Research Article

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Effect of Electrospinning Process on Total Antioxidant Activity of Electrospun Nanofibers Containing Grape Seed Extract

https://doi.org/10.1515/chem-2019-0098
received December 13, 2018; accepted March 19, 2019.

Abstract: Electrospinning is a common technique used for the production of nanofibers, and it is based on the fact that the electrically charged liquid polymer is positioned in a continuous fiber form on a grounded surface. Grape seed is rich in phenolic compounds and can be used as a dietary supplement or as a natural antioxidant source in diet. In this study, grape seed extract of Burdur Dimrit variety (Vitis vinifera L.) was electrospun with gelatin, polyvinyl alcohol (PVA) and PVA/β-cyclodextrin polymers to produce nanofibers with antioxidant activity. The aim of this study was to determine the effect of the electrospinning process on the total antioxidant activity and total phenolic contents of electrospun polymers with grape seed extracts. Total antioxidant activity of samples (by ABTS and DPPH assays) and total phenolic contents (Folin–Ciocalteu method) were determined before and after the electrospinning process of polymers with grape seed extract. Electrospinning with gelatin polymer decreased the antioxidant activity (ABTS assay) of nanofibers containing grape seed extract by 65% and their total phenolic contents by 7%. However, electrospinning treatment with PVA and PVA/β-cyclodextrin had no effect on the total antioxidant activity (ABTS and DPPH) and total phenolic substance contents of grape seed extract nanofibers.

Keywords: Electrospinning, Polyvinyl alcohol, Grape seed, Antioxidant, Gelatin

1 Introduction

Grape is one of the most common cultivated plants around the world because it is not very selective in terms of climate, soil, and its reproduction methods and can be consumed in many forms [1]. Viticulture in Turkey, which is located at 36-42° northern latitude and has very convenient climate for grape quality, has a very important place in total agricultural crop production [2]. Annual estimated production of grapes is about 4.2 million tons in Turkey [3]. Grapes generally consist of skin, pulp and seed parts, and 5-12% of a mature grape is made up of skins, which contain a large part of aroma, color and taste constituents. 80-90% of the grape weight constitutes the pulp part. The number of seeds in each grape ranges between 0-4 and 0-5% of the grape weight is composed of seeds.

Grape seeds are waste by-products of the fruit juice, wine and molasses production, and they are the richest part of grapes in terms of phenolic and antioxidant compounds after the fruits of grapes, and they are used as a food supplement and natural antioxidant source [4]. The composition of the grape seed includes catechin, epicatechin, gallocatechin, epigallocatechin, gallic acid, monomeric flavan-3-ol, procyanidin dimers, trimers and higher polymerized procyanidins [5]. Grape seed extract is known as a powerful antioxidant source that protects the human body against diseases such as Alzheimer’s and cancer [6]. In a study comparing the total phenolic content and antioxidant activities of the waste grape seeds obtained from the production of wine and molasses, it was reported that the grape seed obtained from the molasses production had a higher total phenolic content and antioxidant activity than that obtained from the wine production [7]. Linoleic acid (C18:2) content (67.63%) was the highest in the fatty acid composition of grape seed

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Electrospun nanofibers with grape seed oils, followed by oleic (C18:1), palmitic (C16:0) and stearic acids (C18:0) [8].

Encapsulation refers to a technology in which bioactive components are completely enveloped, coated and protected by a physical barrier, and particles of several nanometers to several millimeters in diameter are produced [9]. By the use of the encapsulation process, bioactive components are protected, and these components are recovered from the negative effects of the environment and the release of the encapsulated substance can also be achieved while stabilizing the foodstuff by encapsulation [10]. Recently, electrospinning has been widely used for encapsulation purposes in nano size.

