Food Research International 54 (2013) 1730–1737

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Okra extracts as emulsifiers for acidic emulsions

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ARTICLE INFO

Article history:
Received 8 July 2013
Accepted 30 September 2013

Keywords:
Okra
Pectins
Emulsions
Rheology
Ostwald ripening
Microscopy

ABSTRACT

Extracts rich in pectins were isolated by aqueous extraction at pH 4.0 (OE4) and 6.0 (OE6) from okra (*Abelmoschus esculentus*) pods. They were examined in terms of their composition and emulsion stabilizing capacity in model acidic emulsions (hexadecane-in-water at pH 3.0). Extracted polysaccharides were assessed using size exclusion chromatography (SEC) and FTIR spectroscopy. The properties and stability of the resulting emulsions were examined by means of droplet size distribution and ζ-potential measurements, viscometry, fluorescence microscopy and protein interface concentration. As emulsifiers, both extracts produce emulsions of initially monomodal size distribution and of similar average droplet size, while ζ-potential was negative for both. The emulsions prepared using OE6 are relatively stable in terms of droplet size distribution and average droplet size after 30 days of storage, while emulsions prepared with OE4 increase in droplet size, in part attributable to Ostwald ripening. Determination of the protein interface coverage Γ suggests that the increased stability of the OE6 emulsions should be attributed to the higher protein load of the interfaces prepared using this extract, resulting to increased rigidity of the interface. Viscosity of emulsions containing OE6 was two times greater than OE4 while aging increases the viscosity of both emulsions. The present work suggests that okra extracts can be strong candidates for emulsification in acidic environments.

1. Introduction

Primary cell wall and middle lamella in higher plant tissues are predominantly composed of pectic substances which are covalently bonded to other biopolymers, such as hemicellulose and cellulose (McCann & Roberts, 1991). Pectins are heteropolysaccharides and their backbone is mainly composed of D-galacturonic acid units (D-GalA) bonded with α-(1 → 4) glycosidic linkages. A number of carboxyl groups are methyl esterified whereas some hydroxyl groups can be acetylated. Linear GalA chains are typically interrupted by rhamnose (Rha) residues, which are covalently linked with neutral sugar side-chains composed mainly of arabinose, galactose and xylose. The number of methyl-esterified groups and proportion of neutral sugars are primarily governed by the pectin source and extraction protocol (Kjøniksen, Hiorth, & Nyström, 2005; Turquois, Rinaudo, Taravel, & Heyraud, 1999).

Pectins are widely utilized in the food industry for their gelling, stabilizing and thickening properties. While most of the plant tissues contain pectic substances (0.5–4.0% on wet basis of plant material), its commercial production is limited to a small range of raw materials, such as citrus peel and apple pomace (Kashyap, Vohra, Chopra, & Tewari, 2001; Sakai, Sakamoto, Hallaert, & Vandamme, 1993; Willats, Knox, & Mikkelsen, 2006) and the exploration of alternative sources of pectins is ongoing (Iagher, Reicher, & Ganter, 2002; Williams, Sayers, Viebke, & Senan, 2005).

High emulsification capacity is usually attributed to proteins whereas polysaccharides typically demonstrate negligible surface activity at the o/w interface due to their hydrophilic character and are, therefore, not so useful as emulsifying agents. In some cases, nanocrystals of certain polysaccharides act as emulsion stabilizing agents (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). Similarly to most polysaccharides, pectins are not normally considered as emulsifying agents except the acetylated pectin from sugar beet. It has been shown that it possesses greater surface activity than commercially produced low- or high-methoxyl pectins and is capable of producing and stabilizing fine o/w emulsions (Dea & Madden, 1986). The emulsifying properties of sugar beet pectins were attributed to the presence of acetyl groups (4–5%), co-extracted protein fraction and ferulic acid moieties covalently attached to the pectin molecule (Dea & Madden, 1986; Endreß & Rentschler, 1999; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). Citrus pectins with low molecular weight of about 60–70 g mol^{-1} and high degree of methoxylation were found also to be good emulsifying agents (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002).

Okra (*Abelmoschus esculentus* (L.) *Moench*) originates from Africa and is now widely spread in many areas such as Asia, Middle East, India and the southern states of the USA. Currently, it is suggested that water extracted okra polysaccharides can be used as a diverse food ingredient (BeMiller, Whistler, & Barbalowm, 1993; Costantino & Romanchick-Cerpoviez, 2004; Romanchik-Cerpovicz, Costantino, & Gunn, 2006; Romanchik-Cerpovicz, Tilmon, & Baldree, 2002; Woolfe, Chaplin, & Otchere, 1977). Okra pectins are found to be acidic, random coil

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0963-9969/$ – see front matter.
http://dx.doi.org/10.1016/j.foodres.2013.09.051

polysaccharides composed of galactose, rhamnose and galacturonic acid. The repeating unit was reported to be α-(1-2)-rhamnose and α-(1-4)-galacturonic acid residues including disaccharide side chains (Tomada, Shimada, Saito, & Sugi, 1980) and they form viscous solutions that exhibit pseudoplastic behavior (Georgiadis et al., 2011; Kontogiorgos, Margelou, Georgiadis, & Ritzoulis, 2012; Sengkhamparn et al., 2010). Furthermore, they differ greatly from those extracted from apple, citrus and beet in terms of protein and acetyl contents, indicating their greater hydrophobicity and therefore substantial surface activity at the o/w interface suggesting that pectin derived from okra can be used as an effective emulsifying agent (Kravtchenko, Voragen, & Pilnik, 1992; Levigne, Ralet, & Thibault, 2002; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009; Thibault, 1988).

The aims of present work, therefore, were to obtain okra extracts rich in pectins using different extraction protocols and determine their emulsifying capacity in model o/w emulsions under acidic conditions.

