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Effect of ultrasound treatment on the properties of nano-emulsion films obtained from hazelnut meal protein and clove essential oil

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ABSTRACT

Hazelnut meal protein (4% (w/v)) and clove essential oil (CEO) (3% (v/v)) were homogenized with ultrasound (US) at different times (2, 4 and 6 min) and amplitudes (50, 75 and 100%) to obtain nano-emulsion films. Film forming nano-emulsions (FFNs) were analyzed for average particle size (D_z) and zeta potential, and edible film characterization were evaluated depending on US treatment, as well as antibacterial and antioxidant activities. D_z values and zeta potential of FFNs decreased with increasing acoustic energy delivered to nano-emulsion system. Thickness and water solubility of films significantly decreased with increasing US treatment. Films became more transparent depending on US treatment probably due to particle size reduction. Tensile strength (TS) of films significantly increased with US treatment, while elongation at break (EAB) slightly increased. Microstructure of films became more homogeneous after US treatment and caused to lower water vapor permeability. Enrichment with CEO has given the films antibacterial activity against *L. monocytogenes*, *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*, and antioxidant activity, and US application has improved these activities. US technology can be used to improve mechanical, barrier and antimicrobial properties of hazelnut meal protein based edible films enriched with CEO.

1. Introduction

In recent years, there is a particular interest focused on proteins which are obtained from defatted meals of oil industry. For instance, functional and bioactive properties of defatted meals such as sunflower, soybean, canola and rapeseed have been widely investigated [1–4]. Besides, hazelnut meal proteins extracted with different solvents and temperatures have been evaluated in terms of functional, bioactive and edible film making properties [5]. Hazelnut (*Corylus avellana* L.) is very popular tree nut all over the world and Turkey is the largest producer of hazelnut with almost 85% of world requirement [6]. Hazelnut is mostly used in confectionery and hazelnut cream in food industry, but recently, oil production from hazelnut has seen an increasing demand due to similar fatty acids profile with olive oils [7]. Hazelnut meal has nutritive value and high protein content, therefore it has widely used for animal feeding [8,9]. Moreover, Tatar, Tunç and Kahyaoglu [10] investigated the functional and rheological properties of Turkish tombul hazelnut protein concentrate and reported that defatted hazelnut flour and cake are suitable functional properties which could be used as an ingredient in the food industry.

Edible films have received considerable interest in recent years to replace with synthetic packaging materials due to prevent environmental pollution. Protein and polysaccharide based films have good mechanical and gas barrier properties, but they are poor against water vapor [11]. On the other hand, lipid based films have good water barrier properties. The combination of these materials in the form of emulsions can ensure that the films to be produced are capable of meeting the requirements of the food packaging industry. Edible films obtained from emulsions may be used as an active packaging because they have capable to carry active agents such as antimicrobials and antioxidants. Antimicrobial films are of particular interest because of their ability to prevent the development of pathogenic microorganisms in ready-to-eat foods [12].

The reduction in particle size of emulsion droplets improves the accessibility of bioactive agents such as antimicrobials and/or antioxidants [11,13]. In addition, particle size reduction could enhance the mechanical and water barrier properties of films [14]. High speed mixing, high pressure homogenization and ultrasound can be used for producing nano-emulsions which has particle size lower than 200 nm [15]. Ultrasonic emulsification was described by Kentish, Wooster, Ashokkumar, Balachandran, Mawson and Simons [16] with two mechanism. Firstly, unstable interfacial waves could be produced by the

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application of acoustic field and the oil phase could be dispersed into particles by bursting in the water phase. Secondly, low-frequency ultrasound application could cause the formation of microbubbles with pressure fluctuations of a simple sound wave and subsequent collapse. The collapse of each bubble causes an implosion on a macroscopic scale and extremely localized turbulence. The turbulent micro explosions act as an effective method for separating primary droplets of dispersed oil into droplets at sub-micron size. Ultrasound (US) technology has been widely used for emulsification [17,18], protein modification [19,20], particle size reduction of whey proteins [21] and curing [22]. Moreover, Borah, Das and Badwaik [23] used US technology for development of potato peel and sweet lime film, and reported that increasing ultrasound treatment times gives better result in film properties. Rodrigues, Cunha, Brito, Azeredo and Gallão [24] observed that surface hydrophilicity and tensile strength of mesquite seed gum and palm fruit oil emulsion films increased due to uniform droplet dispersion.

To the best of our knowledge, there is just one study about edible film production from hazelnut meal proteins [5]. However, there are no researches about both lipid incorporation and ultrasonic nano-emulsification of hazelnut meal protein films. Therefore, the objectives of this study were to form nano-emulsion films from hazelnut meal proteins and clove essential oil (CEO) with US at different times and amplitudes, and to evaluate the effect of US treatment on film forming nano-emulsions and final film properties.

