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Effect of oligosaccharides from agricultural waste as prebiotic in animal feed

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Abstract This study investigated the effects of oligosaccharide derived from sugar palm peel, coconut meal and spent tea as prebiotic for animal feed. The chemical compositions were significantly different between treatments ($P<0.05$). Enzyme-treated cashew nut testa and coconut meal had lower crude fiber and higher gross energy than other agricultural wastes. Furthermore, reducing sugar contents were significantly different between treatments ($P<0.05$). Enzyme-treated sugar palm peel had higher amount of reducing sugar than other agricultural waste. Oligosaccharides were analysis by thin-layer chromatography (TLC) found that all treatments resulted in the release of oligosaccharides. The results revealed that the sugar product from all experimental treatments could increase the growth of *Lactobacillus plantarum* except the enzyme-treated Brewer's grain and spent tea leaves. Besides, enzyme-treated sugar palm peel, coconut meal and cashew nut testa could decreased the growth of *Escherichai coli* (500 µg/ml for 24 hrs). In summary, it was advised that enzyme-treated sugar palm peel, coconut meal and cashew nut testa can be used as prebiotics and antimicrobial agents. Enzyme-treated spent tea leaves can be used as only prebiotics. However, enzyme treated Brewer's grain cannot be used as prebiotics and antimicrobial agents.

Keywords: Agricultural waste, Enzyme and prebiotics

Introduction

Prebiotics are carbohydrate compounds, mainly oligosaccharides, which is a class of non-digestible carbohydrates by the digestive enzymes and is able to be passed to the small intestine. Functions of prebiotics are to stimulate the growth of *Lactobacilli* and *Bifidobacteria* which are probiotics (Al-Sheraji *et al.,* 2013). The most common prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), manno-oligosaccharides (MOS), xylo-oligosaccharides (XOS) and pectic-oligosaccharides (POS). Sources of prebiotics are generally found in plant fiber such as oats, barley, onion, garlic and flexseed (Kaur *et al.,*

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2021). In addition, agricultural by-products are also prebiotics sources. They are obtained from coconut meal, peel of sugar palm, used tea leaves, Brewer's grain and cashew nut testa. The most abundant component of agricultural by-products are lignocellulose or non-starch polysaccharides (NSP). Hemicellose and cellulose comprise 40-60% of NSP (Mckendry, 2002). The hemicellulose is found in copra meal (27%), sugar palm peel (23%), spent tea leaves (18.59%), Brewer's grain (35.62%) and cashew nut testa (26.95%) (Chimtong *et al.,* 2016; Nikiema *et al.,* 2020). The hydrolysed products of NSP with enzymes could be used to release oligosaccharides (Gibson, 2004). According to research of earlier studies, Chen *et al.* (2015) noticed that enzymatic hydrolysis of palm kernel expeller can release oligosaccharide and can stimulate growth of nonpathogenic bacteria (*Lactobacillus and Bifidobacterium*). Similarly, Chimtong *et al.* (2016) also informed that pretreated spent tea leave with enzyme could enhance the growth of *L. acidophilus* and decrease the growth of *E. coli.* Recent study by Hui *et al.* (2022) advised that pretreated guava by-products with an enzyme has the potential to be used as prebiotics because it could enhance the growth of probiotics (*L. Plantarum, L. Rhamnosus and L. Brevis*).

Therefore, the aim was to improve the quality of agricultural by-products of sugar palm peel, coconut meal, spent tea leaves, Brewer's grain and cashew nut testa by enzymatic degradation to be used as prebiotics in animal feed.

Materials and methods

Preparation of enzyme treatment

In the first experiment, sugar palm peel, coconut meal and spent tea leaves were obtained from Phetchaburi province. Brewer's grain and cashew nut testa were obtained from Chonburi province, Thailand. The experiment was comprised of 5 treatments and 5 replicates per treatment. The experiment included five treatments with five replicates for each treatment. Samples were mixed and treated with a commercial enzyme (Sclase A®) in a 1:1 ratio and dissolved in a 0.05M phosphate buffer (pH=4). The enzyme-treated peels were incubated at 40°C for 19 hours. Subsequently, the samples were centrifuged at 10,000 rpm at 25°C for 10 minutes. The supernatant was collected for analyzing reducing sugars and oligosaccharides, while the solid samples were dried in a hot air oven at 60°C for 48 hours and then stored in a refrigerator for proximate analysis.

