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Original article

# Soluble peptidoglycan production from the waste peels of pineapple *Ananas comosus* (L.) Merr.

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## Summary

*Introduction* – **The growing area of pineapple in Taiwan has reached 9797 hectares that annually produce 410,000 tons of pineapples and roughly contributed an amount of waste peels 119,583.5 tons. Whether the reclaim of the soluble polysaccharide fractions (SPF) from these waste peels are worth for green technology, we performed this study.** *Materials and methods* – **Cultivars TN-3 and TN-17 were used for analysis.** *Results and discussion* – **SPF differs in composition depending on the pineapple cultivars regarding the ash, carbohydrate, crude fiber, sugar, carotenoids and bromelain. At average, SPF of TN-3 and TN-17 consisted of carotenoids 4652 kg/Y and bromelian $2.117\times10^{11}$ units/Y. SPF was peptidoglycan in nature having average molecular weight below 500 kDa. The monosaccharide composition of SPF covered a spectrum of rhamnose, fucose, arabinose, xylose, ribose, fructose, mannose, galactose, glucose, and myo-inositol. The amino acid composition consists of mostly essential amino acids. SPF exhibited strong DPPH-, hydroxyl radical-, and superoxide anion radical-scavenging capability, but entirely ineffective as ferrous ion chelating agent. In cell mode, TN-3-2 behaved stronger cytotoxicity than TN-17-2. Based on the average amount of pineapple peels (119,583.5 tons), the reclaimable soluble peptidoglycans reached 6433.59 tons amounting up to 5.38% of the total waste peels.** *Conclusion* – **Using the improved novel green technology, it is possible to recover much of the valuable constituents present in the waste pineapple peels, in particular, the peptidoglycans that exhibit a diversity of biomedical significance.**

**Keywords**
antioxidative capability, cytotoxicity, green technology, polysaccharide fractions

## Significance of this study

*What is already known on this subject?*

- The growing area of pineapple in Taiwan has reached 9797 hectares that annually produce 410,000 tons of pineapples and roughly contributed an amount of waste peels 119,583.5 tons.

*What are the new findings?*

- The monosaccharide composition of the soluble polysaccharide fractions (SPF) from the pineapple peel waste covered a spectrum of rhamnose, fucose, arabinose, xylose, ribose, fructose, mannose, galactose, glucose, and myo-inositol. The amino acid composition consists of mostly essential amino acids. SPF exhibited strong DPPH-, hydroxyl radical-, and superoxide anion radical-scavenging capability.

*What is the expected impact on horticulture?*

- A variety of valuable components are present in the waste pineapple peels, hence green technology is suggested to reclaim the soluble polysaccharide fractions from the pineapple peel waste.

## Introduction

Taiwan is famous for its economic pineapple cultivation. The subtropical climate of Taiwan, mostly the middle southern area, is rather feasible for growing pineapples (*Ananas comosus* L. Merr.). The pineapples in Taiwan can be classified into three categories: the native 'Queen species', the Cayenne species, and the hybrid (Hume and Miller, 1904). The Cayennes are species transplanted from the overseas outside Taiwan. In 2013, the growing area for pineapples in Taiwan reached 9797 hectares from which 410,000 tons of pineapples had been produced, yielding a market value of 2.6 million US$ (Agriculture & Food Agency Council of Agriculture, Executive Yuan [Taiwan], 2015). The original cultivated varieties are TN-1, TN-2, and TN-3, which has high Brix (16.0 °Brix) and moderate acidity 0.35% (Weekender, 2011), usually getting matured in May to August. Recently, the newly developed species like TN-11, TN-13, TN-16, and TN-17, moderately acidic and sweet with thick and juicy pulp, have gained much reputation and become favorite tropical fruits

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(Southern Region Branch, Agriculture & Food Agency, Council of Agriculture, *n.d.*). According to a survey made in 1953, the most widely distributed type is 'Collar of Slips', exhibiting a proportion of 95.15 %, 'Normal Smooth Cayenne', 4.51 %; 'Elevated eyes', 0.3 %; and 'Sarawak', 0.04 % (Wang and Chang, 1957). For decades, the majority of economic environments have been greatly changed; as a consequence, the export of pineapples has been severely reduced and most of the pineapples are sold in domestic market and eaten fresh, with a minority as ornamental (Southern Region Branch, Agriculture & Food Agency, Council of Agriculture, *n.d.*). Among the produced pineapples, about 70% is eaten fresh and 30% is squeezed for juice production. Approximately, the total waste pineapple peel may contribute 1/4 to 1/3 of the total production weight (Guerout, 1975; Huang *et al.*, 2011), *i.e.*, roughly 102,500 to 136,667 tons (or an average of 119,583.5 tons). The pineapple peel contains an appreciable amount of insoluble fiber-rich fraction (FRF) (41.8–48.0 g 100 $g^{-1}$) including insoluble dietary fiber (IDF), alcohol-insoluble solids (AIS), and water-insoluble solids (WIS), which primarily consists of cellulose, pectic substances and hemicellulose. These fractions also contain notable proportions of lignin (60.7–65.8 g 100 $g^{-1}$) (Huang *et al.*, 2011). Pineapple peel fiber (PPF) was saponified and subjected to cross-linking with succinic anhydride to produce modified pineapple peel fibers. Such kind of fibers exhibit strong adsorption capability for metallic ions like $Cu^{2+}$, $Cd^{2+}$, and $Pb^{2+}$, being favoring the green technology (Hu *et al.*, 2010). Compared with cellulose, PPF exhibits greater water- and oil-holding capacities (7.94–12.3 mL $g^{-1}$ and 5.84–8.64 g $g^{-1}$, respectively), swelling properties (10.6–18.4 mL $g^{-1}$), and cation-exchange capacities (102–120 mEq $kg^{-1}$) (Huang *et al.*, 2011). Pineapple residue was also used as co-substrate to produce vinegar by fermentation (Lin *et al.*, 2009) (patent CN 101531965 A, 2009). Peels of pineapple is often discarded without reclaim. It is supposed to exhibit many nutritional and health benefits, such as strengthening the immune system and reducing inflammation. In addition, pineapple peels are also nutritious, containing vitamin C and bromelain, an enzyme which acts as a natural anti-inflammatory. The insoluble fiber-rich fraction of pineapple peels has been linked to healthy digestion and use as functional food ingredients for reduction of calories or dietary fiber enrichment and reduced risk of type 2 diabetes (Huang *et al.*, 2011). In addition, the waste contains a huge amount of soluble polysaccharides (Cheng, 2008). Literature emphasizing the biomedical activity of peptidoglycans are emerging. Considering there could be present much of peptidoglycans in pineapple peels, we performed this study. TN-3 and TN-17 were used as samples and their proximate composition was examined first, with subsequent fractionation to obtain different molecular size peptidoglycans. Their chemical composition and biological activity were examined to evaluate the order of quality, which included the monosaccharide and amino acid compositions, the average molecular weight, the antioxidative capability, the FTIR spectrum as well as the MTT assay to examine its cytotoxicity.

## Materials and methods

### Source of waste pineapple peels

Fresh waste pineapple peels of cultivar TN-3 and TN-17, each weighing 50 kg, were provided by the Pineapple Mount of Three-Beauties-in-One Co. (Taichung, Taiwan). The fresh pineapple peels were transported while frozen.

### Sample pretreatment

The fresh pineapple peels (2 kg) were immediately cut into pieces with a knife-equipped blender (Taichung Super Machine No. 202). The chopped-up pieces were then transferred to the homogenizer (Japan Seiki, Osaka) and homogenized for 20 min with the aid of ultrasonication. The sample mass was frozen fresh for use.

### Proximate compositional analysis

The proximate compositional analysis was carried out according to methods of CNS and AOAC: water content (CNS5033), ash (CNS5034), crude fat (CNS5036), crude protein (CNS5035), saccharides (CNS12634), and crude fiber (A.O.A.C.955.29).

The carbohydrate content was calculated as:

$$\%\ \text{carbohydrate} = 100 - (\%_{\text{water}} + \%_{\text{ash}} + \%_{\text{crude fat}} + \%_{\text{crude protein}}).$$

Because the yields and absolute amount reclaimable in other samples were too low, only samples TN-3 and TN-7 were used in this experiment.

### Determination of carotenoids

The pineapple peels were homogenized in a blender and 25 mg of the homogenate was transferred into the flask. BHA acetone solution (30 mL) was added. The mixture was ultrasonicated for 30 min and left to stand at ambient temperature for 1 h with constant stirring. The mixture was centrifuged at 3000 rpm for 30 min; the supernatant was collected. The extraction was repeated 3 times. The supernatants were combined and made to 100 mL with fresh BHA–acetone solution, mixed well, and filtered through the 0.45 μM micropore. The OD of the filtrate was read at 450 nm against the blank solvent.

### Determination of bromelain activity

The activity of bromelain was determined according to Soares *et al.* (2012) with slight modification. Briefly, to sample solution (0.1 mL) and 0.1 mL of 0.1 M PBS (pH 7.5, containing 2% casein) (as the control) 1.25 mL of 0.1 M PBS (pH 7.5, containing 2% casein) was added respectively. The mixture was incubated at 37 °C for 10 min in a water bath. TCA 2.5 mL was added and left to stand in ambient temperature for 10 min. The reaction mixture was centrifuged at 3000 rpm for 30 min. The supernatant was separated and the OD was read at 280 nm.

