ORIGINAL ARTICLE

Micro-encapsulation of ozonated red pepper seed oil with antimicrobial activity and application to nonwoven fabric

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Significance and Impact of the Study: This is the first report on the antimicrobial action of RPSO after ozonation process. These findings suggest that ozonated red pepper seed oil (ORPSO) may be a useful and effective antimicrobial agent against the micro-organisms with antibiotic resistance. Therefore, as a natural product, RPSO represents a sustainable alternative to the use of synthetic antimicrobial agents. To our knowledge, this is also the first time that ORPSO has been micro-encapsulated for the preparation of functional textile material with significant antimicrobial activity.

Abstract

In recent years, functional fabrics possessing antimicrobial activity have drawn significant interest because antibiotic resistance is becoming widespread among pathogenic micro-organisms. The aim of this study was to produce microcapsules incorporating ozonated red pepper seed oil (ORPSO) with antimicrobial properties and apply them to nonwoven fabrics to prepare functional textiles. Red pepper seed oil (RPSO) was ozonated and micro-encapsulated via a complex coacervation method using gelatin (GE) and gum arabic (GA) as wall materials. While micro-encapsulation yield and oil loading decreased with increases in the amount of surfactant, the mean particle size increased. The antimicrobial activity of the oil was tested via the disc diffusion method. The microcapsules were also tested using the agar well method. While RPSO had no effect on the test micro-organisms, the ORPSO and microcapsules containing ORPSO were found to be active against the test micro-organisms. The microcapsules were then applied to nonwoven fabric using the padding method to produce a disposable functional textile. The microcapsule-impregnated functional fabrics provided a 5 log decrease in 1 h. It is therefore possible to functionalize nonwoven fabrics to have antimicrobial activity against antibiotic-resistant micro-organisms, using microcapsules containing ORPSO.

Introduction

Recently, antimicrobial textiles have been widely used in home textiles and personal care products. Currently, disposable nonwoven textiles with antimicrobial properties are widely used in the production of functional textiles. There are various methods to extend the antimicrobial properties of textiles. One of these is the use of micro-encapsulated antimicrobial agents within the fibre matrix (Ramachandran *et al.* 2004; Bonin 2008; Tavaria *et al.* 2012).

Micro-encapsulation is a technique of surrounding solid particles, liquid droplets or gas bubbles with a continuous film or polymeric material. Microcapsules range in size from 1 μm to 1 mm (Benita 1996; Mayya *et al.* 2003). A variety of techniques have been developed for micro-encapsulation. Coacervation, based on the phase separation of one or many hydrocolloids, is one technique. Coacervation is divided into simple and complex coacervation. While simple coacervation involves the use of a single polymer in organic or aqueous media, complex

coacervation involves the use of two oppositely charged polymeric materials (Benita 1996; Aguilera and Gómez-González 2008; Leclercq *et al.* 2009). Complex coacervation, firstly used in carbonless copying paper by Green *et al.* (1957), is the oldest and most commonly preferred micro-encapsulation method. It is also simple and technically easy. The complex coacervation technique involves five steps to obtain microcapsules: dissolution of the polymer, emulsification, coacervation, hardening and rinsing/filtering/drying (Cosco *et al.* 2007; Harrington and Morris 2009; Bansode *et al.* 2010).

Proteins and polysaccharides, particularly gelatin (GE) (type A) and gum arabic (GA), are generally used for complex coacervation because of their natural, environmental- friendly and biodegradable properties (Leclercq 2008). A variety of materials have been packaged by micro-encapsulation. GE/GA-based microcapsules have been studied using several core materials such as vetiver oil (Prata *et al.* 2008), shikonin (Huang *et al.* 2007), camphor oil (Chang *et al.* 2006), violet, lemon and peach perfumes (Ovez and Yuksel 2002), orange oil (Vahapzadeh *et al.* 2004), paraffin oil (Mayya *et al.* 2003; Onder *et al.* 2008), *N*-benzylmorpholine (Bukar *et al.* 2008), indomethacin (Daniels and Mittermaier 1995), limonene and menthol powder (Leclercq *et al.* 2009), capsaicin (Xing *et al.* 2004) and flavor oil (Yeo *et al.* 2005).

