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Antioxidant and Prebiotic Activity of Enzymatically Hydrolyzed Lychee Fruit Pulp

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Summary

The optimal extraction of bioactive compounds from lychee fruits using commercial Pectinex® Ultra SP-L pectinase hydrolysis of the fruit pulp homogenate was evaluated. The lychee fruit pulp was treated with the pectinase at volume per mass ratios between 0.5 and 2.5 % for 0.5–5 h. The degree of hydrolysis (DH), monitored as the level of released reducing sugar, increased with the increase of enzyme volume per mass ratio and time up to 2 % and 4 h, respectively, to give a maximum DH of 19 %. The antioxidant activity in lychee pulp hydrolysates increased with increasing DH, with the highest DH of 19 % displaying an EC_{50} of 0.25 μg of fresh mass (FM) per μg of diphenyl-(2,4,6-trinitrophenyl)-iminoazanium and 53.17 μM of Trolox equivalents per g of FM. The lychee hydrolysate with the DH of 19 % had the highest level of total phenolic acids (165.8 mg of gallic acid equivalents per 100 g of FM) and flavonoids (70.8 mg of catechin equivalents per 100 g of FM), and the highest prebiotic activity score of 1.68 and 0.82 of *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5, respectively. In accordance with the level of phenolic acids and flavonoids, the lychee hydrolysate with the DH of 19 % revealed a reasonably good level (72–77 %) of lipid peroxidation inhibition in soya bean, lard and olive oil. The lychee hydrolysate with the DH of 19 % increased the number of detected volatile compounds to 37, with 11 new compounds that were not found in the lychee hydrolysate with the DH of 0 %. Moreover, the particle size and insoluble fibre level were reduced with a concomitant increase in the soluble fibre level.

Key words: pectinase, lychee pulp, antioxidation, prebiotics

Introduction

Lychee (*Litchi chinensis* Sonn.) is a subtropical to tropical fruit of the Sapindaceae family, native to Southeast Asia. Lychee fruits contain antioxidant compounds, such as phenolic acids and flavonoids, in the pericarp, seeds and flowers (*1–5*). In general, phenolic acids and flavonoids exhibit powerful antioxidant activities (*6*) that inhibit key enzymes in mitochondrial respiration, offer protection against coronary heart diseases, and have anti-inflammatory, antitumour and antimicrobial activities (*7–9*).

Whilst some bioactive compounds, such as polyphenols, are found at high concentrations in plants, others are only found at very low levels and may only be synthesized in specialized cell types or during particular growth stages or under specific conditions. This makes their extraction and purification quite difficult. Many extraction techniques, such as hot water or other less polar solvent extractions, ultrahigh pressure, ultrasonic and microwave-assisted extractions (*10-13*) have been widely used to extract bioactive compounds from plants. Plant cell walls and some internal structures contain polysaccharides, such as cellulose, hemicellulose and pectins, which

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act as barriers to the release of intracellular substances. Moreover, many bioactive compounds bind to these components and pectinases can depolymerize these polysaccharides and degrade the cell wall structure, facilitating the release of linked compounds (*14*,*15*).

This study evaluates the use of pectinase-based enzyme-assisted extraction to increase the yield of extracted bioactive compounds. The effect of the degree of hydrolysis (DH) on the level of soluble dietary fibre (SDF), volatiles, prebiotic activity, and inhibitory activity of lipid peroxidation of the lychee fruit pulp hydrolysates was also monitored.

Materials and Methods

Preparation of materials

Mature (ripe) lychee fruits (*Litchi chinensis* Sonn. cv. Jukkapat) were obtained from a local market in Bangkok, Thailand. They were peeled and the seed was removed to leave the pulp which was then homogenized in a blender. To inhibit the enzymatic browning reaction, the crude homogenate was blanched at 85 °C for 1 min and then cooled rapidly to and kept at 4 °C until use. The commercial pectinase Pectinex® Ultra SP-L was purchased from Novozymes Co., Ltd. (Bagsværd, Denmark). All other chemicals and solvents used in the experiment were of analytical grade and were purchased from Sigma Chemicals Co., Ltd. (St. Louis, MO, USA) or Sigma-Aldrich Co., Ltd. (Steinheim, Germany).