### Fractionation of the polysaccharide fractions from the pineapple peels

The method of Ker *et al.* (2010) was followed with a slight modification. In brief, to fresh pineapple peels (1 kg) 1 L distilled water was added, homogenized, and centrifuged at 12,000 rpm for 10 min. The supernatant was separated and lyophilized (sample lyophilized soluble pineapple peel fraction, LPP). To LPP 2 L distilled water was added and the extraction was carried out at 90 °C for 2 h, centrifuged at 12,000 rpm for 30 min, and the supernatant was collected. The extract was repeated for 3 times. The supernatants were combined and lyophilized (sample residue 1, R1). To R1 residue, 2 L 2% NaOH was added and heated at 80 °C for 2 h, centrifuged at 12,000 rpm for 30 min, and the supernatant was collected. The extract was repeated for 3 times. The supernatants were combined (supernatant 1, S1) and the residue was kept for use (residue 2, R2). To the residue R2, 2 L 10% KOH was added and the solution was heated at 80 °C for

2 h, centrifuged at 12,000 rpm for 30 min. The supernatant was collected. The extract was repeated for 3 times and the supernatants were combined (supernatant 2, S2). S1 and S2 were adjusted to the isoelectric point pH 4.0 with con. sulfuric acid, the solutions were left to stand for 3 h to facilitate the precipitation. The solutions were centrifuged at 12,000 rpm for 30 min. The supernatant was separated and lyophilized (sample S3 and S4, respectively). The residues (R3 and R4, respectively) were dialyzed for 48 h and lyophilized (Ker *et al*., 2010).

**Alcohol precipitation**

To the lyophilized residues R1, R2, and R3 500 mL of distilled water was added to redissolve the residue. Ethanol (3-fold volume) was added and the solution was left to stand at ambient temperature to facilitate the precipitation. The solution was centrifuged at 12,000 rpm for 30 min, and the supernatant was discarded. The precipitate was subjected to dialysis for 48 h and lyophilized to obtain respectively powder samples R1A, R2A, and R3A. These samples were weighed and the percent yield was calculated. The polysaccharide fractions obtained from both TN-3 and TN-17 pineapples were designated respectively as fraction 1: hot water extract with 3-fold volume ethanol precipitate; fraction 2: 2% NaOH extract with pI precipitate; fraction 3: NaOH extract with 3-fold volume ethanol precipitate; fraction 4: 10% KOH extract with pI precipitate; and fraction 5: 10% KOH extract with 3-fold volume ethanol precipitate (Ker *et al*., 2010).

**Determination of the carbohydrate and peptide content in each polysaccharide fraction**

**1. Analysis for the carbohydrate content.** The analysis was carried out according to Dubois *et al*. (1956). In brief, 5 mg of the polysaccharide fraction was transferred into sample vial, 1 N NaOH 4 mL was added and subjected to ultrasonication for 30 min. The mixture was heated at 100 °C for 2 h and made to 5 mL with 1 N NaOH. An aliquot 0.5 mL was accurately measured and 0.5 mL phenol was added, agitated to mix well. 2.5 mL sulfuric acid was added to facilitate the development of orange yellow color and the optical density (OD) was read at 490 nm. The determination was repeated in triplicate.

**2. Analysis for the peptide content.** Method of Bradford (1976) was followed to carry out the determination. Briefly, sample polysaccharide faction 5 mg was accurately measured and transferred into sample vial. NaOH (1 N) 4 mL was added and subjected to ultrasonication for 30 min. The mixture was heated at 150 °C for 2 h and 1 N NaOH was added to make volume of 5 mL. An aliquot of 0.1 mL was measured to which 2.5 mL Dye Reagent Concentrate was added to facilitate the development of bluish-brown coloration. The solution was mixed well and the OD was read at 595 nm. The determination was repeated in triplicate.

**3. Gel permeation chromatography of polysaccharides**. The method of Wang *et al*. (2012) was followed with slight modification. Briefly, 10 mg of polysaccharide fraction was accurately measured and transferred into the 20-mL sample vial. 1 mL NaOH (1 N) was added, capped well, and subjected to ultrasonication for 30 min until completely dissolved. 2 mL of distilled water was added and agitated to mix well. An aliquot of 0.5 mL was measured and injected into the gel column ($\ell$ × id = 76 × 1.6 cm, packed with Superdex 200). The flow rate of mobile phase was controlled at 0.3 mL min$^{-1}$. The eluent was collected with the fraction collector, 2 mL in each. A total of 60 tubes were collected. Each tube was treated with the phenol-sulfuric acid coloration method and the OD was read at 490 nm. Simultaneously, the OD at 280 was also measured.

**4. Analysis of the monosaccharide composition.** The method was carried out by following Blakeney *et al*. (1983) with a slight modification. Briefly, 5.0 mg of polysaccharide fraction sample was accurately measured and transferred into a 3-mL reaction vessel. TFA (2 M) 3 mL was introduced. The mixture was heated on the derivation heater at 120 °C for 24 h until completely hydrolyzed. During heating the reaction vessel was removed from the heater and agitated to mixed well. The reaction solution was lyophilized. To the residue, 100 μL pyridine was added with agitation until completely redissolved. The entire solution was transferred into 2 mL derivation vial, 100 μL BSTFA was added, agitated well and heated at 70 °C on the derivation plate for 50 min, with frequent agitation every 10 min. After the derivation reaction was completed, the mixture was left to stand at the ambient temperature until cooled down. The content was transferred to sample vial and blown to almost dry under the nitrogen flow. The sample was subjected to GC analysis. The analytical conditions were: column, Restek Rtx 225 ($\ell$ × id = 30 m × 0.32 mm); temperature program: 60 °C for 1 min, 8°C min$^{-1}$ up to 220 °C, kept for 20 min. The temperature at Inj/Det = 230°C/230 °C. The carry gas use was $N_2$ and its flow rate was set at 1.0 mL min$^{-1}$.

**5. Analysis of the amino acid composition.** The method of Namera *et al*. (2002) was followed with slight modification. In brief, the polysaccharide fraction 5 mg was accurately measured and transferred in to a 3-mL reaction vessel. HCl (6 M, 3 mL) was added. The mixture was heated at 110 °C on the derivation heated for 24 h and lyophilized to obtain amino acid powder. HCl (0.01 M) 0.3 mL was added until completely dissolved. 0.01 mL of the internal standard norleucine (10 mg mL$^{-1}$) was added with agitation. 0.05 mL ethyl chloroformate was added and agitated to mix well. 0.5 mL alcohol-pyridine and 1 mL chloroform were added, capped, and the solution was agitated for 1 min to facilitate the derivation and extraction. The reaction system was left to stand at ambient temperature for 5 min to facilitate phase separation. Water (0.7 mL) was added, agitated for 30 min, and left to stand for 5 min to facilitate the phase separation. The upper layer was discarded. Anhydrous sodium sulfate was added to the bottom layer (the chloroform layer) for dehydration. The dehydrated chloroform layer was transferred into the sample vial and blown to almost dry under the nitrogen stream. The product was subjected to GC analysis. The analytical conditions were: column, HP-5MS ($\ell$ × id = 30 m × 0.2 mm); film thickness, 0.25 μm; temperature program: 50 °C for 1 min, 10 °C min$^{-1}$, 300 °C for 6.5 min. The Inj/Det temperature: 300 °C/305 °C; the carry gas used was $N_2$ and its flow rate was set at 0.8 mL min$^{-1}$.

**Determination of antioxidative capability**

**1. Preparation of stock solution.** 100 mg of the fractionated polysaccharide was dissolved in 10 mL 0.01 M PBS (pH 7.4) to make the stock solution and serially diluted to prepare sample solutions with concentration 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 mg mL$^{-1}$, respectively.

**2. Determination of 2,2-diphenyl-1-picryl-hydrazyl radical scavenging capability.** According to Thaipong *et al*. (2006). The stock solution was serially diluted to the required experimental concentration as indicated. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) test was carried out. The solution was left to react at ambient temperature for 30 min

**Table 1.** The proximate composition, carotenoid and bromelain contents obtained from the pineapple peels TN-3 and TN-17 cultivars.*

| Sample | Water content | Ash | CHO* | Crude fat | Crude protein | Crude fiber | Sugar | CRTN* | Bromelain |
|---|---|---|---|---|---|---|---|---|---|
| | | | | (%) | | | | (mg 100 $g^{-1}$) | (U $mg^{-1}$) |
| TN-3 | 85.1±0.1 | 0.7±0.1 | 5.2±0.1 | 0.3±0.1 | 5.6±0.1 | 3.3±0.1 | 4.6±0.1 | 4.3±0.1 | 2.0±0.2 |
| TN-17 | 84.4±0.1 | 0.7±0.1 | 6.5±0.1 | 0.3±0.1 | 5.4±0.1 | 2.7±0.1 | 5.1±0.1 | 3.5±0.1 | 1.6±0.1 |

*CHO: Carbohydrate; CRTN: Carotenoids. Values are expressed in mean ± S.D. ($n$ = 3).

avoiding direct sunlight. The OD was read at 517 nm. A blank was similarly conducted as the control. The experiment was repeated three times and the data obtained were statistically treated.