Micro-encapsulation technology has been used in numerous fields including textiles, agriculture, pharmaceuticals, food and cosmetics for multiple purposes (Nelson 2002; Augustin and Hemar 2009). In the textile industry, many different core materials such as fragrances (Hong and Park 1999; Rodrigues *et al.* 2009), fire retardants (Giraud *et al.* 2005), insect repellents (N'Guessan *et al.* 2008), dyes (El-Zawahry *et al.* 2007; Leelajariyakul *et al.* 2008), phase change materials (Qingwen *et al.* 2007; Deveci and Basal 2009) and drugs (Ma *et al.* 2009) have been successfully encapsulated. Microcapsules can be applied to textile materials by padding, spraying, coating and exhaust techniques (Badulescu *et al.* 2008). There is also a rising interest in using plant and vegetable products such as extracts and oils in the medical industry. Some plant-originated products with anti-oxidant and antimicrobial activities represent an important segment of the literature (Hammer *et al.* 1999). *Capsicum annuum* L. has been widely used as red pepper powder. Amongst the pepper-producing countries, Turkey ranks third after China and Mexico (Fıratlıgil-Durmuş and Evranuz 2010). Red pepper seeds can be considered as byproducts of red pepper powder production. Red pepper seeds contain protein, oil and fibre along with several amino acids (El-Adawy and Taha 2001a; Fıratlıgil-Durmuş and Evranuz 2010). Currently, red pepper seeds are a subject of research, and it has been found that the red pepper seed is rich in anti-oxidants (Sim and Sil 2008; Song *et al.* 2010). Recently, more attention has been focused on the characterization and utilization of red pepper seed oil (RPSO) (El-Adawy and Taha 2001a; Li *et al.* 2011).

Ozone treatment of vegetable oils is also being investigated for medical applications. Upon ozone treatment, ozone gas dissolves in vegetable oils and forms ozonides (Cronheim 1947; FAO 2008; Zanardi *et al.* 2008). Ozonides are generally believed to be responsible for the broad biological activities of ozonated vegetable oils. Ozonated oils are used in the medical and pharmaceutical industries and in general health care (Holmes 2008). To the best of our knowledge, no report concerning the use of micro-encapsulated ozonated red pepper seed oil (ORPSO) and its application to nonwoven fabric to produce a disposable antimicrobial textile material has been published in the literature.

In this study, ORPSO with antimicrobial activity was micro-encapsulated using GE and GA as wall material by complex coacervation. Optimum processing parameters, including amounts of RPSO and surfactant, were studied and their effects on yield, particle size, microcapsule loading and release were investigated. Microcapsules obtained under optimum conditions were then applied to nonwoven fabric using the padding method to produce a disposable medical textile with antimicrobial properties.

Results and discussion

Characterization of RPSO and ORPSO

Red pepper seed has been found to be rich in its oil (24·4%) and protein (25·91%) content (Fıratlıgil-Durmuş and Evranuz 2010). RPSO is also reported to be rich in unsaturated fatty acids; linoleic acid (74%) and oleic acid (13%) which are reported to be major acids in RPSO (Jung *et al.* 1999; El-Adawy and Taha 2001a,b; Duarte *et al.* 2004; Li *et al.* 2011). In this study, the oil yield was found to be 21% under the defined conditions and this value matched well with the previously reported yield values. The major saturated fatty acids in oil were reported to be palmitic and stearic acids. The total saturated fatty acid content of seed oil is reported to be 17·6% in the literature (El-Adawy and Taha 2001a; Fıratlıgil-Durmuş and Evranuz 2010; Li *et al.* 2011).

In RPSO, the most abundant unsaturated free fatty acids (UFFA) were reported to be linolenic acid and oleic acid. The fatty acid content and degree of unsaturation of RPSO show similarities to those of sunflower and safflower oils, which have linoleic acid as the most abundant fatty acid, followed by oleic acid. This reveals a high degree of unsaturation in RPSO.

Ozone treatment of vegetable oils (Maritza *et al.* 2006) is also a new field for medical, pharmaceutical and dermocosmetic applications due to its antimicrobial activity. It has been shown that ozone treatment of unsaturated fatty acids can cause the conversion of olefinic bonds to ozonides (Harrison and Murphy 1996). The chemical reactions occurring during ozone treatment of oil are very complex. These reactions cause changes in functional groups during ozonation, which can be explained by the well-known Criegee mechanism such as the formation of ozonides from alkenes and ozone (Soriano *et al.* 2003a,b). In this study, the effects of ozonation on the chemical composition of RPSO were analyzed using Gas chromatography-mass spectrometry (GC-MS) and FT-IR analyses.

Gas chromatography-mass spectrometry analysis

The ozonated RPSO sample was subjected to GC coupled with a mass spectrometer to determine its constituents. A literature search was performed to compare the selected mass spectra with the spectra of reference compounds. Using the GC/MS spectral results, the aldehydes 1-nonanal (nonaaldehyde or pelargon aldehyde, 14·88% in abundance), 2-nonenal (0·33% in abundance), 4-nonenal (31·96% in abundance), and 1-hexanal (hexanaldehyde, 10·93% in abundance) were identified as the major volatile components. Carboxylic acids such as nonanoic acid (pelargonic acid, 7·23% in abundance), hexanoic acid (11·32% in abundance) and 8-nonenoic acid (6·47% in abundance) were also detected in our ORPSO samples.

FTIR spectroscopy

In the analysis of RPSO and ORPSO with IR spectroscopy, wavelengths corresponding to primary aldehydes and carboxylic acids were evaluated. The spectra were checked for double bonds, C=C stretching (1654 cm^{-1}) and =C–H stretching (3009 cm^{-1}) (Vlachos *et al.* 2006), as well as ozonide CO stretching (1105 cm^{-1}) by unsaturated fatty acid moieties (Soriano *et al.* 2003a,b; John *et al.* 2004). As seen in Fig. 1, RPSO samples showed strong absorbance at approximately 1700, 2900 and 3500 cm^{-1} infrared (IR). The presence of free fatty acids in RPSO can be identified from its FT-IR spectrum.