The electrospinning process is a technique for producing polymer/biopolymer-based fibers composed of nanofibers or other nano-structures and based on the principle that the electrically charged liquid polymer is positioned in a continuous fiber form on a grounded surface [11]. The electrospinning system consists of four components: a high voltage power supply, feeding unit, a grounded collector and liquid polymer. A simple electrospinning assembly is shown in Figure 1. After the polymer to be electrospun is dissolved in a suitable solvent, it is placed in the syringe in the feeding unit and nanofiber production is carried out by applying high voltage between the metal tip and the collector plate [12]. Solution properties, process parameters and environmental conditions influence the electrospinning process [13].

Polyvinyl alcohol (PVA), cyclodextrin (CD) and food-borne polymers like starch and gelatin can be used to obtain nanofibers by electrospinning. PVA is a water-soluble polymer prepared by the hydrolysis of polyvinyl esters (polyvinyl acetate). PVA is odorless and nontoxic and has very good chemical resistance, physical and mechanical properties as well as flexibility [15]. CDs are enzymatically modified starches composed of glucopyranose units [16]. Functionally, CDs can be considered as empty capsules and the most important properties of these are the effective protection of each molecule encapsulated compared to conventional encapsulation methods [17]. CDs are used as a processing aid in applications such as increasing the solubility of various vitamins and colorants in the food industry, preserving and increasing the shelf-life of heat-, light- and oxygen-sensitive food components, masking unwanted taste and odor substances, stabilizing the aroma, vitamins and essential fatty acids against undesired changes, the controlled release of some food components, and removing cholesterol from milk and dairy products. They can be used as a process aid removing cholesterol from milk and dairy products [18]. Gelatin is a natural biopolymer used in electrospinning process and is an animal protein produced from collagen by controlled hydrolysis. Collagen is found in the connective tissues of animals such as skin, bone, cartilage and tendon. Gelatin has functional properties such as gelling, water-binding, surface tension reducing, film-forming and it is used in microencapsulation [19]. Electrospun nanofibers may have functions such as the use of functional and/or renewable polymers resulting from very large surface-to-volume ratios or encapsulation of bioactive compounds [20].

The electrospinning process has been used to produce nanofibers for food industries in medical and pharmaceutical applications, encapsulation purpose, packaging and new sources for biopolymers [21] but studies on the effect of this process on the health beneficial constituents of foods are limited. In this study, grape seed extract of Burdur Dimrit variety (Vitis vinifera L.) was electrospun with gelatin, PVA and PVA/β-CD polymers in order to produce nanofibers with antioxidant activity. The aim of this study was to determine the effect of the electrospinning process on the total antioxidant activity and total phenolic contents of electrospun polymers with grape seed extracts.

2 Materials and Methods

2.1 Materials

Fresh grapes (Vitis vinifera L.) of Dimrit variety was obtained from local producers in Burdur, Turkey. PVA and gelatin were purchased from A&D Chemical Industry and Trade Inc. (Istanbul, Turkey) while β-CD was
obtained from Merck (Germany). Trolox® (Fluka, USA), DPPH (Fluka, USA), ABTS (Sigma, Germany), potassium persulphate (Merck, Germany), Folin-Ciocalteu reagent (Merck, Germany), sodium carbonate (Riedel-de Haen, Germany), gallic acid (Fluka, USA), chromatographic grade ethanol (Merck, Germany) and methanol (Sigma-Aldrich, Germany) were used in analyses.

2.2 Preparation of freeze-dried grape seed extract

Seeds were manually removed from fresh grapes and dried at 40°C for 24h in a conventional oven (Wisecube, Daihan, Korea). Dried grape seeds were ground by a commercial coffee grinder (SCM2934, Sinbo, Turkey), and a sieve (U-1967, 35mesh, Loyka, Turkey) was used to obtain ground seed powder of a standard size. Grape seed powder was mixed with aqueus ethanol (70% by volume) at a ratio of 1:10 (w/v). After the mixture was sonicated for 10 minutes in an ultrasonic water bath (Wise Clean WSC-D06H, Daihan, Korea), it was shaken for 15 minutes using an orbital shaker (SHO-1D, Daihan, Korea) at 200 rpm. Samples were transferred into Falcon tubes and centrifuged at 8000 rpm for 10 minutes at 10°C (NF 800, Nüve, Turkey). Then, supernatants were taken into a flask, and the tube was exposed to the same procedure again. This was repeated for each sample. Clear supernatants were stored in amber bottles at -24°C.