**3. Determination of ferrous ion chelating capability.** The method described by Saito and Ishihara (1997) was followed. Briefly, the stock solution was serially diluted to prepare sample solutions suitable for experiment. The ferrous ion – Ferrozine chelation test was carried out. The solution was mixed well and left to stand at ambient temperature to facilitate reaction avoiding direct sunlight. The OD was taken at 562 nm. A blank was similarly conducted. The experiment was repeated three times and the data obtained were statistically treated.

**4. Determination of the formation of hydroxyl free radicals.** The method of Ghiselli *et al*. (1998) was followed to determine the level of hydroxyl free radicals. The reaction solution was left to stand at ambient temperature for 10 min and the OD was read at 532 nm. A blank was similarly carried out. The experiment was repeated three times and the data obtained were statistically treated.

**5. Determination of the scavenging capability for superoxide anion radicals.** According to Robak *et al*. (1988), the stock solution was serially diluted to prepare sample solutions with required concentrations. The phenazine methosulfate (PMS) method was performed. The OD was read at 560 nm. A blank was similarly treated. The experiment was repeated three times and the data obtained were statistically treated.

**6. FTIR spectral analysis.** The polysaccharide fractions that exhibited better antioxidative capability were selected and to 2 mg of which KBr (IR grade) 200 mg was added and rapidly mixed to avoid moisture adsorption. The powder was compressed for 2–3 min at 300–500 kgf $cm^{-2}$ to prepare KBr tables. The sample tablet was subjected to FTIR (Jusco, FT/IR-460) scanning.

**7. Cell viability assay-MTT assay.** The method of Mosmann *et al*. (1983) was followed to perform MTT assay. In brief, RAW264.7 cells were seeded at $1 \times 10^5$ cells $mL^{-1}$ onto 24 well-plate. On the other hand, caco-2 cells were seeded at $1 \times 10^4$ cells $mL^{-1}$. The culture was incubated at 37 °C for 24 h. The cells were rinsed twice with PBS. The stock solution was added to wells at a dose of 1, 0.5, 0.1, 0.05, and 0.01 mg $mL^{-1}$ and left to stand for 24 h. The cells were rinsed twice with PBS and 300 μL of MTT dilute solution was added. The plate was enwrapped and covered with aluminum foil to avoid direct sunlight and incubated for 1 h under $CO_2$ atmosphere. The MTT solution was sucked off. 200 μL of DMSO was added with gentle shaking to dissolve the precipitated formazan. The sample control (cells without treatment with drug) and solvent control (dimethylsulfoxide, DMSO only) were similarly performed in parallel. The solubilized formazan in DMSO was pipetted into a 96-well plate and the OD was read at 570 nm against the DMSO blank. The cell viability was calculated as

$$\% \text{ cell viability} = (OD_{570\text{sample}} - OD_{570\text{DMSO}})/(OD_{570\text{ control}} - OD_{570\text{DMSO}}) \times 100$$

**Statistical analysis**

Data obtained in the same group were analyzed by Student's $t$-test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL, USA). ANOVA software statistical system was used with Tukey's testing to analyze the variances and significances of difference between paired means. Significance of difference was judged by a confidence level of $p < 0.05$.

## Results and discussion

**The proximate composition and contents of carotenoids and bromelain**

The proximate compositional analysis showed that TN-3 cultivar exhibited contents of ash, 0.71 ± 0.01%; carbohydrate, 5.17 ± 0.04%; crude fat, 0.24 ± 0.03%; crude protein, 5.55 ± 0.07%; crude fiber, 3.25 ± 0.02%; sugar, 4.63 ± 0.10%; carotenoids, 4.32 ± 0.05 mg 100 $g^{-1}$; and bromelain (1.96 ± 0.15 U $mg^{-1}$) ($p < 0.05$). As contrast, TN-17 showed higher content of carbohydrate (6.51 ± 0.05%), and sugar (5.09 ± 0.14%), with less amount of carotenoids (3.46 ± 0.16 mg 100 $g^{-1}$) and bromelain (1.58 ± 0.10 U $mg^{-1}$) ($p < 0.05$) (Table 1). Based on the average amount of pineapple peels (119,583.5 tons), the reclaimable carotenoids are estimated to be 5166 kg, and that of bromelain, $2.344 \times 10^{11}$ IU.

Bromelain has long been used as beef and meat tenderninzing agent (Dubey *et al*., 2012)). Recently, Pavan *et al*. (2012) have released a review on bromelain, indicating its fibrinolytic, anti-edematous, antithrombotic, and anti-inflammatory activities.

While carotenoids, a highly unsaturated zwitterionic surfactant, are very promising antioxidant, anti-inflammatory (Ciccone *et al*., 2013), anticancer agents (Linnewiel-Hermoni *et al*., 2015). Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases, such as cancer (Linnewiel-Hermoni *et al*., 2015). The combinations of several carotenoids (*e.g.*, lycopene, phytoene, and phytofluene), or carotenoids and polyphenols synergistically inhibit the androgen receptor activity and activate the EpER/ARE system (Linnewiel-Hermoni *et al*., 2015).

**Yield of soluble polysaccharide fraction from the peels**

The 3-fold ethanol precipitate from 2% NaOH extracts showed the highest yield of polysaccharides, reaching 1.19% and 0.65% respectively for TN-3 and TN-17 cultivars (Table 2). Interestingly, the next are 0.57% from TN-3-1 (the 3-fold ethanol precipitate from hot water extract), and 0.56% from TN-17-2 (the isoelectric precipitate from 2% NaOH extract), although fractions TN-17-2 and TN-17-3 (the 3-fold ethanol precipitate from 2% NaOH extract) were rather com-

**Table 2.** Yield of polysaccharide obtained from the pineapple peels of TN-3 and TN-17 cultivars.*

| Polysaccharide fractions | Weight (g) | Yields (%) | Reclaimable amount (ton) |
|---|---|---|---|
| TN-3-1 | 5.7724 | 0.57 | 681.63 |
| TN-3-2 | 3.1254 | 0.31 | 370.71 |
| TN-3-3 | 12.0358 | 1.19 | 2272.09 |
| TN-3-4 | 3.4546 | 0.34 | 406.58 |
| TN-3-5 | 0.4235 | 0.04 | 47.83 |
| TN-17-1 | 5.1079 | 0.51 | 609.88 |
| TN-17-2 | 5.5680 | 0.56 | 669.67 |
| TN-17-3 | 6.5258 | 0.65 | 777.29 |
| TN-17-4 | 4.5873 | 0.46 | 550.08 |
| TN-17-5 | 0.3970 | 0.04 | 47.83 |
| Total sum | | | 6433.59[z] |

*TN-3-1 and TN-17-1, the 3-fold ethanol precipitate from hot water extracts.TN-3-2 and TN-17-2, the isoelectric precipitate from 2% NaOH extracts. TN-3-3 and TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts. TN-3-4 and TN-17-4, the isoelectric precipitate from 10% KOH extracts, and TN-3-5 and TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

[z] As the average waste peels amount to 119.583.5 tons per year, the reclaimable yield may reach 5.38%.

parable (Table 2). Taking the average amount of peels waste to be 119,583.5 tons, the total reclaimable amount roughly could reach 6433.59 tons, lumping up to 5.38% (Table 2).

### Crude protein and carbohydrate content presents in the soluble polysaccharide fractions

In TN-3, the crude protein content ranged from 13.50 ± 0.16% (for TN-3-4) (the isoelectric precipitate from 10% KOH extract) to 57.34 ± 0.16% (for TN-3-2) (the isoelectric precipitate from 2% NaOH extract), as contrast, ranging from 32.12 ± 0.16% (for TN-17-3) to 56.32 ± 0.01% (TN-17-2) for TN-17 cultivar (Table 3, Figure 1). The carbohydrate content in TN-3 ranged from 41.62 ± 0.21% (TN-3-2) to 57.71 ± 0.04% (TN-3-4), and from 21.52 ± 0.08% (for TN-17-3) to 44.47 ± 0.15% (for TN-17-5) (Table 3, Figure 2), implicating the nature of peptidoglycans with great variation of polysaccharide characteristics in the two cultivars. There is a high diversity in the composition and sequence of the peptides in the peptidoglycan from different species. Furthermore, in several species examined, the fine structure of the peptidoglycan significantly varies with the growth conditions (Vollmer *et al.*, 2008). More importantly, data have revealed the glycan nature of these polysaccharide fractions, implicating strongly its beneficial biological and immunological effects (Verbrugh *et al.*, 1983).