The presence of linoleic acid in RPSO samples was revealed from these results. In the FTIR spectrum of ORPSO, absorptions are sharp and strong around 1750 cm^{-1} due to the carbonyl group. In addition, after ozonation, a peak was observed due to OH that revealed the presence of aldehydes and carboxylic acids. The C=O bond specific to carboxylic acid was observed at 1100 cm^{-1}. The increase in the intensity of this band demonstrates the

formation of carboxylic acids after ozone treatment. Nonanoic acid detected by GS/MS analysis could also be identified in the FT-IR spectra as a carboxylic acid.

It is a known phenomenon that the ozonide and peroxide oligomers formed are unstable and eventually decompose to a mixture of aldehydes and carboxylic acids. The most important industrial use of ozone treatment is the cleavage of oleic acid to nonanoic acid (pelargonic acid) and di-nonanoic acid (azelaic acid) (Baumann *et al.* 1988).

Decomposition of triolein ozonide leads to the formation of aldehydes and acids (pelargonic acid, azelaic acid, caproic acid). Similar ozone treatment products of linoleic acid and oleic acids have also been reported in the literature (Cronheim 1947). It is known that dicarboxylic acids can be produced industrially by ozonolysis of oleic acid to azelaic acid (Hill 2007; Köekritz and Martin 2011).

RPSO primarily contains large amounts of linoleic acid followed by oleic acid. Saturated fatty acids such as palmitic and stearic acids do not react with ozone but remain intact (Tran *et al.* 2005). The ozonation reaction in RPSO occurs mainly through linoleic acid and oleic acid.

Aldehydes are the major volatile reaction products after ozonation of RPSO; 1-nonanal was detected following reaction with oleic acid; 2-nonenal, 4-nonenal and 1-hexanal were detected following reaction with linoleic acid. Azelaic acid was identified as a liquid-phase reaction product following reaction with oleic acid (Moise and Rudich 2002).

Nonanal, also known as nonanaldehyde or pelargonaldehyde, is an alkyl aldehyde detected in ORPSO. It has a
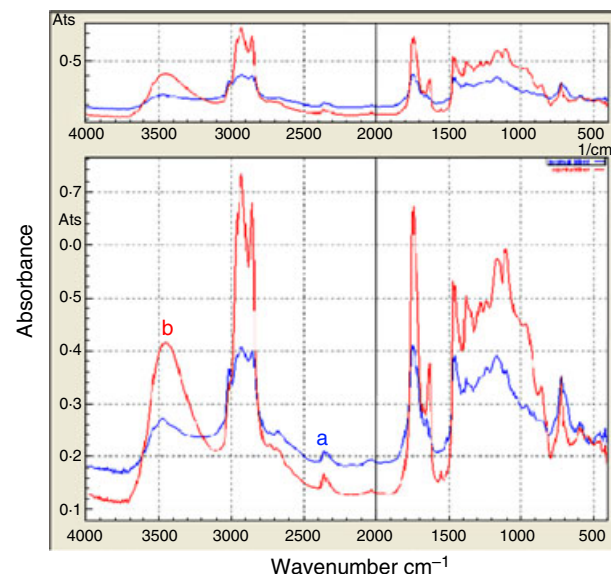

Figure 1 FT-IR spectra of: (a) red pepper seed oil, and (b) ozonated red pepper seed oil.

strong fruity or floral odour and is used as a fragrance in industry. Hexanal (hexanaldehyde), found in ORPSO, is an alkyl aldehyde used in the flavour industry to produce fruity flavours. Its scent is similar to that of freshly cut grass.

Formation of microcapsules

In this study, ORPSO microcapsules with antimicrobial activity were developed and successfully applied to non-woven fabrics for medical use. The aim of developing bio-active textiles is to prevent infections in the case of injury, promote healing and improve health. Therefore, antibiotic-resistant test strains were used for a more realistic simulation of the implementation of the bio-active fabrics.

ORPSO was obtained and encapsulated successfully via a complex coacervation method using GE and GA as wall material. The antimicrobial activity of both the oil and the microcapsules was tested. The shape and formation of the microcapsules were observed under an optical microscope. It can be seen in the microscopic images that the microcapsules have a spherical shape and a smooth wall. In Fig. 2, particles surrounded by a black line are microcapsules. It is apparent that the microcapsules have a spherical shape. While the black lines are the walls of the microcapsules, the materials surrounded by a black line are their cores. The microcapsules have a single and continuous core. Their mean particle size increased with an increase in both, the amount of surfactant and oil.

To optimize the parameters for micro-encapsulation of ORPSO, an experimental design was set up. The effects of varying the processing parameters, including the amount of oil and amount of surfactant, on encapsulation yield, mean particle size, oil loading and oil release were investigated.

