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Research Article

Changes in quality and bioactive properties of concentrated Roselle (*Hibiscus sabdariffa* Linn.) extract

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Abstract: This study aims to monitor quality changes and bioactive properties of concentrated roselle extract in lug cap glass containers as affected by pasteurization and storage conditions. It was found that quality changes and bioactive properties of concentrated roselle extract before and after pasteurization included pH of 2.79 ± 0.00 and 2.80 ± 0.01, total acidity (as malic acid) of 2.24 ± 0.00 and 2.24 ± 0.03 %, total soluble solids of 44.50 ± 0.00 and 44.56 ± 0.01˚Brix, total anthocyanin contents of 382.66 ± 2.49 and 370.72 ± 1.71 mg/l concentrated roselle extract, total phenolic contents of 6.06 ± 0.18 and 5.82 ± 0.01 mg/g concentrated roselle extract, respectively. The antioxidant activities using DPPH assay with BHT standard, expressed as EC_{50} (Efficient Concentration) were 230.01 ± 2.40 and 235.34 ± 0.79 µg/ml, respectively. In addition, concentrated roselle extract had nutrition facts (percent Thai RDI as over 6 years of age) for 1 serving size (70ml) providing 140 kilocalories, 11% carbohydrate, 50% vitamin E (α-Tocopherol equivalent), 15% vitamin A (Retinol equivalent) and 15% vitamin B1. After 90 days storage at 4˚C and 27˚C, concentrated roselle extract in glass container had pH of 2.80 ± 0.01 and 2.80 ± 0.01, total acidity of 2.23 ± 0.04 and 2.25 ± 0.04%, total soluble solids of 44.60 ± 0.28 and 44.64 ± 0.11˚Brix, total anthocyanin contents of 298.25 ± 0.77 and 100.86 ± 1.54 mg/l concentrated roselle extract, total phenolic contents of 3.39 ± 0.41 and 2.58 ± 0.43 mg/g concentrated roselle extract and EC_{50} of 359.96 ± 0.89 and 390.63 ± 4.75 µg/ml, respectively. Both pasteurization and storage conditions significantly affected the bioactive properties of concentrated roselle extract.

Keywords: plants, beverages, food, concentrated roselle extract, antioxidants, bioactive properties, pasteurization, lug cap glass container

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Introduction

Roselle (*Hibiscus sabdariffa* Linn.) is a tropical plant widely cultivated in Thailand and locally known as *krachiap daeng*. Roselle produces red edible calyxes which primarily can be used for making brilliant red colour jam, jelly, preserves and juice (Hirunpanish *et al*., 2006). Since the early 1970s, Roselle has received considerable attention as a potential source of natural food colourant, pharmaceuticals and cosmetics (Mazza and Miniati, 1993). Currently, roselle is also claimed as a Thai traditional medicine for kidney stones and can be used as an antibacterial and an antifungal substance (Farnworth and Bunyapraphatsara, 1992). The calyx contains brilliant red pigments of four anthocyanins including dephinidin 3-sambubioside, cyanidin 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside (Du and Francis, 1973; Wong *et al*., 2002). Roselle anthocyanins can also provide health benefits as a good source of antioxidants, as well as a natural food colourant. Since anthocyanins are derivatives of the basic flavylium cation structure which have an electron deficient nucleus as shown in Figure 1, they generally behave as highly reactive. The reactions usually involve decolourization of the anthocyanin pigments. The rate of anthocyanin destruction depends on many factors such as pH, temperature, intermolecular copigmentation, ascorbic acid, oxygen, etc. The reactions are usually undesirable in juice processing and long-term product storage (Mazza and Miniati, 1993). Therefore, the present study aims to monitor quality changes and bioactive properties of concentrated Roselle extract in lug cap glass containers as affected by pasteurization and storage conditions.

