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Citric Acid Sweet Potato Extraction Beverages Containing Grape Juice and Fermented Glutinous Rice Syrup

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Abstract: Beverages were developed from three cultivars of sweet potato, with orange-purple-and yellow-colored flesh that were mixed with grape juice and with two cultivars of fermented rice beverage (white and black glutinous rice). The beverage with the optimum formula consisted of 44.98% purple-colored flesh of sweet potato juice extraction (extracted with 0.5% citric acid solution), 40% red grape juice, 6% white fermented glutinous rice syrup, 9% sucrose and 0.02% sodium chloride. The product was a pinkish-purple color and cloudy. The total soluble solids, pH, L*, a*, b*,% titratable acidity (as citric acid) and anthocyanin were 16.95°Brix, 3.60, 4.82, 9.08, 6.56, 0.42% and 4.82 unit/g, respectively. The average score from sensory evaluation produced a moderate result. The product was placed in glass bottles at 80-90°C, tightly closed and then steamed with gas stove for 5 min. After storage for 3 month at room temperature, the sensory test of overall liking of the products decreased to little liked. The product was found to be microbiologically safe, but the color and total anthocyanins content had decreased. The product was found to have no measurable anticancer properties on human breast adenocarcinoma, lung carcinoma and epidermoid carcinoma of the oral cavity and had a noncytotoxic effect on the Vero cell line.

Key words: Beverage, sweet potato, grape juice, fermented glutinous rice

INTRODUCTION

Sweet potato (Ipomoea batatas (L.) Lam.) is a dicotyledonous plant. Globally, sweet potato is ranked as second to the potato in economic importance among all the root crops (Horton, 1988). Sweet potato is a crop rich in nutrients (Suda et al., 1999; Woolfe, 1992) and contains many types of bioactive compounds, such as phenolic compounds and carotenoids that act as free radical scavengers and contribute to the distinctive colors of sweet potatoes (Kays et al., 1993). Phenolic compounds include phenolic acids and anthocyanins. which are predominant in purple-fleshed sweet potatoes (Goda et al., 1997). Anthocyanins are the most important group of water-soluble pigments in plants and are responsible for most blue, red and related colors in flowers, fruits, leaves and storage organs (Clifford, 2000). Anthocyanins in the root of sweet potato include 3-O-(6-O-trans-caffeyl-2-O- β -glucopyranosyl- β glucopyranoside)-5-O-β-glucoside of cyanidin and peonidin (Goda et al., 1997). The peonidin: cyanidin ratio in purple-fleshed sweet potato affects the color of the raw and cooked roots as purple-fleshed sweet potatoes

have a higher ratio and a greater degree of redness, but sweet potatoes rich in cyanidin have a greater degree of blueness (Yoshinaga *et al.*, 1999). Carotenoids are compounds that mammals can transform into retinal (vitamin A), with α -carotene, β -carotene and β -cryptoxanthin being predominant in orange-fleshed sweet potatoes (Woolfe, 1992; Kays *et al.*, 1993; Yoshinaga *et al.*, 1999). Mice that were fed on a diet rich in β -carotene had slower rates of cancer cell growth compared to a placebo diet (Dorogokupla and Zdavookhr, 1977).

Grape is a non-climacteric fruit. Anthocyanins are the main phenolics in red grapes, whereas flavan-3-ols are the most abundant phenolics in the white varieties (Cantos *et al.*, 2002). Anthocyanidins in grape rinds seemed effective in the suppression of cell growth (Koide *et al.*, 1996).