Micro-encapsulation yield

The statistical effects of the two parameters, amount of oil and amount of surfactant, on micro-encapsulation yield were calculated together and separately using SPSS statistical analysis software (SPSS Inc., Chicago, IL, USA). As a result of the statistical analysis, it was determined that these parameters, either together or separately, had no statistically significant effect on the micro-encapsulation yield.

Mean micro-encapsulation values obtained from all experiments are displayed in Table 1. Although the statistical analysis showed that the amount of surfactant did not have any effect on the micro-encapsulation yield, it can be seen in Table 1 that the micro-encapsulation yield decreased with increasing amounts of surfactant.

Particle size and particle-size distribution

The effects of the two parameters on particle size were also analyzed, both together and separately, using SPSS. As a result, while the amount of oil had no effect on size,
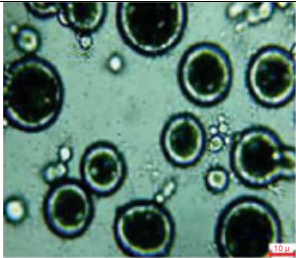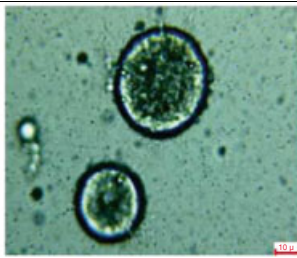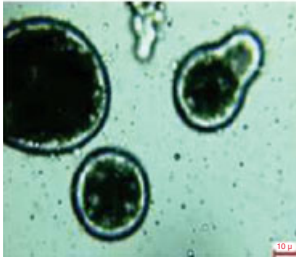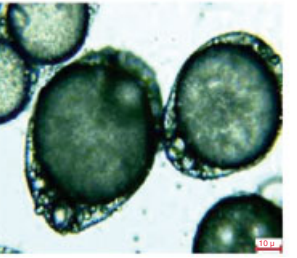
Parameters		Amount of Oil	
		10 ml	20 ml
Amount of surfactant	1·6 ml		
	3·2 ml		

Figure 2 Optical microscope images of microcapsules.

Table 1 Values of micro-encapsulation yield, mean particle size, oil loading and oil release

Experiment number	Amount of oil (ml)	Amount of surfactant (ml)	Micro-encapsulation yield (%)	Particle size (micron)	Oil loading (%)	Oil release (%)
1	10	1·6	87·3	18·7	53·0	42·8
2	10	3·2	68·8	35·5	47·5	47·0
3	20	1·6	76·9	27·3	56·1	49·0
4	20	3·2	75·2	38·7	54·6	42·3

the two parameters together had a significant effect. But as seen in Table 1, when the amount of oil or amount of surfactant increases, the particle size increases as well. The effect of changing the amount of oil in the mixture on the particle size is an expected result. A previous article, by Xing *et al.* (2004), does not support this result. It can be attributed to compositional changes due to oxidation of free fatty acids in RPSO with ozone treatment.

Oil loading/release

The mean values of oil loading and release are given in Table 1. As a result of the statistical analysis, it was concluded that amount of surfactant and oil, either together or separately had no statistically significant effect both on oil loading percentage and oil release. As seen from the results, the use of 10 ml of oil and 1·6 ml of surfactant resulted in a maximum micro-encapsulation yield for the preparation of microcapsules with ORPSO. At these conditions, the micro-encapsulation yield, mean size of microcapsules, oil loading and oil release were determined to be 87·3%, 18·7 μ, 53 and 42·8% respectively.

Impregnated microcapsules in padded fabric

In Fig. 3, dry fabrics with and without microcapsules are shown. The materials with spherical shapes are microcap-
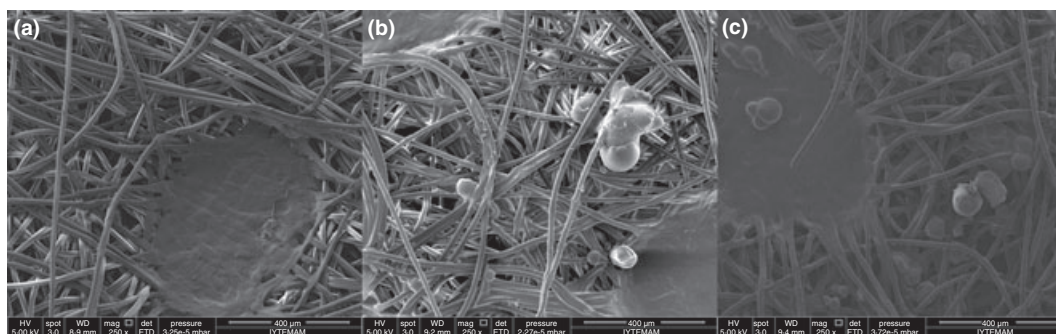

Figure 3 SEM images of fabrics with and without microcapsules: (a) fabric without microcapsules, (b) fabric passed through bath including 20 g l^{-1} microcapsules, (c) fabric passed through bath including 30 g l^{-1} microcapsules.

