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ORIGINAL PAPER

Entrapment of Flaxseed Oil Within Gelatin-Gum Arabic Capsules

S. Liu · N. H. Low · Michael T. Nickerson

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Abstract The aim of this study was to optimize the encapsulation of flaxseed oil within a gelatin-gum Arabic (GA) matrix via complex coacervation. The effect of homogenization rates (3,000–15,000 rpm) and total biopolymer concentrations (1–2% w/v) on emulsion efficiency was studied in order to optimize the wall matrix. The physicochemical properties of the dried powder, and the capsule's ability to inhibit oxidation during storage were assessed. As homogenization rates increased from 3,000 to 9,000 rpm, the structure of the capsule transitioned from a spherical mononuclear-type to irregular-shaped multinuclear capsules. The size of the capsules and amount of non-encapsulated oil was found to increase as the total biopolymer concentration was raised from 1 to 2% (w/v). Subsequently, gelatin-GA capsules were produced with a 1:1 core-to-wall ratio at a total biopolymer concentration of 2% (w/v) and at a homogenization rate of 9,000 rpm. Formed capsules had an encapsulation efficiency of 84% and showed a protective effect against the production of primary and secondary oxidative products versus non-encapsulated oil during 25 days of room temperature storage.

Keywords Flaxseed oil · Gelatin · Gum Arabic · Encapsulation · Oxidative stability

S. Liu · N. H. Low · M. T. Nickerson (✉)
Department of Food and Bioproduct Sciences,
University of Saskatchewan, 51 Campus Dr.,
Saskatoon, SK S7N 5A8, Canada
e-mail: Michael.Nickerson@usask.ca

Introduction

Flaxseed oil offers a rich source of omega-3 fatty acids (α-linolenic acid) increasingly recognized for their role in reducing the risk of diseases and maintaining human health [1]. However, its incorporation into foods is hindered due to its incompatibility (i.e. lack of solubility) in the aqueous food environment and its inherent instability against oxidation [2, 3]. Various approaches have been used to increase the levels of essential fatty acids (EFAs) in the diet. Examples include: (a) the inclusion of flaxseed oils into animal feed, resulting in EFAs accumulating at higher levels in tissues; (b) creation of transgenic plants that are capable of synthesizing higher levels of EFAs than conventional lines; and (c) encapsulation technology [4].

The entrapment of flaxseed oil within micron-sized particles offers a novel means to increase its compatibility in food, and at the same time improve their stability and controlled release properties for optimized bioavailability and dose delivery. The technology involves encasing or coating the core ingredient within a wall material (e.g. polysaccharides or proteins), often with cross-linking to improve mechanical integrity. Depending on the formulation, desirable release and functional properties (e.g. stability, size, and strength) can be achieved to suit a wide range of applications. Although encapsulation technology is commercially available, significant hurdles still limit its widespread use in food.

The coacervation process involves the electrostatic attraction between two biopolymers of opposing charges, and typically occurs over a narrow pH range. Gelatin-gum Arabic (GA) based capsules have been previously studied for a variety of applications. Yeo et al. [5] investigated the effect of polyion concentration, homogenization rates, and

oil release upon heating for entrapped flavor oils within gelatin-GA capsules. Dong et al. [6, 7] studied the effect of processing parameters on the entrapment of peppermint oil within gelatin-GA capsules. Furthermore, Chang et al. [8] and Prata et al. [9] studied the release properties of camphor and vetiver oils, respectively, from gelatin-GA based capsules. Electronic ink [10] and textiles [11] are two other products that use encapsulated materials.

Gelatin is a linear protein derived from the partial hydrolysis of collagen, which contains high levels of hydroxyproline, proline and glycine. It remains advantageous as a delivery matrix due to its biocompatibility and ability to form thermally reversible networks, which melt above 35 °C depending on the gelatin concentration used [12]. In contrast, GA is an anionic arabinogalactan polysaccharide-protein complex comprised of three fractions. The major faction (~89% of the total; ~250 kDa) consists of a β-(1 → 3) galactopyranose (galactan) polysaccharide backbone that is highly branched with β-(1 → 6) galactopyranose residues terminating in arabinose and glucuronic acid and/or 4-*O*-methyl glucuronic acid units [13]. The combination of these two materials produces an encapsulating material. Thus, the objectives of this study were to determine the effect of total biopolymer concentration and emulsifying parameters on capsule formation and to assess the physicochemical properties of the encapsulated flaxseed oil.

