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Original research

Effect of encapsulation of polyphenolic compounds of unripe (sour) grape waste on its quality and stability

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A B S T R A C T

In the present study, to prepare the polyphenolic extract of sour grape waste, the simultaneous effect of enzyme (pectinase, Yieldmash) and ultrasound (for 10, 25 and 40 minutes at sound intensity of 30, 60 and 90%) treatments was used and the best sample was encapsulated with maltodextrin and basil seed gum (BSG). To optimize the preparation of microcapsules, Design-Expert 13.0 and response surface methodology (RSM) were used. This design included 3 independent variables of maltodextrin level, BSG level and spray dryer inlet temperature. Finally, the best sample in encapsulation was examined by scanning electron microscope (SEM) and differential scanning calorimetry (DSC). The results showed that by increasing sound intensity, extraction efficiency, total phenol and antioxidant activity increased significantly ($p \leq 0.01$). The highest factors were observed in the sample extracted by enzyme and ultrasound at 90% sound intensity for 40 minutes ($p \leq 0.01$). In second phase, increasing the inlet temperature of the dryer and the level of maltodextrin as a carrier reduced the encapsulation efficiency and the DPPH radical scavenging activity of the capsules non-linearly. The optimum conditions included 12.129% maltodextrin, 0.5% BSG and the inlet temperature of the dryer of 177.22 °C. The results of DSC showed that the formation of complexes between the compounds led to the formation of heat-stable capsules. The average size of the particles was 1.04 micrometers. According to the results of SEM, the microcapsules had an irregular and quasi-spherical shape. At all the pH and temperatures, the microencapsulated sample had higher stability ($p \leq 0.05$). Over time, the bioavailability and release of phenolic compounds increased significantly ($p \leq 0.05$).

Keywords: Sour grape; Pectinase; Ultrasound; Encapsulation; Basil seed gum

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1. Introduction

According to the reports provided by the Food and Agriculture Organization (FAO), fruits and vegetables waste during the production chain from farm to table is estimated to be 20-40% (FAO, 2011). Agricultural-food waste can be a potential source for extracting bioactive compounds such as phenolic compounds and antioxidants used in the pharmaceutical, food, cosmetic and health industries as well as animal feed (Fontana et al., 2013). Phenolic compounds are the best natural bioactive substances due to their health benefits. However, solubility, low stability, unpleasant taste and limited bioavailability have limited their use in food formulations (Rahman et al., 2019). Sour grape is a rich source of flavonoid compounds, mainly seed and skin tannins, including flavonols and hydroxycinnamic acids. sour grape has less anthocyanin than ripe grapes. Resveratrol is mainly found in the seed coat. The amount of resveratrol in sour grape is high, while its concentration reduces in the ripe fruit. Verjuice is used in salad sauces and processed vegetables (Karapinar & Sengun, 2007; Nik Fardjam, 2008). The amount of phenols in the extracts is significantly affected by the extraction technique and processing variables such as temperature, time and type of solvents used (Setford et al., 2017). Gençdağ et al. (2023) investigated the effect of ultrasonication before the microwave thermal treatments at 60°C, 70°C and 80 °C on the quality characteristics of verjuice and stated that the ultrasound-assisted microwave heating showed a relatively lower browning index at all temperature levels, highest viscosity values and antioxidant capacity (DPPH and ABTS) compared to sole microwave heating and conventional heating. Wei et al. (2022), in the study on 9 diluted samples of biomass of thinned unripe (TUR) grape and ripe grape fruit (RGF) stated that

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the content of total phenolic, flavonoid, tannin and flavan-3-ols of TUR was 4.2-13.5, 3.6-12.3, 4.3-62.8 and 1.5-7.6 times RGF, respectively. At the same time, the antioxidant capacity of TUR was significantly higher than RGF. Turkmen et al. (2017) showed that the TP content of Verjuice varies from 436.36 and 758.52 mg/L. Guler et al. (2018) on the changes in the phenolic compounds of Verjuice during the production of concentrate, by HPLC, reported the polyphenolic compounds of gallic acid, catechin, epigalocathechin, vanillic acid, epigalocathechin gallate (EGCG), epicatechin, caftaric acid, caffeic acid, p-cumaric, ferulic acid, cinapic and quercetin. Sour grape waste makes up about 20% of fresh weight of the primary fruit (Mattivi et al., 2006). Encapsulation of bioactive compounds leads to their protection against degradation during processing and storage (Domínguez et al., 2021). Using this process, micro-sized particles are covered by wall/coating/shell materials to protect these particles under environmental conditions (Gómez et al., 2018). The features of such coverage determine the kinetics of release of bioactive or functional substances under certain conditions. In other words, encapsulation is a process in which active ingredients are packed inside a secondary material (wall) (Domínguez et al., 2021). Shell or wall materials should not react to active materials, have excellent film production capability, and have desired protective properties against various environmental conditions (Shishir et al., 2018). There are many conventional and advanced encapsulation techniques that are suitable for all kinds of food (Domínguez et al., 2021). The choice of encapsulation depends on the size, bioavailability, and biodegradability of microparticles, the physical and chemical properties of the core and coating materials, the application of microcoated materials, and the release of active substances (Nesterenko et al., 2013). Release or controlled release provides the possibility to create release, health and sensory benefits of active substances by using compounds with specific permeability properties. Controlled release is a process by which the active component (smell and flavor or bioactive compounds) trapped in the network is released to achieve the desired effects at a speed or in a certain amount. Two important types of release are delayed release and sustained release (Lactis, 2007). Bayram et al. (2023) stated during the encapsulation of verjuice with maltodextrin (MD) and pectin (PEC) that the percentage of encapsulation efficiency was significantly affected by the values of MD and PEC. In verjuice and encapsulated powder, the most phenolic composition was fumaric acid, caffeic acid, gallate, catechin and epicatechin and the encapsulation led to the preservation of more polyphenols in the verjuice powder. Estevinho and Rocha (2022) during the encapsulation of several polyphenolic compounds by the spray drying process stated that the encapsulating agents used had an effect on the type of particles obtained and their properties. Ricci et al. (2022) on the extraction and encapsulation of red wine polyphenolic compounds reported the optimal spray drying of 7 g per 100 ml of maltodextrin and inlet temperature of 110 °C. Considering the importance of developing new effective methods to recover bioactive compounds from agricultural-food waste and preservation of this compounds in different processing conditions and the passage of time, in the present study polyphenolic extract of sour grape waste was extracted by pectinase and different ultrasound pretreatment and the effect of polyphenolic encapsulation of sour grape waste on its quality and stability was investigated.

Table 1. Real and coded values of independent variables used for CCD.

Independent variables	Code	levels of variables	Unit	Independent variables	Code
		-1	0	1	
Maltodextrin	A	15	10	5	%
BSG	B	1.5	1	0.5	%
Inlet temperature	C	140	160	180	°C

Table 2. Treatments investigated in the optimization stage of the production of grape extract powder.

Run	Factor 1 A: Maltodextrin (%)	Factor 2 B: BSG (%)	Factor 3 C: Inlet temperature (°C)
1	10	1	160
2	10	1	126.364
3	10	1	160
4	10	1	160
5	5	1.5	180
6	5	0.5	180
7	15	0.5	140
8	10	1	160
9	1.59104	1	160
10	5	1.5	140
11	10	1	160
12	5	0.5	140
13	15	1.5	140
14	10	1	193.636
15	10	0.159104	160
16	15	1.5	180
17	18.409	1	160
18	10	1.8409	160
19	10	1	160
20	15	0.5	180

Table 3. Comparison of antioxidant capacity mean of sour grape waste extracts.