Determination of reducing sugar

A volume of 0.5 ml supernatant was placed into a sample tube and combined with 0.5 ml of dinitrosalicylic acid (DNS) reagent. The mixture was then boiled for 10 minutes and subsequently cooled by submerging the sample tube in cold water. Following this, 5.0 ml of water was added and mixed thoroughly. The absorbance of the sample was measured at 540 nm, using distilled water as a blank (Miller, 1959). Various concentrations of glucose (0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml) were prepared to create a glucose standard curve.

Determination of oligosaccharides

Thin-layer chromatography (TLC) is utilized for the analysis of oligosaccharides, as outlined by Cabrera and Van Cutsem (2005). The sample supernatant was applied near the bottom of a silica gel plate (Merck & Co., Inc. art. No.1.05554, size 20×20 cm). The TLC plate was subsequently immersed in a shallow solvent pool (2-propanol: ammonium hydroxide: distilled water, in a 7:1:2 ratio) within a developing chamber, with the bottom of the plate submerged in the liquid. This solution serves as the mobile phase, gradually ascending the TLC plate through capillary action. A 10% sulfuric acid solution in ethanol was utilized to spray dry the TLC plate within a fume hood, then heated at 100°C until fully dry. The spots on the TLC plate was compared to the standards of glucose and cellobiose.

Determination of chemical compositions

The solid samples that were centrifuged and dried were assessed for dry matter (DM), crude protein (CP), ash, crude fiber (CF), and ether extract (EE) following the AOAC guidelines (1990). Additionally, gross energy was measured using a bomb calorimeter (CAL 2K, South Africa).

Prebiotic and antibacterial activities

In the second experiment, the products resulting from enzyme treatment were utilized to assess their effectiveness in promoting the growth of beneficial bacteria (*Lactobacillus plantarum)* as well as pathogenic bacteria (*Escherichia coli*). Overnight culture broths obtained from the stock culture were diluted to an optical density (OD) of 0.5 using nutrient broth (NB) (0.05% cysteine) for L. plantarumand NB for E. coli. The bacterial cultures were incubated in a medium enriched with oligosaccharides at sugar concentrations of 0, 500, 1,000, 1,500,

2,000, and 2,500 µg/ml, maintained at 37°C. The optical density at 660 nm of L. plantarumand E. coligrowth was monitored every 6 hours using a spectrophotometer. SPECTROstar Nano, Germany) (Lee et al., 2002; Rousseau et al., 2005).

Statistical analysis

The experimental data were subjected to analysis of variance and Tukey's test of means using R-studio software . Differences were considered significant at 5% probability.

Results

Chemical compositions

The data revealed that the chemical compositions were significantly different among treatments ($P<0.01$). Enzyme-treated copra meal had lower fiber content than other treatments ($P<0.01$). Moreover, enzyme-treated copra meal was higher in gross energy than other treatments ($P<0.01$) (Table 1).

Reducing sugar

Reducing sugars contents of enzyme treatments are displayed in Table 1. The data showed that enzyme-treated sugar palm peel had a higher content of reducing sugar than other treatments ($P<0.01$) (Table 1).

Table 1. Chemical compositions and reducing sugar of treatments (% on dry matter basis)

	Treatments					
Item (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Moisture	8.17^{b}	30.10^{a}	7.51^{b}	3.79^{d}	5.50^{c}	0.09
Ash	1.17^{d}	1.08^{e}	4.77^{a}	2.80^{b}	2.16^{c}	0.01
Ether extract	0.72^{d}	26.45^{a}	3.00^{c}	10.10^{b}	8.88^{b}	0.24
Crude protein	3.60^{d}	4.85^{d}	19.48^{b}	27.73^{a}	16.19^{c}	0.16
Crude fiber	53.28^{a}	37.54^{b}	27.83^{c}	20.18^{d}	16.57^{d}	0.41
Gross energy (kcal/kg)	$4,568.62^{d}$	$5,974.79^{a}$	$4,939.96^{c}$	$5,232.71^{b}$	$5,326.33^{b}$	15.30
Reducing sugar (mg/ml)	$14,023.34^{a}$	$4,266.92^{b}$	$3,741.28^{b}$	$3,036.15^{b}$	$13,895.13^{a}$	201.35

^{a,b,c,d,e} Means with varying superscripts in the same row are significantly different ($P<0.01$), T1= Enzyme-treated sugar palm peel, T2= Enzyme-treated coconut meal, T3 =1% Enzyme-treated spent tea leaves, T4=1% Enzyme-treated Brewer's grain, T5= 5% Enzyme-treated cashew nut testa.

