EFFECT OF PECTIC OLIGOSACCHARIDES FROM FRUIT PEELS AS PREBIOTIC IN ANIMAL FEED

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Abstract:

The objective of this study was to improve fruit peels (pomelo peel, lime peel and mango peel) with enzyme (Hemicell®) as prebiotics in animal feed. There were 2 experiments designed in completely randomized design (CRD). In the experiment 1, it consisted of 6 treatments with 3 types of fruit peels (pomelo peel, lime peel and mango peel) and 2 levels of enzymes (0 and 1% (w/w)) with 3 replications. The samples were taken to measure chemical compositions, reducing sugar content and oligosaccharides analysis. The results were showed that chemical compositions were significantly different among treatments ($P<0.01$). Enzyme treated lime peel had higher ash, crude protein and ether extract content but lower cellulose than other treatments ($P<0.01$), 4.98, 6.40, 8.87% and 9.92%, respectively. Enzyme treated and untreated mango peel contained higher gross energy than other treatments ($P<0.01$), 4,100.54 and 4,069.14 kcal/kg, respectively. In addition, reducing sugar content was significantly different among treatments ($P<0.05$). Enzyme treated mango peel contained higher reducing sugar content than other treatments ($P<0.05$). There were 42.04, 28.86, 177.06, 77.28, 37.09 and 265.91 mg/g, respectively. Oligosaccharides analysis by Thin layer chromatography method showed that all treatments releasing oligosaccharides. In experiment 2, it was examined prebiotic properties (concentration of sugar 1.5 mg/ml), the results showed that glucose product from all treatments could increase growth of *Lactobacillus plantarum* but enzyme treated lime peel could decrease growth of *Escherichai coli*. In conclusion, lime peel treated enzyme can be used as prebiotics as it could increase probiotics and decrease growth of pathogenic bacteria.

Keywords: Fruit peels; Enzyme; Oligosaccharides; Prebiotics

Introduction

Pectin is one of the most complex carbohydrates and mostly found in the plant cell wall. The pectin is broken by enzyme and possible to release pectic oligosaccharides (POS). POS works as prebiotics because its increase the growth of *Lactobacilli* and *Bifidobacteria* and decrease the growth of *Escherichai coli*. Source of POS mainly obtained from the peels of citrus, apple and sugar beet pulp obtained from agricultural by-products (Baldassarre *et al.,* 2018). Thailand is one of the most abundant sources of tropical fruits and mainly export in the world. Therefore, agricultural by-products are available and low price such as pineapple, orange and mango peel. The pectin of citrus peel contains 35% from pomelo peel, 32% from lime peel and 21% from mango peel (Gullon *et al.,* 2013; Huang *et al.,* 2014; Gragasin *et al.,* 2014). Thus, the aim of this study was to improve fruit peels (pomelo peel, lime peel and mango peel) with enzyme (Hemicell®) as prebiotics in animal feed.

Materials and Methods

Preparation of enzyme treated fruit peel

Fruit peels (pomelo, lime and mango) were obtained from the market at Cha-am city. The peels were cut and dried at 60°C for 1-2 days in hot air oven. After that, the peels were grind to be the size of 1×1 mm. This experiment consisted of 6 treatments and 3 replicates. The fruit peels were treated with commercial enzyme (Hemicell@, endo-1,4 β-mannanase) at 2 levels (0 and 1% w/w) dissolved with phosphate buffer (pH 7). The peels were incubated at 55°C for 6 hours. Then they were centrifuged with speed of 10,000 rpm for 10 mins. The supernatant sample was collected for reducing sugar and oligosaccharides analysis. While, the solid sample was dried in hot air oven at 60°C for 24 hours and stored in a refrigerator for proximate analysis.

Determination of reducing sugar

Approximately 0.5 ml of the supernatant was transferred into sample tube in which 0.5 ml of dinitrosalicylic acid (DNS) reagent was added into each tube. The mixture was boiled for 10 mins and left to be cool by immersing the sample tube into cold water immediately. The absorbance was read at 540 nm with distilled water as blank (Miller, 1959). Different concentrations of glucose (0.15, 0.20, 0.25 and 0.30 mg/ml) were prepared using similar procedure described above to develop a glucose standard curve for the above assay.

