International Journal of Agricultural Technology 2017 Vol. 13(7.1): 1403-1412
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Effect of Process Conditions and Shelf life on ORAC (Oxygen Radical Absorbance Capacity) Value of Supplement Mangosteen Juice

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Benjamas, D., Varith, J., Ariyadet, C and Thechatakerng, P. (2017). Effect of Process Conditions and Shelf life on ORAC (Oxygen Radical Absorbance Capacity) Value of Supplement Mangosteen Juice. International Journal of Agricultural Technology 13(7.1): 1403-1412.

The fruit of *Garcinia mangostana* Linn. (mangosteen) is a popular fruit in Thailand. The fruit rind contains antioxidants of which a major constituent are anthocyanin, polyphenol and xanthones. These are influent to ORAC (oxygen radical absorbance capacity) value content. The ORAC value has recently been proposed as a quality index of total antioxidant in supplement juice. This study was undertaken to evaluate the content of total anthocyanin (mg/100g), total polyphenol (mg eq GA/100g) and ORAC (μmoles TE/100ml) in fresh and dried mangosteen rind in 4, 5 and 6 ripe levels extracted. Study the stability of ORAC on supplement mangosteen juice in different process conditions by varies pH value at 3.0, 3.5 and 4.0 and temperature at 105, 110 and 115°C degree. Study shelf life at 0, 4, 8 and 12 months. The process of this study was used plate heat exchanger method. Fresh rind extracted in 4, 5 and 6 ripe levels the total anthocyanin were 4.06±0.17, 4.04±0.24 and 5.09±0.18, total polyphenol were 632.73±21.14, 686.59±29.06 and 707.39±29.73 and ORAC were 17,063.36±883.25, 20,958.61±725.08 and 24,744.62±784.78 respectively. All of value was increased when the rind was more ripe level. Dried rind extracted the total anthocyanin were 1.10±0.01, 0.99±0.03 and 0.17±0.01, total polyphenol were 922.87±6.11, 1,010.03±42.95 and 798.37±6.97 and ORAC value were 23,550.05±690.88, 26,634.41±1132.74 and 33,802.98 ±1,374.38 respectively. The total anthocyanin and total polyphenol were decreased when the rind was more ripe level but ORAC was increased. The temperature was influented to ORAC value but pH and interaction of pH and temperature were not. The ORAC value was decreased in 12 months shelf life, the value start at 2,183.56 ±51.47 at 0 month and decreased to 2,077.474±12.87, 2,057.05±49.64 and 1,945.58±10.98 at 4, 8 and 12 months or decreased from 100% to 95.15% 94.23 and 89.10% respectively.

Keywords: supplement magosteen juice, ORAC shelf life, antioxidant in process conditions, ORAC in process condition, total anthocyanin, total polyphenol, ORAC content.

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Introduction

Garcinia mangostana Linn. (mangosteen) is a popular fruit in Thailand. It is commonly cultivated in Thailand, Malaysia, and Indonesia. Most of mangosteen is consumed fresh or exported to foreign market. It contains high nutritional values. In essence, it is a rich source of vitamins, minerals and fibers such as vitamin C, E, folate, calcium, potassium and magnesium (Limpisathian, 2008) Mangosteen pericarp has been used in traditional Thai medicine for treating skin infections, wounds, and diarrhea for many years (Mahabusarakam *et al.*, 1987). The fruit rind contains antioxidants of which a major constituent are anthocyanin, polyphenol and xanthones. The major anthocyanin in mangosteen was cyanidin-3-sophoroside (Du and Francis, 1977). Several researchers recognized phenolics and anthocyanin for their antioxidant properties (Robards *et al.*, 1999; Karalaya *et al.*, 2001; Rossi *et al.*, 2003; Davalos *et al.*, 2005; Balasundram, 2006). These are influent to ORAC (oxygen radical absorbance capacity) value content. The ORAC value has recently been proposed as a quality index of total antioxidant in supplement juice. In Thailand, the rind extracted is used in products such as herbal cosmetics and pharmaceutical. Recently, mangosteen rind extracted is used in mangosteen juices or dietary supplements mangosteen juices. It has begun to be widespread around the world supplement.

