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Comparative evaluation of natural vinegar produced from mature coconut water and coconut inflorescence sap

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ABSTRACT

The investigation aimed to compare the natural vinegar produced from mature coconut water and partially fermented coconut inflorescence sap. pH, total soluble solids and titratable acidity of the coconut water and inflorescence sap were 5.30±0.1, 2.40±0.3ºB and 0.3 ±0.1% and 5.27±0.06, 12.17±0.06 ºB and 0.62±0.1% respectively. Total soluble solids of mature coconut water was increased to 10ºB by incorporating refined sugar (12%). Significant influence ($P< 0.05$) of the two substrates and the period of storage on the qualitative attributes were observed. Results showed a clear evidence of dominance of alcoholic fermentation in the mature coconut water during the initial 10 days of fermentation. However, in the later stages, acetic acid fermentation in the sap was faster and the concentration of acid produced in sap based vinegar was significantly higher than mature coconut water (5.87% and 4.20% respectively). Total sugar dropped steadily, while reducing sugar increased constantly but quickly decreased after 10 days in both the cases.

Keywords: vinegar, acetic acid fermentation, mature coconut water, coconut inflorescence sap, total sugars

INTRODUCTION

Vinegar is an acidic liquid containing acetic acid as the main component, made from fermentable carbohydrate source within two steps: alcoholic fermentation and acetification (Adams, 1998). FAO (1987) has defined it as " a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars, or starch and sugars by the process of double fermentation, alcoholic and acetous, and contains a specified amount of food additive than synthetic vinegar as it carries nutrients, vitamins, amino acids of fruit source and is reported to act as medicine for many illnesses. The acetic acid in vinegar elicits beneficial effects by altering metabolic processes in the gastrointestinal tract and in the liver (Johnston and Gaas, 2006). Vinegar is normally used in house cooking and it is widely applied in dressing industry with enormous products and in food industry as acidulant or flavoring agent (Huskin, 2008). Traditionally, vinegar has been applied as a food preservative as it inhibits the microbial growth and contributes to sensory properties to a number of foods such as sauces, mayonnaise, etc. It is still used for preserving pickles and for applications such as cleaning, sanitizing, and deodorizing. There are many sources for the natural production of vinegar. Any material containing at least 10% of fermentable sugar and starch is suitable for the production of vinegar. Among them, mature coconut water and partially fermented coconut inflorescence sap are two best substrates which are presently underutilized.

Coconut water, the liquid endosperm, is a common by-product of the coconut industry. Tonnes of coconut water are wasted each year in India and the amount is increasing due to the increasing demand for coconut oil, coconut milk, virgin coconut oil etc. (Prades *et al*., 2012). Virgin coconut oil production from 1,-50,000 nuts give 15,000 litres of water (Manikantan *et al*., 2016). Interestingly, the perception and utilization of coconut water has evolved over the years owing to its unique chemical composition of sugars, vitamins, minerals, amino acids, enzymes and phyto hormones that play different functional roles in the human system (Yong *et al*., 2009). Processing coconut water into various products such as squash, ready to serve, lemonade, vinegar etc. could be considered as practical ways of waste management from coconut industry.

Coconut inflorescence sap on the other hand is utilized as fresh/ unfermented drink. Once the pH of sap falls less than 6, it would not be suitable for consumption as a fresh drink. In that stage either it can be utilized for vinegar production or it can be allowed to ferment completely for making toddy as it is rich in sugars which give most ideal condition for the growth of microorganisms. Partially

fermented sap is exclusively utilized for making vinegar in some places such as Lakshadweep (Surendran, 2015). The present investigation was undertaken to compare the fermentation behaviour of mature coconut water and partially fermented coconut inflorescence sap for the production of vinegar.

