LWT - Food Science and Technology 60 (2015) 630–636

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Effect of emulsifiers on microstructural changes and digestion of lipids in instant noodle during in vitro human digestion

S.J. Hur [a], S.J. Lee [a], S.Y. Lee [a], Y.Y. Bahk [b], C.G. Kim [b, *]

[a] Department of Animal Science and Technology, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi-do, 456-756, Republic of Korea
[b] Department of Biotechnology, Konkuk University, Chungbuk, Chungju, 380-701, Republic of Korea

ARTICLE INFO

Article history:
Received 22 November 2011
Received in revised form
17 June 2014
Accepted 24 July 2014
Available online 2 August 2014

Keywords:
In vitro human digestion
Emulsified lipids
Instant noodle
Confocal microscopy

ABSTRACT

This study was conducted to investigate the impact of emulsifiers on the digestion of lipid in instant noodles as they passed through a model gastrointestinal (GI) tract. To produce an instant noodle, wheat flour, starch and sodium chloride were mixed with water and then steamed and fried in 990 g of palm oil with 10 g of emulsifiers (T1: soybean lecithin, T2: yolk lecithin, T3: saponin, T4: Tween 20 and T5: caseinate). The largest change in the microstructure of instant noodle occurred when the instant noodle moved from the simulated stomach to the small intestine. Moreover, the value of free fatty acids and thiobarbituric acid reactive substances (TBARS) also significantly increased after small intestine digestion. Various emulsifiers added to stabilize the instant noodle lipids induced different digestion of lipids during in vitro human digestion. The size or amount of instant noodle lipids prepared with yolk lecithin were smaller than those prepared with the other emulsifiers, which suggests that yolk lecithin was more effective at producing small droplets during digestion.

1. Introduction

Emulsifiers, which are widely used in bread, pasta and noodle products, are surface-active compounds that possess both lipophilic and hydrophilic properties (Choy, Hughes, & Small, 2010). Accordingly, it is possible that the nature of emulsifier molecules that initially coat lipid droplets in food would affect the behavior of emulsifiers in the gastrointestinal (GI) tract. Moreover, the nature of the emulsifier will impact the susceptibility of the lipid droplets to coalescence and degradation within the GI tract, thereby altering the total surface area of the lipid exposed to lipase (Hur, Decker, & McClements, 2009). When a lipid is ingested it may undergo changes in its chemistry and structural organization as it passes through the GI tract (Johnson, 2001; McClements, Decker, & Park, 2007). Steamed and fried instant noodles represent a rapid growing product reflecting a growing market in Asian countries (Liu & He, 2008; Shin & Kim, 2003). However, the high residual oil content in instant noodles is disadvantageous for food manufactures due to high cost, and for consumers due to its association with obesity, cardiovascular disease and other health disorders (Goel, Singhal, & Kulkarni, 1999; Wu, Aluko, & Corke, 2006). The associated oxidative rancidity of oils is another major problem associated with fried foods and a major cause of food deterioration (Rho, Seib, & Chung, 1986; Wu et al., 2006). Many studies of the toxicity of oxidized fat and oil have shown that oxidized fats and oils are toxic (Gotoh et al., 2006). Khalil (1999) reported that the oil content of commercial instant noodles ranged from 16% to 24% in a survey of samples collected from eight Asian countries. For this reason, instant noodles are high fat foods, and the oil content in steamed and fried instant noodles has now become a major health concern (Wu et al., 2006). Thus, a better understanding of structural changes in ingested lipids would enable the food industry to design foods to increase, decrease or control lipid digestion and absorption within the human GI tract. Therefore, this study was conducted to determine the effect of different emulsifiers on the in vitro human digestion of lipids in instant noodles. To accomplish this, we utilized scanning electron microscopy and confocal scanning fluorescence microscopy to provide information regarding these structural changes.

2. Materials and methods

2.1. Materials

Potassium chloride, potassium hydroxide, potassium persulfate, sodium sulfate, sodium hydrogen carbonate, hydrogen chloride,

* Corresponding author. Tel.: +82 43 840 3614; fax: +82 43 852 3616.
E-mail address: changil.kim@kku.ac.kr (C.G. Kim).

http://dx.doi.org/10.1016/j.lwt.2014.07.036
0023-6438/

Table 1
Constituents and concentrations of the various synthetic juices of the in vitro digestion model representing fed conditions.