Mangosteen contains a substance called xanthone, which is a flavonoid and polyphenon compound. It was found that xanthone extracted from the mangosteen rind, called alpha-mangostin, beta-mangostin and garcinone B can inhibit *Mycobacterium tuberculosis* (Suksamrarn *et al.*, 2002). Alpha-mangostin is a major component which possesses anti-inflammatory and antibacterial activities against methicillin-resistant *Staphylococcus aureus*, *S. epidermidis* and *Propionibac-terium acnes* which is the critical eticologic agent in acne (Di-ngam, 2009; Sikhamchum, 2009). According to the report of Sakagami (2005), xanthone has antioxidant, anti-cancer and anti-bacterial properties, respectively. A recent study by Sukma *et al.* (2011) also claimed that alpha-mangostin is anti-inflammatory, reduce inflammation in NG 108-15 cells.

Because of mangosteen has high beneficary for human, supplements factories are focusing on dietary supplements mangosteen juices. But processing method has an impact on phenolics anthocyanins and total antioxidant value. Heating has varied effect on several products depending on heating temperature and process conditions. But no report on effect of process conditions on total antioxidant as ORAC value. ORAC is not including antioxidant only anthocyanin and polyphenol but included strong antioxidant as

xanthones. In this study will be reported the antioxidant value from different mangosteen ripe level, effect of process conditions and shelf life on ORAC value.

Material and methods

This study is used organic mangosteen fruits from Chanthaburi province in different ripe levels (4, 5 and 6). Specifications of mangosteens were selected by mangosteen harvest and post-harvest guide book from officer of Agricultural research and Development regions, Chanthaburi, Thailand. The rind of fruit was separated from white pulp, grinding and divided into 2 parts (1) fresh grinded rind and (2) made the dried rind at 60°C, 12 hours in prior of extraction.

Each part of fresh and dried mangosteen rinds were immersed in 95 % ethanol at 1,000g.: 3,000ml. ratio for 7 days, extracted 2 times. The extracted solutions were combined together and evaporated to 1,000ml. by rotary evaporator. The antioxidants from the rind were dissolved in liquid extraction such as anthocyanin, polyphenol and xanthones.

Anthocyanin analysis

The total anthocyanin content was determined by using of capillary electrophoresis (CE) analysis under acid condition has significantly increased peak resolution and improved the detection limits by several orders of magnitude. CE offers the advantage of economies of very small sample size, very small solvent consumption, and short analysis times along with the future possibility of being combined with MS defection. This study was analyzed at Food and Nutrition Laboratory, Institute of Nutrition, Mahidol University.

Polyphenol analysis

The total phenolic in mangosteen extracted content was determined according to the Fo-Lin-Ciocalteu spectrophotometric method. The extracted solution was determined at 760 nano meter (nm) absorbance. The measurement was compared to standard curve of prepared gallic acid (GA) solution, and the total phenolic content was expressed as milligrams of gallic acid equivalents (QAE) per gram of day weight (mg eg GAE/g). This analysis was conducted at Food and Nutrition Laboratory, Institute of Nutrition, Mahidol University.

ORAC analysis

ORAC value was used by Cao et al method which measures antioxidants scavenging activity against peroxyl radical induced by fluorescein (FL) (3',6'-dihydroxy spiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one) as the fluorescent probe. The FL oxidized products induced by peroxyl radical have been identified by LC/MS, and the reaction mechanism was determined to proceed as a classic hydrogen atom transfer (HAT) mechanism. Unlike other popular antioxidant activity methods, the improved $ORAC_{FL}$ assay provides a direct measure of hydrophilic chain-breaking antioxidant capacity against peroyl radical. (Ou *et al.* 2001). This study was analyzed at Food and Nutrition Laboratory, Institute of Nutrition, Mahidol University.

