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Ellagitannins loaded maltodextrin and lecithin nanoparticles: antioxidant, antimicrobial, antidiabetic activity, and bioaccessibility under in-vitro digestion

Mit Ellagitanninen beladene Maltodextrin- und Lecithin-Nanopartikel: Antioxidative, antimikrobielle und antidiabetische Aktivität sowie Bioverfügbarkeit bei in-vitro-Verdauung

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Summary

In recent years, the evaluation of by-products from agricultural and food processing has become very important. However, the biological activity of these components decreases as a result of the losses that occur during the applied processes and metabolism activities. In this study, antioxidant, and antimicrobial activity, antidiabetic properties, and bioaccessibilities of pomegranate peel ellagitannins nanoencapsulated with maltodextrin and soy lecithin were investigated. Results showed that significant increases were obtained in the solubility in pH 6.8 phosphate buffer. There was a decrease in the total phenolic content, antimicrobial and antioxidant activity values of the nanoparticles compared to the core material due to the nanoencapsulation process. In addition, all samples showed higher antidiabetic activity compared to acarbose. As a result, the nanoparticles have the potential to be a natural resource that can be used in the pharmacology, food, and cosmetics industries with their high ellagic acid (EA) bioaccessibility, antidiabetic activities, and increased solubility.

Keywords: Pomegranate, nanoencapsulation, antimicrobial activity, bioaccessibility, antidiabetic activity

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Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and has been cultivated and consumed since 3000 BC (Galaz et al., 2017). In recent years, consumptions of pomegranate products have increased with the understanding of its benefits to human health, and pomegranate peel has become one of the most common wastes in the food industry (Okumuş and Bakkalbaşı, 2021). The large volumes of waste materials of the food industry have the potential to create natural resources, especially in the pharmacology, food, and cosmetics industry (Amyrgialaki et al., 2014). Therefore, many studies have been conducted on the biological activities and chemical properties of the pomegranate peel (Demiray et al., 2018; Okumuş and Bakkalbaşı, 2021). In some scientific studies on pomegranate peel extract, its anti-inflammatory, heavy metal removal, antioxidant, antimicrobial, anti-infective, antimutagenic, hepatoprotective (liver protective) properties were determined (Bachoual et al., 2011; Hayrapetyan et al., 2012; Ismail et al., 2012; Shaban et al., 2013; Ventura et al., 2013). In addition, thanks to these properties it was stated that pomegranate peel is one of the most valuable wastes of the food industry (Zhu et al., 2015).

Pomegranate peels are rich in tannins containing approximately 28–30% (Al-Zoreky, 2009). Due to the potential beneficial effects of the phenolic compounds contained in the pomegranate peels on health, it is used as a food supplement or medicine in many countries (Wang et al., 2010). Ellagitannins (punicalagin and punicalin) are hydrolyzable tannins in pomegranate peel (Gil et al., 2000) and they show high antioxidant activity (Akhtar et al., 2015). However, the sensory acceptability of pomegranate peel is very low due to its bitter taste. The tannin content in pomegranate peel causes its bitter taste (Ismail et al., 2014; Sharma et al., 2014). Despite its significant nutritional and pharmacological potential, bitterness is an important limiting factor in the use of pomegranate peel, especially in the food industry (Akhtar et al., 2015). Today, in order to benefit from the health benefits of pomegranate peel, capsule, tablet, and gel formulations of pomegranate peel extracts are prepared to minimize its bitter taste (Akhtar et al., 2015).

Nanoencapsulation is a method used to protect bioactive compounds such as phenolic acids, anthocyanins, flavonols, and flavan-3-ols against unsuitable conditions and to increase their bioavailability. Thanks to the nanoencapsulation method, many factors that limit the application of ingredients with low stability and poor solubility to food are improved (Esfanjani et al., 2018). The functional properties of these compounds such as antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial properties preserve by nanotechnology application, and their solubility and bioavailability increase. Another advantage of nanoencapsulation is the controlled release of bioactive compounds (Katouzian et al., 2017; Akhavan et al., 2018).