Ethanolic phase of the extract was removed by rotary evaporator under vacuum (RE100-Pro, Scilogex, Connecticut, USA) at 40°C and the aqueous phase was dried in a freeze-drier (BW-10B, Vacuum Freezing Dryer, Bluewave, China). Freeze-dried powder was used in the encapsulation processes.

2.3 Nanoencapsulation by electrospinning process

2.3.1 Preparation of polymer solutions

Gelatin (20% w/v) was prepared with aqueous acetic acid while PVA (6%, w/v) was prepared with water. Equal mixtures of PVA and β-CD (1%) were prepared with distilled water. Freeze-dried Dimrit seed extract (0.5%, w/v) was added to each polymer solution before electrospinning. To determine the effect of electrospinning process on the antioxidant activity of electrospun polymers, part of the polymeric solutions was carefully spread on aluminum foils and dried in an oven for 2 hours at 40°C. Then, nanofibers containing grape seed extract were carefully separated from the foil.

2.3.2 Electrospinning unit

In electrospinning, a high voltage applied to a polymeric fluid induces electric charge within the fluid, and a fluid jet erupts from the droplet at the tip of the needle. This results in the formation of a Taylor cone at a certain critical charge value. Parameters such as the voltage, flow rate, type and temperature of the polymer, type of collector, diameter of needle and distance between the needle tip and collector have a significant influence on the electrospinning processing as well as the morphology of electrospun fibers [22]. The Taylor cone shape is the most important parameter, which can be used to determine the electrospinning stability [23]. In this study, based on the stable Taylor cone shape, optimum conditions for the nano-encapsulation of grape seed extract powder in a laboratory scale electrospinning unit (Eraktek Innovation, Konya, Turkey) such as voltage, flow rate and distance were determined by preliminary studies. The electrospinning system used in the experiments is shown in Figure 2, and process parameters applied to the polymer solutions containing freeze-dried grape seed extract powder during the electrospinning process are given in Table 2.

2.4 Antioxidant Activity Assays

Dried polymeric solutions or their nanofibers (0.1g) were mixed with distilled water (10mL) on a magnetic stirrer (WiseStir, Daihan, Korea) at 500 rpm at room temperature and time for polymers, solvents and the speed of magnetic stirrer (Wisestir, Daihan, Korea) are given in Table 1. After the electrospinning process of polymeric solutions, wet nanofibers deposited on the collector of aluminum foil were dried in an oven for 2 hours at 40°C. Then, nanofibers containing grape seed extract were carefully separated from the foil.

| Polymer (w/v) | Solvent                  | Temperature-Time (°C-minute) | Speed (rpm) |
|--------------|--------------------------|-----------------------------|-------------|
| Gelatin (20%)| Aqueous acetic acid (20%, v/v) | 40-4                        | 900         |
| PVA (6%)     | Water                    | 40-2                        | 400         |
| PVA (6%)/β-CD (1%) | Water                | 40-2/40-1                  | 900         |

Table 1: Preparation conditions of polymeric solutions containing freeze-dried grape seed extract.
Electrospun nanofibers with grape seed

2.4.1 DPPH assay

The working solution was obtained by diluting the stock solution (24 mg/100mL in methanol) with methanol to a final absorbance of 1.20±0.02. The linear region for the calibration curve of Trolox® (12.5mg/25mL) was less than 50 mM. The sample or standard (150mL) was mixed with the DPPH working solution (2850mL) in test tubes, and the reaction was continued in dark for 30 minutes. At the end of this period, absorbances were recorded by a spectrophotometer (Optizen Pop, Optizen, South Korea) at a wavelength of 734 nm.

