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# Effect of pectinase on volatile and functional bioactive compounds in the flesh and placenta of 'Sunlady' cantaloupe

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**Abstract:** The effect of commercial pectinase, Pectinex® Ultra SP-L (10,292 PGU/ml), on volatile and functional bioactive compounds of flesh and placenta of fully ripe cantaloupe cv. Sunlady was studied. It was found that enzymatic degradation of cantaloupe flesh and placenta until the reducing sugar contents as 57.09-58.48 and 39.12-40.44 mg glucose/g FM, respectively, had more bioactive compounds than other conditions significantly ($p \leq 0.05$). Antioxidant activities of flesh and placenta detected by DPPH method were 9 and 4 times higher than the undegraded samples (control) (1.57, 0.66 µg FM/µg DPPH); and those measured by ABTS method were 3 and 2 times higher than undegraded samples (5.12, 7.88 µg Trolox equivalents/g FM). Total phenolic contents were 8 and 3 times higher than the undegraded samples (14.95, 39.72 mg gallic acid equivalents/100 g FM). Total flavonoid contents were 10 and 2 times higher than the undegraded samples (2.25, 12.24 mg catechin equivalents/100 g FM). Beta-carotene had increased from 68.15 to 76.71 µg/100 g FM in flesh and 97.23 to 181.89 µg/100 g FM in placenta. Dietary fiber changed from 0.65 to 0.76 g/100 g FM in flesh and 0.32 to 0.52 g/100 g FM in placenta. Prebiotic activities resulted from probiotic type *L.acidophilus* La5 in flesh and placenta were 0.15 and 0.14, respectively, and ones from *B.lactis* Bb12 were 0.34 and 0.33, respectively.. Furthermore, the main volatile compounds found in enzymatic degraded flesh were nonanal, cis-6-nonen-1-ol, and geranyl acetone as well as those found in placenta were ethyl acetate, nonanal, cis-3-nonen-1-ol, and cis-6-nonen-1-ol, etc. Therefore, fully ripe cantaloupe flesh and placenta extracted by enzyme can be a good material to be developed as food colorant with bioactive compound and to be used instead of synthetic agent in the future.

**Keywords: Cantaloupe cv. Sunlady, pectinase, bioactive compounds, volatile compounds, prebiotic, antioxidant activities**

## Introduction

Cantaloupe (*Cucumis melo* var. *cantalupensis* Naud) (Kourkoutas *et al*., 2006) is a kind of melon fruit belonging to the family, *Cucurbitaceae* which can be cultivated in the tropical area all over the world (De melo *et al*., 2000). It is classified as a climacteric fruit (Villanueva *et al*, 2004; Obando-Ulloa *et al*., 2008). In Thailand, cantaloupe is widely grown in Srakaew province, and it is one of the most famous fruits in this province with characteristic fragrant, sweet taste and orange color of the flesh (De Melo *et al*., 2000; Villanueva *et al*., 2004). For industry use, cantaloupe fragrance is one of popular flavor for snack and dessert. The sweet aroma of cantaloupe comes from its volatile substances such as 2-methylbutyl acetate (fruity, sweet), hexyl acetate (apple, cherry), isobutyl acetate (sweet, fruity), ethyl hexanoate (fruity, apple), isoamyl acetate, ethyl butyrate (fruity, sweet) ,ethyl 2-methylbutylrate and (Z)-3-hexenyl acetate (green, fruity) etc. (Fallik *et al*., 2001; Kourkoutas *et al*., 2006; Saftner *et al*., 2006). In addition, cantaloupe is rich in vitamin A, vitamin C, folic acid, potassium and ß-carotene, a pigment in carotenoid group with antioxidant activity (Setiawan *et al*., 2001; Vouldoukis *et al.*, 2004; Lester and Hodges, 2008; Saftner, 2009). These orange-yellowish carotenoid and sweety substances reside in the cell wall structure of polysaccharide such as pectin, cellulose and hemicelluloses (Fu *et al*., 2008; Najafian *et al*., 2009). There are many methods to extract these bioactive compounds. One example is the extraction with solvents such as ethanol, acetone, hexane and toluene. However, this method changes carotenoid pigment to water soluble form and oxidizes easily. However, carotenoid extraction by enzyme from sweet potato keeps natural state in which carotenoid molecules still bound to proteins, leading to tolerate the oxidative activity (Cinar, 2005). There are many examples of enzyme application in extraction which can increase the amount of bioactive compounds from many types of fruits. For example, there were an increasing of phenolic compounds quantity and stability of strawberry and raspberry (Versari *et al.*, 1997), and the increasing total phenolic compound, oil yield and color of olive oil (Najafian *et al*., 2009). Increasing 40% of total phenolic amount of pine including greater antioxidation in *Pinus taiwanensis* than *Pinus morrisonicola* (Lin *et al*., 2009) and increasing flavonoid compounds such as luteolin and apigenin of pigeonpea leaves (Fu *et al*., 2008) were also studied. Furthermore increasing carotenoid yield

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extracted from marigold flower (Delgado-Vargas and Paredes-López, 1997), including increasing carotenoid, antioxidation, water soluble dietary fiber and volatile substance from bael fruit syrup (Charoensiddhi and Anprung, 2010) were scrutinized. These represent the benefit of enzyme degradation.