Enzymatic hydrolysis of lychee pulp

The lychee pulp homogenate was treated with Pectinex® Ultra SP-L (enzymatic activity of 10 292 polygalacturonase units (PGU) per mL at the controlled reaction temperature of (30±2) °C) at six different ratios (0, 0.5, 1, 1.5, 2 and 2.5 %, by volume per mass) at various times (0, 1, 2, 3, 4 and 5 h). The hydrolysis reaction was then stopped by heating at (100±5) °C for 5 min. The DH of each obtained lychee hydrolysate was determined from the amount of reducing sugar (as glucose equivalents) using the Nelson-Somogyi method (*16*,*17*), as follows:

$$\mathrm{DH}=\left(\frac{m(\text{reducing sugar})/\text{mg}}{m(\text{total dietary fibre})/\text{mg}}\right)\cdot 100 \qquad /1/$$

Determination of the antioxidant activity

Samples were prepared according to the modified method of Velioglu *et al*. (*18*). Lychee pulp was ground and extracted with 95 % ethanol in the dark at 25 °C for 4.5 h. The free radical-scavenging activity of each lychee hydrolysate was measured using the DPPH assay according to the method of Maisuthisakul *et al*. (*19*). The antioxidant activity, as a percentage of the scavenging activity on the DPPH radical, was evaluated using the following equation:

$$\text{DPPH radical-scavenging activity}=\left(\frac{[A_0-(A_1-A_S)]}{A_0}\right)\cdot 100 \qquad /2/$$

where A_1 and A_s are the absorbance (measured at 517 nm) of the diluted sample with and without DPPH, respectively, and A_0 is that of the DPPH solution without any lychee hydrolysate (control) and methanol as the blank. The percentage of DPPH radical-scavenging activity was calculated and plotted against the sample extract concentration (μg/mL) to determine the amount of the extract capable of decreasing the DPPH radical fraction by 50 % (EC_{50}).

The ferric reducing antioxidant power (FRAP) assay was performed on each lychee hydrolysate as reported previously (*20*) with some modifications. Briefly, the fruit extracts (50 μL) were allowed to react with 950 μL of the FRAP solution for 4 min in the dark. The standard curve was linear between 82 and 625 μM of Trolox. The results were expressed in μM of Trolox equivalents (TE) per g of fresh mass (FM).

Determination of the total phenolic and flavonoid contents

Samples were prepared in the same way as for the determination of antioxidation activity. Total phenolic content was determined by the Folin-Ciocalteu assay (*21*). Gallic acid (50–500 mg/L) was used for calibration of the standard curve. The results were expressed in mg of gallic acid equivalents (GAE) per 100 g of FM.

Total flavonoid content was measured by aluminium chloride colourimetric assay (*22*). Measurements were calibrated to a standard curve of prepared catechin solution (20–100 mg/L). The results were expressed in mg of catechin equivalents (CE) per 100 g of FM.

Determination of the dietary fibre content

The total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) contents were analyzed according to the standard AOAC method (*23*), and the results were expressed in g per 100 g of FM.

Determination of the prebiotic activity

Lactobacillus acidophilus La5 and *Bifidobacterium lactis* Bb12 (Christian Hansen, Hørsholm, Denmark) were used as the representative probiotic cultures (P), while *Escherichia coli* (ATCC 25922) (Culture Collection Unit, King Chulalongkorn Memorial Hospital, Bangkok, Thailand) was used as the representative enteric species (E). Inulin, a commercial prebiotic, was used as the reference standard for comparison. Each assay was performed in triplicate, measuring the number of viable colony forming units (CFU) per mL before (P^0 and E^0) and after (P^{24} and E^{24}) incubation for 24 h on 1 % (by mass per volume) glucose (P_G and E_G), 1 % (by mass per volume) test lychee hydrolysate or 1 % (by mass per volume) inulin as the prebiotic (P_x and E_x) as reported previously (*20*). The prebiotic activity score (PA) was then determined using the following equation:

$$\mathrm{PA}=[(\log P_X^{24}-\log P_X^0)/(\log P_G^{24}-\log P_G^0)]-[(\log E_X^{24}-\log E_X^0)/(\log E_G^{24}-\log E_G^0)] \qquad /3/$$

Analysis of volatile compounds

Volatile compounds were isolated using solid-phase microextraction (SPME) as reported previously (*24*). Com-

pounds were identified by matching the mass spectra (quality match >80 %) against the mass spectral library (*25*).

Particle size measurement

Particle size was measured using a Mastersizer 2000 analyzer (Malvern Instruments Ltd, Malvern, UK) as reported earlier (*26*). The results are reported as volume weighted mean, $D_{[4,3]}$ (μm). Each analyzed sample was replicated four times.