Materials and Methods

Materials

Porcine gelatin (Type A, 300 Bloom, 9.47% moisture) was purchased from Sigma-Aldrich Co. (Oakville, ON, Canada), whereas GA (Gum Arabic FT Pre-Hydrated, Lot #: 11229, 2007) was kindly donated by TIC Gums (Belcamp, MD). The chemical composition of GA powder was determined to be: 9.56% moisture, 0.86% protein (N × 6.25), 0.11% lipid, 84.28% carbohydrate and 5.19% ash (mineral content (w/w): 0.50% sodium, 0.24% potassium, 1.03% calcium, 0.12% magnesium). Chemical analyses of all materials were performed according to Association of Official Analytical Chemists [14] Methods 925.10, 923.03, 920.87, and 920.85 for moisture, ash, crude protein and lipid (% wet weight basis), respectively. Carbohydrate content was determined based on the percentage differential from 100%. Flaxseed oil (Lot#: 804183, 2009) used in this study was kindly supplied by the Bioriginal Food and Science Corp. (Saskatoon, SK, Canada). All chemicals used in this study were reagent grade, and purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

Encapsulation of Flaxseed Oil

Gelatin-GA microcapsules were prepared according to a modified method published by Yeo et al. [5] at a 1:1 biopolymer mixing ratio and 1:1 core-to-wall ratio. Emulsion formation was studied systematically: first, as a function of the homogenization rate (3,000, 6,000, 9,000, 12,000 and 15,000 rpm) at a 2.00% (w/v) total biopolymer concentration and, second as a function of total biopolymer concentration (1.00, 1.25, 1.50, 1.75, and 2.00%, w/v) at a constant homogenization rate (9,000 rpm). Stock gelatin solutions were prepared by dispersing the powder in 150 mL of Milli-Q^{TM} water (Millipore Corporation, MA, USA) under constant mechanical stirring at 500 rpm (Heidolph MR 3003 control stir plate, Heidolph Instruments GmbH & Co., DEU) for 10 min at a constant temperature (50 °C, maintained using a water bath). Stock solutions of GA were prepared in a similar manner except at room temperature (~23 °C). Emulsions were prepared by homogenizing 6.0 g of flaxseed oil into 150 mL of a gelatin dispersion at pre-determined rates (rpm) for 3 min using an Omni Macro Homogenizer (Omni International, Inc., Marietta, GA, USA). Homogenization took place at room temperature using the pre-heated mixture (50 °C), and then the emulsion was placed back into the water bath at 50 °C to maintain the temperature. To visualize the entrapped oil by light microscopy, Nile Red, a lipid-soluble dye, was used (1 mg/g flaxseed oil) to provide sufficient contrast from the aqueous biopolymer phase. The GA solution (150 mL) was then added dropwise to the gelatin-stabilized emulsion to a final aqueous volume of 300 mL (pH 5.09). The emulsion was then allowed to stir for an additional 5 min at 50 °C, followed by acidification to pH 4.0 by the dropwise addition of 10% (w/w) acetic acid, to induce complex coacervation. The heating unit of the water bath was then turned off, and the mixture was allowed to slowly cool from 50 °C to room temperature over time (~1.5 h) under constant mechanical stirring at 500 rpm using a stir bar. Once at room temperature, stirring was terminated so as to afford the phase separation of an upper aqueous-rich and a lower coacervate-rich (with entrapped oils) layer. The upper aqueous-rich phase was removed using a pipette, whereas the remaining coacervate-rich phase was then freeze-dried (Labconco Corporation, MO, USA) for 3 days to yield a free flowing yellowish powder.

Emulsion Morphology

The coacervate-rich lower layer (i.e. prior to freeze drying) was visualized as a function of homogenization rates and

total biopolymer concentration using a light microscope (Zeiss, West Germany) under an objective magnification of 10× and imaged using a Nikon COOLPIX 990 camera (Japan).