**Table 3.** Crude protein and carbohydrate content of polysaccharide fractions obtained from pineapple peels of TN-3 and TN-17 cultivars.*

| Polysaccharide fractions | Crude protein (%) | Carbohydrate (%) |
|---|---|---|
| TN-3-1 | 46.23±0.25$^{c}$ | 44.64±0.14$^{c}$ |
| TN-3-2 | 57.34±0.16$^{a}$ | 41.62±0.21$^{d}$ |
| TN-3-3 | 54.23 ±0.05$^{b}$ | 45.19±0.03$^{c}$ |
| TN-3-4 | 13.50±0.16$^{h}$ | 57.71±0.04$^{a}$ |
| TN-3-5 | 44.64 ±0.11$^{d}$ | 52.63±0.09$^{b}$ |
| TN-17-1 | 40.75±0.03$^{e}$ | 32.69±0.14$^{e}$ |
| TN-17-2 | 56.32±0.01$^{a}$ | 43.66±0.16$^{c}$ |
| TN-17-3 | 32.12±0.17$^{g}$ | 21.52±0.08$^{f}$ |
| TN-17-4 | 43.38±0.06$^{d}$ | 39.48±0.23$^{d}$ |
| TN-17-5 | 39.23±0.02$^{f}$ | 44.47±0.15$^{c}$ |

* TN-3-1 and TN-17-1, the 3-fold ethanol precipitate from hot water extracts.TN-3-2 and TN-17-2, the isoelectric precipitate from 2% NaOH extracts. TN-3-3 and TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts. TN-3-4 and TN-17-4, the isoelectric precipitate from 10% KOH extracts, and TN-3-5 and TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

Values are expressed as mean ± SD from triplicate determinations. Different superscripts in lower case in the same column indicate significantly different.

### Mean molecular weight of the soluble polysaccharides

The polysaccharide fraction obtained from TN-3 cultivar exhibited average molecular weight ranging from 37.91 to 492.80 kDa. The highest was 492.80 kDa for TN-3-4 while the lowest was 37.91 kDa (for TN-3-1), contrasting to 93.69 kDa (for TN-17-4) (the isoelectric precipitate from 10% KOH extract) to 1222.40 kDa (for TN-17-1) (the 3-fold ethanol precipitate from hot water extract) (Table 4, Figures 1 and 2), again implicating the great variation of polysaccharide composition from cultivar to cultivar, and physicochemically, these polysaccharides are soluble in nature due to their low molecular weight. Soluble glycans play the role of excellent immunity-enhancer (Verbrugh *et al.*, 1983).

### The monosaccharide composition of the soluble polysaccharides

The two cultivars all showed similar monosaccharide constituent consisting of rhamnose, fucose, arabinose, xylose, ribose, fructose, mannose, galactose glucose, and myo-inositol. For rhamnose, TN-3-5 showed the highest content (4.05 mol%) contrasting to 3.64 mol% of TN-17-1 (Table S1). And the other fractions that showed the highest content of monosaccharide were: fucose 6.18 mol% (TN-3-1) and 6.06 mol% (TN-17-3); arabinose 4.89 mol% (TN-3-1) and 3.53 mol% (TN-17-5); xylose 5.13 mol% (TN-3-1) and 4.04 mol% (TN-17-5); ribose 8.03 mol% (TN-3-3) and 10.41 mol% (TN-17-1); fructose 3.57 mol% (TN-3-3) and 5.08 mol% (TN-17-5); mannose 5.83 mol% (TN-3-5) and 6.94 mol% (TN-17-5); galactose 64.18 mol% (TN-3-2) and 78.27 mol% (TN-17-2); glucose 12.54 mol% (TN-3-4) and 13.58 mol% (TN-17-4); and myo-inositol 40.15 mol% (TN-3-3) and 42.28

mol% (TN-17-1) (Table S1). To our astonishment, the high content of myo-inositol would play the role of antioxidant, signalling messengers, and immunity enhancer (Gao and Wang, 2007). Phosphatidylinositol signaling mediates Wnt3a action in the canonical pathway, acting to generate inositol pentakisphosphate, a key second messenger of Wnt3a (Gao and Wang, 2007). Combined therapy of myo-inositol plus D-chiro-inositol is effective in improving endocrine and metabolic parameters in young obese PCOS-affected women (Benelli *et al.*, 2016). Inositols belong to the polyols family and form nine different stereoisomers. These molecules – mainly the stereoisomer called myo-inositol – have gained momentum during the last decades as novel promising treatment for a wide range of pathological conditions namely gynecological diseases, insulin resistance, respiratory distress syndrome in newborns, psychiatric illness, and even cancer (Unfer *et al.*, 2011). A number of studies showed that significantly higher concentrations of myo-inositol in the follicular fluid were correlated with good quality oocytes, thereby promoting the development of equally high-quality embryos, in subfertile patients (Unfer *et al.*, 2011). Myo-inositol and D-chiro-inositol, intracellularly incorporated into inositolphosphoglycans (IPGs), play the pivotal role as second messengers of insulin (Unfer *et al.*, 2016). Myo-inositol and D-chiro-inositol, which is another type of stereoisomers, exert a key role in controlling glucose

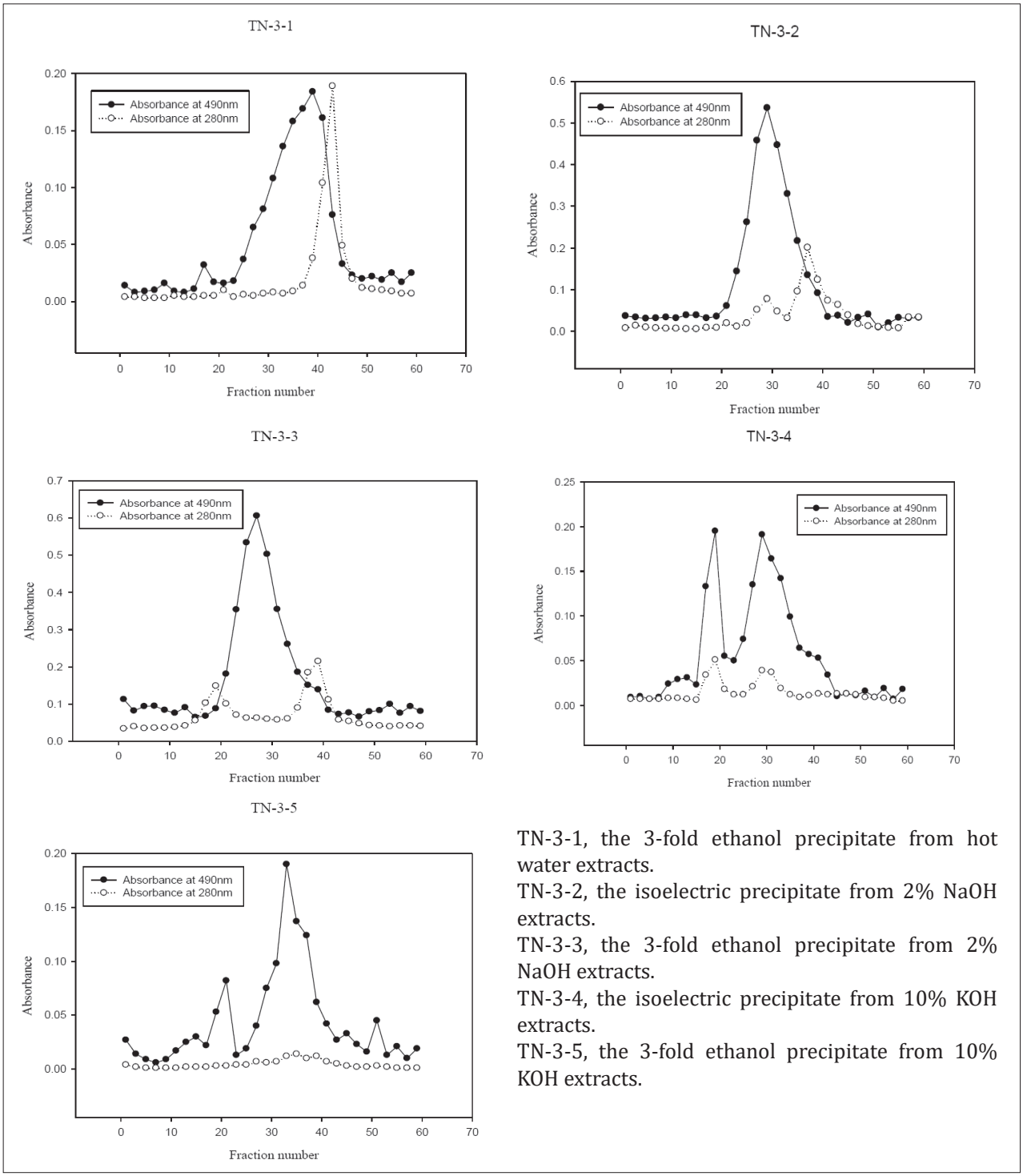
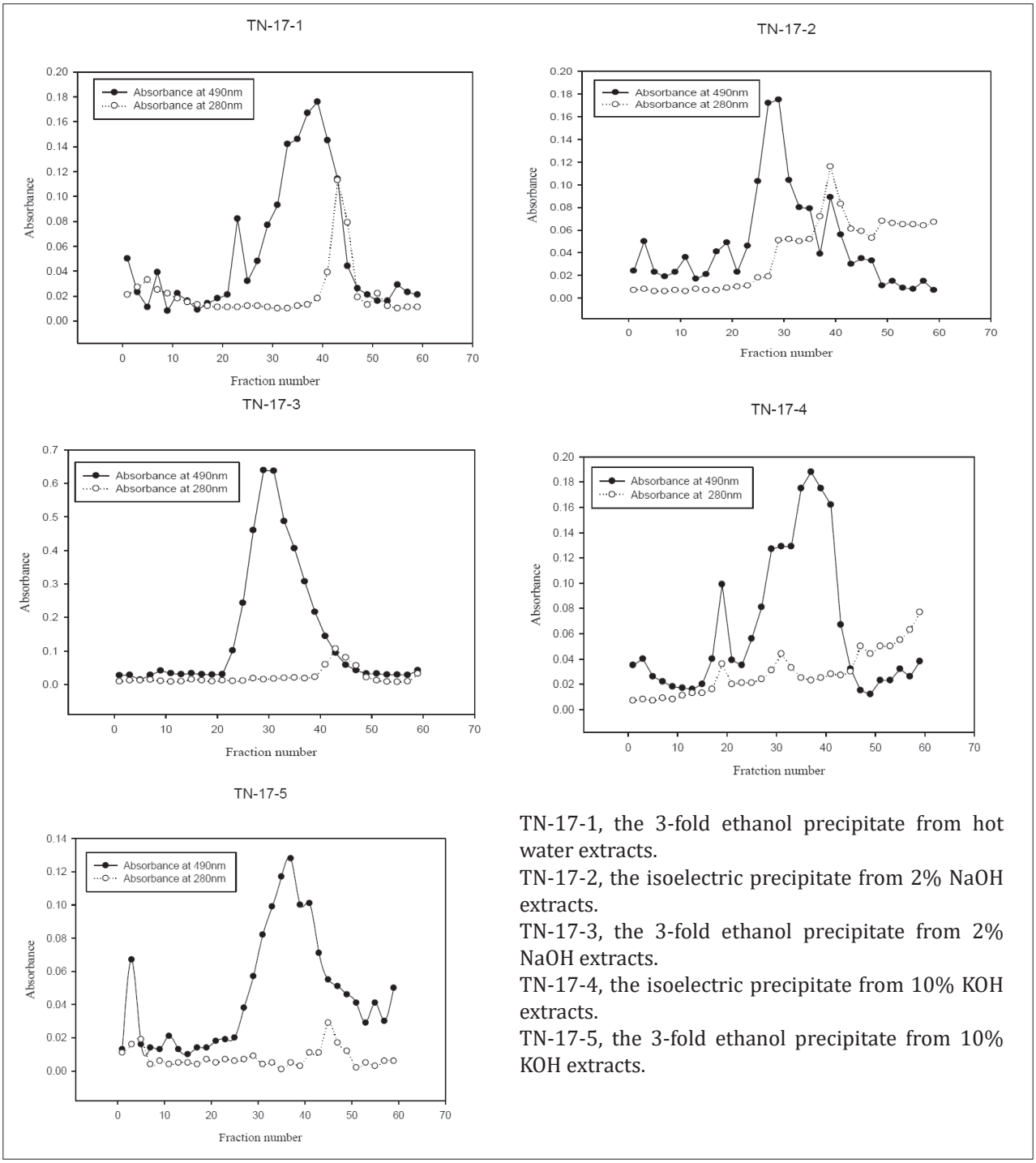