Determination of the inhibition of lipid peroxidation activity

Each lychee hydrolysate was screened for the ability to inhibit the lipid peroxidation of soya bean, lard and olive oil using the modified thiobarbituric acid reactive substances (TBARS) assay (*27*) with some modifications. Briefly, 0.1 mL of ferrous sulphate and 0.1 mL of ascorbic acid were added to 1 mL of the sample or butylated hydroxatoluene (BHT) with oil (vegetable oil, olive oil and lard). Test samples were incubated at 37 °C for 45 min with continuous shaking. The reaction was stopped and TBARS were analyzed using 1 mL of 0.5 % thiobarbituric acid (TBA) in 20 % trichloroacetic acid (TCA). The products used in the samples were added to the blank. After agitation, the samples were incubated at 100 °C for 15 min and then centrifuged at 1000×*g* for 15 min at 4 °C. The amount of produced TBARS was measured as the spectrophotometric absorbance of the supernatant at 593 nm. The standard curve was obtained using malondialdehyde-*bis*-diethyl-acetate, whilst 1 mg/mL of BHT was used as a comparative standard. The results were expressed in mg of malondialdehyde (MDA) per g of FM.

Statistical analysis

Statistical analyses were performed using the SPSS v. 19 software. Results are shown as the mean values± standard deviation (S.D.). The significance between different means was tested using one-way analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT), with significance accepted at $p \leq 0.05$ level.

Results and Discussion

The effect of enzyme hydrolysis

The level of released reducing sugar and DH significantly increased with the increase of pectinase ratio from 0.5 up to 2.5 % (by volume per mass) and hydrolysis time from 0.5 up to 4 h (Fig. 1). Further increase in either the enzyme concentration (to 2.5 %) or the reaction time (to 5 h) had no significant effect on the obtained DH (maximum at DH of 19 %). The increased reducing sugar level represented the hydrolysis of the glycosidic bonds in the lychee hydrolysate. With pectinase at 2.0 % (by volume per mass), the DH of lychee pulp of 6, 11, 14 and 19 % were obtained after 1, 2, 3 and 4 h of digestion, respectively (Fig. 1), and these hydrolysates were selected, along with the hydrolysate with the DH of 0 % (no enzyme treatment), to screen for bioactive compounds.

Antioxidant activity

The DH of 0, 6, 11, 14 and 19 % of lychee hydrolysates were screened for antioxidant activity using the DPPH and FRAP assays, with the results summarized in Table 1. The level of antioxidant activity significantly increased in a dependent manner with the increase of DH of the hydrolysate, which was twofold higher at the DH of 19 % compared to the DH of 0 %. Thus, the EC_{50} values decreased from 0.50 at DH of 0 % to 0.25 μg of FM per μg of DPPH at the DH of 19 %, whilst the FRAP increased from 25.3 to 53.2 μM of TE per g of FM, respectively. Therefore, pectinase-assisted extraction of plant homogenates can improve the extraction efficiency of bioactive compounds and give higher yields (*4*).

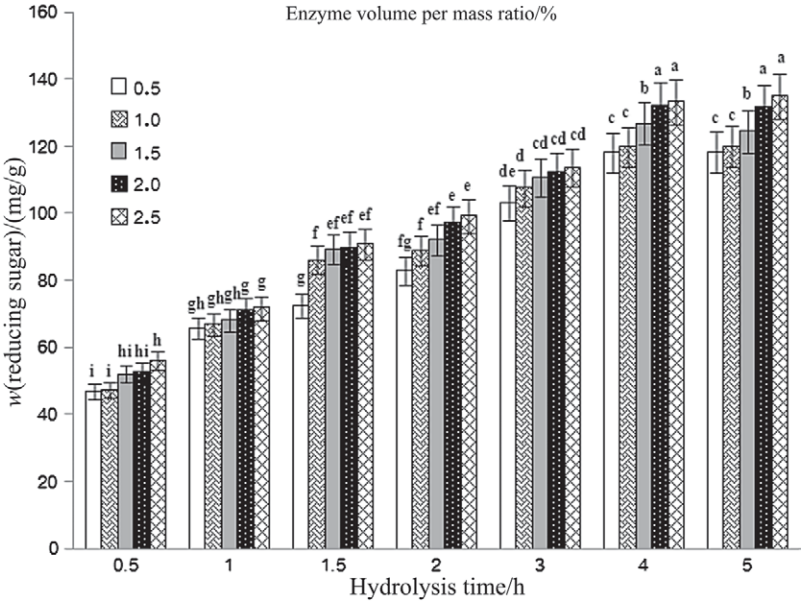

Fig. 1. Changes in the reducing sugar (glucose) content in lychee fruit hydrolysates during pectinase hydrolysis. Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values with different letters are significantly different ($p<0.05$)

