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Contribution of okra extracts to the stability and rheology of oil-in-water emulsions

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ABSTRACT

Three okra polysaccharide extracts were isolated and studied in terms of their composition and their capacity to affect the rheology and stability of emulsions. HBSS (hot buffer soluble solids, extracted at 70 °C, pH = 5.2) comprised of charged (zeta potential −21.5 mV) polysaccharides sizing between 5 kDa ($d \sim 3$ nm) and 50 kDa ($d \sim 200$ nm), and a population of very large molecules (MW >> 1.4 MDa). Upon addition in Tween 20-stabilized emulsions, HBSS caused flocculation and enhanced creaming at low concentrations (0.125%), while at higher concentrations (1.25%–2.50%) it drastically reduced creaming due to its increase of the continuous phase viscosity.

CHSS (chelating agent soluble solids, extracted at 70 °C, pH = 5.2) comprised of distinct polysaccharide populations between 12 kDa ($d \sim 10$ nm) and 70 kDa ($d \sim 80$ nm), with a negative zeta potential (−14.3 mV) and a high (31%) protein content. Upon addition in Tween 20-stabilized emulsions, CHSS induced flocculation, shear-thinning rheology, and rapid creaming at concentrations above 0.5%. DASS (diluted alkali soluble solids, extracted at 0 °C) comprised of polymers 12 kDa ($d \sim 20$ nm) and 70 kDa ($d \sim 130$ nm), and had a low zeta potential (−6.2 mV). Adding small amounts (0.25%) of DASS in emulsions induced flocculation, development of shear-thinning rheology and fast creaming. At higher DASS concentrations (above 1.65%), viscosity increase of the aqueous phase delayed creaming.

1. Introduction

Okra (*Abelmoschus esculentus L.*), also known as lady's fingers, gumbo, bamya/bamia, or bhindi, is a flowering plant of the *Malvacae* family. Originally from Africa, it is an integral part of the diet of that continent, as well as of Middle East, southeast Europe, India, Pakistan, the southern United States, the Caribbean, Philippines and Japan, among other countries. Its composition, functional and nutritional properties have been the subject of studies for many years (Karakoltsidis & Kostantinides, 1975; Whistler & Conrad, 1954; Woolfe, Chaplin, & Otchere, 1977). Okra flour has been found to possess antioxidant activity which increases by roasting, while *in vitro* digestibility studies showed that most of its antioxidative activities are available in the intestinal phase of gastrointestinal tracts (Adelakun, Oyelade, Ade-Omowaye, Adeyemi, & Van de Venter, 2009). Okra seeds are considered a high-protein oilseed crop to be used to complement with other protein sources (Bryant, Montecalvo, Morey, & Loy, 1988). Okra pods are rich in phenolic compounds (mainly composed by oligomeric catechins and flavonol derivatives), while the skins' polyphenolic profile is composed principally by hydroxycinnamic and quercetin derivatives (Arapitsas, 2008).

Of primary interest is the thick and slimy texture of okra water extracts; this is due to its polysaccharide content (*i.e.* BeMiller, Whistler, & Barbalowm, 1993). In early works, the latter were identified as acidic polysaccharides consisting of galactose, rhamnose and galacturonic acid (Whistler & Conrad, 1954), while partial acetylation of the acidic polysaccharides has been reported (Tomoda, Shimada, Saito, & Sugi, 1980). Lengsfeld, Titgemeyer, Faller, and Hensel (2004) and Deters, Lengsfeld, and Hensel (2005) reported that okra polysaccharides consist of the sugars rhamnose, galacturonic acid, galactose, glucose and glucuronic acid. In order to elucidate the structure–function relations in okra polysaccharide extracts, the rheology of such systems in solution has been the subject of a number of studies (Meister, Anderle, & Merriman, 1983; Ndjouenkeu, Goycoolea, Morris, & Akingbala, 1996; Ramadas Bhat & Tharanathan, 1987; Woolfe, Chaplin, & Otchere, 1977). Hot sodium acetate extracts ("hot buffer soluble solids", HBSS) were found to consist of highly branched rhamnogalacturonan I containing high levels of acetyl groups and short galactose side chains, while hot EDTA extracts ("chelating agent

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soluble solids", CHSS) were found to contain pectin with less rhamnogalacturonan I regions and slightly longer galactose side chains (Sengkhamparn et al., 2009a). Oscillation rheological measurements have shown that concentrated HBSS solutions are mainly elastic, while CHSS ones are primarily viscous (Sengkhamparn et al., 2010).

Due to the rheological properties described above, okra polysaccharides are of putative interest for applications in the food industry as thickeners, viscosity enhancers, gelling agents and texture modifiers in food emulsion products such as dressings, sauces, low-calorie spreads, and mayonnaise substitutes, among others. Proper utilization of okra extracts towards such applications requires the understanding of their effect on the behavior of emulsion systems. This work investigates the composition and structure of okra polysaccharide extracts, and the effects of their addition to the rheology and stability of low-viscosity model oil-in-water emulsions stabilized by a model non-ionic emulsifies. Extracts were obtained after subsequent application of three protocols in defatted and lyophilized okra skins.

