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# STUDY ON THE EFFECTS OF LEMON JUICE ON CHEMICAL AND FUNCTIONAL PROPERTIES OF SOME FRUIT JUICES

– Short communication –

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**Abstract**: Natural antioxidants from fruit juices have been interesting in terms of safety and low risk of side effects. Lemon juice was added to apple, grape, orange, pineapple and watermelon juices to obtain various concentrations. The blends were assayed for total phenolic content and total antioxidant capacity as well as superoxide dismutase (SOD)-like activity. The highest phenolic content was present in 50% (v/v) lemon juice in pineapple blend (425.1±5.1 µg/ml) in comparison to original juice. The total antioxidant capacity and SOD-like activity in the juice blends increased with increasing the proportion of lemon juice when compared with original juice. Our study indicated that lemon juice enhanced health benefits in terms of total phenolic content, total antioxidant capacity and SOD-like activity.

**Keywords**: Total phenolic content, Total antioxidant capacity, SOD-like activity, Lemon juice, Juice blends

## INTRODUCTION

Oxidative stress plays an important role in developing and progression of human degenerative diseases (Rajendran et al., 2014). The overproduction of free radicals can damage biomolecules such as proteins, lipids and nucleic acids leading to the development of complications in many diseases (McCord, 2000). Antioxidants are very important in attenuation oxidative stress and protect cells from free radicals. There is an increasing interest in consumption of fruit juices to prevent and reduce risk of developing oxidative stress in several diseases (Zheng et al., 2017). For this reason, many researchers have been trying to improve quality in terms of antioxidant capacity of juice beverages (Vilaplana et al., 2012; Bamidele & Fasogbon, 2017).

Lemon (*Citrus aurantifolia*) juices is known as a drink for health benefits and commonly used to make soft drink and cocktail (Zou et al., 2016). In addition to lemon juice, other fruit juices such as watermelon (*Citrullus lanatus*), pineapple (*Ananas comosus*), apple (*Malus domestica*), orange (*Citrus reticulate Blanco*) and grape (*Vitis vinifera L.*) are popular to drink for health values. Fruit juices naturally contain many bioactive compounds that act as antioxidants (Pisoschi et al., 2009). The advantages of antioxidant intake from natural sources such as fruit juices are safety and low risk of side effects. In this study, lemon is considered to be rich source of phytochemicals and used to increase total antioxidant capacity in fruit juices for health benefits. Natural antioxidants of fruit juices have relationship with phenolic compounds (Park et al., 2015). Some phenolic compounds act as superoxide dismutase (SOD)-like activity that can scavenge superoxide radicals (Valcheva-Kuzmanova et al., 2007). The aim of this work is to assess total antioxidant capacity including, total phenolic content and SOD-like activity of the juice blends of lemon juice and original fruit juices.

## MATERIALS AND METHODS

*Chemicals and reagents:* Folin-Ciocalteu reagent, gallic acid, antioxidant assay kit and SOD determination kit were obtained from Sigma-Aldrich (St. Louis, MO, USA) company. All other chemicals used in this study were of analytical grade.

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**Sample preparation:** Lemon, apple, grape, orange, pineapple and watermelon were collected from the local market in Bangkok, Thailand. Fruit juices were prepared by squeezing fruits and centrifuged at 12,000 x g for 10 min to obtain clear juices. The clear juices were then filtered through 0.2 µm Supor® membrane to obtain particle free fruit juices. Lemon juice was added to apple, grape, orange, pineapple and watermelon juices to obtain final lemon concentration of 5%, 10%, 20%, 30%, 50% (v/v) in the mixed fruit juices. The blends were stored at -20ºC until further analysis. The controls were prepared using 0.2 M citric acid buffer (pH 3.0) instead of lemon juice at the same proportion.

**Total phenolic content determination:** Total phenolic content was assayed by using the Folin-Ciocalteu reagent which described by Singleton & Rossi (1965). Briefly, 10 µl of juice or mixed juice was added to 40 µl Folin-Ciocalteu reagent (diluted with distilled water 1:5; v/v). After 3 min, 200 µl of 7.5% $Na_2CO_3$ was added to reaction mixture and stand for 30 min at room temperature in the dark. The absorbance was measured with a microtiter-plate reader, EZ Read 2000 (Biochrom) at 725 nm. All samples were assayed in triplicate and the results were averaged. The total phenolic content was calculated as µg gallic acid equivalents (GAE) in µg /ml using standard curve of gallic acid.

**Total antioxidant capacity determination:** Total antioxidant capacity in the juices and mixed juices were quantified using antioxidant assay kit (Sigma-Aldrich). All samples were assayed in triplicate according to the manufacturer's protocol. Total antioxidant capacity value is determined as Trolox equivalent antioxidant capacity and calculated in the millimolar (mM) concentration.