Table 1. Bioactive compounds and antioxidant capacity of lychee hydrolysates measured on fresh mass basis with different degrees of hydrolysis (DH)

Antioxidant activity and bioactive compounds	DH/%				
	0	6	11	14	19
DPPH radical scavenging activity, EC_{50}/(μg/μg)	(0.5±0.0)^a	(0.400±0.002)^b	(0.40±0.002)^c	(0.310±0.014)^d	(0.250±0.016)^e
FRAP as TE/(μM/g)	(25.3±1.8)^e	(33.4±1.8)^d	(38.3±1.8)^c	(45.8±1.8)^b	(53.17±1.8)^a
w(TP as GAE)/(mg/100 g)	(104.1±0.2)^e	(118.5±0.3)^d	(128.7±0.1)^c	(142.1±0.6)^b	(165.8±0.2)^a
w(TF as CE)/(mg/100 g)	(23.8±0.1)^e	(43.3±0.2)^d	(51.1±0.3)^c	(62.02±0.05)^b	(70.8±0.2)^a

Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values in the same row with a different lower case letter in superscript are significantly different ($p<0.05$). FRAP=ferric reducing antioxidant power, TE=Trolox equivalent, TP=total phenolics, GAE=gallic acid equivalent, TF=total flavonoids, CE=catechin equivalent

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents in the lychee fruit hydrolysates increased in a dose-dependent manner with increasing levels of the DH of the hydrolysate, reaching total phenolic and flavonoid contents per 100 g of fresh mass of 165.8 mg of GAE and 70.8 mg of CE, respectively, in the hydrolysate with the DH of 19 % (Table 1), *i.e.* 1.6- and 3.0-fold higher than those in the hydrolysate with the DH of 0 %, respectively. Thus, the lychee fruit hydrolysates at 0 and 19 % of DH were selected to evaluate the effect of pectinase treatment on the dietary fibre, prebiotic activity score, volatile compounds, lipid peroxidation inhibition and particle size.

Dietary fibre

The TDF, SDF and IDF levels in the lychee hydrolysates with the DH of 0 and 19 % are shown in Table 2. The TDF levels in the lychee hydrolysates with the DH of 0 and 19 % were not significantly different ($p>0.05$), whereas the SDF was increased by the enzyme treatment to 0.150 g per 100 g of FM (2.67-fold), whilst the IDF was correspondingly reduced to 0.170 g per 100 g of FM (1.83-fold). This is the result of the cleavage of the pectin α-1,4-glycosidic bonds to form smaller more soluble pectins and carbohydrates (*20,28*), which increased the SDF level and concomitantly decreased the IDF level.

Table 2. Total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) levels in the untreated (DH of 0 %) and pectinase-treated (DH of 19 %) lychee hydrolysates

w(dietary fibre)/(g/100 g)	DH/%	
	0	19
TDF	(0.46±0.01)$^{n.s.}$	(0.46 ±0.01)$^{n.s.}$
SDF	(0.09±0.00)^b	(0.24±0.00)^a
IDF	(0.38±0.01)^a	(0.21±0.01)^b

Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values in the same row with a different lower case letter in superscript are significantly different ($p<0.05$); n.s.=not significant, DH=degree of hydrolysis

Prebiotic activity score

The prebiotic activity scores of *L. acidophilus* La5 and *B. lactis* Bb12 were significantly higher (8.15- and 16.4--fold, respectively) in the lychee hydrolysate with the DH of 19 % than the corresponding values for the untreated hydrolysate (DH of 0 %), and only 1.04- and 1.46-fold lower than that for inulin, a commercial prebiotic (Fig. 2 and Table 3). Indeed, the prebiotic activity score observed here of the lychee hydrolysate with the DH of 19 % containing *B. lactis* Bb12 was higher than that reported for optimally digested mangosteen pulp or banana (*20,29*). Presumably the enzyme-cleaved polysaccharides in the lychee hydrolysate (DH of 19 %) were smaller soluble oligosaccharides of which some are prebiotic compounds (*30,31*). Thus, the lychee hydrolysate with the DH of 19 % has a better potential as a prebiotic than the optimally digested mangosteen pulp or banana.

