

Proximate composition of the edible part of purple passion fruit and santol and *in vitro* prebiotic activity of crude polysaccharide extracts

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Abstract

This study aimed to evaluate the functional and prebiotic properties of the edible portions of certain tropical fruits in Thailand, passion fruit and santol. The proximate composition of passion fruit and santol were analysed which total carbohydrate was the major component in both samples. Total dietary fibre of passion fruit and santol was 44.81 and 26.82% (w/w), respectively, with the majority of insoluble dietary fibre. Soluble dietary fibre of both fruits was extracted by hot water and water-extractable polysaccharide was yielded at 16.47% and 25.24% (w/w) for passion fruit and santol, respectively. The functional properties of fruit fibre were informed, with the satisfactory oil holding capacity of santol polysaccharide. The effect of both fruit polysaccharides on proliferation number at 24 hrs was not different from inulin. Prebiotic activity score of fruit polysaccharide was calculated from the growth of *Lactobacillus acidophilus* and *Bifidobacterium longum* compared with the growth of the enteric pathogen, *Escherichia coli* corresponding to -0.25 and -0.23 for passion fruit and 0.10 and -0.01 for santol, respectively. In conclusion, both passion fruit and santol polysaccharide showed a distinct effect on the supportive growth of probiotic bacteria which may be potential candidate ingredient incorporated in probiotic food. From this evidence, the development of fruit-based synbiotics from passion fruit and santol might be affordable.

1. Introduction

Fresh tropical fruits are consumed daily in the local diet as a delightful refreshment. Moreover, are considered a healthy food because they contain bioactive and nutritional constituents such as polysaccharides, vitamins, natural antioxidants, phytochemicals and are likely the cause of prevention of some diseases and cancers (Maria do Socorro *et al.*, 2010; Quirós-Sauceda *et al.*, 2014). Many plant-based foods especially fruit and vegetable favour a variety of health benefits according to their dietary fibre content. Dietary fibre (DF) is the edible part of plants or carbohydrate polymers that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Brownlee, 2011). It exerts physiological function by the modulation of gut microbiota called prebiotic effects. These prebiotics deliberate certain soluble fermentable fibre which provides selective utilization by the beneficial host microorganisms and support health (Gibson *et al.*, 2017). Several studies demonstrated that the edible parts of fruit have shown

prebiotic properties including date palm (Al-thubiani and Khan, 2017), bambangan (Al-Sheraji *et al.*, 2012), guava (Thuaytong and Anprung, 2011; Lim *et al.*, 2018), lychee (Fasawang and Anprung, 2014), and longan (Thitiratsakul and Anprung, 2014), pitaya and jackfruit (Wichienchot *et al.*, 2015).

Among the desired tropical fruits, passion fruit is rich in several bioactive compounds such as carotenoids, vitamins, phenolic compounds, and fibres. There are two popular types of passion fruits namely the yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Deg) and purple passion fruit (*Passiflora edulis* f. *edulis*) (Corrêa *et al.*, 2016; Lascano *et al.*, 2020). Though most studies are based on yellow passion fruits on the available data of their chemical, physico-chemical, technological, and pharmacological activities such as antibacterial and antioxidant properties (Martínez *et al.*, 2012; López-Vargas *et al.*, 2013). While the purple passion fruit, which is commonly freshly eaten in Thailand, has limited study. Santol (*Sandoricum koetjape* Merr.) is another popular seasonal fruit in Thailand. Santol is an

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ethnobotanical plant used as a folk medicine for anti-inflammatory and anti-colic activities using root and stem bark decoction (Nassar *et al.*, 2010). Santol fruits and juices have antioxidant and antibacterial activities (Anantachoke *et al.*, 2016; Toobpeng *et al.*, 2017). However, the study on the edible portion of santol fruit is limited especially on the effects on beneficial bacteria or probiotics. Conventionally, prebiotic effects refer to selectively enhancing the growth of bifidobacteria or lactobacilli which is expected to be profitable to produce substrates as prebiotic (Verspreet *et al.*, 2016). Recently, Lascano *et al.* (2020) have developed the synbiotic powder containing *Lactobacillus plantarum* and yellow passion fruit juice powder with probiotic stability and sensory acceptability. Nevertheless, most studies on santol and passion fruit have focused on dietary fibre from waste and by-products but no information about edible portion as potential prebiotic yet.