Enzyme type	UltraSound intensity (%)	Time (min)	Extraction efficiency (%)	DPPH (mg/mL)	Total phenol (mg/mL)
Without Enzyme	30	10	7.26 ± 0.08 ^{g}	53.27±0.01 ^{a}	5.11±0.02 ^{u}
		25	7.57 ± 0.32 ^{pq}	41.14±0.35 ^{c}	5.23±0.03 ^{t}
		40	8.05 ± 0. 12 ^{o}	36.94±0.49 ^{d}	5,44±0.03 ^{r}
	60	10	7.89 ± 0.06 ^{op}	44.24±1.45 ^{b}	5.32±0.02 ^{s}
		25	8.47 ± 0.07 ^{n}	36.29 ±0.27 ^{de}	5.51±0.03 ^{q}
		40	9.24 ± 0.08 ^{k}	3234 ±0.58 ^{gh}	5.73±0.03 ^{o}
	90	10	8.03 ± 0.10 ^{o}	40.74±0.60 ^{c}	5.60±0.04 ^{p}
		25	8.65 ± 0.01 ^{m}	33.25±0.46 ^{g}	5.97±0.02 ^{n}
		40	9.26 ± 0.12 ^{j}	30.11±0.33 ^{j}	6.18±0.04 ^{m}
Pectinex YildMASH.	30	10	9.69 ± 0.17 ^{j}	35.98±0.26 ^{e}	8.30±0.02 ^{j}
		25	10.66 ± 0.19 ^{f}	31.53±0.20 ^{i}	8.63±0.02 ^{i}
		40	11.08 ± 0.10 ^{de}	27.18±0.14 ^{l}	9.12±0.03 ^{f}
	60	10	10.07 ± 0.09 ^{hi}	32.11±0.36 ^{h}	8.83±0.03 ^{h}
		25	11.24 ± 0.06 ^{d}	26.20±0.50 ^{m}	9.32±0.06 ^{d}
		40	11.76 ± 0.08 ^{b}	25.43±0.25 ^{n}	9.50±0.04 ^{c}
	90	10	10.81 ± 0.30 ^{ef}	30.21±0.18 ^{j}	9.21±0.04 ^{e}
		25	11.48 ± 0.10 ^{c}	23.25±0.11 ^{o}	9.88±0.05 ^{b}
		40	12.08 ± 0.14 ^{a}	23.05±0.10 ^{o}	9.99±0.04 ^{a}

Different small letters indicate statistically significant differences ($p \leq 0.05$).

2. Material and Methods

2.1. Materials

Sour grape waste was obtained from Verjuice production factories. All chemicals used for chemical analysis were prepared from Merck (Germany). The equipment used are HPLC (Zwick, Germany), vacuum oven (Memmert, Germany), spectrophotometer (UNICO, America), ultrasonic cleaner (Baker, China), rotary evaporator under vacuum (EYELA, Japan), and pH meter (Hanna HI98127, China).

2.2. Preparation and drying of sour grape waste

Sour grape waste was collected immediately after the dewatering process, which was kept frozen until drying. It was dried at room temperature for approximately 48 hours until their moisture content reached 10%. Then, it was crushed and sieved to select particles with dimensions of 0.6-1 mm (Öncül et al., 2015).

2.3. Extraction by enzyme and ultrasound

Extraction was done by pectinase enzyme (Pectinex Yieldmash, Novozymes, Denmark) and ultrasound. In this way, sour grape waste affected by the ultrasound at a frequency of 25 kHz for 10, 25 and 40 minutes, and sound intensity of 30, 60 and 90. Then it was subjected to 30 mg/kg of pectinase enzyme. In order to increase the enzyme efficiency, it was placed in incubator for 40 minutes at the optimum temperature of its activity (55 °C). After the incubation, the samples were placed at 80 °C for 5 minutes to deactivate the pectinase enzyme. Then, the extract was filtered under vacuum, packed in dark glass containers and stored in a refrigerator at 5 °C. The filtered extract was first concentrated by a vacuum rotary evaporator, at a temperature of 40 °C, Then, dried in a vacuum oven at a temperature of 45 °C until reaching a constant weight. The dried extracts were kept at -18 °C until use (Oszmiański et al., 2011; Dehghan Tanha et al., 2018).

2.4. Tests of sour grape waste extracts

2.4.1. Determination of extraction efficiency

The extraction efficiency was based on the total polyphenolic mass present in 100 g of sour grape waste. For this purpose, after removing the solvent from the ethanolic extract, it was mixed with water and filtered using whatman paper (No.1) to remove its suspended particles. Then, the phenolic compounds were measured according to the Folin-Ciocalteu method. The absorbance was read by a spectrophotometer at 765 nm and a calibration curve was drawn using gallic acid as a standard. The phenolic compounds in the sample were reported based on gallic acid equivalents (Wootton-Beard et al., 2011).

2.4.2. Antioxidant activity (DPPH radical scavenging activity)

The antiradical property of the extract was measured based on the ability to donate hydrogen atoms or electrons in ethanolic extracts or the decolorization of 2-diphenyl-1-picrylhydrazyl (DPPH radical scavenging activity) in methanol (Öncül et al., 2015).

2.4.3. Identification of the extracted compounds in the sample

Quantitative and qualitative investigation and identification of phenolic compounds present in the extract of sour grape waste were based on a high performance liquid chromatography (HPLC). ODS C_{18} column (5 µm and 4.6 mm × 250 mm) was used for analytical separation and diode array detector (DAD) in HPLC (Agilent 1260 infinity). For the detection and quantification of phenolic

compounds, standard concentrations of 2.5, 5, 10, 20 and 40 mg/L were used for the calibration curves of gallic acid, vanillic acid, caffeic acid, caffeic acid, p-cumaric acid, ferulic acid, cinapic acid, quercetin and epicatechin. The concentrations of 5, 10, 20, 40 and 60 mg/L were used for calibration curves of catechin, epigalocathechin and EGCG. The extracts were diluted with methanol and filtered through a 0.45 µm PTFE syringe filter before HPLC. Then, the samples were directly injected into HPLC (Guler et al., 2018).

2.5. Encapsulation

Maltodextrin (at three levels) and BSG (at three levels) were added to the best sample of extracted sour grape waste. Then, the prepared solutions were dried with spray drying. The air flow rate of spray dryer was 600 liters per hour and the feeding pump speed of 10 milliliters per minute (Buchi Laboratoriums-Technik, Switzerland Model B-191). Design-Expert 13.0 and RSM were used in order to optimize the preparation of microcapsules of sour grape waste extract. This design included 3 independent variables (Table 1) maltodextrin level, BSG level and spray dryer inlet temperature. To select the best combination of dependent variables for the production of extract capsules, the responses of encapsulation efficiency, antioxidant activity, and solubility in water were considered. The design of the test was in the form of a central composite design (CCD) and 4 replications at the central point. The average values of the tests were used as a continuous variable or response, and the results of RSM were drawn as three-dimensional Figures. Table 2 shows the treatments studied in this part of the research.

2.6. Tests of produced microcapsules

2.6.1. Encapsulation efficiency

To obtain the efficiency, the weight of the powder obtained from the spray dryer was divided by the amount of solids of sour grape waste extract fed to the device. To obtain the amount of solids of sour grape waste extract fed to the device, the weight of the extract should be multiplied by its Brix and the weight of the additives should also be added to it. To measure the remaining phenolic compounds in the produced encapsulated powder, the Folin-Ciocalteu method was used (Ordoñez et al., 2006).