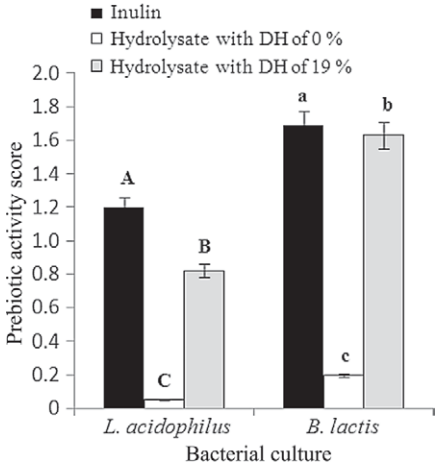

Fig. 2. Prebiotic activity scores of bacterial cultures grown in MRS supplemented with 1 % (by volume per mass) of the lychee hydrolysate with DH of 0 % (no enzyme treatment) or DH of 19 % compared to that with 1 % (by mass per volume) inulin as a prebiotic. Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values with different letters are significantly different ($p<0.05$)

Volatile compounds

In total, 41 volatile compounds were found in the lychee hydrolysates with the DH of 0 and 19 %, including an ester, an alcohol, aldehydes, ketones and other compounds (Table 4). These compounds are important for the fruit aroma, and the major compounds found were geraniol, ethanol, 1-octen-3-ol, β-citronellol and acetaldehyde. The pectinase-mediated hydrolysis of pectin used in this extraction is somewhat similar to the natural fruit

Table 3. Increase in the cell density from 0 to 24 h of growth of bacterial cultures in MRS supplemented with the indicated prebiotics and the untreated (DH of 0 %) or pectinase-treated (DH of 19 %) lychee hydrolysates at 1 % (by mass per volume)

Bacterial strain	Cell density			
	N/(log CFU/mL)			
	Glucose	Inulin	DH/%	
			0	19
L. acidophilus La5	(8.79±0.01)^{b}	(8.87±0.03)^{a}	(7.55±0.02)^{d}	(8.71±0.03)^{c}
B. lactis Bb12	(10.10±0.06)^{b}	(10.33±0.06)^{a}	(9.42±0.01)^{c}	(10.29±0.01)^{a}
E. coli ATCC 29922	(9.96±0.01)^{a}	(8.91±0.01)^{b}	(8.92±0.02)^{b}	(8.62±0.01)^{c}

Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values in the same row with a different lower case letter in superscript are significantly different ($p<0.05$); DH=degree of hydrolysis, MRS=de Man, Rogosa and Sharpe agar

ripening where an increase in the activity of the cell-wall-hydrolyzing enzymes, such as pectinases and pectinesterases, is noted (*32*) along with the volatile profiles of lychee (*33*). This is because they were released from the cell wall, leading to changes in the proportion of volatile compounds, and to the subsequent isomerization of some of the volatile compounds *via* oxidation (*34*,*35*).

The lychee hydrolysate with the DH of 19 % contained 11 compounds that were not found in the lychee hydrolysate with the DH of 0 %, as opposed to only four in the opposite case, which is in agreement with the fact that postharvest fruits have more volatile compounds than the preharvested fruits (*36*). The increased level of some volatiles observed in the lychee pulp with the DH of 19 % is likely due to the presence of both free and glycosidically bound aroma compounds, and when treated with the pectinase, the latter were released, whilst some of the former were unstable and easily oxidized, causing the formation of new aroma compounds and the loss of the old ones (*34*,*35*).

Particle size

The lychee hydrolysate with the DH of 19 % had an average particle size of (128±2.98) μm ($D_{[4,3]}$), which was 1.75-fold smaller than that of the lychee hydrolysate with the DH of 0 % ((225±2.98) μm). This may be due to the degradation of the polysaccharide components of the plant cell wall, such as pectin, cellulose and hemicelluloses, by enzyme treatment. In addition, the higher degradation level in lychee pulp with the DH of 19 % can cause the smallest particle size formation, which may effectively inhibit pulp droplet aggregation or coalescence, leading to the increase of cloud stability.

Lipid peroxidation

From the TBARS assay, the lipid peroxidation of lard, soya bean and olive oil was found in all cases to be significantly inhibited by BHT (positive control) at 95–96 % of the MDA control. The lychee hydrolysate with the DH of 19 % inhibited the lipid peroxidation of lard, soya bean and olive oil by 77, 76 and 72 % of the MDA control, respectively, better than the hydrolysate with the DH of 0 % (Fig. 3). The decreased lipid peroxidation was likely due to the total phenolic and flavonoid contents that neutralized (reduced) the free radical molecules (*36–40*). Accordingly, the lychee hydrolysate with the DH of 19 % had the highest levels of phenolics and flavonoids (Table 1).

