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Effect of biopolymer encapsulation on the digestibility of lipid and cholesterol oxidation products in beef during *in vitro* human digestion

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ABSTRACT

In this study, beef patties were encapsulated with 3% chitosan, pectin, onion powder, or green tea powder and the beef patties were then passed through an *in vitro* human digestion model. The total lipid digestibility was lowest ($p < 0.05$) in beef patties encapsulated with chitosan and pectin after digestion in the small intestine. Thiobarbituric acid reactive substance (TBARS) values were significantly lower ($p < 0.05$) for beef patties encapsulated with chitosan and pectin, when compared with the control, after digestion in the small intestine. In contrast, the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical–scavenging activity was highest ($p < 0.05$) in beef patties encapsulated with onion powder and green tea powder after digestion in the small intestine. The total cholesterol oxidation product (COP) content was significantly lower ($p < 0.05$) in beef patties encapsulated with biopolymers than in the control after digestion in the small intestine.

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1. Introduction

Because meat and meat products are some of the most important sources of dietary fat, modifying the lipid profile of such products could reduce their nutritional quality (Trindade, Mancini-Filho, & Villavicencio, 2010). The consumption of oxidised lipids or cholesterol plays an important role in both human health and meat quality. In general, many cholesterol oxidation products (COPs) are cytotoxic, atherogenic, mutagenic and carcinogenic (Hur, Min, Nam, Lee, & Ahn, 2013a). COPs are present in low amounts in raw foods of animal origin, but their concentration increases dramatically after high-temperature treatment in highly processed food products containing cooked meat, and after exposure to light, metals, natural sensitizers and oxygen (Boselli, Rodriguez-Estrada, Fedrizzi, & Caboni, 2009). Several studies have demonstrated the role of COPs in the rapid progression of coronary heart disease and atherosclerosis; COPs cause endothelial cell damage, which initiates a complex series of pathological changes that ultimately lead to plaque formation (Li & Mehta, 2005; Poli, Sottero, Gargiulo, & Leonarduzzi, 2009; Sevanian, Hodis, Hwang, McLeod, & Peterson, 1995).

In addition, several studies have reported that biopolymers can be hypocholesterolemic in both animal and human models. It has been suggested that inhibiting cholesterol absorption in the gastrointestinal tract is a major mechanism underlying the cholesterol-lowering activity of biopolymers. The cholesterol-lowering effect of chitosan is one of its most extensively studied bioactivities (Anrakua et al., 2011). Data suggests that chitosan not only exerts cholesterol-lowering effects but also enhances resistance to oxidative stress (Anrakua et al., 2011). Santhosh, Sini, Anandan, and Mathew (2006) also reported that the administration of chitosan prevented the oxidation of hepatotoxic lipids in rats. Pectin has a gel-forming capacity and therefore binds to cholesterol and bile acids in the gut and promotes their excretion; thus, it exerts cholesterol-lowering effects (Mudgil & Barak, 2013). Marounek, Volek, Synytsya, and Copikova (2007) reported that pectin and other gel-forming polysaccharides increase viscosity and affect the processes of digestion and absorption in the small intestine. Several studies have reported the production of oxidised lipids and cholesterol in cooked meats. However, little is known about the effects of biopolymer encapsulation on lipid or cholesterol oxidation during digestion. Therefore, the aim of this study was to investigate the effects of biopolymer encapsulation on the digestibility of lipids and COPs in beef patties during *in vitro* human digestion.

2. Materials and methods

2.1. Sample preparation

All experiments were performed using five replicates from 10 different patty samples. Chitosan (10% w/w) was dissolved in 90 g

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acetate buffer solution (100 mM acetic acid: sodium acetate, pH 3.0, 0–150 mM NaCl); 10% (w/w) pectin, onion powder and green tea powder, were dissolved in phosphate-buffered solution (2 M monobasic sodium phosphate and 2 M dibasic sodium phosphate, pH 7.0). All solutions were stirred for 12 h using a magnetic stirrer.

A beef chuck roll was ground twice through a 3-mm plate, and patties were prepared without additives. Ground beef was mixed with 20% fat for 5 min, and biopolymer encapsulation was then performed by mixing biopolymer solution (a final volume of 3% v/w) and beef patties for 30 min using a meat mixer. Patties were approximately 9 cm in diameter, 1.5 cm in thickness and weighed 100 g. The patties were placed in a glass dish and cooked (the patty was turned over twice between cycles) in a microwave oven (3000 MHz) to an internal temperature of 8590 °C. The core temperature of the samples was measured using a flexible internal thermometer (Temp 300; Thermo Scientific, MA, USA). Cooked samples were vacuum-packed in oxygen-impermeable PVDC bags and stored at 04 °C until use.

2.2. In vitro human digestion model

The *in vitro* human digestion model used was a modified version of that described previously (Hur, Decker, & McClements, 2009; Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005). Briefly, the protocol was as follows:

I. *Pre-ingestion*: Beef patties.
II. *Mouth*: Beef patties weighing ~5 g were mixed with 6 ml of simulated saliva fluid (pH 6.8) and stirred for 5 min at 37 °C.
III. *Stomach*: Approximately 12 ml of simulated gastric fluid (pH 2) was added and mixtures were stirred for 2 h at 37 °C.
IV. *Small intestine*: Approximately 12 ml of duodenal juice, 6 ml of bile juice and 2 ml of bicarbonate solution (pH 6.57) were added, and the mixtures were stirred for 2 h at 37 °C.

