

The kinetic behavior of antioxidant activity and the stability of aqueous and organic polyphenol extracts from navel orange peel

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Abstract

The kinetic behavior of the antioxidant activity of aqueous/organic polyphenol extracts from navel orange peel was evaluated using DPPH and FRAP assays to assess their capacity to scavenge DPPH and ferric reducing antioxidant power. The stability of polyphenol extracts, including the influencing factors of temperature, pH, light, and the correlation of total phenolic content and antioxidant activity were also investigated. The kinetic reaction results showed that the antioxidant activity i.e. DPPH radical scavenging capacity and ferric reducing antioxidant power depended on concentration and reaction time. Its stability in aqueous phase and organic phase was influenced by temperature, pH and light in varying degrees. 50 °C was a key temperature point above which the total polyphenol content and antioxidant ability decreased remarkably. The phenolic compounds were not stable at pH 7, but showed a strongest DPPH radical scavenging capacity in both aqueous and organic phases. Acidic conditions seemed to be better for maintaining antioxidant ability of extracts from navel orange peel than alkaline conditions in aqueous and organic phases. Avoiding light kept the extracted phenolic compounds stable. There is a significant correlation between total phenolic content and antioxidant activity. These results provide some basic understanding of extracts from navel orange peel and promote the application of the extracted polyphenol from navel orange peel as antioxidants.

Keywords: navel orange peel; polyphenol; antioxidant activity; stability.

Practical Application: Aqueous/organic polyphenol extracted from navel orange peel was investigated, for the kinetic behavior of the antioxidant activity, physicochemical stability and the correlation of total phenolic content and antioxidant activity. This study provides a theoretical basis for the application of navel orange peel polyphenols as antioxidants.

1 Introduction

There are more recent evidences that free radicals induce oxidative damage, which has been implicated in ageing and in several human pathologies and other diseases (Gliszczyńska-Swiągł, 2006). Several studies indicate that adequate intake of fruits and vegetables prevent these chronic diseases caused by oxidative stress (Zhang & Tsao, 2016; Tabart et al., 2009). Numerous studies have shown that plant polyphenol have good effects on anti-mutagenesis, anti-aging, anti-cancer, anti-inflammatory, anti-diabetes, weight loss and neuroprotection (Braicu et al., 2013; Hano, 2020; Williams & Spencer, 2012; Zaveri, 2006), and have certain therapeutic or preventive effects on some diseases that seriously harm human health, such as hypertension, heart disease, cancer and so on (Hertog et al., 1993; DuPont et al., 2002). These protective effects have been given credit for the antioxidant substances, such as polyphenolic compounds, carotenoids, vitamins C and E. Antioxidants have capability against scavenging reactive oxygen and nitrogen species and prevent oxidative damage to crucial biological macromolecules, such as DNA, lipids, and protein (Singh et al., 2018; Szeto et al., 2004). Natural antioxidants have been found to be safer than many synthetic ones and can provide additional nutritional value (Ajila et al., 2007). This has led to increasing development of natural antioxidants, especially from fruits and vegetables.

Citrus fruits are the most abundant fruits grown throughout the world containing valuable antioxidant phytochemicals (Hou et al., 2019; Satari & Karimi, 2018). Navel orange is a type of citrus which is among the most popular orange varieties. It has good economic value and is widely cultivated in countries such as China, Brazil, the United States, and Spain. By the end of 2017, Guangzhou has been the largest production area of navel oranges in China with the total planting areas of navel orange 103.2 thousand hectares, and the annual output of around 1.24 million tons (Zhang et al., 2018). Navel orange is a commonly consumed product because of its high nutritional value and desirable sensorial characteristics, which are attributed to a large supply of antioxidant compounds mainly vitamin C and polyphenols as well as dietary fiber content (Nicolle et al., 2004; Serafini et al., 2002). With the consumption of orange pulp, huge amounts of orange peel are wasted, which account for about 30% of the weight of the whole fruits (Rezzadori et al., 2012). Traditionally, orange peel is commonly used as a substrate to produce animal feed, organic fertilizer, and biofuel (Santos et al., 2010).

The navel orange peel residue is a source of phenolic compounds, including antioxidant active substances such as

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phenolic acids and flavonoids (Rafiq et al., 2018). It is worth noting that the polyphenolic compounds from orange peel have aroused great research interests worldwide, due to their structure and biodiversity that present antioxidant, antibacterial, enzyme inhibition, anti-inflammatory, and antitumour activity (Shirisha et al., 2019; Smeriglio et al., 2019; Mandalari et al., 2007; Bobo-García et al., 2015; Klangpetch et al., 2016). The application of natural antioxidants as health-promotion supplements or as a food preservative has currently gained much attention.

The antioxidant activity is normally evaluated at a fixed time-point, which can hardly avoid instrumental errors. Besides, this evaluation method is used without considering if the reaction is extensively completed. Therefore, some researchers have proposed a kinetic approach in determining the antioxidant activity of citrus juices, sesame extracts (Suja et al., 2004) and red wines (Magalhães et al., 2012). The polyphenols with hydroxyl groups, especially ortho phenolic hydroxyl groups, are active and can be easily affected by enzymes, oxygen, light, moisture, and other factors. The stability of phenolic compounds needs further investigation to assess their antioxidant ability under various temperatures and pH conditions.