The role of Pectinex Ultra SP-L enzyme was studied with respect to release functional bioactive compounds in structure of cantaloupe flesh and placenta. Also increasing the value of cantaloupe cv. Sunlady which is not appropriate for consumption when it's fully ripe. Moreover the enzymatic treatment would result in natural form of extract with higher concentration, leading to be used as functional food colorant instead of artificial substance in the future.

## Material and Methods

### *Sample preparation and enzymatic treatment*

Cantaloupe cv. Sunlady (*Cucumis melo* var. *cantalupensis*) from a farm in Srakaew, Thailand, was harvested not later than 60 d after full bloom. The fruits with the weight between 1300-1600 g were incubated at 30±2$^0$C for one week to meet fully ripe stage. Then they were separated into two parts: flesh and placenta (around seed). Each part was blended for 3 min and controlled browning reaction by adding ascorbic acid in 0.2 and 0.1% (w/w), heating until reaching 85$^0$C at the center and holding for 3 and 2 min in flesh and placenta, respectively. After that, the samples were degraded by commercial pectinase enzyme (Pectinex® Ultra SP-L, Novozymes Switzerland AG, Dittengen, Switzerland) produced from *Aspergillus aculeatus* which had enzymatic activity of 10,292 PGU/ml. The enzyme concentrations were varied into 7 levels (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/w)), and the degradation times were varied into 9 levels (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6 h). The enzyme reaction was stopped by heating at 95±5$^0$C for 5 min. Then, the degradation efficiency was measured by the derived amount of reducing sugar using the method of Nelson (1944). All chemicals and solvents used in this experiment were analytical grade, purchased from Sigma Chemical Co., Ltd (St,Louis, MO,USA) and Sigma Aldrich Co., Ltd (Steinheim, Germany).

### *Determination of antioxidant activities*

#### *Sample preparation for antioxidant activity determinations*

Samples were prepared to determine the antioxidant activity, and total phenolic and total flavonoid content by the method modified from Velioglu *et al*. (1998). Cantaloupe flesh and placenta samples (60 g) were mixed with 300 ml of 95% ethanol, kept in the dark at 25°C for 4.5 h, and stored in air-tight amber bottles at -18°C until further analysis.

#### *Antioxidant activity determinations*

The antioxidant activities of the sample extracts were evaluated using the DPPH and ABTS assays. The DPPH radical-scavenging activity was adjusted from the method of Leong and Shui (2002) and Maisuthisakul *et al*. (2007). Sample extracts were first prepared in different concentrations; 4.9 ml of each dilution was mixed with 100 µl of 5 mM DPPH in methanol, and set aside at room temperature for 30 min. The absorbances of three samples, sample with DPPH ($A_1$), diluted sample without DPPH ($A_s$), and DPPH solution alone without sample ($A_0$), were measured at 517 nm using a spectrophotometer and methanol as blank. The decreased content of DPPH radical-scavenging activity in each concentration of sample extract could be calculated by the formula:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{[A_0 - (A_1 - A_s)]}{A_0} \times 100$$

The decreased content of DPPH radical-scavenging activity was plotted against the extracted concentration from cantaloupe flesh and placenta to determine the amount of extract capable to decrease DPPH radical concentration by 50% ($EC_{50}$). The unit of antioxidant activity was defined as $1/EC_{50}$ representing as µg fresh mass (FM)/ µg DPPH.

For ABTS assay derived from the method of Thaipong *et al*. (2006), the stock solutions which contained 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution were prepared. The working solution was then prepared by mixing the two stock solutions in equal quantities and setting at room temperature for 24 h. The solution was then diluted by mixing 2 ml of ABTS solution with 60 ml of methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using methanol as blank. Sample extracts from different concentrations (150 µl) were allowed to react with 2850 µl of the derived ABTS solution for 2 h in a dark condition, then the absorbance was measured at 734 nm using the spectrophotometer and compared with the standard curve of 100-500 µM Trolox. The results were expressed in µg Trolox equivalents (TE)/g FM.

### *Determination of total phenolic content*

Total phenolic content was determined by Folin-Ciocalteau colorimetry following Marinova *et al*.,

2005 and Waterhouse, 2005. The extracts from flesh and placenta were prepared, then mixed 1 ml of sample extract with 9 ml of deionised water and 1 ml of Folin-Ciocalteu's phenol reagent in 25 ml volumetric flask, kept at room temperature for 5 min, mixed with 10 ml of 7% $Na_2CO_3$ solution, adjusted the volume with deionised water, then mixed again and set aside at room temperature for 90 min. The absorbance of the solution was measured at 750 nm wavelength using deionised water as blank. The phenolic content could be calculated from standard curve that was made from gallic acid at different contents (20-100 mg/l). The values were expressed as mg gallic acid equivalent (GAE)/100 g FM.