2.6.2. Solubility

To check the solubility, 100 milliliters of water was poured into the beaker and 1 g of finely coated powder was added to it. The resulting mixture was stirred with a magnetic stirrer at high speed for 5 minutes and then centrifuged at 3000 g for 5 minutes. 25 ml of the surface liquid was transferred to a petri dish and dried in an oven at 105 °C for 5 hours. The solubility percentage was calculated using the difference in weights (Tonon et al., 2009).

2.6.3. Morphology of microcapsules

The external surfaces and surface morphology of the produced capsules were examined by imaging using SEM (EM3200-KYKY, KYKY, China) (the morphology of the best capsule was examined). For this purpose, the samples were fixed on the aluminum stubs of the device and covered with gold: palladium (60:40). To investigate the shape and surface properties (fracture, depression, wrinkling, etc.), the image of the surface of the microcapsules was provided (Carneiro et al., 2013).

2.6.4. Particle size

The particle size distribution and average diameter were measured by the particle size analyzer based on the laser light diffraction method (Ersus & Yurdagel, 2007).

2.6.5. DSC

This method was used to investigate the heating curve, glass transition temperature and melting enthalpy. 5 mg of each sample was examined in the experiment. The temperature of the sample was increased from 25 to 300 °C at 10 rpm. It should be noted that DSC was performed on the sample (Abdel-Aty et al., 2023).

2.6.6. Release of phenolic compounds in the small intestine

Simulated intestinal fluid (SIF) was prepared by the methods of previous studies (Salvia-Trujillo & McClements, 2016; Zhang et al., 2016). 30 ml of the final mixture was transferred to a 100 ml glass beaker and placed in a water bath at 37 °C. 1.5 ml of SIF and 3.5 ml of bile salt (46.87 mg/ml) dissolved in phosphate buffer were added to the mixture. The pH of the solution was adjusted to 7. Then, 2.5 ml of lipase suspension (24 mg/ml) in phosphate buffer solution and 3 ml of pancreas solution (16.25 mg/ml) were added to the solution. The solution was incubated at 37 °C for 2 hours and 100 rpm. During this period, the pH of the solution was kept constant at pH = 7 using NaOH (Charpashlo et al., 2020). Sampling was done for 2, 5, 10, 20, 40, 80 and 120 minutes.

2.6.7. Release of phenolic compounds

The concentration of phenolic compounds was determined according to previous studies with some changes (Charpashlo et al., 2021; Zhang et al., 2016). To determine the concentration and study the release of phenolic compounds, the digested samples of the digestive tract were mixed with 15 ml of hexane containing 0.05% weight/volume (BHT) and vortexed twice for 10 seconds. Then, the resulting mixture was centrifuged (15 minutes, 320 g) and 3 ml of deionized water was added to it. The mixture was left at room temperature for 5 minutes until the two phases were completely separated. The absorption of phenolic compounds in the upper layer was measured at 472 nm (λmax) by a spectrophotometer (Bramley, 2000). Pure hexane was used as reference absorption or blank. The amount of phenol was calculated by the following equation.

$$Polyphenol\ (mg/100g) = \frac{Abs427 \times M_w \times V_{hexane}}{\varepsilon \times L \times g_{sample}} \times 100 \quad (1)$$

where Abs472 is absorbance of the upper layer at 472 nm, Mw is the average molecular weight of phenolic compounds, Vhexane is the volume of hexane used (10 ml), ϵ is the extinction coefficient of phenolic in hexane (17.2 × ۱۰^4 M/cm^{-1}) and L is the length cuvette (1 cm) and sample gram (0.1 g) (Salvia-Trujillo & McClements, 2016).

2.6.8. Bioavailability of polyphenols

The bioavailability of polyphenols was determined by previous methods with some changes (Salvia-Trujillo & McClements, 2016; Zhang et al., 2016). For this purpose, a small amount of digested raw sample was centrifuged after the small intestine simulation for 40 minutes at 2600 × g and 25 °C. The collected supernatant, in which polyphenol was dissolved, was considered as "micelle fraction" (Charpashlo et al., 2021). In other words, micellar fraction refers to the milligram of polyphenol released after digestion in the simulated digestive tract, which was determined exactly by the previous method. The bioavailability of polyphenol was calculated by the following equation.

$$Bioaccessibility\ (\%) = \frac{C_{micelle}}{C_{tri} - layered} \times 100 \quad (2)$$

$C_{micelle}$, C_{tri} and layered are the concentration of polyphenol in micelle fraction, the concentration of polyphenol in the wall material and the polyphenol released in the small intestine, respectively.

2.6.9. Thermal stability of total phenol content

The phenolic content of the polyphenolic extract of micro-coated waste micro-coated was determined beforehand (at room temperature), and the samples were subsequently exposed to high temperature conditions. These parameters were selected to simulate the common heat treatment processes that these microcapsules were selected when used as nutrients in food: a) sterilization process (autoclave at 121°C and 15 psi, for 15 minutes) and b) baking process (temperature 180-185 °C, for 25 minutes) (López de Dicastillo et al., 2019).

2.6.10. pH stability of polyphenols

The phenolic extract of Sour grape waste was provided at pH = 4, 7 and 9 by aqueous buffers of sodium acetate, sodium phosphate and Tris, respectively, by evaluating the total antioxidant capacity. The test started 30 minutes after the extract was dissolved in the buffer. The total antioxidant capacity is based on the reduction of 6-valent molybdenum to 5-valent molybdenum in an acidic medium, which is associated with the formation of a green complex of 0.1 ml of extract solution by 1 phosphomolybdenum with maximum absorption at 695 nm. 0.1 ml of the reagent (0.6 M of sulfuric acid, 28 mM of sodium phosphate and 4 mM of ammonium molybdate) was poured into the Eppendorf tube and after tightening the Eppendorf tube was placed in a water bath for 1.5 hours at 95 °C. After cooling, the absorbance of the samples was read at 695 nm (Arabshahi et al., 2007).

2.7. Statistical analysis

The data of the experiments were compared by one-way ANOVA. Statistically significant differences between mean values (in cases where the effect of treatments was significant) were determined using Duncan's multiple range test. The results were obtained using SPSS 26. For the comparison of all data, a significance level of $p \leq 0.01$ was considered. Encapsulation parameters were optimized using the RSM. For this purpose, the central composite design was used. Data were analyzed by Design expert 7, 5, 1.

3. Results and Discussion

3.1. Extraction efficiency

The results (Table 3) of the present study showed that the highest extraction efficiency belonged to the sample extracted by enzyme and ultrasound at a sound intensity of 90% for 40 minutes ($p \leq 0.01$), followed by the sample extracted by enzyme and ultrasound at 60% sound intensity for 40 minutes ($p \leq 0.01$). The sample without enzyme but ultrasound at 30% sound intensity for 10 minutes had the lowest extraction efficiency ($p \leq 0.01$). It has been reported in the articles that ultrasound intensity had a positive effect on the yield of polyphenols, which is attributed to the collision of molecules during the increase in ultrasound power, facilitating the release of phenolic compounds. Teh and Brich (2014) concluded that ultrasound-assisted extraction for 20 minutes resulted in a high yield of phenolics and flavonoid content from rapeseed cakes followed by a reduction in content over time. But in the present study, by increasing ultrasound time from 10 to 40 minutes, the extraction efficiency increased significantly. The results of the present study were consistent with the reports of Lv et al. (2015) who stated that during the extraction of corn cob polyphenols, by increasing ultrasound power, the extraction efficiency increased, which proves that ultrasound power and frequency play a dynamic role in extraction of polyphenols. According to the findings of the researchers, the cavitation effect increased due to the increase in ultrasound intensity in *Nephelium lappaceum* fruit peel extract and in grape seeds when the sound intensity increased from W50 to W150 led to an increase in total phenol content (Puri et al., 2012; Maran et al., 2017).