In this study, the edible portions of passion fruit and santol were yielded for dietary fibre by hot water extraction. The fruit fibre was evaluated for the proliferative effect of selected probiotic strains compared to the common enteropathogenic bacteria. Moreover, its technological properties offered functionality to the host's health such as water-holding capacity (WHC), oil-holding capacity (OHC), and cation exchange capacity (CEC) was determined. The fruit fibre extraction that focuses on soluble fermentable polysaccharide may indicate the potential of passion fruit and santol to value-added as a prebiotic ingredient or used as a probiotic carrier in functional food development.

2. Materials and methods

2.1 Sample preparation

Fresh fruits of santol and passion fruit were purchased from the local market and store in Bangkok, Thailand. The fruits were cleaned with water and manually separated for their edible parts. Passion fruit was cut in half and spooned for its seed and pulp. For santol, the peel was removed and its inner flesh without the seed was collected. Then, the edible portions were dried in a hot air oven at 60°C. The dried samples were grounded with a food homogenizer and kept in a desiccator until analysis was performed.

2.2 Proximate analysis and dietary fibre content assay

The moisture, ash, fat, and protein content of the edible part of the passion fruit and santol were determined by the Association of Official Analytical Chemists (AOAC) method of analysis and expressed in percentage (wet weight basis) (Horwitz and Latimer Jr., 2012). Fresh fruits were analysed for moisture content at

105°C using a moisture analyser (MA50.R model, Radwag, Poland). Total ash was determined as weight loss after incineration at 550°C for 5 hrs. Crude protein was estimated by the Kjeldahl method with a factor of 6.25. Crude fat was determined using solvent extract apparatus and petroleum ether as solvent. Total carbohydrate was calculated by difference using the equation:

$$\text{Total carbohydrate} = 100 - (\text{weight in grams} [\text{protein} + \text{fat} + \text{water} + \text{ash}] \text{ in } 100 \text{ g of food})$$

Insoluble, soluble, and total dietary fibre of moisture-free, and fat-free and its fat content exceed 10% (w/w), were determined according to the enzymatic-gravimetric method of the AOAC using Megazyme assay kit. Total dietary fibre was calculated by summation of insoluble and soluble dietary fibre. This commercial kit is based on AOAC method 985.29.

2.3 Crude polysaccharide extraction

Fruit fibre was retrieved by hot water extraction according to Tadayoni *et al.* (2015) with minor modification. Briefly, the sample was mixed with water at a ratio 1:3 and stirred on a hot plate magnetic stirrer. Then, the sample was extracted with hot water at 90°C for 3 hrs. The fruit polysaccharide was filtered using layers of cotton cheesecloth, then submitted to concentrate by rotary evaporator under reduced pressure of 60°C. The water-soluble polysaccharide was dried by lyophilization and then the powder was weighed to calculate the yield of extraction and stored in a desiccator until the experiment.

2.4 Functional properties of fruit polysaccharide

Water-holding capacity (WHC), oil-holding capacity (OHC), and cation exchange capacity (CEC) were analysed for the functionality of fruit polysaccharide (Requena *et al.*, 2016). For WHC, the sample was mixed with distilled water at a ratio of 1:10 and left for 24 hrs, then centrifuged at 1,006×g for 30 mins. Volume was measured and WHC was expressed as g of water held by g of sample. OHC was determined by mixing 0.1 g of sample with 1 mL of commercial olive oil, then the suspensions were centrifuged. OHC was expressed as g of oil held per g of sample. CEC was determined by mixing 0.5 g of sample with 5 mL of 2N HCl and incubated at room temperature for 24 hrs, then centrifuged at 2,200×g for 30 mins and washed with saturated NaCl solution. The residue was suspended and titrated with 0.1 N NaOH. The titrant was calculated in milliequivalents (mEq) and the CEC was expressed in mEq per g.

