Kinetic of total phenolic content and profile of antioxidant activity during the roasting of peanut kernel

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Abstract. In this study, the functional properties, which include total polyphenol content and antioxidant activity of peanut during roasting was investigated. The main focus was to evaluate the kinetic of total phenolic content during peanut roasting using various temperature-time combinations of 120, 150 and 170°C, at 15, 30, 45 minutes, respectively. The kinetic was assessed using the Arrhenius equation for determining kinetic parameters including kinetic order and activation energy. Furthermore, the presence or absence of the correlation antioxidant activity with total phenolic content was also studied. The results showed that total phenolic content changed positively with increasing temperature and roasting time. Meanwhile, the kinetic of phenolic content, which followed zero-order reaction within the activation energy of reaction was 113.07 kJ/mol. It was also discovered that there is a positive correlation between total phenolic content and antioxidant activity. Furthermore, the increase in total phenolic content of peanut induced by roasting made the capability of peanut in scavenging radicals stronger.

1. Introduction

Peanut produced worldwide is usually applied in meal as snack or confectionary. It not only has a delicious taste but also enormous beneficial effects for human body. Its macronutrient contains balance composition, i.e. 16% carbohydrate, 25% protein and 50% lipid [1]. This composition provides enough energy for human and usable to manage malnutrition problem. The major portion of its carbohydrate is composed of sucrose and starch, with reducing sugar as minor constituent. Interestingly, the carbohydrate constituent accounts for the 14-scale glycemic index ranking of peanut [2]. In regards to its lipid component, peanut is dominantly composed of monounsaturated and saturated fatty acid by which it is possible to control cardiovascular system and blood glucose. Oleic acid becomes the dominant portion of peanut’s unsaturated fatty acid that is able to interact with the hormone present in the intestine, in order for its fullness to be prolonged [1]. Meanwhile, peanut contains protein consisting of some essential amino acids. Arginine, which occupies the biggest portion of amino acids, has greatly contributed to human body. Several metabolic mechanisms of human body, such as protein, urea, and creatine synthesis, involve the use of arginine. Available and long-term administration of arginine is
capable of stimulating the production of insulin by the pancreas and reduces cardiovascular disease as well [3]. In addition, it plays a role in maintaining immunity and repairing tissues in the human body.

Superiority of peanut as functional food is not only considered in terms of its macronutrient but micronutrient side as well. Peanut is well known as dietary fiber source which is dominantly located in its skin. Dietary fiber is well-known as one of components, which play an important role in digestive system. However, the other health benefits associated with peanut skin have not been studied. Meanwhile, it is a potential source of phenolic compound [1]. The activities of the phenolic compound of peanut skin includes antioxidant, anti-inflammatory and anti-carcinogenic. Phenolic compound of peanut skin has various types, which include the free, esterified and bound or polymer form. Free phenolic acids (caffeic, ferulic, p-coumaric acid) contribute a minor portion [4]. Meanwhile, major phenolic compound owned by proanthocyanidin contributes 17% by weight of the skin [5]. Unfortunately, several years ago, the peanut skin was usually rejected from peanut-based product in order to reduce bitter aftertaste and get well-visual aspect. However, with increasing knowledge and awareness, some peanut-based food products have been developed by enclosing the peanut skin into the products.

Roasting as one of the high-temperature processing methods is capable of decreasing some qualities of peanut. The physical parameter, peanut’s colour, is one of the quality parameters that was reported to have decreased during roasting and the kinetic of decreased coloration during roasting has been studied by some researchers [6,7]. Conversely, the functional qualities of peanut, phenolic content and antioxidant activity, increased during roasting [1]. Furthermore, this high-temperature treatment liberated bound or polymer type into free phenolic compound. The cell wall of peanut may also be disturbed and the solubility of phenolic compounds increased as well. During roasting process, the presence of sugar and protein induce non-enzymatic browning (Maillard) reaction. The products of Maillard reaction, like tetrahydrofuran (THF), melanoidin, pyrazine and other heterocyclic compounds have antioxidant activity [1].

Although, the increase in phenolic content and antioxidant activity during roasting has been reported, evaluation of kinetic of phenolic content during peanut roasting was limitedly published. Through this study, the kinetic of phenolic content was evaluated, in order to obtain the kinetic parameters including reaction order and activation energy by applying Arrhenius equation. Furthermore, the parameters are usable in predicting the phenolic content as dependence factor of roasting time and temperature. To complete this study, the antioxidant activity was evaluated by correlating it with phenolic content.