3.2. Antioxidant activity (DPPH radical scavenging activity)

The results (Table 3) of the present study showed that the highest antioxidant activity (DPPH radical scavenging activity) belonged to the samples extracted by enzyme and ultrasound at 90% sound intensity at 40 minutes ($p \leq 0.01$). The sample extracted without enzyme but ultrasound at 30% sound intensity for 10 minutes had the lowest antioxidant activity ($p \leq 0.01$). According to the results, by increasing sound intensity, the antioxidant activity increased significantly ($p \leq 0.01$). The reason for this can be attributed to the phenolic compounds. Polyphenols are known for their ability to perform a series of oxidation and reduction reactions and local resonance effects in phenyl rings. Polyphenols are considered more efficient antioxidants due to their diverse chemical structures, from simple to complex (Xu et al., 2017). Several studies have been conducted on the antioxidant activity of plant polyphenols extracted by ultrasound treatment (Wijesinghe & Jeon, 2012). The TEAC value of *Limonium sinuatum* flower extract obtained from ultrasound was higher than soaking and soxhlet (Xu et al., 2017). According to the reports of Teh and Birch (2014), phenols and flavonoids extracted by ultrasonic waves from fat-free flax cakes and rapeseeds had twice the antioxidant potential of conventional extraction methods. These results are consistent with the preference of ultrasound treatment for the extraction of polyphenols from plants compared to traditional methods.

3.3. Total phenol

The results (Table 3) of the present study showed that the highest total phenol belonged to the samples extracted by enzyme and ultrasound at 90% sound intensity for 25 and 40 minutes ($p \leq 0.01$). The sample without enzyme but ultrasound at 30% sound intensity for 10 minutes had the lowest total phenol ($p \leq 0.01$). According to the results, total phenol increased significantly ($p \leq 0.01$) by increasing sound intensity. The results of various studies have shown that different enzymes are suitable to be used in different phenolic matrices and the use of these enzymes has only improved phenolic compounds (Zimman et al., 2002, Álvarez et al., 2006). One of the reasons for the low extraction of phenolic compounds from grapes to wine, even when using some enzymes, is the interaction between the extracted phenolic compounds and the cell walls of the skin and suspended pulp. Cell wall components show a high tendency to phenolic compounds and absorb them in their structure (Osete-Alcaraz et al., 2020, Osete-Alcaraz et al., 2021). The structural polysaccharides that have the highest combination affinity with phenolic compounds include pectin, hemicellulose and partially cellulose (Ruiz-García et al., 2014). Therefore, by crushing grapes, phenolic compounds are extracted, but a large amount of suspended cell wall material is also produced. Balasubramaniam et al. (2019) stated that total polyphenol content increased significantly by increasing enzyme concentration up to 1500 U and then the phenolic levels reduced, which was attributed to substrate limitation at higher enzyme concentrations. The objective of enzyme treatment is to disrupt the structure of the plant cell wall by weakening/decomposing the cellulose-phenolic network to enable the release and recovery of phenolic bioactive substances.

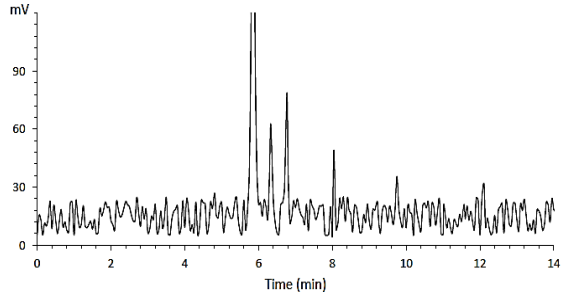

Fig. 1. HPLC spectrum of phenolic compounds of of sour grape waste extracts.

Table 4. The results of the type and amount of phenolic compounds of sour grape waste extracts.

Type of compound (µg/g.dw)	Quantity (%)	Rt (min)
Gallic acid	1.23	4.81
Caftaric acid	46.65	5.85
Catechin	4.85	6.33
Epigalocathechin	6.34	6.77
Vanillic acid	2.47	8.04
Caffeic acid	1.08	8.41
Epigalocathechin gallate	1.27	9.22
Epicathechin	1.79	9.75
p-Cumaric acid	0.15	10.24
Ferolic acid	0.27	11.02
p-Cinapic acid	0.24	11.57
Querictin	0.35	12.11

3.4. Optimal sample for encapsulation

The results showed that by increasing sound intensity, extraction efficiency, total phenol and antioxidant activity increased significantly ($p \leq 0.01$). So that the highest factors were observed in the sample extracted by enzyme and ultrasound at 90% sound intensity for 40 minutes ($p \leq 0.01$). It was selected as the best treatment in the first phase.

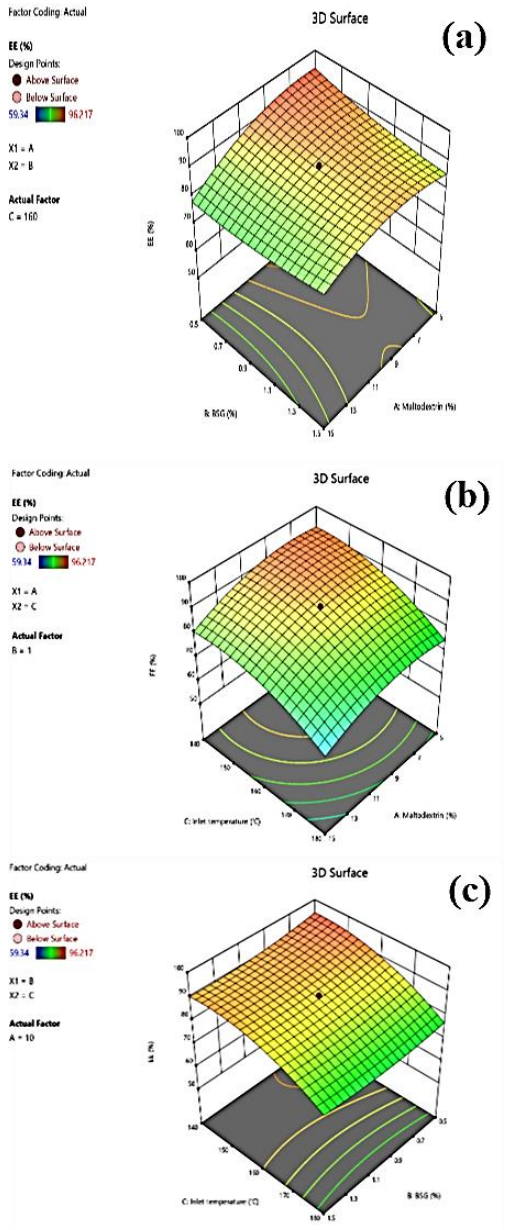

Fig. 2. (A): Interaction between maltodextrin and BSG, (B): Interaction between maltodextrin and the dryer inlet temperature, (C): Interaction between BSG and the temperature of the dryer inlet on the efficiency of the encapsulation of the extract powder of Sour grape waste.