Oligosaccharides

Glucose products found enzyme treatment (column 2 to 6) and lighter than glucose (column 1) and cellobiose molecule (column 7). The glucose products might be as oligosaccharides (Figure 1).

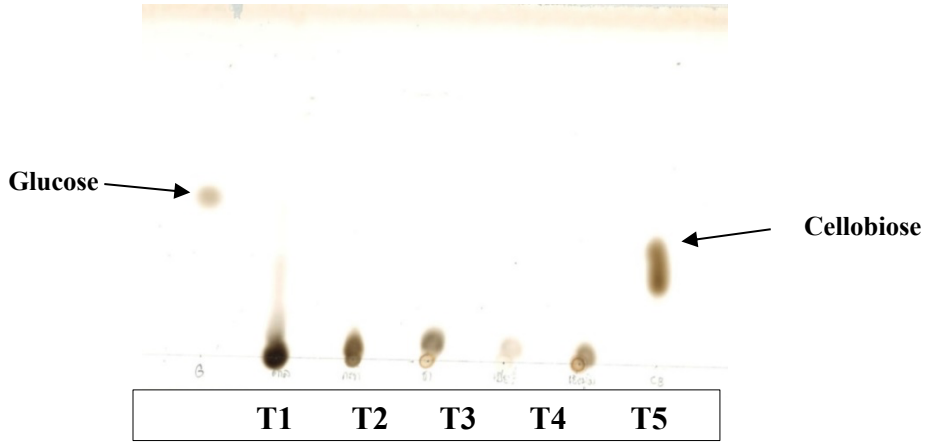

Figure 1. Oligosaccharides of enzyme treatments: T1= Enzyme-treated sugar palm peel, T2= Enzyme-treated coconut meal, T3 =1% Enzyme-treated spent tea leaves, T4=1% Enzyme-treated Brewer's grain, T5= 5% Enzyme-treated cashew nut testa

Prebiotic properties

The sugar product from all treatments were able to increase the growth of *L. plantarum* except the enzyme-treated Brewer's grain spent tea leaves. Besides, enzyme treated sugar palm peel, coconut meal and cashew nut testa decreased the growth of *E. coli* at all concentrations (Figure 2 and 3).

Discussion

The enzyme (Sclase A®) is a crude enzyme and has been shown to be effective in breaking down plant fiber in all agricultural waste especillay copra meal and releasing sugar and oligosaccharides. According to the previous study of Khanongnuch *et al.* (2006), copra meal treated with enzyme showed a reduced CF amount from 34.53% to 20.53%. Similarly, Corredor *et al.* (2007) also reported that enzymatic hydrolysis of sorghum bran had lower hemicellulose content than raw sorghum bran from 17.5% to 12.3% respectively. Conversely, CP and ash content of enzyme-treated copra meal were higher than raw copra meal. Similarly, Kraikaew *et al.* (2020) mention that fermented copra meal with

enzyme and yeast had higher CP (8.32-8.78%) than raw copra meal (4.60%). The fermented copra meal had 64% increased in CP and 50% decreased in CF. The result showed that the enzyme was effective and able to break down the plant cell wall and releasing more reducing sugar. Chimtong *et al.* (2016) reported that spent tea leaves that have been treated with enzyme were the most hydrolyzed as shown by the higher reducing sugar content than other types of agricultural waste (sugar palm peel, pineapple peel, spent coffee ground, Brewer's spent gain, copra meal and rice straw). On the other hand, Saenphoom *et al.* (2020) reported that enzyme treated sugar palm peel had a higher reducing sugar than enzyme treated coconut meal.

