Beta-carotene stability in extruded snacks produced using interface engineered emulsions

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Abstract
Snack-type extrudates were produced using single-layer (SL) and layer-by-layer (LBL) emulsions (0.05% β-carotene in sunflower) to deliver beta-carotene in extrudates. The dry feed was composed of wheat flour (60% w/w), maltodextrin (20% w/w), and lactose (20% w/w). The extrudates were stored at 20, 40 and 60°C and analysed using HPLC (C30 column and diode array detector at 450 nm). The results showed rapid loss of β-carotene during the first 6 days of storage at all temperatures and gradual levelling off at 15 days. LBL emulsion may enhance protection of bioactive compounds in extrusion.

Keywords: Extrusion; beta-carotene; HPLC; layer by layer emulsion; microencapsulation

1. Introduction
Extrusion in food processing is used to produce breakfast cereals, ready to eat snack foods as well as other textured foods. By using extrusion, raw materials can be converted to various intermediate and finished food products [1]. The increased production and consumption of snack foods has led to expanding choices of products being made available to consumers [2]. Extrusion processes enable continuous operation in cooking and shaping [3]. The high mechanical shear in the twin screw extrusion process may result in breaking of covalent bonds in biopolymers. The structural disruption and mixing promotes changes in functional properties of food ingredients and affects texture [4]. The advantages of an extrusion process include the continuous production of high quality products, the capability to produce products with textural advantages, such as crispiness and desired mouth-feel, low operating cost, high productivity and reduced cooking time [5,6]. The gelatinization of starch increases the digestibility of the extrudates [7].

Generally, 70% of the vitamin A in human diet comes from carotenoids. Due to its high antioxidant capacity as well as provitamin A activity, β-carotene has received more attention compared to other carotenoids [8]. Beta-carotene occurs naturally in plants either in crystalline form (carrots) or non-crystalline form (mangoes) [9]. However, the beneficial biological activity of carotenoids can be lost when they are exposed to low pH, high temperature, light and oxygen through isomerization, oxidation and fragmentation [10]. Bioavailability of β-carotene can be improved by incorporating β-carotene in the lipid phase of an oil-in-water (O/W) emulsion [8]. The lipophilic β-carotene can be dissolved into the oil before homogenization to form an O/W emulsion [11]. The stability of an emulsion may be increased with the application of layer by layer (LBL) technology on protein coated oil particles. LBL emulsion has better stability towards changes in pH, ionic strength and heat in thermal processing and drying, lipid oxidation, freeze thaw cycles and high salt concentrations [12,13]. The thicker interfacial layer provides the particles with higher resistance towards disruptions [14].

Oil droplets in emulsions form particles covered by protein (SL) and protein and polyelectrolyte (LBL) layers and the particles become entrapped within a continuous glass-forming wall matrix during extrusion. The objectives of the present study were to produce snack-type extrudates and to investigate their ability to encapsulate and protect beta-carotene from liquid feed emulsions using single layer (SL) and layer by layer (LBL) interface structures.

2. Materials and Methods

2.1 Materials
A mix of wheat flour (Musgrave Retail Partners, Cork, Ireland; 14.3% H2O), α-lactose monohydrate (Sigma-Aldrich, Inc., St. Louis, Mo., U.S.A.; 2.4% H2O) and maltodextrin (MD250, DE 23-27 GPC, U.S.A.; 6.3% H2O) was used as a solids feed. Whey protein isolate (WPI, Isolac, Carbery Food Ingredients, Balineen, Ireland) was used as an emulsifier. Gum Arabic (Sigma Aldrich G9752 Stenheim, Germany) was used as a
polyelectrolyte. Sunflower oil (Spain) was used as the lipid phase and the solvent for β-carotene (crystalline Type II, synthetic, 95% HPLC, Sigma-Aldrich, U.S.A.). All other chemicals were purchased from Sigma-Aldrich, Inc (St. Louis, Mo., U.S.A.).