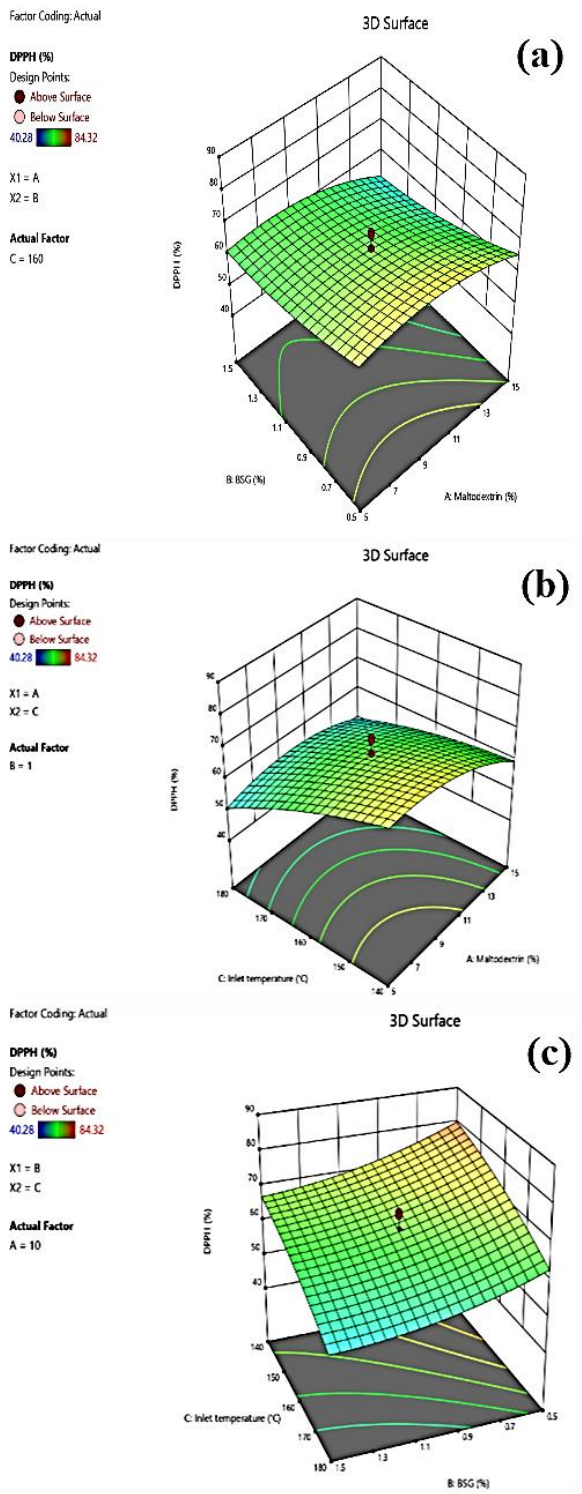

Fig. 3. (A): Interaction between maltodextrin and BSG, (B): Interaction between maltodextrin and the dryer inlet temperature, (C): Interaction between BSG and the temperature of the dryer inlet on the DPPH radical scavenging activity of the extract powder of Sour grape waste.

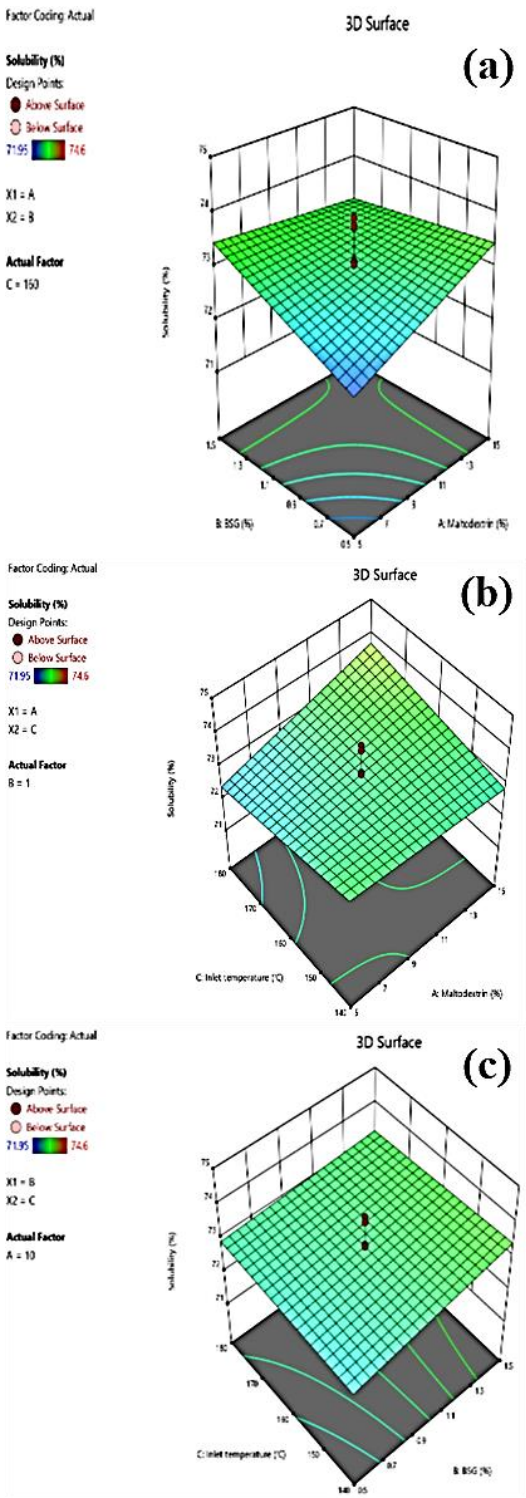

Fig. 4. (A): Interaction between maltodextrin and BSG, (B): Interaction between maltodextrin and the dryer inlet temperature, (C): Interaction between BSG and the temperature of the dryer inlet on the solubility of the extract powder of Sour grape waste.

3.5. *Analysis of phenolic compounds in the extract of sour grape waste by HPLC*

Extracted extracts were injected into HPLC to identify phenolic compounds and the amount and type of phenolic compounds were determined. Table 4 and Fig. 1 show phenolic compounds of the extracts along with the frequency percentage of each component. The study results of the phenolic compounds of the extracts showed that 12 phenolic compounds were identified in the extract of sour grape waste, including gallic acid (1.23%), caftaric acid (46.65%), catechin (4.85%), epigalocathechin. (6.34%), vanillic acid (2.47%), caffeic (1.08%), EGCG (1.27%), epicathechin (1.79%), p-cumaric acid (15 0.0%), ferulic acid (0.27%), p-cinapic acid (0.24%), querictin (0.35%). Guler *et al.* (2018) investigated the changes in phenolic compounds and antioxidant activity of sour grape waste during concentrate production, by HPLC, and reported polyphenolic compounds of gallic acid, catechin, epigalocathechin, vanillic acid, EGCG, epicatechin, caftaric acid, caffeic acid, p-cumaric acid, ferulic acid, cinapic and quercetin.