Physicochemical Properties of Gelatin-GA Capsules

The physicochemical properties of the freeze-dried capsules were studied for the optimal matrix design (i.e. 9,000 rpm homogenization rate and 2.00% total polymer concentration) based on emulsion morphology visualized by light microscopy. Conditions were selected to produce small spherical-like droplets with no visible free oil. Water activity (A_w) was determined using an AquaLab CX-2 (Decagon Devices, Inc., Washington DC, USA), whereas % moisture was determined gravimetrically after drying in a oven at 105 °C for ~12 h according to Klaypradit and Huang [15]. Surface oil on the dried capsules was determined based on the modified methods of Heinzelmann et al. [16] and Sottitantawat et al. [17], whereby 1 g of capsules was dispersed in 30 mL of hexane followed by vigorous shaking for 30 s. The solvent was filtered (Whatman #41), and then allowed to evaporate overnight in a fume hood. Surface oil was then determined gravimetrically, after heating the beaker at 105 °C for 30 min to remove any residual solvent. Due to the porous nature of the biopolymer wall, it is assumed that minor amounts of interior oil may have been extracted during the process. In contrast, total oil (encapsulated and surface) was determined using the Röse-Gottlieb method [14]. Briefly, 1 g of capsules was initially dispersed in 20 mL of water at 65 °C under constant mechanical stirring for 15 min, followed by the addition of 2 mL of 28–30% (w/w) NH_4OH for an additional 15 min. The mixture was then cooled to room temperature, and transferred to a separatory funnel. Ten milliliters of 95% ethanol was added and mixed gently. Gas was released before adding 25 mL each of diethyl ether and hexane. The flaxseed oil was extracted into the organic phase by inverting the separatory funnel for 30 s. After separation, the lower aqueous phase was removed. The extraction step was subsequently repeated an additional two times with a mixture of 5 mL of ethanol and 15 mL each of diethyl ether and hexane. The organic phases were combined and filtered through anhydrous Na_2SO_4 to removed residual water, and then evaporated under a steady stream of nitrogen to a constant weight. Total oil was then determined gravimetrically. Encapsulation efficiency (EE) was determined based on the ratio between surface to total oil [EE = (Total−Surface)/Total) × 100%] [16]. Three replicates were measured on duplicate batches of capsules.

Oxidative Stability

Oxidative stability, before (bulk oil) and immediately after encapsulation, and during storage at room temperature over a 25 day period was characterized using the conjugated diene (CD) [18, 19], peroxide value (PV) [18, 19] and ρ-anisidine value (ρ-AV) [20] methods. Gelatin-GA capsules (3-4 g/bottle) or bulk oil (50 mL) were stored within sealed nitrogen-flushed 60-mL amber glass bottles for storage stability studies. Oxidative testing was carried out every 5 days, using a separate unopened bottle of capsules and oil. Extraction of flaxseed oil from the gelatin-GA capsules followed the same procedure as that used for total oil determination. Three replicates were measured on duplicate batches of capsules.

Statistical Analysis

A one-way analysis of variance using the Scheffe post-hoc test was used to test for differences in oxidative stability between free and entrapped oils during the encapsulation process (i.e. before and after encapsulation) and over 25 days of storage. Statistics were applied to data from duplicate batches of prepared capsules (note: for each batch, assays were performed in triplicate). Statistical analysis was performed using Systat software (SPSS Inc., Ver. 10, 2000, Chicago, IL, USA).

Results and Discussions

Encapsulation of Flaxseed Oil

Successful encapsulation of flaxseed oil was achieved using the gelatin-GA matrix, based on light micrographs of the coacervate-rich phase. In Fig. 1, the effect of homogenization rates on capsule formation was observed at a constant total biopolymer concentration (2.00%, w/v) and core-to-wall ratio (50:50). Morphology at low homogenization rates (3,000 rpm) revealed the formation of mononuclear gelatin-GA capsules surrounding a single large oil droplet (stained with Nile red). Free oil and capsules without entrapped oil were also prevalent. As homogenization rates increased, multiple smaller oil droplets became entrapped within the gelatin-GA capsules as a consequence of the higher mechanical energy and droplet surface area in the system (Fig. 1b). At rates ≥9,000 rpm, large multinuclear capsules developed with several tiny oil droplets enclosed. It was also observed that the degree of aggregation (and capsule size) increased with the homogenization rate, which was presumed to be associated with a rupturing of the capsule membrane. The possible exposure of the entrapped core