**Figure 1.** Gel permeation chromatography of polysaccharide fractions obtained from TN-3 pineapple variety.

homeostasis. Myo-inositol can be converted into D-chiro-inositol by the epimerase, which is an enzyme stimulated by insulin (Unfer *et al.*, 2016). Myo-inositol exhibits the potential to improve insulin resistance, metabolic syndrome and their allied disorders (diabetes, polycystic ovary syndrome [PCOS]), autoimmunity and certain malignancies (Benvenga and Antonelli, 2016).

**Amino acid composition of the soluble polysaccharides**

The amino acid that appeared in the polysaccharide fractions consisted of alanine, glycine, valine, leucine, isoleucine, proline, glutamic acid, methionine, aspartic acid, hydroxyproline, phenylalanine, cysteine, lysine, histidine, and tyrosine. The top three amino acids in each fraction were TN-3-1 (isoleucine, alanine, and valine), in which isoleucine reached 37.27 wt%; TN-3-2 (tyrosine, glutamic acid, and histidine), in which the content of tyrosine reached 18.08 wt%; TN-3-3 (leucine, isoleucine, and tyrosine) in which isoleucine reached 13.49 wt%; TN-3-4 (tyrosine, histidine, and isoleucine); and TN-3-5 (cysteine, glutamic acid, and isoleucine) (Table S2). For TN-17, the corresponding data were: TN-17-1 (cysteine, lysine, and methionine); TN-17-2 (tyrosine, lysine, and proline); TN-17-3 (cysteine, aspartic acid, and lysine); TN-17-4 (proline, methionine, and lysine); and TN-17-5 (hydroxyproline, methionine, and isoleucine) (Table S2). As can be seen, the polysaccharide fractions present in the pineapple peels were all enriched with abundant essential amino acids, which are beneficial




TN-17-1, the 3-fold ethanol precipitate from hot water extracts.
TN-17-2, the isoelectric precipitate from 2% NaOH extracts.
TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts.
TN-17-4, the isoelectric precipitate from 10% KOH extracts.
TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

**Figure 2.** Gel permeation chromatography of polysaccharide fractions obtained from TN-17 pineapple variety.

**Table 4.** Mean molecular weight of different polysaccharide fractions obtained from the peels of TN-3 and TN-17 pineapple cultivars.*

| Polysaccharide fractions | Mean molecular weight (kDa) |
|---|---|
| TN-3-1 | 37.91 |
| TN-3-2 | 187.75 |
| TN-3-3 | 190.02 |
| TN-3-4 | 492.80 |
| TN-3-5 | 136.66 |
| TN-17-1 | 1222.40 |
| TN-17-2 | 368.49 |
| TN-17-3 | 274.86 |
| TN-17-4 | 93.69 |
| TN-17-5 | 310.05 |

* TN-3-1 and TN-17-1, the 3-fold ethanol precipitate from hot water extracts.TN-3-2 and TN-17-2, the isoelectric precipitate from 2% NaOH extracts. TN-3-3 and TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts. TN-3-4 and TN-17-4, the isoelectric precipitate from 10% KOH extracts. And TN-3-5 and TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

to enhancing immunity and strengthening the health. These amino acids could serve as the peptide bridging between the polysaccharide molecules, as commonly known for structure of peptidoglycans (Vollmer *et al*., 2008). The glycan strands are made up of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues linked by β-1→4 bonds. The D-lactoyl group of each MurNAc residue is substituted by a peptide stem whose composition is most often L-Ala-g-D-Glu-meso-$A_2$pm (or L-Lys)-D-Ala-D-Ala ($A_2$pm, 2,6-diaminopimelic acid) in nascent peptidoglycan, the last D-Ala residue being lost in the mature macromolecule (Robak and Gryglewski, 1988). Cross-linking of the glycan strands generally occurs between the carboxyl group of D-Ala at position 4 and the amino group of the diamino acid at position 3, either directly or through a short peptide bridge (Vollmer *et al*., 2008). Peptidoglycan contributes to the maintenance of a defined cell shape and serves as a scaffold for anchoring other cell envelope components such as proteins (Dramsi *et al*., 2008) and teichoic acids (Neuhaus and Baddiley, 2003). It is intimately involved in the processes of cell growth and cell division. Thus, the presence of abundant peptidoglycans in the pineapple peels may implicate its relevant role associated with the fruit growth and maturation. Chyau *et al*. reported the peptidoglycan SC2 (MW 841 kDa) obtained from *Schisandra chinensis* (Turz Baill) (*S. chinensis*) (SC) fruit assisted the transportation of schisandrin B and deoxyschisandrin across cell membrane (Chyau *et al*., 2014). Taken together the above mentioned results (Tables 3 and S3), the peptidoglycans were composed of different peptide moieties linked to different heteropolysaccharides. In TN-3, the heteropolysaccharides respectively were TN-3-1 (galactoribosan), TN-3-2 (galactoglucosan), TN-3-3 (galactoglucosan), TN-3-4 (galactoglucosan), and TN-3-5 (galactoglucosan); and in TN-17, TN-17-1 (galactoribosan), TN-17-2 (galactoglucosan), TN-17-3 (galactoribosan), TN-17-4 (galactoglucosan), and TN-17-5 (galactoribosan). Thus, both the TN-3 and TN-17 all contained a profound amount of essential amino acids associated precious bioactive peptidoglycans, implicating the possible nutraceutical role of the polysaccharides present in TN-3 and TN-17 (Verbrugh *et al*., 1983). Peptidoglycan is the rigid, but flexible, macromolecule that defines an organism's shape and anchors protein complexes and extracellular organelles to the cell surface (Young, 2011). By definition, it also exists in a diversity of plant cells. Peptidoglycan fragments trigger neighbouring microorganisms to grow or to modify their own walls, serve as maturation signals for vertebrate immune systems and may be used to manipulate the immune system for either the benefit of pathogenic organisms (Young, 2011) or the hosts.