3.6. *Optimizing the preparation of microcapsules of the extract of Sour grape waste by RSM*

3.6.1. *Efficiency of the encapsulation of the capsules of the extract of sour grape waste*

The results of the statistical analysis of the data showed that the linear effect of all three independent variables on the encapsulation efficiency of the extract capsules was significant ($p < 0.05$). The effect of the interaction between maltodextrin and BSG, as well as the exponential effect of maltodextrin and temperature in the dryer was also significant ($p < 0.05$), but the interaction between maltodextrin and temperature, and BSG and temperature, as well as the exponential effect of BSG, was not significant ($p > 0.05$). The value of the coefficient of determination (R^2) of this model was 95.00 and its adjusted R^2 (R^2adj) was 90.50 and the lack of fit of the model was insignificant, indicating a good fit of the model to the experimental data.

$$Efficiency = 88.13 - 4.67A - 1.00B - 7.63C + 3.13AB + 0.8794AC + 1.16BC - 4.68A^2 + 1.52B^2 - 4.91C^2 \quad (3)$$

The encapsulation efficiency of the capsules of the extract of sour grape waste was in the range of 73.52-96.22%. Fig. 2a shows that the increase in both factors caused a nonlinear reduction in the encapsulation efficiency of the capsules. Fig. 2b shows the interaction between maltodextrin level and spray dryer inlet temperature. Both increasing the temperature of the dryer inlet and increasing the level of maltodextrin as a carrier reduced the encapsulation efficiency of the capsules in a non-linear manner. Fig. 2c shows that increasing the temperature of the dryer and the level of the BSG carrier led to a non-linear reduction in the encapsulation efficiency of the extract capsules. The effect of dryer temperature on the efficiency of encapsulation was higher than the level of maltodextrin and BSG carriers, and among these two carriers, the percentage of maltodextrin showed a higher effect. The efficiency of encapsulation is an important parameter of improving the encapsulation, regardless of the encapsulation processes and materials. In this study, the results showed that by increasing in the percentage of maltodextrin and BSG carriers, the encapsulation efficiency of the extract reduced, which is probably related to the dilution of the feed nutrients entering the dryer due to the increase in the carrier level. The inlet temperature of the spray dryer also showed a significant effect on the encapsulation efficiency of the extract powder of Sour grape waste, so that the encapsulation efficiency reduced significantly due to the destruction of phenolic compounds due to their sensitivity to heat. Malekizadeh et al. (2017) observed a reduction in the efficiency of the encapsulation of capsules due to the increase in the temperature of the dryer. The study results of Abdel-Aty et al. (2023) were consistent with the results of the present study. These researchers found that for the encapsulation of *Lepidium sativum* phenolic extract, increasing maltodextrin reduced the encapsulation efficiency.

3.6.2. *Antioxidant activity of the capsules of the extract of sour grape waste*

The results of statistical data analysis showed that the linear effect of all three independent variables on DPPH radical scavenging activity of extract capsules was significant ($p < 0.05$). The exponential effect of maltodextrin was also significant ($p < 0.05$), but all three binary interactions as well as the exponential effects of BSG and dryer inlet temperature were not significant ($p < 0.05$). The value of the R^2 of this model was 90.28 and its R^2adj was 82.54 and the lack of fit of the model was insignificant, indicating a good fit of the model to the experimental data.

$$DPPH = 64.84 - 3.56A - 5.92B - 8.01C - 1.15AB + 3.03AC + 0.6075BC - 4.76A^2 + 2.44B^2 - 1.82C^2 \quad (4)$$

The DPPH radical scavenging activity of the capsules of the extract of sour grape waste was in the range of 40.22-84.32%. Fig. 3a shows that increasing maltodextrin caused a non-linear and partial reduction in the percentage of DPPH radical scavenging activity of the capsules, but due to the increase in the level of BSG, it increased the antioxidant activity. Fig. 3b shows that increasing both the inlet temperature of the dryer and the level of maltodextrin as a carrier reduced the DPPH radical scavenging activity of the capsules nonlinearly, but the effect of the dryer temperature was significantly higher. Fig. 3c shows the interaction between BSG levels and spray dryer inlet temperature on the antioxidant activity of the capsules. Increasing the dryer temperature caused a non-linear reduction and increasing the BSG carrier level led to a non-linear increase in the DPPH radical scavenging activity of the extract capsules. The DPPH is the basis of the study of the antioxidant capacity of plant compounds, because it is a convenient and quick method to evaluate the antioxidant activity of the given products. As a result of the increase in the inlet temperature of the spray dryer, the antioxidant activity of the extract powder of sour grape waste showed a reduction, because phenolic compounds are bioactive compounds that are sensitive to external conditions, and therefore high temperature causes their thermal degradation, polymerization and transformation. Increasing the level of maltodextrin as a carrier reduced the antioxidant activity of the capsules, because the increase in viscosity increases the time for the formation of droplets and the mixing of core materials during the drying process. Therefore, the efficiency of the encapsulation and

the antioxidant activity reduced due to the reduction in the content of phenolic compounds. However, by increasing BSG, the antioxidant activity of the capsules was slightly improved. In general, BSG contains significant amounts of phenolic compounds and exhibits antioxidant activity. Hence, due to the increase in the level of BSG as a coating factor, due to the increase in the content of phenolic compounds, the antioxidant activity of the produced powders increased. In a study by Hajiaghaei and Sharifi (2022), consistent with the results of the present study, a reduction in the antioxidant activity of instant powder based on red beet and fruit extracts was observed due to the increase in the percentage of maltodextrin carrier. In a study by Lourenco et al. (2020), consistent with the results of the present study, a reduction in the DPPH radical scavenging activity of pineapple peel extract powder due to increasing the temperature of the spray dryer from 150 to 190 °C was reported.

3.6.3. *Water solubility of the microcapsules of the extract of Sour grape waste*

The results of statistical data analysis showed that the linear effect of all three independent variables on the solubility of extract capsules was significant ($p < 0.05$). But all three interactions were not significant ($p > 0.05$). The value of R^2 of this model was 77.43 and its R^2adj was 73.32, and the lack of fit of the model was insignificant, indicating a relatively good fit of the model to the experimental data.

$$Solubility = 73.04 + 0.2578A + 0.2571B + 0.0170C - 0.4000AB + 0.4125AC - 0.1250BC \quad (5)$$

Water solubility of the capsules of the extract of sour grape waste was in the range of 71.95-74.60%. Fig. 4a shows that the increase in both factors increased the solubility of the capsules. Fig. 4b shows that increase in both the temperature of the inlet of the dryer and the level of maltodextrin as a carrier increased the solubility of the capsules. Fig. 4c shows that increasing the temperature of the dryer and the level of the BSG carrier led to an increase in the solubility of the extract capsules. Among the variables examined in this study, the biggest effect was related to dryer inlet temperature, maltodextrin and BSG, respectively. Solubility in water expresses the behavior of the product in the aqueous phase and is a general criterion for determining the quality of powder regeneration. For consumers, quick and complete regeneration of powdered products is one of the main indicators of quality. Solubility of powders can be affected by various parameters including the initial composition of the raw material for spray drying, type of carrier or wall, compressed air flow rate and low feed rate (Jafari et al., 2017). Moisture content and particle size are parameters that affect water solubility of powders, and as a result of reducing moisture content and increasing particle size, the powder dissolves faster in water and the solubility increases (Cegledi et al., 2022). Since moisture content of the capsules reduced by increasing the levels of carriers and the inlet temperature of the dryer, increasing solubility of these capsules was not far from expected. On the other hand, increasing solubility of powders due to increasing the level of maltodextrin can be due to the fact that maltodextrin contains a high number of hydroxyl groups, which facilitates the dissolution process (Fernandes et al., 2014). By increasing the level of maltodextrin, the number of hydroxyl groups and the solubility of the powder increases. Arepally and Soswami (2019) also stated that the hydrophilicity of maltodextrin is responsible for its higher solubility. Abdel-Aty et al. (2023) stated that the solubility of *Lepidium sativum* phenolic extract powder prepared by *Lepidium sativum* plant gum was lower than that of powder prepared with maltodextrin. In a study by Sablania and Bosco (2018), by increasing the temperature of the spray dryer and the levels of maltodextrin and acacia gum, an increase in the solubility of the extract powder of the leaves of Murraya koenigii was observed, which was consistent with the results of the present study. Lourenco et al. (2020) found that increasing the temperature of the spray dryer from 150 to 190 °C led to a significant increase in water solubility of pineapple peel extract powder.