At present, there were many studies mainly focused on the extraction of active ingredients from navel orange peel (Guo et al., 2012; Yang et al., 2009; Lux et al., 2019). However, limited data are available on the antioxidant activity kinetic behavior of different phase phenolic extracts from navel orange peel. In the present study, the reaction kinetics of aqueous/organic phase polyphenol extracts were clarified for the antioxidant properties by DPPH radical scavenging assays and ferric reducing-antioxidant power (FRAP) assay. Meanwhile, the stability of aqueous/organic phases polyphenol extract, and the correlation between the content changes of polyphenol and antioxidant activity under the different factors were also investigated. They could contribute to the value-added utilization of this orange peel in the food, cosmetic, and pharmaceutical industries.

2 Materials and methods

2.1 Chemicals and reagents

Gallic acid, Folin phenol reagent, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,4, 6-Tis-2,4,6-tripyridyl-2-triazine (TPTZ) were purchased from Sigma-Aldrich GmbH (Sternheim, Germany), Na₂CO₃, ethyl alcohol, Butylated hydroxytoluene (BHT), sodium acetate, acetic acid, methanol, concentrated hydrochloric acid, acetone, ethyl acetate, n-hexane were obtained from Yima Chemical Reagent Factory (Guangzhou, China). All solvents were analytical grade.

2.2 Plant material

Navel oranges were purchased from Carrefour Supermarket (Guangzhou, China). Navel orange peels were peeled off from the fresh fruits by hand, and dried at 50 °C in electric constant temperature drying oven (Flyde, Guangzhou, China) until constant weight was obtained, then crushed by using a high speed pulverizer (Ruian, Yonglu Pharmaceutiical Co.,LTD, Zhejiang,

China). The powder was passed through a mesh 40 and stored at room temperature after sealing.

2.3 Preparation of phenolic extracts

The polyphenols extraction was performed on samples according to Yan et al. (2012). 0.5 g of sample that has been sampled by the quartile method was mixed with 10 mL of ethanol/water (50:50 v/v) for 2 h at 60 °C in the dark. The mixture was then centrifuged (13,000 g, 25 °C) for 20 min. The supernatant obtained was concentrated under vacuum at 40 °C by using a rotary evaporator (Gongyi Yuhua Instrumeny Co.,LTD, Henan, China), and defatted with hexane (samples/solvent, 1:1, v/v) for six times, then extracted with ethyl acetate/anhydrous ether (samples/solvent, 1:1, v/v) for six times. Then hexane phase/ethyl acetate phase /anhydrous ether phase was evaporated respectively at 30 °C by rotary evaporation until dried. The rest of aqueous phase was freeze-dried at -46 °C in a vacuum freeze dryer (Boyikang Lab Instrument Co.,LTD, Beijing, China). The dried hexane phase/ethyl acetate phase /anhydrous ether phase was adjusted to a final volume of 5 mL with ethanol solution (50%, v/v) to determine the polyphenol content respectively.

2.4 Total phenolic content (TPC)

Total phenol content (TPC) in each extract was determined using the FC method described by McDonald et al. (2001), with slight modifications. The calibration curve was established using gallic acid (0~45 µg/mL). Phenol extract (0.5 mL) was transferred into 5 mL volumetric flasks, added distilled water (3 mL) and FC reagent (0.25 mL), and mixed thoroughly for 3 min. 20% Na₂CO₃ solution (0.75 mL) was added to the mixture and the mixture was diluted to 5.0 mL with distilled water. The mixture was incubated in water bath at 75 °C for 10 min and rapidly cooled in ice bath, then centrifuged at 4000 r/min for 10 min. The absorbance of the mixture was measured at 760 nm using a spectrophotometer (BECKMAN COUCTER, USA). The blank consisted of all reagents and solvents without samples. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight (mg GAE/g). Determination of TPC was conducted in a duplicate.

2.5 DPPH radical scavenging assay

The DPPH free radical scavenging rate of extracts were determined by the method of Calliste et al. (2005) with minor modifications. Briefly, 0.2 mL of sample was added into 4 mL DPPH ethanol solution (0.1 mM). The absorbance was measured at 517 nm using a UV~VIS spectrophotometer every 10 min from 0 min to 60 min. The percentage inhibition of radicals was calculated using the following formula (Equation 1):

$$\%inhibition = (A_{control} - A_{sample}) \times 100 / A_{control} \quad (1)$$

where A_{control} is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of sample with DPPH solution. The DPPH radical scavenging capacity is inhibition or EC₅₀

value. The half-maximal inhibitory concentration (EC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50% (Do et al., 2014). A lower absorbance or EC_{50} value indicates a stronger capability to scavenge DPPH radicals. All tests were performed at least in triplicate, and graphs were plotted using the average of three determinations.

2.6 Ferric reducing-antioxidant Power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was conducted according to the procedures described by Benzie & Strain (1996) with some modifications. The FRAP reagent contained 2.5 mL of 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6, and was prepared freshly and warmed at 37 °C prior to use. The FRAP reagent (3600 μ L), prepared and warmed to 37 °C, was mixed with distilled water (360 μ L) and Polyphenol extract (120 μ L) of navel orange peel. The absorbance was measured at 593 nm every 10 min from 0 min to 60 min against a blank without samples (120 μ L distilled water instead). A standard curve was prepared using different concentrations of $FeSO_4 \cdot 7H_2O$ (0 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM). The evaluation index of ferric reducing antioxidant power is absorbance or EC_1 value (the dose of an antioxidant (mmol Fe^{2+} /g) has the same antioxidant capacity as 1 mM $FeSO_4 \cdot 7H_2O$). The FRAP is stronger with the higher of absorbance or EC_1 value. Increased absorbance or EC_1 value of the reaction indicated increased ferric reducing-antioxidant Power.