The fermented glutinous rice beverage is an indigenous food of Thailand and is known locally as 'Khaomak'. It is prepared from steamed glutinous rice and mixed with a starter culture called 'lookpang', prepared from rice flour, spices and required organisms, such as mold

(Rhizopus, Mucor, Chlamydomucor, Pennicilium and Aspergillus) and yeast (Hansenula and Saccharomyces) to digest the rice starch (Chatisatienr, 1977). Syrup is produced at about 30-40° Brix after 3d of fermentation, giving a partially liquefied, rice paste and a sweeter end product, with little alcohol. Khaomak is consumed as a dessert or snack item without further processing or is used as an ingredient in other fermentation products, including vinegar, fermented fish and alcoholic beverages. Khaomak abounds in zinc that helps to make the skin glow and to cure pimples. The ancients believed that Khaomak could help to cure ulcers and blood, especially maintain the women (Prakitchaiwattana, 2011).

A beverage is a liquid that is specifically prepared for human consumption. In addition to filling a basic human need, beverages form part of the culture of human society. Fruits and vegetables contain high levels of biologically active components that impart health benefits and nutritional value (Larson, 1988). Nowadays, global warming has become a problem, with the Earth's surface temperature having increased 0.74±0.18°C between the start and the end of the 20th century (Solomon et al., 2010). Consequently, beverages are a very appropriate food, especially in a tropical country such as Thailand, because they could help beverage consumers to abate the effects of heat and produce a fresh feeling after drinking. However, there is a trend by consumers to require any new product line to be natural and healthy. Thus, the objective of this research was to develop a nutritious and innovative beverage from sweet potato mixed with grape juice and fermented glutinous rice

MATERIALS AND METHODS

Raw materials: Three cultivars of sweet potato were selected-namely, cv. Mon-lueng from Suphan Buri province with Yellow-Colored Flesh (YCF), cv. Mon-khaii from Ayutthaya province with Orange-Colored Flesh (OCF) and cv. Mon-tor-pauk from Phetchabun province with Purple-Colored Flesh (PCF). Black grape cv. Popdam and white grape cv. White malaga from Ratchaburi province were also chosen. White glutinous rice cv. Sanpatong and black glutinous rice cv. Leum puua were sourced from Phetchabun province. Khaomak lookpang, sucrose and salt were purchased from a local market. Anhydrous citric acid (food grade) was purchased from the Thai Food and Chemical Co. Ltd. in Thailand. All raw materials were collected in Thailand during 2007 (February-May).

Characteristics of three sweet potato cultivars: The sweet potatoes were measured for size and washed, peeled and chopped into small pieces for color analysis with a spectrophotometer (Spectraflash 600 plus, Datacolor International, USA) that measured the CIE color values, recorded as L* = lightness (0 = black, 100 = white), a*(-a* = greenness, +a* = redness) b* (-b* = 100 cm size of the sweet potatoes and washed).

blueness, +b* = yellowness) and proximate analysis as moisture (T-CM-002 based on AOAC (2000) 925.45), fat (T-CM-075 based on AOAC (2000) 989.05), protein (T-CM-003 Kjeldahl method, based on AOAC (2000) 991.20, using 6.25 as the conversion factor), crude fiber (T-CM-077 based on AOAC (2000) 978.10), ash (T-CM-001 based on AOAC (2000) 938.08) and carbohydrate contents (using the calculation 100-% moisture-% fat-% protein-%ash), with two replications.

Extracted sweet potato, white Grape Juice (WG) and black Grape Juice (BG) preparation: The peeled sweet potatoes were blended using an electric blender (National) for about 30 sec with 0.5% citric acid solution, 10 times its weight. The juice was filtered through fine nylon cloth and left 5 min for starch precipitation. Separate samples of white and black grapes were rinsed with water and the seeds separated out following blending using an electric blender (National) for about 30 sec and then filtering the juice through fine nylon cloth.

Fermented white rice syrup (WK) and black rice syrup (BK) preparation: One kilogram each of white rice and black rice was rinsed separately with water and then soaked with water for 30 min and 3 hr, respectively. Then, each sample was drained and cooked in a steamer for 15 min and 1 hr, respectively. The steamed rice was left uncovered to cool at room temperature and washed with water until the washed water was clear. The lookpang for Khaomak, 1% by weight of rice weight before soaking, was thoroughly mixed into the cooled rice. The mixture was transferred into a container with a lid and set aside for 3d to ferment. The syrup was filtered through fine nylon cloth. The alcohol content was measured using an ebulliometer, Dujardin-Salleron, No. 81117, Paris, France.