MATERIALS AND METHOD

Quality evaluation of substrates

Mature coconut water obtained during the processing of virgin coconut oil was taken for the production of vinegar. Coconut inflorescence sap was collected by tapping the unopened spadix. To initiate fermentation, fresh sap was kept at ambient temperature. Partially fermented coconut inflorescence sap (less than 6 pH) and matured coconut water were analysed for the basic biochemical qualities such as moisture, TSS, pH and titratable acidity. The chemicals used were of analytical grade and purchased from Merck, New Delhi, India.

Production of vinegar from mature coconut water

Mature coconut water obtained during the production of virgin coconut oil was filtered using a muslin cloth. Standardization was made on the quantity of refined sugar to be added to the coconut water to get the total soluble solids to a minimum of 10ºB. It was then pasteurized at 90ºC for 15 min and transferred to an amber coloured glass bottle once after it had cooled down to ambient temperature (33 ºC) followed by addition of baker's yeast at 0.15%. Care was taken to fill three forth of the bottle and one forth was kept vacant for the froth and air bubbles formed during fermentation and the bottle was capped tightly. A sample was taken on 10th day and after taking the observations of biochemical parameters, the entire liquid was siphoned off and filled in another glass bottle after the addition of mother vinegar (which is the sediment containing live bacteria of the previous batch) at 10% of the liquid and covering the lid with muslin cloth so as to promote the aerobic fermentation and kept for acetic fermentation. The mean temperature during the 30 days of experiment was 33.8 ±1.5ºC. Experiment was carried out in three replications.

Vinegar production from coconut inflorescence sap

Partially fermented coconut inflorescence sap (less than 6 pH) was used as the substrate and kept in an amber coloured glass bottle filling three forth capacity which was capped loosely for 48 h for the lactic acid fermentation followed by tightly capping the bottle for inducing anaerobic fermentation for alcoholic production. It was then kept for acetic fermentation after observing the quality on the 10th day by covering the lid with muslin cloth so as promote the aerobic fermentation and stored for 30 days. Samples were analysed at 10 days intervals. The mean temperature during the 30 days of experiment was 33.8 ±1.5ºC. Experiment was carried out in three replications.

Effect of substrates and period of fermentation on the quality attributes of vinegar

pH of the fermented liquid was measured using a digital a pH meter (ECPHWP15002K, Eutech, Singapore) calibrated with 4, 7 and 10 pH buffers. Total soluble solids (TSS) was measured using a hand held refractometer (0-32%, Erma, Japan.). Titrimetry method was used to estimate the titratable acidity and expressed as in terms of the organic acid present (Ranganna, 1986). Mass by volume method was used to calculate the specific gravity. Total sugar and reducing sugar were estimated using phenol sulphuric acid and Nelson-Simogyi method respectively (Sadashivam and Manickam,1992).

Statistical analysis

The experimental design was laid out in factorial complete randomized design with two substrates and period of fermentation as factors. The quality parameters were subjected to analysis of variance and significant difference among means were worked out at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

Quality characteristics of substrates

Table 1 shows the basic quality characteristics required for vinegar production of mature coconut water and partially fermented inflorescence sap. A comparatively lesser TSS present in mature coconut water indicated the necessity of addition of refined sugar to increase the sugar or total solids content of the mature coconut water for favouring the growth of yeast for alcoholic fermentation. TSS content in sap was 12.17°B. Sap is a rich source of sugar (Hebbar *et al*., 2018) which facilitates a contagious environment for the growth of microorganisms. Though both the samples have the same pH levels, the corresponding titratable acidity was different. Titratable acidity of sap was almost double than that of mature coconut water.