2.5 Analysis for prebiotic properties of the fruit polysaccharide

The prebiotic activity of fruit polysaccharide was evaluated *in vitro* by the growth of intestinal bacteria associated with health as described by Fissore *et al.* (2015). Bacterial cultures used in this study were standard JCM strains (equivalent to ATCC strains) which from human origin: *Lactobacillus acidophilus* JCM 1132 (=ATCC 4356^T), *Bifidobacterium longum* JCM 1217 (=ATCC 15717^T), and *Escherichia coli* ATCC 11775^T. Bacteria were streaked onto MRS agar for *L. acidophilus*, MRS agar supplemented with 0.05% L-cysteine HCl for *B. longum* and tryptic soy agar (TSA) for *E. coli*, then incubated at 37°C for 24-48 hrs. For *B. longum*, the culture was incubated in an anaerobic chamber (Oxoid) under an anaerobic atmosphere (Anaerocult A, Merck). After that, a single colony of *L. acidophilus* and *B. longum* was transferred into 5 mL of MRS and 5 mL of MRS broth supplemented with 0.05% L-cysteine HCl, respectively, while a single colony of *E. coli* was grown in 5 mL of tryptic soy broth (TSB), and incubated at the same condition as stated above. The assay was performed by adding 1% (v/v) of overnight culture to another tube containing specific media broth with 1% (w/v) glucose or 1% (w/v) fibre samples. Specific media broth was MRS broth, MRS broth supplemented with 0.05% L-cysteine HCl, and M9 minimal medium for *L. acidophilus*, *B. longum*, and *E. coli*, respectively. At 0 and 24 hrs of incubation, samples were enumerated in duplicate using the serial dilution method and the results were calculated as colony forming units per milliliter (CFU/mL). Each assay was replicated a minimum of three times.

To assess the selectivity of the substrates, prebiotic activity score (PAS) was determined according to the equation of Huebner *et al.* (2007). Inulin was used as the standard for comparison.

Prebiotic activity score (PAS) = $[\Delta(\log\text{CFU/mL}) \text{ probiotic at 0 and 24 hrs in prebiotic}] / [\Delta(\log\text{CFU/mL}) \text{ probiotic at 0 and 24 hrs in glucose}] - [\Delta(\log\text{CFU/mL}) \text{ non-probiotic at 0 and 24 hrs in prebiotic}] / [\Delta(\log\text{CFU/mL}) \text{ non-probiotic at 0 and 24 hrs in glucose}]$

2.6 Statistical analysis

Experiments were carried out in triplicate. Data were presented as mean value \pm standard deviation. For statistical analysis, IBM SPSS statistics 21 was used to perform the analysis of variance (ANOVA) followed by the least significant difference (LSD) test at a 5% level of significance.

3. Results and discussion

3.1 Proximate analysis and dietary fibre content of passion fruit and santol

Table 1 shows the proximate composition of the edible part of the passion fruit and santol. Apart from water content, total carbohydrate and fat were the main compositions of passion fruit at 15.44 \pm 1.18% and 10.24 \pm 0.29% wet basis. Whereas total carbohydrate was also a major component of santol fruit which was 12.75 \pm 0.21% fresh weight with few ash and protein content. When compared to the most studied yellow passion fruit (*Passiflora edulis* f. *flavicarpa*), purple passion fruit had more protein and fat content as previously reported (López-Vargas *et al.*, 2013; Corrêa *et al.*, 2016; dos Reis *et al.*, 2018). Whereas this is the first report on the proximate composition of Thai nung cultivar. Santol fruit showed similar ash and protein content to those reported with slightly more fat content (Janick and Paull, 2008).

Table 1. Proximate analysis of the edible portions of passion fruit and santol

g per 100 g wet basis	Passion fruit	Santol
Moisture	70.991.20	82.46 \pm 0.28
Ash	0.89 \pm 0.13	0.52 \pm 0.01
Protein	2.44 \pm 0.11	0.47 \pm 0.00
Fat	10.24 \pm 0.29	3.81 \pm 0.16
Total carbohydrate	15.44 \pm 1.18	12.75 \pm 0.21

The fibre content of both selected fruits is shown in Table 2. Total dietary fibre (TDF) by summation was 44.81 \pm 0.77% and 26.82 \pm 1.05% of dry weight for passion fruit and santol, respectively. Insoluble dietary fibre (IDF) represents a dominant constituent compared to the soluble dietary fibre (SDF) in both fruits. However, santol fruit fibre is nearly equal between IDF (14.00 \pm 2.20%) and SDF (12.82 \pm 2.03%) which gave the IDF: SDF ratio 1.09. This ratio in the range of 1.0 to 2.3 represents the balance of both types of fibre on the physiological effect in the intestine as whole fruits and fruit by-products are well recognized for their fibre content (Wanlapa *et al.*, 2015). Both passion fruit and santol are rich in dietary fibre compared with other tropical fruits such as sapota (*Achras sapota*), guava (*Psidium guajava*), and date (*Phoenix dactylifera*) (Ramulu and Rao, 2003). Though the total fibre content of passion fruit was higher than santol, passion fruit was