***Determination of total flavonoid***

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al*. 1999). The extracts from flesh and placenta were prepared, then mixed 1 ml of sample extracts with 4 ml of deionised water in 10 ml volumetric flask, added 0.3 ml of 5% $NaNO_2$ and stood for 5 min, then added 0.3 ml of 10% $AlCl_3$ and stood for 6 min, after that added 2 ml of 1 M NaOH, and adjusted the volume with deionised water. The obtained solution was measured for the absorbance value at 510 nm wavelength using deionised water as blank. The flavonoid content could be calculated from standard curve that was made from catechin concentrations in range of 20-100 mg/l. The values were expressed as mg catechin equivalent (CE)/100 g FM.

***Determination of ß-carotene***

The ß-carotene content was determined by the spectrophotometric method adapted from Ranganna (1986). The blended cantaloupe flesh and placenta samples (5 g) were extracted with acetone and then filtered via Whatman No.1 in several times until colourless. The filtrate was then transferred to separating funnel and added 10-15 ml of petroleum ether. The sample would be in petroleum ether phase and acetone would be removed by 5% sodium sulfate solution. Extraction was repeated with further portions of petroleum ether until there was no yellow color in acetone phase. The extract in petroleum phase was filtered through Whatman No.1 into 50 ml volumetric flask and adjusted the volume with petroleum ether. The absorbance values were measured at 450 nm using spectrophotometer and then compared with the standard curve (0.5-10.0 µg/ml), representing the results in µg/100g FM.

***Determination of dietary fiber***

Total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) contents were analyzed according to the standard AOAC methods (AOAC, 2006).

***Prebiotic activity determinations***

*Bacterial strains and prebiotic activity assay adapted from Huebner* et al. *(2007)*

*L. acidophilus* La5 and *B. lactis* Bb12 (Christian Hansen,Denmark) were prepared by streaking on MRS agar and incubated at 37°C for 24–48 h with the atmospheric condition for *L. acidophilus* La5 but anaerobic condition in anaerobic jar for *B. lactis* Bb12. A single colony from each plate was transferred into 10 ml of MRS broth and incubated at 37°C for 24 h in ambient atmosphere. Then 1% (v/v) of prepared *L. acidophilus* La5 and *B. lactis* Bb12 cultures was transferred to MRS Broth with 1% (w/v) glucose or 1% (w/v) samples.

*Escherichia coli* ATCC 29922 (Culture Collection Unit, Chulalongkorn hospital) was prepared by streaking on Tryptic Soy Agar (TSA) and incubated at 37°C for 24–48 h at ambient atmosphere. A single colony was transferred into 10 ml of Tryptic Soy Broth (TSB) and incubated at 37°C for 24 h in ambient atmosphere. Then 1% (v/v) of prepared *E. coli* ATCC 29922 culture from TSB was transferred into 10 ml of Minimal Medium broth (Atlas, 1993) and incubated at 37°C for 24 h in ambient atmosphere. Then 1% (v/v) of *E. coli* ATCC 29922 culture was transferred in Minimal Medium broth with 1% (w/v) glucose or 1% (w/v) samples and incubated at 37°C in ambient atmosphere. After 0 and 24 h of incubation, samples were enumerated on MRS agar for *L. acidophilus* La5 and *B. lactis* Bb12 and on TSA for *E. coli* ATCC 29922, and the prebiotic activity score was determined as:

=$\{$(probiotic log cfu $ml^{-1}$ on the prebiotic at 24 h
- probiotic log cfu $ml^{-1}$ on the prebiotic at 0 h)/
(probiotic log cfu $ml^{-1}$ on glucose at 24 h
- probiotic log cfu $ml^{-1}$ on glucose at 0 h)$\}$
- $\{$(enteric log cfu $ml^{-1}$ on the prebiotic at 24 h
- enteric log cfu $ml^{-1}$ on the prebiotic at 0 h)/
(enteric log cfu $ml^{-1}$ on glucose at 24 h
- enteric log cfu $ml^{-1}$ on glucose at 0 h)$\}$.

***Volatile compounds analysis***

Volatile compound preparation using solid-phase microextraction (SPME) method was modified from Kourkoutas *et al*. (2006) and Fallik *et al*. (2001). The volatile compounds from flesh and placenta in headspace area of 25 ml vial were absorbed by fibre (50/30 µm Divinylbenzene /Carboxen /

Polydimenthylsiloxane, Supelco) at 35ºC for 30 min. The samples were desorbed at 200ºC for 5 min, then fed into GC-MS (Agilent 6890 GC, Agilent Technologies) using capillary column, 30 m length, 0.25 mm diameter, 0.25 µm thickness (HP-Innowax), and helium as carrier gas with flow rate of 13.5 ml/ min. The temperature of injector and detector was 250ºC by starting the system at 40ºC for 10 min, then increasing to 260ºC with the rate of 15ºC/min and remaining for 10 min. The MS (Agilent 5973 mass-selective detector, Agilent Technologies) with electron multiplier voltage 70 eV was used and the mass range m/z was analysed in range of 30-400 with the ion source and quadrupole temperature of 230°C and 150°C, respectively. The types of volatile compounds were analysed and classified by comparing the derived mass spectra with Wiley 7n Spectral Library.

### *Statistical analysis*

The experimental data obtained were analyzed statistically for analysis of variance. Mean values were evaluated for statistical significance ($p \leq 0.05$) by Duncan's new multiple range test and T-test using Statistic Package for the Social Science (SPSS version 11.5, USA). All experiments were replicated three times.