Table 1: Quality characteristics of substrates used for vinegar production

Parameters	Mature coconut water	Partially fermented sap
Moisture (%)	95.83+0.6	96.72+0.3
TSS (ºB)	2.40+0.3	12.17+0.06
pH	5.30+0.1	5.27+0.06
Titratable acidity (%)	0.3±0.1	0.62 ±0.1

Values are mean of three replications

Effect of raw materials and period of fermentation on pH, TSS, titratable acidity and specific gravity

Significant differences were observed in all the parameters (Table 2). Comparison of pH values of between coconut water and partially fermented inflorescence sap over the period of fermentation revealed that reduction in pH was significantly faster in coconut water than sap. Results of pH, TSS and titratable acidity could be correlated. Added yeast in mature coconut water acted faster than that of wild yeast naturally present in sap which started consuming the sugars and releasing alcohol affecting the pH, TSS and titratable acidity. pH values reduced from 5.30 to 2.33 and 5.27 to 2.90 for mature coconut water and inflorescence sap respectively. Correspondingly there was a gradual increase in the titratable acidity from 0.3% to 4.2% and 2.10 to 5.87%. It is very clear that maximum changes are occurring in the initial 10 days. Atputharajah *et al.* (1986) isolated 166 isolates of yeasts and 39 isolates of bacteria from coconut inflorescence sap. Offiong and Akpan (2017) reported that *Saccharomycces cerevisiae* dominating among the yeast species while *Lactobaccilus platrium* and *Lacoristoic mensenteroides* were the lactic acid bacteria present in palm sap. Kalaiyarasi *et al*. (2013) isolated and identified the microflora and reported that *Schizosaccharomyces pombe* ferments sucrose and glucose of coconut inflorescence sap and during the later stages *Pichia angophorae* ferments sucrose followed by the fermentation of trace elements such as fructose, galactose by *Kloeckera apiculata* and *Candida glabrata.* The decrease in pH value during alcoholic fermentation was due to the presence of carbon dioxide and hydrogen ions. Yeast transformed sugar added or present in coconut water and sap into alcohol and carbon dioxide gas during alcoholic fermentation (Fathima and Mishra, 2015). At the later stages (20th and 30th day) the increasing concentration of acetic acid also caused in lowering the pH in both vinegars. Fermentation process in mature coconut water involves alcoholic followed by acetic acid fermentations while in sap it consists of initial lactic acid fermentation (within 48 h) then alcoholic fermentation and followed by acetic fermentation (Xia *et al*., 2011; Nwaiwu *et al*., 2016.). At each stage, the microbial activity helps the activity of the micro-organisms in the next stage (Atputharajah *et al*., 1986). Lactic and alcoholic fermentation in sap was completed by 10th day.

Drastic decrease in TSS during 10th day of fermentation indicated the completion of alcoholic fermentation by 10th day. About 87% and 83% reduction in the TSS was observed on the 10th day from mature coconut water and inflorescence sap respectively. The refractometer showed zero reading in the reading scale for 20th day and 30th day mature coconut water and partially fermented inflorescence respectively, which justified that alcoholic fermentation in mature coconut water was faster than inflorescence sap.

There was a slight increase in percent titratable acidity during the initial days of fermentation. Acid production progressed after 20th day of fermentation. It is interesting to note that though the alcoholic fermentation was faster in mature coconut water, the acetic fermentation was taken over by the inflorescence sap (5.87 % in sap where as 4.2% in water).

A non significant effect (P>0.05) of specific gravity was observed for the interaction of substrates and fermentation days. The values were close to one. However, specific gravity was found reducing towards the end of fermentation.

Effect of substrates and period of fermentation on total and reducing sugars

Fig. 1 and 2 show the changes of total sugar and reducing sugar during the fermentation of mature coconut water and coconut inflorescence sap. Significant effect was observed in the percent total sugars (P<0.05). Inversion of sugars occurred during fermentation. Faster rate of alcoholic fermentation during the initial days in mature coconut water was clearly visible from Fig. 1 as only 5% total sugar was observed in the initial days. 78% and 53% of the total sugars present in coconut water and sap were found to be hydrolysed in 10 days. Sekar and Mariappan (2005) reported that half of the total sugars were fermented during first 24 hours. Concentration of sugars had a linear relationship with the reading of TSS.

