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Development of non-alcoholic wines from the wastes of Mauritius pineapple variety and its physicochemical properties

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Abstract

The accumulation of large quantities of pineapple wastes has become a world-wide environmental issue due to its high acidity and the high concentrations of biodegradable organic matter. In this study the proximate composition and some other physicochemical properties of the 1st peel, 2nd peel and core of Mauritius pineapple variety were determined. Results from the proximate analysis showed the moisture content, Titratable Acidity, Total Soluble Solids was highest in 2nd peel. The crude protein content was highest in the 1st peel. The carbohydrate content of core was the highest. The developed Non-alcoholic wines (NAW) from the above pineapple wastes were analyzed after 5 and 56 days of fermentation. All the developed NAWs showed alcohol strengths below the required 0.5% (v/v) level. Methanol was not detected in any of the NAWs developed. Therefore there's a possibility to utilize pineapple wastes successfully as a source for the production of NAWs.

Keywords: Non-alcoholic Wine, 1st peel, 2nd peel, core.

Introduction

The pineapple (*Ananas comosus*) is one of the most important fruits in the world and is the leading edible member of the family *Bromeliaceae* [1]. It is found to grow throughout the tropical and subtropical regions of the world [2]. Pineapple is one of the most widely grown fruit variety in Sri Lanka. According to Department of Agriculture, Sri Lanka, the main pineapple varieties cultivated are Mauritius and Kew and Mauritius is the widely grown variety. Pineapples are commonly processed into canned fruit and juice, in addition to being eaten fresh [3]. Pineapple waste contains high concentration of biodegradable organic material and suspended solid [4]. The increase of the global production of pineapple and the increase of production of different end products has remarkably increased the pineapple waste accumulation. Processing residuals of pineapple ranges between 45 to 65%, an indication of serious organic-side streams disposal challenges, which causes environmental pollution if not successfully utilized [5]. Rosma *et al.* (2005) [6] reported that pineapple waste which consists of peel, core and unwanted parts of pineapples contain up to 6.14% of carbohydrate, minerals especially magnesium and 0.6% of crude protein.

The wastes from pineapple canneries have been used as the substrate for bromelain, organic acids, ethanol, etc. since these are potential source of sugars, vitamins and growth factors [7, 8]. [9, 10]. Wine preparation from pineapple peel was considered a bioconversion method that can facilitate easy removal of these wastes from the environment. This production of wine from pineapple peel may provide alternatives to the already established wine production raw material such as grapes [11]. From the point of consumers, high alcohol content has negative effect on human health and is not appreciated by a majority of consumers drinking responsibly. Therefore, various techniques and technologies are developed to reduce the alcohol content of wines while conserving the organoleptic properties, flavor and the overall quality. Wines with a reduced alcohol content can be classified as dealcoholized or no-alcohol (<0.5% v/v), low-alcohol (0.5–1.2% v/v) or reduced-alcohol (1.2% to 5.5–6.5% v/v). This classification system is loosely based on labelling and legislative requirements, and can vary between countries [12]. There are different techniques to acquire low alcohol contents in wine. This study was aimed on accessing the possibilities to develop Non-alcoholic wine (NAW) from different wastes of pineapple processing, by reducing the available fermentable sugars of the must and analysis of the physicochemical properties of the developed NAWs as a solution for the removal of the large quantities of pineapple wastes accumulated during processing and analysis of the proximate composition and some important physicochemical parameters of three selected pineapple wastes.