3.7. *Tests performed on the optimal capsule*

3.7.1. *DSC test*

Fig. 5 shows DSC thermogram related to sour grape extract, BSG and capsules containing sour grape extract, maltodextrin and BSG.

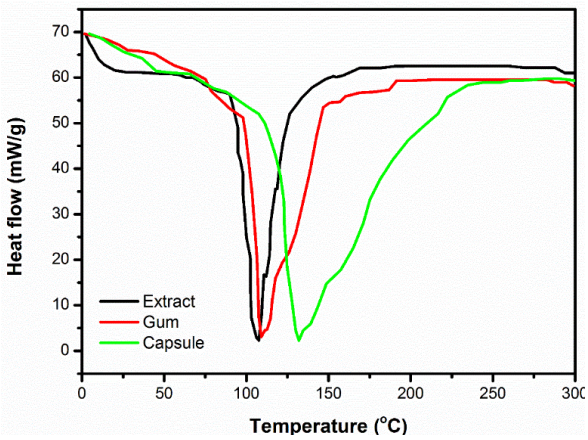

Fig. 5. DSC thermograms related to sour grape extract, BSG and capsule containing sour grape extract and BSG.

According to the thermogram, an endothermic peak was observed in all three samples at temperatures less than 80 °C, which is related to the evaporation of water and other volatile solvents in the structure of the samples (Dai et al., 2006; Rivero et al., 2006). It is found that the intensity of this peak is higher in the extract sample than the other two samples, which can be due to the remaining solvent in the extraction process. Next, in the sample of sour grape extract, a strong endothermic peak at a peak temperature of about 107.61 °C is observed, which is caused by the thermal decomposition of the polyphenolic compounds present in the extract, which is close to the thermal decomposition temperature of these compounds in other studies (Cordero Castaño et al., 2015; Stoica et al., 2017; Pruchnik et al., 2016). The temperature of the beginning and end of the process as well as the enthalpy were 89.85 and 126.40°C, and 29.94 J/g, respectively. As shown in Fig. 5, BSG also has an endothermic peak at a temperature of about 146.70 °C, which according to a study by Nazir et al. (2021)

corresponds to the glass transition temperature of the chemical compounds in the gum (such as glucomannan, xylosyl and glucan). The temperature of the beginning and end of the process as well as the enthalpy were equal to 97.97 and 146.70 °C and 37.65 J/g, respectively. After the encapsulation of these compounds, it is found that the peak temperature increased significantly, so that the temperature at the beginning of the process has increased by 107.61 °C, the peak temperature by 131.98 °C and the temperature at the end of the process by 175.13 °C, indicating a significant increase in the thermal stability of the sample after the encapsulation of the compounds of phenolic extract, BSG and maltodextrin. Also, the enthalpy was about 51.80 J/g, indicating an increase of about 73 and 37% compared to the values obtained for the samples of Sour grape extract and BSG. The results showed that the creation of a complex between the compounds in Sour grape extract, BSG and maltodextrin has resulted in a heat-stable capsule.

3.7.2. Examining encapsulated sample by SEM

For the surface morphology and microstructure of the samples, SEM was used, micrographs of which are shown at different magnifications in Fig. 6. Image J was used to measure visible particles in these images.

As shown in Fig. 6a, micrometer particles were observed on the surface, which can be caused by the presence of capsules containing sour grape extract, BSG and maltodextrin in this sample. The average particle size was 1.04 micrometers. It was also found that these microcapsules have irregular and quasi-spherical shapes that are similar to the particles obtained from a study by Esmaeilzadeh Kenari et al. (2022) on the encapsulation of phenolic compounds obtained from Portulaca extract in *Trigonella foenumpgraecum* seed gum. In general, differences in particle morphology can be caused by differences in conductivity, solution concentration, and capsule content (Naji-Tabasi et al., 2018). In addition, deep holes were observed in this micrograph, which may be due to the presence of hydrophilic groups in the extract and gum compounds and the creation of hydrogen bonds between these compounds (Shaygannia et al., 2021). Also, the presence of these holes can be due to the freeze drying process of the samples according to a study by Pasrija et al. (2015) on the encapsulation of phenolic compounds of green tea extract.

3.7.3. Dynamic light scattering (DLS)

The quantitative results of this measurement (Table 5), shows the value of the polydispersity index (PDI) was obtained from the square of the standard deviation divided by the mean. The hydrodynamic diameter of the particles started from about 70 nm and continued until about 1100 nm. It should be noted that in the SEM, mostly larger particles are observed, and the size of these particles was close to the maximum size of the particles obtained from the results of the DLS. It is also found that the distribution of these particles is normal and narrow, and the largest number of particles have a diameter of about 260 nm. According to Di (50%) index, about 50% of the particles have a particle size less than 259.21 nm, and according to Di (90%) index, only 10% of the particles have a size larger than 568.88 nm. Also, the PDI value equal to 0.477 has been obtained, indicating the narrowness of the particle size distribution and their closeness to the average value and monodispersity of the measured particles. The results showed that in the colloidal state, the minimum size of the hydrodynamic diameter of the particles was more than 100 nm, which could be due to the small agglomeration of the particles in this situation. In other words, due to interactions (such as hydrogen bonds and/or electrostatic forces) between particles, these particles are slightly stuck together and have formed relatively larger agglomeration with a diameter of about 300 nm.

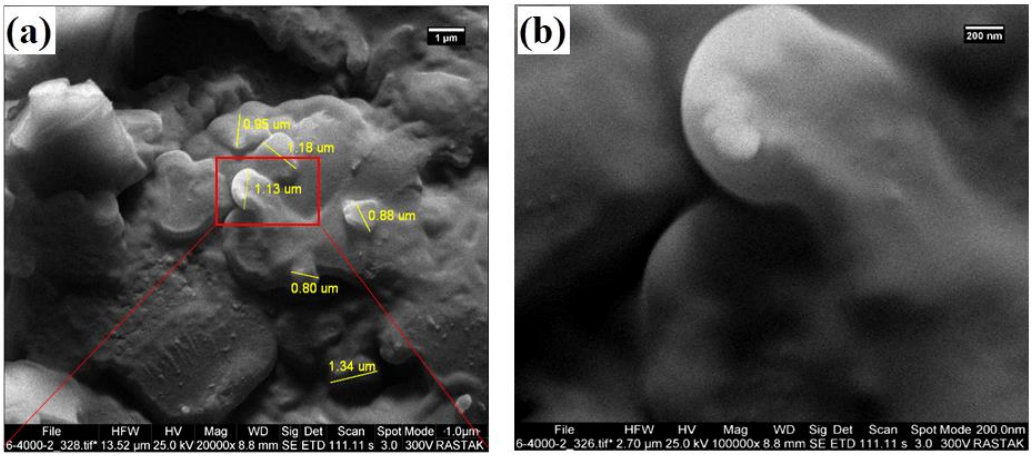

Fig. 6. Micrographs related to the encapsulated sample containing Sour grape extract, BSG and maltodextrin at two magnifications a. 20,000, b. 100,000.

Table 5. Quantitative values obtained from DLS test.

Sample	Average particle size (nm)	Di (10%)	Di (50%)	Di (90%)	PDI
Capsule	315.85	118.11	259.21	568.88	0.477

Table 6. Bioavailability and Release of polyphenols in the encapsulated sample.