Determination of oligosaccharides

Oligosaccharides analysis was performed using thin-layer chromatography (TLC) described by Srinang *et al.* (2008). The supernatant sample was spotted near the bottom of silica gel plate (Merck & Co., Inc. art. No.1.05554 size 20×20 cm). Then, the TLC plate was placed in a shallow pool of a solvent (2-propanol: ammonium hydroxide: distilled water, 7:1: 2) in a developing chamber so that only the very bottom of the plate was in contact with the liquid. This liquid would act as the mobile phase which would slowly rise up the TLC plate by capillary action. After thoroughly drying the TLC plate with 10% (v/v) sulfuric acid in ethanol solution in an operating hood, and heated at 100°C until dry. The spot on TLC plate was compared with pectin.

Determination of chemical compositions

Dried solid samples from centrifuge were analyzed for dry matter (DM), crude protein (CP), ash, crude fiber (CF), ether extract (EE) (AOAC, 1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Goering and Van Soest, 1970). Gross energy was determined using bomb calorimeter (CAL 2K, South Africa).

Prebiotic properties

The glucose products of treated fruit peel were used to evaluate its ability to support growth of beneficial microbes (*Lactobacillus plantarum*) and pathogenic microbes (*Escherichia coli*). The concentration of glucose used was 0.5, 1.0, 1.5 and 2.0 mg/ml respectively. The *L. plantarum* and *E. coli* were inoculated into nutrient broth and incubated at 37°C. The growth of *L. plantarum* was measured at 0, 6, 12, 18, 25 and 48 hrs while the growth of *E. coli* was measured at 0, 3, 6, 9, 12, 15 and 18 hrs by spectrophotometer and reading absorbance at 600 nm.

Statistical analysis

The data were analyzed the variance procedures by using SAS software (SAS, 1998). A significance level of $P < 0.05$ was used to differentiate between means.

Results and Discussion

Chemical compositions

Chemical compositions of untreated and enzyme treated fruit peels are shown Table 1. The results showed that chemical compositions were significantly different among treatments ($P<0.01$). Enzyme treated lime peel had higher ash, crude protein and ether extract content but lower cellulose than other treatments ($P<0.01$). They were 4.98, 6.40, 8.87% and 9.92%, respectively. Enzyme treated and untreated mango peel consisted of higher gross energy than other treatments ($P<0.01$). The gross energy value was 4,100.54 and 4,069.14 kcal/kg, respectively. However, enzyme treated fruit peels increased CP and decreased CF content indicating that the enzyme (Hemicell@) is crude enzyme and effective in breaking down fiber in fruit peels. The above results were in agreement with Khanongnuch *et al.* (2006) who reported that enzyme treated copra meal reduced crude fiber up to 14%. Similarly, previous studies found that enzyme treated (Hemicell@) reduced hemicellulose in taro leaves and cellulose in coconut meal in comparison to untreated enzyme. They were 14.55 vs 15.15% and 26.33 vs 39.57% respectively (Saenphoom *et al.*, 2016; Saenphoom *et al.*, 2020).

Reducing sugar

Reducing sugars content of untreated and enzyme treated fruit peels are shown in Table 1. The results showed that enzyme treated mango peel had higher reducing sugar content than other treatment ($P<0.05$). They were 42.04, 28.86, 177.06, 77.28, 37.09 and 265.91 mg/g, respectively because mango peel had higher soluble dietary fiber (19.45%) than pomelo peel (6.43%) and lime peel (19.20%) (Figuerola *et al.*, 2005; Arumugam & Manikandan, 2011). In addition, enzyme treated fruit peels had higher reducing sugar content than untreated (Figure. 2) due to the activity of enzyme. The enzyme can break down fiber into monosaccharide sugars. Similarly, Saenphoom *et al.* (2020) found that enzyme treated (Hemicell@) can reduce the increase of reducing sugar in coconut meal comparing to untreated enzyme. They were 0.71 vs 3.79 mg/g, respectively.