2. Material and Methods

2.1. Material
The sun-dried peanut kernel (var. Kancil) separated from the shell was obtained from Gunungkidul district after harvesting. Furthermore, water (CV. General Labora, Indonesia) and methanol (Merck Millipore, USA) were used for extraction. Meanwhile, chemicals used for analyzing the total phenolic content and antioxidant activity fulfilled analytical grade.

2.2. Methods

2.2.1. The roasting of peanut. Initially, peanut kernel with skin was sorted manually based on its colour, size, form, presence/absence of damage and density when it was soaked into water. Furthermore, it was washed with tap water, dried using cabinet dryer until the weight was constant. The peanut was roasted in batch rotary oven according to Lin et al. (2016) with modification [8]. Approximately 40g of each sample was roasted in an oven at temperatures of 120, 150 and 170°C, each for 15, 30 and 45 minutes. During the roasting process, each sample was laid in a perforated cylindrical stainless-steel tray which was coupled into the oven. Subsequently, the roasted peanut was cooled slowly by bringing it in contact with ambient air. It was further milled to obtain fine sizes using a blender. Finally, grounded peanut was packed into plastic and stored in cooler at a temperature of 4°C prior to analysis.
2.2.2. Aqueous extraction of bioactive phenolic and antioxidant compounds. Phenolic compounds of peanut were extracted from ground roasted peanut following the Lemos et al. (2012) method with modifications [9]. Approximately 4.0 g of fine-ground roasted peanut was suspended in 20 ml of pure methanol. The next step was to incubate the suspension, which was carried out in the rotary shaker at a temperature of 30°C for 30 min. Afterwards, 20 ml of methanol was added and mixed with the suspension and it was further subjected to sonication for 30 min. The liquid was separated from the solid part by centrifuging for 15 min at 5000 rpm. For proper separation, filtration of the centrifuged liquid was carried out using filter paper Whatman No.1. The clear liquid extract was further analyzed for its phenolic content and antioxidant activity.

2.2.3. Assay for estimating total phenolic content. Spectrophotometric method was used to determine the total phenolic content [10]. It was expressed as gallic acid equivalent (GAE), therefore gallic acid was used as the standard solution for making standard curve. The gallic acid was dissolved by aquadest resulting in various concentrations, which ranged from 1.56 to 100 µg/ml. Meanwhile, 4-fold dilution of the sample extract using aquadest was carried out to obtain absorbance in the range of standard curve. Furthermore, diluted extract solution (or standard solution) was mixed with aquadest and 10% Folin-Ciocalteu’s reagent with volume ratio of 1:1:1 respectively. After 3 minutes, the mixture was mixed with 1 M Na₂CO₃ solution at volume ratio of 3:1 to stop the reaction. The mixed solution was incubated with no light condition at room temperature for 30 min. After incubating, the absorbance of the sample and standard was measured using Ultraviolet-visible light (UV-Vis) spectrophotometer (Dynamica HALO RB-10, UK) at wavelength of 750 nm. The interference of the sample’s colour was corrected by replacing both 10% Folin- Ciocalteu’s reagent and 1 M Na₂CO₃ solution with aquadest. Meanwhile, blank solution contained aquadest only. By following the formula, total phenolic content (TPC) expressed as mg gallic acid equivalent (GAE) per 100 g of dry basis of peanut was obtained [11].

\[
\text{TPC (mg GAE per 100 g of dry basis of peanut) = } \frac{AxDFxV}{Wx10} \quad \text{Eq. 1}
\]

Where: A = sample’s concentration based on standard curve (µg/ml), DF = dilution factor (4), V = volume of extraction (40 ml), W = dry-based weight of peanut (g).

2.2.4. Assay for quantifying antioxidant activity. DPPH (2-diphenyl-1-picrylhdrazyl) radical-scavenging activity was utilized for quantifying antioxidant activity. The assay followed Brand-William et al. (1995) method with slight modification [12]. Initially, DPPH diluted into various concentrations (0-100 µM) were measured for the absorbance and the DPPH calibration curve was further constructed.