Table 4. Volatile compounds in the untreated (DH of 0 %) and pectinase-treated (DH of 19 %) lychee hydrolysates

Peak no.	RI	Compound name	Peak area/%	
			DH/%	
			0	19
1	714	acetaldehyde	7.59	3.28
2	807	ethyl acetate	3.42	n.d.
3	857	athanol	20.98	18.88
4	877	hexaldehyde	n.d.	1.16
5	990	β-myrcene	2.66	2.66
6	1035	DL-limonene	2.17	1.77
7	1155	isoamyl alcohol	0.90	0.80
8	1240	3-buten-1-ol	0.73	0.73
9	1276	α-terpinolene	2.17	1.55
10	1286	octanal	n.d.	0.76
11	1299	1-octen-3-one	n.d.	1.60
12	1313	2-buten-1-ol	3.27	3.06
13	1358	*trans*-rose oxide	n.d.	0.39
14	1368	3-octanol	1.23	1.52
15	1430	2-octenol	n.d.	1.95
16	1455	1-octen-3-ol	8.37	10.75
17	1488	α-copaene	0.92	n.d.
18	1529	α-gurjunene	0.52	n.d.
19	1544	linaloon	2.26	2.88
20	1551	1-octanol	0.54	1.33
21	1558	β-cubebene	0.44	n.d.
22	1579	4-terpineol	0.47	0.52
23	1652	(E)-2-decen-1-ol	n.d.	0.94
24	1658	Z-citral	0.52	0.48
25	1669	α-terpineol	2.05	1.94
26	1149	α-muurolene	0.75	0.42
27	1686	E-citral	1.05	1.09
28	1715	carvone	n.d.	0.67
29	1737	β-citronellol	6.11	6.35
30	1752	7-methyl-3-methylene-6-octen-1-ol	0.65	0.81
31	1782	benzoic acid	n.d.	0.29
32	1825	(Z)-3,7-dimethyl-2,6-octadien-1-ol	2.30	2.42
33	1857	geraniol	23.65	22.78
34	1884	benzyl alcohol	0.78	0.68
35	1915	isopropyl myristate	0.34	0.44
36	1958	octanoic acid	n.d.	0.39
37	2017	acrylaldehyde	n.d.	0.42
38	2202	nonanoic acid	1.49	1.90
39	2215	eugenol	0.41	1.18
40	2243	2,4-di-tert-butylphenol	0.86	0.46
41	2517	dodecanoic acid	n.d.	0.49

RI=retention index, n.d.=not detected

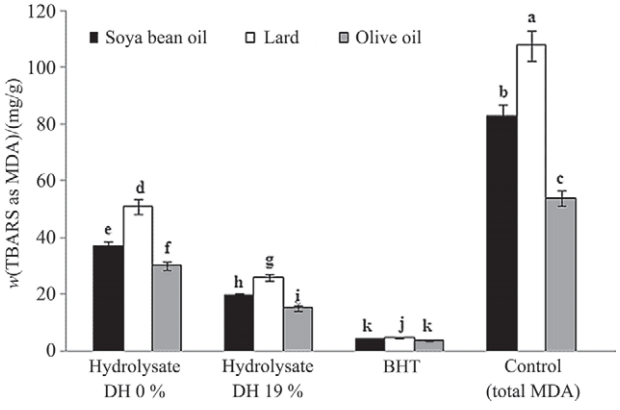

Fig. 3. Thiobarbituric acid reactive substances (TBARS) formation in oil upon treatment with the lychee hydrolysate with DH of 0 or 19 % or BHT (positive control) compared to that with MDA (no inhibition control). The lychee hydrolysate with DH of 0 % contained 104.1 mg of GAE and 23.8 mg of CE per 100 g of fresh mass, whilst the one with the DH of 19 % had 165.8 mg of GAE and 70.8 mg of CE. Data are shown as the mean values±S.D. and are derived from three independent repeats. Mean values with different letters are significantly different ($p<0.05$). DH=degree of hydrolysis, BHT=butylated hydroxytoluene, MDA=malondialdehyde, GAE=gallic acid equivalent, CE=catechin equivalent

The particle size, dietary fibre, prebiotic activity score, lipid peroxidation and volatile compounds of lychee hydrolysates were studied at the highest cleavage level of glycosidic bonds or at the degree hydrolysis of 19 %. Under these conditions, lychee hydrolysate should show notably different characteristics from the nonenzymatically treated sample (DH of 0 %).