## Results and Discussion

### *Enzymatic treatments and functional bioactive compounds*

The degradation of polysaccharides in middle lamella with pectinase enzyme, Pectinex® Ultra SP-L, such as antioxidant, phenolic, total flanovoid, ß-carotene and vitamin C. It was found that increasing the intensity of enzyme and degradation time could affect to polysaccharide degradation rate in both flesh and placenta shown by higher reducing sugar content significantly ( $p \leq 0.05$) as shown in figure 1 and 2. It could be explained that Pectinex Ultra SP-L degraded glycosyl bonds of pectin, cellulose and hemicellulose at the cell wall of the fruit tissue to release reducing group (Grohman and Baldwin, 1992; Multu *et al*,1999). Determination of the quantity of functional bioactive compounds after enzymatic degradation of cell wall structure was done by selecting the degraded samples in which have reducing sugar content in between 36.14-58.48 (flesh) and 22.13-40.44 mg glucose/g FM (placenta) to compare with control set (undegraded samples). The results were reported according to significant difference ($p<0.05$) of enzymatic hydrolysis stages (shown in different RS content) and using code for cantaloupe flesh as F0 (control)-F6 and placenta as P0 (control)-P5.

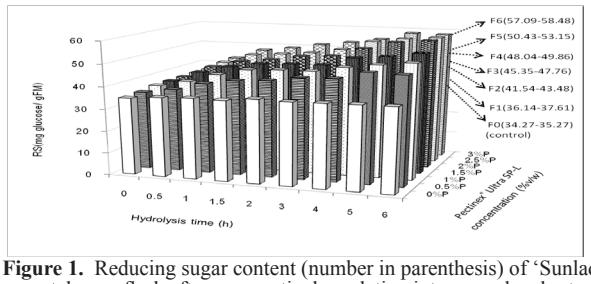


**Figure 1.** Reducing sugar content (number in parenthesis) of 'Sunlady' cantaloupe flesh after enzymatic degradation into many levels start from F0 (control) to F6

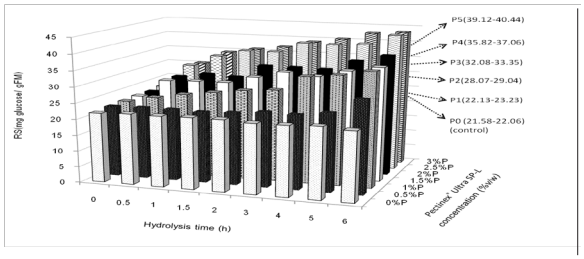


**Figure 2.** Reducing sugar content (number in parenthesis) of 'Sunlady' cantaloupe placenta after enzymatic degradation into many levels start from P0 (control) to P5

### *Antioxidant activities*

From Table 1 and 2, it showed that antioxidant activities in cantaloupe flesh and placenta tend to increase as determined by DPPH and ABTS method. It can be noticed that samples with the highest hydrolysis stage (F6, P5) had antioxidant activities than other stages significantly ($p<0.05$) in both cantaloupe flesh and placenta samples. The ones determined by DPPH method were 9 and 4 times higher than control (1.57, 0.66 µg FM/µg DPPH) for flesh and placenta respectively, while the ABTS method showed 3 and 2 times higher than control (5.12, 7.88 µg Trolox equivalents/g FM). It can be calculated that the antioxidant activity determined by ABTS method in cantaloupe flesh was 1.26 times higher than placenta sample. The $EC_{50}$ values at the highest hydrolysis stage of flesh and placenta were 0.17 and 0.15 µg FM/ µg DPPH, respectively. Furthermore there was a research reported that the enzymatic extraction gave higher antioxidant activities than methanol extraction (Tachakittirungrod *et al*. (2007)). It showed that the antioxidant in guava leaves extracted by enzyme was 3 times higher activity than one extracted by methanol.

### *Total phenolics and total flavonoids*

The analysis of total phenolics and flavonoids revealed that samples with the highest hydrolysis stage (F6, P5) had total phenolic content of 8 and 3 times higher than control (14.95, 39.72 mg GAE/100 g FM) and total flavonoid content of 10 and 2 times higher than control (2.25,12.24 mg CE/100g FM) as shown in Table 1 and 2. It can be calculated that

**Table 1.** Bioactive compounds and antioxidant activity of the various flesh samples of 'Sunlady' cantaloupe

| Bioactive compounds/ Code | Flesh | | | | | | |
|---|---|---|---|---|---|---|---|
| | F0 | F1 | F2 | F3 | F4 | F5 | F6 |
| DPPH ($EC_{50}$: µg FM [A]/ µg DPPH) | 1.57[a] ±0.09 | 1.50[a] ±0.006 | 0.61[b] ±0.001 | 0.54[c] ±0.001 | 0.51[d] ±0.003 | 0.40[e] ±0.001 | 0.17[f] ±0.001 |
| ABTS (µg TE [B]/g FM) | 5.12[e] ±0.17 | 4.10[f] ±0.04 | 13.65[d] ±0.07 | 16.95[c] ± 0.10 | 17.86[b] ±0.04 | 18.50[a] ±0.06 | 18.49[a] ±0.09 |
| Total phenolics (mg GAE[C]/100g FM) | 14.95[g] ±0.13 | 63.70[f] ±0.55 | 67.71[e] ±0.82 | 73.82[d] ±0.51 | 75.64[c] ±0.77 | 109.66[b] ±0.41 | 116.37[a] ±0.45 |
| Total flavonoids (mg CE[D]/100g FM) | 2.25[f] ±0.11 | 9.38[e] ±0.17 | 14.96[d] ±0.10 | 19.15[c] ±0.17 | 24.43[b] ±0.04 | 24.93[a] ±0.12 | 24.96[a] ±0.14 |
| β-carotene (µg /100g FM) | 68.15[c] ±0.19 | 66.98[d] ±0.78 | 68.48[c] ±0.35 | 70.92[b] ±0.35 | 76.69[a] ±0.45 | 76.60[a] ±0.64 | 76.71[a] ±0.90 |