Table 2 The results of antimicrobial disc diffusion tests for red pepper seed oil (RPSO) and ozonated red pepper seed oil (ORPSO)

	Inhibition zone (mm)*									Antibiotics	
	%10 DMSO			%25 DMSO			%50 DMSO			G (10 μg per disc)	N (100 U per disc)
Micro-organisms	C	RPSO	ORPSO	C	RPSO	ORPSO	C	RPSO	ORPSO		
Escherichia coli O157H7	0	0	13	0	0	13·5	0	0	15	21	Nt
Pseudomonas aeruginosa ATCC 27853	0	0	12	0	0	11	0	0	14	25	Nt
Methicillin-resistant *Staphylococcus aureus*	0	0	23	0	0	23	0	0	20	23	Nt
Vancomycin-resistant *Enterococcus faecium*	0	0	27	0	0	23	0	0	15	19	Nt
Candida albicans	0	0	16	0	0	15	0	0	15	Nt	18

Nt, not tested; C, DMSO controls corresponding to 10%, 25%, and 50% (v/v) concentrations; G, Gentamycin; N, Nystatin.
*The values are the mean of three experiments. Zones of inhibition include the diameter of the disc.

sules. It can also be observed that the microcapsules have a smooth surface.

The difference between dry fabrics passed through baths containing 20 or 30 g l^{-1} of microcapsules is shown in Fig. 4. It is clear from the figure that more microcapsules were impregnated in the fabric applied to the 30 g l^{-1} microcapsule bath.

Antimicrobial activities of RPSO, ORPSO, microcapsules and fabrics

The antimicrobial activities of RPSO and ORPSO were found to be quite different. The antimicrobial activities of the RPSO and ORPSO are shown in Table 2. Different concentrations of DMSO and RPSO showed no antagonistic activity against the test strains, but ORPSO samples showed considerable activity compared with those of antibiotics. This effect can be attributed to the ozone treatment. In a previous study, Agaoglu *et al.* (2007) similarly found that crushed red pepper was ineffective against a panel of test micro-organisms including *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. In another research, capsaicin, the major metabolite of red pepper, was also not found to be very active against *E. coli* and *Saccharomyces cerevisiae* (Torres *et al.* 1999). However, in another study, the authors reported that a combination of *C. annuum* extract and sodium chloride had an inhibitory effect against *Salmonella typhimurium* and *Pseudomonas aeruginosa* in meat samples (Careaga *et al.* 2003). In our study, the antimicrobial effect of ORPSO can be explained by the presence of antimicrobial compounds such as aldehydes and carboxylic acids (e.g. azelaic and pelargonic acids) formed by the ozonation process. Azelaic acid inhibits the growth of skin bacteria that cause acne, and keeps skin pores clear. Its antimicrobial activity is attributed to the inhibition of

microbial cellular protein synthesis (Leeming *et al.* 1986; Porro 1987). Nonanoic acid is used in the food industry, particularly for its antifungal properties (Mirabal *et al.* 2003; Sahin *et al.* 2006).

The results in Table 3 show that the encapsulated ozone treated with oil retained its antimicrobial activity in the microcapsule form. This prolonged activity is important for the production of functional textiles.

Microcapsules produced under optimum conditions were impregnated into the nonwoven fabric using two different baths with either 20 or 30 g l^{-1} of microcapsules and the antimicrobial activities of the fabrics were tested. The bath containing 20 g l^{-1} of microcapsule was sufficient to confer antimicrobial activity to the fabric. The efficacy of the bioactive fabrics is shown in Table 4. Microcapsule-impregnated fabrics are clearly more effective than the untreated fabrics ($P < 0.05$).

Although a statistically significant decrease was found between initial microbial counts (IMC) and final microbial counts (FMC) ($P < 0.05$) in the control fabrics, they still had biologically significant (10^4–10^5 CFU ml^{-1}) microbial counts. However, biologically active fabrics eliminated the test micro-organisms, showing up to 5-log decrease in 1 h. Both bio-active fabrics impregnated with 20% (w/w) bio-active microcapsules (BAF1) and bio-active fabrics impregnated with 30% (w/w) bio-active microcapsules (BAF2) were found to be very active against the antibiotic-resistant test micro-organisms. Compared to other bioactive fabrics containing silver ions (Mariscal *et al.* 2010), ozone-treated oil-containing microcapsules provided more effective and rapid protection against bacteria.

