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Enhancing the antibacterial efficacy of isoeugenol by emulsion encapsulation

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ABSTRACT

Food spoilage and foodborne illnesses are two global challenges for food manufacturers. Essential oils are natural antibacterials that could have a potential for use in food preservation. Unfortunately high concentrations are needed to obtain the desired antibacterial effect, and this limits their use in food due to their adverse organoleptic properties. Encapsulation could make essential oils more effective by concentrating them in the aqueous phase of the food matrix where the bacteria are present. Here we tested encapsulation of the essential oil isoeugenol in spray-dried emulsions as a means of making isoeugenol a more effective antibacterial for use in food preservation. We used β-lactoglobulin and *n*-OSA starch as emulsifiers, and some emulsions were coated with positively charged chitosan to promote the contact with bacteria through electrostatic interactions. The antibacterial efficacy was quantified as the minimal bactericidal concentration in growth media, milk and carrot juice. The emulsion encapsulation system developed in this study provided high loading capacities, and encapsulation enhanced the efficacy of isoeugenol against Gram-positive and -negative bacteria in media and carrot juice but not in milk. Chitosan-coating did not enhance the efficacy further, possibly due to the aggregation of the chitosan-coated emulsions. The encapsulation system is easy to upscale and should be applicable for encapsulation of similar essential oils. Therefore, we believe it has potential to be used for natural food preservation.

1. Introduction

Two major problems concerning microorganisms in food are food spoilage and foodborne illnesses. It has been estimated that roughly one third of foods go to waste every year globally (Food and Agricultural Organization United Nations, 2011). This is a huge waste of resources in a world where not everyone has access to food every day. The Centers for Disease Control and Prevention estimate that 48 million citizens in the United States of America contract foodborne illnesses every year (Centers for Disease Control and Prevention, 2011). These numbers make it clear that much can still be achieved by improving food safety by better control of microorganisms in food products. Control of microorganisms in food is typically sought by the addition of chemically synthesized preservatives, but a consumer trend towards natural foods has spurred an interest in using natural antibacterials as food preservatives.

Many natural antibacterials can be found among essential oil compounds. Essential oils are volatile compounds extracted from plants, often as part of the plants' natural defense against microorganisms (Burt, 2004). There is still much to be learned about the mode of action for essential oils, but a common and important feature is their hydrophobicity, which enables interaction with the cell membrane, leading to leakage of important ions and other compounds (Burt, 2004). The hydrophobicity is, however, problematic for the antibacterial efficacy in food matrixes because essential oils partition into the lipid phase whereas bacteria thrive in the aqueous phase (Robins and Wilson, 1994). Higher concentrations of essential oils are therefore usually needed in food systems to achieve the same antibacterial effect as obtained growth media (Smith-Palmer et al., 2001). A solution to this problem could be encapsulation of the essential oil into a water soluble matrix.

The aim of this study was to enhance the antibacterial efficacy of the essential oil isoeugenol through emulsion encapsulation. Isoeugenol is a phenylpropene, which is a subgroup of the essential oils (Hyldgaard et al., 2012). It has the chemical name 2-methoxy-4-(1-propenyl)phenol (Thompson et al., 1983) and can be produced by a range of plants, e.g., the petunia flower (Koeduka et al., 2006). The Food and Drug Administration in the United States of America has approved it for use in food (21CFR172.515).

Essential oils can be encapsulated in many different systems, such as emulsions, liposomes, polymer-particles and micelles. We focused on emulsion encapsulation followed by spray-drying, using two types of

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emulsifiers: β-lactoglobulin and *n*-OSA starch. β-Lactoglobulin is a major constituent of whey protein. Its emulsifying properties are pH dependent (Losso and Nakai, 2002) with a maximum in emulsion droplet size around its isoelectric point at pH 5.2 (Tcholakova et al., 2005; Mounsey et al., 2008). *n*-OSA starch is short for *n*-octenylsuccinate-derivatised starch, meaning that the starch has been modified with octenyl-substituted succinic acid. This modification allows for a material with high emulsifying properties that is also food-approved (Drusch and Schwarz, 2006).

Encapsulation in a water soluble matrix should itself enhance the efficacy of the essential oil by changing the distribution between the aqueous phase and the lipid phase. However, the antimicrobial efficacy could potentially be increased further by promoting direct contact between the encapsulated oil and the bacterial cells. Bacteria have a negative surface potential, and application of a positively charged surface coating to the oil-loaded emulsions could promote the direct contact through attractive electrostatic forces. Chitosan was chosen in this study as a means of obtaining a positive surface charge of emulsions. Chitosan is the *N*-deacetylated derivative of chitin, which is a naturally abundant glycosaminoglycan found in the exoskeleton of e.g., crustaceans and insects (Kumar, 2000). With a pKa value of 6.3–7, it is a positively charged polymer below neutral pH (Klinkesorn and McClements, 2009). The polymer is biocompatible and biodegradable and has been shown to have antimicrobial effects against a range of microorganisms. However, the antimicrobial activity is dependent on pH, solvents, concentration and molecular weight and deacetylation degree of the chitosan tested (Kong et al., 2010).

The antibacterial efficacy of isoeugenol emulsions was tested against two model organisms in this study: The Gram-negative *Escherichia coli K12* and the Gram-positive *Listeria monocytogenes*, which are both common foodborne pathogens. We compared the antibacterial efficacy of encapsulated and unencapsulated isoeugenol, and evaluated the potential for further enhancing the antibacterial effect by targeting the emulsions to bacterial cells via positively charged chitosan coatings.