2. Materials and methods

2.1. Materials

The soft and mature okra pods of *A. esculentus* were grown in Meliki (Imathia, Greece) and purchased from the local market. Pods were frozen and kept at −20 °C until handling. *n*-Hexadecane, sodium azide, citric acid monohydrate, sodium citrate dihydrate, monosodium phosphate and acetic acid (all analytical grade reagents) were obtained from Sigma-Aldrich (St. Louis, MO). Petroleum ether was obtained from Mallinckrodt Baker BV (Deventer, Holland). De-ionized water was used throughout the extraction experiments. Dextrans (GPC M_p 1–1400 kDa) were purchased from Fluka (Sigma-Aldrich, Buchs, Switzerland).

2.2. Isolation of alcohol-insoluble solids

Extraction of okra AIS (alcohol insoluble solids) has been performed according to the technique established by Vierhuis, Schols, Beldman, & Voragen (2000) and which was further adapted to okra by Georgiadis et al. (2011) and Sengkhamparn et al. (2009). In brief, seeds and calyces were removed and the remaining okra pods were freeze-dried. These were then defatted using Soxhlet extraction (Velp Scientifica, MOD 148, Milan, Italy) with petroleum ether (bp 40–65 °C). The lipid-free material (20 g) was then extracted twice using 300 mL ethanol (70% v/v) at 40 °C for 1 h. Filtration followed (2 times, 150 mL), during which insoluble residue (alcohol insoluble solids, AIS) was washed with acetone and air-dried for 24 h.

2.3. Pectin extraction

AIS (20 g) were used to extract okra pectins at pH 6.0 (600 mL for 30 min (three times) at 70 °C using 0.05 M phosphate buffer, pH 6.0). This sample was labeled "OE6". A second batch of AIS (20 g) was used for the extraction of okra polysaccharides at pH 4.0 (600 mL for 30 min (three times) at 70 °C with 0.05 M acetate buffer, pH 4.0). This sample was labeled "OE4". In both samples, the soluble biopolymer was separated from the insoluble residue by means of centrifugation (5000 ×*g* for 25 min at 25 °C, Sorvall, RC 28C, Newtown, CT) and freeze-dried.

2.4. Preparation of okra extract solutions and emulsions

Preliminary experiments on the optimum concentration of okra extracts towards emulsion stability showed that okra extracts at concentration 1.44% w/v with dispersed phase volume fraction $\varphi = 0.2$ (*n*-hexadecane) and under acidic conditions (pH 3.0) produce fine emulsions. The aqueous phases of the emulsions were prepared by means of dissolving okra extract powders at 1.8% w/v concentration in citric buffer (0.01 M, pH 3.0) at room temperature. Okra extract solutions were characterized at 1.8% w/v concentration. For 1.44% w/v emulsion preparation, the above aqueous phases were magnetically stirred with hexadecane for 3 min in order to produce emulsion pre-mixes with oil volume fraction $\varphi = 0.2$ and 1.44% w/v final extract concentration in the entire emulsion. This pre-mix was immediately homogenized (IKA T18 basic, Ultra-Turrax, Germany) for 1 min. For the determination of the long-term stability all emulsions were stored in an incubation chamber at 25 °C.

2.5. Determination of particle droplet distribution

Droplet size distribution was measured immediately after the emulsion preparation and after 5, 10, 20 and 30 days of storage using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) laser diffraction particle size analyzer using the small volume sample dispersion unit Hydro 2000SM (Malvern Ltd., UK). Refractive index of hexadecane and dispersion medium (citric buffer, 10 mM, pH 3.0) was set to 1.435 and 1.333, respectively. Consequently, droplet size was described using the surface-weighted mean diameter (d_{32}) and volume-weighted mean diameter (d_{43}). The measurements were performed in duplicates in three different emulsion preparations yielding in total six replicates for each sample.

2.6. Rheological measurements of okra extracts and emulsions

Rheological properties of samples were measured using a Bohlin Gemini 200HR Nano rotational rheometer (Malvern Instruments, Malvern, UK) equipped with cone-and-plate geometry (40 mm diameter, cone angle 4°) and Peltier temperature controller. All measurements were performed in a steady shear mode in the range 0.01–1000 s^{-1} at 25 °C. Viscosity measurements were conducted immediately after preparation of okra extract solutions and emulsions and after 5, 10, 20 and 30 days of storage. All measurements were performed in duplicates.

2.7. Determination of interfacial protein concentration

Okra extract stabilized o/w emulsions were centrifuged at 2727 ×*g* for 5 min (Centrifuge 5702, Eppendorf, Hamburg, Germany) in order to separate the dispersed phase (oil droplets) from the continuous phase and serum was then carefully collected using a syringe. Interfacial protein concentration (Γ, mg m^{-2}) was calculated as the protein concentration difference in the extract and serum solutions divided by the specific surface area of the oil droplets:

$$\Gamma = \frac{\text{mg of adsorbed protein}}{\text{SSA} \times \text{mL of oil in emulsion}} \quad (2)$$

where specific surface area (SSA), m^2/mL was obtained by the result analysis report of the instrument. Protein was measured in both solutions and serum of centrifuged emulsions according to the Bradford method (Bradford, 1976) using Quick Start™ Bradford Protein Assay kit. Calibration curve was constructed using bovine serum albumin (BSA) and absorption was measured at 595 nm. All measurements were performed at least six times.

2.8. Determination of ζ-potential

All ζ-potential measurements were performed using a ZetaSizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 °C. Emulsions were diluted 1000 times in buffer solutions in order to avoid multiple scattering effects. All measurements were performed in duplicates immediately after emulsion preparation and after 5, 10, 20 and 30 days of storage.