The composition of the simulated saliva, gastric, duodenal and bile fluids are listed in Table 1. For the *in vitro* human digestion model, samples were swirled (60 rpm) in a shaking water bath to simulate the motility of the gastrointestinal tract (Model HB-205SW; Hanbaek, Co. Bucheon, Korea).

2.3. Total lipid digestibility

The total lipid digestibility in the beef patties during simulated human gastrointestinal digestion was determined by assessing the infiltration rates in dialysis tubing and was expressed as the percentage of total lipids inside and outside the tubes.

2.4. Total lipid content

Total lipids were extracted using chloroform and methanol, as described by (Folch, Lees, and Stanley (1957). Each beef patty sample (5 g) was combined with 50 ml of Folch solution (chloroform:methanol, 2:1, v/v) and 50 ml of BHA (butylhydroxyanisole, 10%) and was homogenised using a Polytron homogenizer (IKA, Model T25; Staufen, Germany) for 10 s. Homogenates were filtered using Whatman No. 1 filter paper. The residue and filter paper were then mixed with 50 ml of Folch solution and re-filtered. Distilled water (25 ml) was added to the filtered solution and centrifuged at 500×*g* for 10 min. The upper layer (methanol and water) was removed by aspiration, and the bottom layer (chloroform containing the lipid extract) was passed through anhydrous sodium sulphate (Na_2SO_4). The Na_2SO_4 was then rinsed with 30 ml of chloroform, and the extracts were concentrated using an evaporator (Zymarkturbovap 500; Hopkinton, MA, USA) at 40 °C.

2.5. Free fatty acid content

The free fatty acid content was determined using a modified version of the AOAC method (AOAC, 1995) and weighed by titrimetry. Five grams of each beef patty was weighed in 50 ml test tubes; these 5-g samples were then homogenised with 15 ml of deionized distilled water using a Polytron homogenizer at 1000×*g* for 10 s. Two millilitres of beef patty homogenates were transferred to a 300 ml flask, and 100 ml of ether/ethanol solution (ether:ethanol, 1:1) was added. Several drops of phenolphthalein were added and the free fatty acids were titrated with 0.1 M KOH. The free fatty acid content was then calculated using the following equation:

$$\text{Free fatty acid (KOH/g)} = 5.611 \times A \times F/\text{sample weight (g)}$$

where A = ml of 0.1 M KOH solution and F = KOH titer

2.6. Thiobarbituric acid-reactive substances (TBARS)

TBARS values were determined using a modified version of the method described by Buege and Aust (Buege & Aust, 1978). Five-gram beef patties samples were weighed pre- and post-digestion in 50 ml test tubes and homogenised in 15 ml of deionized distilled water using a Polytron homogenizer at 1000×*g* for 10 s. One-millilitre samples of beef patty homogenates were transferred to

Table 1
Components of the synthetic juices used in the *in vitro* human digestion model.

	Saliva	Gastric juice	Duodenal juice	Bile juice
Inorganic components	10 ml KCl[a] (89.6 g/l)[b] 10 ml KSCN (20 g/l) 10 ml NaH_2PO_4 (88.8 g/l) 10 ml $NaSO_4$ (57 g/l) 1.7 ml NaCl (175.3 g/l) 20 ml $NaHCO_3$ (84.7 g/l)	15.7 ml NaCl (175.3 g/l) 3.0 ml NaH_2PO_4 (88.8 g/l) 9.2 ml KCl (89.6 g/l) 18 ml $CaCl_2{\cdot}2H_2O$ (22.2 g/l) 10 ml NH_4Cl (30.6 g/l) 6.5 ml HCl (37 g/l)	40 ml NaCl (175.3 g/l) 40 ml $NaHCO_3$ (84.7 g/l) 10 ml KH_2PO_4 (8 g/l) 6.3 ml KCl (89.6 g/l) 10 ml $MgCl_2$ (5 g/l) 180 µl HCl (37 g/l) 9 ml $CaCl_2{\cdot}2H_2O$ 22.2 g/l	30 ml NaCl (175.3 g/l) 68.3 ml $NaHCO_3$ (84.7 g/l) 4.2 ml KCl (89.6 g/l) 150 µl HCl (37 g/l) 10 ml $CaCl_2{\cdot}2H_2O$ 22.2 g/l
Organic components	8 ml Urea (25 g/l) 15 mg Uric acid	10 ml Glucose (65 g/l) 10 ml Glucuronic acid (2 g/l) 3.4 ml Urea (25 g/l) 10 ml Glucosamine hydrochloride (33 g/l) 1 g BSA	4 ml Urea (25 g/l) 1 g BSA	10 ml Urea (25 g/l) 1.8 g BSA 30 g Bile
Enzymes	290 mg α-Amylase 25 mg Mucin	2.5 g Pepsin 3 g Mucin	9 g Pancreatin 1.5 g Lipase	
pH	6.8 ± 0.2	1.30 ± 0.02	8.1 ± 0.2	8.2 ± 0.2

[a] The numbers are the concentration of chemicals to make digestive juices.
[b] The number in parentheses are the concentration of inorganic or organic components per litre distilled water. After mixing all ingredients (inorganic components, organic components and enzymes), the volume was increased to 500 ml with distilled water.

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