บทความวิจัย

EFFICIENCY IMPROVEMENT OF BIOACTIVE COMPOUNDS EXTRACTION FROM CANTALOUPE AND MUSKMELON BY FREEZE-THAWING AND PEF

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ABSTRACT

This study aimed to determine the effect of freeze-thawing cycle and pulsed electric field (PEF) pretreatment on extraction efficiency of bioactive compounds from cantaloupe and muskmelon peels. The peels were treated by either 0-5 cycles of freeze-thawing or 0-10,000 pulses of PEF at E = 143 V/cm, f = 100 Hz, and the pulse width of 50 μs prior to extraction by aqueous acetone or aqueous ethanol solvent. Antioxidant activity was determined by ferric reducing power (FRP) and DPPH methods. It was found that the acetone based extract of cantaloupe skin pretreated with 5 cycles of freeze-thawing gave the highest antioxidant activity (2,031.28±3.01 mg AAE/100 g and 400.39±93.94 mg AAE/100 g for FRP and DPPH methods, respectively) with 2.96% extraction yield. On the other hand, the acetone based extract of cantaloupe skin pretreated with PEF for 10,000 pulses gave the antioxidant activity of 1,613.19 ± 37.61 mg AAE/100 g and 327.54±6.06 mg AAE/100 g for FRP and DPPH methods, respectively) with 2.37% extraction yield. Although the PEF treatment gave the extract with less antioxidant activity and less yield, the time used by this method was much shorter than that of freeze-thawing, i.e., 1 min 40 s for PEF and 5 days for freeze-thawing. Thus, PEF is considered to be more energy efficient and economically sound than freeze-thawing

pretreatment. In addition, PEF pretreatment could yield greater extraction efficiency if higher electric field strength or number of pulses is applied.

Keywords: antioxidant activity, cantaloupe, freeze-thawing, muskmelon, pulsed electric field

Introduction

Fresh-cut fruit is a rapidly growing segment of the produce industry and is increased in value from 3.3 billion USD in 1999 to 15.5 billion USD in 2007 (Cook, 2009). Cantaloupe *(Cucumis melo var. cantalupensis)* and muskmelon *(C. melo var. acidulous)* pieces are common components of fresh-cut fruit products and are available year-round throughout Thailand. Consumer preference for this fruit is determined largely by its sweetness (i.e sugar content), flavor or aroma, and texture. In addition, these fruits are considered as a rich source of phytonutrients. They are rich in ascorbic acid, carotene, folic acid, and potassium as well as a number of other human health-bioactive compounds (Lester, 2008; Menon & Raman, 2012). The trend of fresh-cut fruit is continuously growth due to the busy consumer life-style leading to increasing amount of food waste generation. The unwanted parts that are peeled, trimmed and cut from the fresh fruits during fresh-cut preparation are generally disposed and commonly used as composts, or low value animal feed (Hossain et al., 2015). In the United Kingdom alone, the volume of food wastes was about 6.7 million tons, which is disposed as landfills each year (James and Ngarmsak, 2010). Disposal of this waste imposes high costs on food processors and burdens the environment. Therefore, the industry is keen to utilize these wastes to make high value ingredients that have potentials for pharmaceutical or nutraceutical product development.

Cantaloupe and muskmelon peels are considered to be one of the most attractive fruit by-products. They contain high content of antioxidants, such as, phenolic compounds, ascorbic acid and carotenoids, which can be useful alternatives to synthetic antioxidants. These antioxidants can also be used as food additives and nutraceuticals among other applications (Sabino et al., 2015; Singh et al., 2016). Moreover, cantaloupe peel is also a great source of minerals and fatty acids (Sabino et al., 2015). To create value-added products, it is important to develop extraction methods, which can recover

these compounds from cantaloupe and muskmelon peels. Conventional solvent extractions of bioactive compounds from plant materials are time and solvent consuming and may involve toxic chemicals. For example, the extraction of phenolic content from cantaloupe peels and muskmelon fruit was generally performed at 25°C for about 20 h (El-Din Ibrahim & El-Masry, 2016; Sasi Kumar et al., 2014-15). Owing to the need to improve the conventional extraction methods, many researchers have been exploring non-conventional extraction methods to reduce extraction time, solvent and energy consumption, as well as to achieve higher extraction yield.