2.7 Investigation of the stability of polyphenol extract

Effect of temperature on the total phenolic content and antioxidant activity

The aqueous/organic phase polyphenol extract were added to centrifuge tubes and stored in warm bath at 30 °C, 40 °C, 50 °C, 60 °C and 70 °C for 30 min. The TPC, EC_{50} and EC_1 of each sample was measured respectively.

Effect of pH on the total phenolic content and antioxidant activity

Buffer solutions used were disodium hydrogen phosphate and citric acid buffer, pH 3.0, 4.0 and 5.0; disodium hydrogen phosphate buffer, pH 6.0, 7.0 and 8.0; Tris-HCl buffer, pH 9.0 and 10.0. The aqueous/organic phase polyphenol extract were mixed with equivalent buffer respectively in centrifuge tubes and incubated at room temperature for 1 h. The TPC, EC_{50} and EC_1 of each sample was measured respectively.

Effect of light on the total phenolic content and antioxidant activity

The aqueous/organic phase polyphenol extract were added to centrifuge tubes and irradiated for 0, 12, 24, 48, 72 and 96 h under simulated natural light (25 W incandescent lamp). The TPC, EC_{50} and EC_1 of each sample was measured respectively.

2.8 Data statistics and analysis

All the analyses were carried out in triplicates. The obtained results were presented as mean (c) \pm (standard deviation (SD)). All data were disposed using SPSS software, and analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences. The correlation of the variables was analysed by Pearson correlation test. Differences were considered significant at $p < 0.05$ or very significant at $p < 0.01$.

3 Results and discussion

3.1 DPPH radical-scavenging activities

The DPPH radical is a stable radical with maximum absorption at 517 nm that can readily undergo reduction by an antioxidant (Lu & Foo, 2000). DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Tohma & Gulçin, 2010). As shown in Figure 1, the DPPH radical scavenging capacity increased with increasing phenolic content and reaction time. The results indicated that the aqueous/organic phases polyphenol extract both have strong and lasting DPPH radical-scavenging activities at the concentration above 100 mg GAE/g DW. The absorption value decreased rapidly in the first 10 mins, then reached a plateau after 50 mins. The research group from Slovenia (Terpinc et al., 2009) reported a similar conclusion on the polyphenol antioxidant activity indicating that phenolic compounds exhibited a concentration-dependent increase of scavenging rate. It is consistent with our results that the DPPH radical scavenging capacity of aqueous /organic phase polyphenol extract depends on concentration and reaction time, while our results showed a higher scavenging capacity at a similar concentration. Besides, both aqueous phase and organic phase showed a similar trend of DPPH radical-scavenging activities, no significant difference.

3.2 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power of aqueous/organic phase polyphenol extracts were shown in Figure 2. It suggested a similar conclusion with DPPH radical scavenging capacity that aqueous/organic phase polyphenol extracts showed a concentration and time dependent increase in the ferric reducing antioxidant power. The color reaction initiated rapidly in 10 mins, followed by a time-dependent increase during the first 50 min, and a plateau afterwards (Figure 2AB). These values suggested that ferric reducing antioxidant power of aqueous/organic phases polyphenol extract were strong. The results are consistent with previous studies, which demonstrated a strong positive correlation between the total phenolic content and the antioxidant capacity of fruits. (Wang & Lin, 2000; Dai et al., 2007; Bagetti et al., 2011)

3.3 The effect of temperature on polyphenol content and its antioxidant activity

The total phenolic content from navel orange peel extracts in aqueous phases decreased as the temperature of warm bath increased between 30 °C~70 °C (Figure 3A), while no significant loss of total phenolic content in organic phase were observed in the