Diabetes (Diabetes Mellitus) is a chronic metabolic disorder with a rapidly increasing occurrence all over the world. In recent years, scientific studies have clearly demonstrated that diabetes is an important disease and significant progress has been made in its treatment methods. In many studies on the use of medicinal plants in the treatment of diabetes, in addition to the use of drugs, it has been determined that plants contain large amounts of bioactive compounds with free radical scavenging effects such as phenolics, nitrogen compounds, vitamins and other endogenous metabolites (Karadeniz et al., 2015). It has been reported that antioxidants in fruits prevent the destruction of pancreatic ß-cells by inhibiting the peroxidation chain reaction, and therefore may protect against the development of diabetes (Aslan et al., 2010). In addition, it was determined that the application of pomegranate extract contributed to the regulation of hyperglycemia and hyperlipidemia in diabetic rats (Gharib and Kouhsari, 2019).

In this study, the aim was to determine the antioxidant activities, phenolic contents, in vitro bioaccessibility of phenolics, solubility values, and antidiabetic properties of the nanoparticles formed with the ellagitannins extract from pomegranate peels using maltodextrin and lecithin coating materials.

Materials and methods

Samples

The Hicaz pomegranate variety was used belongs to the 2019 harvest year and was obtained from a local market in Van. The peels of the fruits were separated from the seed, lyophilized, and then ground into powder form (between 60–80 mesh) using a mill. Ellagitannins (ET) from pomegranate peel were extracted with ethanol (60%). Nanoparticles were prepared from ET extract using maltodextrin and lecithin as coating materials. The preparation and characterization of the ET nanoparticles and the obtaining of ET extracts from pomegranate peel were presented in our previous study (Okumuş et al., 2021). The initial average molecular diameter of the ET was 47.02 ± 31.0 µm. After the nanoencapsulation process, the average molecular diameters of maltodextrin-coated nanoparticles (ETM) and lecithin-coated nanoparticles (ETL) samples were determined as 371.84 ± 229.75 nm and 339.02 ± 215.29 nm, respectively (Okumuş et al., 2021).

Extraction of nanoencapsulated ET

0.2 g of nanoparticles and 25 mL of methanol:acetic acid:water (50:8:42, v/v/v) were mixed in a falcon tube. This mixture was vortexed for 1 min and then sonicated for 3 min by an ultrasonic homogenizer (Bandelein, Sonopuls HD 3200, Germany). The supernatant was centrifuged for 5 min at 8000xg and stored at 4°C until analysis time (Robert et al., 2010).

Determination of total phenolic content

Total phenolic content (TPC) was determined by the Folin-Ciocalteau method Singleton and Rossi (1965). Results were expressed as gallic acid equivalent (mg GA eq./100 g dw).

Phenolic profiles

The phenolic profiles of samples were determined using the Shimadzu HPLC system (LC-20AD, Shimadzu, Kyoto, Japan). The separation of phenolic compounds was carried out using a Symmetry C18 (250 × 4.6 mm id, particle size 5 µm) column (Waters, USA) at 25°C. The method utilizes a binary mobile phase consisting of 2% acetic acid in water (A) and 0.5% acetic acid in water:acetonitrile (1:1, v/v; B). Gradient program was as follows: 0 min 100% A; 50 min 45% A; 60 min 0% A. The flow rate was 1.0 mL/min. Detection was made at 360 nm for ellagitannins. Ellagic acid, punicalagin A, and punicalagin B, which was appearing in chromatograms, were identified based on their retention times and spectral data by comparison with standards (Uğurlu et al., 2020).

DPPH and ABTS assays

DPPH assay was performed using a spectrophotometric method described by Pyo et al. (2004). The ABTS assay was applied according to the method of Re et al. (1999). The results of both DPPH and ABTS assays were expressed as Trolox equivalent antioxidant capacity (mmol Trol. eq./g dw).

Bioaccessibiliy of nanoparticles

30 mL of distilled water and 1.5 mL of pepsin (20 g/L in 0.1 mol/L HCl) were mixed with 1.5 g of ET or nanoparticle extract. The mixture was adjusted to pH 2 with 6 mol/L of HCl and incubated in a water bath at 37°C for 1 h. Gastric digestion was stopped by adding 1 mol/L $NaHCO_3$ into each mixture to adjust the pH to 7.2. After adding 7.5 mL of bile/pancreatin solution (2 g/L of pancreatin and 12 g/L of bile salt in 0.1 mol/L $NaHCO_3$) and 7.5 mL of NaCl/KCl (120 mmol/L NaCl and 5 mmol/L KCl), the mixture was incubated for another 2.5 h under the same conditions. The mixture was centrifuged at 8000xg for 10 minutes and the supernatant was stored at –24°C until analysis time (Vitali Čepo et al., 2009).

