Phenolic Compound Extraction from Industrial Tomato Waste by Ultrasound-Assisted Extraction

N Sengkhamparn¹* and N Phonkerd¹
¹Faculty of Applied Science and Engineering  Khon Kaen University, Nong Khai Campus Nong Khai, Thailand, 43000
nipaporn@kku.ac.th

Abstract. The tomato fruits have been reported that it contained high nutrient and bioactive compounds. The various Ultrasound-Assisted Extraction (UAE), including extraction temperature of 30, 50 and 70°C and extraction time of 10, 30 and 50 min, were performed in order to extract phenolic compound from tomato waste from industry. The total phenolic compound, total flavonoid content and also retained carotenoid in obtained extract were determined. Moreover, the DPPH radical scavenging activity of extract were studied. The results showed that the extraction temperature was a main factor which affected the amount of extracted bioactive compound. Moreover, the antioxidant activity of the extract was related to the total flavonoid and carotenoid content which can be draw the regression as follows; Y = 55.81 +0.75X1 + 0.96X2 when radical scavenging activity, total flavonoid and carotenoid content was Y, X1 and X2, respectively. This regression analysis showed high significant (R² = 0.74).

Moreover, the UAE condition of 50°C for 50 min was a best condition which gave the highest antioxidant activity and in accordance with the high amount of total flavonoid and carotenoid content.

1. Introduction
Tomato (Solanum lycopersicum L.) is one of the health plants which provide important nutrients such as dietary fiber, protein sugars, mineral (potassium and folate), vitamin (A, E, C) and contain bioactive compound like carotenoids and phenolic compound [1,2]. They are consumed as a fresh vegetable and also processed into many food products like ketchup, paste, sauce, juice. Due to their health properties, tomatoes and also tomato products were increasingly demand for years [2]. During commercial tomato manufacturing, it generally produces a lot of wastes. The component of waste obtained from tomato product industry are varied depending on product processing. These wastes can be used as raw material for bioactive compound extraction such as carotenoids [3,4], pectin [5]. The wastes provided from ketchup industry were consisted of seed, skin and some pulps [3]. Many researches have been studied about the carotenoid contents in tomato by-product however the phenolic contents in by-product have not been limited studied.

Phenolic compounds are found in plants tissue of fruits, vegetables and grains which produced for against microorganism infections and insect injuries [6]. Moreover, they also may reduce risk of prostate, heart diseases and cancer and also have biological effect like anti-inflammatory, anti-allergic and antibacterial [2,6]. There have been reported that the flavanones such as naringenin and flavonols such as quercetin, rutin and kaempferol were the main phenolic compound in tomato [7]. Even, these phenolic compound was be stable during industry processing, but the thermal processing can be also affected to the phenolic content. Crozeir et al [8] reported that the quercetin content in tomatoes was...
decreased during microwaving, boiling and frying. Moreover, Chang and Liu [9] found that the total flavonoid and phenolic content was be declined during hot air drying. In addition, Vallverdú-Queralt et al. [7] found that during step of paste processing, the phenolic and antioxidant activity was differently changed. Kelebek et al [10] reported that the processing of paste by using low temperature (cold break) can increased the phenolic acids more than that using high temperature (hot break).

Ultrasound-assisted extraction (UAE) has been reported that it was an effective method for the extraction of bioactive compounds due to low extraction time and solvent consumption [11,12]. This technique is used the ultrasonic wave that produce a cavitation phenomenon and caused the damage of solid thus the solvent penetration and mass diffusion will be occurred [12]. However, the extraction temperature and time were affected to the yield. Espada-Bellido et al. [12] found that the extraction temperature was the importance factor for anthocyanin and phenolic compound from mulberry (Morus nigra) pulp.

Therefore, this study was aimed to use UAE for phenolic compound extraction from industrial tomato waste. The antioxidant activity of obtained extract was also determined.

2. Material and Methods

2.1 Material
Tomato waste from the tomato paste industry was dried at 60°C for 24 h, then was ground and kept at -18°C before further experimentation.

2.2 Physicochemical of tomato waste powder
The chemical composition including protein, fat, crude fiber, ash, and carbohydrate content of tomato waste powder was determined according to AOAC 2000 [13]. The results are expressed as g per 100 g dry tomato waste powder. The Color of powder was also observed and expressed as L*(lightness), a* (redness) and b* (yellowness).