Conclusion

The pectinase-assisted extraction of lychee fruit pulp significantly increased the level of antioxidant activity, total phenolics, total flavonoids, soluble dietary fibre (SDF), prebiotic activity score and lipid peroxidation inhibition. These were all increased in a DH-dependent manner, the highest being at the highest DH (19 %) of the hydrolysate. This is likely caused by the cleavage at the glycosidic bond in the cell wall as a consequence of hydrolysis. The lychee hydrolysate with the DH of 19 % showed the highest level of lipid peroxidation inhibition, prebiotic activity score and increased SDF with a concomitant decrease in the insoluble dietary fibre (IDF) level and decreased particle size. The concentration of some volatile compounds, such as 1-octen-3-ol, *trans*--rose oxide and eugenol, also increased following the pectinase digestion treatment. Pectinase digestion of lychee fruit pulp may be of benefit to the food industry, such as in the manufacture of energy drinks, fruit essences, functional food products, and as a preservative in food or food additive to enhance flavour. Moreover, it may be able to promote human health and give additional value to lychee fruit.

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References

1. B. Yang, J. Wang, M. Zhao, Y. Liu, W. Wang, Y. Jiang, Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities, *Carbohydr. Res. 341* (2006) 634–638.
2. X. Duan, Y. Jiang, X. Su, Z. Zhang, J. Shi, Antioxidant properties of anthocyanins extracted from litchi (*Litchi chinenesis* Sonn.) fruit pericarp tissues in relation to their role in the pericarp browning, *Food Chem. 101* (2007) 1365–1371.
3. S.C. Liu, J.T. Lin, C.K. Wang, H.Y. Chen, D.J. Yang, Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers, *Food Chem. 114* (2009) 577–581.
4. L. Wang, G. Lou, Z. Ma, X. Liu, Chemical constituents with antioxidant activities from litchi (*Litchi chinensis* Sonn.) seeds, *Food Chem. 126* (2011) 1081–1087.
5. K.N. Prasad, B. Yang, S. Yang, Y. Chen, M. Zhao, M. Ashraf, Y. Jiang, Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (Litchi sinensis Sonn.) seeds, *Food Chem. 116* (2009) 1–7.
6. G. Zheng, Z. Yi, J. Zhang, D. Zhong, Studies on the antioxidative effect of extract from mature and premature litchi pericarp, *Nat. Prod. Res. Develop. 15* (2003) 341–344.
7. E. Tripoli, M. Guardia, S. Giammanco, D.D. Majo, M. Giammanco, Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review, *Food Chem. 104* (2007) 466–479.
8. J.B. Harborne, C.A. Willliams, Advances in flavonoid research since 1992, *Phytochemistry*, *55* (2000) 481–504.
9. L.W. Morton, R.A.A. Caccetta, I.B. Puddey, K.D. Croft, Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease, *Clin. Exp. Pharmacol. Physiol. 27* (2000) 152–159.
10. N. Rangkadilok, S. Sitthimonchai, L. Worasuttayangkurn, C. Mahidol, M. Ruchirawat, J. Satayavivad, Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract, *Food Chem. Toxicol. 45* (2007) 328–336.
11. K.N. Prasad, B. Yang, J. Shi, C. Yu, M. Zhao, S. Xue, Y. Jiang, Enhanced antioxidant and antityrosinase activities of longan fruit pericarp by ultra-high-pressure-assisted extraction, *J. Pharm. Biomed. Anal. 51* (2010) 471–477.
12. K. Zhong, Q. Wang, Y. He, X. He, Evaluation of radicals scavenging, immunity-modulatory and antitumor activities of longan polysaccharides with ultrasonic extraction on in S180 tumor mice models, *Int. J. Biol. Macromol. 47* (2010) 356–360.
13. V.P. Reddy, A. Urooj, Antioxidant properties and stability of *Aegle marmelos* leaves extracts, *J. Food Sci. Technol. 50* (2013) 135–140.
14. J. Moore, Z. Cheng, L. Su, L. Yu, Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran, *J. Agric. Food Chem. 54* (2006) 9032–9045.
15. S. Chen, X.H. Xing, J.J. Huang, M.S. Xu, Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: Improvement effect of flavonol transglycosylation catalyzed by *Penicillium decumbens* cellulose, *Enzyme Microb. Technol. 48* (2011) 100–105.