**The antioxidative capability**

The DPPH scavenging capabilities of TN-3 and TN-17 polysaccharides were only moderately strong (Figure 3a and 3b), although that of TN-3-1 seemed to be a little stronger than that of TN-17 (Table S3). The $IC_{50}$ values (mg $mL^{-1}$) of DPPH scavenging for all TN-3 fractions were: TN-3-1 (0.88), TN-3-2 (0.49), TN-3-3 (5.07), TN-3-4 (13.24), and TN-3-5 (7.59). And those for TN-17 fractions were: TN-17-1 (5.72), TN-17-2 (3.84), TN-17-3 (7.11), TN-17-4 (15.58), and TN-17-5 (10.43) (Table S3). Interestingly, the two polysaccharide fractions were almost entirely ineffective with respect to the ferrous ion chelating capability (Figure 3c and 3d). Ferrozine has served as the basis of numerous assays for the quantification of iron in solution (Stookey, 1970). The ferrozine-chelation assay for iron can be affected by many factors like pH (optimum 5.5), reaction time (optimum 135 min), reaction temperature (optimum 37 °C), and the presence of reducing agent (optimum final concentration 50 mM), and the stoichiometry of ferrozine (final concentration 10 mg $mL^{-1}$ or 20.3 mM) (Jeitner, 2014). We suspect such a negative result for ferrous (or ferric) ions could be possibly due to the reasons: 1) the chelation effect of all fractions in TN-3 and TN-17 for soluble iron being much stronger over that of ferrozine; 2) the bias of reaction pH; 3) the insufficient reaction time; and 4) improper stoichiometry of ferrozine to the strong chelating peptidoglycans like TN-3 and TN-17.

The hydroxyl radical inhibitory ability of TN-3-1, TN-3-2, and TN-17-2 were rather comparable and moderately reaching 60% at 10 mg $mL^{-1}$ (Figure 3e and 3f). As contrast, TN-3-4 and TN-17-4 seemed to be the poorest regarding the inhibition of hydroxyl radical formation (Figure 3e and 3f). The $IC_{50}$ values (mg $mL^{-1}$) for hydroxyl radical inhibitory capability were: TN-3-1 (4.30), TN-3-2 (10.15), TN-3-3 (3.93), TN-3-4 (22.01), and TN-3-5 (8.83). While for TN-17, fractions were: TN-17-1 (7.95), TN-17-2 (3.53), TN-17-3 (4.65), TN-17-4 (12.55), and TN-17-5 6.11) (Table S3). Amazingly, all TN-3 fractions showed very low superoxide anion scavenging capability, while TN-17-3 revealed rather promising scavenging capability (Figure 3g and 3h). As contrast, the fractions TN-3-5 and TN-17-4 were the poorest. The $IC_{50}$ values (mg $mL^{-1}$) for the superoxide anion radical scavenging capability were: TN-3-1 (13.75), TN-3-2 (9.64), TN-3-3 (10.04), TN-3-4 (11.51), and TN-3-5 (12.66), and that for TN-17 were: TN-17-1 (12.82), TN-17-2 (8.99), TN-17-3 (0.31), TN-17-4 (11.39), and TN-17-5 (4.33). Thus, fractions TN-3-2 and TN-3-1 were the two top DPPH scavengers, being stronger than TN-17-2 and TN-17-1; the latter was comparable to TN-3-3 (Table S3). Fractions TN-3-1, TN-3-3 and TN-17-2 revealed to be rather comparable regarding the hydroxyl radical inhibitory capability, and among these fractions the fraction TN-3-4 was the poorest (Table S3).

Amazingly, the fraction TN-17-3 revealed to be the strongest superoxide anion radical scavenger, and the next was TN-17-5. Other fractions were almost very comparable (Table S3).

The reactions to scavenge the superoxide anions are very selective and complicated due to its presence of an unpaired triplet electron. Profound chemicals contribute more or less to the scavenging capability, including rhamnose, ribose, glucose, and myo-inositol (Ker *et al.*, 2010, 2011), flavonoids (Robak and Gryglewski, 1988; Trevithick-Sutton

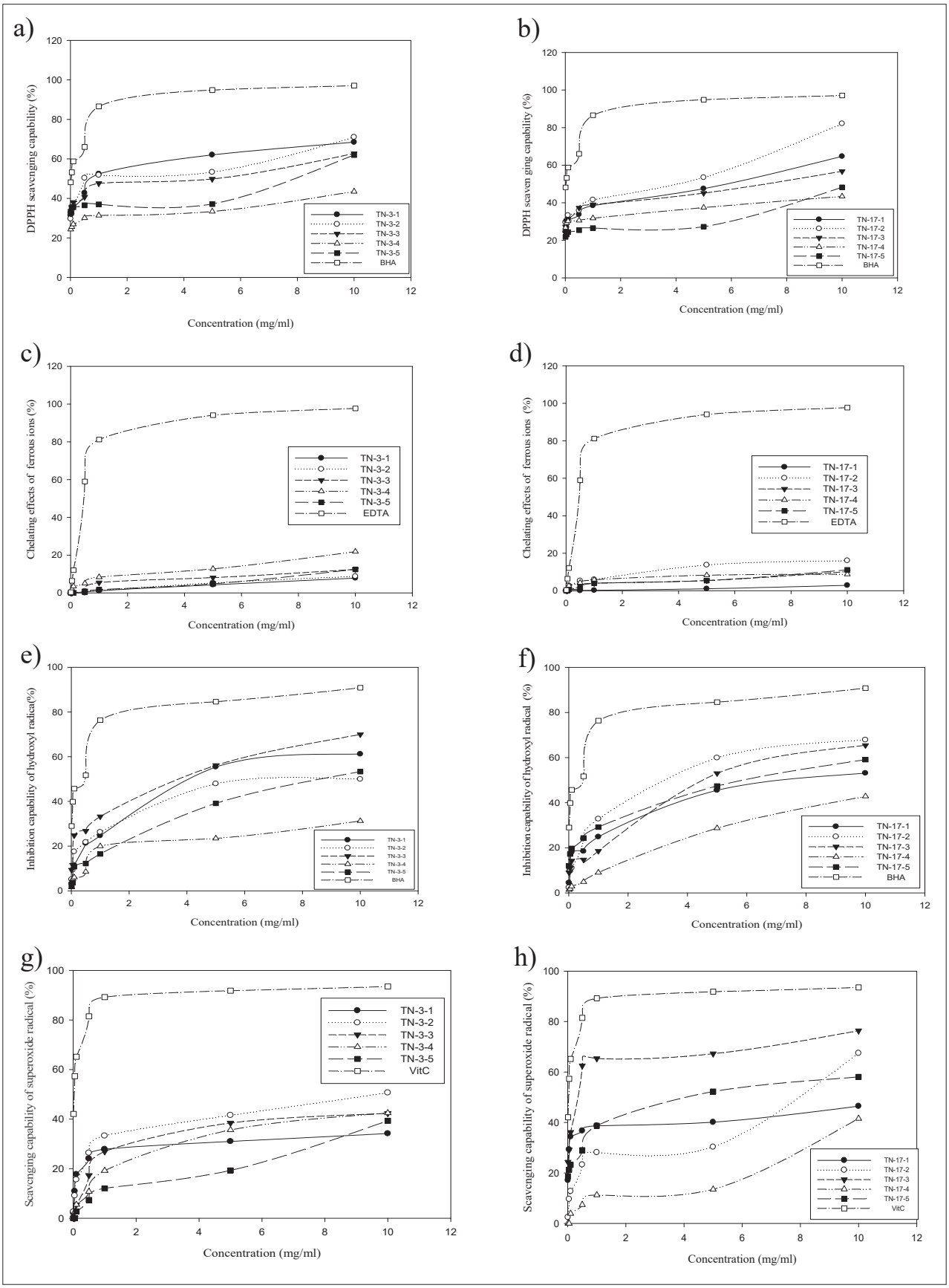



**Figure 3.** DPPH, ferrous ions, hydroxyl radical, and superoxide anion scavenging capability of the polysaccharide fractions obtained from the pineapple TN-3 and TN-17 cultivars.

*et al.*, 2006), and cysteine residues in the polysaccharide fractions (Kitaoka *et al.*, 2005). L-cystathionine and N-acetyl-L-cysteine suppressed fMLP- and PMA-induced superoxide generation by the inhibition of translocation to membrane of p47(phox) and p67(phox). N-acetyl-L-cysteine also had scavenging activity against DPPH radicals and superoxide anion (Kitaoka *et al.*, 2005). As shown, the cysteine residue contents in TN-17-3 and TN-17-5 were 40.98% and 7.65%, respectively (Table S2).

Taken together, the reclaimed polysaccharides may be used as valuable nontoxic anti-oxidative and antimicrobial remedies as well as materials (Coma, 2013). Suggestively, to prevent the negative consequences of food contamination and oxidative processes, these active packaging materials could help minimize the risk of contamination (Coma, 2013), implicating the specially alternative usage of the reclaimed polysaccharides.

