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Potential impact of biopolymers (ε-polylysine and/or pectin) on gastrointestinal fate of foods: *In vitro* study

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ABSTRACT

Food-grade biopolymers, such as proteins and polysaccharides, may impact the gastrointestinal fate of foods through various mechanisms. In this study, we examined the influence of ε-polylysine (an antimicrobial) and pectin (a thickening agent) on the behavior of a standard rodent diet (full-fat and fat-free) in a simulated gastrointestinal tract that included mouth, stomach, and small intestine phases. Powdered biopolymers were incorporated into the standard diet in either individual or complexed form. The presence of the biopolymers altered the microstructure and charge characteristics of the gastrointestinal contents. In particular, the presence of pectin appeared to increase the rate and extent of lipid digestion, which may have been due to its ability to inhibit protein aggregation. Our results do not support the hypothesis that polylysine inhibits lipid digestion, as has been reported previously. Overall, the results of this study may be useful for interpreting animal feeding studies of the influence of biopolymers on the gastrointestinal fate of foods.

1. Introduction

Many consumers are concerned about the utilization of synthetic preservatives in food products, which has stimulated research into the development of effective, safe, natural alternatives (Gyawali & Ibrahim, 2014; Tajkarimi, Ibrahim, & Cliver, 2010). ε-Polylysine (ε-PL) is a natural generally regarded as safe (GRAS) antimicrobial that is effective against various bacteria, yeasts, molds, and bacteriophages (Chang, Lu, Park, & Kang, 2010; FDA, 2011; Geornaras, Yoon, Belk, Smith, & Sofos, 2007; Shima, Matsuoka, Iwamoto, & Sakai, 1984; Yoshida & Nagasawa, 2003). However, the highly cationic nature of this biopolymer limits its implementation in many food products because it has a tendency to interact with anionic components present, thereby promoting precipitation and sedimentation (Chang, McLandsborough, & McClements, 2011b). Additionally, ε-PL has been reported to have a bitter and astringent mouthfeel, which also limits its application in many foods (Kido et al., 2003).

Previous work has shown that electrostatic interactions between cationic ε-PL and anionic pectin lead to the formation of a negatively-charged complex that maintains the antimicrobial properties of ε-PL while minimizing any undesirable interactions with other anionic components that might be present in food matrices (Chang et al., 2011a, 2011b; Lopez-Pena & McClements, 2014). These antimicrobial complexes have been shown to perform successfully in model food systems (Chang, McLandsborough, & McClements, 2012), and are therefore promising all-natural alternatives to synthetic antimicrobials used currently. Nevertheless, it is important that systematic studies are conducted to assess the safety of these complexes prior to their incorporation in food systems. For example, these antimicrobial complexes may alter the normal digestion of macronutrients, or that they may reach the colon and therefore alter the microbial microflora.

Research is therefore being carried out by our group to elucidate the potential influence of ε-PL-pectin complexes on the gastrointestinal fate of foods, and on the composition of the colonic microflora. This overall aim will be achieved using a combination of *in vitro* and *in vivo* studies, focusing on macronutrient digestion, metabolic markers in blood, abnormalities in body and organ weight, and impact on the gut microbiome. In this paper, our objective was to obtain an understanding of the potential influence of ε-PL-pectin complexes on the gastrointestinal fate of foods using a simulated gastrointestinal tract (GIT). This work was carried out because previous studies have shown that polylysine may interfere with lipid digestion (Kido et al., 2003). Previous studies have usually used simple model systems (such as oil-in-water emulsions) to pass through GIT models. In the current study, we used a simulated GIT to study the potential gastrointestinal fate of powdered standard rodent diet (modified AIN-76A) that were used in animal feeding studies so that the results of this *in vitro* study could be directly compared with the results of *in vivo* studies (reported in a later paper). This modified diet contains 20% fat (a mixture of hydrogenated

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Table 1
Composition of animal diet (modified AIN-76A).

Component	Amount (%w/w)	Energy density (Kcal/kg)
Casein	23.5	841.3
Cornstarch	35.7	1285.2
Dextrose	9.02	328.33
DL-methionine	0.35	14
Cellulose	5.9	0
Lipids	20	1800
Beef tallow	*3.2*	
Lard	*2*	
Anhydrous milkfat	*2.4*	
Soybean oil	*6*	
Peanut oil	*1*	
Corn oil	*5.4*	
Mineral mix #200000	4.11	19.32
Vitamin mix $300050	1.18	46.26
Choline bitartrate	0.24	0
Total	100	4334.40

soybean oil, corn oil, beef tallow, lard, butter and peanut oil, see Table 1) that mimics the typical western diet found in the United States (Xiao et al., 2008).

2. Materials and methods

Maltodextrin with a dextrose equivalent of ≈18 (Maltrin® M180) was provided by the Grain Processing Corporation (Muscatine, IA). ε-Polylysine was obtained from Wilshire Technologies, Inc. High-methoxyl pectin was donated by TIC Gums (White Marsh, MD). Full-fat mixed lipid diet and its fat-free equivalent were acquired from Dyets, Inc. (Bethlehem, PA) (Table 1).