Oligosaccharides

Oligosaccharides of untreated and enzyme treated fruit peels were shown in Figure 1. Glucose products was found in both untreated and treated enzyme fruit peels (row 2 to 7) and lighter than pectin molecule. The glucose products might be as galacturonic acid and pectic oligosaccharides (POS). Similarly, Saenphoom & Chimtong (2014) reported that oligosaccharides content was not significantly different between untreated and treated enzyme tea leaves.

Table 1: Chemical compositions and reducing sugar of untreated and enzyme treated fruit peels (% on dry matter basis).

Items	Treatments						SEM
	T1	T2	T3	T4	T5	T6	
Dry matter (%)	94.99^{c}	95.79^{b}	97.11^{a}	94.29^{d}	95.69^{b}	97.04^{a}	0.22
Moisture (%)	5.01^{b}	4.21^{c}	2.89^{d}	5.71^{a}	4.31^{c}	2.96^{d}	0.02
Ash (%)	3.56^{d}	4.85^{b}	2.75^{f}	4.31^{c}	4.98^{a}	3.29^{e}	0.01
Crude protein (%)	3.83^{e}	5.93^{b}	5.26^{c}	4.69^{d}	6.40^{a}	5.45^{c}	0.03
Ether extract (%)	6.67^{b}	6.65^{b}	8.71^{a}	8.10^{a}	8.87^{a}	8.59^{a}	1.87
NDF (%)	67.74^{a}	37.54^{f}	40.40^{e}	60.18^{b}	44.70^{c}	42.27^{d}	0.13
ADF (%)	39.30^{a}	25.14^{c}	25.98^{c}	37.70^{a}	26.83^{b}	26.44^{c}	0.55
ADL (%)	29.61^{a}	3.62^{f}	8.31^{d}	27.07^{b}	16.05^{c}	6.17^{e}	0.07
Hemicellulose (%)	29.06^{a}	12.85^{f}	14.63^{e}	21.98^{b}	18.75^{c}	16.87^{d}	0.03
Cellulose (%)	9.76^{c}	21.52^{a}	17.67^{b}	10.63^{c}	10.78^{c}	20.27^{a}	0.16
Gross energy (kcal/kg)	3,680.72^{b}	3,626.10^{b}	4,069.14^{a}	3,729.20^{b}	3,518.01^{b}	4,100.54^{a}	33.05
Reducing sugar (mg/g)	42.04^{d}	28.86^{f}	177.06^{b}	77.28^{c}	37.09^{e}	265.91^{a}	0.38

^{a,b,c,d,e,f} Means with different superscripts in row are significantly different)P<0.01(, T1= Untreated pomelo peel, T2= Untreated lime peel, T3= Untreated mango peel, T4= Enzyme treated pomelo peel, T5= Enzyme treated lime peel, T6= Enzyme treated mango peel, NDF =Neutral detergent fiber, ADF =Acid detergent fiber, ADL =Acid detergent lignin, SEM= Standard error of mean