Each value represents a mean± standard deviation.
Means with the different letter in the row are significantly difference at $p<0.05$.
[A]FM= fresh mass, [B] TE=Trolox equivalents , [C] GAE=gallic acid equivalents , [D] CE=catechin equivalents.

**Table 2.** Bioactive compounds and antioxidant activity of the various placenta samples of 'Sunlady' cantaloupe

| Bioactive compounds/ Code | Placenta | | | | | |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | P5 |
| DPPH ($EC_{50}$: µg FM [A]/ µg DPPH) | 0.66[a] ±0.02 | 0.72[a] ±0.052 | 0.46[b] ±0.002 | 0.35[c] ±0.001 | 0.23[d] ±0.025 | 0.15[e]±0.002 |
| ABTS (µg TE [B]/g FM) | 7.88[f] ±0.12 | 10.72[e] ±0.05 | 11.68[d] ±0.04 | 14.05[c] ± 0.04 | 14.46[b] ±0.06 | 14.73[a] ±0.25 |
| Total phenolics (mg GAE[C]/100g FM) | 39.72[e] ±0.69 | 66.96[d] ±0.35 | 66.65[d] ±0.35 | 70.56[c] ±0.42 | 85.95[b] ±0.77 | 105.20[a] ±0.54 |
| Total flavonoids (mg CE[D]/100g FM) | 12.24[e] ±0.99 | 17.46[d] ±0.12 | 18.45[c] ±0.12 | 19.20[b] ±0.35 | 23.85[a] ±0.13 | 23.80[a] ±0.18 |
| β-carotene (µg /100g FM) | 97.23[d] ±0.10 | 158.35[c] ±0.58 | 160.92[b] ±0.93 | 182.22[a] ±0.83 | 182.04[a] ±0.93 | 181.89[a] ±0.59 |

Each value represents a mean± standard deviation.
Means with the different letter in the row are significantly difference at $P<0.05$.
[A]FM= fresh mass, [B] TE=Trolox equivalents , [C] GAE=gallic acid equivalents , [D] CE=catechin equivalents.

the total phenolic content in cantaloupe flesh (F6) was 1.11 times higher than placenta sample (P5), while the total flavonoid contents were similar. This was related with the report of Versari *et al*. (1997) which reported that the extraction of ellagic acid and quercetin, phenolic compounds in strawberry and raspberry juices, by pectinolytic enzyme brought about higher compound amount after enzymatic hydrolysis of polysaccharide structure. Furthermore, there were findings of increasing phenolic and flavonoid contents after enzymatic hydrolysis in olive oil (Najafian *et al*., 2009), pine leaves (Lin *et al*., 2009), and pigconpea leaves (Fu *et al*., 2008). It can be explained that enzyme hydrolyzed plant cell wall, leading to release more extracts and phenolic compounds out of the cell.

### *ß-carotene*

The ß-carotene content in cantaloupe flesh and placenta increased from 68.15 to 76.71 and from 97.23 to 182.22 µg/100 g FM for F4 and P3, respectively. The sample P5 had ß-carotene content of 2.37 times higher than F6 as enzyme hydrolyzed polysaccharide structure and pigments were released in the natural state then bounded with proteins through covalent bonding. This bonded structure can prevent oxidation, whereas solvent extraction method will cause oxidation easily (Cinar, 2005). The ß-carotene content increased after enzymatic degradation by pectinase was related with a report which showed the increase of ß-carotene content in marigold flower from 1.2 to 7.4 g/kg dw or 6 times higher than degraded sample (Delgado-Vargas and Paredes-López,1997).

### *Enzymatic treatments and yield of dietary fiber, prebiotic activity score and volatile compounds*

*Dietary fiber*

From Table 3, it was found that total dietary fiber contents in undegraded samples (F0 and P0) compared with F6 and P5 were not different in all samples. The soluble dietary fiber (SDF) content increase from F0 to F6 and from P0 to P5 as 0.65 to 0.76 (increase 17%) and from 0.32 to 0.52 g/100 g FM (increase 70%), respectively as the effect of many enzymes in Pectinex Ultra SP-L® hydrolyze pectin in cantaloupe samples. The pectin esterase (PE) had function to remove methyl group from pectin compound, while polygalacturonase (PG) can hydrolyses a1,4 linkages