	Saliva	Gastric juice	Duodenal juice	Bile juice
Organic and inorganic components	8 ml urea[a] (25 g/L)[b] 15 mg uric acid	1 g bovine serum albumin	9 ml $CaCl_2 \cdot 2H_2O$ (22.2 g/L) 1 g bovine serum albumin	10 ml $CaCl_2 \cdot 2H_2O$ (22.2 g/L) 1.8 g bovine serum albumin 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 one liter distilled water. After mixing the all ingredients (inorganic components, organic components and enzymes), the volume was increased to 500 ml with distilled water.

potassium phosphate monobasic, magnesium chloride, hexane, methanol, acetate, thiobarbituric acid, trichloroacetic acid, phosphoric acid, ferric chloride, hydrochloric acid, sulfuric acid, chloroform, ether and ethanol were purchased from Fisher Scientific chemical company (Pittsburgh, PA, USA). Bicarbonate, potassium thiocyanate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride, calcium chloride, ammonium chloride, urea, glucose, glucuronic acid, glucoseamin, α-amylase, uric acid, mucin, bovine serum albumin, pepsin, pancreatin, lipase, bile salt extraction, butylated hydroxyanisole, phenolphthalein, L-ascorbic acid, malondialdehyde (MDA), phenolphthalein, soybean lecithin, yolk lecithin, saponin and caseinate were purchased from Sigma–Aldrich chemical company (St Louis, MO, USA). Bis-[trimethylsilyl]trifluoroacetamide and trimethylchlorosilane were purchased from Supelco company (St Louis, MO, USA). Wheat flour, starch and palm oil were purchased from commercial markets.

2.2. Instant noodle preparation

Wheat flour (900 g), starch (100 g) and sodium chloride (10 g) were mixed with 180 ml of deionized distilled water (DDW) for 5 min using a food mixer and then stored at room temperature for 10 h. The dough was first formed into a dough sheet by a process of folding and passing the crumbly dough through the rollers of the noodle machine three times and then reduced to a final thickness of 3 mm by five successive passes through a chopper (Fuji, M-22S, Seoul, Korea). The dimension of the resultant noodle strands was 3 mm in diameter and 10 cm in length. The raw fresh instant noodles were then stored at room temperature for 1hr, after which they were steamed in autoclave at 100 °C for 5 min and subsequently fried in palm oil at 145 °C using a domestic electric fryer for 100 s. Before frying, 10 g of soybean lecithin, yolk lecithin, saponin, Tween 20 and caseinate as an emulsifier were added to the 990 g of palm oil. The fried instant noodles were drained for 2 h in an air cabinet and cooled to room temperature. Finally, 120 g of instant noodle were boiled with 500 ml DDW at 100 °C for 4 min and 30 s. Emulsified oil absorption into noodles were confirmed by lipid extraction method (T1:30.34%, T2: 30.28%, T3 30.71%, T4: 30.52% and T5: 31.02%).

2.3. In vitro human digestion model

The in vitro human digestion model used was a modified version of that described by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) and Hur et al. (2009):

I. *Mouth*: About 5 g of cooked instant noodle was mixed with 6 ml of simulated saliva fluid (pH 6.8) and then stirred for 5 min at 37 °C.
II. *Stomach*: About 12 ml of simulated gastric fluid (pH 2) was added, and then the mixture was stirred for 2 h at 37 °C.
III. *Small Intestine*: About 12 ml of duodenal juice, 6 ml of bile juice and 2 ml of bicarbonate solution (pH 6.5~7) were added, and then the mixture was stirred for 2 h at 37 °C.

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

2.4. Scanning electron microscopy (SEM)

A Jeol scanning electron microscope system (JSM-5600LV, JEOL., Tokyo, Japan) was used to capture the SEM images of instant noodle. The whole instant noodle was cut into 10 mm and then placed on the sample holder with the help of double-sided adhesives tape and sputter-coated with gold (2 min, 200 Pa). Finally, each sample was transferred to the microscope where it was observed at 5 kV and vacuum of 9.75 × 0.0001 Pa.

2.5. Confocal microscopy

A confocal scanning fluorescence microscope (Carl Zeiss, LSM 5 Live, GmbH, Germany) with a 20 × objective lens was used to capture the confocal images. Nile red (a lipid fluorescent dye) was excited with 488-nm argon laser line. The fluorescent emitted from the sample was monitored using a fluorescence detector (543 nm) with a pinhole size of 150 μm. The resulting images consisted of 512 × 512 pixels, with a pixel size of 414 nm, and a pixel dwell time of 5 s.

2.6. Fatty acid composition

Fatty acid composition of digested instant noodle mixture was determined by the method of Hur et al. (2004). Lipids of digested instant noodle mixture were extracted with chloroform and methanol as described by Folch, Lees, and Sloane-Stanley (1957). For lipid hydrolysis, an aliquot of lipid extract (30 mg) and 3 ml of 0.25 mmol/L H_2SO_4 in methanol were combined in a screw-capped test tube. The test tube was placed in boiling water (100 °C) for 20 min and subsequently cooled at room temperature. The resulting free fatty acids were methylated with 1 ml of 140 g BF_3 in methanol at room temperature for 30 min. Water (1 ml) and hexane (5 ml) were added. Samples were vortexed and centrifuged at 500 × *g* for 10 min. The upper organic solvent layer was used to determine fatty acids composition. Fatty acid methyl esters were analyzed on a gas chromatograph (Agilent 6890, Wilmington, DE, USA) equipped with an on-column injector port and flame-ionization detector. A Silar capillary column (30 m × 0.32 mm × 0.25 μm) was used for the separation of the fatty acid methyl esters. The gas chromatography oven temperature