2.4.2 ABTS assay

The procedure described by Thaipong et al. [24] was used in the ABTS assay. ABTS was dissolved in water, and mixed with 2.6 mM potassium persulfate at a ratio of 1:1 (v/v). The mixture was allowed to stand at room temperature for 12-16 hours. Stock solution was diluted with methanol to the absorbance value of 1.20±0.02. Calibration curve was prepared with Trolox®. The sample or standard (150mL) was mixed with the ABTS working solution (2850mL) in test tubes, and the reaction was continued in dark for 30 minutes. At the end of this period, absorbances were recorded by a spectrophotometer (Optizen Pop, Optizen, South Korea) at a wavelength of 734 nm.

2.4.3 Total phenolic content

The micro-adapted Folin-Ciocalteu (FC) method was used to determine the total phenolic contents [25]. The FC reagent was diluted with distilled water by a ratio of 1:10 (v/v). To prepare sodium carbonate solution (20%), sodium carbonate (75 g/L) was dissolved by distilled water in a volumetric flask. The linear region of the gallic acid calibration curve was in the range of 5-100 mg/L. A 2 mL sample or standard was mixed with 10 mL of a diluted FC reagent. After 1 to 8 minutes, 8 mL of 20% sodium carbonate was added and the mixture was kept in a dark place for 2 hours. At the end of this period, absorbances were recorded by a spectrophotometer (Optizen Pop, Optizen, South Korea) at 760 nm wavelength.

2.5 Statistical analysis

Data were analyzed using the Statistical Analysis System software (SAS Institute Inc., Cary, North Carolina, USA) to determine the effect of nano-encapsulation conditions on antioxidant activity of electrospun polymers, and differences were determined by using ANOVA and the Duncan multiple comparison test at α=0.05.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

Although three polymeric solutions to be electrospun were prepared in the same manner, initial total antioxidant and antioxidant activity values of dried forms of these polymeric solutions were dramatically different from each other. Mean total phenolic contents of dried gelatin and PVA polymers were about 740 and 9725 mg GAE/g dm, respectively (Table 3). The type of polymers had a significant influence on the antioxidant activity of initial polymeric solutions as well as their electrospun counterparts. Pure solutions of PVA and gelatin showed an activity in the total phenolic content assay, and their total phenolic contents were determined as 36.05±0.23 and
2.83±1.26 mg GAE/kg, respectively. On a dry matter basis, these values and their contribution to the total phenolic contents of polymeric solutions and their electrospun nanofibers seem negligible. Fresh grape seed is a cheap antioxidant source and a perishable by-product of grape processing industry. The use of antioxidants especially in foods with high oil content is a good way to prevent lipid oxidation and increase shelf life [26]. The viniferin and catechin found in grapes produce aspirin and naproxen-like effects by inhibiting the cytochrome oxidase enzyme, and resveratrol in the grape seed inhibits the coagulation by inhibiting thromocyte aggregation, decreasing LDL oxidation and suppressing the pre-inflammatory response [27, 28]. The total antioxidant activity (ABTS and DPPH) values of the polymers containing grape seed extract before and after electrospinning are given in Table 3. The electrospinning process with gelatin polymer decreased the antioxidant activity of grape seed extract nanofibers with ABTS by 65% and total phenolic content by 7%. Electrospinning treatment with PVA and PVA/β-CD did not affect the total antioxidant activity (ABTS and DPPH) and total phenolic content of grape seed extract nanofibers. Perez-Jimenez and Saura-Calixto [29] studied the effect of the solvent type and pH of the media on antioxidant activity values determined by the assays such as ABTS, DPPH, FRAP and ORAC. They found that both the solvent type and pH could influence the results of the assays.

Electrospinning is a new method used in the food industry. In a study [30], the electrospinning process was used to encapsulate gallic acid (at concentrations of 5, 10, 20%) with zein proteins to produce functional nanofibers. As a result of the process, it was observed that the antioxidant activity of encapsulated gallic acid was retained by the DPPH method. In a different study with zein protein, β-carotene was encapsulated with a light-sensitive colorant and antioxidant molecule and it was determined that zein proteins were distributed evenly in the encapsulated compound and the light stability of β-carotene increased [31]. In our study, it was determined that nano-encapsulation can be used in the preservation of grape seed extract powder by using electrospinning and the encapsulated product maintains its functional property.