Steps in beverage production: Using a gas stove, there were five steps in the beverage production: 1) extracted sweet potato juice was mixed with white sucrose and sodium chloride, placed in a pot and heated until it reached 90°C; 2) extracted grape juice was added and heated until it reached 90°C; 3) fermented glutinous rice syrup was added and heated until it reached 90°C; 4) the beverage was poured into sterile glass bottles (steamed with gas stove, for 5 min) at 80-90°C and kept in the refrigerator and 5) for the study on shelf life, the beverage from step 4 was filled with a headspace of about 2.5 cm, tightly closed and then steamed with gas stove for 5 min.

Optimum extracted sweet potato juice cultivars for beverage production: The Completely Randomized experimental Design (CRD) involved three replications of three cultivars of sweet potato juice with each of 49.98% extracted sweet potato juice, 40% WG, 6% WK, 4% white sucrose and 0.02% sodium chloride in the

finished product. Total Soluble Solids (TSS) were measured with a hand refractometer (Atago) and pH with a pH meter (Orion Model 410A). A sensory evaluation of preference (color, aroma, flavor, texture and overall) was used by 30 panelists according to a seven-point hedonic scale (1 = dislike very much and 7 = like very much). The data were processed by analysis of variance and Duncan's New Multiple Range Test (DNMRT) for mean comparisons at the 0.05 significance level, using the SPSS statistical software program (SPSS for windows Ver. 12.0, now a part of IBM Corp.; White Plains, NY, USA).

Optimum fermented glutinous rice syrup cultivars for beverage production: The CRD involved three replications of two cultivars of fermented glutinous rice beverage with each of 6% WK or BK, 49.98% extracted PCF juice, 40% BG, 4% white sucrose and 0.02% sodium chloride. The products were examined using the above procedure.

Optimum white sucrose quantity for beverage production: The CRD involved three replications of three treatments involving 5, 7 and 9% white sucrose in the finished product. The weight of extracted PCF juice was substituted with an equal weight of white sucrose and each treatment used 40% BG, 0.02% sodium chloride and 6% WK. The products were examined using the above procedure. Only one treatment (the most preferred) was selected for the next study.

Cytotoxicity test and cancer cell lines of developed beverage product: A cytotoxicity test was conducted to measure the protein quantity from the growth of the Vero cell line with ellipticine and doxorubicine as the positive control and dimethylsulfoxide as the negative control by plate reader at 510 nm (Skehan et al., 1990). The anticancer properties were estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay that provided an indirect measurement of the number of viable cells (tetrazolium-dye based colorimetric microtitration) by plate reader at 570 nm (Plumb et al., 1989).

Shelf life of the developed beverage product: The beverage products were kept in a dark drawer at room temperature and every month were examined using color, total anthocyanins content, microbiology tests of total plate count (Maturin and Peeler, 2001), yeast and mold (Tournas et al., 2001) and evaluated using the sensory test as detailed as above.

Analysis of total anthocyanins content: The total anthocyanins content was determined using the pH differential method (Fuleki and Francis, 1968). Absorbance was measured in a UV-Vis 1601 Shimadzu spectrophotometer double beam (UV 190-1,100 nm) at 510 nm in buffers at pH 1.0 and 4.5. Results were expressed as unit per 1 g sample by total anthocyanins content using Equation 1:

Total anthocyanins content = $[(OD2)_{OH4.5} - (OD1)_{OH1}]$ * DF/Weight of sample

Where

OD = Optical detector DF = Dilution factor = 30

RESULTS AND DISCUSSION

Characteristics of three sweet potato cultivars: From Table 1, the YCF had the biggest size while OCF was the smallest. The color of PCF was purple, YCF was yellow and OCF was orange. YCF contained the highest protein, lowest fat and the ash content was higher than that of PCF but this was not different to that of OCF.