Determination of α-amylase and α-glucosidase inhibitory activity of nanoparticles

α-amylase inhibitory activity of the samples was determined as described by Kazeem et al. (2013). A total of 250 µL α-amylase (0.05 U/mL) in 0.02 M phosphate buffer (pH 6.9) was mixed with 200 µL of various concentrations of the extract and preincubated at 37°C for 10 min. Thereafter, 250 µL 1% starch solution (0.02 M phosphate buffer, pH 6.9) was added as the substrate and incubated at 37°C for 15 min. The reaction was terminated by adding 500 µL 1% dinitrosalicylic acid and the mixture was then incubated in boiling water for 10 min. The mixture was cooled at room temperature and diluted with 5 mL of distilled water. The absorbance was measured at 540 nm by UV-spectrometer. Acarbose was used as a standard. The α-amylase inhibition activity of the samples was expressed as IC_{50}, which was calculated graphically.

α-glucosidase inhibitory activity was determined with the method described by Zahratunnisa et al. (2017). Firstly, 60 µL α-glucosidase (1 U/mL) in phosphate buffer (0.1 M, pH 6.8) was mixed with 120 µL of various concentrations of the extract and preincubated at 37°C for 10 min. Thereafter, 120 µL 5 mM 4-nitrophenyl α-D-glucopyranoside was added as a substrate and incubated at 37°C for 15 min. The reaction was terminated by adding 300 µL 0.1 M Na_2CO_3. The absorbance of the p-nitrophenol released was recorded at 405 nm. α-glucosidase inhibition activity of the samples was expressed as IC_{50}, which was calculated graphically.

Determination of water activity

The water activity of the samples was measured using the Novasina Labstart-aw (Novasina AG, Lachen, Switzerland) instrument.

Determination of solubility

The method developed by Kuck and Norena (2016) was modified and used to determine the solubility of the samples in different solvents. 100 mL of methanol, water, 0.1 N HCl, acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) were added to 1 g each of lyophilized ET and the nanoparticles obtained from it, they were placed in separate containers, and mixed for 5 minutes in a magnetic stirrer. Then, 25 mL was taken from the mixture, which was centrifuged (Nüve NF 1200R, Turkey) at 3000xg for 15 minutes, and transferred to containers with a constant weight. The samples, which were kept in an oven at 105°C for one day, were cooled in a desiccator and their solubility percentages were determined.

Determination of antimicrobial activity

Disk diffusion method was used to determine antimicrobial activity. In the analysis, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 29212 representing gram-positive bacteria and *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7 ATCC 43894, *Salmonella* Typhimurium ATCC 14028 representing gram-negative pathogenic bacteria were studied. Mueller Hinton Agar (Oxoid) was used for bacteria in the antimicrobial activity test. For the activation of bacteria, bacterial strains were inoculated into Mueller Hinton Broth (Oxoid) and incubated at 37±0.1°C for 24 hours (Bağcı and Dığrak, 1996). Sterilized and cooled to 45–50°C, Mueller-Hinton Agar (MHA, Oxoid) was dispensed into sterile petri dishes with a diameter of 9.0 cm with sterile pipettes at 20 mL. The medium was distributed homogeneously and the solidified agar was kept at room temperature for 2 hours and then stored at +4°C until used. Then, a loopful of bacterial colonies from 18–24 hour fresh bacterial cultures were suspended in sterile physiological saline solution (FTS), and the density of the bacterial suspensions was adjusted according to the 0.5 Mc Farland standard. 100 µL of the adjusted bacterial suspensions were taken and spread on petri dishes with a Drigalski spatula. 20 µL of ET and ET-loaded nanoparticle extracts were placed on empty discs in petri dishes. Petri dishes prepared in this way were kept at 4 °C for 15 minutes and then incubated at 37°C for 24 hours. At the end of the period, the inhibition zones formed on the medium were measured with the help of a digital calliper and evaluated in mm (Perez et al., 1990). Standard antibiotic discs (10 µg ampicillin and 10 µg gentamicin) were used as positive controls for comparison.

Statistical analysis

The data were analysed using the SPSS package program (version 18) for one-way analysis of variance (ANOVA). Duncan's multiple range test procedure was used to identify significant differences ($p<0.05$).