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Material and methods

Plant materials

The fresh pineapple wastes of Mauritius variety, matured and ¼th yellow in colour were obtained from one of the leading pineapple processor in Sri Lanka. The wastes used for this research are of 3 types,

- 1st peel – the outermost peel, with spikes – 1 cm thickness
- 2nd peel – the inner layer with brown eyes – 0.5 cm thickness
- Core – the middle fibrous area

The wastes were free from infestations or any mechanical damage. The 1st peel was washed properly using potable water to remove dust or dirt. The wastes were packed and sealed separately in High Density Polyethylene bags and stored in a deep freezer at -18 °C. The pineapples were from the pineapple cultivation in Gampaha district, Sri Lanka.

$$\text{Carbohydrate \%} = 100 - (\text{crude protein\%} + \text{total ash\%} + \text{crude fat\%} + \text{crude fiber\%}).$$

The mineral content (Calcium, Potassium, Iron and Manganese) of the pineapple wastes were determined according to the standard AOAC method using Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000). The pectin content of the pineapple wastes was determined by using the acid extraction method described by Simpson *et al.* (1984) [14]. The total phenolic content was determined using Folin-Ciocalteu's reagent as described by Singleton & Rossi (1965) [15].

Pineapple wastes were blended separately and filtered through a muslin cloth to obtain the pineapple peel extracts for the analysis of pH, Brix, and Titratable acidity.

The pH of the pineapple waste was determined by using a pH meter (Intelli CAL pH, pHc101) at room temperature, total soluble solids were determined using a Hand-held refractometer (OPTIKA HR-150) at room temperature and the titratable acidity was assessed by AOAC 962.12 method, and expresses as % of anhydrous citric acid.

Development of Non-alcoholic wines (NAW) from 1st peel, 2nd peel and core

Fresh, undamaged pineapple wastes were taken and the 1st peel was washed thoroughly with potable water to remove any dirt. Then each was blended separately to obtain juice. The obtained juice was filtered through a muslin cloth to remove fibrous matter. Then 200 ml of each filtered juice was mixed with 800 ml of potable water to obtain 2^o Brix. pH of the juice was adjusted to 3.4 – 3.5 by adding citric acid. Then the juice was pasteurized at 85 °C for 5 minutes. The juice was then allowed to cool up to about 40 °C and 0.2968 g of sodium metabisulphite (200 ppm) and previously activated *Saccharomyces cereveciae* (2g per Liter) were added and mixed properly. The juice was then filled into clean, previously washed and dried glass bottle keeping a sufficient head space of 1 inch and the mouth of the bottle was covered with a cotton bung to facilitate the removal of produced CO₂ during fermentation. The prepared wine samples were allowed to ferment at room temperature (28 °C) with daily aeration. After 5 days of fermentation, the upper layer was siphoned off to another clean pre-sterilized glass bottle without keeping a head space. Then they were further allowed to ferment for 56 day at room temperature. The same

Analysis of proximate composition and physicochemical parameters of the selected pineapple wastes

Preparation of samples for proximate and other physicochemical parameter analysis for the proximate composition analysis, 1st peel, 2nd peel and the core of pineapples were dried in a dehydrator at 60 °C for 72 hours until constant weight was obtained. Then the dried peel samples were ground in to a powder and passed through 50 µm sieve. This powder was sealed in Low Density Polyethylene bags and stored at room temperature and was used for the proximate analysis in dry basis.

Moisture content, total ash, crude fiber, crude fat and crude protein content of the 1st peel, 2nd peel and core in dry basis were determined by using standard AOAC methods [13] and carbohydrates content was determined by difference as;

procedure was followed to develop NAW from 2nd peel and core. After 5 and 56 days of fermentation the prepared NAWs were chemically analyzed. To increase the effectiveness NAW samples were prepared in triplicates and duplicate measurements were taken from each replicate.