2.2 Emulsion Preparation

WPI was dispersed in deionized water (12 % w/w) at room temperature and stirred for 1 h to enhance hydration of the proteins. pH was adjusted to pH 3.5 using a 10% citric acid (w/w) solution. The oil phase was prepared by dissolving β-carotene (0.05%, w/w) in sunflower oil at 50°C by mixing with magnetic stirrer in a beaker for 2 hours. Light exposure of the oil was avoided by covering the beaker with aluminium foil. The oil phase (500 g) and water phase (500 g) was pre-homogenised using an Ultra-Turrax (T25 Digital, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10,000 rpm for 60 s. The pre-emulsions were subsequently homogenised using a two-stage homogenizer (APV-1000, APV Homogenizer Group, Wilmington, MA, U.S.A.) for 3 cycles at 250 bar (approximately 20% of the total pressure was applied for the second stage). The protein-stabilised primary emulsion was added with 100g of deionized water to obtain the SL emulsion to be fed into the extruder. LBL emulsion was prepared using gum Arabic (0.15% w/w, 0.15g in 99.85g water) that was dispersed in deionized water at room temperature and stirred 1 hour. The solution was then adjusted to pH 3.5 with citric acid solution (10%, w/w). The primary emulsion was mixed with the gum Arabic solution at room temperature for 30 min to form the LBL emulsion.

2.3 Extrusion

A blend of the dry ingredients was prepared by using a mixer (Kenwood KM330, Kenwood Limited, Hampshire, UK) at 60 rpm for 5 min. The dry feed into a twin-screw pilot extruder (MPP model, APV Baker, Peterborough, UK) was 73.4 g/min. The barrel had four heating zones and hosted twin screws with a screw diameter of 19 mm and length to diameter ratio (L/D) of 25:1. The screw speed was 300 rpm. The emulsions were diluted with water at a weight ratio of 4:1 enabling feed with a peristaltic pump (504UK, Watson Marlow Ltd) at a rate of 12.153 g/min. The temperatures in the four zones were adjusted to 105, 120, 145 and 155°C, respectively, and these temperatures were kept constant during processing. The extrudates were allowed to cool to room temperature and ground by a blender (30 s at minimum speed). Approximately 2g of the powdered extrudates were transferred into 10 mL clear glass vials (Schott, Mühlheim, Germany). The vials were sealed and closed with septa under vacuum in a freeze dryer (Lyovac, GT 2, Steris, Hurlt Germany). Closed vials were subsequently sealed in plastic packages (PA/PE 90, Fispak Ltd., Dublin, Ireland) under vacuum (99%) using a vacuum packaging machine (Polar 80 KL, Henkelman B. V., Den Bosch, The Netherlands). Samples were stored at 20°C (cooling incubator, KBP 6151 series 6000, Termarks, Bergen, Norway), 40°C (TS 8136, Termarks) and 60°C (TS 8136, Termarks) and protected from light, water loss and uptake from the environment. The packages with vials retained vacuum during the storage indicating a closed system. Samples were analysed at intervals during storage for up to 15 d.

2.4 HPLC Analysis

Two grams of the extrudates at various intervals of storage were hydrated by suspending in 15mL of deionized water and vortexing (Scientific Industries Inc., G-560E, NY, USA) at room temperature for 5 min to release suspended oil particles. In order to destabilize emulsified droplets and extract beta-carotene, 4mL of methanol:ethylacetate (1:1 v/v) solution containing 0.25% butylated hydroxyl toluene (BHT) was added and vortexed for 30 s. Oil was saponified by adding 1 mL of 2M potassium hydroxide in methanol and vortexed for 30 s to separate the lipid carrier (saponized fraction) from the β-carotene (unsaponised). Finally remaining β-carotene was extracted using 1mL of dichlorometane and the sample was vortexed for 30s. The organic phase was separated by adding 4mL of n-hexane and the sample was further vortexed for 30s. The extracts were left to stand for 30min. The top layer was separated using a pipette and centrifuged (Sigma 1-15, Model 78307, D-37520, Ostenode am Harz, Germany) at 10,000 rpm for 5 min. The supernatant was filtered through a sterile CA- membrane and GF-prefilter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) and transferred into HPLC vials. Injections of 200 μL were used. The β-carotene contents of the extrudates were quantitated using an HPLC (Dionex ICS3000, Sunnyvale, CA, U.S.A.), autosampler (AS-1, Dionex, Sunnyvale, CA, U.S.A.), and photodiode-array detector (PDA ICS Series, Dionex, Sunnyvale, CA, U.S.A.) at 450 nm. The HPLC column was a 250 mm × 4.6 mm i.d., 5 μm, reversed-phase Acclaim C30 analytical column with a 4 mm × 4 mm i.d. guard column of the same material (Dionex, Sunnyvale, CA, U.S.A.). An eluent gradient composed of acetonitrile at 85 to 65%, methanol:ethyl acetate (1:1) at 15 to 35% and 0.5% acetic acid in water was used for separation of carotenoids. The amounts of β-carotene present in the samples were calculated from the standard curve of all-trans β-carotene using freshly prepared SL emulsion.
3. Results and Discussion