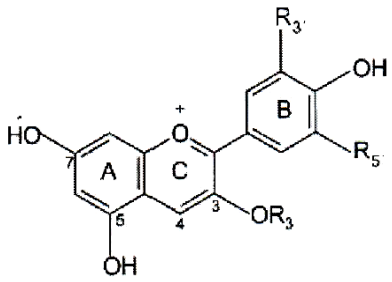

Figure 1 Structure of anthocyanins (Stintzing, 2004).

Materials and Methods

Materials

Fresh Roselle fruit (*Hibiscus sabdariffa* Linn.), cultivar Sudan, were obtained from Amphur Namom, Songkhla Province in Thailand.

Methods

After harvesting, fresh Roselle fruit were washed with tap water three times, the seeds removed and dried in a rotary air dryer at 50^{o}C for 36 hours to obtain 10% moisture content of dried Roselle calyxes.

Dried Roselle calyxes were ground for 3 seconds using a single speed blender (Panasonic, MX-895M). The optimum conditions for water extraction of dried Roselle powder were 1:10 ratio of dried powder to water, with the extraction temperature of 50^{o}C for 30 minutes. Roselle extract was filtered through a cheesecloth bag and concentrated in a

vacuum steam jacket evaporator at 70^{o}C and 44 cmHg until the extract had the total soluble solids of 26.79±0.04^{o} Brix.

Concentrated Roselle extract product was prepared and this consisted of 14% concentrated extract, 36 % water, 32% fructose, 10% honey, 8% oligofructose, 0.0035% vitamin A and 0.0013% vitamin E. The concentrated Roselle extract was subsequently heated to 85^{o}C, followed by hot filling in a lug cap 70ml glass container and capping afterwards. The concentrated extract had pH of 2.80 and was packed in glass containers as thermal processing of the product must meet with food regulations. Thermal processing by pasteurization of concentrated Roselle extract was performed using a steam water spray automated batch retort with the initial product temperature of 32.4^{o}C, 10 minutes increase time, processing temperature of 101.0^{o}C, 5 minutes processing time and cooling until the product temperature reached 40.0^{o}C. The concentrated extract products were analyzed for nutritive composition, which was then listed as the Nutrition Facts.

Concentrated Roselle extract products were stored at 4^{o}C and 27^{o}C for 90 days.

Quality changes and bioactive properties of concentrated Roselle extract in lug cap 70ml glass containers before and after pasteurization as well as 90 days storage at 4^{o}C and 27^{o}C were determined.

Determination of quality changes and bioactive properties included the pH, total acidity as malic acid, total soluble solids contents (A.O.A.C., 2000), total anthocyanin contents (Fuleki *et al*., 1968), total phenolic contents (as gallic acid) by Folin-Ciocalteu method (Miliauskas *et al.,* 2004) and the antioxidant activity, expressed as EC_{50} (Efficient Concentration, µg/ml) according to the DPPH radical scavenging assay using BHT as a standard with EC_{50} of 13.82±0.38 µg/ml (Yamasaki *et al.,* 1994). Determination was done in triplicate.

The experiments were conducted in duplicate. Data were analyzed using a Completely Randomized Design (CRD). Significant level was established at $p \leq 0.05$. Duncan's New Multiple Range Test (DMRT) was used to determine significant difference between treatment means. SPSS for Windows Version 12 was used for statistical analyses.

Results and Discussion

The pasteurization conditions of concentrated Roselle extract in 70ml glass containers are shown in Table 1. In accordance with food safety regulations, acidified food ($pH \leq 4.0$) and food packed in glass containers, must be thermally processed under a temperature of 101^{o}C for 5 minutes.

Table 1 Pasteurization conditions of concentrated Roselle extract in 70ml glass containers.