Analysis of the Non-alcoholic wines

The alcoholic strength % (v/v) of the developed Non-alcoholic wines (NAW) was determined using ebulliometer and a spectrophotometric method described by Caputi *et al.* (1968) [16] by using UV visible spectrophotometer (UVmini – 1240). The methanol content of the NAWs was determined by Gas Chromatographic Method, AOAC 972.11. pH of the NAWs were determined by using a digital pH meter (Intelli CAL pH, pHc101), TSS by hand held refractometer (OPTIKA HR-150) and titratable acidity according to AOAC 962.12. The colour intensity and colour tone of the Non-alcoholic wines were determined according to the spectrophotometric absorbance method proposed by Sudraud (1958) [17]. The absorbance of the NAWs was measures at at wavelengths of 420 and 520 nm using UV visible spectrophotometer (UVmini – 1240). The colour intensity and colour tone were calculated according to the following equations.

$$\text{Colour Intesity} = \text{absorbance at 520 nm} + \text{absorbance at 420 nm}$$

$$\text{Colour Tone} = \frac{\text{Absorbance at 420 nm}}{\text{Absorbance at 520 nm}}$$

After 56 days of fermentation, the total polyphenols of the NAWs was determined by using the method proposed by Šulc *et al.* (2003) [18] based on Singleton and Rossi (1965) [15].

Statistical Analysis

All the experimental measurements were replicated 3 times with duplicate measurements and the gathered data were statistically analysed using One-Way Analysis of Variance (ANOVA) by using Minitab – 17 package at 95% confidence interval. All the values were reported as mean ± Standard deviation (SD) where n=6.

Results and discussion

Proximate and physicochemical analysis of the pineapple wastes

Table 1: Proximate composition of pineapple wastes

Parameter	1 st peel	2 nd peel	Core
Moisture %	86.81 ± 0.11 ^a	87.37 ± 0.34 ^a	84.90 ± 0.23 ^b
Dry matter %	13.19 ± 0.1 ^b	12.63 ± 0.34 ^b	15.10 ± 0.23 ^a
Proximate analysis (g/100 g of dry waste)			
Crude protein	5.04 ± 0.05 ^a	3.52 ± 0.21 ^b	3.67 ± 0.05 ^b
Crude fat	2.78 ± 0.07 ^b	4.78 ± 0.53 ^a	2.35 ± 0.15 ^b
Crude fiber	42.02 ± 1.72 ^a	5.76 ± 0.10 ^c	9.14 ± 0.13 ^b
Ash	6.20 ± 0.11 ^a	4.32 ± 0.09 ^b	1.70 ± 0.15 ^c
Carbohydrates	43.95 ± 1.75 ^b	81.68 ± 0.35 ^a	83.03 ± 0.32 ^a
Minerals (mg/100 g) in dry basis			
K	409.25 ± 1.18 ^a	398.08 ± 15.23 ^a	247.70 ± 32.80 ^b
Ca	48.96 ± 2.13 ^a	22.95 ± 1.53 ^b	8.73 ± 0.34 ^c
Fe	3.43 ± 0.19 ^a	3.87 ± 0.64 ^a	1.61 ± 0.23 ^b
Mn	4.40 ± 0.31 ^a	4.19 ± 0.55 ^b	1.63 ± 0.54 ^b

*Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c letters in same column are significantly different at (p < 0.05) level.

As shown in the Table 1, the highest moisture content was found in the 2nd peel as 87.37% and the highest dry matter content in the core. There is no significant difference (p < 0.05) between the moisture content of 1st peel and 2nd peel, but there is a significant difference between the moisture content of 1st peel and core and 2nd peel and core. The crude protein content of the pineapple wastes range from (5.04 ± 0.05 g/100 g of waste) to (3.52 ± 0.21 g/100 g of 1st peel). As reported by Romelle *et al.* (2016) [19], the protein content of the Smooth Cayenne pineapple variety was 5.11 ± 0.02 (g/100g dry peel). The difference in the protein content may be due to the difference in the pineapple variety. Highest crude fat content in the pineapple wastes was obtained in the 2nd peel (4.78 ± 0.53 %), and lowest in the core (2.35 ± 0.15 %). The crude fiber content of the 1st peel was the highest (42.02 ± 1.72 g/100 g of waste) and was significantly different from the 2nd peel and the core in pair wise comparison at P < 0.05 level of significance. Wijana *et al.* (1991) [20] reported that the pineapple peel contains 20.87 % of crude fiber. This was lower than the crude fiber obtained from the analysis. The lowest amount of crude fiber was obtained from the 2nd peel (5.76 ± 0.10 g/100 g of waste). There was a significant difference (P < 0.05) between the ash content of the 1st peel, 2nd peel and the core. The highest ash content was found in the 1st peel (6.20 ± 0.11 g/100 g of waste). The highest carbohydrate content was obtained in the core (83.03 ± 0.32 g/100 g of waste) making it more suitable for the wine