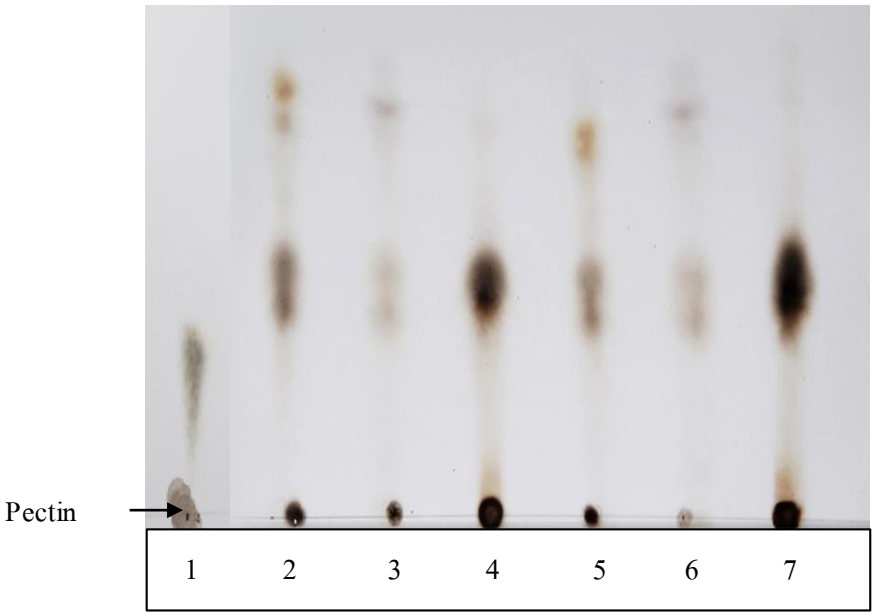

Figure 1: Oligosaccharides of untreated and enzyme treated fruit peels
Row 1 = Pectin, Row 2 = Untreated pomelo peel, Row 3= Untreated lime peel, Row 4 = Untreated mango peel, Row 5 = Enzyme treated pomelo peel, Row 6= Enzyme treated lime peel, Row 7 = Enzyme treated mango peel.

Prebiotics properties

The glucose products from all treatments increased growth of *L. plantarum*. However, enzyme treated lime peel decreased growth of *E. coli* at the concentration of 1.5 mg/ml (Figure 2). Li *et al.* (2013) reported that POS of lime peel decreased growth of *E. coli*. at prolong, stationary and lag phases.

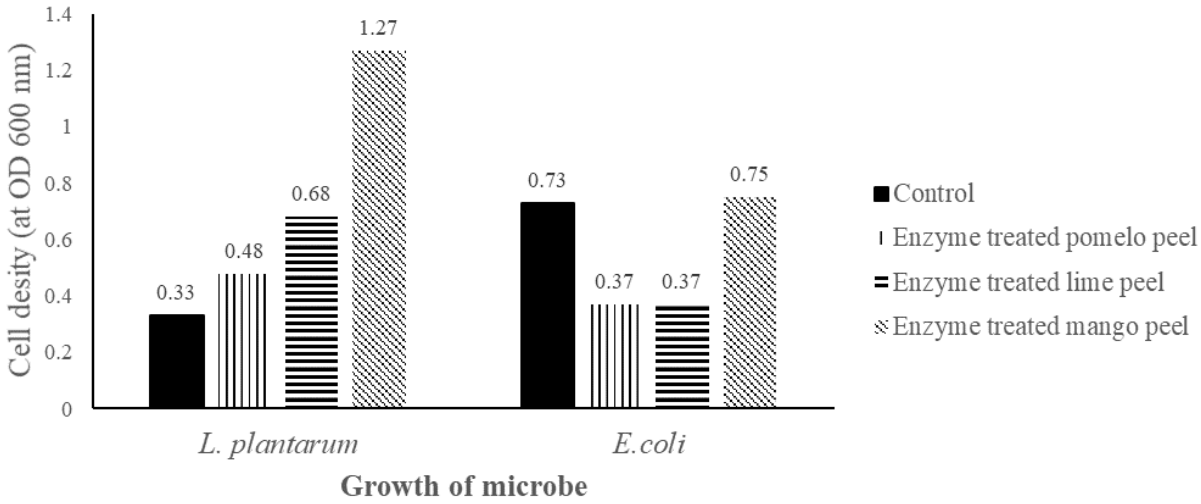

Figure 2: Microbial growth of untreated and enzyme treated fruit peels.

Conclusion

Enzyme hydrolysis effectively breaks down cell wall of fruit peels and releases reducing sugar and oligosaccharides. Moreover, glucose product from all treatments increase growth of *L. plantarum* but enzyme treated lime peel decrease growth of *E.coli*. In conclusion, lime peel treated enzyme can be used as prebiotics because it could increase probiotics and decrease growth of pathogenic bacteria.

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