Freeze-thawing technique has been used since 1994 to separate recombinant protein from *E. coli* cell (Johnson and Hecht, 1994). This type of homogenization uses the effect of ice crystal formation in the tissue during freezing process. The repeated cycles of freezing and thawing disrupt the integrity of membranes and can lead to the formation of transient pores. This results in the release of recombinant proteins without causing total destruction of the cells. This method does not introduce any external impurities into the sample because frozen solution is isolated from the external environment (Johnson and Hecht, 1994; Bodzon-Kulakowska et al., 2007) and no heat is involved making it suitable for extraction of heat sensitive compounds. Freeze–thawing is effective towards most of the bacterial, plant and animal cells in water solution and may be used as an additional or final step after mechanical or ultrasonic homogenization (Bodzon-Kulakowska et al., 2007). However, this technique requires long process time.

Pulsed electric field (PEF) is an emerging technology which has various applications, such as, improving mass transfer process, extending shelf-life, and microbial and enzymatic inactivation without increasing the temperature of samples. The interests have been grown toward the use of PEF for extraction of desired compounds from biological materials as it leads to electroporation of cell membranes, and thus promotes extraction of intracellular compounds (Asavasanti et al., 2011; Hossain et al., 2015; Bouras et al., 2016). PEF pretreatment has a potential to facilitate subsequent extraction of nutritionally valuable compounds combined with other extraction techniques (Parniakov et al., 2016). PEF was recently used for polyphenols recovery from orange peel (Luengo et al., 2013), citrus peels (El Kantar et al., 2017), plum and grape peels (Medina-Meza & Barbosa-Cánovas, 2015), and blueberry by-products (Bobinaite et al., 2015). Despite the increasing interest toward the use of peels as a source of antioxidants,

the use of PEF in cantaloupe and muskmelon peels is scarce, incomplete or absent.

Therefore, the aim of the present study is to determine the effect of freeze-thaw cycles and pulsed electric field (PEF) pretreatment on extraction efficiency of bioactive compounds from cantaloupe and muskmelon peels and antioxidant capacity of the extracts.

Methods

Chemicals and plant materials

All the chemicals and reagents used in this study were ACS grade. Cantaloupe and muskmelon fruits were purchased from local market in Bangkok during November, 2016 – March, 2017. These fruits were washed and air-dried. Around 1 cm from each pole was cut off. The fruits was split and peeled. Pulp and seed were separated. Then, the peel was cut into cubes (1.0 x 1.0 x 1.0 (± 0.3) cm) and kept at 4±2 ^{o}C until use.

Plant material extraction

Seventy grams of peel were mixed with 140 mL of solvent in 250 mL Erlenmeyer flask. The solvent was a mixture between either acetone or ethanol to water in the ratio of 3:4 by volume. The flask was shaken at 200 rpm, 25 ^{o}C for 24 h. The extracts were filtered and the filtrate was concentrated in a rotary evaporator at 40 ^{o}C. The concentrated extracts were used for determination of extraction yield and antioxidant capacity: DPPH radical-scavenging activity (DPPH-RSA) and ferric reducing power (FRP) measurements.

Extraction yield determination

The extraction yield was calculated based on fresh sample weight as follow:

$$\% \text{ Yield} = \frac{(\text{Weight of extract (g)} \times 100)}{(\text{Weight of fresh sample (g)})} \quad (1)$$

Pretreatment methods

- Freeze-thawing (F-T)

Seventy grams of peels and 60 mL of distilled water was mixed and frozen in a freezer at -20 ^{o}C for 24 h. The sample was then thawed at 30 ^{o}C in a water bath for 2 h to reach equilibrium. The freeze-thawing process was repeated for 1, 3 and 5 cycles, respectively. The samples without F-T treatment were considered as untreated samples. Each treatment was carried out in duplicate.