Process conditions and shelf life study

pH conditions

Mangosteen juice was prepared from white pulp, the seed was separated, used only juice and pulp mixed together by colloid miller. The juice is 96% mixed with 4% mangosteen rind extracted solution. Divided the juice to 10 parts, each part was 100kg. First part used as control prepared by heating at 72°C degree 5 minutes with added sodium sorbate 0.04 % (400 ppm), and divided into 6 groups of package to test the shelf life of those antioxidants. The other 9 parts were adjusted pH by citric acid to 3 levels, 3.0, 3.5 and 4.0 respectively, each pH adjusted in 3 parts of mangosteen juice.

Temperature conditions

After adjusted pH of mangosteen juice by citric acid to 3 levels, each pH juice was heat in different temperature at 105, 110 and 115°C degree by plate heat exchanger instrument. Samples from each temperature were divided into 6 groups to test ORAC shelf life.

Shelf life study

After prepared the juices by different pH and heated in different level, the juices were keeping for ORAC shelf life study at 0, 4, 8 and 12 months.

Experimental design and statistics methods

Study of anthocyanin, polyphenol and ORAC content in different mangosteen ripe level was experiment designed by CRD (complete randomized design) method one way ANOVA was used for determination of differences between

mangosteen ripe level and process with SPSS V.14.0. The Duncan multiple range test was used to compare means value. A probability level of $p \leq 0.05$ was considered as significant. Study of process conditions on ORAC value was used in experiment of factorial in CRD method mixed conditions were 9 treatments from main factors pH and temperature, pH were used 3.0, 3.5 and 4.0 and temperature were used 105, 110 and 115°C degree. Effect of conditions on ORAC value was determined by 1 way ANOVA from main factors, and interaction factor. Any interaction contrast will be written as a linear.

Results and Discussion

Total anthocyanin, polyphenol and ORAC value in different freshness and ripe level, dried mangosteen rinds were extracted from outer and inner part of shell. The total polyphenol, anthocyanin and ORAC content in each ripe level are showed in table 1

Table 1 The total polyphenol, total anthocyanin and total ORAC content in different mangosteen ripe level.

Mangosteen rind extracted	Total anthocyanin mg/100g	Total polyphenol (mg eq GA)/100g	Total ORAC (μmole TE/100ml)
Fresh rind extracted			
Ripe in level 4	4.06±0.17[a]	632.73±21.14[a]	17,063.36±883.25[a]
Ripe in level 5	4.04±0.24[a]	683.58±29.63[b]	20,958.61±725.08[b]
Ripe in level 6	5.09±0.18[b]	707.39±29.73[b]	24,744.65±787.78[c]
Dried rind extracted			
Ripe in level 4	1.10±0.10[c]	922.87±6.11[b]	23,550.05±690.88[a]
Ripe in level 5	0.99±0.03[b]	101.03±42.95[c]	26,634.41±1132.74[b]
Ripe in level 6	0.17±0.10[a]	798.37±6.97[a]	33,802.96±1,374.38[c]

Total anthocyanin

Anthocyanin in different freshness of rinds 4, 5 and 6 ripe levels extracted were 4.06 ± 0.17, 4.04 ± 0.24 and 5.09 ± 0.18 mg/100g. respectively and significant difference ($p<0.05$) (Table 1). The trend of anthocyanin was increasing when the rind was riper. Dried extracted, total anthocyanin were 1.10 ± 0.1, 0.99 ± 0.03 and 0.17±0.1 mg/100g respectively and significant difference ($p<0.05$). It was decreased when the rind was riper. Heat in dried rind process destroyed anthocyanin content. Anthocyanin from mangosteen fruit is use fresh rind for extraction better than using dried rind.

Total polyphenol

Polyphenol in table 1 are showed that fresh rind extracted the polyphenol content were 632.73±21.14, 683.58±29.63, and 707.39±29.73 (mg eq GA/100g) in 4, 5 and 6 fruit ripe level respectively. The content in each ripe has significant difference ($p<0.05$). Polyphenol was higher when the fruit was riper. In dried rind, polyphenol were 922.87 ± 6.11, 1010.03 ± 42.95 and 798.37 ±6.97 (mg eq GA/100g) in 4, 5 and 6 fruit ripe level. The content was higher than fresh rinds extract (W/W). Total polyphenol in mangosteen ripe level 5 was strong with other level and significant difference ($p<0.05$). The polyphenol from mangosteen fruit was found in dried rind, this research has confirmed that whether needed to extract the antioxidants from mangosteen fruits, the dried rind is good chance for user because the polyphenol antioxidants are still consist and easier to storage.