Pasteurization	Values
Bottle size	70 BG.
Net content	70 cc.
Initial temperature	32.4˚C
Come up time	10 min
Processing temperature (retort)	101˚C
Processing time (retort)	5 min
Coldest point processing temperature and time	85˚C and 5 min
Cooling temperature	40˚C
Sterility test	Negative

Following pasteurization, the nutrition facts were determined, based on the percent Thai RDI as over 6 years of age, for 1 serving size of 70ml concentrated Roselle extract product providing 140 kilocalories, 11% carbohydrate, 50% vitamin E (α-Tocopherol equivalent), 15% vitamin A(Retinol equivalent) and 15% vitamin B1, as shown in Table 2. This study also indicated that the concentrated Roselle extract contained a rich source of vitamin E , as well as a small amount of vitamins A and B1.

The quality and bioactive properties of the product before and after pasteurization, and 90 days storage are shown in Table 3. Pasteurization and storage conditions significantly affected the total anthocyanin contents and EC_{50} of the product. Storage at 27^{o}C for 90 days also significantly effected the reduction of total anthocyanin contents and total phenolics. Antioxidant activity expressed as EC_{50} was also less at 27^{o}C compared to that of storage at 4^{o}C.

Table 2 Nutrition Facts of concentrated Roselle extract product (1 serving size = 70 ml).

Nutritive Values	Value/1 serving size	% Thai RDI*
Energy (kilocalories)	140	-
Carbohydrate (add Dietary fiber) (g)	34	11
Sugar (g)	30	-
Sodium (mg)	10	-
Vitamin A (µg RE**)	105	15
Vitamin B1 (mg)	0.21	15
Vitamin B2 (mg)	0.01	-
Calcium (mg)	33	4
Iron (mg)	0.8	6
Vitamin E (mg α-TE***)	4.9	50

Note: *Thai RDI (Percent Thai Recommended Daily Intakes for population over 6 years of age are based on a 2,000 kilocalories diet.)
** RE = Retinol equivalent, 1 RE = 1 µg retinol
*** α-TE = α-Tocopherol equivalent, 1 α-TE = 1 mg D-α-Tocopherol

Table 3 Quality and bioactive properties of concentrated Roselle extract product before and after pasteurization and storage at 4˚C and 27˚C for 90 days.

Quality and bioactive properties	Before pasteurization	After pasteurization	Storage for 90 days	
			4˚C	27˚C
pH	2.79±0.00[b]	2.80±0.01[a]	2.80±0.01[a]	2.80±0.01[a]
Total acidity, as malic acid (%)	2.24±0.00[a]	2.24±0.03[a]	2.23±0.04[a]	2.25±0.04[a]
Total soluble solids (˚Brix)	44.50±0.00[a]	44.56±0.01[a]	44.60±0.28[a]	44.64±0.11[a]
Total anthocyanin contents (mg/l concentrated extract)	382.66±2.49[a]	370.72 ±1.71[b]	298.25±0.77[c]	100.86±1.54[d]
Total phenolic content, (mg gallic acid /g concentrated extract)	6.06±0.18[a]	5.82±0.01[a]	3.39±0.41[b]	2.58±0.43[c]
EC_{50} (µg/ml)	230.01±2.40[d]	235.34±0.79[c]	359.96±0.89[b]	390.63±4.75[a]
Vitamin A(µg/ml, RE*)	182.00	105.00	-	-
Vitamin E (mg/ml, α-TE**)	5.14	4.90	-	-

Note: Means±standard deviation in each row with the same letters are not significantly different (p>0.05)
EC_{50} : The concentration of dried Roselle extract (µg/ml) needed for 50% decreasing in the initial DPPH concentration
*RE = Retinol equivalent, 1 RE = 1 µg retinol
**α-TE = α-Tocopherol equivalent, 1 α-TE = 1 mg D- α- Tocopherol

Conclusions

Pasteurization at 101^{o}C for 5 minutes with a water spray automated retort and storage at 4^{o}C and 27^{o}C for 90 days caused changes in quality and bioactive properties of concentrated Roselle extract in lug cap 70ml glass containers. The concentrated Roselle extract contained a rich source of vitamin E. This study suggested that the storage at 4^{o}C provided greater retention of the bioactive properties of the products compared to storage at 27^{o}C.

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