulated powder on the production day (112.185 mg gallic acid per 100 mL) and the lowest was observed in the

control cake sample containing free extract (26.95 mg). The cake sample contained 6% microencapsulated extract. All samples had a statistically significant difference in terms of phenolic compound content ($P \leq 0.05$). The results of the study by Sari et al. (2012) also showed that the presence of copigments such as phenolic acids in the beverage food model led to an increase in phenolic content compared to samples without copigment [59]. Statistical analysis of the data showed that all studied samples had statistically significant differences from each other in terms of phenolic compound content ($P \leq 0.05$). In addition, the effect of storage time on the content of phenolic compounds was evaluated. The results indicated that with increasing storage period of the cakes, the amount of phenolic compounds decreased, with the lowest amount observed on the seventh day of storage. These findings are consistent with the results of the study by Sari et al. (2012), which showed that the presence of copigments such as phenolic acids in beverage food models leads to an increase in phenolic content compared to samples without copigment

3-14. Investigation of the Antioxidant Activity of the Cake

The cake sample containing 6% microencapsulated powder on the production day had the highest antioxidant activity at 82.69%, which had a statistically significant difference with the cake sample containing free extract at 32.34% ($P \leq 0.05$). The lowest antioxidant activity was observed in the control sample, while the samples containing microencapsulated powder showed higher antioxidant activity due to better preservation of bioactive compounds. The amount of antioxidant activity in the samples and its decreasing trend during storage were consistent with the results obtained for phenolic compounds and anthocyanins in this study, with the lowest amount observed on the seventh day of storage. The findings of this research are consistent with the findings of Metini et al. (2018) regarding the increase in antioxidant activity and total phenolic compound content of yogurt samples containing microencapsulated red grape extract [60]. Mildner-Szkudlarz et al. (2009) reported high amounts of phenolic compounds and high antioxidant properties of compounds extracted from red grape pomace extract [61].

3-15. Investigation of the Anthocyanin Content of the Cake

The use of red grape skin extract and the microencapsulated powder of this extract had a significant effect on the amount of anthocyanin in the cake ($P \leq 0.05$). The highest amount of anthocyanin was observed in the cake sample containing 6% microencapsulated powder on the first day after production (110.160 mg/mL). The results showed that the application of microencapsulated powder was effective in preserving the anthocyanin content. The highest amount of anthocyanin was observed in the microencapsulated samples. This amount had a statistically significant difference with the cake sample containing free extract ($P \leq 0.05$). Also, the anthocyanin content decreased significantly during the storage period of the cake samples ($P \leq 0.05$), with the highest amount recorded on the first day of production and the lowest on the seventh day of storage. It should be noted that Drosou et al. (2015) and Marand et al. (2019) also reported high amounts of phenolic compounds, phenolic acids, and anthocyanins in red grape pomace in their studies [62, 63].

4- Conclusion

This study comprehensively investigated the effectiveness of microencapsulating red grape pomace anthocyanin extract using Zedo gum and basil mucilage, and subsequently, its application in a red velvet cake formulation. The findings of this study demonstrated that microencapsulation of the red grape pomace anthocyanin extract components, particularly with a weight ratio of 2:1 of Zedo gum to basil mucilage (2% Zedo gum, 1% basil mucilage), led to a significant increase in stability and

preservation of high levels of anthocyanins compared to the free extract. This confirms the effective protective role of the encapsulating materials against factors such as oxidation and thermal degradation during the drying and storage processes. The cake samples containing the microencapsulated powder showed significantly superior levels of anthocyanin, total phenolic compounds, and antioxidant activity compared to the control cake sample (containing free extract). Another key finding of this research was the positive effect of microencapsulation on the stability of bioactive compounds over the product's shelf life. In contrast to the control sample, which experienced a significant loss of its active compounds, the cake containing the microencapsulated powder demonstrated better preservation of these compounds during the storage period. Overall, the results of this study indicate that microencapsulation of red grape pomace extract using Zedo gum and basil mucilage is an efficient and stable method for increasing the concentration of antioxidants and natural pigments in food products. This technique not only helps preserve and enhance bioactive compounds but can also serve as a healthy and natural alternative to synthetic colors and additives in industries such as baking and confectionery, beverages, dairy products, and other food items. Therefore, the development and commercialization of this technology could represent a significant step towards producing healthier food products with higher nutritional value and greater visual appeal.

Suggestions for Future Research

1. Investigating the effect of different levels of coating materials and their different ratios on microencapsulation characteristics.

2. Evaluating the effect of different thermal processes (e.g., baking at higher temperatures) on the stability of microcapsules.

3. Studying the sensory effects (taste and aroma) resulting from the addition of microencapsulated powder to various food products.

4. Comparing the efficiency of this microencapsulation method with other common industrial techniques.

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Conflict of Interest

There are none to declare.

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بررسی شرایط استخراج آنتوسیانین از انگور قرمز با استفاده از روش ریز پوشانی و کاربرد آن در کیک ردولوت

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چکیده

تأثیرکاربرد عصاره ریزپوشانی شده پوست انگور قرمز با صمغ فارسی و موسیلاژ دانه ریحان بر خصوصیات فیزیکو شیمیایی (فنول کل، درصد رادیکالDPPH ، میزان آنتوسیانین، وزن سنجی حرارتی و طیف‌سنجی فروسرخ) با هدف بهبود پایداری و به‌کارگیری آن در کیک ردولوت انجام شد. نتایج نشان داد که افزودن عصاره ریز پوشانی شده انگور قرمز به کیک ردولوت منجر به افزایش و حفظ میزان فنل کل و فعالیت آنتی‌اکسیدانی در نمونه کیک ردولوت شد. همچنین عصاره ریز پوشانی شده موجب افزایش پایداری ترکیبات فنولی و آنتی‌اکسیدانی در عصاره ریز پوشانی شده نسبت به عصاره آزاد شد. نتایج به‌دست‌آمده بیانگر آن بود که میزان فنل کل و درصد رادیکال DPPH عصاره تحت تأثیر ریز پوشانی تغییرات معنی‌داری داشتند ($P\leq$۰/۰۵). به‌طوری‌که با افزودن ٦ درصد عصاره ریز پوشانی شده به کیک ردولوت میزان ترکیبات فنل کل و درصد رادیکال DPPHنسبت به عصاره آزاد افزایش یافته و تا روز هفتم نگهداری، مقدار بیشتری از ترکیبات فنل کل و درصد رادیکال DPPH در کیک ردولوت نسبت به عصاره آزاد مشاهده شد. همچنین نمونه‌های ریز پوشانی شده با نسبت ۲:۱ (۲ درصد صمغ زدو، ۱ درصد موسیلاژ ریحان) به میزان قابل‌توجهی موجب افزایش پایداری عصاره انگور قرمز و همچنین موجب افزایش راندمان در آزمایشات مربوط به پایداری در برابر تغییرات pH ، تأثیر رطوبت محیط و همچنین رفتار در حلالیت نسبت به نمونه شاهد عملکرد بهتری نشان دادند. در این تحقیق ، نمونه شماره ٤ که حاوی (۲ درصد صمغ زدو ، ۱ درصد موسیلاژ دانه ریحان) بود به عنوان تیمار برتر معرفی گردید. این انتخاب بر اساس کسب بالاترین امتیازات در ارزیابی‌های انجام شده صورت گرفت. قابلیت نگهداری کیک ردولوت در این نمونه به مدت ۷ روز در دمای یخچال، بدون افت کیفی قابل توجه، مورد تائید قرار گرفت.