Time	Release (mg/g)	Bioavailability (%)
2 min	0.64±0.13 [f]	9.70 ± 0.24 [f]
5 min	1.43±0.28 [e]	21.65 ± 0. 32 [e]
10 min	4.32±0.01 [d]	65.34 ± 0.03 [d]
20 min	5.55 ±0.02 [c]	84.08 ± 0.40 [c]
40 min	6.07 ±0.06 [b]	91.80 ± 0.89 [b]
80 min	6.39±0.04 [a]	96.65 ± 0.42 [a]
120 min	6.44±0.04 [a]	97.33 ± 0.41 [a]

Different small letters indicate statistically significant differences ($p \leq 0.05$).

3.7.4. *Release of phenolic compounds*

The study results of the release of polyphenols (Table 6) showed that over time, the release increased significantly ($p \leq 0.05$), so that the highest release belonged to 80 and 120 minutes and the lowest release was observed for 2 minutes ($p \leq 0.05$). Vulić et al. (2019) on the effect of encapsulation of phenolic compounds isolated from red pepper waste on its bioavailability and bioactivity acknowledged that the release of bioactive compounds was significantly affected by pH during the digestion process. Researchers have reported differences in the release of bioactive compounds from capsules exposed to simulated digestive fluids (Šaponjac et al., 2016; Fredes et al., 2018). Since these capsules are designed for possible incorporation into food products, it is necessary to control the release by wall matters during gastrointestinal digestion, as they retain their biological activity and protect against degradation (Vulić et al., 2019). Estevinho and Rocha (2022) stated that the release time of microencapsulated polyphenol with different encapsulating agents was different and polyphenolic microparticles prepared by spray drying showed higher potential for encapsulating and protecting sensitive bioactive compounds for food-related applications. Lee et al. (2020) in a study on the encapsulation of *Lepidium meyenii* leaf polyphenolic extract in a mixture of maltodextrin (MD) and neutral polysaccharides extracted from *Lepidium meyenii* root (NPMR) stated that powder microcapsules coated with MD-NPMR released less polyphenolic compounds than MD coated microcapsules in simulated gastric and intestinal fluids.

3.7.5. *Bioavailability of polyphenols*

The study results of the bioavailability of polyphenols (Table 6) showed that over time, the bioavailability increased significantly ($p \leq 0.05$). So that the highest bioavailability was related to 120 and 80 minutes and the lowest bioavailability was observed for 2 minutes ($p \leq 0.05$). The bioavailability of phenolic compounds and antioxidants depends on release from the food matrix during the digestion process (Pastoriza et al., 2011; Attard, 2013) and the most important point in relation to the application of health-giving effects is bioavailability. It was found that the application of TPPs in food is limited by several factors. On the one hand, the poor stability of TPPs against temperature, light, pH, and oxygen greatly accelerates their degradation during long-term storage (Su et al., 2003). On the other hand, due to the harsh GI tract and the low permeability of intestinal membranes, only a small fraction of TPPs remain for absorption in the human body after consumption, leading to low bioavailability of TPPs (Sang et al., 2006). Encapsulation is an effective (Rein et al., 2012). Zoo et al. (2014) stated that the stability of EGCG in SIF after encapsulation by nanoliposome significantly increased. Norcao et al. (2016) acknowledged that black rice microcapsules obtained from spray drying by pure whey protein released large amount of anthocyanins and total phenolic compounds during laboratory intestinal digestion and showed a significant increase in antioxidant activity. Microencapsulated polyphenols can remain more stable against digestion, protected against exposure to reactive oxygen species (ROS) and alkaline pH in the digestive tract, and as a result, their stability in the intestine increases (Lipinski et al., 2012; Yang et al., 2018).

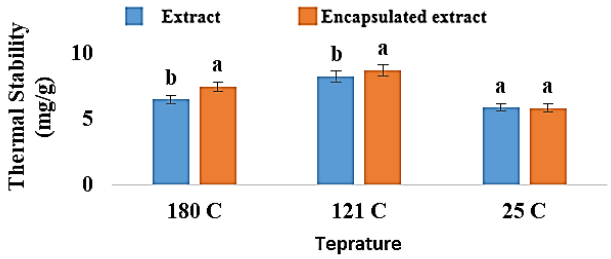

Fig. 7. Comparison of thermal stability between encapsulated sample and extract. Different small letters indicate statistically significant differences ($p \leq 0.05$).

3.7.6. *Thermal stability of polyphenols*

The study results of thermal stability of polyphenols (Fig. 7) showed that at all the temperatures, thermal stability of microencapsulated samples was significantly higher than the extract sample ($p \leq 0.05$). Based on the total phenol, the highest thermal stability was observed at 121 °C and the lowest at 25 °C ($p \leq 0.05$). According to the researchers' findings, reduction in the size of the molecule caused that small molecules can better access the site of the DPPH (Kim et al., 2000). In the present study, it seems that at higher temperatures, the breaking rate of phenolic molecules was higher, and on the other hand, it reacted more by the Folin-Ciocalteiu method and the amount of total phenol was higher. Although at higher temperatures (180°C), some phenolic molecules probably changed their nature and total phenol values were lower than the autoclave temperature (121 °C). Zanon et al. (2020) acknowledged that encapsulation of *Palla Rossa* extract, improved the retention of polyphenols and antioxidant capacity in thermal processes. Rajapaksha and Shimizu (2020) stated that an amorphous powder with the highest retention of phenolic compounds (94.28%) was produced during storage at 45°C for 40

days during the encapsulation of polyphenolic extract from tea waste. Ricci et al. (2022) reported that microcapsules showed high stability when exposed to heat-stress test during the encapsulation of polyphenolic compounds recovered from red wine. Memshlo et al. (2013) showed no significant change in the antioxidant activity of the *Mespilus germanica* extract at 50 °C. Above 100 °C, the activity reduced to about 76%. This reduction at 100 °C was attributed to the loss of natural antioxidants present in the extract or to the formation of new peroxide compounds at this temperature.

3.7.7. pH stability of polyphenols

The study results of pH stability of polyphenols (Fig. 8) showed that the highest stability was at pH = 7 and the lowest stability was at pH = 9 ($p \leq 0.05$). At all pH, the encapsulated sample had higher stability ($p \leq 0.05$). Memshlo et al. (2011) on the effect of pH and temperature on antioxidant activity and total phenol content of *Mespilus germanica* fruit phenolic extract acknowledged that it had the highest stability at pH = 5 and the highest reduction in antioxidant activity was observed at pH = 9. The reduction in antioxidant activity at alkaline pH has been attributed to the loss of antioxidant activity of phenolic extract or the increase in lipid peroxidation at the pH (Mansour & Khalil, 2000).

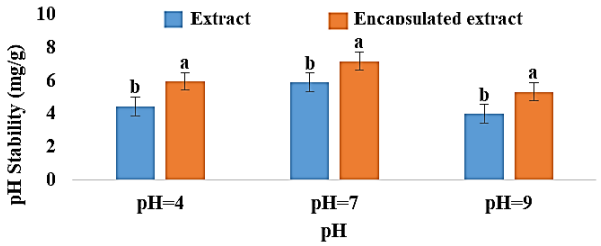

Fig. 8. Comparison of pH stability between encapsulated sample and extract. Different small letters indicate statistically significant differences ($p \leq 0.05$).

4. Conclusion

The results of the present study showed that by increasing sound intensity, the extraction efficiency and antioxidant activity increased significantly, so that the highest factors were observed in the sample extracted by the enzyme and ultrasound at 90% sound intensity for 40 minutes. According to the results, the optimum encapsulation conditions were 12.129% maltodextrin, 0.5% BSG and the inlet temperature of the dryer of 177.22 °C. As a result, heat-stable encapsulation was created, and on the other hand, at all pH and temperatures, the encapsulation sample had higher stability.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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