3.1 Extrudates and Beta-carotene Retention

Extrudates were successfully produced with either SL or LBL emulsion as the wet feed (Figure 1). The combination of wheat flour, lactose and trehalose was used as the carbohydrate sources for glass forming wall material that could provide the extrudates with better stability and protection of beta-carotene. The glass transition temperature (onset) for both extrudates was found to be approximately 2°C. Matrix stability in the glassy state plays an important role in the prevention of degradation of dispersed lipophilic compounds in hydrophilic solids [9].

![Figure 1. Extrudates and ground samples for storage in vials.](image)

Three main peaks of β-carotene isomers were found in the HPLC chromatograms. The β-carotene isomers were taken as 15-cis-β-carotene (19.2±0.8 min), 13-cis-β-carotene (23.5±0.7 min), and all-trans-β-carotene (27.3±0.9 min) (Figure 2). Significant amount of isomerisation was found in both SL and LBL extrudates producing isomers 15-cis-β-carotene and 13-cis-β-carotene. It can be observed that isomerization occurred in both SL and LBL samples and at all storage temperatures. It was found that retention (%) of β-carotene was dependent on temperature and decreased with increasing storage temperature (Figure 3). The degradation of β-carotene was more rapid during the first six days of storage, after which the degradation rate of β-decreased, as is typical of first-order kinetics. During the first six days of storage, β-carotene losses in SL extrudates were 23.9%, 28.2%, and 62.2% at 20, 40, and 60°C, respectively. The β-carotene losses in LBL extrudates (16.7%) were less than in the SL system at 20°C during the first six days. Beta-carotene losses at 40°C were almost the same for both extrudates while higher β-carotene losses occurred in extrudates with LBL emulsion (72.6%) at 60°C during the first six days. Oxidation is a major cause of β-carotene degradation [9,15,16]. The higher loss of β-carotene in the first 6 days could be due to the oxygen trapped in the particles and headspace. The porous structure of extrudates could entrap oxygen that could further enhance β-carotene losses during storage. After nine days of storage, the amount of the β-carotene showed a constant rate for all storage temperatures. Upon storage for an additional six days, 7.2%, 16.5%, and 10.7% β-carotene losses for SL extrudates were found at 20, 40, and 60°C, respectively. However, comparable amounts of β-carotene losses were found for extrudates with LBL emulsion upon additional six days of storage.
Figure 2. Three main β-carotene peaks in SL and LBL extrudates stored at 20, 40 and 60°C.

Although the retention (%) of β-carotene in SL extrudates was higher than in LBL extrudates, the amount of β-carotene in LBL extrudates was higher at all storage temperatures. The higher retention of β-carotene in LBL emulsion after the extrusion process gave LBL extrudates higher initial β-carotene content. The β-carotene losses after the extrusion process were 50.3% and 37.7% from the theoretical amount for extrudates with SL and LBL emulsions, respectively. These losses were lower than the results of Guzman-Tello and Cheftel (1990) and Emin et al. (2012) who reported 70-73% reduction in β-carotene during the extrusion process, respectively. The results showed that the encapsulation of β-carotene in the oil droplets increased the retention. LBL emulsions gave a better protection of β-carotene during the extrusion process. This could be due to the thicker interfacial layer of LBL particles that provided the particles with a higher resistance towards disruptions [14]. However, during storage the degradation of β-carotene was found to be faster in LBL extrudates.

Figure 3. The percentage retention of β-carotene in SL and LBL extrudates stored at 20, 40 and 60°C.

4. Conclusion

Extrudates were successfully produced using SL and LBL emulsions as the wet feed. It was noted that the β-carotene present in LBL extrudates exceeded that of SL extrudates indicating that the use of LBL emulsion in extrusion could prevent β-carotene losses during the process. However, the amounts of β-carotene present over storage in LBL were generally higher than in SL at 20, 40, and 60°C. Isomerization occurred in both samples at all storage temperatures producing isomers of 15-cis-β-carotene and 13-cis-β-carotene.
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References