Results and discussion

Water activity and solubility

Water activity (aw) is a measure of the amount of free water in food and is an indicator of how tightly water is structurally and chemically bound in food products. The aw values of the ET and nanoparticle-formed samples are given in Table 1. The aw values of the ETM and ETL samples were close to each other at 0.31 and 0.32, respectively, compared to the initial water activity value of the ET sample (0.31). In another study, different ratios of maltodextrin and chitosan were used as coating materials (Mayasari et al., 2018). It was stated that the aw values of the nanocapsules ranged between 0.45 to 0.35. In addition, they concluded that the use of maltodextrin could increase the content of the aw value (Mayasari et al., 2018). While the water activity value of durable foods such as dried fruit and crackers varies in the range of 0.2–0.3, fruits and

Journal of Food Safety and Food Quality 73, Heft 6 (2022), Seiten 169–194

vegetables have higher water activity values such as 0.9–0.99. For this reason, in food applications of the obtained structures, the desired water activity value in the final product should be taken into account and the change in water activity value of the product by nanoparticles should be considered.

In vitro solubility tests are generally used to predict the bioavailability of the compound used by humans measured in vivo. In the solubility test, the rate of dissolution of solid drug-like chemicals in an environment such as an artificial stomach or intestine is determined under certain experimental conditions. For this purpose, three different buffer solutions with pH between 1–8 are used. Preferred pH values are 1.2, 4.6 and 6.8, which represent the pH values of the stomach, small intestine and large intestine, respectively (Iskit, 2014). The solubility of ET and the formed nanoparticles was determined in methanol, water, and biological buffers (0.1N HCl (pH 1), pH 4.5 acetate buffer and pH 6.8 phosphate buffer) (Table 1).

The solubility of the samples in methanol and water was determined as ET>ETL>ETM and ET≥ETM>ETL, respectively. The ET sample was found to have the highest solubility in water and methanol. The solubility of the samples in 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer were determined as ETM>ET>ETL. The solubility of all samples was found to be the highest in pH 6.8 phosphate buffer ($p<0.05$). The solubility of ETM and ETL sample in pH 6.8 phosphate buffer was quite close to ET sample ($p>0.05$). As a result, the solubility of ETM and ETL samples in methanol and water decreased compared to ET. However, there was an increase in the solubility rate of ETM nanoparticles in the stomach (pH 1) small intestine (pH 4.5), and large intestine (pH 6.8). ETL samples had the lowest solubility in all medium except methanol. ETM was a more successful application in terms of solubility since it increases the solubility in biological buffers compared to ET and ETL. A nanoemulsifying drug system (SNEDDS) based on the phospholipid complex technique was developed by Avachat and Patel (2015) to increase the oral bioavailability of ellagic acid. In the study, the solubility of ellagic acid phospholipid complex (EAPL) was determined in n-octanol, water, 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer, and it was reported that EAPL increased in solubility in all pH conditions compared to EA (Avachat and Patel, 2015).

Phenolic content and antioxidant activity

In Table 2, punicalagin A, punicalagin B, ellagic acid contents which are dominant phenolics in pomegranate peel, TPC, and antioxidant activity values of samples were given at the initial and after digestion. The highest amount of punicalagin A in the samples at the initial stage and after digestion was determined in the ET sample and the lowest in the ETL sample. The highest bioaccessibility value was also found in the ET sample. As with the punicalagin A content, the initial punicalagin B content and bioaccessibility value were highest in the ET samples and the lowest punicalagin B and bioaccessibility values were found in the ETL ($p<0.05$). Kamiloglu et al. (2014) stated that antioxidants are degraded by alkaline pH, so after in vitro digestion, there is a general loss of phenolic compounds, which are antioxidant sources. However, there was a significant decrease in the bioaccessibility of punicalagin A and punicalagin B by nanoencapsulation compared to the core (ET) sample ($p<0.05$). As a results of nanotechnology applications, it may be due to the decrease in resistance of the particles against to digestive enzymes by the effect of the reduction in sizes.