In this article, the preparation of ORPSO containing microcapsules by complex coacervation and their application to nonwoven fabrics to prepare functional textiles has been tested. While RPSO does not possess significant

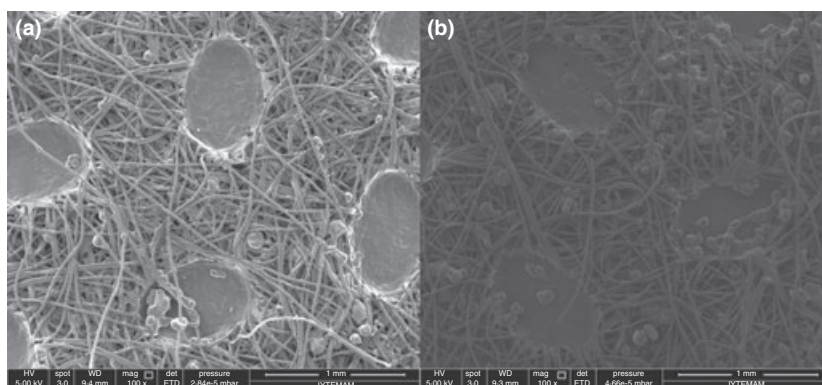**Figure 4** Difference between dry fabrics passed through baths including different amount of microcapsules: (a) fabric passed through bath including 20 g l^{-1} microcapsule, (b) fabric passed through bath including 30 g l^{-1} microcapsule.

antimicrobial activity, ORPSO has a considerable antimicrobial activity against test micro-organisms with antibiotic resistance. ORPSO can be readily encapsulated by using complex coacervation to produce microcapsules. It has been possible to obtain functional textiles by applying ORPSO-loaded microcapsules to nonwoven fabrics.

Materials and methods

GE (from porcine skin, type A) and GA (from the acacia tree) were purchased from Sigma–Aldrich (Munich, Germany) and used as wall materials. Red pepper (*Capsicum annuum*) seed was a gift of Memişoğlu Spices Co. (Kahramanmaras, Turkey). RPSO was obtained using a cold press (KYP20D) Köprülü Machine Co. (Izmir, Turkey). RPSO was processed and ozonated in the Natural Products

Table 3 Agar well antimicrobial test results on microcapsules containing ozonated red pepper seed oil (ORPSO)

	Inhibition zone (mm)[a]		
Microorganisms	Microcapsules with ORPSO	Gentamycin (10 μg per disc)	Nystatin (100 U per disc)
Escherichia coli O157H7	21·50	20·0	Nt
Pseudomonas aeruginosa	19·50	25·0	Nt
Methicillin-resistant *Staphylococcus aureus*	22·50	23·0	Nt
Vancomycin-resistant *Enterococcus faecium*	18·50	17·0	Nt
Candida albicans	17·0	Nt	18·0

Nt, not tested.
[a]The values are the mean of three experiments. The zones of inhibition include the 6 mm diameter of the wells.

Research Development Unit (NPRDU/DUAG Ltd Co.) (Izmir Technology Development Zone, Izmir, Turkey). Tween® 20 ($C_{58}H_{114}O_{26}$), sodium hydroxide (NaOH), hydrochloric acid (37%, purity HCl) and 2-propanol were purchased from Sigma–Aldrich. Polypropylene SMS (spunbond-meltblown) 100% nonwoven fabric weighing, 40 g m^{-2}, was provided by Kisbu Textile (Izmir, Turkey). Deionized water was used in all the experiments.

Characterization of RPSO and RPSO

Gas chromatography-mass spectrometry analysis. A Hewlett-Packard 5890 including FID and HP innowax capillary column (60 m × 0·32 mm i.d.; 0·25 μm) combined with a mass spectrometer was used in the GC–MS analyses. The injector was set at 240°C with a splitting ratio of 1 : 50. Samples were diluted with *n*-hexane (40/100) prior to analysis. Helium was used as the carrier gas at 35 ml min^{-1}. The column temperature was maintained at 50°C for the first 5 min and then raised to 260°C at a rate of 5°C per minute, and then kept at 260°C for 5 min. The GC-MS analyses were performed at Arge Far (Ege University, Izmir, Turkey).

FTIR spectroscopy. The FTIR spectra of RPSO and ORPSO samples on KBr discs were recorded using an FT-IR spectrophotometer (Digilab FTS 3000 Mx, Randolph, MA, USA). A regular scanning range of 400–4000 cm^{-1} was used at a spectral resolution of 4 cm^{-1}.

Preparation of microcapsules

Microcapsules containing ORPSO were prepared by a complex coacervation method according to a modified method published by Jouzel *et al.* (2003). Solutions of 2% (w/v) GE and GA were prepared. Four grams of polymer were dispersed into 200 ml of deionized water at room

Table 4 The efficacy of bio-active fabrics functionalized by impregnation with ozonated red pepper seed oil (ORPSO) containing microcapsules against three selected micro-organisms