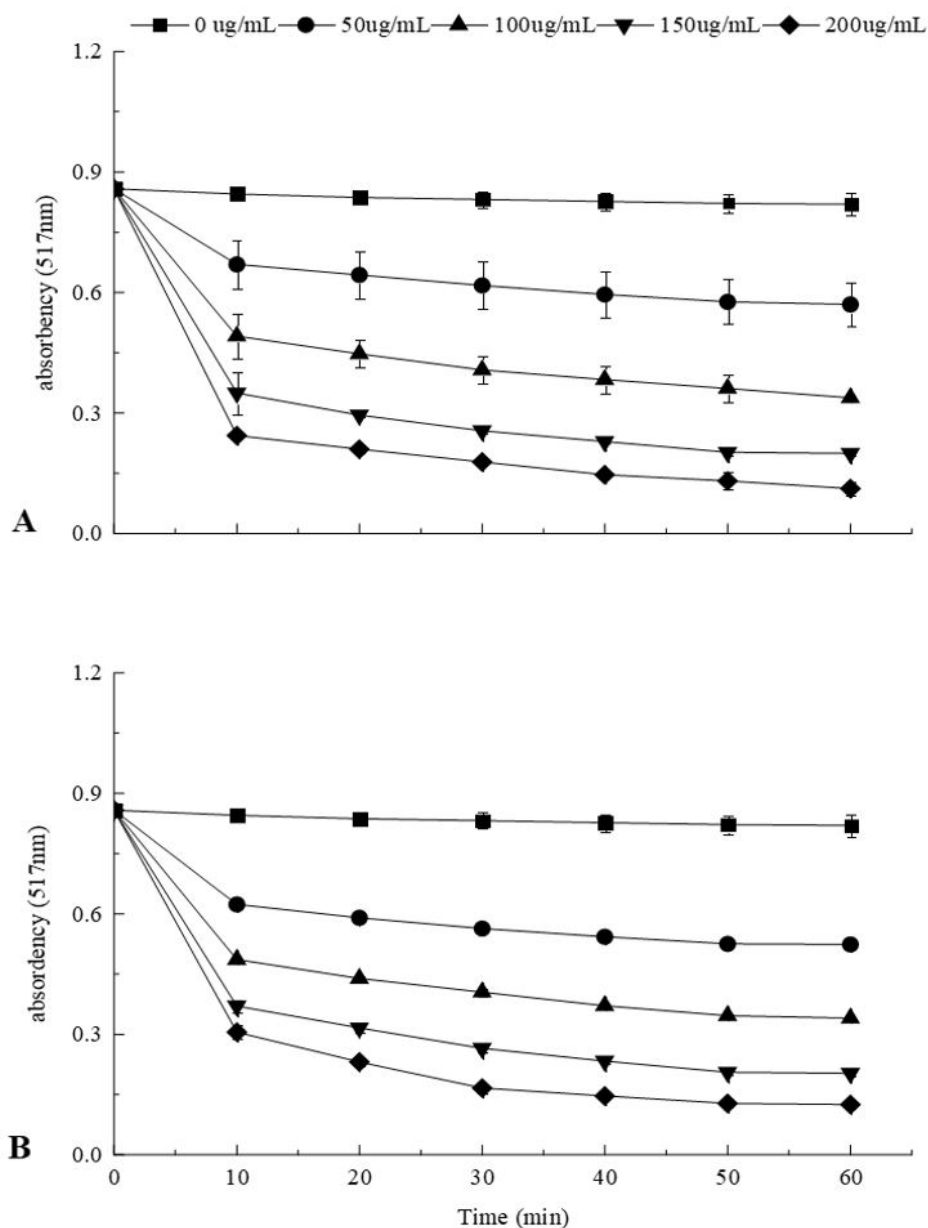


Figure 1. Reaction kinetics of phenolic extract in aqueous phase (A) or organic phase (B) with DPPH assay.

same temperature range from 30 °C to 60 °C. Significant loss (around 10% of total phenolic content) only occurred above 70 °C, which suggested that total phenolic content in organic phase are relatively stable under high temperature conditions. By contrast, phenolic content in aqueous phase was not as stable as those in organic phase. There was a 16% reduction of total phenolic compounds in aqueous phase at 70 °C. It has been reported that total phenolic content and free radical scavenging activities decreased with increasing extraction temperatures. (Akowuah et al., 2009). It is worth to note that total phenolic content was twice more extracted from water than that from organic solvent at both low and high temperature.

Temperature exerted different effects on the DPPH radical scavenging capacity of polyphenol extracts in Figure 3B. As shown in Figure 3B, the low EC_{50} was only observed below 50 °C

and there was no significant difference between aqueous and organic phase, indicating that the strong free radical-scavenging capacity of the polyphenol extract can be maintained up to 50 °C. However, EC_{50} remarkably increased above 50 °C with a 48% increase in aqueous phase and 100% increase in organic phase (Figure 3B). These changes in free radical-scavenging capacity were consistent with changes in total phenolic content.

As shown in Figure 3C, EC_1 showed slightly increase in organic phase and decrease in aqueous phase, suggesting that temperature had positive effects on the ferric reducing antioxidant power of polyphenol extract in organic phase, but caused a 13.5% reduction in aqueous phase at 70 °C.

All the results suggested that 50 °C was a key temperature to maintain the high antioxidant activity of polyphenol extracts,