In the initial stage and after digestion, while the ET sample had the highest ellagic acid content, ETL had the lowest ellagic acid content ($p<0.05$). However, ET sample had the lowest bioaccessibility values. ETM and ETL samples obtained with nanotechnological applications increased the bioaccessibility for EA. Especially the ETM sample had the highest bioaccessibility result with 49.23% ($p<0.05$). It is thought that EA is better coated with ETM thanks to its small molecular weight and is better preserved during the digestion process, and it provides high bioaccessibility thanks to its good dissolution in biological buffers. Similar to our results for ellagic acid, there were some studies indicating that nanocarriers are a good method of improving the bioavailability of phenolics and bioactive compounds (Liang et al., 2017; Araiza-Calahorra et al., 2018).

The initial content and % bioaccessibility results belong to TPC and antioxidant activity were given in Table 2. The total phenolic content and antioxidant activity values of all sample decreased after in-vitro digestion. The decrease in the amounts of TPC is due to the transformation of these compounds into different structural forms during digestion (Bermúdez-Soto et al., 2007). Similarly, the decrease in TPC content in nanoencapsulated sam-

TABLE 2: *Punicalagin A, punicalagin B, ellagic acid contents (mg/100g dw), TPC, and antioxidant activity of samples and bioaccessibility (%) after in vitro digestion.*

	ET*	ETM	ETL
Punicalagin A	27810.36±82.10^{c}	17284.69±233.98^{b}	10218.24±124.81^{a}
GID-Punicalagin A	21310.52±32.72^{c}	3303.20±66.05^{b}	1633.73±24.63^{a}
Bioaccessibility (%)	76.63±0.34^{c}	19.11±0.12^{b}	15.99±0.44^{a}
Punicalagin B	23745.67±727.77^{c}	9765.48±471.62^{b}	5913.10±428.76^{a}
GID-Punicalagin B	6634.91±75.92^{c}	671.12±29.43^{b}	306.65±7.39^{a}
Bioaccessibility (%)	27.96±1.18^{b}	6.89±0.63^{a}	5.20±0.50^{a}
Ellagic acid	964.94±1.31^{c}	232.05±3.33^{b}	220.84±1.78^{a}
GID-Ellagic acid	127.76±1.42^{c}	114.25±2.59^{b}	89.44±0.70^{a}
Bioaccessibility (%)	13.24±0.13^{a}	49.23±0.41^{c}	40.50±0.64^{b}
TPC (mg GAE/100g dw)	28611.11±78.57^{c}	17666.67±117.85^{b}	9319.44±137.49^{a}
GID-TPC (mg GAE/100g dw)	12086.96±115.29^{b}	12314.67±80.70^{b}	6078.26±23.06^{a}
Bioaccessibility (%)	42.25±0.40^{a}	69.71±0.46^{c}	65.22±0.25^{b}
DPPH (mmol Trol. eq./g dw)	214.45±0.22^{c}	185.94±1.08^{b}	170.61±0.65^{a}
GID-DPPH (mmol Trol. eq./g dw)	125.73±0.51^{c}	111.16±1.37^{b}	91.19±1.83^{a}
Bioaccessibility (%)	58.63±0.24^{b}	59.78±0.74^{b}	53.45±1.07^{a}
ABTS (mmol Trol. eq./g dw)	166.89±1.89^{c}	51.50±0.65^{b}	47.07±0.43^{a}
GID-ABTS (mmol Trol. eq./g dw)	48.98±1.12^{c}	21.63±0.20^{b}	16.22±0.11^{a}
Bioaccessibility (%)	29.35±0.67^{a}	42.00±0.38^{c}	34.47±0.24^{b}

Data are expressed as mean±standard deviation. a, b, c Different superscript lowercase letters show differences between the samples ($p<0.05$). ET*: Referenced from our previous study (Okumuş and Bakkalbaşı, 2021). GID: Gastrointestinal digestion.

TABLE 1: *Water activity and solubility values in different solvents (%).*

	a_w	Solubility (%)				
		Methanol	Water	0.1 N HCl	pH 4.5	pH 6.8
ET	0.31±0.00^{a}	82.60±1.13^{cB}	78.00±1.41^{bA}	91.10±0.99^{bC}	92.80±0.57^{bC}	97.50±1.56^{aD}
ETM	0.31±0.00^{a}	37.00±0.85^{aA}	75.90±0.42^{bB}	98.10±0.42^{cD}	95.30±1.27^{bC}	98.80±0.85^{aD}
ETL	0.32±0.01^{b}	54.80±1.13^{bA}	64.90±0.99^{aB}	55.20±0.57^{aA}	74.80±1.13^{aC}	96.30±1.84^{aD}

