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Orignal article

Chemical characterization of arabic gum- chitosan-propolis beads and determination of α-amylase inhibition effect

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Summary. Propolis is a resinous mixture collected by honey bees from various plant secretions, tree bark and leaves. It has been used as a natural and supplementary food, increasing the body's defense mechanism against infections. It has many biological activity such as antioxidant, antimicrobial, antitumor, anti-inflammatory and antidiabetic effect etc. Because of its resinous structure, propolis should be used as extracts and ethanol/water solution (70%) is the best solvent for extraction of raw propolis. The ethanol solution limits the consumption of this valuable natural product in foods and apitherapy. Propolis extract was microencapsulated using Arabic gum and chitosan to reduce the limiting effect of ethanol. Inhibition effect of microcapsules on α-amylase enzyme was investigated. The encapsulation efficiency was found 73.46% and the Arabic gum-chitosan-propolis capsules (beads) size was found 784.1 nm in average. Antioxidant capacity of propolis extract was found 347.16 μg Trolox/g. Release properties of capsules were determined and capsules were released in the pH 7.4 and pH 1.5. Inhibition effect on α-amylase enzyme was found (IC_{50}) 0.55 mg/mL. It is clear that Arabic gum-chitosan-propolis capsules could be used as a supplementary food in the treatment of Diabetes Mellitus.

Key words: Diabetes Mellitus, Arabic gum, chitosan, encapsulation

Introduction

Diabetes Mellitus is a metabolic disease in protein, carbohydrate and fat metabolism. It is related to absolute or relative failure of insulin secretion or insulin resistance of body leading to hyperglycemia (1). It causes acute metabolic complications as well as chronic complications like vascular, renal, retinal, and neuropathic. It is a disease with high morbidity and mortality and widespread worldwide (2).

The pharmacological treatment of diabetes mellitus depends on hypoglycemic drugs and insulin. Due to the side effects of these therapeutic agents, there is an increasing interest in herbal and synthetic treatment methods as an alternative. Therefore, there has been a great interest in herbal medicine. In herbal medicine researches, the new approach to finding new drugs (ethnomedical approach) based on folk medicine is seen as a practical, cost-free and logical approach. This approach increases the chance of finding new drugs that can be used for diabetic patients. It is known that the World Health Organization (WHO) has gave a lot of importance to medicinal plant research as well (3).

Propolis is a resinous mixture collected by honey bees from various parts of plants, tree bark and leaves and is the only natural mixture used by honey bees to protect their hives physically and chemically. Raw propolis consists of 50% resin, 30% wax, 10% essential oils, 5% pollen, various phenolic compounds and organic acids. Because propolis is known to prevent decay since ancient times, it has been used as a natural remedy in mummification, increasing the body's defense mechanism (strengthening immune system) against infections and healing wounds (4,5).

Propolis is a pharmaceutical bioactive natural bee product, showing many biological activity like antioxidant, antimicrobial, antitumor and anti-inflammatory properties. It has been reported that propolis contains many different compounds depending on its botanical origin like volatiles, phenolic acids, flavonoids and terpenes. It is also stated that these compounds are responsible for the biological activity of propolis (6,7). Because of its resinous structure, propolis should be used as extracts (8). Ethanol/water solution (70%) is the best solvent for the extraction of propolis. However, the negative effects of alcohol on human health (9) led people to find different solvents like olive oil, glycerol, polyethylene glycol, DMSO. Propolis extracts prepared with many different solvents are commercially available. But solvent-independent forms of propolis extract were obtained by using microencapsulation techniques due to the non-standardization and harmful effects of the solvents used.

The technique of microencapsulation was discovered about 60 years ago (10). Solid, liquid or gaseous active ingredients are encapsulated into a support material and controlled release of these components under special conditions with the help of capsules. Encapsulation techniques are used in many fields such as food, chemistry, agriculture, medicine, pharmacy, veterinary and biotechnology (11). The support material from which the capsules are made may consist of a mixture or a single material. Carbohydrates, proteins, lipids and synthetic polymers can be used as encapsulating agents. Arabic gum and chitosan are high molecular weight polysaccharides and they are used as encapsulation agent.

In this study, solvent-free Arabic gum-chitosan-propolis beads were obtained. The chemical characterization, release properties and inhibition effect on α-amylase enzyme were investigated.