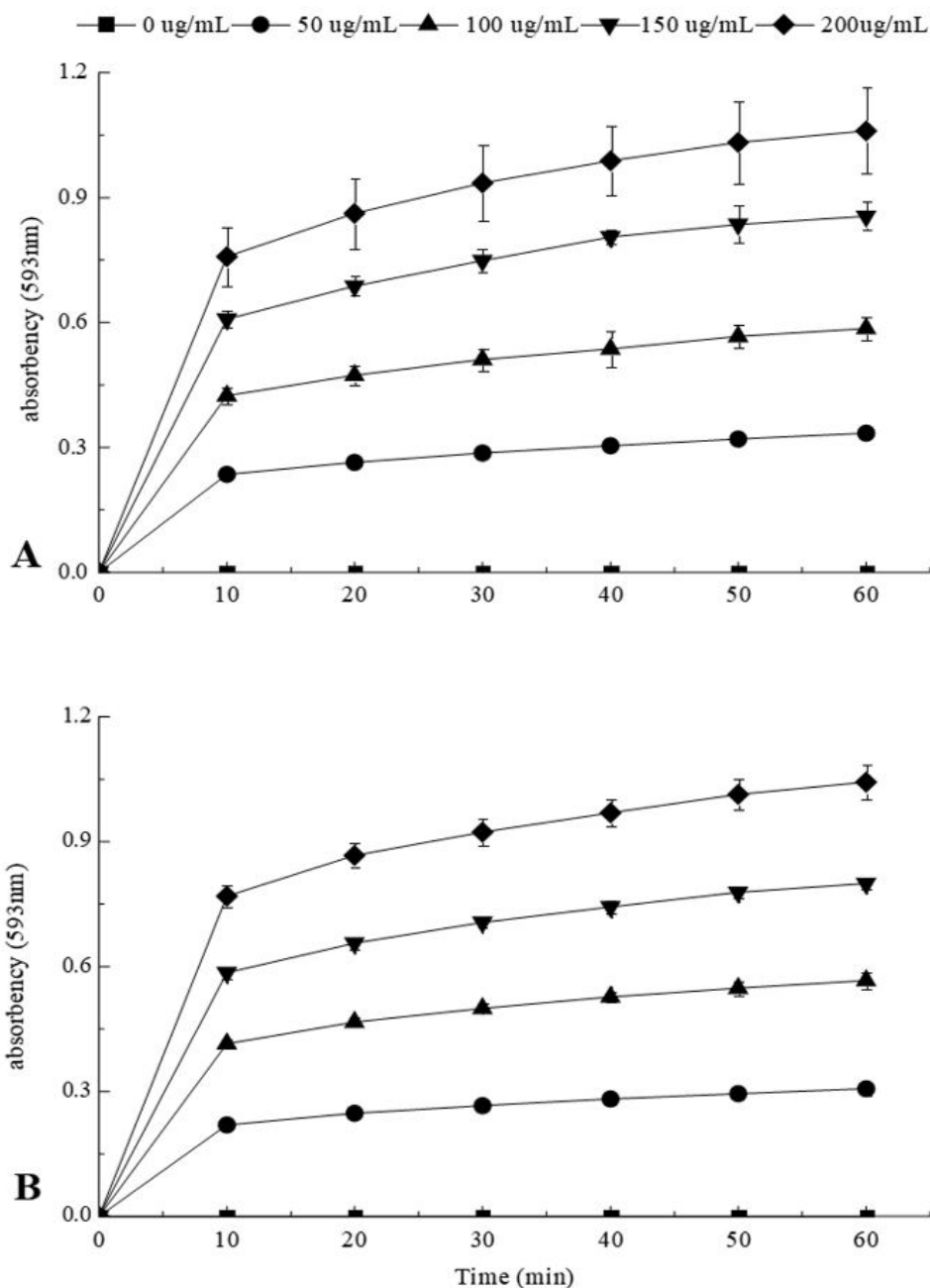


Figure 2. Reaction kinetics of different concentrations of navel orange peel phenolic extract in aqueous phase (A) or organic phase (B) with FRAP assay.

which could be due to the combined effect of nonenzymatic reaction and anthocyanin stability (Reyes & Cisneros-Zevallos, 2007). Temperature significantly affected the stability of total phenolic content and its antioxidant activity in aqueous phases compared with that in organic phase. The differences in temperature sensitivity of polyphenol extract between aqueous phase and organic phase, maybe due to the structures of phenolic compounds, i.e. mainly oligomer and monomer in organic phase and multimer in aqueous phase. The antioxidant ability of navel orange peel extracts was not determined by total polyphenol

content but the specific structures of phenolic compounds that related to their stability at high temperature.

3.4 The effect of pH on polyphenol content and its antioxidant activity

The influence of various pH on the content and its antioxidant activity of aqueous/organic phases polyphenol extract were investigated. As it was shown in Figure 4A, the aqueous phase polyphenol content was significantly higher at pH 3.0~7.0 than

