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Storage Effect on Quality Attributes of Combine Mild Heat and Ultraviolet Irradiated Roselle (*Hibiscus Sabdariffa*) Juice Compare with Thermal Treatment

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Roselle juice is high in vitamin C and anthocyanin contents. Commercial thermal pasteurization affect the quality of roselle juice especially the anthocyanin color pigment and heat sensitive vitamin C. Extraction with mild heating able to extract more yields with clear juice to meet limitation of UV-C. Combine mild heat extraction (60 °C, 30 minutes) and UV-C treatment (65.86mJ/cm²) show significantly no difference in anthocyanin after treatment. Color of roselle juice more acceptable in appearance compare with thermally pasteurized roselle juice. Other properties of physicochemical (pH, titratable acidity, total soluble solid and turbidity) also shows promising result for combine mild heat and UV-C treatment. Ascorbic acid of roselle juice remains higher compare to thermally pasteurized (90°C for 2 minutes) roselle juice. Microbiologically, the combine treatment managed to extend the shelf life of roselle juice for 6 weeks of storage at 4±2°C for 8 weeks.

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Introduction

Ultraviolet irradiation-C (UV-C) at wavelength range from 254 nm to 264 nm primary mechanism of inactivation is by creation of pyramiding dimmers which prevent microorganism from replicating and rendering them from causing infection (Koutchma, 2009). UV-C light penetration depth through liquid surface is limited unless for clear liquid. Transmittance of UV light through juice low compared to water due to its high optical density (Koutchma, 2009). Turbidity of fruit juice affects the UV-C performance the most as suspended solid causing juice to be turbid and blocked the UV light (Koutchma, 2008).

Roselle (Hibiscus sabdariffa Linn) belongs to Malvaceace family and widely cultivated in Asia and tropical Africa (Lee, Hadi, Zainal Abidin and Mazni, 2015). Roselle is an annual erect, bushy plant with typical red stem (Ismail, Khairul, Ikram and Nazri, 2008), edible leave with rhubarb taste (Fasoyiro, Bababola and Owosibo, 2005) and roselle flower which contain edible red petal known as calyces and capsule of seed (Luvonga, 2012). Roselle juice was extracted from its calyces which rich in anthocyanins, ascorbic acid and hibiscus acid, with excellent red colour (Abou-Arab, Abu-Salem and Abou-Arab, 2011).

Quality of roselle juice greatly depends on extraction method. Extraction is a technique of phase changes of targeted compound (Dean, 2009) includes, hot water extraction, hot and cold water blending, screw press, microwave oven and enzymatic extraction (Quek, Chin and Yusof, 2012). Wong, Yusof, Mohd Gazhali and Che Man (2003) found, hot water extraction retained higher anthocyanin content compare to cold water blending. Extraction method also affects the efficiency of ultraviolet irradiation treatment as different methods of extraction result with different turbidity value. However, up to date there was no reported study on the effect of mild heat extraction method on the quality of roselle juice ultraviolet irradiated. Thus, this study aim to evaluate the performance of ultraviolet irradiation treatment on mild heat extracted roselle juice compare with thermal stored at 4±2°C for 8 weeks.

Materials and Methods

Roselle juice preparation

Fresh roselle at commercial maturity obtained from farm in Puchong, Selangor. The capsule of seed was removed to obtain only the calyces (Ismail, Khairul Ikram and Mohd Nazri, 2008). Extraction was done by soaking calyces in mild temperature water (60°C, 30 minutes) in water bath (Waterbath, Memmert, Germany) with ratio of 1gram roselle calyces to 10ml water. Cooled extract then, filtered using muslin cloth to separate the calyces. Further filtration was done using filter paper (Filter paper, Whatman No. 1, UK) to obtain clear juice color to meet the limitation of ultraviolet irradiation treatment.

Ultraviolet irradiation (UV-C) and heat pasteurization treatment

Ultraviolet pasteurization (Malaysian Patent PI201203186) was used in this study. Cleaning, operating and UV-C dosage determination of juice using the unit follows Chia, Shamsudin, Mohd Adzahan and Wan Ramli (2012), Mansor, Shamsudin, Mohd Adzahan and Hamidon (2014) and Mohd Hanif, Shamsudin and Mohd Adzahan (2016). UV-C dosage was measured as mJ/cm². The same filtered juices undergo heat pasteurization at 90°C for 2 minutes in waterbath (Waterbath, Memmert, Germany). Roselle juice then stored in glass bottled covered with aluminium foil and refrigerated (4±2°C) for 8 weeks.