2.1. Powder production and characterization

In this section, the method used to convert the biopolymer solutions into powders that could be incorporated into rodent diet are described.

2.1.1. Liquid feed preparation

Four different solutions were prepared to be subjected to spray drying: (i) 20% w/w maltodextrin; (ii) 0.1% w/w maltodextrin & 1% w/w pectin (MR 1:10); (iii) 0.1% w/w maltodextrin & 1% w/w pectin & 0.05% w/w ε-polylysine (MR 10:20:1); (iv) and 20% w/w maltodextrin & 2% w/w ε-polylysine (MR 10:1). The maltodextrin and ε-polylysine solutions were prepared by dispersing the corresponding amounts of reagents in double distilled water. Solutions of HCl and NaOH at varying concentrations were used to adjust the solutions to a final value of pH 3.5. Pectin stock solutions (2% w/w) were prepared by dispersing powdered high methoxyl pectin into hot double-distilled water, and then stirring at 550 rpm under heated conditions (plate surface temperature of 135 °C) until the solution started to boil. The solution was then allowed to stir overnight at room temperature to ensure full dispersion, adjusted to pH 3.5, and brought to the appropriate volume the next day. Corresponding volumes of the maltodextrin or maltodextrin-ε-polylysine solutions were combined with the pectin stock solution, and thoroughly stirred to ensure homogeneity.

2.1.2. Spray drying conditions

The liquid feeds were subjected to spray drying using two different spray dryers. Maltodextrin (MD), maltodextrin-ε-polylysine (MD + PL), and some of the maltodextrin-pectin (MD + P) and maltodextrin-pectin-ε-polylysine (MD + P + PL) were processed in a Büchi B-290 Spray Dryer (Büchi Laboratorium-Tecnik, Flawil, Switzerland) under the following experimental conditions: inlet temperature 120 °C, outlet temperature 67–72 °C, Q-flow 40, pump flow 30% (spray flow feed rate 9 ml/min), aspirator 100%, and nozzle diameter 1.5 mm. Due to the large quantities of feed that needed to be processed, large volumes of the solutions containing pectin were processed using a Niro Atomizer Versatile Utility Spray Dryer (NGEA Process Engineering A/S, Søborg, Denmark). The inlet temperature was 120 °C, and the flow rate 5.55 l/h. Samples were stored in a desiccator after production.

2.1.3. Powders characterization

Prior to use, the moisture content of the powders was determined following the standard gravimetric method described by the International Dairy Federation (1993) and the FAO (1997). The procedure consisted of weighing 1 to 3 g of the sample into previously dried aluminum capsules, and storing the sample in an oven at 102 ± 2 °C for 2 h. The samples were stored in a desiccator for 30 min to allow them to cool, and were then weighed. The samples were then returned to the oven for 1 h for further drying, and weighed after spending 30 min in the desiccator. This process was repeated until the difference between measurements was 0.5 mg or less. The moisture content (% w/w) was obtained through the following formula:

$$\%Moisture = \frac{100 \times (M_1 - M_2)}{M_1 - M}$$

where: M is the mass of the empty capsule (g); M_1 is the initial mass of the capsule with the sample (g); and M_2 is the final mass of the capsule with sample after drying (g).

2.1.4. Estimation of biopolymer levels in diets

In a parallel study, we will examine the influence of the two biopolymers on the *in vivo* gastrointestinal fate of powdered chow fed to animals. In the current study, we wanted to use powdered chows with the same compositions as those used in the animal studies, and therefore we estimated the levels of the biopolymers required. The amount of each biopolymer powder incorporated into the rodent diet was calculated based on the estimated annual average consumption of soft drinks in the United States per capita (Mintel, 2014). Soft drinks were selected because this is one of the major potential applications for polylysine as an antimicrobial agent in the food industry. The maximum amount of ε-polylysine that can be used in soft drinks is 0.025%w/w (FDA, 2011), and therefore a level equivalent to this was used. Previous studies have shown that a mass ratio of 20:1 pectin-to-polylysine leads to electrostatic complexes that retain their antimicrobial efficacy while inhibiting precipitation (Chang et al., 2011a, 2012, 2011b; Lopez-Pena & McClements, 2014), and therefore an equivalent of 0.5%w/w pectin was used in this study. The daily dosage (g/kg body weight) of the powdered samples that would be fed to the mice was based on the average body weight of Americans (U.S. Department of Health and Human Services, 2008) and on the average *per capita* annual consumption of soft drinks in the United States (Mintel, 2014), which was in turn used to calculate the exposure levels for the mice based on a subchronic toxicology analysis for 13 weeks.

2.2. Simulated gastrointestinal tract

2.2.1. Preparation of simulated GIT fluids

The simulated GIT used in this study included oral, gastric, and intestinal phases, and was adapted from the method described by Li, Hu, and

Table 2
Measured moisture content of the powders containing maltodextrin (MD), polylysine (PL) and pectin (P) used in the rodent diet.

Powder composition	Moisture content (w/w%)
MD	3.61 ± 1.12%
MD + PL	3.60 ± 0.28%
MD + P	4.12 ± 0.16%
MD + P + PL	3.97 ± 0.16%