2. Materials & methods

2.1. Materials

Chitosan (Sigma-Aldrich, 448869, lot# SLG1673V), *n*-OSA starch 12635 (Cargill, C*Emcap 12635, batch no. R13M085A), glucose syrup powder (Cargill, C*Dry GL 01934, batch no. 02120671), β-lactoglobulin (BiPro, lot# JE099-2-420), isoeugenol (Sigma-Aldrich, Kosher, W246808-1KG-K, Lot# MKBJ9376V), tween 80 (Fluka analytical, Sigma-Aldrich, 59924, lot # BCBJ6978V), sodium acetate (NaOAc), acetic acid (AcOH), thymol (Sigma-Aldrich, T0501, Batch# 126K0140), diethylether (Sigma-Aldrich, 32203N, Lot: SZBD0180V), acetone, milk (Milsani, minimum 3.5% milk fat, UHT treated, homogenized), carrot juice (Biotta, lactofermented, pasteurized, organic).

Tryptic Soy Broth (TSB, CASO) (3.0% (w/v), pH 6, Merck 1054590500), Yeast Extract (Fluka Analytical, 92144, lot: BCBH5306V), Ringer's solution (RINGER tablets, Merck 1.15525.0001), peg lids (Thermo Scientific, Nunc-TSP, cat. no.: 445497), 96 well plates (Nunclon™ surface, Nunc, cat. no.: 161,093), Phosphate buffered saline (PBS, Amresco, 20× concentrate, pH 7.5, code: E703, lot: 1323C322), Dialysis tube (Pur-A-Lyzer Maxi 12000, Sigma-Aldrich, PURX12005-1KT, MWCO 12–14 kDa).

2.2. Methods

2.2.1. Preparation of spray-dried emulsions

Emulsions containing isoeugenol were made by homogenization followed by spray-drying. All materials were weighed off according to Table 1. Emulsifier (*n*-OSA starch 12635 (Cargill, Germany, C*Emcap 12635) or β-lactoglobulin (Davisco Foods International, USA)) and glucose syrup (Cargill, Hamburg, Germany, C*Dry GL 01934) were added during magnetic stirring for 45 min until all materials were dissolved.

Table 1
Composition solution for encapsulation of isoeugenol.

Material	Mass (%)
Isoeugenol	18
Emulsifier (β-lactoglobulin/*n*-OSA)	3
Demineralized water	55
Glucose syrup powder	24

Finally, isoeugenol (Sigma-Aldrich, Copenhagen, Denmark, W246808) was added and pre-emulsions were made with a high-shear homogenizer (Ystral, Germany, X 10/25, Masch-Nr: 508082) at 24,000 rpm for 1 min. These pre-emulsions were then passed through a high-pressure homogenizer (Niro Soavi S.p.A., Type NS1001L2K S.N. 4810) with a stage 1 pressure at 250 bar and a stage 2 pressure at 50 bar. Emulsions were taken from the second pass.

Some emulsions were coated with chitosan (Sigma-Aldrich, Copenhagen, Denmark, 448869). Chitosan (0.6% (w/v)) was dissolved in 0.172 M/0.028 M NaOAc/AcOH buffer (pH 5.4) and passed through a 20 μm filter (Merck Millipore, Germany, 475855) before adding powderous glucose syrup (79.9% (w/w)) and stirring for 1.5 h. The chitosan solution was then mixed 1:1 with the emulsion solution, magnetically stirred for ca. 10 min, and passed through the high-pressure homogenizer with stage 1 at 50 bar through one pass.

Emulsions were spray-dried on a pilot plant spray-dryer at 180/70 °C inlet/outlet temperature and 4 bar with rotary atomization (Mobile Minor 2000, Niro A/S, Copenhagen, Denmark with a rotary atomizer (SL 24–50/M, Niro A/S, Copenhagen, Denmark)).

2.3. Characterization of emulsions

Emulsions were characterized to obtain information about size, structure, surface charge, loading capacity, and release profiles.

2.3.1. Droplet size and zeta potential

The droplet size distribution for emulsions in liquid suspension was measured before and after spray-drying using a LA-95OV2 particle size distribution analyzer from Partica, Retsch Technology. Approximately 50 μL of emulsion suspension or spray-dried powder was added to a standing cell containing demineralized water along with a magnet (note that the emulsion concentration is not relevant for the accuracy of this measurement). Measurements were performed at 25 °C with an equilibration time of 60 s. Results are reported as averages of triplicates of the 50th (D50) and 90th (D90) percentiles of the size distributions.

2.3.2. Structure of spray-dried emulsions

Scanning electron microscopy images were taken of spray-dried encapsulated isoeugenol made with *n*-OSA starch using a Nova NanoSEM (FEI) with a Low Vacuum Detector (LVD) at 5.00 kV. Spray-dried emulsion powder was placed on an aluminum sample holder with carbon tape. The same spray-dried emulsion powders were dissolved in demineralized water and air-dried directly on a sample holder. Control samples were pure glucose syrup powder and glucose syrup powder dissolved in demineralized water.

Table 2
Material for release profiles.

Sample	Amount (mg)
β-Lactoglobulin no coat	34.5
β-Lactoglobulin chitosan	77.8
n-OSA no coat	38.4
n-OSA chitosan	71.4
Pure isoeugenol	13.6