production by fermentation. But in this analysis the carbohydrate content was determined by calculation. Therefore, the calculated results also include amount simple sugars such as monosaccharides and disaccharides [21]. According to a study conducted by Siti-Roha *et al.* (2013) [22], the main sugars detected by HPLC in pineapple wastes were fructose, glucose and sucrose. According to his findings fructose (%) of peel and core reported were 1.98 ± 0.16 and 2.00 ± 0.07 respectively. The glucose (%) of peel and the core were 2.18 ± 0.09 and 2.31 ± 0.10. The sucrose (%) was 2.58 ± 0.08 and 8.37 ± respectively.

In this analysis, there was a significant difference (P < 0.05) between the Potassium content of the 1st peel and core and 2nd peel and core. And also, there was a significant difference in Ca content between all the three pineapple wastes and the 1st peel had the highest Ca content. There was also a significant difference between the Fe content in 1st peel and core and 2nd peel and core. When Mn content was considered, there was a significant difference between 1st peel and 2nd peel and 1st peel and core, but no significant difference between 2nd peel and core. There was a significant difference between the mineral content in the various pineapple wastes. This was further confirmed by the studies of Leterme *et al.* (2006) [23], who had reported that several factors like variety, state of ripeness, soil type, soil condition, and irrigation regime may cause variation in the mineral and trace elemental contents in different types of fruits as well as within different parts of the same fruit.

Table 2: Physicochemical parameters of pineapple wastes

Parameter	1 st peel	2 nd peel	Core
Titrateable Acidity (% anhydrous citric acid)	0.58 ± 0.01 ^b	0.71 ± 0.01 ^a	0.47 ± 0.03 ^c
pH	3.99 ± 0.01 ^b	3.85 ± 0.01 ^c	4.11 ± 0.02 ^a
Total Soluble Solids (^o Brix)	7.07 ± 0.12 ^b	10.13 ± 0.12 ^a	9.47 ± 0.12 ^c
Pectin (g/100 g)	0.30 ± 0.02 ^a	0.15 ± 0.03 ^b	0.09 ± 0.01 ^c
Total polyphenols (Gallic Acid Equivalents – ppm)	341.36 ± 1.36 ^a	120.44 ± 0.24 ^c	168.78 ± 5.61 ^b

* Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c letters in same column are significantly different at (p < 0.05) level.

According to Table 2, TA of the pineapple wastes showed a significant difference at P < 0.05 level. The highest TA was found in 2nd peel 0.71 ± 0.01. Dull (1971) [24] also had reported that the titrateable acidity of the pineapple fruit ranges from 0.6 – 1.62 % of anhydrous citric acid. As described by Hemalatha and Anbuselvi [4], the titrateable acidity of the wastes of Indian pineapple variety was 1.86%. The predominant acid in the citrus fruits is citric acid, so the titrateable acidity of the pineapple was expressed as % of anhydrous citric acid. But there was a difference in the TA

values of this study and the previous studies. This may be due to the differences in the maturity stages of the pineapple fruits, variety of the pineapple used and the storage environmental conditions. The lowest pH for the pineapple waste extract was found in the 2nd peel, followed by 1st peel and the core. There was a significant difference between all the three pineapple wastes at P < 0.05 level of significance. According to Quijandria (1997) [25], the residues of the pineapple have an acidic pH between 3 and 4. But the core had a higher pH (4.11 ± 0.02) than the above level. Due to the