**SOD-like activity determination:** SOD-like activity in juices and mixed juices were determined using SOD determination kit (Sigma-Aldrich). All samples were assayed in triplicate according to the manufacturer's protocol. 1 U of SOD-like activity is defined as the amount of superoxide radical scavenging which inhibits the decrease of a water-soluble formazan dye by 50% under the assay conditions.

**Statistical analysis:** Microsoft Excel version 2010 software (Microsoft Corporation, Redmond, WA, USA) was used for statistical analysis. All data were expressed as mean ± SD (standard deviation) of three replicate samples. Differences among groups were compared for significant by Student's paired two-tailed *t*-test. A p value below 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

### Total phenolic content

The total phenolic content of different proportion of lemon juice in the juice blends was assayed by Folin-Ciocalteu reagent and the results are shown in Figure 1. In previous study, phenolic compounds and flavonoids of lemon juice were characterized and reported as the amount of different types of active compounds in the juice (Gironés-Vilaplana et al., 2012). The concentrations of phytochemicals in lemon depend upon various conditions (González-Molina et al., 2010). In our study, the phenolic content of lemon juice was 319.3±6.0 µg/ml. After adding lemon juice to apple juice at different proportion, the results showed significant increasing in phenolic content at proportion of 30% and 50% (v/v) of lemon juices in the blends ($p<0.05$) compared to that of 100% apple juice. Lemon juice had higher phenolic content and may effect to increase amount of phenolic content at those proportion of the blends. The mixture between orange and lemon juices at proportion of 50% lemon juice had significant increase in phenolic content ($p<0.05$) compared to pure orange juice, whereas other blend ratios of lemon and orange juices had no significant difference in phenolic content ($p>0.05$).

In case of pineapple juice, there has been significant increase in phenolic content in the juice blends with increasing proportion of lemon juice (Figure 1). The highest phenolic content was found in 50% (v/v) lemon juice in pineapple juice blend (425.1±5.1 µg/ml) in comparison to 100% lemon juice (319.3±6.0 µg/ml) and 100% pineapple juice (287±6.2 µg/mL). The synergistic increase in total phenolic content of juice blends may be due to the hydrolyzation of polyphenol glycosides by protease (bromelain) from pineapple juice (Hale et al., 2005). Attri et al. (2017) demonstrated that total phenolic content of fruit juices increased significantly after digestion with protease. In the juice blends of lemon and watermelon, the adding of lemon juice in the blends increased total phenolic content with increasing of blending ratio compared to 100% watermelon juice.

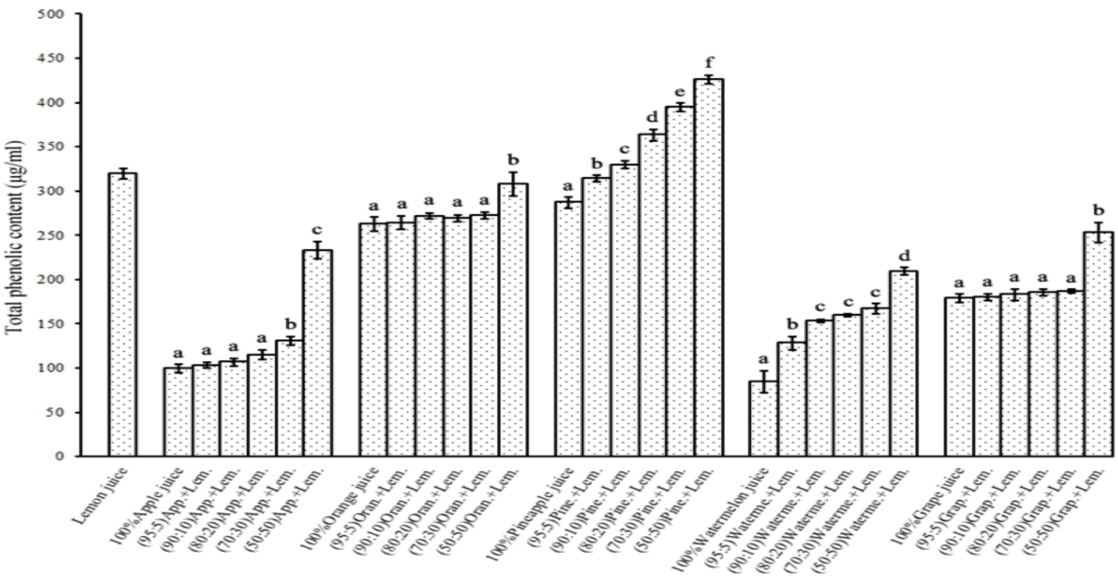


Figure 1. Total phenolic content in original fruit juices and juice blends of fruit juices and lemon juice. Data are expressed as mean ± SD of three samples. Different letters above the average bars between groups denote significantly differences at $p<0.05$. Abbreviations Lem., Lemon juice; App., Apple juice; Oran., Orange juice; Pine., Pineapple juice; Waterme., Watermelon juice; Grap., Grape juice