- Pulsed electric field (PEF)

Seventy grams of peels and 60 mL of distilled water was mixed and filled into a Superlene® sample holder with two parallel stainless steel electrodes. The setup of PEF treatment system was shown in Figure 1. The sample was treated with PEF at the electric field strength *(E)* of 143 V/cm, frequency *(f)* of 100 Hz, and pulse width of 50 μs. The number of pulses was varies at 0, 1,000, 10,000 pulses, respectively. Each treatment was conducted in duplicate at room temperature (28±2 °C).

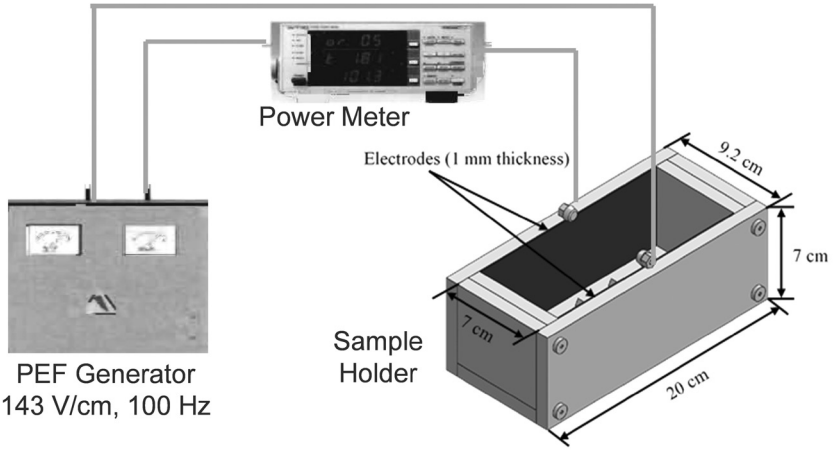

Figure 1 Pulsed electric field treatment system

Determination of tissue integrity

In order to study the effect of pretreatment methods on integrity of the peels, degree of tissue permeabilization (Z) was determined. The electrical resistance of the sample both before and after pretreatment was measured. Then, the degree of tissue permeabilization after F-T and PEF treatment was estimated based on the electrical conductivity of cantaloupe and muskmelon peel suspensions. The Z value was calculated from (Vorobiev & Lebovka, 2010):

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i} \quad (2)$$

where σ is the electrical conductivity of sample (S/m) and the subscripts i and d refer to intact and completely damaged tissues, respectively. The Z value varies

from 0 to 1, where 0 and 1 represent intact and completely damaged tissues, respectively. The electrical conductivity was measured using a conductivity meter InoLab pH/cond Level 1, WTW (Weilheim, Germany) at the temperature of 28±2 °C.

Antioxidant capacity

- DPPH radical scavenging activity

The method used was as described by Metrouh-Amir et al. (2015). DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in methanol. The samples were diluted with methanol until the absorbance in the range of 0.023 – 0.420 was reached. The reaction was initiated by adding 300 µL of a suitable dilution of sample extract to 2.7 mL of DPPH colored radical solution (0.06 mM) (Sigma–Aldrich, Steinheim, Germany). The sample was incubated for 20 min at room temperature (T = 28±2 °C). Absorbance was measured at the wavelength of 517 nm. The antioxidant activity was expressed as milligram ascorbic acid equivalent per 100 gram fresh weight (mg AAE/100 g).

- Ferric reducing power assay

The reducing power of the extracts was determined by the method of Singhal et al. (2011). The samples were diluted with distilled water until the absorbance was less than 0.952. One mL of a suitable dilution of sample extract was mixed with 0.2 M phosphate buffer pH 6.6 (2.5 mL) and 1% (w/v) potassium ferricyanide (2.5 mL). The mixture was incubated at 50 °C for 20 min. Aliquots of 10% (w/v) trichloroacetic acid (2.5 mL) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 milliliter) was mixed with distilled water (2.5 mL) and a freshly prepared 0.1% (w/v) ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Ascorbic acid (20 µg/mL) was used as standard. A blank was prepared using distilled water instead of the extract. The reducing power was expressed as milligram ascorbic acid equivalent per 100 gram fresh weight (mg AAE/100 g).