Color properties

Color properties of L* (lightness form black, 0 to white, 100,) a*(greenness, -a* to redness, +a*) and b* (blueness, -b* to yellowness, +b*) of roselle juice obtained using color Spectrophotometer UltraScanPro (D65, Hunter Lab, USA) and result obtained used to calculate the hue and chroma color properties. Hue angle (h*) and chroma color properties describe the color visual sensation according to area similar to one or proportions of two color (red, yellow, green or blue) (Luvonga, 2012).

$$\text{Hue angle } (h^*) = \tan^{-1} (b^*/a^*) \quad \text{(Eq. 1)}$$

$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})} \quad \text{(Eq. 2)}$$

Anthocyanin color pigment that act as natural antioxidant contained in roselle extract measured using UV spectrophotometer (Ultraspec, 3100, Amersham) at wavelength 517 nm and 700 nm following pH differential method of Chew, Soh, Liew and Teh (2007).

Physicochemical properties

pH and titratable acidity

High pH condition provide comfortable living environment to microorganism compare to acidic condition (Tasnem et al., 2010). pH of roselle juice measured using digital autotitrator meter (785 DMP Titrino, Metrohm, Switzerland). Titratable acidity (TA) measured the total acid contained in fruit juice (Bamise and Oziegbe, 2013) using AOAC (1995) method and expressed as percentage of Malic acid (Scott, Clavero, and Troller, 2001).

Total soluble solid

Total soluble solid obtained directly from digital refractometer (AR-2008, Krus, Germany) and expressed as unit °Brix. Optical properties of freshly extract fruit juice often result with soluble and suspended solid that affect the optical properties of the juice (Koutchma, Keller, Chirtel and Parisi, 2004).

Turbidity

Turbidity of liquid caused scattering of UV-C light reduced its efficiency (Fredericks, du Toit and Krugel, 2011). Turbidity of juice measured using turbidimeter (TN-100, Eutech, Singapore) in unit of Nephelos Turbidity unit (NTU). Calibration of the turbidimeter was done using standard solution of 0.02, 20.0, 100.0 and 800.0 NTU.

Vitamin C

Vitamin C is an essential nutrient that easily destroyed from exposure to heat and oxygen during processing (Nielsen, 2010). Vitamin C content in roselle juice was determined using titration method of AOAC 967.21 (AOAC, 2007) expressed in mg ascorbic acid/100g.

Microbiological analysis

Total plate count (TPC) and yeast and mould counts (YMC) were the microbiological analysis conducted. Plate count agar (PCA) (Merck, Germany) was used to determine TPC (Maturin and Peeler, 2001) while for YMC, Dichloran Rose-Bengal chloramphenicol agar (DRBC) (Condalab, Spain) was used (AOAC, 2002). 0.1 ml of sample from each serial dilution (10^{-1} to 10^{-5}) spread onto the solidified agar for both microbe tests. The PCA were incubated for 2 days at 35±2°C whereas for the YM it incubated for 5 days at 25±2°C and expressed as log cfu/ml (Cheng, Soh, Liew and Teh, 2007).

Statistical analysis

The data include in this study were analysed by Turket HSD test ($P<0.05$) using SPSS software (IBM 22, USA) for analysis of variance (One way ANOVA).

Results and Discussion

UV-C dosage determination

Determination of UV-C dosage was based on microbial count of total plate count (TPC) and yeast and mould count (YMC) in the juice. Table 1 shows the UV-C dosage effect on the TPC and YMC counts in roselle juice. UV-C dose of 65.86 mJ/cm^2 chosen as the amount was adequate to inactive the microbial counts in roselle juice. Since, roselle juice rich in anthocyanin content that easily affected by light, lower dosage was chosen as precaution and to minimize the anthocyanin degradation in Roselle juice. Mohd Hanif, Shamsudin and Mohd Adzahan (2016) study on effect of UV-C dosage on lime juice found that lower UV-C dose of 22.76mJ/cm^2 able to maintain most of the physicochemical properties of the juice.