2. Materials and methods

2.1. Materials

Tween 20 (polyoxyethylene sorbitan monolaurate), Trizma base (2-amino-2-(hydroxymethyl)-1,2 propanediol), sodium azide, sodium borohydride and EDTA disodium salt were all obtained from Sigma (St Louis, MO). Petroleum ether was obtained from Mallinckrodt Baker BV (Deventer, Holland). Olive oil was obtained from the local super market. De-ionized water has been used for all formulations. Ultrapure water obtained by a Direct Q apparatus (Millipore, MA) was used for HPSEC (high pressure size exclusion chromatography), size distribution and zeta potential measurements. Dextran standards (molecular weights 1 kDa–1.4 MDa) for HPSEC were purchased from Fluka (Sigma–Aldrich, Steinheim, Switzerland). Kits (LCK 338) for nitrogen determination were purchased from Hach Lange (Düsseldorf, Germany).

2.2. Isolation of okra extracts

The soft and mature okra pods of *A. esculentus L.* (6–9 cm in length) were grown in the area of Meliki (Imathia), Greece, and were obtained from the local market in August 2009. Pods were immediately frozen and kept at −20 °C until handling.

2.3. Isolation of alcohol-insoluble solids

After removal of the seeds and calyxes, the okra pods were freeze dried and defatted by Soxhlet extraction with petroleum ether (bp 40°–65 °C). Subsequently, the lipid-free material (20 g) was extracted with 70% (v/v) aqueous ethanol at 40 °C for 1 h (2 × 300 mL). After filtration, the insoluble residue was washed with acetone and air dried (Alcohol-Insoluble Solids, AIS).

2.4. Sequential extraction of okra AIS

AIS were treated according to the method developed by Vierhuis, Schols, Beldman, and Voragen (2000), as modified for okra by Sengkhamparn, Verhoef, Schols, Sajjaanantakul, and Voragen (2009b), and is as follows: 20 g of AIS were sequentially extracted with 600 mL of the following extractants; 0.05 M sodium acetate buffer, pH 5.2 (three times) at 70 °C for 30 min (hot buffer soluble solids, HBSS); 0.05 M EDTA and 0.05 M sodium acetate in 0.05 M sodium oxalate, pH 5.2 (two times) at 70 °C for 30 min (chelating agent soluble solids, CHSS); and 0.05 M sodium hydroxide and 20 mM $NaBH_4$ (two times) at 0 °C for 30 min (diluted alkali soluble solids, DASS). After each extraction, solubilized polymer was separated from the insoluble residue by centrifugation (5000 g for 25 min) and freeze dried.

2.5. High pressure size exclusion chromatography (HPSEC)

The HPSEC system comprised of: a SpectraSystem SCM 1000 degasser (Thermo Separation Products, San Jose, CA); a SpectraSystem P 2000 chromatographic pump (Thermo Separation Products, San Jose, CA), followed by an μm frit (Idex, Oak Harbor, WA); a GPC/SEC PL-Aquagel-OH 50 × 7.5 mm guard column (8 μm) (Varian Inc, Palo Alto, CA); three tandem GPC/SEC PL-Aquagel-OH 300 × 7.5 mm columns (Varian Inc, Palo Alto, CA); and an ERC 7515 refractive index detector (Rigas Labs, Thessaloniki, Greece). Samples (2.5 mg mL^{-1}) were eluted with ultrapure water containing 0.02% sodium azide with a flow rate of 1 mL min^{-1}. EZChrom™ software (Scientific Software Inc, Pleasanton, CA), was used for data acquisition and handling.

2.6. Protein content determination

Nitrogen content was assessed spectroscopically using a Hach Lange DR 2800 spectrometer (Hach Lange, Düsseldorf, Germany), while reactions were held in a Hach Lange LT 200 thermoreactor (Hach Lange, Düsseldorf, Germany). LCK 338 kits (Hach Lange, Düsseldorf, Germany) have been used for the experiments. The principle of the determination is as follows: Nitrogen is oxidized to nitrate; nitrates then react under acid with 2,6-dimethylphenol to form nitrophenol; the latter is then photometrically assessed. The protein content was indirectly estimated by means of multiplication by the generic factor 6.25.

2.7. Measurement of particle radius and zeta potential

Size distributions of the polymer particles have been measured using a Malvern Zetasizer Nano S (Malvern, Worcestershire, UK) using a backscattering technique (measurement at 173°). A refractive index of 1.46 has been used for the dispersed phase, assuming that dissolved polysaccharides are hydrated systems. The viscosity values were taken from the rheological data of the present work. Zeta potential has been measured using a Brookhaven ZetaPlus apparatus (Brookhaven, NY).

2.8. Emulsion preparation

All emulsions under study were prepared by means of mixing equal volumes of a stock emulsion (A) and an okra extract solution (B). Individual formulations were manufactured as follows:

2.8.1. Preparation of stock emulsions (A)

An aqueous buffer solution containing of 0.025 M Trizma and 0.025% sodium azide was prepared, and the pH was set to 7.0. Non-ionic surfactant Tween 20 was subsequently dissolved in order to obtain a 4% solution. This is a concentration adequate for the stabilization of fine emulsion droplets, but not adequate for promoting any kind of depletion effects (Dickinson, Ritzoulis, & Povey, 1999). This was then magnetically stirred with olive oil in order to prepare a crude pre-mix with an oil volume fraction $\varphi = 0.5$. This pre-mix was subsequently homogenized using a laboratory ultrasonic homogenizer (Hielscher UP-100H, Germany) for 4 min. The droplet size and distribution were measured using a Malvern laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd, Worcesteshire, UK), in order to make sure that all formulations had the same average