Statistical analysis

The experimental data were analyzed and presented as mean with standard deviation values. The differences between the means were established using Tukey's multiple range test at a significance level of 95% ($p < 0.05$). The statistical program MINITAB (version 16) was used to perform all statistical analysis.

Results and Discussion

Effect of freeze-thawing and PEF pretreatment on tissue integrity

The electrical conductivity of aqueous suspension of cantaloupe and muskmelon peels before and after F-T and PEF treatment was shown in Table 1 and 2. The degree of tissue permeabilization after each treatment was calculated using Eq.2. The untreated samples gave Z = 0, while Z = 0.3 to 1 were obtained for the pretreated peels. It was found that 3 cycles of freeze-thawing could completely damage the tissue structure of cantaloupe and muskmelon peels (Z = 0.9-1.0). Further increase in number of freeze-thawing cycle from 3 to 5 did not significantly increase the Z value ($p > 0.05$). On the other hand, 1,000 pulse-PEF resulted in partially damage of cantaloupe and muskmelon peels (Z = 0.3-0.4).

Table 1 The conductivity and Z value of the peel extracts after 0-5 cycles of freeze-thawing

Fruits	Cycle	Before treatment		After treatment		Z
		Resistance (Ω)	Conductivity (μS/cm)	Resistance (Ω)	Conductivity (μS/cm)	
Cantaloupe	0	860.25 ± 54.85	544.78	860.25 ± 54.85	544.78	0.0
	1	858.23 ± 62.76	546.07	167.78 ± 36.74	2,793.22	0.6
	3	874.88 ± 28.39	535.68	103.72 ±4.64	4,518.58	1.0
	5	849.35 ± 37.55	551.77	113.46 ± 2.05	4,130.44	0.9
Muskmelon	0	311.29 ± 27.84	1,505.52	311.29 ± 27.84	1,505.52	0.0
	1	324.60 ± 17.47	1,443.78	135.97 ± 11.04	3,446.75	0.6
	3	329.45 ± 3.32	1,422.52	107.49 ± 8.09	4,359.79	0.9
	5	308.18 ± 7.95	1,520.73	109.21 ± 8.74	4,291.30	0.9

Table 2 The conductivity and Z value of the peel extracts after application of PEF

Fruits	Pulses (n)	Before treatment		After treatment		Z
		Resistance (Ω)	Conductivity (μS/cm)	Resistance (Ω)	Conductivity (μS/cm)	
Cantaloupe	0	852.33 ± 2.25	549.85	852.33 ± 2.25	549.85	0.0
	1,000	848.58 ± 8.03	552.30	462.29 ± 7.52	1,013.88	0.1
	10,000	810.72 ± 51.95	579.26	213.67 ± 8.19	2,194.96	0.4
Muskmelon	0	500.86 ± 1.26	935.69	500.60 ± 3.34	936.20	0.0
	1,000	486.71 ± 39.76	966.11	310.74 ± 18.31	1,510.78	0.1
	10,000	493.89 ± 22.34	949.86	219.63 ± 4.61	2,134.34	0.3

Effect of F-T and PEF pretreatments on extraction yield and antioxidant capacity

After each treatment, the extraction yield was calculated using Eq.1. The antioxidant capacity of the cantaloupe and muskmelon peel extracts was determined by two different assays: ferric reducing power (FRP) and DPPH radical scavenging activity. The results were shown in Table 3 and 4.