Table 1. UV-C dosage effect on microbiological (TPC and YMC)

Exposure time (mins)	UV-C dosage (mJ/cm²)	TPC (log cfu /ml)	YMC (logcfu / ml)
22.94	67.42	0^a	0^a
21.62	65.86	0^a	0^a
20.88	64.40	3.602	3.647

Color properties

Color properties of L* indicate the lightness of juice color. Based on Fig. 1 (Lightness, L* of roselle juice at different treatment during storage), L* value of mild heat extracted roselle juice (HE) stable until week 5 and start to degrade significantly (p<0.05) after. Combine treatment UV-C and mild heat extraction (HEU) resulted with lower L* values (32.2 at week 0) but not significantly different from HE (34.59 at week 0). L* of HEU roselle juice remains stable until week 6. In the meanwhile, thermal treatment of mild heat extracted roselle juice (HEH), result with massive L* color properties decrement after treatment and become unstable after week 3 of storage. Klim and Nagy (1988) stated that heating cause the accumulation of dark color compound in juice and consistently decreased the L* value, proved later by Yeom et al., (2000) in which the thermally pasteurized blended orange and carrot juice lightness decreased significantly during storage at 22 °C. Thus, combine mild heat extraction and UV-C able to retain roselle juice lightness throughout the 8 weeks of storage.

Fig. 1 (Hue angle of roselle juice at different treatment during storage) showed, hue of HE was stable during storage. Hue of HEU significantly unchanged for 7 weeks but increased suddenly in week 8. Sudden increased in hue of HEU indicate colour of juice become less red but studies by Ramirez et al., (2010) state that desirable hue of roselle juice range from 33.82 to 35.89. HEH roselle juice not significantly differed from HE during first 4 weeks of storage but start to decrease (p<0.05) at week 5. Lower hue value resulted with darker red color of HEH juice.

Chroma is the intensity of the color (Crisosto, Bremer, Ferguson, and Crisosto, 2010). Fig. 1 (Chroma of roselle juice at different treatment during storage) shows, HE, HEU and HEH illustrated similar decrement trend. Decreased in chroma caused less intense red color of roselle juice. Supposed HEU able to maintain its chroma, but maybe due to mild heat exraction (60°C, 30 minutes), color pigment in roselle juice already destruct as mention by (Rattanathanalerk, Naphaporn, and Srichumpoung, 2005), pigment destruction caused by non-enzymatic browning results in darker pineapple juice color. According to Esteve and Frigola (2007) heating, air and light cause color pigment to undergo oxidation, cis-trans changes and alteration in exipode rings as a function of storage that lead to color changes in juice.

Color degradation in roselle was a result of anthocyanin degradation as chroma and hue are dependent on conditions inside and outside the vaculoles in which anthocyanin pigment accumulate (Muriithi, et al., 2009). Refereing to Fig. 1 (Anthocyanin, m untg-cy-3-glu/g roselle juice at different treatment during storage), anthocyanin content of HEU (20.04 m untg-cy-3-glu/g) not significantly differed with HE (19.85 m untg-cy-3-glu/g) at week 0. Anthocyanin content of HEU degrade starting at 5th week of storage (might due to mild heat treatmnet), while anthocyanin of HEH low until 4th week compare with HEU, but slightly increased at week 7. From this study it shows that heat main contribution in the degradation of anthocyanins content in roselle juice. Magnitude and duration of heating has a strong influence on anthocyanins stability (Ankit, et al., 2010).

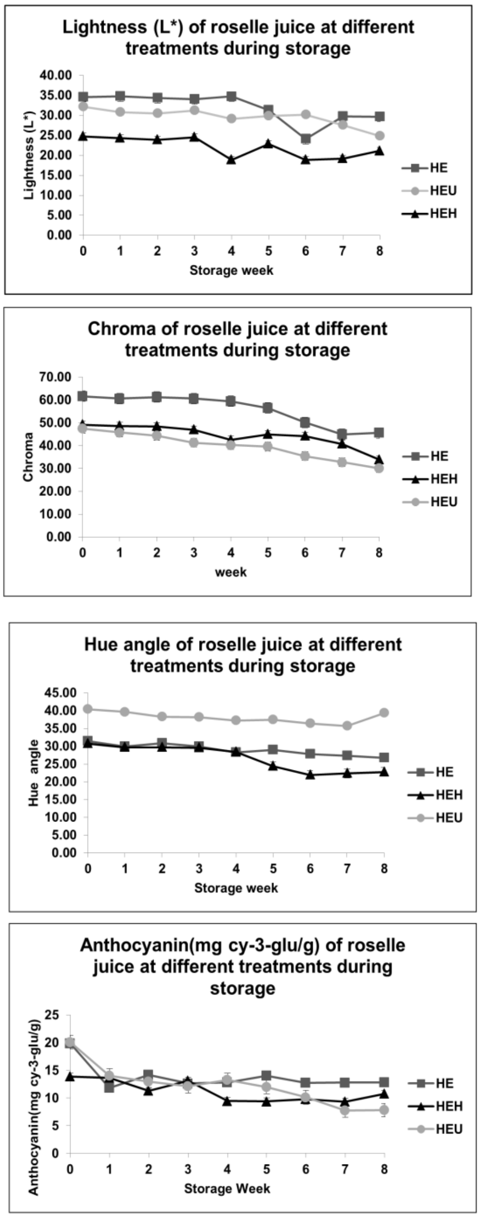


Fig. **1**. Color properties of lightness (L*), hue angle, chroma and anthocyanin (mg cy-3-glu/g) of roselle juice at different treatments (mild heated, HE; combine mild heated and UV-C, HEU; combine mild heated and thermal pasteurization, HEH) during 8 weeks storage