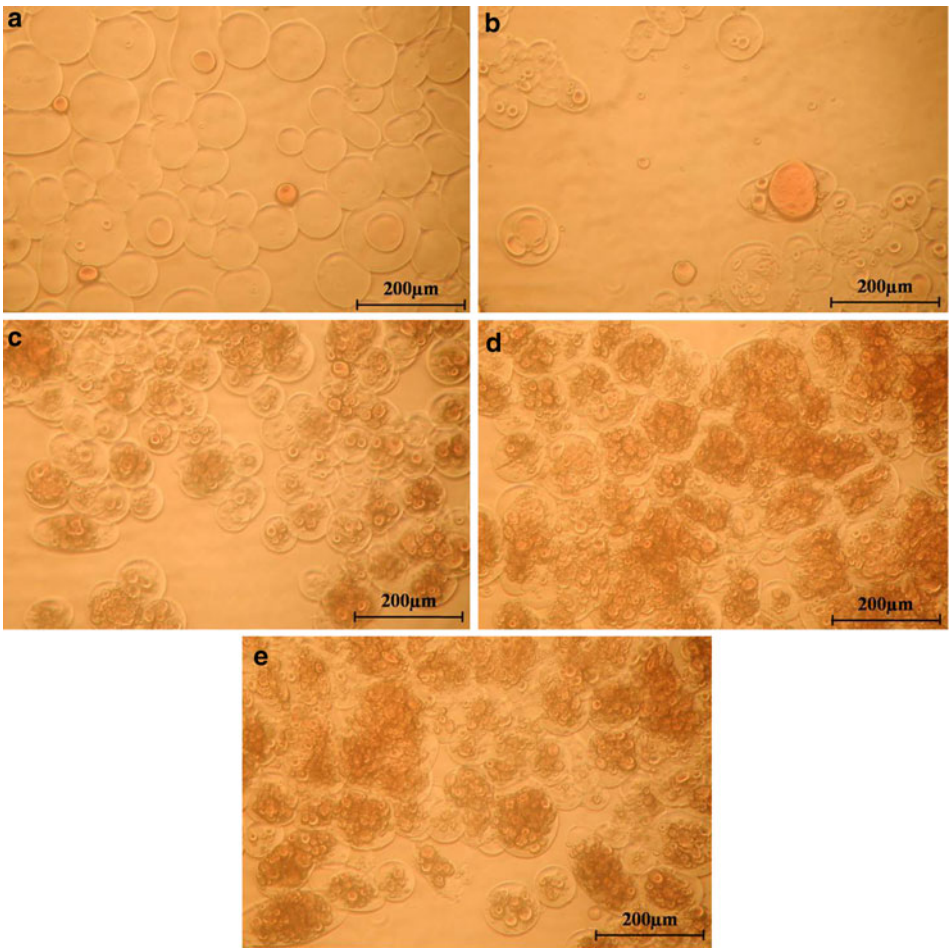

Fig. 1 Gelatin-GA coacervates observed by light microscopy as a function of homogenization rates: **a** 3,000 rpm; **b** 6,000 rpm; **c** 9,000 rpm; **d** 12,000 rpm and **e** 15,000 rpm. Coacervates were formed at a constant total biopolymer concentration (2%, w/v) and core-to-to wall ratio (50:50)

could allow for increased hydrophobic interactions and clustering of neighboring capsules (Fig. 1c–e). The effect of total biopolymer concentration on flaxseed oil entrapment was observed at a constant homogenization rate (9,000 rpm) and a 1:1 core-to-wall ratio by light microscopy (Fig. 2). At all biopolymer levels, multinuclear capsules were observed, however, the size of aggregated capsule clusters decreased substantially as the biopolymer levels were raised from 1.00% to 2.00% (w/v); as did the amount of non-encapsulated oil present (Fig. 2). Furthermore, the thickness of the outer gelatin-GA wall increased with total biopolymer concentration. After homogenization ceased, emulsions containing 2.00% (w/v) biopolymers were thought to be more stable than those at the 1.00% (w/v) level due to a presumed rise in continuous phase viscosity and greater biopolymer alignment at the oil–water interface (as evident from the thicker capsule wall). The trend leads to fewer hydrophobic interactions between neighboring droplets and smaller capsules at the 2.00% (w/v) biopolymer level. Based on these findings, optimal conditions for gelatin-GA capsule formation, occurred using a homogenization rate of 9,000 rpm and a total biopolymer concentration of 2.00% (w/v).