Table 2. Dietary fibre content (dry basis) of the edible portions of passion fruit and santol

Dietary Fibre % (w/w)	Passion fruit	Santol
Insoluble fibre (IDF)	43.09 \pm 2.34	14.00 \pm 2.20
Soluble fibre (SDF)	1.71 \pm 0.54	12.82 \pm 2.03
Total dietary fibre	44.81 \pm 0.77	26.82 \pm 1.05
IDF:SDF ratio	25.2	1.09

responsible for insoluble fibre content as previously reported of *Passiflora edulis* var *flavicarpa* (Chau and Huang, 2004). This may be due to its consumable seed coat mixed in puree or juice.

3.2 Fruit polysaccharide extraction

Extraction by hot water presented in this study yielded in its dry basis were 16.47% and 25.24% for passion fruit and santol, respectively. In this study, passion fruit was defatted in ethanol because lipid is able to interfere with water permeation (Azmi *et al.*, 2012). Moreover, to mimic the edible fruit, the ethanol precipitation step was not included while the purification step could be applied to minimize those characteristics of dietary fibre in the furthered experiment.

3.3 Functional properties of fruit polysaccharide

The functional properties were shown in Figure 1. The hydrating possessions of water-soluble polysaccharide were in small amounts with undetected swelling capacity (data not presented). Santol fruit polysaccharide displayed an oil holding capacity of 4.43 ± 0.93 g of oil held per g of the sample compared with the passion fruit polysaccharide which was 2.62 ± 0.10 g of oil held per g of sample. There are some technological properties that offer physiological functionality including WHC, OHC, and CEC (Elleuch *et al.*, 2011). Water-extractable polysaccharide from passion fruit seed and pulp depicts lower WHC and higher OHC than dietary fibre powder (López-Vargas *et al.*, 2013). Santol fruit polysaccharide had superb OHC at 4.43 g oil per g fibre when compared to other tropical fruits such as mango at 1.6, pineapple at 0.7, and guava at 0.7 g oil per g fibre (Martínez *et al.*, 2012). Oil-holding capacity is the amount of oil retained by the fibre; thus, fibre can bind with fat resulting in the

prevention of fat loss during food processing and reducing serum cholesterol levels (Luo *et al.*, 2017).

3.4 Prebiotic properties of fruit polysaccharide

Figure 2 shows the prebiotic activity score (PAS) derived from the number of bacterial cells enumeration by viable plate count (Table 3). The proliferation of probiotic strains was excellent on passion fruit polysaccharide with a maximum value of 3.45 log CFU/mL differing between 0 hr and 24 hr for *B. longum* and 3.29 log CFU/mL for *L. acidophilus*, though it also supports the growth of *E. coli*. Hence the key qualifier for a prebiotic effect is the selectivity of fermentable dietary fibre. Prebiotic activity score (PAS) was developed to evaluate preliminarily selectivity of the substrate in promoting the growth of intestinal bacteria associated with health and pathogenic bacteria (Huebner *et al.*, 2007). The PAS values of passion fruit polysaccharide were the lowest score of -0.25 on *L. acidophilus* and of -0.23 on *B. longum*. Whereas the santol polysaccharide selectively supports the growth of probiotic bacteria and resulted in the highest PAS at 0.10 on *L. acidophilus*. The score was used to assess the prebiotic effect of the variety of fruit dietary fibre for example guava displayed the PAS at 0.13 on *L. acidophilus*, enzyme hydrolysate of longan displayed the PAS at 1.69 and 1.44 for *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12, respectively (Thuaytong and Anprung, 2011; Thitiratsakul and Anprung, 2014). In this study, PAS of hot water fibre-derived extraction may be the effect of sugar, which are more preferable and non-selective on established microorganisms. Hence, the fibre extracts are able to promote *E. coli* as well.