Total ORAC content

ORAC content in fresh rind 4, 5 and 6 ripe levels extracted were 17,053.36 ±883.25, 20,958.61±725.08 and 24,744.65±787.78 μmoles TE/100 ml respectively and significant difference ($p<0.05$). The trend of ORAC was increasing when the rind becomes riper. In dried rind extracts, the rind ORAC content were 23,550.05± 690.88, 26,634.41± 1132.74 and 33,802.96± 1374.38 μmoles TE/100 ml. and significant difference ($p<0.05$). It was increasing when the fruit was riper. This result has shown that the total antioxidants are depending on the ripeness of fruits. In commercial antioxidant extraction is possible to use the most ripest of fruits and it is easier for user to select the fruits for extraction because the color of fruits is most purple-dark color. In dried rind extracted the most ORAC value of this research is depending on polyphenol and xanthones content it is not depending on anthocyanin because anthocyanin will be decreasing when the fruit was riper.

Effect of process conditions and shelf life

pH and temperature conditions on ORAC value

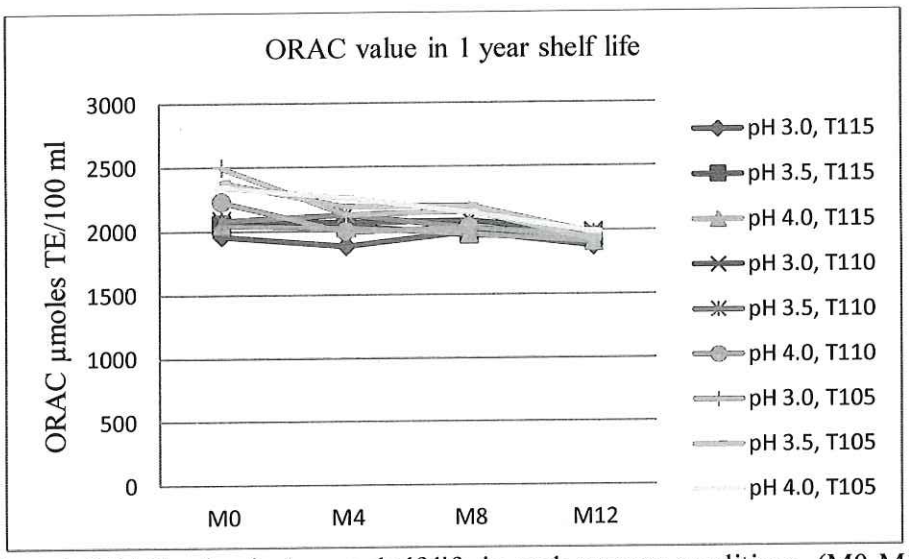
Table 2 the mangosteen juice was added 4% of rind extracted before pH adjusting and heating process. Process of this study was used plate heat exchanger method. The ORAC value was start at 2,386.69±80.87 μmole TE/100ml and reduced to 2,183.55±51.47, after treated by process conditions.

Table 2 ANOVA of main factors, temperature and pH affected on ORAC content after process.

Source	Sum of Squares	df	Mean Square	F	Sig
Corrected Model	2434411.471(a)	8	304301.434	1.522	.218
Temp	2338866.435	2	1169433.218	5.851	.011*
pH	17015.462	2	8507.731	.043	.958
Temp * pH	78529.574	4	19632.394	.098	.982
Error	3597813.770	18	199878.543		
Total	139481494.876	27			