acidic nature of pineapple wastes, they acidify the soil in a significant way and attract insects, which turn pineapple waste as not suitable for its decomposition in an organic way. Pineapple peel extract contained weak acids like citric acid and malic acid and sodium, potassium and calcium salts [26]. Highest total soluble solids are found in the 2nd peel (10.13 ± 0.12 °Brix), then in core and the lowest was in the 1st peel. There is a significant difference between the total soluble solids in all the 3 waste types. The pectin content of the 1st peel was 0.30 ± 0.02 g/100 g dry weight according to the acid extraction method proposed by Simpson *et al.* [14]. The pectin content of the 2nd peel and core were 0.15 ± 0.03 and $0.09 \pm$

0.01 g/100 g dry weight. There was a significance ($P < 0.05$) difference between the pectin contents of the pineapple wastes. There was a significant difference between the total polyphenol content all the three pineapple wastes. The highest quantity of the total polyphenols was found in 1st peel (341.36 ± 1.36 ppm). Larrauri *et al.* (1997) [7] had identified phenolic such as myricetin, salicylic acid, tannic acid, *trans*-cinnamic acid and *p*-coumaric acid from the high dietary fiber powder of pineapple shell.

Analysis of the developed NAWs

Table 3: Physicochemical parameters of developed NAWs after 5 days of fermentation

Parameter	NAW from 1 st peel	NAW from 2 nd peel	NAW from Core
% of ethanol (w/v)			
Ebulliometer	0.37 ± 0.06^a	0.33 ± 0.06^a	0.33 ± 0.06^a
pH	3.51 ± 0.01^a	3.31 ± 0.01^b	3.38 ± 0.01^c
TSS	1.47 ± 0.01^a	1.43 ± 0.01^a	1.47 ± 0.01^a
Titrateable Acidity(% of citric acid)	0.74 ± 0.00^a	0.85 ± 0.01^b	0.55 ± 0.00^c
Colour tone	1.07 ± 0.00^b	1.30 ± 0.03^a	1.28 ± 0.01^a
Colour intensity	0.81 ± 0.01^a	0.47 ± 0.02^b	0.45 ± 0.00^b

* Data presented as mean values for triplicates with duplicate measurements in each replicate \pm S.D (n=6). a,b,c letters in same column are significantly different at ($p < 0.05$) level.

Table 4: Physicochemical parameters of developed NAWs after 56 days of fermentation

Parameter	NAW from 1 st peel	NAW from 2 nd peel	NAW from Core
% of ethanol (w/v)			
Ebulliometer	0.47 ± 0.06^a	0.47 ± 0.06^a	0.43 ± 0.06^a
Methanol (ppm)	n.d	n.d.	n.d.
pH	3.60 ± 0.01^a	3.61 ± 0.01^a	3.53 ± 0.00^b
TSS	1.00 ± 0.00^a	1.00 ± 0.00^a	1.01 ± 0.01^a
Titrateable Acidity (% of citric acid)	0.48 ± 0.02^a	$0.39 \pm 0.05^{a,b}$	0.36 ± 0.01^b
Free SO ₂ (ppm)	34.13 ± 1.48^a	37.40 ± 1.73^a	29.87 ± 1.48^b
Colour tone	3.26 ± 0.30^a	2.36 ± 0.13^b	3.00 ± 0.12^a
Colour intensity	0.10 ± 0.00^b	0.11 ± 0.00^a	0.07 ± 0.00^b
Total polyphenols (in Gallic acid equivalents-mg/L)	108.54 ± 1.95^b	88.91 ± 0.85^b	85.20 ± 1.79^b

* Data presented as mean values for triplicates with duplicate measurements in each replicate \pm S.D (n=6). a,b,c letters in same column are significantly different at ($p < 0.05$) level.