Multinuclear capsules are generally recognized in the literature as having better controlled release properties than mononuclear designs. The multinuclear-type capsules are capable of releasing their contents slowly over time upon complete or partial degradation of the matrix, whereas the mononuclear-type capsules generally release their contents in a single burst [13]. Yeo et al. [5] reported a similar trend in morphology for entrapped baked flavor oil within gelatin-GA coacervates at different homogenization rates (3,000 vs. 9,000 rpm) and biopolymer concentrations (0.5, 1 and 2%, w/v). The authors reported that the release behavior of flavors from mononuclear-type structures were less stable relative to a multinuclear design in response to heat (i.e. released a greater quantity of oil). Oil released from their gelatin-GA capsules was inhibited at low temperatures (e.g., 4 °C and −20 °C), but was promoted when exposed to 100 mM of NaCl due to disruption of the electrostatic linkages between the two biopolymers. Dong et al. [6] found that when the biopolymer concentration or the core-to-wall ratio increased, the morphology of the multinuclear structures average size increased and the shape changed from spherical to irregular.

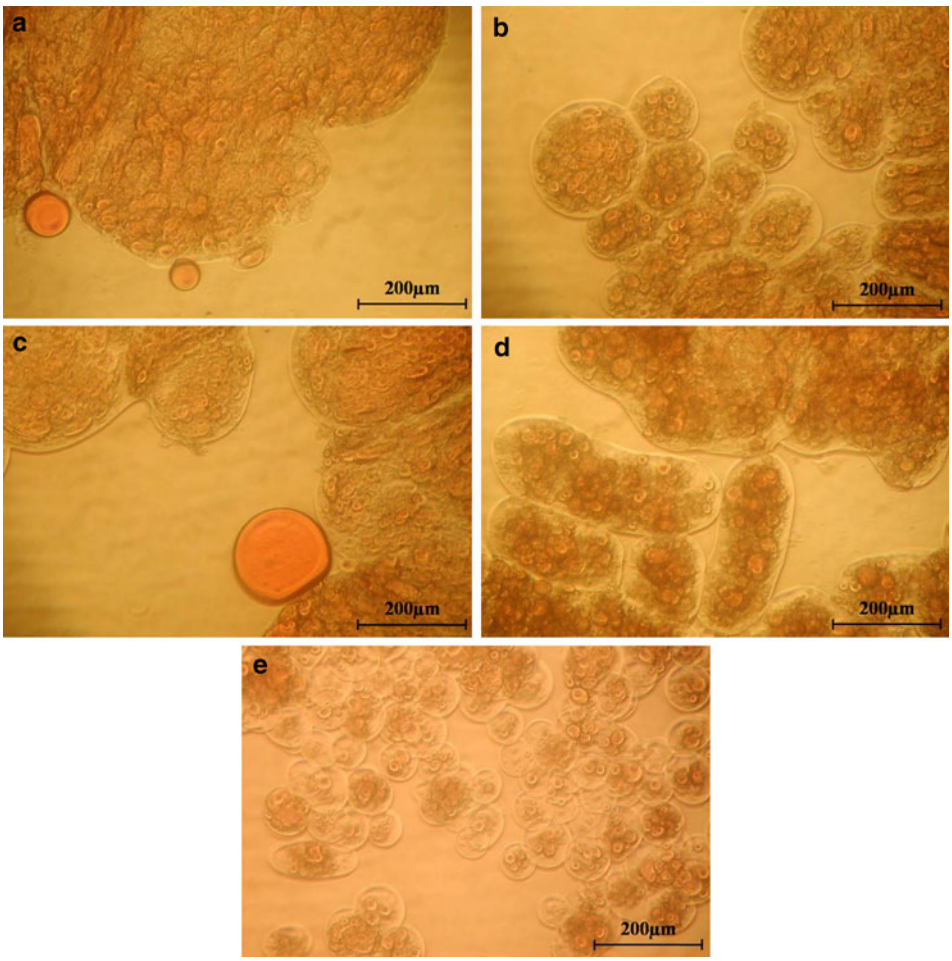

Fig. 2 Gelatin-GA coacervates observed by light microscopy as a function of total biopolymer concentration (% w/v): **a** 1.00%; **b** 1.25%; **c** 1.50%; **d** 1.75 and **e** 2.00%. Coacervates were formed at a constant homogenization rate (9,000 rpm) and core-to-to wall ratio (50:50)

Physicochemical Properties

Freeze drying of the gelatin-GA capsules with entrapped flaxseed oil produced a free-flowing yellowish powder with low moisture (3.17 ± 0.08%) and A_w (0.18 ± 0.00). In the food industry, the maximum moisture specification for most dried powders is 3–4%, with an A_w close to 0.3 [15]. Flaxseed oil encapsulation efficiency reached 84.0 ± 0.7%, with low amounts of oil extracted from the surface (7.7 ± 0.5%). The high entrapment efficiency suggests the majority of α-linolenic acid will be partitioned from the oxygen-rich aqueous phase to hinder oxidation from occurring. Total oil associated with the gelatin-GA capsules was measured at 48.1 ± 0.7%. Yeo et al. [5] reported a similar surface oil content in the coacervate-rich phase, but did not freeze-dry their material into a powder-form. Although the %EE of the designed capsules are comparable to others in literature, the total polymer concentration is significantly less when using freeze-drying versus spray drying. For instance, Bylaitë et al. [21] entrapped caraway essential oil within a whey protein concentrate-maltodextrin matrix and obtained a 85% EE value with a 30% total polymer concentration. A high polymer concentration (40%) was also used to form spray-dried bixin capsules to achieve an EE value of 86% [22]. The use of lower concentrations of polymer to achieve the same %EE is considered one of the major advantages of using the complex coacervation technique.