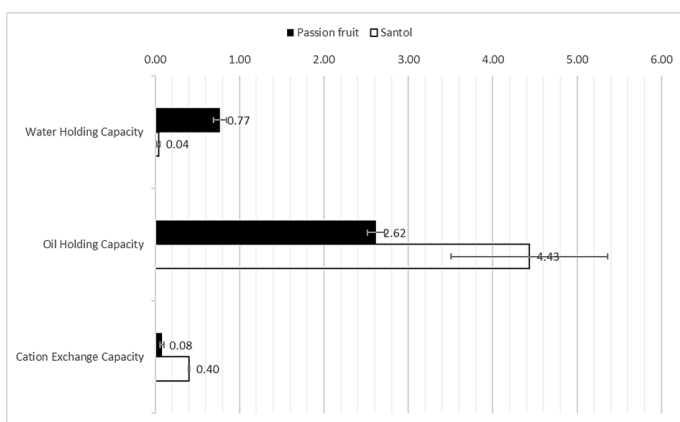


Figure 1. Functional properties of fruit polysaccharide from passion fruit (■) and santol (□) are represented as water holding capacity, oil holding capacity, and cation exchange capacity. The polysaccharides of santol have better oil holding and cation exchange capacity than the polysaccharide of passion fruit, however it less preferable for oil holding capacity.

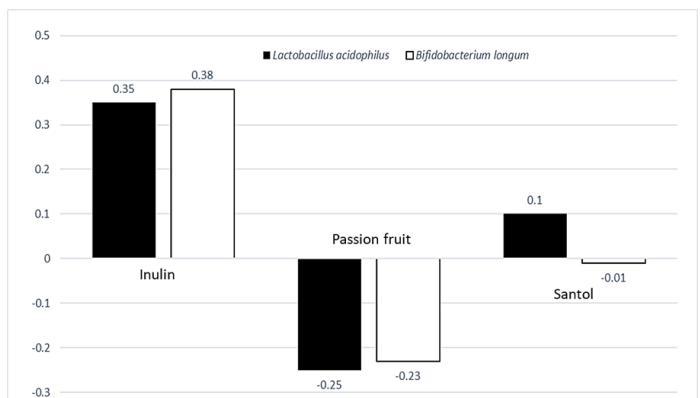


Figure 2. Graphical representation of prebiotic activity scores of *L. acidophilus* (■) and *B. longum* (□) on passion fruit and santol polysaccharide.

Accordingly, passion fruit polysaccharide can support the proliferation of all tested microorganisms in a great manner including on *E. coli*. As a result, passion fruit polysaccharide may not be suitable for prebiotic effects, but it has the potential to be a probiotic carrier where further research needed. While santol fibre can be used to support the selective growth of *L. acidophilus* not

statistically different from inulin, the commercial prebiotic. However, the same substrates may be highly specific to a different genus of microorganisms, future research should be conducted to evaluate the dynamics of intestinal microbiota (Bindels *et al.*, 2015; Figueroa-Gonzalez *et al.*, 2019).

Table 3. Proliferation of tested microorganisms on fruit polysaccharide, difference in bacterial growth between 0 hr and 24 hrs (log CFU/mL)

Bacterial culture	Inulin	Passion fruit	Santol
<i>Escherichia coli</i>	1.39±0.69 ^b	2.64±0.39 ^a	1.82±0.64 ^a
<i>Lactobacillus acidophilus</i>	3.29±0.42 ^a	3.29±0.40 ^a	3.16±0.63 ^a
<i>Bifidobacterium longum</i>	3.46±0.54 ^a	3.45±0.36 ^a	2.87±0.54 ^a

Values are expressed as mean±SD. Values with different superscript within the same row are significantly different ($p < 0.05$).

4. Conclusion

Fruit dietary fibre is attractive as a conventional substance for its prebiotic effect. The present study demonstrated the supportive effect of passion fruit and santol polysaccharide on the growth of *L. acidophilus* and *B. longum* as well as inulin. The prebiotic activity score of santol polysaccharide indicated its selectivity to promote the growth of *L. acidophilus*. While passion fruit polysaccharide can promote the growth of tested microorganisms with a low selectivity rate, this may be appropriate for auxiliary probiotic strains as food matrix in probiotic food formulation. Moreover, the oil holding capacity of water-extractable dietary fibre from both types of fruits showed the potential use in absorbing the fat content. These results may ensure the fruit fibre, as a whole or by-product, is an appropriately functional ingredient in modern cuisine.

Conflict of interest

The authors declare no conflict of interest.

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