Materials and Methods

Materials

Arabic gum, ethanol, $CaCl_2$ and Na_2CO_3 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Folin Ciocalteu phenol reagent was purchased from Fluka Chemie GmbH (Switzerland). Other chemicals used in the study were off analytical grade.

Preparation of Propolis Extract

Raw propolis samples were collected from the city of Bilecik, Turkey. 1:10 (g/v) ratio was used for the extraction. Frozen propolis sample was powdered by grinding and 2 g of this fine powder was mixed with 30 mL of 70% (v/v) ethanol solution and shaken for 24 h under controlled speed. Then the mixture was filtered and the obtained clear filtrate was used as propolis extract.

Preparation of Arabic Gum- Chitosan- Propolis Beads

Chitosan solution was prepared 0.7% (w/v) in acetic acid (1%) under continuous stirring, during 60 min. 0.3 % (w/v) Arabic gum solution was prepared in 30% ethanol solution (12), mixed with propolis extract and stirred until obtaining homogenous mixture. This mixture was added into chitosan solution by syringe, under continuous stirring, during 2 hours to obtain Arabic gum- chitosan-propolis beads. The beads were separated by centrifugation in 4000 rpm, for 60 min. Then the beads were filtered and stored at -20^{0}C (13).

Characterization of Physical and Chemical Parameters of the Beads

The amount of total polyphenol and antioxidant capacity of propolis extract and filtrate were determined to identify the chemical composition of the beads. Polyphenolic composition of the propolis extract was determined by Gas chromatography (GC/MS). The size and morphology of Arabic gum- chitosan -propolis beads were determined by Scanning Electron Microscopy (SEM).

Determination of Total Polyphenol

The amount of total polyphenol of propolis extract and filtrate were determined according to Folin–Ciocalteu method (14). Gallic acid was used as a standard. The results were expressed as mg of Gallic acid equivalents per mL sample. The total phenolic content of the propolis extract and filtrate obtained after capsulation were determined and encapsulation efficiency was calculated by using the formula given below.

Encapsulation efficiency %= (A- B)/A*100 where; A is the amount of total polyphenol as mg GAE/ mL in crude propolis extract B is the amount of total polyphenol as mg GAE/ mL in filtrate.

Determination of antioxidant capacity

The antioxidant activity of propolis extract and Arabic gum- chitosan- propolis beads were measured by using ferric reducing antioxidant power (FRAP) (15). Trolox was used as a positive control in order to construct a reference curve.

Chemical Composition of Propolis Extract by GC/MS

Main chemical composition of ethanol propolis extract was determined with gas-chromatography coupled with mass spectrometry. Derivatization of propolis extracts was carried out by using N-Methyl-N-(trimethylsiliyl)-trifloroacetamide (MSTFA). Shortly, propolis extracts were dried by using a rotary evaporator and 5 mg of dried residue was mixed with 50 µL of dry pyridine and 75 µL of MSTFA. This reaction mixture was heated at 80°C for 20 min. GC-MS analysis was applied with an Agilent 7890A GC system equipped with HP5-MS capillary column (30 m* 0.25 mm * 0.5 mm). The oven temperature was programmed from 75 to 325°C at a rate of 5°C/min, and a 15 min hold at 325°C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:50, the injector temperature 300°C, and the ionization voltage 70 eV (16). Identification of the compounds was performed using commercial libraries like Wiley.

Release of Alginate-Propolis Beads in vitro Digestive System

In vitro digestive system was formed by using simulated gastric and simulated intestinal fluids. For gastric system pH 1.50 HCl/ KCl buffer and for intestinal system pH 6.80 and 7.40 phosphate buffer with 1% Tween 80 solutions were prepared respectively (17). Known amount of beads was put in 30 mL of these solutions separately. The obtained mixtures were put in a bath at 37 °C. Once every 30 min, 2 mL of solution was sampled out from the mixtures individually. The amount of total polyphenol in the samples was determined and the releases of beads were calculated as a percentage value respectively (12).

Determination of α-Amylase Inhibition

The activity of α-amylase was assayed in the presence of soluble starch as substrate. Reducing ends were determined according to the DNS method described by Bernfeld (18) as glucose equivalent. The reaction mixture containing 300 µL of 1% soluble starch and 300 µL of enzyme solution was incubated at 35°C for 30 min. Equal volume of DNS reagent was added into tubes and kept in a boiling water bath. The absorbance of the tubes was recorded at 550 nm against a blank sample. All characterization assays were performed in triplicate. IC_{50} value of the extracts was determined at five different extract concentrations under standard assay conditions and a dose- response curve was generated. Acarbose was used as a reference inhibitor (19).