Oxidative Stability

One of the key roles for an effective encapsulation matrix for encapsulating EFA containing oils is to delay autoxidation, which causes off-flavors and off-odors, as well as nutrient loss. Primary oxidative products, such as peroxides, are highly reactive and readily breakdown into free radicals. These can then degrade further into secondary oxidative products, such as aldehydes and ketones to give additional loss to product sensory quality and EFA bioavailability. The oxidative stability indices (i.e. CD, PV, and ρ-AV) of flaxseed oil, before and immediately after the encapsulation process were not statistically different ($p > 0.05$). Conjugated diene, peroxide and ρ-anisidine values for the bulk oil were found to be 10.7 ± 1.6 µmol/g,

8.44 ± 1.17 mequiv active O_2/kg, and 1.52 ± 0.72 (unit less), respectively. Following encapsulation the value were 10.0 ± 1.9 μmol/g, 11.0 ± 1.8 mequiv active O_2/kg, and 1.89 ± 0.71 (unit less) for CD, peroxide and ρ-anisidine values, respectively. These findings suggest that the encapsulation process had no effect on flaxseed oil oxidative products.

During room temperature storage (25 days), changes to the conjugated diene, peroxide and ρ-anisidine values over time were measured for both bulk and encapsulated oil samples (Fig. 3). Peroxide values began to rise at day 15 in the bulk oil ($p < 0.05$), whereas the encapsulated oil remained unchanged over the 25 day storage period ($p > 0.05$), indicate the production of significant levels of primary oxidative products (peroxides) occurred only in the bulk oil. These products are odorless and colorless, and only indicating their potential for adverse sensory attributes [23]. However, peroxides are highly labile and can degrade via enzymatic or nonenzymatic processes to a variety of secondary products, such as aliphatic aldehydes, alcohols, ketones and hydrocarbons which adversely affect sensory quality [23]. Other evidence of primary oxidative products were observed after 20 days of storage, where a significant ($p < 0.05$) rise in conjugated diene values occurred in the bulk oil relative to the entrapped oil. In the present study, ρ-AV methods indicated that the increase of secondary oxidative products in the bulk oil occurred at day 20 ($p < 0.05$), whereas the encapsulated oil remained unchanged over the entire 25 days period ($p > 0.05$). Constant data from CD, PV and ρ-AV methods over the entire storage period for the entrapped oils indicates that the gelatin-GA capsule design provided adequate protection against oxidative degradation.