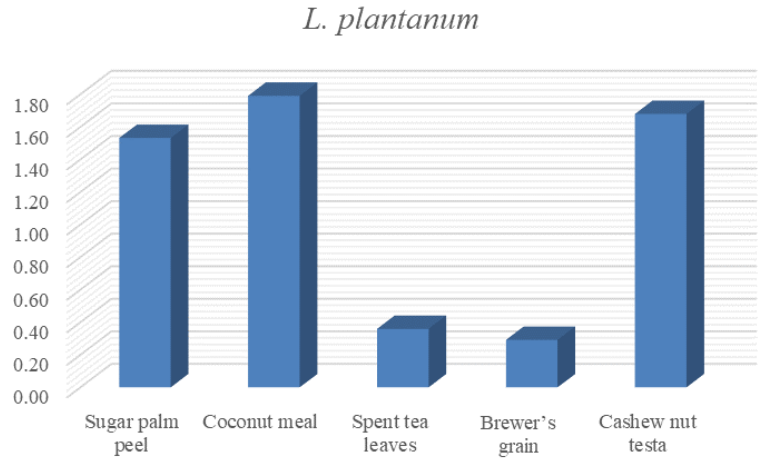

Figure 2. Effect growth of *L. plantarum* at 500 µg/ml for 24 hrs

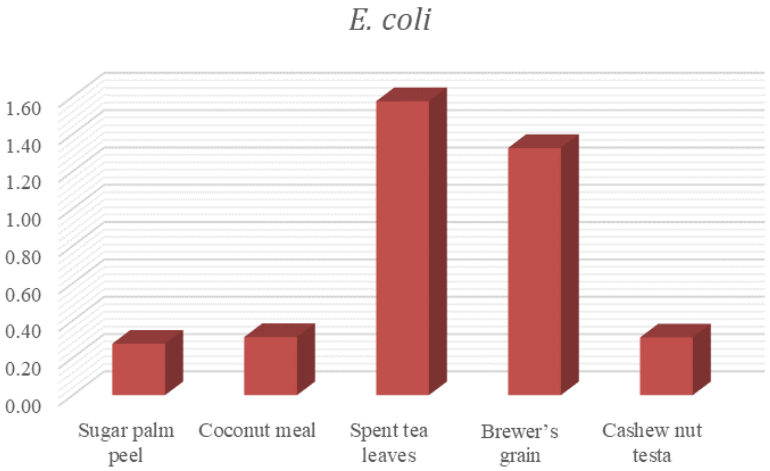

Figure 3. Effect growth of *E. coli* at 500 µg/ml for 24 hrs

For oligosaccharides, the hydrolyzed products after treated enzyme might be as oligosaccharides. Similarly, Chimtong *et al.* (2016) reported that enzyme (Pentozyme@) was effective in the hydrolysis of spent tea leaves in order to produce oligosaccharides. Study of Chen *et al*. (2015) noticed that enzyme treated palm kernel expeller (PKE) could release more manan oligosaccharides (28.91 g/kg PKE) compared to untreated PKE (20.93 g/kg PKE). In addition, the sugar product from all treatments were able to successfully increase the growth of *L. plantarum* except the enzyme-treated Brewer's grain spent tea leaves. Besides, enzyme treated sugar palm peel, coconut meal and cashew nut testa could decrease the growth of *E. coli*. Enzyme was effective for producing the oligosaccharides, as Titapoka *et al.* (2008) reported that the hydrolyzed products of copra mannan (MOS) enhanced growth of *L. reuteri* and inhibited growth of *E. coli.* Similarly, Kraikaew *et al.* (2020) also found that fermented copra meal with enzyme and yeast had increased MOS which act as prebiotics. Meanwhile, Saenphoom *et al.* (2020) reported that enzyme-treated coconut meal also can increase growth of *L. acidophilus* but cannot inhibit growth of *E. coli*

In conclusion, the process of enzymatic hydrolysis is effective in breaking down the plant's cell wall of agricultural waste, thus releasing the reducing sugar and oligosaccharides. Moreover, glucose product from enzyme-treated sugar palm peel, coconut meal and cashew nut testa could stimulates *L. plantarum* growth while at the same time inhibits *E. Coli* growth. Our experimental data indicated that sugar palm peel, coconut meal and cashew nut testa-treated enzyme can be utilized as a source of prebiotics, due to its ability to increase probiotics and decrease the growth of pathogenic bacteria.

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