2.3 Ultrasound-assisted extraction
The tomato waste powder (5 g) was added to 95 % ethyl alcohol at a solid/solvent ratio of 1/20 w/v and sonicated at 30, 50, and 70°C for 10, 30, and 50 min each. Then, the separation was performed by using Hexane containing 0.1 % BHT for fractionation of hydrophobic and hydrophilic part. The hydrophilic part was collected and concentrated by using rotary evaporator. The crude extracted was stored at -18°C for further analysis.

2.4 Total phenolic content
The total phenolic content of the crude extract was determined using Folin-ciocaltau method. The 0.4 mL of crude extract was mixed with 4 mL of 2% Na₂CO₃ and 0.2 mL of Folin-ciocaltau reagent. Then, the mixed solution was left for 30 min and the absorbance of the solutions was measured at 750 nm. Results are expressed as micrograms of gallic acid equivalent per g of dry weight of tomato waste powder.

2.5 Total flavonoid content
The total flavonoid content of the crude extract was determined. The 500 µL of extract was mixed with AlCl₃ and left for 10 min. the absorbance of the solution was measured at 430 nm and the results were expressed as micrograms of catechin equivalent per g of dry weight of tomato waste powder.

2.6 Carotenoid content
The carotenoid content of crude extract was determined using spectrophotometer at 503 nm. The results were expressed as mg beta-carotene equivalent per g of dry weight of tomato waste powder.
2.7 DPPH radical scavenging activity
The 2 mL of crude extract was mixed with 2 mL of 0.2 mM DPPH in ethanolic solution. The solution was left in the dark for 10 min. the absorbance of the solution was measured at 515 nm. The ethanol was used as a control solution instead of the extract. The DPPH radical scavenging activity was calculated with the following equation: Radical scavenging activity (% ) = (Acontrol - Asample)x100 / Acontrol

2.8 Statistical analysis
All results were done in triplicate and expressed as the mean with standard deviation and the treatment comparison was done by Duncan’s New Multiple Range Test at significance level of p<0.05. Moreover, the correlation and regression analysis were performed at significance level of 0.05.

3. Material and Methods

3.1 Physicochemical of tomato waste powder
The tomato waste from industry was dried and chemical composition and physical properties were determined. The results showed that the tomato waste powder contained of protein, crude fat, crude fiber and carbohydrate of 22.81±0.24, 11.89±0.56, 54.61±0.78, 2.75±0.02 and 7.88±1.12 % (% dry basis), respectively. Moreover, it exhibited the lightness, redness and yellowness as 51.30±0.03, 22.45±0.18 and 39.87±0.56, respectively.

3.2 Total phenolic content
The total phenolic content of crude extract is showed in figure 1. The results showed that the extraction time was not significantly affected to the total phenolic content, while the extraction temperature was significantly affected the total phenolic yield. The extraction temperature of 50°C gave the lowest yield, while that of 70°C and 30°C were comparable.

3.3 Total flavonoid content
The total flavonoid content of crude extract is showed in figure 2. The results showed that the higher extraction temperature, the higher flavonoid yield. Moreover, the extraction time of 30 min and 50 min were comparable.
3.4 Carotenoid content
Due to the extract showed an orange solution, therefore the carotenoid content was determined and compared to beta-carotene. The results are shown in table 1. These results showed that even though the fractionation was performed, still carotenoid was found to retain in the extract. Moreover, the higher extraction time, the higher carotenoid content was found.

3.5 DPPH radical scavenging activity
The phenolic compound has been reported to be an antioxidant compound which found in natural plant. Therefore, the DPPH radical scavenging activity of extract were determined as shown in table 1. The results exhibited that the higher extraction temperature, the higher activity was observed.

Table 1. Carotenoid content and radical scavenging activity of the extract.

| Extraction condition | Carotenoid content (mg β- carotene/g DM) | Radical scavenging activity (%) |
|----------------------|------------------------------------------|---------------------------------|
| 30 °C/10 min         | 52.35 ± 15.93b                          | 61.84 ± 8.17c                  |
| 30 °C/30 min         | 53.01 ± 10.85b                          | 67.73 ± 2.08e                  |
| 30 °C/50 min         | 48.37 ± 14.05b                          | 66.69 ± 5.58c                  |
| 50 °C/10 min         | 78.19 ± 9.37bc                          | 71.52 ± 8.25bc                 |
| 50 °C/30 min         | 100.06 ± 68.41bc                        | 72.59 ± 12.41bc                |
| 50 °C/50 min         | 134.30 ± 29.32a                         | 85.87 ± 1.37a                  |
| 70 °C/10 min         | 117.95 ± 12.78a                         | 81.73 ± 2.51a                  |
| 70 °C/30 min         | 58.31 ± 28.11b                          | 79.84 ± 9.52a                  |
| 70 °C/50 min         | 84.50 ± 14.03ab                         | 86.19 ± 0.53a                  |

* Different letter in each column shows the significant difference (p<0.05) of the values.