For sample analysis, serial dilution was carried out. The diluted sample in various concentration were poured into 96-microwell. Furthermore, 75 µM DPPH solution was mixed into diluted sample with similar volume. A control, DPPH solution which was eliminated of any antiradical substances, was made by mixing ethanol 80% with 75 µM DPPH solution in similar volume also. The colour of sample solution was corrected by creating a mixture of ethanol 80% and sample solution, ethanol 80% as blank.

The absorbance of solutions was measured using Elisa Reader (Thermo Scientific Multiskan GO Type 1510, Finland). Initially, microwell was shaken for 10 seconds to homogenize solution. Immediately, absorbance was determined at wavelength of 515 nm. It was assumed that the measurement was carried out at initial time (t=0 min), therefore the absorbance of control was used for calculating initial DPPH. The next step was incubation with no light condition for 30 min to allow the scavenging of DPPH radical by antioxidant present in the sample. After incubation was completed (t=30 min), the microwell was shaken immediately and the absorbance of the solution was read at a wavelength of 515 nm. The absorbance value was used to determine concentration of remaining DPPH.

The next step was to calculate the percentage of remaining DPPH according to the following formula [12]:

...
Each sample concentration had certain remaining DPPH percentage. The correlation of the two parameters was described at graphical plot, which was used to determine the concentration of sample capable of reducing the concentration of DPPH by 50%. The sample concentration is usually stated as effective concentration (EC50). For clarity, antiradical power (ARP=1/EC50) was preferably used because it has positive correlation with antioxidant activity [13].

2.2.5. Determination of moisture content. Moisture content data was used in calculating total phenolic content, because it was stated in dry bases of peanut. The thermogravimetric method according to AOAC, 950.46 (AOAC, 1995) was used as protocol in the determination of moisture content [14].

2.2.6. Kinetic of phenolic compound during roasting. The kinetic of increasing total phenolic content during roasting was approached and simplified through the chemical reaction kinetics.

\[
\text{Phenolic (in esterified, bound, polymeric form)} \rightarrow \text{Free phenolic compound} \\
\text{A} \rightarrow \text{P}
\]

The rate of product (P) was written as follows:

\[
(rp) = kC_p^n \quad \text{Eq. 3}
\]

By a batch system approach, kinetics of increasing phenolic content was evaluated as follows:

\[
\frac{dC_p}{dt} = kC_p^n \quad \text{Eq. 4}.
\]

Using integral method, equation (4) was converted to:

\[
C_p^{1-n} - C_{p0}^{1-n} = (1 - n)kt \quad \text{Eq. 5}
\]

Where, \(C_{p0}\) is initial concentration of phenolic content, \(t\) is reaction (production) time, \(C_p\) is concentration of phenolic content at time \(t\) and \(n\) is reaction order

Exception for first reaction order (\(n=1\)) which the equation (4) is changeable to:

\[
\ln C_p = \ln C_{p0} + kt \quad \text{Eq. 6}.
\]

To obtain the value of the kinetic constant of the rising of total phenolic content by roasting (k) and to ascertain whether the k value is a function of temperature or not, using the Arrhenius equation as follows:

\[
k = Ae^{-\frac{E}{RT}} \quad \text{Eq. 7}.
\]

Using linearization, it is possible to change equation (5) to:

\[
\ln k = \ln A - \left(\frac{E}{R} \right) \left(\frac{1}{T}\right) \quad \text{Eq. 8}.
\]

Where A= pre-exponential factor (frequency factor), \(T\)=temperature, \(E\)= activation energy

2.2.7. The assessment of kinetic’s accuracy. Kinetics of increasing phenolic content was evaluated using zero, first and second-order reaction. For evaluating the accuracy, kinetic constant (k) was searched by minimizing Root Mean of Sum Square Error (RMSE). The RMSE value is formulated as follows [15]:

\[
\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (C_{mi} - C_{ci})^2} \quad \text{Eq. 9}
\]

Where \(C_{mi}\) is measured phenolic content, \(C_{ci}\) is calculated phenolic content, and \(N\) is the number of experimental points. Smaller RMSE value indicates better order kinetic.
3. Results and Discussion

Critical parameters of roasting, which include time and temperature affected total phenolic content as shown in Fig 1 (a).