	IMC		FMC (log 10 CFU ml^{-1}) in 1 h				FMC (log 10 CFU ml^{-1}) in 3 h			
			C				C			
Test Organisms	Mean CFU ml^{-1}	Log 10 CFU ml^{-1}	Mean CFU ml^{-1}	Log 10 CFU ml^{-1}	BAF1	BAF2	Mean CFU ml^{-1}	Log 10 CFU ml^{-1}	BAF1	BAF2
Escherichia coli	1·0·106 ± 2·1·104	6·0	2·0·105 ± 5·2·103	5·3	0	0	5·4·104 ± 1·5·103	4·7	0	0
Staphylococcus aureus	3·5·106 ± 7·3·104	6·5	2·6·105 ± 8·0·103	5·4	0	0	6·4·104 ± 1·4·103	4·8	0	0
Candida albicans	4·0·106 ± 7·9104	6·6	4·2·105 ± 1·4·104	5·62	0	0	6·7·104 ± 3·1·103	4·8	0	0

CFU, colony forming units; C, control fabric; BAF1, bioactive fabric impregnated with 20% (w/w) bioactive microcapsules; BAF2, bioactive fabric impregnated with 30% (w/w) bioactive microcapsules; IMC, initial microbial counts; FMC, final microbial counts.

temperature (~25°C) for 30 min. The temperature of the aqueous solutions was increased to 40°C to dissolve the polymer, and the pH of the solutions was adjusted to 7·0 by adding 3 mol l^{-1} NaOH. The GE solution was then added to a three-neck flask equipped with a mechanical stirrer. Different amounts of ORPSO (10 or 20 ml) were emulsified in GE solution by stirring at a speed of 350 rev min^{-1} at 40°C. Different amounts of Tween® 20 (1·6 or 3·2 ml), as a surfactant were added to the three- neck flask and the emulsion was stirred at 350 rev min^{-1} at 40°C for 30 min to form a stable oil-in-water emulsion. GA solution was added to the emulsion drop-wise and it was stirred again at 350 rev min^{-1} for 15 min. The pH of the emulsion was adjusted to 4·0 with 1 mol l^{-1} HCl solution to stabilize the polymers. The coacervation was allowed to continue by stirring at 350 rev min^{-1} for 90 min. At the end of the coacervation, the heater was switched off and 600 ml of cold, deionized water was added to the coacervate to reduce the temperature of the coacervate to room temperature and also prevent agglomeration. The temperature of the coacervate was then reduced to 5–10°C with an ice bath. Consequently, GE and GA covered the solidified oil droplets and formed microcapsules. The system was stirred continuously at 350 rev min^{-1} for 2 h. After 2 h of hardening, microcapsules in the aqueous phase were transferred to a refrigerator overnight. Finally, the microcapsules were collected, rinsed with 2-propanol, filtered and dried at room temperature.

In the present study, the amount of oil (ml) and amount of surfactant (ml) were chosen as experimental parameters in the preparation of the microcapsules. All other parameters were kept constant. For this purpose, four different treatments were prepared. The amounts of oil and surfactant used were varied from: 10 and 1·6, to 10 and 3·2, to 20 and 1·6, to 20 and 3·2 ml for treatments 1–4 respectively. All treatments were carried out in duplicate.

Morphology of microcapsules

The structure, shape and formation of microcapsules were observed using an optical microscope (Bresser® LCD Micro, Rhede, Germany) connected to a digital camera.

Determination of micro-encapsulation yield

The micro-encapsulation yield of ORPSO was determined by the following equation:

$$\text{Micro-encapsulation yield}(\%) = (W_1/W_2) \times 100$$

W_1: weight of the microcapsules obtained
W_2: initial weight of the materials used (combined weight of core and wall materials).

Determination of particle size

Images of microcapsules obtained by optical microscopy at the rinsing step of the micro-encapsulation process were used to measure the size of the microcapsules. Sizes of the microcapsules were measured using IMAGE J software (NIH, Bethesda, MD, USA) as pixels and transformed to microns using a scale. Seventy measurements were taken for each treatment and mean particle size was obtained from values of diameter of 70 different microcapsules. Particle-size distribution was determined by SPSS 15.0 statistical analysis software.

Determination of oil loading

Oil loading was determined using a centrifuge (Hettich, Tuttlingen, Germany) and UV-VIS spectrophotometer (Thermo Multiskan Spectrum, Barrington, IL, USA) with the following method. First, five different specific amounts of the oil were dissolved in 750 μl of 2-propanol and a calibration curve was established with UV-VIS spectrophotometer at 275 nm. Then, 0·05 g of microcapsules were dispersed in 750 μl of 2-propanol.

Microcapsules in 2-propanol were shaken in a centrifuge to release the encapsulated oil completely. The concentration of the oil dissolved in 2-propanol was obtained by a UV-VIS spectrophotometer at 275 nm. All determinations were carried out in triplicate and mean values were calculated.

Determination of oil release with mechanical rupture

The oil in microcapsules impregnated onto the fabric can be released by mechanical rupture of the capsule wall. For this reason, oil release was determined with the BioSpec mini bead-beater (Bartlesville, OK, USA). Microcapsules in 2-propanol were shaken with a BioSpec mini bead-beater to release the encapsulated oil. A UV-VIS spectrophotometer was also used to determine the oil release (%) just as in the case of the oil loading determination method. All determinations were carried out in triplicate and mean values were calculated.