3.6 Regression analysis
The multiple regression coefficients analysis for radical scavenging activity found that both total flavonoid and carotenoid content was found to be significant (p<0.05). Moreover, the multiple regression coefficient analysis and the ANOVA for this regression are showed in table 2 and 3 with the correlation coefficient of 0.74. The relation of residual and radical scavenging activity observed was plotted (figure 3) and showed that no irregular values was found.

Table 2. Coded regression coefficients, standard error and p values for building the model.

| Coefficients | Standard Error | t Stat | P-value |
|--------------|----------------|--------|---------|
| Intercept    | 55.81          | 2.47   | 22.59   | 1.11x 10^{-17} |
| flavonoid    | 0.75           | 0.17   | 4.50    | 0.000148       |
| carotene     | 0.96           | 0.39   | 2.46    | 0.02159        |

Table 3. ANOVA for the regression

|                | df | SS       | MS       | F       | Significance |
|----------------|----|----------|----------|---------|--------------|
| Regression     | 2  | 2081.81  | 1040.907 | 39.06762| 2.83 x 10^{-8}|
| Residual       | 24 | 639.45   | 26.64373 |         |              |
| Total          | 26 | 2721.26  |          |         |              |
4. Discussion

During the commercial processing of tomato paste, the large amount of waste was produced. This waste has been used as a raw material for bioactive compound extraction [3-5]. The tomato waste was obtained from tomato industry in Nongkhai, Thailand. The chemical composition of tomato waste powder was determined. The results showed that the tomato waste powder contained mainly crude fiber which was 54.61 % which was lower than that of reported by Kanyakam and Uriyapongson [14]. This probably due to the method, this study has used the hot acid and alkali solution to digest while Kanyakam and Uriyapongson [14] used the enzyme which more specific for determine the fiber content. The tomato waste powder showed higher redness and yellowness pointed to lycopene content in the sample. However, the phenolic content has been also reported that it found in tomato and the research in this point of view was limited.

The UAE method has been reported that it was an efficiency method due to lower time and solvent consumption. Therefore, the phenolic compound was extracted from industrial waste by UAE. The various UAE condition were performed and the results showed that the extraction temperature was more important factor than extraction time for total phenolic content (figure 1). The extraction temperature of 50°C yielded the lowest phenolic compound this was probably due to the activity of polyphenol oxidase (PPO). The activity of PPO in tomato has been reported that it has highest activity at 55°C [15], hence the phenolic compound was degraded higher than others. Manieddine et al [16] reported that some phenolic compound in tomato has been degraded by heat treatment for example hydroxycinnamic acid, while some phenolic such as caffeic acid and chlorogenic acid has be found to be increased. Moreover, the extraction temperature of 70°C was comparable to that of 30°C. This may be explained by the cavitation effect during extraction. The cavitation effect was a phenomenal concerning the collapse of bubbles near by the surface of material, hence caused the damage of cell wall. This effect was interrupted during higher temperature due to the high-pressure vapor of solvent. Therefore, the cavitation effect at temperature of 70°C was lower than that of 30°C so the phenolic yield could be declinable extracted at temperature of 70°C.

Similar to phenolic yield, the extraction temperature was more important factor. The results showed that the higher extraction temperature, higher flavonoid was extracted (figure 2). This can be explained by the increasing of flavonoid solubility to the solvent at the higher extraction temperature. Moreover, the cavitation effect seems to less affected to the flavonoid yield than phenolic yield. In addition, the flavonoid content in the extract was higher than phenolic content in accordance to Vallverdú-Queralt et
and al [7] which reported that the main phenolic compound was flavanones and flavonols. However, the results were variated due to the tomato waste powder was consisted of seed, skin and pulp.

The obtained extract showed red-yellow liquid, this may be because of the retain of carotenoid compound. The carotenoid compound in the extract was determined and compared to the beta-carotene standard. The results (table 1) showed that the extraction temperature of 50 and 70°C was comparable and the extraction temperature of 50°C for 50 min gave the highest carotenoid content. This can be explained by the cavitation effect during sonication. This effect enhanced the temperature of solvent and also the micro-jet on material surface which caused the higher solubility of bioactive compound. At higher extraction temperature (70°C) the cavitation phenomenon was affected by vapor of solvent; hence the lower yield of carotenoid was found. Moreover, this results also pointed that even though the fractionation of the crude extract by Hexane, still some carotenoid retained in the hydrophilic part. This carotenoid compound can be affected to antioxidant activity of the extract.