16. N. Nelson, A photometric adaptation of the Somogyi method for determination of glucose, *J. Biol. Chem. 153* (1944) 375–380.
17. M. Somogyi, Notes on sugar determination, *J. Biol. Chem. 195* (1952) 19–23.
18. Y.S. Velioglu, G. Massa, L. Gao, B.D. Oomah, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, *J. Agric. Food Chem. 46* (1998) 4113–4117.
19. P. Maisuthisakul, M. Suttajit, R. Pongsawatmanit, Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants, *Food Chem. 100* (2007) 1409–1418.
20. S. Sangthawan, P. Anprung, Prebiotic activity and bioactive compounds of the enzymatically depolymerized Thailand-grown mangosteen aril, *J. Food Res. 1* (2012) 268–276.
21. A.L. Waterhouse: Determination of Total Phenolics. In: *Handbook of Food Analytical Chemistry; Pigments, Colorants, Flavors, Texture, and Bioactive Food Components*, R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz *et al.* (Eds.), John Wiley and Sons, New Jersey, NY, USA (2005) pp. 463–481.
22. J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem. 64* (1999) 555–559.
23. Official Methods of Analysis of the AOAC International, Association of Official Analytical Chemists (AOAC), Washington, DC, USA (1995).
24. J.L. Chen, S. Yan, Z. Feng, L. Xiao, X.S. Hu, Changes in the volatile compounds and chemical and physical properties of yali pear (*Pyrus bertschneideri* Reld) during storage, *Food Chem. 97* (2006) 248–225.
25. Wiley Registry™ of Mass Spectral Data 7th edition with NIST 2005 Spectral Data, F.W. McLafferty (Ed.), Wiley/NIST, John Wiley & Sons, Inc., Hoboken, NJ, USA (2005).
26. S. Worrasinchai, M. Suphantharika, S. Pinjai, P. Jamnong, β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise, *Food Hydrocolloids, 20* (2006) 68–78.
27. J.P. De La Cruz, L. Quintero, M.A. Villalobos, F. Sánchez de la Cuesta, Lipid peroxidation and glutathione system in hyperlipemic rabbits: Influence of olive oil administration, *Biochim. Biophys. Acta, 1485* (2000) 36–44.
28. K. Karunasawat, P. Anprung, Effect of depolymerized mango pulp as a stabilizer in, oil-in-water emulsion containing sodium caseinate, *Food Bioprod. Process. 88* (2010) 202–208.
29. S. Thaiphanit, P. Anprung, Physicochemical and flavor changes of fragrant banana (*Musa acuminata* AAA group 'Gross michel') during ripening, *J. Food Process. Preserv. 34* (2008) 1745–4549.
30. W. Grajek, A. Olejnik, A. Sip, Probiotics, prebiotics and antioxidants as functional foods, *Acta Biochim Pol. 52* (2005) 665–671.
31. B. Gullón, B. Gómez, M.M. Sabajanes, R. Yánez, J.C. Parajó, J.L. Alonso, Pectic oligosaccharides: Manufacture and functional properties, *Trends Food Sci. Technol. 30* (2013) 153–161.
32. A. Chourasia, V.A. Sane, P. Nath, Differential expression of pectate lyase during ethylene-induced postharvest softening of mango (*Mangifera indicavar* Dashehari), *Physiol. Plantarum, 128* (2006) 546–555.
33. H.G. Chidley, R.S. Kulkarni, K.H. Pujari, A.P. Giri, V.S. Gupta, Spatial and temporal changes in the volatile profile of Alphonso mango upon exogenous ethylene treatment, *Food Chem. 136* (2013) 585–594.
34. C.C. Chyau, P.T. Ko, C.H. Chang, J.L. Mau, Free and glycosidically bound aroma compounds in lychee (*Litchi chinensis* Sonn.), *Food Chem. 80* (2003) 387–392.
35. R.F.A. Moreira, C.A.B. De Maria, M. Pietroluongo, L.C. Trugo, Chemical changes in the volatile fractions of Brazilian honeys during storage under tropical conditions, *Food Chem. 121* (2010) 697–704.
36. J. Song, F. Bangerth, The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production, *Postharvest Biol. Technol. 8* (1996) 259–269.
37. B. Halliwell, J.M. Gutteridge, C.E. Cross, Free radicals, antioxidants and human disease: Where are we now?, *J. Lab. Clin. Med. 119* (1992) 598–620.
38. S. Khennouf, S. Amira, L Arrar, A. Baghiani, Effect of some phenolic compounds and *Quercus* tannins on lipid peroxidation, *World Appl. Sci. J. 8* (2010) 1144–1149.
39. N. Thitilertdecha, A. Teerawutgulrag, J.D. Kilburn, N. Rakariyatham, Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities, *Molecules, 15* (2010) 1453–1465.
40. L.Y. Wu, H. Xiao, W.J. Zhao, H. Shang, M.Z. Zhang, Y.D. Lin *et al.*, Effect of instant tea powder with high ester-catechins content on shelf life extension of sponge cake, *J. Agr. Sci. Tech. 15* (2013) 537–544.