![Fig 1](image)

Fig 1. The influence of time and temperature of roasting on total polyphenol content of peanut

From Fig 1 (a), it is clearly described that the roasting process represented by time and temperature increased the measured total phenolic content. It was in agreement with other studies on roasting of peanut [1], heating of honey [16] and roasting of grape seed [17], which also resulted in the increase of total phenolic content. In food matrix, the phenolic compounds covalently bounded to cell wall, protein, pectin and fiber in esterified and polymeric forms which are predominantly insoluble [18]. The high temperature of roasting led to the liberation mechanism. Therefore, occurrence of the breakdown of polymeric and esterified polyphenols, resulted in free phenolic compounds. The other reason is peanut’s cell wall breakage by which the extracted total phenolic content extracted from the solvent increased. Furthermore, the increase in phenolic extractability improved the phenolic content measured spectrophotometrically as well [8]. This result was also in accordance with the discoveries of Talcott et al. (2005) which exhibited that roasting enhanced phenolic acids as free forms of phenolic substances including p-hydroxybenzoic, chlorogenic and p-coumaric [19]. Mandari et al. (2010) indicated that the phenolic acids may be classified as cell-wall bound phenolic compounds [20]. Nevertheless, roasting alter them to free form compounds.

Meanwhile, data of phenolic content kinetic of peanut kernel simulated by some reaction orders is shown in Table 1.

| Temperature (°C) | Zero-order k | RMSE | First-order k | RMSE | Second-order k | RMSE | Ea (kJ/mol) by zero-order |
|------------------|--------------|------|---------------|------|----------------|------|-----------------------|
| 120              | 0.0233       | 2.053 | -1.4354x10^10 | 31.888 | -1.268x10^13 | 31.888 |
| 150              | 0.5401       | 2.092 | -1.4354x10^10 | 46.801 | -4.837x10^12 | 46.801 | 113.07 |
| 170              | 1.0008       | 3.962 | -1.4354x10^10 | 59.672 | 3.627x10^4   | 8.874  |

Note: k=kinetic constant of total phenolic content of roasted peanut; Ea= activation energy of increasing in peanut’s total phenolic content; RMSE= root of mean of square error

From Table 1, it was seen that zero-order kinetic yielded the lowest Root Mean of Square Error (RMSE). This implies that zero order is more suitable in depicting total phenolic content profile during peanut roasting. This is in accordance with the study of total phenolic content of honey during heating [16]. By zero-order kinetic, the increase in total phenolic content and change of roasting time were in positive correlation. This implies that the kinetic of the enhancing of phenolic content was not dependent on concentration. The activation energy (Ea) of increasing of peanut’s total phenolic content during roasting was in same range to other heat-treated food commodities, such as boiled grape juice and heated
honey with Ea of 132 kJ/mol and 64.7 kJ/mol, respectively. It means that peanut was more sensitive compared to heat grape juice, but had lower sensitivity compared to honey. The difference may be related to food matrix structure.

Meanwhile, the effect of roasting on antioxidant activity of roasted peanut kernel is shown in Fig 1 (b). It is firmly show that roasting temperature and antioxidant activity are in direct proportion. Roasting at 150°C exhibited a sharp increase in antioxidant activity compared to at 120°C, but the difference in antioxidant activities at roasting temperature of 150°C and 170°C was not significant for all time of roasting. In accordance with the influence of roasting time on antioxidant activity, roasting in 15 min improved antioxidant activity significantly. However, prolonged roasting above 15 min towards 45 min (after 15 until 45 min), altered the increase in antioxidant activity insignificantly.

The increase in antioxidant activity by roasting was regarded with total phenolic content and also the non-enzymatic browning reaction (Maillard reaction). The responsibility of total phenolic content to antioxidant activity is shown in Fig 2.

**Fig 2.** Correlation of total phenolic content and antioxidant activity

From Fig 2, it is seen that the rising of total phenolic content by roasting process caused an increase in antioxidant activity. This result indicates that total phenolic content has a strong positive correlation with antioxidant activity. Phenolic compounds of roasting peanut exhibited antioxidant properties by which the oxidation of substrate is preventable or delayed [21]. The mechanism of phenolic compound as antioxidant include directly scavenging free radical, chelating metal ions as pro-oxidant, donating electron or hydrogen followed with formation of stable hydroperoxide substances [22].