Data are expressed as mean±standard deviation. a, b, c Different superscript lowercase letters show differences between core and nanoparticles ($p<0.05$). A, B, C, D Different superscript uppercase letters show differences between solvents in the same sample ($p<0.05$).

ples was also reported by Ali et al. (2020). The decreases in the antioxidant activities also may be due to the decomposition of antioxidant compounds during digestion. However, nanoparticles (ETM and ETL) had the higher the bioaccessibility of TPC and antioxidant activity than that of pomegranate peel extracts (ET). The TPC and the antioxidant activity of the nanoparticles coated with maltodextrin were higher than that of lecithin ($p<0.05$). The highest amount of TPC and % bioaccessibility after digestion was found in the ETM sample with 12314.67 mg GAE/100 g dw and 69.71%, respectively ($p<0.05$). According to the DPPH and ABTS assays after in vitro digestion, the highest % bioaccessibility was determined in the ETM sample, and they were 59.78% and 42.00%, respectively ($p<0.05$).

Antimicrobial activity

In Table 3, the zone diameters of ET extract and nanoparticles against *Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis* representing gram-positive bacteria and *Escherichia coli, Escherichia coli* O157:H7, and *Salmonella* Typhimurium pathogen bacteria representing gram-negative bacteria were given. The effects of the obtained zone diameters on the same gram-negative and gram-positive bacteria were compared using the antibiotics ampicillin (10 µg) and gentamicin (10 µg) (Table 3). The zone diameters of ampicillin and gentamicin against to selected bacteria were higher than those of samples (ET, ETM, and ETL). Results showed that all sample have antimicrobial activity against only *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 7644. The highest zone diameter formed on the *S. aureus* ATCC 25923 strain was 17.25 mm in the ET sample. In the results obtained from the nanoparticles, the effect of the coating material on the *S. aureus* strain was found to be insignificant ($p>0.05$). The zone diameters of ET, ETM, and ETL samples against Listeria monocytogenes were close to each other, and the difference between them was statistically insignificant ($p>0.05$). It was determined that there was a significant decrease in the antimicrobial activity values of the nanoparticles obtained as a result of the nanoencapsulation process compared to the ET sample ($p<0.05$). The nanoparticles formed lost their antimicrobial activity properties. It is due to the low core material ratio in the nanoparticle. Only the ET sample showed antimicrobial activity against *Enterococcus faecalis* ATCC 29212, *Escherichia coli,* and *Escherichia coli* O157:H7 ATCC 43894. The zone diameters of ET against these bacteria were 14.25, 9.75 and 9.50 mm, respectively. The antimicrobial activity in any of the samples was not detected against *Salmonella* Typhimurium ATCC 14028 gram-negative pathogenic bacteria. Donsì et al. (2012) reported that a carvacrol nanoemulsion prepared with pea protein and soy lecithin did not effectively inhibit *Escherichia coli*. Similar to our results, the study reported a decrease in the antimicrobial activity of the nanoparticle-formed components. In addition, Kanatt et al. (2010) reported that 0.1% concentration of pomegranate peel extract was ineffective against *Escherichia coli* and *Salmonella* Typhimurium. The antimicrobial activity analysis of pomegranate peel extract was performed by Demir et al. (2019). The inhibition zone diameters were found to be 23.0 mm for *S. aureus,* 18.5 mm for *Enterococcus faecalis,* and 16.5 mm for *Escherichia coli*. Our results are lower than the findings of Demir et al. (2019). It may be due to the different extraction methods, antimicrobial analysis methods, and pomegranate variety.

TABLE 3: *Average zone diameters of samples (mm).*

	Diameter of inhibition zone (mm)					
	S. aureus	*L. monocytogenes*	*Enterococcus faecalis*	*Escherichia coli*	*Escherichia coli* O157:H7	*Salmonella* Typhimurium
ET	17.25±1.06^{b}	13.25±1.06^{a}	14.25±1.06^{a}	9.75±0.35^{a}	9.50±0.71^{a}	–
ETM	14.25±0.35^{a}	11.75±0.35^{a}	–	–	–	–
ETL	14.25±0.35^{a}	12.00±0.00^{a}	–	–	–	–
Ampc	43.50±0.71^{d}	32.50±0.71^{c}	28.25±0.35^{c}	17.25±1.06^{b}	22.75±1.06^{b}	24.00±0.00^{a}
Gent	23.75±0.35^{c}	29.50±0.71^{b}	21.50±0.71^{b}	23.75±0.35^{c}	26.00±1.41^{c}	25.50±0.71^{a}

Data are expressed as mean±standard deviation. $^{a, b, c, d}$ Different superscript lowercase letters show differences between the samples ($p<0.05$). Ampc: ampicillin. Gent: gentamicin.