Optimum extracted sweet potato juice cultivars for beverage production: This beverage was modified from the patent of Shigeo (2004). The main ingredients by weight for this beverage were extracted sweet potato juice with citric acid solution because it gave an attractive color and was cheap compared with other ingredients. Extraction with citric acid solution assisted the higher total soluble solid content and acidity, imparting

Table 1: Size and color of three sweet potato cultivars

	Size			Color of peeled sweet potato		
Culti∨ar	Weight (g)	Length (cm)	 Width (cm)	L*	a*	b*
PCF	204±44.77°	16.4±2.95	15.4±2.46	44.95±3.12	11.26±2.44	6.32±0.85
YCF	394±78.61	16.8±2.78	23.2±3.19	78.88±1.12	5.01±0.98	38.37±0.55
OCF	98±24.40	11.1±1.29	13.0±1.53	73.27±1.52	16.90±0.41	29.87±0.72

Average ± standard deviation, n = 5

Table 2: Proximate analysis (%) of three peeled sweet potato cultivars by fresh weight

Culti∨ar	Moisture	Carbohydrate	Protein	Fat (%)	Ash	Crude fiber
PCF	65.63±2.14b	33.18±2.27°	0.61±0.08b	0.31±0.03b	0.27±0.02 ^b	1.47±0.04ª
YCF	72.42±2.21 ^a	24.86±2.43b	1.44±0.12°	0.27±0.07a	1.01±0.03°	1.14±0.05 ^a
OCF	78.07±1.59 ^a	20.09±1.79 ^b	0.60±0.10 ^b	0.32±0.04b	0.92±0.06°	1.47±0.04°

Average \pm standard deviation, n = 5

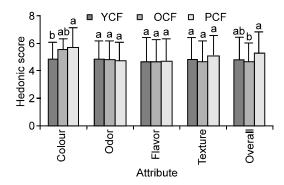


Fig. 1: Sensory evaluation of beverages from three sweet potato cultivars. For each attribute, the same lower case letters above columns indicate that those mean values were not significantly different (p>0.05) (T indicates the upper range of the mean plus the standard deviation)

sourness to the beverage. WG and WK were selected at first because their color did not conceal the color of the sweet potato. WG also provided a sweetness (TSS 17°Brix) and acidity (about pH 3.70). WK provided an innovative flavor from the fermentation esters and sweetness (TSS 32° Brix). However, WK constituted only 6% because after heating, its flavor changed from that of fresh (unheated) fermented glutinous rice that is popularly consumed. The WK yield is about 50% of raw glutinous rice and has pH 3.70-3.85 from the lactic acid (Chatisatienr, 1977) but it was less than the quantity of its sugar so the product tasted sweet. There was only a little (about 1%) alcohol in WK. The pH (3.59-3.65) and TSS (13.20-13.22) of the three products were not significantly different, but color differed according to the cultivar. The vellow and orange color of YCF and OCF. respectively, is due to β-carotene (Purcell and Walter, 1968). The purple color of PCF is due to anthocyanins pigments (Clifford, 2000). The acidity of the PCF beverage resulted in more stable anthocyanins because anthocyanins are pH sensitive and are stabilized by the trance formation of the flavylium cation structure in acidic aqueous solution (Hayashi et al., 1996; Palakajornsak, 2004). The sensory evaluation is shown in Fig. 1. The beverages from PCF gained the highest score for color (5.68) and overall (5.26) so it was selected for the next beverage study. However, the scores for this beverage were only "like slightly", especially for the flavor (4.63), as some panelists commented that it was too sour. This problem could be addressed by one of two choicesnamely, to reduce the quantity of extracted sour sweet potato or by increasing the sweetness. As the main raw material in this research was the extracted sweet potato it was decided to add sugar.