As shown in the Table 3 and 4, after 5 and 56 days of fermentation, the alcohol content of all the NAWs was below 0.5% (w/v) which is the basic legal requirement for the NAW and there was no significant difference between alcohol content among the three NAWs. The highest alcohol content was found in the 1st peel NAW after 56 days of fermentation. After 56 days of fermentation, methanol was not detected in any of the NAWs developed. Methanol is one of the most important components in wine, but its production is not desirable because of its toxicity [27]. This may be due to the relatively low amount of pectin found in the three pineapple peel extracts.

The TSS level of all the NAWs was reduced from the initial value of 2⁰ Brix to during the fermentation period. The value was further reduced after 56 days of fermentation. The decrease in TSS was due to the metabolism of the inoculated yeast. During alcoholic fermentation, yeasts degrade sugars, producing alcohol and CO₂. The reduction in total soluble solids was due to the assimilation of the sugars by the yeast [28]. This reduction of TSS during the fermentation was also observed in the analysis of pineapple wine conducted by Chanprasartsuk *et al.* [29].

There was a significant difference ($p < 0.05$) between the pH of the NAW after 5 days of fermentation. But after 56 days of fermentation the pH values were higher than the pH after 5 days of fermentation. Akin [30], had reported that the

metabolism of yeast during alcoholic fermentation induces a continuous change of the environment and the consumption of carbon and nitrogen substrates is following by acid metabolites production. The same author has showed that for white wine fermentation, first phase characterized by a decrease (about 0.2 pH units) of the pH, followed by a second phase characterized by an increase of pH until the end of fermentation. According to Perrin [31], the pH of the wines after fermentation is generally 2.0 to 4.0. The different results among studies could be due to the fact that the acidity and ethanol content of wine depend on several factors, including type of fruit, type of yeast used, initial TSS in must and methods of wine production [32].

There was also a significant difference ($P < 0.05$) between the colour intensity of the 3 NAW samples. With the progress of the fermentation, the colour intensity was reduced and the lowest colour intensity of the 1st peel NAW, 2nd peel NAW and core NAW (0.10 ± 0.00 , 0.11 ± 0.00 , 0.07 ± 0.00 nm) were recorded after 56 days of fermentation. Various wavelengths (in nanometers) are used in the colour determination. 420 nm is used to determine the yellow/brown pigments while 520 nm is used to determine red pigments [33]. There was a significant difference ($P < 0.05$) in the colour tone of all the NAW samples. The highest colour tone, also known as the colour hue, was found in 1st peel NAW (3.26 ± 0.3) after 56 days of fermentation.

From the three pineapple wastes, after 56 days of fermentation, the highest amount of polyphenols was recorded in the 1st peel NAW as 108.54 ± 1.95 ppm of Gallic Acid Equivalents. The total poly phenol content of 2nd peel non-alcoholic wine and core non-alcoholic wine were 88.91 ± 0.85 and 85.20 ± 1.79 ppm. At 0.05 level of significance, there was a significant difference between the 1st peel and core and 1st and 2nd peel non-alcoholic wine in related to total polyphenol content, but no significant difference between 2nd peel and core non-alcoholic wines. According to Ibegbulem *et al.* (2014) [34], pineapple must and pineapple wine contains saponins, tannins and flavonoids. The number of polyphenols in wine is influenced by many factors, including climatic and pedological conditions of vineyard, temperature and length of maceration, variety of grapes, yeast species, winemaking technology, SO₂ and alcohol content, pH, etc. [35,36].

Conclusion

From the results obtained in this study it can be concluded that the 1st peel, 2nd peel and core of Mauritius pineapple can be successfully utilized for developing various end products depending on their proximate composition and physicochemical characters. The analysed physicochemical characters of the developed NAWs satisfy the basic legal requirements for wine. Further sophisticated research is required to qualitative and quantitative analysis of the phytochemicals of the developed NAWs. Since the consumer demand for the NAWs is increasing world-wide, the development of NAWs can be considered as a low cost and effective method for removal of large quantities of pineapple wastes generated daily during the pineapple processing for all the pineapple growing countries.

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