Application of microcapsules to nonwoven fabric

Microcapsules prepared at the optimum conditions were applied to 100% poly propylene nonwoven fabric using a laboratory type foulard (Ernst Benz 8153, Rümlang, Switzerland) in the finishing process of textile fabrication. Different amounts of microcapsules with ORPSO (20 or 30 mg ml^{-1}) were suspended in a 500 ml bath containing only deionized water. The following equation was used to determine the pick-up ratio of the fabric.

$$Pick\text{-}up\ ratio(\%) = (mass\ of\ bath\ solution\ taken$$
$$by\ the\ fabric/mass\ of\ dry\ fabric) \times 100$$

The foulard with two rollers was used at 7 m min^{-1} of working speed and 6 bar of pressure of rollers and the pick-up ratio of the fabric was fixed at 150%. A fabric of 20 cm width and 60 cm length was passed through the bath and subsequently through squeeze rollers. The padded fabrics were dried for 1 day at room temperature (~25°C).

Analysis of microcapsules impregnated on the padded fabric

Microcapsules in the padded fabric and their structures were also observed by scanning electron microscopy [SEM; Philips (FEI) XL30-SFEG, Eindhoven, the Netherlands].

Antimicrobial activities of RPSO, ORPSO and micro capsules with ORPSO

The antimicrobial activity of the RPSO was determined by means of the disc diffusion method described by the National Committee for Clinical Laboratory Standards (NCCLS 2003). Several antibiotic-resistant test strains were used for experiments: *E. coli* O157H7, *Ps. aeruginosa* ATCC 27853, methicillin-resistant *Staph. aureus* RSKK 95047, vancomycin-resistant *Enterococcus faecium* DSMZ 13590 and *C. albicans* DSMZ 5817. All tests were performed in Mueller–Hinton agar (MHA; Oxoid, Basingstoke, UK) and Sabouraud Dextrose agar (SDA; Difco, Detroit, MI, USA) for bacteria and *C. albicans* respectively. For the disc diffusion assay, fifteen microlitres of ozone- treated and untreated RPSO samples were injected into sterile discs of 6 mm in diameter (Murray *et al.* 1995). Gentamycin (10 μg) and nystatin (100 U) were used as positive reference standards to determine the sensitivity of the tested strains. All experiments were done in triplicate.

The antimicrobial activities of the microcapsules with ORPSO were tested using the agar well method (Bell and Grundy 1968). One hundred microlitres of suspension containing 10^6 CFU ml^{-1} bacteria and 10^5 CFU ml^{-1} yeasts were spread onto sterile petri dishes containing MHA and SDA respectively. Six-mm wells were excised aseptically and filled with 50 mg microcapsules containing ORPSO. The plates were kept at 4°C for 2 h and then incubated at 35°C for 24 h for bacteria and 48 h for yeasts. The results were recorded by measuring the zones of growth inhibition surrounding the wells. All experiments were done in triplicate.

Antimicrobial activity of the nonwoven fabrics. Three selected antibiotic-resistant test microorganisms (methicillin-resistant *S. aureus* RSSK 232, *E. coli* O157:H7 and *C. albicans* DSMZ 5817) were used to assess the antimicrobial effectiveness of the fabrics. The modified method of Mariscal *et al.* (2010), with modifications was used. Briefly, bacteria were grown overnight in Mueller–Hinton broth (MHB; Oxoid) and *C. albicans* was grown in Sabouraud Dextrose Broth (SDB; Difco) at 37°C and collected by centrifugation. These were then rinsed twice in PBS buffer and resuspended to obtain 10^6 CFU ml^{-1} viable micro-organisms detected by plating on MHA and SDA for determination of IMC.

The nonwoven test fabric was used as a control, and nonwoven fabrics loaded with microcapsules containing ORPSO were used as bio-active fabrics. All test materials were sterilized with ethylene oxide before the assay by using an Ethylene Oxide sterilization system (Axis, Izmir, Turkey). Pieces (5 cm^2) of the sterilized fabrics were placed in sterile plastic petri dishes and inoculated with 100 μl of the microbial suspension. The plates were kept at room temperature in a 60% humidified incubator for 1 and 3 h respectively. The fabrics were then removed using sterile pincers, and immersed in a sterile test tube containing 10 ml of 0·2% Tween 80 in PBS buffer. The tubes were subjected to vigorous vortexing for 3 min. One hundred-μl aliquots from each tube were serially diluted in 900 μl of sterile saline solution (0·85% w/v of NaCl) and then plated on agar media MHA and SDA. After incubating for 24 h at 37°C, FMC were calculated. The CFU data were analyzed using Tukey's and LSD tests in ANOVA (Michelson and Schofield 2002). A *P*-value < 0·05 was considered significant. All tests were done in triplicate.

Statistical analysis

Experimental results were analyzed by SPSS 15.0 statistical analysis software for statistical analysis of means, standard deviations and significant differences. Effects of amount of oil, amount of surfactant parameters and their interaction were analyzed at a 95% confidence interval.

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