TABLE 4: *α-amylase and α-glucosidase inhibitory activities of samples.*

	IC50(α-amylase) (mg/mL)	IC50(α-glucosidase) (mg/mL)
ET*	0.14±0.03^{a}	3.50±0.02^{b}
ETM	1.38±0.01^{c}	2.56±0.12^{a}
ETL	1.04±0.02^{b}	3.00±0.27^{ab}
Acarbose	2.44±0.11^{d}	31.12±0.82^{c}

Data are expressed as mean±standard deviation. a, b, c, d Different superscript lowercase letters show differences between the samples ($p<0.05$). ET*: Referenced from our previous study (Okumuş and Bakkalbaşı, 2021).

Antidiabetic activity of nanoparticles

Type 2 diabetes is a chronic and progressive syndrome characterized by metabolic abnormalities such as insulin resistance and decreased pancreatic ß-cell function, which alters energy-sensing processes in the body (Velingkar et al., 2009). However, many studies have shown that phenolic phytochemicals inhibit the activity of α-amylase and α-glucosidase, which are responsible for the postprandial increase in blood glucose level as a result of the development of Type 2 diabetes (Andlauer and Furst, 2003; McCue and Shetty, 2004). In this study, α-amylase and α-glucosidase enzyme inhibitions of the samples were given in Table 4 as IC_{50} values.

The highest α-amylase inhibition efficiency belonged to the ET sample and the highest α-glucosidase inhibition efficiency belonged to the ETM sample ($p<0.05$). The α-amylase enzyme inhibitions of the samples decreased with the encapsulation process. It was determined that there was no significant change between IC_{50} activities of α-glucosidase enzyme inhibitions of ET and ETL samples. On the other hand, α-amylase and α-glucosidase enzyme inhibitions of all samples showed higher α-amylase and α-glucosidase inhibition activities than the positive control acarbose ($p<0.05$). Similar to our results, Çam et al. (2014) stated that the pomegranate peel showed antioxidant and α-glucosidase inhibition activities in ice creams enriched by adding microencapsulated pomegranate peels. Barathikannan et al. (2016) reported that pomegranate peel extract has a significant effect on reducing the long-term complications of diabetes patients due to their α-glucosidase inhibition activity.

Conclusion

Pomegranate peels are one of the most valuable by-products of the food indus-

Journal of Food Safety and Food Quality 73, Heft 6 (2022), Seiten 169–194

try. They are a rich source of bioactive compounds that are effective on health. In order to better protect the phenolic and antioxidant activity of the pomegranate peel, studies are increasing day by day that nanoencapsulation processes are found to be more advantageous than conventional processes. In this study, changes in solubility, antioxidant activity, antimicrobial activity, antidiabetic properties, and their bioaccessibilities of pomegranate peel ellagitannins as a result of encapsulation with maltodextrin and soy lecithin were investigated. It was determined that the solubility of pomegranate peel extracts by nanoparticles formed with maltodextrin as a coating material may be increased in the stomach, small intestine, and large intestine. There was a decrease in the bioaccessibility values of punicalagin A and punicalagin B components of the formed nanoparticles, but a significant increase in the bioaccessibility values of ellagic acid, TPC and antioxidant activity. In addition, ETM sample have high bioaccessibility values and α-glucosidase inhibitory activity than ETL sample. As a result, it was determined that the type of coating material used was an important parameter and maltodextrin in terms of analyzed parameters is a more suitable coating material than lecithin. The possibilities of using ellagitannins nanoparticles encapsulated with maltodextrin and lecithin in commercial applications, food, cosmetics, and pharmacology can be improved, and pomegranate peels can be processed into high value-added products.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Availability of data and material

All data and materials are available.

Code availability

Not applicable.

CRediT authorship contribution statement

Emine Okumuş: Conceptualization, Methodology, Writing – original draft, Data curation, Visualization, Investigation, Formal analysis. Emre Bakkalbaşı: Conceptualization, Methodology, Validation, Visualization, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

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