Optimum fermented glutinous rice syrup cultivars for beverage production: This experiment selected the BG

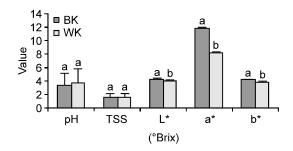


Fig. 2: pH, TSS and color of beverage made BK and WK. For each parameter, the same lower case letters above columns indicate that those mean values were not significantly different (p>0.05). (T indicates the upper range of the mean plus the standard deviation)

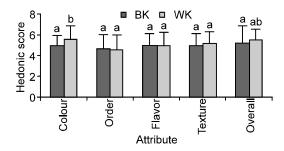


Fig. 3: Sensory evaluation of beverage made from BK and WK. For each attribute, the same lower case letters above columns indicate that those mean values were not significantly different (p>0.05). (T indicates the upper range of the mean plus the standard deviation)

(TSS 13° Brix, pH 3.89) to replace WG because it has higher colored pigment levels, especially anthocyanins (Cantos et al., 2002). On the other hand, its color blended well in the juice made from PCF juice that was selected. Only the color of the beverages in Fig. 2 was affected, as the beverage from BK was darker than that of WK because of the color of black glutinous rice. On the other hand, BK had higher total soluble solids (35°Brix) and higher fiber content, making it difficult to filter and thus producing a lower syrup portion with a 20% yield. The sensory evaluation (Fig. 3) revealed that the beverage from WK had a higher score than that of BK in color and overall evaluation so the WK was selected for the next experiment. However, the odor score (4.55) and flavor score (5.0) showed that it was only "little liked" because the beverage had no smell and remained very sour. The sourness was a result of the acid extracted from the PCF juice and also from the BG so the white sucrose was more added.

Optimum white sucrose quantity for beverage production: The pH (3.53) and color (pinkish purple) of

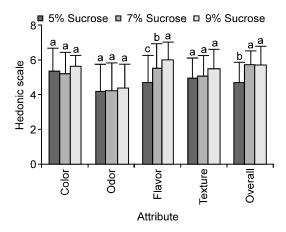


Fig. 4: Sensory evaluation quality of beverages with different sucrose levels. For each attribute, the same lower case letters above columns indicate that those mean values were not significantly different (p>0.05). (T indicates the upper range of the mean plus the standard deviation)

the three beverages were not different but TSS was different and increased with values of 12.50, 14.50 and 16.50°Brix for 5, 7 and 9% white sucrose in the formula due to white sucrose was dissolve in water. For the sensory test (Fig. 4), the beverage containing 9% white sucrose had the highest score (5.9) for flavor (moderately liked) and overall score (5.65) indicating that the sourness may have been suppressed by the level of white sucrose, Thus, the optimum 9% sucrose sample was selected for the shelf life study however, if the extracted sour PCF was reduced, the quantity of white sucrose may be lower than this.

Cytotoxicity and anticancer activity of the developed beverage product: As the beverage was a new product made from many raw materials that may be adversely changed during processing, the safety properties as determined by a cytotoxicity test were of great interest. For anticancer, as this beverage had anthocyanins that have high antioxidant properties (Teow, 2005; Wang et al., 1997) from extracted PCF and BG. PCF have a high proportion of acylated anthocyanins, so it was more stable with low levels of acylation, such as those found in strawberry, raspberry, apple and soybean with black seed coats, even though the beverage was heated that was measured by the percentage residual of the anthocyanins of the three sweet potato cultivars at 80°C for 18 hr was 75% for Kankei 55, 71% for Yamagawa murasaki and 63% for Tanegashima murasaki (Hayashi et al., 1996). As antioxidant properties can act as agents to protect human health, especially against cancer (Loliger, 1991; Nijveldt et al., 2001). The result of this beverage showed no anticancer properties (Table 3) on human breast adenocarcinoma, lung carcinoma and

Table 3: Cytotoxicity test of samples on normal and cancer cell lines

Type of cells	Results
Human lung carcinoma	Inacti∨e
Human epidermoid carcinoma of cavity	Inactive
Human breast cancer	Inacti∨e
African green monkey kidney Vero cells	Noncytotoxic

epidermoid carcinoma of the oral cavity because it have high moisture content (83.05%) so it may have low amounts of important bioactive compounds, especially anthocyanins. However, it was safe to consume because it had no cytotoxic effect on the Vero cell line.