Physicochemical properties

pH and titratable acidity

The effect of storage on pH content in roselle juice during storage of 8 weeks shown in Fig. 2 (pH of roselle juice at different treatments during storage). HE started to decreased at week 3 and remains stable afterward. HEH show similar trend by start to decreased in pH at week 2. As for HEU, pH remains significantly unchanged during storage. Fasoyiro, Bababola and Owosibo (2005) found similar finding as pH of refrigerated roselle juice was unchanged for the first 3 days of storage with only little changes at 15 days.

Titratable acidity (TA) of HE, HEU and HEH illustrated in Fig. 2 (Titratable acidity of roselle juice at different treatments during storage). Starting week 2 of storage, there were no significant different (p>0.05) of TA between HE and HEU, HE and HEH of roselle juice. Usually lower pH value will results in higher TA of sample. Result obtained deviate from previous study by Chia, Shamsudin, Mohd Adzahan and Wan Ramli (2012) in which TA of pineapple juice increased with decreased of pH. According to Jolicoeour (2015) total titratable acidity responsible for tasting sensation in which too much acidity may render the juice or cider too sharp and unpleasant to drink, while pH related to biochemical reaction that occur in fermenting cider. Therefore, this study shows that although, roselle juice was lower in pH, the taste of the juice was not acidic and desirable.

Total soluble solid

Total soluble solid (TSS) of roselle juice is shown in Fig. 2 (Total soluble solid of roselle juice at different treatmnets during storage). TSS of HE decreased tremendously at week 3 might due to the presence of microorganism. Yeom et al., (2000) claimed, untreated juice began to present fermented smell on third week of storage in which the microorganisms causing the fermentation can utilise the soluble solids present in juice and change it °Brix. HEU roselle juice decrease throughout the 8 weeks of storage. TSS of HEH of roselle juice more stable and higher than HEU with no significant change until 7 weeks of storage. Tandon et al.,(2003) reported similar trend of hot-fill pasteurized apple cider, indicated the biological stability of samples throughout their storage period compare to UV-treated.

Turbidity

Fig. 2 (Turbidity of roselle juice at different treatments during storage) shows turbidity of HE increased suddenly to 34.44 (week 2) and reduced significantly after. While, for HEH, turbidity not significantly changed for 5 weeks. Fluctuation of turbidity observed in HEU during storage deviate from previous study by Mohd Hanif, Shamsudin and Mohd Adzahan (2016) in which turbidity of lime juice decreased after UV-C treatment. Besides, turbidity of HEU was higher compare to HEH throughout storage. Abdul Karim Shah, Shamsudin, Abdul Rahman and Mohd Adzahan (2014) stated that turbidity of juice affected by the suspended solid. However, TSS of HEU roselle juice decrease during storage thus, other factor might influence the inconsistency result of juice turbidity.

Vitamin C

Initial vitamin C of 135. 162 mg ascorbic acid/ 100g(HE), reduce about 61.1% (82.86mg ascorbic acid/ 100g) and 42.0% (56.962mg ascorbic acid/ 100g) for HEU and HEH respectively (refer Fig. 3). Vitamin C of HE, HEU and HEH not significantly change during storage may due to stability of the ascorbic acid component and suitable storage condition. Studies by Murtaza, et al., (2004) reported minimal changes of vitamin C observed in the strawberry drink stored at 4 to 6° C compare to 25±2°C. At end of storage time (week 8) 54.36% of vitamin C retained in HEU roselle juice. Although the vitamin C content in roselle juice decreased in HEU, decrement in HEH were more pronounced as supported by Chia, Shamsudin, Mohd Adzahan and Wan Ramli (2012), ascorbic acid of pineapple juice remains higher compare to thermally pasteurized juice during 13 weeks of refrigerated storage. This show ultraviolet irradiation treatment reserved better quality of roselle juice.