High temperature induced by roasting causes the reaction between substrates of amino acid and reducing sugar by which some product substances having antioxidant activity are exhibited, one of which is melanoidin. Furthermore, it increased antioxidant activity, which is seen at temperatures of 120°C and 150°C. However, the increase was restricted when the data at temperatures 150°C and 170°C were compared. The concentration of the substrate might have restricted the Maillard reaction. It was also possible that the substrates almost reacted completely at 150°C.

Similar results were also discovered in other works. The roasting process increased phenolic content and antioxidant activity in the case of sesame seed [23]. Higher antioxidant activity by roasting was also obtained in both peanut flour with skin or without skin [1]. All the researchers concluded that the increase in antioxidant activity was due to the phenolic content and presence Maillard reaction product. However, the increase in phenolic content and antioxidant activity should be compromised with the colour also affected by roasting. Meanwhile, higher temperature and prolonged duration of roasting induced darker colour of peanut.

4. Conclusions
From the results, it was seen that higher temperature and prolonged duration time of roasting increased total phenolic content and antioxidant activity of peanut kernel with skin. The increase in polyphenol
content during roasting followed zero-order reaction and the activation energy of reaction was 113.07 kJ/mol. Furthermore, there was a positive correlation between total phenolic content and antioxidant activity. The increase in total phenolic content of peanut, made the capability of peanut in scavenging radicals stronger. In regards to the higher temperature and prolonged duration of roasting, which include a darker peanut colour, its positive effect in terms of increasing the phenolic content and antioxidant activity should be compromised with the colour parameter.

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References
[1] Win M M, Abdul-Hamid A, Baharin B S, Anwar F and Saari N 2011 Eur. Food Res. Technol. 233 599
[2] Arya S S, Salve A R and Chauhan S 2016 J. Food Sci. Technol. 53(1) 31
[3] Gad M Z 2010 J. Adv. Res. 1 169
[4] Yu J, Ahmedna M and Goktepe I 2005 Food Chem. 90 199
[5] de Camargo A C, Regitano-d’ Arce M A B, Gallo C R and Shahidi F 2015 J. Funct. Foods 12 129
[6] Schneider C, Sanders T H and Davis J P 2011 Proc. 14th Int. Symp. on Olfaction and Electronic Nose (American Institute of Physics) p 81
[7] Shi X, Sandeep K P, Davis J P, Sanders T H and Dean L L 2016 J. Food Process Eng. 1
[8] Lin J, Liu S, Hu C, Shyu Y, Hsu C and Yang D 2016 Food Chem. 190 520
[9] Lemos M R B, Siqueira E M A, Arruda S F and Zambiasi R C 2012 Food Res. Int. 48 592
[10] Pawar S S and Dasgupta D 2018 J. King Saud Uni. – Sci. 30 293
[11] Siddiqui N, Rauf A, Latif A and Mahmood Z 2017 J. Taibah Uni. Med. Sci. 12(4) 360
[12] Brand-Williams W Cuvelier M E and Berst C 1995 Lebensm.-Wiss.u.-Technol 28 25
[13] Mishra M, Ojha H and Chaudury N K 2012 Food Chem. 130 1036
[14] AOAC Methods : 965.33, 940.28. Official Methods of Analysis of AOAC International, 16th ed. 1995. The Association of Analytical Chemists.
[15] Ruhanian S and Movagharnejad K 2016 Engineering in Agriculture, Environment, and Food 9 84
[16] Molaveisi M, Beigbabaei A, Akbari E, Noghabi M S and Mohamadi M 2019 J. Heliyon e01129
[17] Kim S Y, Jeong S M, Park W P, Nam K C, Ahn D U and Lee S C 2006 Food Chem. 97 472
[18] Shahidi F and Yeo J D 2016 Molecules 21 1216
[19] Talcott S T, Passeretti S, Duncan C E and Gorbet D W 2005 Food Chem. 90 379
[20] Mandari G, Tomaino A, Arcoraci T, Martorana M, Lo V and Cacciol F 2010 J. Food Compo. Ana. 23 166
[21] Subramanian R., Subbramaniyan P and Raj V 2013 Springerplus 2:28
[22] Mathew S, Abraham T E and Zakaria Z A 2015 J. Food Sci. Technol. 52(9) 5790
[23] Jeong S M, Kim S Y, Kim D R, Nam K C, Ahn D U and Lee S C 2004 J. Food Sci. 69 C377