Scanning Electron Microscopy (SEM)

SEM analysis was performed in Bilecik Şeyh Edebali University. Arabic gum-chitosan-propolis beads were coated with gold and then analysed by using a ZEISS EVO LS 10 scanning electron microscope at 15 kV. The SEM images of the beads were observed and the size of the beads were determined as well.

Results and Discussion

The encapsulation efficiency of the capsules prepared according to the mentioned method was found to be 73.46 ± 0.28 percentages. The total phenolic content and antioxidant capacity of propolis extract were 58.88 ± 0.47 mg GAE / mL and 347.16 ± 12.48 µg Trolox / mL, respectively. The total phenolic content and antioxidant capacity of the filtrate were 16.08 ± 0.45mg GAE / mL and 110.24 ± 7.61 61g Trolox / mL, respectively. The α-amylase inhibition values of the ethanolic propolis extract and beads are given in Table 1. The propolis extract was found to have an inhibition effect of 0.27 ± 0.001 (mg/mL).

The chemical composition of propolis extract was given in Table 2. The propolis extract was found to be

Table 1. Biochemical characterization of Arabic gum- chitosan-propolis beads

	Total phenolic content mg GAE	FRAP µg Trolox	α-amylase Inhibition IC_{50} (mg/mL)
Propolis extract	58.88± 0.47	347.16± 12.48	0.27±0.001
Arabic gum-chitosan-propolis beads	43.25±0.51	255.77± 7.61	0.55± 0.001

rich in vanillin, quinoline, guaiacol, eugenol, carvacrol and benzyl benzoate contents. The chemical composition of four different propolis samples obtained from Anatolia was reported to be determined by using GC-MS. Their results showed that propolis samples were rich in aromatic acids, fatty acids, aromatic aldehydes, flavone and flavanones (20). The SEM images of the obtained Arabic gum-chitosan and Arabic gum-chitosan–propolis capsules are given in Figure 1.

The major problem of using propolis that has an extremely high biological activity is its solubility. Due to its resinous structure it should be extracted. The bioavailability of raw propolis is low (8) and therefore propolis is treated with various solvents and consumed as extracts (12). Encapsulation techniques provide many advantages like process and storage stability, dose control, oxidation protection etc. For this reason, in the food industry, especially in the production of functional foods, the use of encapsulation techniques has gained momentum in recent years. The use of bio or biocompatible polymers is particularly common in the food industry. However, the use of propolis, a natural preservative, is very limited in food applications. This is a natural consequence of the resinous nature of propolis as well as its own unique smell and aroma. Another important parameter that limits the use of propolis in the food industry is its solubility in ethyl alcohol. Propolis also has an important place in the health industry. Treatment with bee products has gained a rising momentum all over the world in recent years. The expected benefit from these products is possible by providing the active ingredients to the target organ in sufficient amounts. By encapsulation, the active ingredients can be targeted to the desired region of the digestive tract and precisely released at the right location.

In this study, the bioactive components of propolis were taken into ethanol phase and encapsulation was carried out. The resulting capsules were freeze-dried. Although there are many studies in which the active components of propolis are encapsulated by using different encapsulants, it is determined that there are limited studies about the inhibition property of these capsules on α-amylase. When the literature is examined, it is seen that the encapsulation efficiency of capsules and gels obtained by using different biopolymers is quite different from each other. Capsules

Table 2. Chemical composition of propolis extract

Compounds	**%**
Aldehydes	
2-Methyl-2-pentenal	0.02
Cinnamaldehyde	0.01
Phenylacetaldehyde	0.02
Vanillin	0.22
Total	**0.27**
Alcohols	
Benzyl alcohol	17.46
Farnesol	0.30
Lauryl alcohol	0.92
Phenethyl alcohol	2.48
Carvacrol	2.98
Eugenol	0.51
Guaiacol	0.85
Total	**25.50**
Carboxylic Acids and Esters	
Benzoic acid	1.45
Benzyl acetate	0.13
Benzyl benzoate	0.10
Benzyl cinnamate	0.35
Cinnamil acetate	0.05
Ethyl cinnamate	0.04
3-Phenylpropionic acid	0.13
Acetanisole	0.06
Nonyl acetate	0.18
Ferulic acid	2.56
Caffeic acid	4.21
p-coumaric acid	3.48
Vanillic acid	4.16
Total	**16.90**
Fatty Acids	
Palmitic acid	0.73
Total	**0.73**
Other Compounds	
Methyl phenylethyl ether	0.28
Methyl-gamma-Ionone (isomer 1)	0.47
Quinoline	0.70
Valencene	0.33
Total	**1.78**