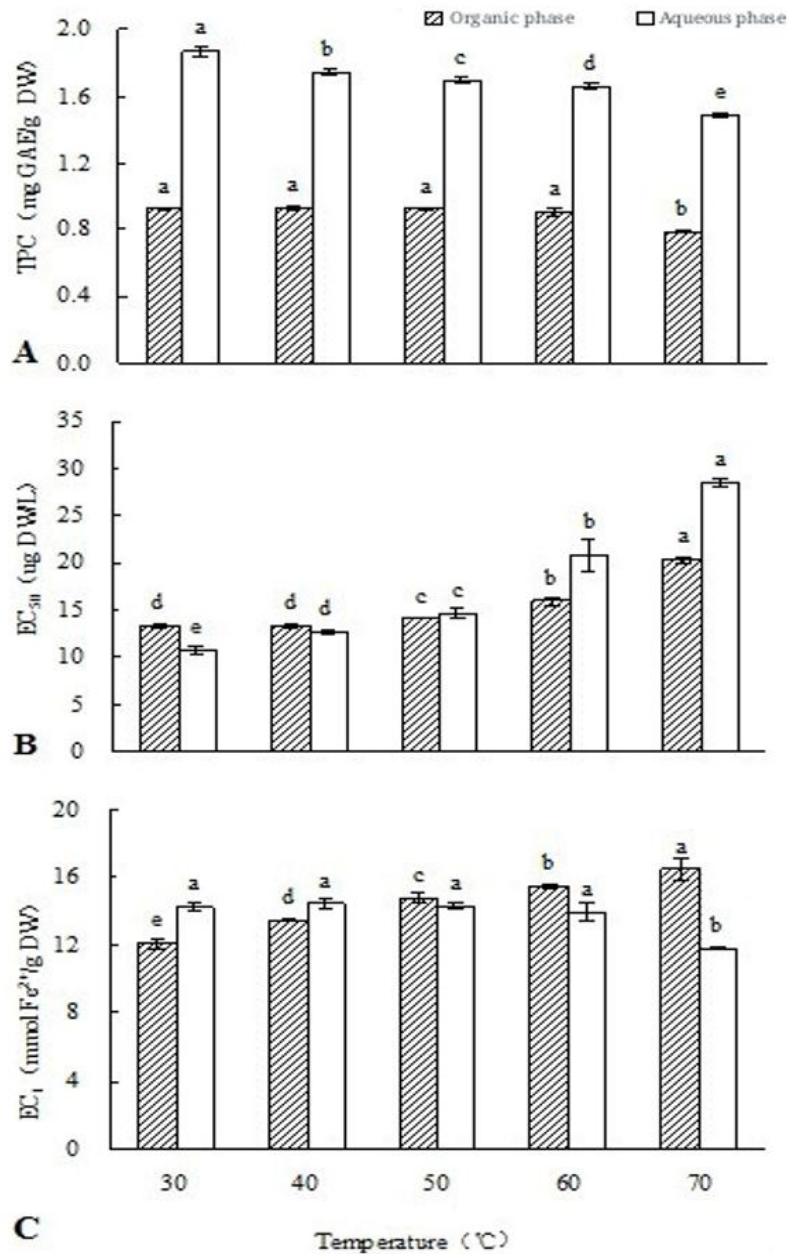


Figure 3. Effects of temperature on TPC and the antioxidant activity of navel orange peel phenolic extract. In each column, the data having different letters are statistically different ($p < 0.05$).

that at pH 8.0~10.0 ($p < 0.05$). The aqueous phase polyphenol was relatively stable at pH 3.0~7.0. It is interesting to note that the content of total phenolics in organic phase was lowest at neutral condition (pH 7), while polyphenols in organic phase were stable in acidic and alkaline conditions (Figure 4A). This result is consistent with EC₅₀ (Figure 4B) and EC₁ (Figure 4C).

Figure 4B shows that DPPH radical scavenging capacity of organic phases polyphenol extract at different pH can be ranked in the order pH 7.0 > pH 6.0 > pH 5.0 > pH 10.0 > pH 3.0 > pH 8.0 > pH 9.0 > pH 4.0. In aqueous phases, it can be ranked in the

order pH 7.0 > pH 4.0 > pH 5.0 > pH 3.0 > pH 9.0 > pH 8.0 > pH 6.0 > pH 10.0. The results neutral condition (pH 7) facilitates to scavenge DPPH radicals and the DPPH radical scavenging capacity was stronger in acidic conditions than that in alkaline conditions in both organic and aqueous phases.

As shown in Figure 4C, the ferric reducing antioxidant power of aqueous phase polyphenol extract was much higher at pH 8.0~10.0 ($p < 0.05$) than that at pH 3.0~7.0, and was lowest in neutral conditions. However, it was significantly higher in organic phase at pH 7.0 ($p < 0.05$) than at other pH values, and

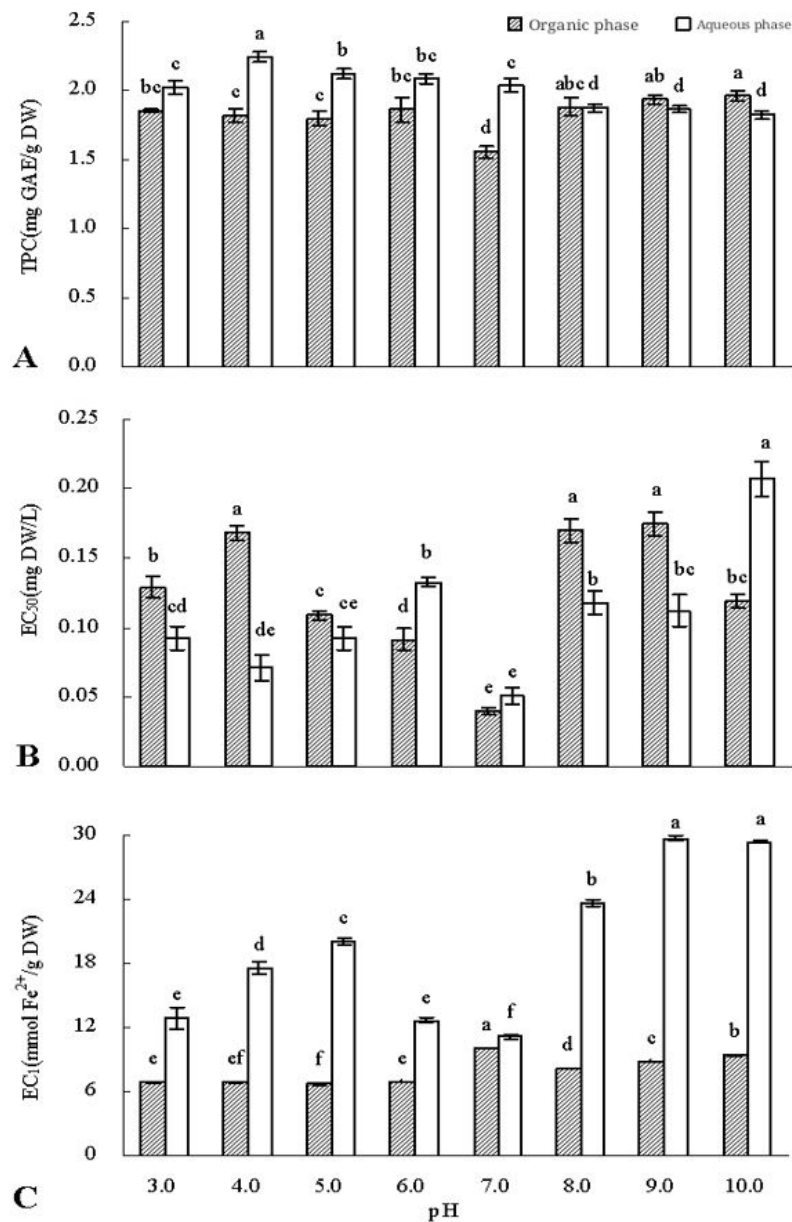


Figure 4. Effects of pH on TPC and the antioxidant activity of navel orange peel phenolic extract. In each column, the data having different letters are statistically different ($p < 0.05$).

it was higher in alkaline conditions than in acid conditions. pH variation in acid conditions. On the polyphenol extracts resulted in a lower amount of the functional substances. These results demonstrate that various pH significantly influenced the content and its antioxidant activity of aqueous/organic phases polyphenol extract, which could be due to the polyphenol stability. It was reported that the pH significantly influenced polyphenol content and the total antioxidant ability (Ruenroengklin et al., 2008; Yuan, 2014).