**FTIR**

The FTIR spectra of TN-3-2 and TN-17-2 were very similar. The absorption peak assignments to TN-3-2 were 3421.1 $cm^{-1}$ ($\nu_{O-H}$) (broad, s, intermolecular hydrogen bonding; and amide A band N-H stretching, 3300 H-bonded), 2917.77 $cm^{-1}$ ($\nu_{C-H}$, s, $\nu$-$_{CH2}$); 900–1500 $cm^{-1}$ (covers $\nu_{c-o}$, $\nu_{c-c}$, $\nu_{-CH2}$, and $\nu_{-COO}$); 1547.8 $cm^{-1}$, s, $\delta_{N-H}$, or $\nu_{C-N}$ (amide II band, sec-amide, -$CONH_2$, coupled C-N stretching and N-H bending); 1636.3~1732.73 $cm^{-1}$ ($\nu_{C=O}$, s, amide I band, sec-amide, $\nu_{-CONH2}$ coupled C-N stretching and N-H bending, asymmetric $\nu_{C=O}$, which could stand for the absorption due to the amide linkage of peptide moiety, or nonconjugated, may be uronic acid); 1385.9 $cm^{-1}$, s, $\delta_{CH3}$; 1250 $cm^{-1}$, s, $\delta_{C-N}$; 1230–1000 $cm^{-1}$ (the *sec*-cyclic alcohols of β-pyranoside). The absorption peaks appearing within 1108.5–1041.6 $cm^{-1}$, s, $\nu_{C-O}$ (hint: 1275–800 $cm^{-1}$ indicating the $\nu_{C-O-C}$ in monosugars like glucose or galactose; or changes in the endocyclic torsion angles of the furanose ring); and 896.74 $cm^{-1}$, m, (β-glycosidic linkage); 800.4 $cm^{-1}$, s, $\nu_{C-O-C}$, m, (monosaccharide units); 800–640 $cm^{-1}$, multiple absorption peaks, s, out of plane $\delta_{N-H}$ (Figure 4) (Chiu *et al.*, 2014). Similar pattern was found for TN-17-2. The main absorption peaks were: 3437.49 $cm^{-1}$ ($\nu_{O-H}$) (broad, s, intermolecular hydrogen bonding; and amide A band N-H stretching, 3300 $cm^{-1}$ H-bonded; 2917.77 $cm^{-1}$ ($\nu_{C-H}$, s, $\nu$-$_{CH2}$), 900–1500 $cm^{-1}$ (covers $\nu_{c-o}$, $\nu_{c-c}$, $\nu_{-CH2,}$ and $\nu_{-COO}$), 1636.3~1772.26 $cm^{-1}$ ($\nu_{C=O}$, s, amide I band, sec-amide, $_{-CONH2}$ coupled C-N stretching and N-H bending, asymmetric $\nu_{C=O}$, which could stand for the absorption due to the amide linkage of peptide moiety, or nonconjugated, may be uronic acid, asymmetric $\nu_{C=O}$); The absorption peaks appearing within 1108.5–1041.6 $cm^{-1}$, s, $\nu_{C-O}$ (hint: 1275–800 $cm^{-1}$ indicating the $\nu_{C-O-C}$ in monosugars like glucose or galactose; or changes in the endocyclic torsion angles of the furanose ring); and 897.701 $cm^{-1}$, m, (β-glycosidic linkage); 800–640 $cm^{-1}$, multiple absorption peaks, s, out of plane $\delta_{N-H}$ (Figure 4) (Chiu *et al.*, 2014). Taken together, these characteristics of IR absorption spectra together with the compositional analysis (Tables 3 and S2) have revealed the soluble polysaccharide fractions of TN-3 and TN-7 to be typical peptidoglycans.

**Cytotoxicity test**

Assuming the dose that still retains a cell viability over than 85% to be nontoxic at all, the results from MTT assay indicated that TN-3-2 was more toxic than TN-17-2. The toxicity of TN-3-2 occurred at dose ≥0.1 mg $mL^{-1}$, contrasting with the dose 1 mg $mL^{-1}$ of TN-17-2 (Figure S1). Apparently, the cytotoxicity may be ascribed to the presence of bioactive peptidoglycans.

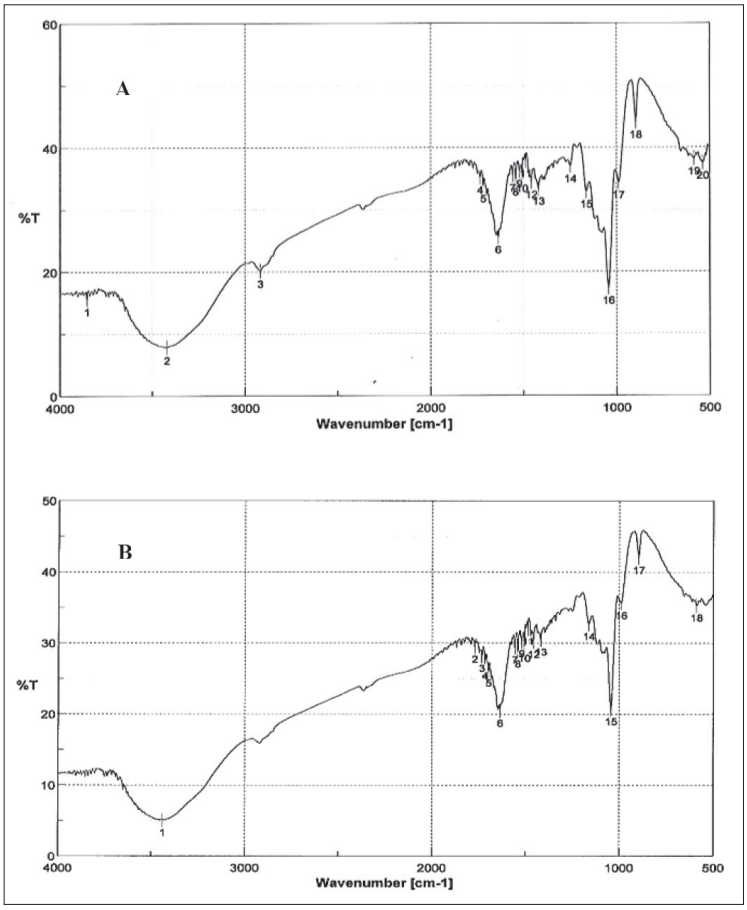



**Figure 4.** FTIR of polysaccharides obtained from TN-3 and TN-17 pineapple cultivar. A: TN-3-2. B: TN-17-2.

## Conclusions

Based on the average amount of annual pineapple peel waste (119,583.5 tons $y^{-1}$), the reclaimable carotenoids are estimated to be 4652 kg $y^{-1}$, and that of bromelian, 2.117 × $10^{11}$ units $y^{-1}$. And the reclaimable soluble polysaccharide fractions from these two cultivars could reach 6433.59 tons amounting up to 5.38% of the total waste peels. The polysaccharide fractions of pine apple peels differs in composition depending on the pineapple cultivars, TN-3 and TN-17, regarding the ash, carbohydrate, crude fiber, sugar, carotenoids and bromelain. The soluble polysaccharides of pineapple peels are peptidoglycan in nature. The most average molecular weight of these polysaccharides is below 500 kDa, implicating small soluble molecules. The monosaccharide composition covers a spectrum of rare bioactive identities like rhamnose, fucose, arabinose, xylose, ribose, fructose, mannose, galactose, glucose, and myo-inositol. The amino acid composition consists of alanine, glycine, valine, leucine, isoleucine, proline, glutamic acid, methionine, aspartic acid, hydroxyproline, phenylalanine, cysteine, lysine, histidine and tyrosine. The polysaccharide fractions exhibit strong DPPH scavenging-, hydroxyl radical-, and superoxide anion radical scavenging capability, but entirely ineffective as ferrous ion chelating agent, and interestingly, TN-3-2 has stronger cytotoxicity than TN-17-2. Taken together, the reclaimable soluble polysaccharide fractions from these two cultivars could amount up to 5.38% of the total waste peels of 119,583.5 tons. Most of the components are valuable bioactive constituents, like essential amino acids, peptidoglycans, and much bioactive monosaccharides. Thus this improved novel green technology is highly recommended to recover much of the valuable constituents present in the waste pineapple peels, in particular, the peptidoglycans, that exhibit a diversity of biomedical significance.

## Acknowledgments

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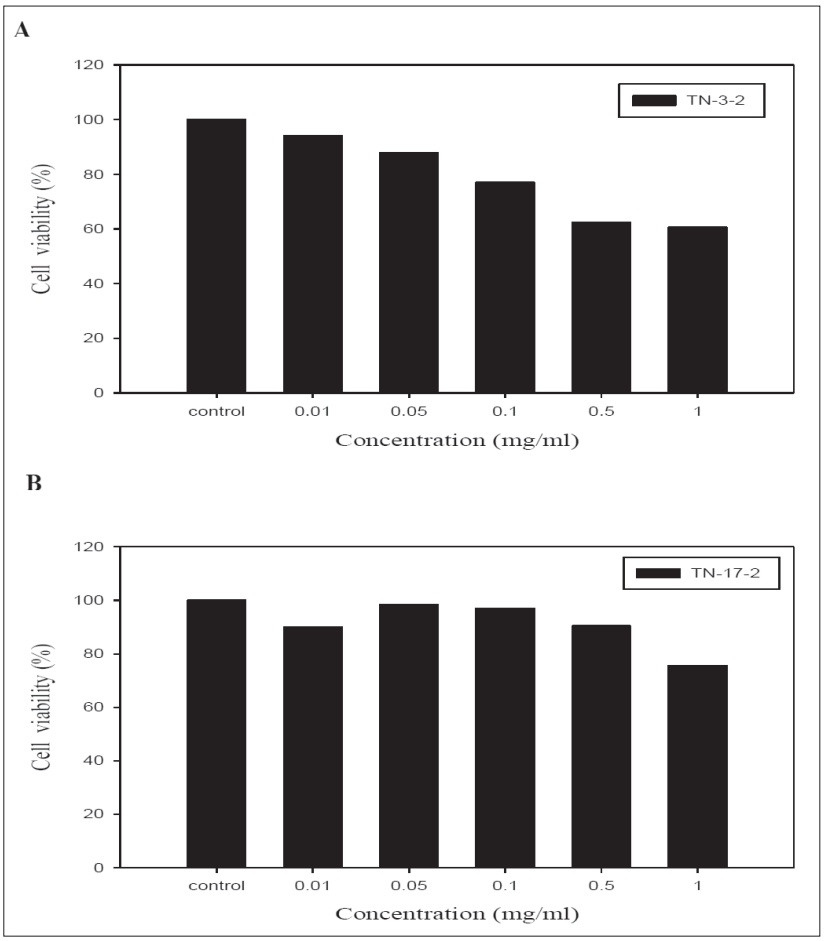


**Figure S1.** Cytotoxicity of polysaccharides TN-3-2 and TN-17-2 obtained from the pineapple TN-3 and TN-17 cultivars. (2% NaOH extract, PI precipitate).