The amount of phenolic content in lemon juice may increase total phenolic content in the blends. There was slightly increase in the level of phenolic compounds in the blends of grape and lemon juices with increase in the ratio of lemon juice 5% to 30% in the blends, whereas 50% of lemon juice in the blends showed significant increasing in total phenolic content compared to that of 100% grape juice ($p<0.05$).

**Total antioxidant capacity**

The total antioxidant capacity of the juice blends is shown in Figure 2. The total antioxidant capacity of lemon juice in our study was 6.69±0.14 mM. The total antioxidant in the juice blends significantly increased with increasing the proportion of lemon juice when compared with original fruit juices. The mixture of lemon juice and pineapple juice at the proportion of 50:50(v/v) showed the highest of total phenolic content (Figure 1), whereas the total antioxidant capacity of the blends had no synergistic effect. Some juice blends had higher phenolic content but lower total antioxidant capacity which were present in the blends of lemon and pineapple juices, for instance. The results demonstrated that the amount of phenolic compounds in the juices could not always anticipate the level of total antioxidant capacity. There has been reported that phenolic compounds with different structure showed different capability in radical scavenging activity (Shahidi & Naczk, 1995). The ability of radical scavenging activity also depends upon the interaction between phenolic compounds (Gramza-Michalowska & Członka-Matyasik, 2011). Our findings, we found pro-oxidant activity in watermelon juice, which could oxidize ABTS reagent. From previous study, under certain conditions, flavonoids can act as pro-oxidants and enhance oxidation to other compounds (Procházková et al., 2011). Our results indicated that the activity of antioxidants released by watermelon juice may be less than those of pro-oxidants in the juice. However, the lemon juice can attenuate the effect of pro-oxidants in the watermelon juice (Figure 2). The juice blends of 10% lemon juice in watermelon juice showed total antioxidant capacity of 0.9±0.1mM and significantly increased with increasing the proportion of lemon juice up to 50% lemon juice in the blends.

**SOD-like activity**

Superoxide anion is one of the important reactive oxygen species (ROS) leading to the pathogenesis of many diseases. SOD is a first line antioxidant enzyme of defense against oxidative stress and catalyzes the dismutation of superoxide anion (Nozik-Grayck et al., 2005). There have been reported that phenolic compounds and flavonoids are able to function on superoxide anion scavenging activity (Farombi & Fakoya, 2005; Sun et al., 2011). Yu et al. (2005) demonstrated bioactive compounds presenting in lemon juice had SOD-like activity. In our study, SOD-like activity presenting in lemon juice was 94.7±0.6 U/ml, whereas the level of SOD-like activity of other fruit juices were hardly different compared to that of lemon juice except that in orange juice (61.0±1.0 U/ml) (Figure 3). The blends of lemon juice and orange juice showed significant increasing in SOD-like activities when increased the ratio of lemon juice from 20% to 50% in final concentration of the blends in comparison to 100% orange juice. It may be possible that a higher level of SOD-like activity in lemon juice was added to the blends.