Friedman & Jürgens (2000) reported that the stability of phenolic substances strongly depends not only on pH but also

on the phenolic substance structure. Each radical group in the phenolic compounds reacts in different ways with the media pH variation, which can be affected by electron-donating or withdrawing and sterically hindering of substituent on the aromatic ring (Ganapathi et al., 2001).

Harbourne et al. (2009) studied the effect of pH on the extraction of total phenols from meadow sweet (*Filipendula ulmaria* L.) using water as solvent and concluded that when increasing the pH from 3.9 to 6.4, total phenols content was 33% higher. On the other hand, Yoshida et al. (1999) studied the efficiency of phenolic compounds extraction from green

tea and observed a decrease in the extraction efficiency when pH was increased, reaching a 30% less extraction at pH 6.0 and 15% at pH 7.6.

3.5 The effect of light on polyphenol content and its antioxidant activity

The polyphenol content of aqueous and organic phases decreased significantly with increasing illumination time in a similar pattern as observed in Figure 5A. In general, the

polyphenol content was higher in aqueous phase than that in organic phase.

As shown in Figure 5B, the influence of DPPH radical scavenging capacity was higher significant. All types of illumination time significantly ($p < 0.05$) influenced the DPPH radical scavenging capacity of aqueous phase polyphenol. However, there was no significant difference on DPPH radical scavenging capacity of organic phase polyphenol in 0~12 h range. It decreased remarkably between 12 h and 96 h ($p < 0.05$).

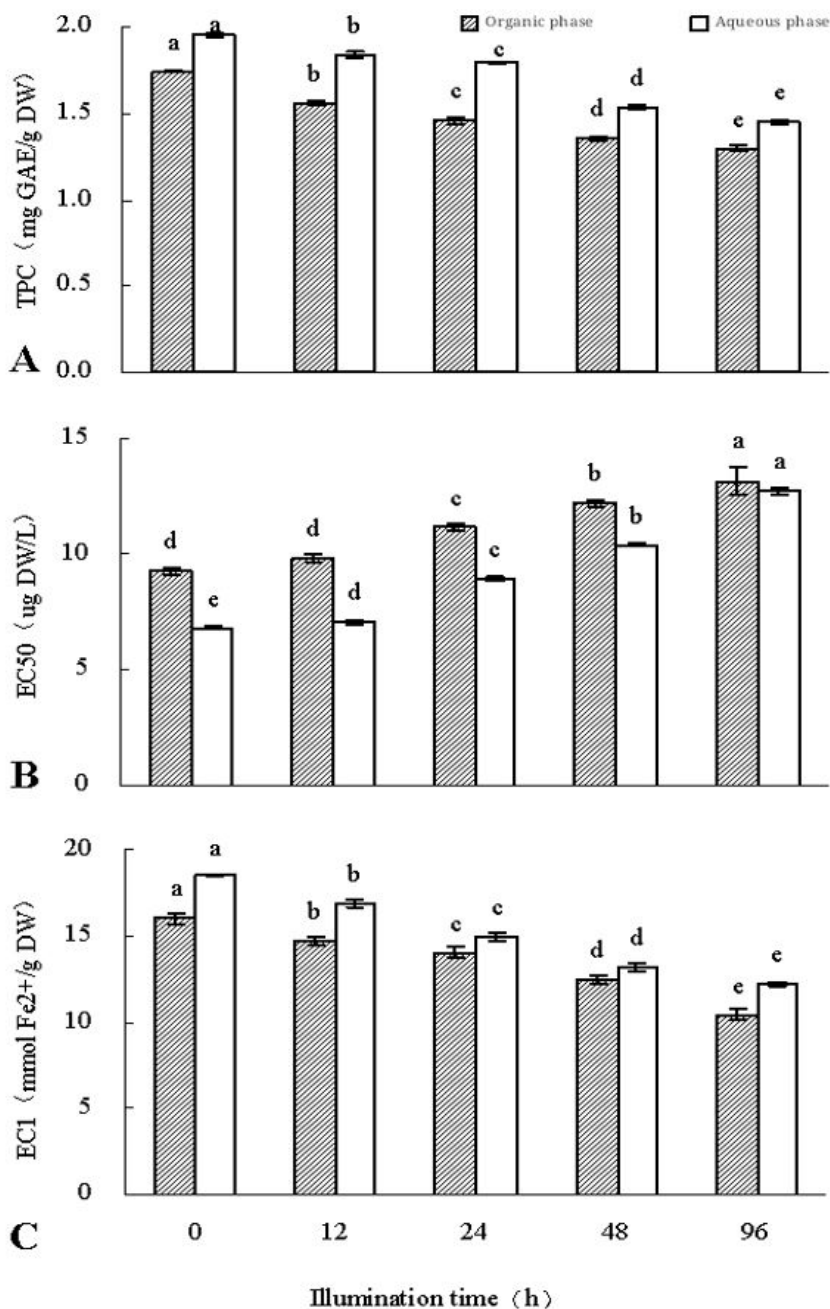


Figure 5. Effects of light on TPC and the antioxidant activity of navel orange peel phenolic extract. In each column, the data having different letters are statistically different ($p < 0.05$).