Shelf life of the developed beverage product: Shelf life was studied from May to September 1990, when the average temperature was rather high (about 30±2°C). The beverages were pasteurized in hot-filled glass bottles to kill microbes and produce a vacuum in the bottle. Then, the closed bottles were steamed to extend the shelf life that this process may be higher bioactive compounds in the beverage because the previous studies of sweet potatoes, steam treatment increased 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging (Wang et al., 1997) and heat treatment significantly increased the polyphenolic content in the purple-fleshed sweet potato clone (Teow, 2005). The results after 3 months showed that the pH, TSS and citric acid content of the products were not significantly different with a pH range of 3.58-3.67, a TSS range of 16.95-17.25°Brix and a citric acid range of 0.42-0.46%. Figure 5 indicates that the color clearly changed, as shown by the decrease in L* and a*, corresponding to a decrease in the anthocyanins content (from 4.82 to 0.33 unit/g), especially in the third month. The anthocyanins content at the start of the experiment (month = 0) was made up of components from the PCF extraction (13.77 unit/g) and the black grape juice (10.90 unit/g). The stability of the anthocyanins decreased as the storage time increased, especially in the high temperatures experienced in Thailand because anthocyanins at low temperature (4±3°C) were more stable than those at high temperature (30±3°C) (Palakajornsak, 2004). On the other hand, the beverage product might be impacted by UV irradiation, even though it was kept in a dark drawer due to Hayashi et al. (1996) found that the UV irradiation stability of anthocyanins expressed as a percentage of the residual color ratio in the three sweet potato cultivars after 18 hr was 80% for Kankei 55, 74% for Yamagawa murasaki and 68% for Tanegashima murasaki. The likeness score (Fig. 6) decreased with every extra month of storage (from 6.0 to 5.13). However, the product was still microbiologically safe of yeast and mold (Table 4) after 3 months because it was acidified food as pH < 4.6 that will inhibit the growth and formation of toxins from the bacteria that cause botulism so this pasteurized process can kills yeast and mold spores on

Table 4: Microbial sampling of products from the shelf life study

Month	Total plate count, cfu.g ⁻¹	Yeast and mold, cfu.g ⁻¹
0	None	Negati∨e
1	None	Negati∨e
2	None	Negati∨e
3	None	Negati∨e

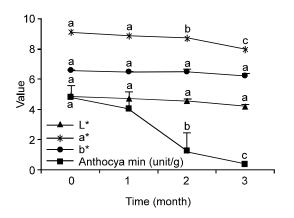


Fig. 5: Effect of beverage storage time on color and anthocyanins. For each attribute, the same lower case letters above line graphs indicate that those mean values were not significantly different (p>0.05). (T indicates the upper range of the mean plus the standard deviation)

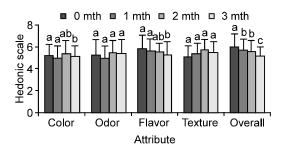


Fig. 6: Sensory evaluation of beverage after 0, 1, 2 and 3 months. For each attribute, the same lower case letters above columns indicate that those mean values were not significantly different (p>0.05). (T indicates the upper range of the mean plus the standard deviation)

the product and the another important point as it was also kept in hermetic container that the microbes could not get insight (NC state University, 2011; USFDA, 1997).

Conclusion: The citric acid sweet potato extraction beverages containing grape juice and fermented glutinous rice syrup was moderately liked for only lab scale panelists with high anthocyanins and kept at room temperature for at least 3 months so it should be further studied for consumer scale acceptance and storage at a lower temperature for prolongation shelf life.

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