- Extraction yield

For freeze-thawing treatment, the extraction yield significantly increased as the number of cycle increased from 0 to 5 cycles ($p < 0.05$). The effect of F-T cycle on extraction yield was slightly different from tissue integrity results; although the *Z* value was comparable for the samples with 3 and 5 cycles of F-T, the average extraction yield of the peels with 5 cycles of F-T was higher than that obtained from 3 cycles of F-T. Considering the solvent use, aqueous acetone solvent gave slightly higher extraction yield than the aqueous ethanol solvent. The maximum extraction yield of 2.96±0.33% and 5.50±2.61% were obtained from aqueous acetone extracts of cantaloupe and muskmelon peels after freeze-thawing pretreatment, respectively. For PEF treatment, the maximum extraction yield of 2.37±0.12% and 7.25±0.17% were obtained from aqueous acetone extracts of cantaloupe and muskmelon peels after PEF treatment for 10,000 pulses, respectively.

Table 3 Antioxidant capacity of the peel extracts after 0-5 cycles of freeze-thawing

Fruit	Cycle	Z	Solvent	% Yield	Antioxidant activity	
					FRP (mg AAE/100 g)	DPPH (mg AAE/100 g)
Cantaloupe	0	0.0	ethanol	1.43 ± 0.03	955.74 ± 49.65	159.36 ± 0.25
			acetone	1.50 ± 0.02	1,046.17 ± 102.30	196.93 ± 6.06
	1	0.6	ethanol	1.61 ± 0.01	1,090.85 ± 3.01	294.07 ± 2.02
			acetone	1.88 ± 0.17	1,173.83 ± 15.04	321.93 ± 0.00
	3	1.0	ethanol	1.70 ± 0.10	2,011.06 ± 25.58	342.54 ± 21.21
			acetone	2.26 ± 0.54	2,023.83 ± 22.57	358.07 ± 17.42
	5	0.9	ethanol	2.09 ± 0.90	2,028.09 ± 19.56	388.07 ± 0.76
			acetone	2.96 ± 0.33	2,031.28 ± 3.01	400.39 ± 93.94
Muskmelon	0	0.0	ethanol	3.38 ± 0.20	100.04 ± 1.05	136.50 ± 15.91
			acetone	3.60 ± 0.02	105.36 ± 1.65	140.43 ± 17.93
	1	0.6	ethanol	4.30 ± 1.09	133.45 ± 2.56	141.86 ± 4.29
			acetone	4.59 ± 0.96	158.55 ± 3.16	157.37 ± 0.47
	3	0.9	ethanol	4.44 ± 1.70	152.55 ± 18.05	232.29 ± 36.87
			acetone	4.74 ± 2.99	655.74 ± 43.63	301.93 ± 10.10
	5	0.9	ethanol	5.21 ± 1.33	991.91 ± 16.55	372.00 ± 5.30
			acetone	5.50 ± 2.61	1,047.23 ± 7.52	397.18 ± 12.12

Table 4 Antioxidant capacity of the peel extracts after 0-10,000 pulses of PEF pretreatment

Fruit	Pulses (n)	Z	Solvent	% Yield	Antioxidant activity	
					FRP (mg AAE/100 g)	DPPH (mg AAE/100 g)
Cantaloupe	0	0.0	ethanol	1.43 ± 0.03	1,145.11 ± 43.63	202.64 ± 1.01
			acetone	1.50 ± 0.02	1,237.66 ± 12.04	206.57 ± 0.50
	1,000	0.1	ethanol	1.95 ± 0.01	1,255.74 ± 19.56	293.79 ± 3.79
			acetone	2.24 ± 0.01	1,346.17 ± 9.03	295.79 ± 3.79
	10,000	0.4	ethanol	1.99 ± 0.01	1,580.21 ± 12.04	315.21 ± 15.91
			acetone	2.37 ± 0.12	1,613.19 ± 37.61	327.54 ± 6.06
Muskmelon	0	0.0	ethanol	3.38 ± 0.20	179.30 ± 3.61	162.29 ± 6.57
			acetone	3.60 ± 0.02	182.81 ± 0.75	164.07 ± 1.01
	1,000	0.1	ethanol	7.01 ± 0.01	234.47 ± 25.58	166.93 ± 14.14
			acetone	7.06 ± 0.02	265.32 ± 21.06	185.14 ± 5.56
	10,000	0.3	ethanol	6.76 ± 0.22	297.23 ± 39.12	202.64 ± 4.04
			acetone	7.25 ± 0.17	339.79 ± 12.04	201.21 ± 2.02