Table 1. Correlations between TPC and antioxidant activity of phenolic in navel orange peel at different factors.

Factors	Samples	Correlation coefficients against EC ₅₀	Correlation coefficients against EC ₁
Temperature	aqueous phase	-0.939**	0.808 **
	organic phase	-0.926**	-0.686**
pH	aqueous phase	-0.619**	-0.663**
	organic phase	0.669**	-0.226
Light	aqueous phase	-0.954**	0.969**
	organic phase	-0.951**	0.940**

All results were expressed as mean \pm SD of three parallel measurements. ** indicated very significant correlations at $p < 0.01$ (two-tailed).

Effect of light on ferric reducing antioxidant power of aqueous/organic phases polyphenol was shown in Figure 5C. The ferric reducing antioxidant power of polyphenol extract in aqueous/organic phases was changed observably in the range of 0~96 h, and it decreased significantly ($p < 0.05$) as the illumination time increased. It demonstrated aqueous/organic phases polyphenols content and its antioxidant activity was sensitive to light. Some investigations had shown that the polyphenol content and its antioxidant activity was significantly affected on the light (Yin et al., 2015; Yuan, 2014), which was in accordance with our results.

3.6 The correlation between TPC and antioxidant activity under different conditions

There was a correlation between the polyphenol content and its antioxidant activity under different conditions as shown in Table 1.

In terms of temperature, aqueous phase polyphenol content was significantly negatively correlated with its corresponding EC₅₀ and positively correlated with its corresponding EC₁ ($p < 0.01$). Moreover, there was a significantly negative correlation between organic phase polyphenol content and its corresponding EC₅₀ and EC₁ ($p < 0.01$). The results suggested that the effect of temperature on antioxidant activity of aqueous and organic phase polyphenols was significant due to polyphenol sensitive to temperature. Temperature had a significant effect on polyphenol content ($p < 0.05$), because it might induce chemical reaction of polyphenol. However, the extracted polyphenols were mainly oligomer and monomers in organic phase that were resistant to temperature, therefore temperature had minor effects on the ferric reducing antioxidant power of organic phase extracts. except for 70 °C. Miguel et al. (2010) found a negative correlation between phenols, flavones and flavonols and antioxidant activity in Portuguese propolis extracts.

At different pH, significant negative correlations between aqueous phase polyphenol content and its corresponding EC₅₀, EC₁ ($p < 0.01$) were shown in Table 1. Positive correlations between organic phase polyphenol content and its corresponding EC₅₀ were also demonstrated in Table 1 ($p < 0.01$). On the contrary, there was no significantly negative correlation with its corresponding EC₁ ($p < 0.05$). The results indicated that there was no uniform regularity in the influence of pH on the change of DPPH radical scavenging capacity, ferric reducing antioxidant power and polyphenol content, which can cause the different effects on specific polyphenol stability.

In terms of light, navel orange peel polyphenol content in aqueous and organic phases and its corresponding EC₅₀ showed a negative correlation ($p < 0.01$). Moreover, a significant positive correlation was also obtained with its corresponding EC₁ ($p < 0.01$). These results suggested that the effect of light on navel orange peel polyphenol antioxidant activity was mainly due to polyphenol content under different light conditions. Lin & Yang (2019) have reported that the illumination was not conducive to polyphenols stability and the condition of avoiding light can protect the polyphenols stability. Therefore, it is necessary to avoid direct light during the storage and application of navel orange peel polyphenols.

Thaipong et al. (2006) reported high positive correlations between polyphenols and FRAP in guava fruit juices. Gardner et al. (2000) have demonstrated the similar behavior in certain pure fruit juices, such as orange, pineapple, apple and grapefruit.

4 Conclusion

The kinetic behavior of antioxidant activity of aqueous/organic polyphenol extracts from navel orange peel were evaluated by using DPPH and FRAP assays. The effects of various factors (temperature, pH, light) on polyphenol content and its antioxidant activity were investigated. The kinetic reaction of polyphenol extracts on DPPH radical scavenging capacity and ferric reducing antioxidant power in aqueous and organic phases was characterized by the dual dependence between concentration and reaction time. The absorption value decreased rapidly in the first 10 min, and 50 min was required to reach a plateau. The kinetic approach may give a more comprehensive understanding about the behavior of antioxidants. The stability of aqueous and organic phases polyphenol was influenced by temperature, pH and light. Polyphenol content and its antioxidant activity in aqueous and organic phases from navel orange peel were negatively affected by temperature change from 30 °C~70 °C. Aqueous polyphenol content was stable under the condition of pH 3.0~5.0. However, in organic phase, it was stable under the acidic and alkaline conditions. Polyphenol content and its antioxidant activity was gradually decreased when light time increased from 12 h~96 h. There is a significant correlation between total phenolic content and antioxidant activity. These results would provide comprehensive information on the total antioxidant capacity and influence factors of polyphenol.

Ethical approval

This article does not contain any studies with human or animal subjects.

Conflict of interest

The authors have no conflict of interest to declare.

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