**Table S1.** The monosaccharide composition of polysaccharide fractions obtained from the peels of TN-3 and TN-17-3 pineapple cultivars.

| Sugar (mole %) | TN-3-1 | TN-3-2 | TN-3-3 | TN-3-4 | TN-3-5 |
|---|---|---|---|---|---|
| Rhamnose | 3.37 | 1.02 | 2.88 | 2.25 | 4.05 |
| Fucose | 6.18 | 0.96 | 5.15 | 0.54 | 3.78 |
| Arabinose | 4.89 | 0.54 | 4.05 | 1.16 | 2.91 |
| Xylose | 5.13 | 2.03 | 3.75 | 0.89 | 4.24 |
| Ribose | 7.94 | 2.13 | 8.03 | 3.12 | 5.83 |
| Fructose | 2.64 | 2.79 | 3.57 | 1.74 | 3.17 |
| Mannose | 2.97 | 0.89 | 4.36 | 3.87 | 5.83 |
| Galactose | 22.86 | 64.18 | 16.63 | 60.14 | 27.76 |
| Glucose | 7.28 | 8.25 | 9.41 | 12.54 | 8.54 |
| Myo-inositol | 36.74 | 17.21 | 40.15 | 13.75 | 33.89 |
| | TN-17-1 | TN-17-2 | TN-17-3 | TN-17-4 | TN-17-5 |
| Rhamnose | 3.64 | 0.18 | 3.18 | 0.59 | 2.97 |
| Fucose | 5.11 | 0.47 | 6.06 | 1.02 | 5.84 |
| Arabinose | 3.10 | 0.67 | 2.96 | 0.95 | 3.53 |
| Xylose | 2.94 | 0.27 | 3.75 | 0.33 | 4.04 |
| Ribose | 10.41. | 0.51 | 9.24 | 1.88 | 8.76 |
| Fructose | 4.75 | 0.33 | 4.27 | 0.74 | 5.08 |
| Mannose | 4.95 | 0.10 | 5.83 | 0.87 | 6.94 |
| Galactose | 17.98 | 78.27 | 20.74 | 69.15 | 19.65 |
| Glucose | 4.84 | 5.93 | 5.41 | 13.58 | 3.41 |
| Myo-inositol | 42.28 | 13.27 | 38.56 | 10.89 | 39.78 |

* TN-3-1 and TN-17-1, the 3-fold ethanol precipitate from hot water extracts.TN-3-2 and TN-17-2, the isoelectric precipitate from 2% NaOH extracts. TN-3-3 and TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts. TN-3-4 and TN-17-4, the isoelectric precipitate from 10% KOH extracts, and TN-3-5 and TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

**Table S2.** The amino acid composition of polysaccharide fractions obtained from the peels of TN-3 and TN-17 pineapple cultivars.

| Polysacc. fractions | Wt (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ala | Gly | Val | Leu | Ile | Pro | Glu | Met | Asp | Hyp | Phe | Cys | Lys | His | Tyr |
| TN-3-1 | 11.8 | 0.39 | 11.0 | 10.0 | 37.3 | 6.22 | 0.43 | 2.04 | 0.48 | 4.01 | 0.72 | 0.16 | 3.08 | 4.19 | 8.21 |
| TN-3-2 | 0.86 | 2.35 | 3.02 | 5.24 | 3.27 | 4.77 | 15.8 | 1.91 | 7.74 | 9.91 | 9.15 | 1.62 | 2.67 | 13.7 | 18.1 |
| TN-3-3 | 0.58 | 5.06 | 6.41 | 14.7 | 13.5 | 1.03 | 6.60 | 0.92 | 0.43 | 11.8 | 5.77 | 9.04 | 7.87 | 3.07 | 13.2 |
| TN-3-4 | 0.88 | 2.33 | 4.96 | 2.88 | 10.3 | 5.82 | 7.19 | 5.25 | 0.77 | 4.18 | 5.28 | 7.95 | 6.66 | 15.6 | 20.0 |
| TN-3-5 | 0.79 | 9.77 | 6.07 | 3.98 | 14.2 | 0.71 | 15.2 | 4.86 | 2.25 | 1.73 | 6.08 | 17.5 | 4.24 | 10.0 | 2.41 |
| TN-17-1 | 0.39 | 1.51 | 4.46 | 4.97 | 11.3 | 0.18 | 8.56 | 12.8 | 2.71 | 0.65 | 0.21 | 27.4 | 23.2 | 0.43 | 1.32 |
| TN-17-2 | 3.19 | 1.42 | 4.97 | 11.8 | 7.19 | 15.6 | 7.02 | 1.23 | 1.03 | 3.78 | 0.73 | 3.73 | 15.8 | 13.0 | 18.1 |
| TN-17-3 | 2.36 | 5.41 | 4.83 | 2.76 | 1,69 | 2.61 | 2.35 | 5.88 | 11.3 | 2.73 | 6.04 | 41.0 | 8.30 | 1.59 | 1.20 |
| TN-17-4 | 0.39 | 0.07 | 2.33 | 4.88 | 8.46 | 25.1 | 9.78 | 19.9 | 1.54 | 8.86 | 2.79 | 0.96 | 11.5 | 0.58 | 2.28 |
| TN-17-5 | 0.43 | 0.79 | 2.95 | 2.22 | 11.7 | 2,06 | 6.63 | 15.8 | 4.72 | 16.1 | 10.8 | 7.65 | 1.29 | 7.42 | 9.49 |

* TN-3-1 and TN-17-1, the 3-fold ethanol precipitate from hot water extracts.TN-3-2 and TN-17-2, the isoelectric precipitate from 2% NaOH extracts. TN-3-3 and TN-17-3, the 3-fold ethanol precipitate from 2% NaOH extracts. TN-3-4 and TN-17-4, the isoelectric precipitate from 10% KOH extracts, and TN-3-5 and TN-17-5, the 3-fold ethanol precipitate from 10% KOH extracts.

**Table S3.** $IC_{50}$ of the DPPH, ferrous ion, hydroxyl radical, and superoxide anion scavenging capability of polysaccharides obtained from the peels of TN-3 and TN-17 pineapple cultivars.

| Sample | $IC_{50}$ (mg $mL^{-1}$) | Sample | $IC_{50}$ (mg $mL^{-1}$) |
|---|---|---|---|
| DPPH scavenging capability | | | |
| TN-3-1 | 0.88 | TN-17-1 | 5.72 |
| TN-3-2 | 0.49 | TN-17-2 | 3.84 |
| TN-3-3 | 5.07 | TN-17-3 | 7.11 |
| TN-3-4 | 13.24 | TN-17-4 | 15.58 |
| TN-3-5 | 7.59 | TN-17-5 | 10.43 |
| BHA | 0.02 | | |
| Ferrous ion chelating capability | | | |
| TN-3-1 | 67.75 | TN-17-1 | 88.21 |
| TN-3-2 | 67.50 | TN-17-2 | 51.33 |
| TN-3-3 | 53.08 | TN-17-3 | 55.84 |
| TN-3-4 | 33.47 | TN-17-4 | 66.72 |
| TN-3-5 | 53.19 | TN-17-5 | 54.34 |
| EDTA | 0.42 | | |
| Hydroxyl radical inhibitory capability | | | |
| TN-3-1 | 4.30 | TN-17-1 | 7.95 |
| TN-3-2 | 10.15 | TN-17-2 | 3.53 |
| TN-3-3 | 3.93 | TN-17-3 | 4.65 |
| TN-3-4 | 22.01 | TN-17-4 | 12.55 |
| TN-3-5 | 8.83 | TN-17-5 | 6.11 |
| BHA | 0.39 | | |
| Superoxide oxide anion radical scavenging capability | | | |
| TN-3-1 | 13.75 | TN-17-1 | 12.82 |
| TN-3-2 | 9.64 | TN-17-2 | 8.99 |
| TN-3-3 | 10.04 | TN-17-3 | 0.31 |
| TN-3-4 | 11.51 | TN-17-4 | 11.39 |
| TN-3-5 | 12.66 | TN-17-5 | 4.33 |
| VitC | 0.03 | | |