- Ferric reducing power (FRP)

Reducing power is a mechanism which measures the conversion of a Fe^{3+}/ ferricyanide complex to the ferrous form (Metrouh-Amir et al., 2015). The results of this study showed that all extracts had a good ferric reducing power and were significantly different ($p < 0.05$) according to solvent used and the fruit cultivar. The samples with higher *Z* value resulted in extracts with higher reducing power. From all treatments, aqueous acetone solvent gave extracts with slightly higher reducing power than the ones using aqueous ethanol solvent. The weakest capacity to reduce iron (III) (approx. 100-180 mg AAE/100 g) was obtained from the aqueous ethanol extracts of the muskmelon peel without any pretreatment. Aqueous acetone extract of the peels showed a highest reducing capacity, i.e., 2,031.28±3.01 and 1,047.23±7.52 mg AAE/100 g for cantaloupe and muskmelon peels with 5 cycles of freeze-thawing pretreatment, respectively. On the

other hand, using 10,000-pulse PEF pretreatment gave the extracts with lower reducing power than the ones obtained from freeze-thawing pretreatment; the maximum reducing power of 1,613.19±37.61 mg AAE/100 g was obtained from the aqueous acetone extracts of the cantaloupe peel.

- DPPH activity

1,1-diphenyl-2-picrylhydrazyl (DPPH·) is a stable free radical, which is reduced by reacting with an antioxidant to 1,1-diphenyl-2-picrylhydrazine (DPPH-H) (Molyneux, 2004). The ability of plant extracts to scavenge the DPPH radical varied significantly ($p < 0.05$) according to the solvent used and the fruit cultivar. Similar to FRP results, the aqueous ethanol and aqueous acetone extracts of cantaloupe peels exhibited the best DPPH scavenging capacity, followed by aqueous ethanol and aqueous acetone extracts of muskmelon peels. The weakest DPPH scavenging capacity was obtained in the aqueous ethanol extracts of muskmelon pulp without any pretreatment.

Singh et al. (2016) have investigated the polyphenolic content and antioxidant capacity of muskmelon peel used the solvent system of ethanol and acetone at three different concentrations in distilled water (50, 70, and 100%) and 100% distilled water. They found that the highest extraction was by 50% acetone in case of peels and 50% ethanol in case of pulp. The best solvent was 50% acetone as it gave highest yield as well as showed highest correlation between various assays. The polyphenolic content and the antioxidant activity were high in peels than pulps. The last was also the same for cantaloupe (*Cucumis melo*) methanolic extracts (Ismail et al., 2010). Other reports also mentioned that fruit-peel extracts had markedly higher antioxidant capacity than the pulp extract and might be rich sources of natural antioxidants (Guo et al., 2003; Li et al., 2006; Xue et al., 2009; Manzoor et al., 2013; Aquino et al., 2016).

Conclusion

The acetone based extract of cantaloupe skin pretreated with 5 cycles of F-T gave the highest antioxidant activity (2,031.28±3.01 mg AAE/100 g and 400.39±93.94 mg AAE/100 g for FRP and DPPH methods, respectively) with 2.96% extraction yield. On the other hand, the acetone based extract of cantaloupe skin pretreated with PEF for 10,000 pulses gave the antioxidant activity of 1,613.19±37.61 mg AAE/100 g and 327.54±6.06 mg AAE/100 g for FRP and DPPH methods, respectively) with 2.37% extraction yield.

Although the PEF treatment gave the extract with less antioxidant activity and less yield, the time used by this method was much shorter than that of F-T, i.e., 1 min 40 s for PEF and 5 days for F-T. Thus, PEF is considered to be more energy efficient and economically sound than F-T. In addition, PEF pretreatment could yield greater extraction efficiency if higher electric field strength or number of pulses is applied.

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