**Table 3.** Total dietary fiber of the various flesh and placenta samples of 'Sunlady' cantaloupe

| Total dietary fiber (g / 100g FM) | Flesh | | Placenta | |
|---|---|---|---|---|
| | F0 | F6 | P0 | P5 |
| Total dietary fiber | $1.02^{a}$ ±0.22 | $1.05^{a}$ ± 0.05 | $0.82^{b}$ ±0.31 | $0.86^{b}$ ± 0.03 |
| - Soluble dietary fiber | $0.65^{b}$ ±0.35 | $0.76^{a}$ ± 0.03 | $0.32^{d}$ ±0.50 | $0.52^{c}$ ± 0.01 |
| - Insoluble dietary fiber | $0.37^{b}$ ±0.47 | $0.29^{b}$ ± 0.08 | $0.50^{a}$ ±0.34 | $0.32^{b}$ ± 0.01 |

Each value represents a mean± standard deviation. FM;fresh mass.
Means with the different letter in the row are significantly difference at $P<0.05$.

**Table 4.** Increasing of cell density between 0 and 24 h. for bacterial cultures grown in various carbohydrates

| Bacterial culture | Cell density [$\log_{10}$(cfu/ml)] | | | | | |
|---|---|---|---|---|---|---|
| | Glucose | Inulin | Flesh | | Placenta | |
| | | | F0 | F6 | P0 | P5 |
| *L. acidophilus* La5 | $1.99^{c}$±0.01 | $2.22^{a}$±0.01 | $2.14^{b}$±0.03 | $2.21^{a}$±0.02 | $2.02^{c}$±0.02 | $2.12^{b}$±0.04 |
| *B. lactis* Bb12 | $2.01^{d}$±0.06 | $2.66^{a}$±0.03 | $2.54^{b}$±0.02 | $2.63^{a}$±0.04 | $2.40^{c}$±0.05 | $2.53^{b}$±0.03 |
| *E. coli* ATCC 29922 | $2.03^{a}$±0.02 | $1.71^{c}$±0.05 | $1.91^{c}$±0.04 | $1.95^{b}$±0.03 | $1.79^{d}$±0.02 | $1.88^{c}$±0.01 |

Each value represents a mean± standard deviation.
Means with the different letter in the row are significantly difference at $P<0.05$.

in the polygalacturonic acid component of the cell walls. Moreover, pectin in insoluble form would change to soluble form because enzyme helped to hydrolyze it into hydroxyl group form (Kashyap *et al*., 2001), relating with the experiment in ripe bael fruit degraded by enzyme (Charoensidhi and Anprung, 2009).

*Prebiotic activity score*

Prebiotic compounds are bioactive compounds such as inulin,oligofructrose or other carbohydrates, supporting the growth of healthy bacteria or probiotics, *L. acidophilus* La5 and *B. lactis* Bb12. The comparative results of both microbial cell amount after growing in media with inulin, media with cantaloupe flesh and placenta samples, and the media without prebiotic (glucose) for 24 h as shown in Table 4. It was found that the amount of *L. acidophilus* La5 and *B. lactis* Bb12 grown in inulin media and sample F6 were higher than other conditions significantly ($p<0.05$). Moreover, the amount of pathogen (*E. coli*) grown in media without prebiotic was higher than one in media with prebiotic significantly ($p<0.05$). The data in Table 4 were calculated for prebiotic activity score as shown in Figure 3, it was found that prebiotic activity scores of *B. lactis* Bb12 was higher than one of *L. acidophilus* La5 in all cases.

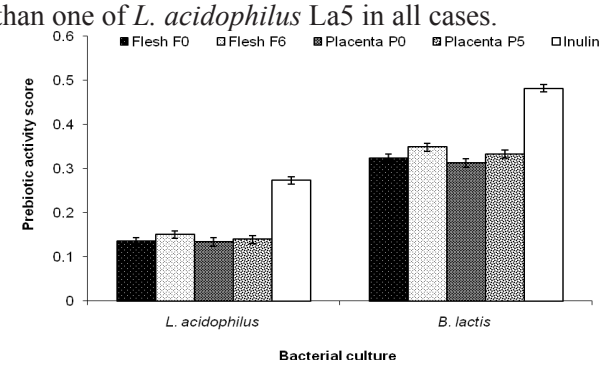


**Figure 3.** Prebiotic activity score of various bacteria grown on Inulin, Flesh F0, Flesh F6 ,Placenta P0 and Placenta P5

Therefore, it can be indicated that the differences in the metabolic capacity apparently existed with the utilization of prebiotics that required the different presence of specific hydrolysis and transport systems (Huebner *et al.*, 2007). It can be noticed that prebiotic activity scores in all samples degraded by enzyme were higher than ones without enzymatic hydrolysis, and the enzyme degradation affected to the increase of both probiotic cell amount significantly ($p<0.05$).