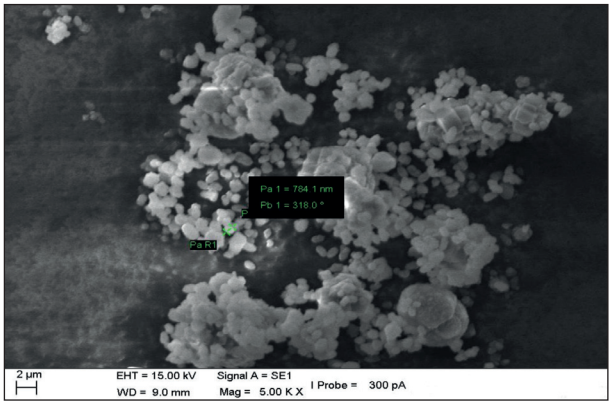

Figure 1. SEM images of beads

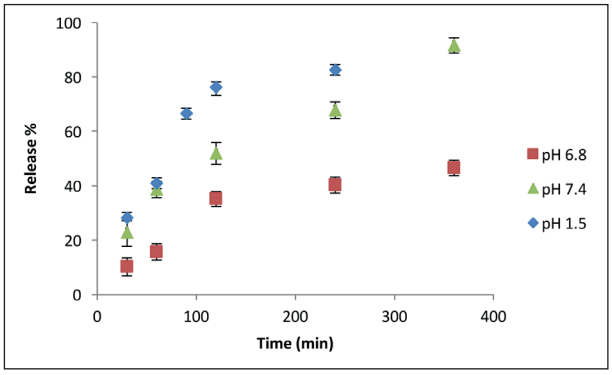

Figure 2. Release properties of bead in pH 1.5, pH 6.8 and pH 7.4

of propolis extracts were obtained using poly (epsilon / caprolactone) emulsion technique. Although they achieved very small capsule sizes, they found their encapsulation efficiency around 25% (21). The capsules of propolis were obtained using a spray- drying method a commercially available encapsulation process, and the effect of the capsules on microbial degradation in meat products was investigated. The capsule efficacy was reported to be 76% (22). Encapsulation of Indonesian propolis with casein micelles was reported. The obtained capsules were declared to be micro- and nano-sized. The encapsulation efficiency was 94% for total flavonoids and 36% for total phenolic content (6). In another study it was reported that propolis capsules with Arabic gum and starch were obtained by using spray- drying technique. The size of obtained capsules was reported to vary between 15-24 microns. The binding effect was reported to be 85%, with an average loss of 3% phenolic component (23).

The release properties of the capsules were given in Figure 2. In a study it is reported that the active ingredients of propolis were encapsulated with Arabic gum and chitosan. Encapsulation efficiency was reported as 71.22 ± 2.98 and it was stated that the capsules were able to release about 50% of the core components in pH 7.4 medium. The use of glutaraldehyde as a crosslinker resulted in a decrease in release efficiency (24).

In a study the effect of aqueous ethanol propolis extracts on α-glucosidase enzyme was reported. It was stated that IC_{50} (µg/mL) values of propolis extracts containing different percentage of ethanol ranged from 7.24 ± 1.16 to 20.1 ± 1.54 (25). Another study revealed that 70% ethanol propolis extract inhibited alpha amylase enzyme. IC_{50} (mg/mL) values were reported to range between 0.09 ± 0.01 to 0.52 ± 0.01 (26). The data obtained in the present study was found to be compatible with the literature.

Conclusion

In recent years propolis has gained increasing usage in apitherapy application as well as in food industry. Because of its resinous nature propolis should be extracted. The extraction of raw propolis using different solvents limits the application of this valuable natural product in food industry. In this study, bioactive compounds of ethanol propolis extract were encapsulated by using Arabic gum and chitosan. A release property of obtained capsules in simulated gastric system was clarified. α-amylase inhibition effect of capsules was also investigated. It could be concluded that obtained capsules have medical importance and can be used as supplementary food in the treatment of Diabetes Mellitus.

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