Antioxidant activity of extract was performed using DPPH radical scavenging assay. The results (table 1) showed the extraction temperature still was an important factor. Moreover, the extraction condition at 50°C for 50 min and at 70°C for all extraction gave the high radical scavenging activity. This probably due to at this condition, the bioactive compound especially flavonoid and carotenoid was extracted. Moreover, the correlation coefficient of each bioactive compound and antioxidant activity was 0.83 and 0.75 for flavonoid and carotenoid content, respectively.

The Multiple regression coefficients for antioxidant activity showed that only the total phenolic compound was not significant at a significance level of 0.05. Therefore, both the total flavonoid and carotenoid content were used for regression analysis and the coefficient was showed in table 2 and the regression was significant, by the significant F was lower than 0.05 (table 3). It can be drawn the regression equation (R² = 0.74) as follow;

\[ Y = 55.81 +0.75X_1 + 0.96X_2 \]

when Y was radical scavenging activity, \(X_1\) and \(X_2\) was total flavonoid and carotenoid content, respectively. The residual plot versus radical scavenging measured exhibited that the values are low and does not appear to show any regular trend. It can be concluded that extraction condition at 50°C for 50 min was the best condition due to it gave high flavonoid content and also carotenoid content which showed the highest antioxidant activity.

5. Conclusion
The tomato waste from industry was used as raw material for phenolic compound extraction. The various UAE condition were performed. The results showed that the extraction temperature was the main factor that affect to the yield of bioactive compound. The higher extraction temperature, the higher flavonoid and carotenoid content was found. The total phenolic content was found lesser than flavonoid content. Moreover, radical scavenging activity of extract was related to the amount of flavonoid and carotenoid in the extract. This can be concluded that the extraction condition of 50°C for 50 min assist with ultrasonication was the best condition.

6. Acknowledgement
The researchers gratefully thank the Research and Technology Transfer Affairs of Nong Khai Campus, Khon Kaen University (KKU-NKC60-009), for their financial support. Moreover, we are also thankful to Supalak Prasertsar and Pimchanok Haputon for their help in experimentation.
References

[1] Coyago-Cruz E, Corell M, Moriana A, Mapelli-Brahm P, Hernanz D, Stinco C M, Beltrán-Sinchiguano E and Meléndez-Martínez A J 2019 Food Chem. 277 480–9

[2] Katırc N, Işık N, Güpür Ç, Ozge Guler H, Gursoy O and Yılmaz Y Journal of the Saudi Society of Agricultural Sciences, https://doi.org/10.1016/j.jssas.2018.11.003

[3] Kaur D, Wani A A, Oberoi D P S and Sogi D 2008 Food Chem. 108 711–8

[4] Poojary M M and Passamonti P 2005 Food Chem. 188 84–91

[5] Grassino A N, Brnčić M, Vikić-Topić D, Roca S, Dent M and Brnčić S R 2016 Food Chem. 198 93–100

[6] Hongyan L, Zeyuan D, Tao W, Ronghua L, Steven L and Rong Tsao H 2012 Food Chem. 130 928–36

[7] Vallverdú-Queralt A, Medina-Remón A, Casals-Ribes I, Andres-Lacueva C, Waterhouse A L and Lamuela-Raventos R M 2012 LWT - Food Sci. Technol. 47 154–60

[8] Crozier A, Lean M E J, McDonald M S and Black C 1997 J. Agr. Food Chem. 45 590–5

[9] Chang C H and Liu Y C 2007 J. Food Sci. 72 E532–40

[10] Kelebek H, Selli S, Kadiroğlu P, Kola O, Kesen S, Uçar B and Çetiner B 2017 Food Chem. 220 31–41

[11] Ma Y-Q, Chen J-C, Liu D-H and Ye X-Q 2009 Ultrasound. Sonochem. 16 57–62

[12] Espada-Bellido E, Ferreiro-González M, Carrera C, Palma M, Barroso C G and Barbero G F Food Chem. 219 23–32

[13] AOAC 17th ed. 2000 (USA: Washington DC)

[14] Kanyakam K and Uriyapongson J 2010 Agr. Sci. J. 41(3/1)(Supplement) 289–92

[15] Shahriar saeidian and Bahaaldin Rashidzad 2013 IRJABS. 4 (11) 3306–11

[16] Mahieddine B, Amina B, Faouzi S M, Sana B and Wided D 2018 AOAS. 63 135–9