*Volatile compoumds*

The analytical results of the type and amount of volatile compounds showed that the sample F6 had 28 types of volatile compounds while there were only 6 types of in sample F0. Moreover, the sample P5 had 21 types of volatile compounds and sample P0 had 18 types as shown in Figure 4, 5 and Table 5. The results indicated that enzyme can hydrolyze polysaccharide structure in cantaloupe flesh and placenta, so the volatile compounds can largely be released but some were easy to be oxidized or changed to other compounds (Beaulieu and Grimm, 2001). Most of volatile compounds in cantaloupe can be classified into ester, aldehyde and alcohol groups. These compounds were derived from component of phytonutrient such as fatty acids, amino acids, carotenoids, phenolics and terpenoids. The volatile compound in ester group came from the esterification of alcohol by alcohol acetyltransferase during ripening stage (Obando-Ulloa *et al*., 2008). Table 5 showed the main volatile compounds in sample F6 which are methyl acetate, ethyl acetate, methyl butanoate, isobutyl acetate, methyl 2-methylbutanoate, butyl acetate, ethyl butanoate and nonanol. The main volatile compounds in sample P5 were methyl acetate, ethyl acetate, isobutyl acetate, (Z)-3-nonen-

**Table 5.** Volatile compounds of the various flesh and placenta samples of Sunlady' cantaloupe with SPME/GC/MS

| Peak No. | Cas No. | LRI[a] | Compound | %area | | | | Flavor description[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | Control | Flesh | Control | placenta | |
| 1 | 000079-20-9 | 826 | Methyl acetate | 0.10 | 1.49 | nd | 0.83 | Unknown |
| 2 | 000141-78-6 | 889 | Ethyl acetate | nd | 0.98 | 0.36 | 3.34 | Pineapple,ethereal |
| 3 | 000554-12-1 | 908 | Methyl propanoate | nd | 0.52 | nd | 0.10 | Fruity |
| 4 | 000590-86-3 | 913 | 3-methylbutanal | nd | 0.10 | nd | 0.11 | Fatty, Fresh, Pungent |
| 5 | 000547-63-7 | 927 | Methyl isobutanoate | nd | 0.12 | nd | 0.08 | Unknown |
| 6 | 000110-62-3 | 935 | Pentanal | nd | 0.14 | nd | nd | Unknown |
| 7 | 000105-37-3 | 953 | Ethyl propanoate | nd | 0.08 | nd | 0.05 | Rum,pineapple |
| 8 | 00019-60-4 | 969 | Propyl acetate | nd | 0.39 | nd | 0.13 | Fruity,pear ,raspberry-like |
| 9 | 000623-42-7 | 980 | Methyl butanoate | nd | 1.12 | nd | 0.13 | Fruity,apple-like |
| 10 | 000868-57-5 | 1014 | Methyl 2-methylbutanoate | nd | 1.48 | nd | 0.44 | Sweet,fruity,apple-like |
| 11 | 000110-19-0 | 1017 | Isobutyl acetate | nd | 2.96 | nd | 0.86 | Sweet,fruity |
| 12 | 000105-54-4 | 1039 | Ethyl butanoate | nd | 0.88 | 0.62 | 0.23 | Apple,sweet |
| 13 | 000123-86-4 | 1044 | Butyl acetate | nd | 1.91 | 0.25 | 0.42 | Fruity |
| 14 | 000106-36-5 | 1049 | Propyl propanoate | nd | 0.05 | nd | nd | Fruity,apple,banana |
| 15 | 007452-79-1 | 1055 | Ethyl 2-methylbutanoate | nd | 0.12 | nd | 0.07 | Green,fruity |
| 16 | 000066-25-1 | 1087 | Hexanal | nd | nd | nd | 0.47 | Fruity,green |
| 17 | 000624-41-9 | 1118 | 2-methylbutyl acetate | 0.19 | nd | 0.77 | nd | Fruity,banana |
| 18 | 000539-82-2 | 1132 | Ethyl valerate | nd | nd | 0.23 | nd | Fruity,apple |
| 19 | 000628-63-7 | 1176 | Amyl acetate | nd | 0.11 | 0.35 | nd | Banana,ethereal |
| 20 | 000106-70-7 | 1190 | Methyl hexanoate | nd | 0.18 | nd | nd | Pineapple,ethereal |
| 21 | 000123-66-0 | 1236 | Ethyl hexanoate | nd | 0.14 | 0.52 | nd | Fruity,apple |
| 22 | 000142-92-7 | 1275 | Hexyl acetate | nd | 0.47 | 1.40 | nd | Apple,cherry |
| 23 | 000124-13-0 | 1286 | Octanal | nd | 0.11 | nd | 0.09 | Fruity, citrus |
| 24 | 003681-71-8 | 1308 | (*Z*)-3-hexenyl acetate | nd | nd | 0.16 | nd | Fruity,green |
| 25 | 000106-30-9 | 1333 | Ethyl heptanoate | nd | nd | 0.19 | nd | Wine-like,fruity |
| 26 | 000111-27-3 | 1348 | 1-Hexanol | nd | 0.49 | nd | 0.20 | Green leaf |
| 27 | 000112-06-1 | 1370 | Heptyl acetate | nd | nd | 0.27 | nd | Woody,oily |
| 28 | 054340-70-4 | 1378 | (*E*)-4-Ethyl heptenoate | nd | nd | 0.33 | nd | Unknown |
| 29 | 000124-19-6 | 1401 | Nonanal | 0.13 | 0.43 | 0.16 | 0.40 | Fatty, melon |
| 30 | 002277-20-5 | 1448 | (*E*)-6-Nonenal | 0.39 | nd | 0.17 | nd | Unknown |
| 31 | 000513-85-9 | 1533 | 2,3-Butanediol | nd | nd | nd | 0.95 | Unknown |
| 32 | 000111-87-5 | 1552 | 1-Octanol | nd | 0.30 | nd | nd | Fatty,green herbal |
| 33 | 000557-48-2 | 1580 | (*E,Z*)-2,6-nonadienal | nd | 0.21 | nd | 0.19 | Cucumber,green |
| 34 | 028473-21-4 | 1657 | Nonanol | 0.42 | 0.93 | 2.08 | 0.65 | Unknown |
| 35 | 0T0340-23-5 | 1682 | (*Z*)-3-Nonen-1-ol | nd | nd | 4.80 | 1.26 | Melon |
| 36 | 035854-86-5 | 1712 | (*Z*)-6-Nonen-1-ol | 1.51 | 0.42 | 1.18 | 0.74 | Melon |
| 37 | 000140-11-4 | 1725 | Benzyl aceate | nd | nd | 0.15 | nd | Sweet,fruity |
| 38 | 003796-70-1 | 1851 | Geranyl acetone | 0.21 | 0.16 | nd | nd | Fresh,rosy |