In a study, during an increase in the drying temperature from 40 to 60°C, grape seeds have retained antioxidant activity but their total phenolic content decreased [32]. The minimum reduction was determined to be 44.1% at 40°C and therefore, it was reported that the temperature should be kept below 40°C in order to prevent the loss of phenolic compounds during drying and storage of grape seeds. The current study was conducted at room temperature, and electrospinning process had no effect on the total phenolic content and antioxidant activity of the grape seed extract by encapsulation with PVA and PVA/β-CD polymers.

In a study by Weng et al. [33], electrospun nanofibers with antimicrobial agents were added to the food packaging material and the PVA/cinnamon essential oil/β-CD with an average diameter of 240±40 nm was used to increase the shelf life of fresh strawberries by integrating this into a packaging material. Results indicated that fresh strawberries had an increased shelf life when compared with other packaging material. In our study, nanofibers containing grape seed extract powder can have a potential to be incorporated into packaging materials to increase the shelf life of foods by preventing the oxidation reactions occurring in foods.

Electrospinning is a technique that has a potential to be used in the food industry. Studies on the use of electrospinning in the preparation and preservation of foodstuffs are mostly focused on areas such as preservation of functional food components by encapsulation and

| Type of Polymer | Processing Step | Total Phenolic Content* (mg GAE/g dm) | ABTS (µmol TE/g dm) | DPPH (µmol TE/g dm) |
|-----------------|----------------|--------------------------------------|---------------------|---------------------|
| Gelatin         | Initial        | 739.89±25.74<sup>a</sup>             | 738.12±93.65<sup>a</sup> | 71.83±8.08<sup>a</sup> |
|                 | Nanofiber      | 686.86±52.06<sup>b</sup>             | 255.48±48.21<sup>b</sup> | 77.01±7.75<sup>a</sup> |
| PVA             | Initial        | 9724.64±795.98<sup>a</sup>           | 2325.79±176.12<sup>a</sup> | 874.96±126.45<sup>a</sup> |
|                 | Nanofiber      | 9411.18±332.81<sup>a</sup>           | 2299.64±92.18<sup>a</sup> | 985.09±100.12<sup>a</sup> |
| PVA/β-CD        | Initial        | 5941.01±123.56<sup>a</sup>           | 2264.82±97.23<sup>a</sup> | 915.59±70.53<sup>a</sup> |
|                 | Nanofiber      | 5663.10±150.54<sup>a</sup>           | 2285.20±133.25<sup>a</sup> | 923.26±61.34<sup>a</sup> |

*Different superscripts within a column for each polymer indicate statistical significances at α = 0.05 level.
incorporation of nanofibres obtained from antimicrobial materials into food packaging materials [34-36]. Recently, food polymers in the structure of carbohydrates (alginate, dextran, chitosan, starch, cellulose) and proteins (gluten, gelatin, zein) have been used in the production of nanofibers. The nanofibers with these polymers were produced (i) to obtain consistency and viscosity enhancing additives in foods (ii) to develop antimicrobial packaging material and (iii) to transport, preservation and controlled release of bioactive substances [37-40] and successful results were obtained in these studies.

4 Conclusion

Electrospinning is a novel method that can be used for encapsulation purposes in the food industry. In this study, grape seed extract powder was nano-encapsulated by the electrospinning process using different polymers. Results indicated that a new product was developed as a raw material for the food industry. The effect of the electrospinning process and polymer type on the total phenolic substance content and antioxidant activity of the encapsulated product was determined. The type of polymer had a significant influence on the total phenolic content and antioxidant activity of the final nanofibers. Nano-encapsulation with gelatin polymer significantly reduced the antioxidant activity of the final product determined by the ABTS method. However, the polymer of PVA, which is widely used in electrospinning, had no significant effect on the total phenolic content and antioxidant activity of the final product. Further studies are required to determine the release of antioxidant constituents of nano-encapsulated fibers into actual food media.

Conflict of interest: Authors declare no conflict of interest.

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