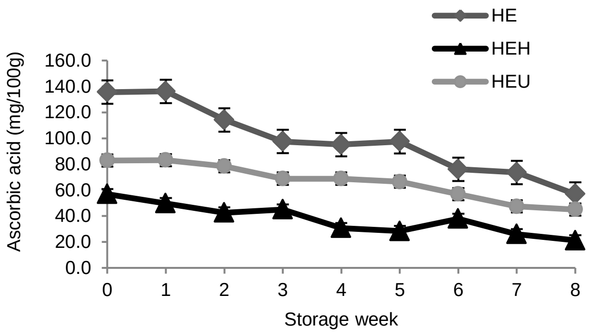


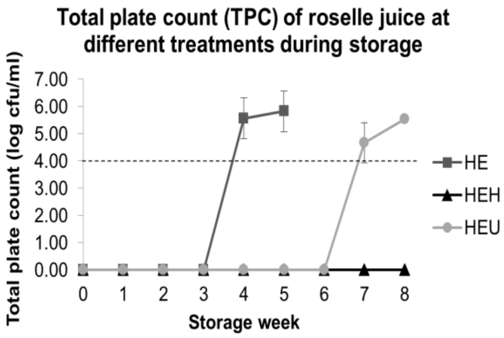
Fig. **3**. Ascorbic acid content (mg/100g) of roselle juice at different treatments (mild heated, HE; combine mild heated and UV-C, HEU; combine mild heated and thermal pasteurization, HEH) during 8 weeks storage

Microbiological analysis

TPC test were done on roselle juice to estimate population level able to survive in the juice throughout the storage time. HE, HEU and HEH show no microbial counts during first 2 weeks of storage. At week 3 the TPC count of HE roselle juice rise up to 5.56 log cfu/ml, while, for HEU roselle juice, the microbial counts started to increase at week 8 (4.67 log cfu/ml). HEH roselle juice remains 0 log cfu/ml throughout the storage time. Al-Jedah and Robinson (2002) stated that maximum count anticipated for total colony count was 5.0×10^{3} per ml (3.7 log cfu/ml) of juice with maximum count permitted was 1.0×10^{4} (4 log cfu/ml).

Yeast and mould count (YMC) may occur in fruit juice due to mispractice during processing, preservation process, poor hygiene and low-quality of raw ingredients (Wareing and Davenport, 2004). YMC in roselle juice (HE, HEU and HEH) shows similar trend as TPC. YMC of HE and HEU exceed the permissible limit of 3 log cfu/ml for YMC (Al Jedah and Robinson (2002) at week 4 and 7 respectively. Noted that as the microbial counts increased sharply at week 4, the pH of HE roselle juice also become more acidic (2.83). Supposed juice in acidic range (pH 2.5- 3.8) able to inhibit most bacteria, but not yeast as fruit juice have lower oxygen level that promote organism growth caused by facultative anaerobes, (Wareing and

Davenport, 2004). The efficiency of the microbial inactvation in ultraviolet irradiation depends on the UV-C dosage used. Study by Mohd Hanif, Shamsudin, Mohd Adzahan shows that the higher the UV-C dosage, the higher microbial inactivation in lime juice. In short, combine mild heat and UV-C on roselle juice microbiologically safe for consumption up to 6 weeks of storage. This study shows promising UV-C effect on roselle juice microbiological safety. The shelf of roselle juice may be improved by taking hygenic precaution and using higher UV-C dosage.



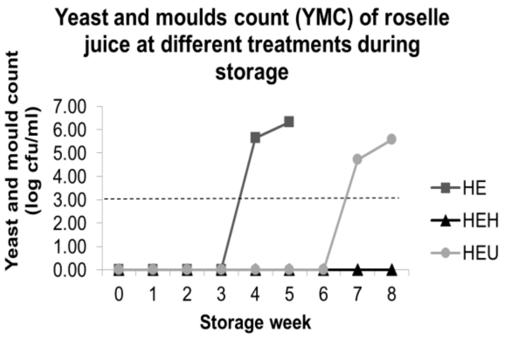


Fig. **4**. Total plate count and Yeast and moulds count of roselle juice at different treatments (mild heated, HE; combine mild heated and UV-C, HEU; combine mild heated and thermal pasteurization, HEH) during 8 weeks storage.

Conclusions

Roselle juice anthocyanin and vitamin C contents degradation minimized after combine treatment of mild heat extraction and ultraviolet irradiation throughout the storage. Color properties of roselle juice also well preserved with physicochemical properties of pH, titratable acidity and total soluble solid shows minimum deviation compare to mild heated roselle juice. Microbiologically, ultraviolet irradiation promotes positive results with extension of roselle juice shelf life for 6 weeks.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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