[a]LRI : Linear retention index calculated against $C_{10}$-$C_{25}$ *n*-alkanes on HP-Innowax column. nd : not detected.
[b]Flavor description from Saftner *et al*. (2006); Chin *et al*.(2007); Mahattanatawee *et al*.(2007).

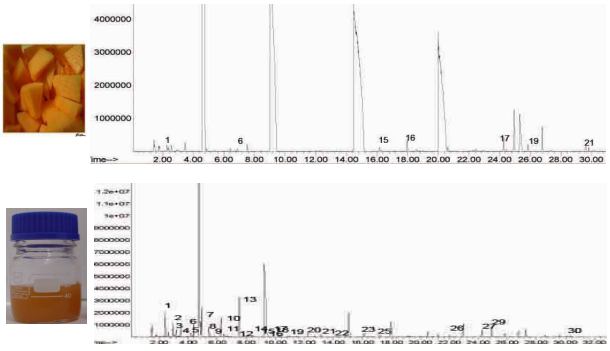

**Figure 4.** Chromatogram of volatile compounds of F0-F6 samples of 'Sunlady' cantaloupe

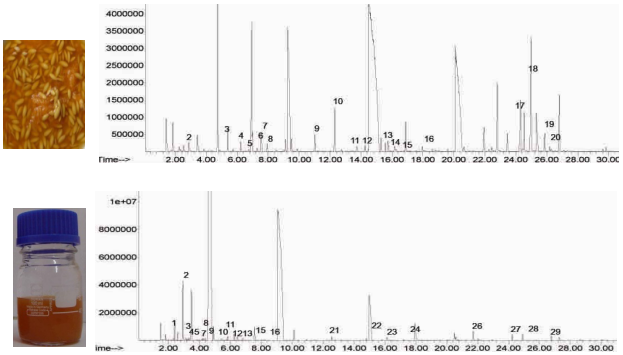

**Figure 5.** Chromatogram of volatile compounds of P0-P5 samples of 'Sunlady' cantaloupe

1-ol, (Z)-6-nonen-1-ol and nonanol. Furthermore, it was found that the main volatile compounds in both sample F6 and P5 had flavor descriptive as pineapple, fruity, melon, apple, banana and sweet (Saftner *et al*., 2006; Chin *et al*., 2007; Mahattanatawee *et al*., 2007). The comparison of volatile compound in cantaloupe with other reports did for volatile compounds in other melons (Kourkoutas *et al*., 2006; Fallik *et al*., 2001) showed that cantaloupe had particular volatile compound, fragrant with honeydew temptation and galia, including comprising of 2-methylbutyl acetate, hexyl acetate, butyl acetate, isobutyl acetate, ethyl 2-methyl butanoate and isoamyl acetate.

## Conclusion

The application of commercial pectinase enzyme, Pectinex® Ultra SP-L, to degrade cell walle structure in both cantaloupe flesh and placenta led to the highest reducing sugar contents of 57.09-58.48 (flesh) and 39.12-40.44 mg glucose/g FM (placenta). It can be concluded that cantaloupe flesh had higher bioactive compounds than placenta, except for carotenoid content. The antioxidant value measured by ABTS method of flesh was 1.26 times higher than placenta. Total phenolic content of flesh was 1.11 times higher than placenta. Total flavonoid content of flesh was similar to placenta. The beta-carotene content of placenta was 2.37 times higher than flesh. The increasing rate of soluble dietary fiber content of placenta was 1.82 times higher than flesh. Furthermore, prebiotic activity score of prebiotics in both flesh and placenta showed the same ability to increase the microbial cell. The main volatile compounds in flesh were more than placenta (methyl acetate, isobutyl acetate and nonanol). The flesh degraded by enzyme can be a good source of bioactive compound except for carotenoid that was high in placenta. Therefore, the flesh and placenta degraded by enzyme were potential to develop as colorant, flavoring agent, dietary fiber with antioxidant property and prebiotic, and it is also a way to add value for fully ripe cantaloupe and

compensation the synthetic compound in the future.

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