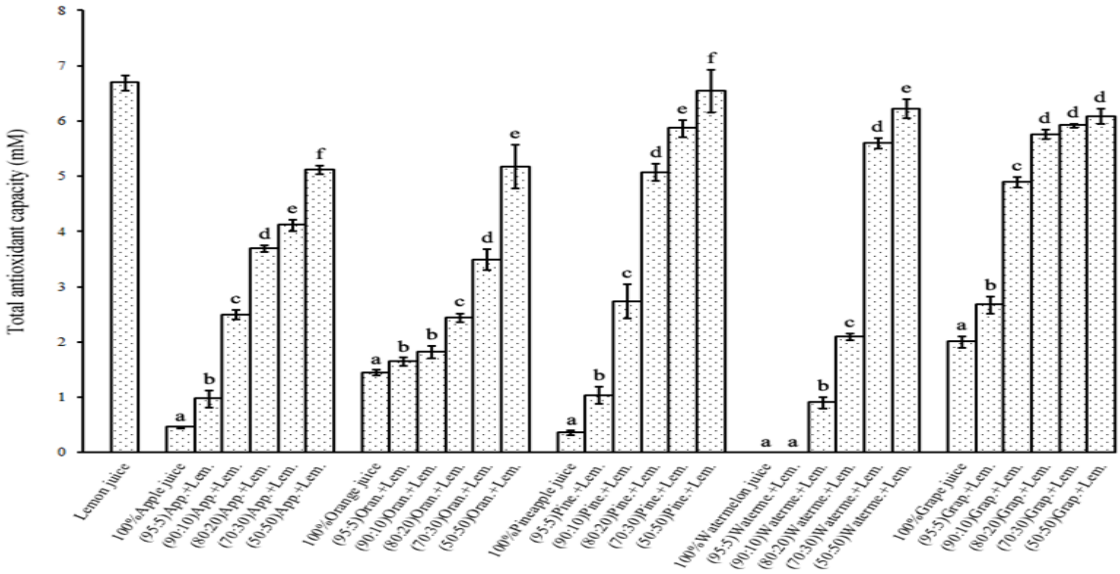


Figure 2. Total antioxidant capacity in original fruit juices and juice blends of fruit juices and lemon juice. Data are expressed as mean ± SD of three samples. Different letters above the average bars between groups denote significantly differences at $p<0.05$. Abbreviations Lem., Lemon juice; App., Apple juice; Oran., Orange juice; Pine., Pineapple juice; Waterme., Watermelon juice; Grap., Grape juice

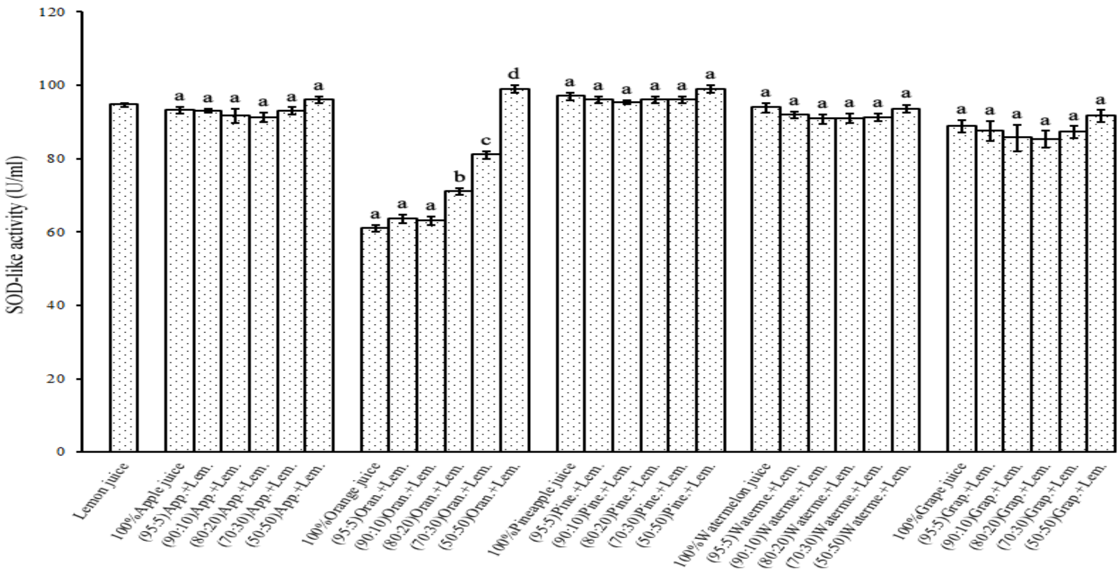


Figure 3. SOD-like activity in original fruit juices and juice blends of fruit juices and lemon juice. Data are expressed as mean ± SD of three samples. Different letters above the average bars between groups denote significantly differences at $p<0.05$. Abbreviations Lem., Lemon juice; App., Apple juice; Oran., Orange juice; Pine., Pineapple juice; Waterme., Watermelon juice; Grap., Grape juice

## CONCLUSIONS

Lemon is one of the citrus fruits that are rich source of nutrients and bioactive compounds. The increase in the ratio of lemon juice in the blends enhanced total phenolic content, especially in the blends of pineapple juice. The mixture of lemon juice and pineapple juice in the proportion of 50:50 (v/v) had the highest phenolic content. The total antioxidant capacity in the juice blends significantly increased with increasing the proportion of lemon juice compared with 100% pure squeezed fruit juice. In addition, the lemon juice can attenuate the effect of pro-oxidants presenting in watermelon juice. These pro-oxidants need to be identified for further study. The addition of lemon juice in orange juice enhanced SOD-like activity in the blends, whereas other fruit juices had no significant difference. The present study showed health benefits that obtained from various lemon juice ratios in the juice blends in terms of total phenolic content, total antioxidant capacity and SOD-like activity.


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