2. Materials and methods

Hazelnuts (*Corylus colurna*) were purchased from Gürsoy Hazelnut Production Factory (Ordu, Turkey) as removed shells-brown skin and the hazelnut oil was extracted from hazelnut by using a headed cold press machine (Ekotok 1, Izmir, Turkey). The resulting cold press hazelnut cake including 8.68% moisture, 43.77% protein, 25.20% carbohydrate, 17.38% lipid and 4.97% ash was used for hazelnut protein extraction. Clove (*Syzygium aromaticum* L.) essential oil (heavier than water) was extracted with a proper Clevenger-type hydro distillation for 3 h at a ratio of plant/water 1/10. Essential oils were collected in aluminum foil wrapped amber vials, and then stored at +4 °C. All chemicals used were of analytical grade.

2.1. Extraction of hazelnut protein

The extraction of hazelnut protein was performed according to Tatar, Tunç and Kahyaoglu [10] with slight modifications. Defatted hazelnut cake was mixed with water at room temperature at cake/water ratio of 1/12 (w/w). The suspension pH was adjusted to 12 with 5 M NaOH and stirred using a magnetic stirrer for 1 h, followed by centrifuging at 3000*g* for 15 min at 4 °C. The precipitate was collected separately and the extraction procedure was repeated 3 times. The supernatant was adjusted to pH 4.5 with 5 M HCl and then it was centrifuged at 3000*g* for 10 min at 4 °C. The supernatant was discarded and precipitate was dried at 40 °C in air circulated oven. Crude protein content of precipitate was determined as 98.38%. Hazelnut proteins were grounded using Waring blender and sieved from 212 μm and stored in glass jars at 4 °C until analyses.

2.2. Preparation of film-forming nano-emulsions (FFN)

Hazelnut protein dispersions were prepared by dissolving 4% w/v in distilled water, and then pH was adjusted to 12 with 5 M NaOH. Dispersion was held 45 min on a magnetic stirrer at 90 °C for protein denaturation. After cooled to room temperature, coarse emulsions were prepared by mixing glycerol (30% w/w based on protein content), Tween 80 (2% v/v of essential oil) and clove essential oil (3% v/v) in a high speed homogenizer at 18,000 rpm for 2 min (IKA T25 model, Staufen, Germany). The resulting coarse emulsions were then treated with an ultrasonic processor (VCX 750, Sonics & Materials, Inc., USA) at 750 W. Different amplitudes (0, 50, 75 and 100%) and sonication times (0, 2, 4 and 6 min) were applied with a titanium probe at 13 mm diameter immersed 1 cm below of the liquid surface. The emulsion temperature during sonication was kept at 25 ± 2 °C with an iced water bath [25]. Control sample was prepared without ultrasound application.

Table 1
Ultrasound parameters and applied acoustic energy to emulsions.

Treatment name	Amplitude (%)	Time (min)	Energy (W/cm^2)
C	0	0	0
US-502	50	2	9.42
US-504	50	4	18.78
US-506	50	6	27.94
US-752	75	2	16.01
US-754	75	4	31.23
US-756	75	6	61.02
US-1002	100	2	25.06
US-1004	100	4	48.26
US-1006	100	6	70.13

C: Control without ultrasound treatment; US-502: Ultrasound treated with 50% amplitude at 2 min; US-504: Ultrasound treated with 50% amplitude at 4 min; US-506: Ultrasound treated with 50% amplitude at 6 min; US-752: Ultrasound treated with 75% amplitude at 2 min; US-754: Ultrasound treated with 75% amplitude at 4 min; US-756: Ultrasound treated with 75% amplitude at 6 min; US-1002: Ultrasound treated with 100% amplitude at 2 min; US-1004: Ultrasound treated with 100% amplitude at 4 min; US-1006: Ultrasound treated with 100% amplitude at 6 min.

Ultrasonic intensity applied to emulsions was given in Table 1 and calculated by dividing the total energy to ultrasound time and probe area. Total energy was displayed as joule in the screen of ultrasonic processor.

2.3. Nano-emulsion characterization

2.3.1. Droplet size and polydispersity index of FFN

The mean droplet size (D_z) and polydispersity Index (PdI) was analyzed with a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). Average droplet size of FFN was measured with dynamic light scattering (DLS) at 25 °C and 633 nm. Samples were diluted 1:20 with ultrapure water to avoid multiple scattering effects. Duplicate nano-emulsions were measured in triplicate [26].

2.3.2. Zeta (ζ) potential

Zeta potential of the FFNs was analyzed by measuring the electrophoretic mobility of emulsions using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA). All measurements were carried out at 25 °C and samples were diluted 1:20 with ultrapure water to minimize the effects of multiple scattering.

2.4. Film formation

Fifty mL of FFN were poured into 15 cm acrylic plates in diameter, held 30 min in a vacuum oven (NUVE, EV 018 Model, Ankara, Turkey) to remove air bubbles, and then, oven-dried at 40 °C under air circulation (JSR, JSOF-50 model, Gongju-City, Korea) for 16 h. Then, samples of dried films were taken out of the plates and conditioned to 54% relative humidity (by using a solution of saturated magnesium nitrate) within a desiccator at room temperature for 3 days. All films were prepared as triplicates.

2.5. Characterization of edible films

2.5.1. Physicochemical properties

A digital micrometer (Insize, 3101-25A model, Jiangsu, China) with