Effect of process conditions, main factor temperature was influented to ORAC content ($p<0.05$) after process. pH and interaction factors pH and temperature were not influentened to ORAC content value. In this result showed that the ORAC was strong in pH but varies with temperature. When treated high temperature ORAC value will be loosing more. Heating has a varied effect on several products depending on heating temperature. Sterilization reduced total phenolic, procyanidin monomer, dimer, trimer, tetramer, pentamer, and hexamer of canned peach and Brownmiller *et al* (2008) report that thermal processing resulted in marked losses in total anthocyanins (28% to 59%) and $ORAC_{FL}$ values (43% to 71%) in all products, with the greatest losses occurring in clarified juices and the least in non-clarified juices. Patrasa *et al* (2010) found that anthocyanin pigments readily degrade during thermal processing which can have a dramatic impact on colour quality and may also affect nutritional properties. This review attempts to summarize some important aspects of anthocyanin degradation during thermal processing. Conclusions regarding the mechanisms and kinetics of anthocyanin degradation during heat treatment are postulated based on current findings. Luke (2012) report that the steps in processing where significant losses of polyphenols occur, anthocyanins and procyanidins are also degraded in processed products stored at ambient temperature with losses accompanied by increased polymeric pigments (PPs). Elizabeth (2006) was studied the effects of processing on hydrophilic antioxidant capacity of black beans and found that there was a significant effect of thermal processing black beans at 110° C for 10 and 30 min. Compared to uncooked beans (229.18 μmol TE/g), there was a decreased of 91% in antioxidant capacity for cooked beans (19.13 μmol TE/g). Junpatiw *et al* (2017) study heat teatment on purple vegetable antioxidant and found that the anthocyanin contents were decreased in steaming with water vapor and boiling in water at 98-100°C treatments more than uncooked samples. The anthocyanin contents decreasing percentage in boiling method were higher than steaming.

The decreasing percentage in boiling and streaming at 14.69 and 4.14 % were found in purple sweet potatoes, 72.65 and 71.98 % in purple eggplants and 18.89 and 4.47 % in purple cabbages, respectively.



Figue 1 ORAC value in 1 year shelf life in each process conditions. (M0-M12 are the month were storing)

Effect of Shelf life on ORAC value

Shelf life of supplement mangosteen juice in each 9 conditions was stored at room temperature and test ORAC value at 0, 4 8 and 12 months after process. The ORAC value was started at 2,386.69±80.87 μmole TE/100ml before process; every condition was slightly reduced after process and more shelf life storage. The average of ORAC value in each month was decreased to 2,183.55±51.47, 2,077.74±12.87, 2,057.05+49.64 and 1,945.58+10.98 or decreased from 100% before process to 91.49% 87.05% 86.21% and 81.52% after process at 0, 4, 8 and 12 months shelf life respectively. This result is consistent with Brownmiller *et al.* (2008) The $ORAC_{FL}$ values showed little change during storage, indicating that the formation of polymers compensated for the loss of antioxidant capacity due to anthocyanin degradation. Methods are needed to retain anthocyanins in thermally processed blueberries. Wang *et al* (2004) report that the antioxidant capacity of honeys was reduced after 6 months of storage with no impact of storage temperature or container type detected at the end point of the storage period. Thankkar (2012) was studied effect of processing on the antioxidant activity and other quality parameter of

muscadine grape juice, the results showed no significant change in total phenolics and ORAC after process but during storage, total phenolics and ORAC value decreased with time. Junpatiw *et al* (2017) study shelf life of antioxidant on purple vegetable and found that the storing of purple vegetables in various temperatures found that the decreasing of anthocyanin contents in 10 and 15 °C treatments were lower than stored at room temperature.

Summary

The total antioxidants; anthocyanin, polyphenol and ORAC value are increase when fresh mangosteen fruits are riper. In dried mangosteen rind extracted the total antioxidants were increasing varies with higher ripe only anthocyanin decreased when the rind become riper.

The effect of process conditions found that temperature was influented to ORAC value; it was reduced when used high temperature. But it was stronger in pH and interaction of pH and temperature.

Shelf life of supplement mangosteen juice will affected to ORAC value changing. The ORAC will be decreased when shelf life storage is longer.

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(Received: 20 October 2017; accepted: 25 November 2017)

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โปรดระบุหมายเลข ISSN หรือชื่อของวารสารที่ต้องการทราบผลประเมิน : ค้นหา

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