The effectiveness of encapsulation at inhibiting oxidation varies tremendously in the literature depending on the type and sensitivity of the core material, properties of the wall matrix, and the encapsulation process. Kolanowski et al. [24] found oxidative degradation occurred at much slower rates in bulk fish oil during storage relative to those entrapped within modified cellulose capsules using a spray drying process. Turchiuli et al. [25] found that after 8 weeks, the presence of conjugated dienes was significantly lower for oils encapsulated within an acacia gum and maltodextrin matrix.

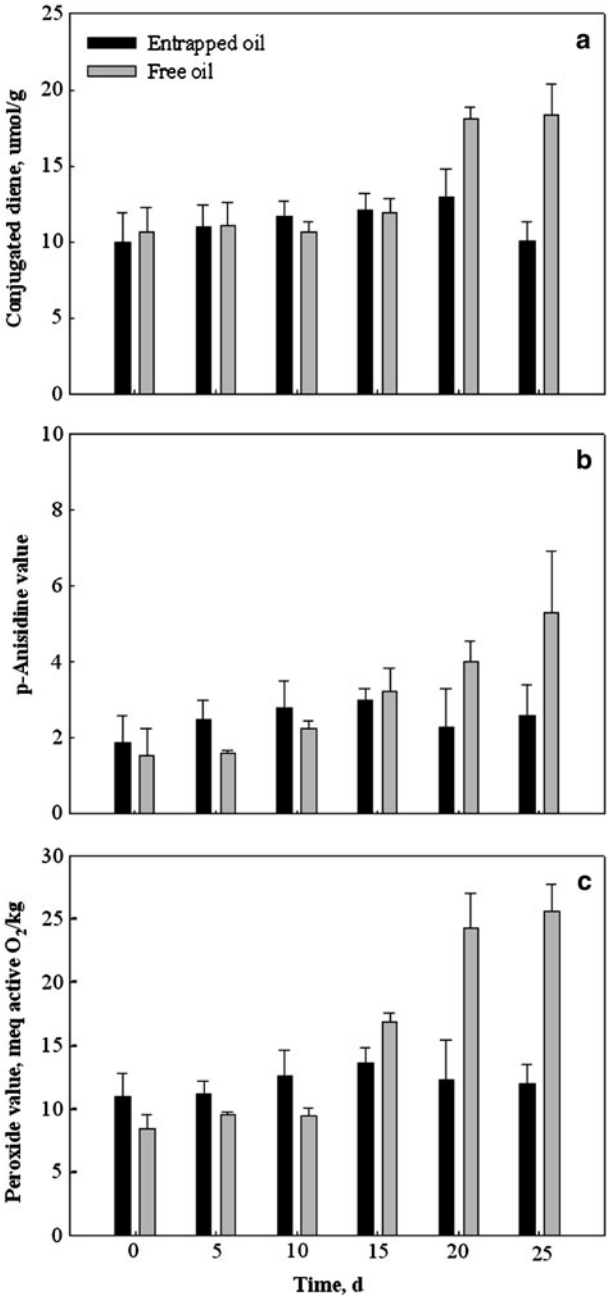

Fig. 3 Changes to **a** conjugated dienes, **b** peroxide and **c** ρ-anisidine values for bulk and entrapped flaxseed oil under storage conditions. Data represent the means ± one standard deviation of duplicate batches (three replicates per batch)

Conclusion

Gelatin-GA capsules for carrying and protecting EFA in flaxseed oil were designed to yield a free flowing powder that acted to inhibit the production of primary and secondary oxidative products during 25 days of storage at room temperature, relative to the bulk oil. Capsules formed by complex coacervation had sufficient stability through electrostatic attraction to maintain their structure without the need for toxic cross-linking agents (i.e. glutaraldehyde). The use of this encapsulation design could lead to increased utilization of flaxseed oil in aqueous food systems, so as to contribute to the health and well-being of consumers.

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