Table 2: Effect of substrates and period of fermentation on pH, TSS, titratable acidity and specific gravity

Parameters	Mature coconut water based vinegar Days				Partially fermented sap based vinegar Days			
	0	10	20	30	0	10	20	30
pH	5.30^{a} ± 0.1	3.10^{c} ± 0.0	2.80^{d} ± 0.0	2.33^{e} ± 0.1	5.27^{a} ± 0.06	3.23^{b} ± 0.1	3.1^{c} ± 0.1	2.9^{d} ± 0.01
TSS	10.33^{b} +0.1	1.0^{e} ± 0.0	0± 0.0^{f}	0.0± 0.0^{f}	12.17^{a} ± 0.06	2.1^{c} ± 0	1.17^{d} ± 0.2	0.0^{f} ± 0.0
Titratable acidity	0.3^{g} ± 0.1	1.8^{e} ± 0.1	3.7^{c} ± 0.1	4.2^{b} ± 0.3	0.75^{f} ± 0.3	2.1^{d} ± 0.1	3.87^{c} ± .1	5.87^{a} ± 0.2
*(non significant) Specific gravity	0.98 ± 0.01	0.98 ± 0.01	0.99± 0.01	0.86± 0.1	0.98 ± 0.01	0.98 +0.01	0.95 ± 0.01	0.91± 0.1

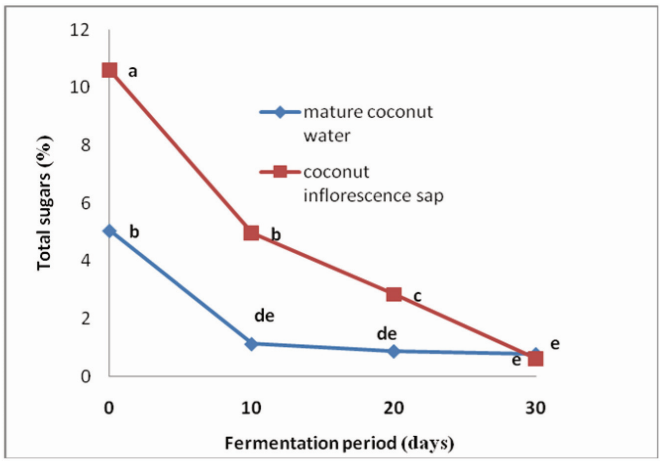

Fig. 1: Effect of substrates and period of fermentation on total sugars (%)

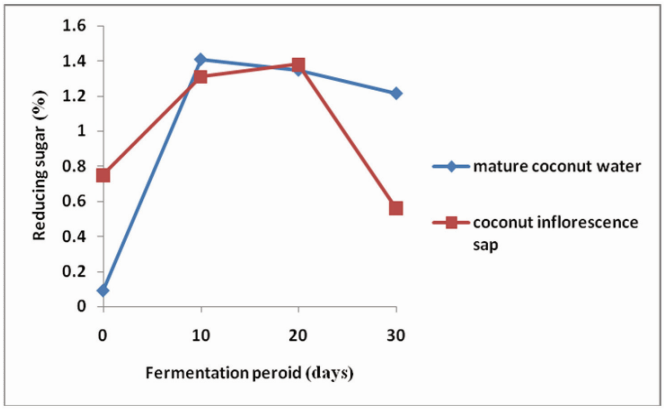

Fig.2: Effect of substartes and period of fermentation on reducing sugar (%)

Total sugar dropped steadily, while reducing sugar increased constantly but quickly decreased after 10 days in both the cases. This is due to sucrose being converted into fructose and glucose during initial fermentation, and the reducing sugar was consumed by microorganisms at the later stage. Similar observations were reported by Xia *et al.* (2011). However, the interaction effects of both the treatments were not significant (P>0.05).

CONCLUSION

Table 3 depicts the overall comparison of vinegar produced from mature coconut water, inflorescence sap and market available synthetic vinegar. Naturally produced vinegar was yellow in colour. Synthetic vinegar available in market is colourless. Among the two naturally processed vinegars, inflorescence sap based one was lighter yellow in colour and had a sweet flavour. The grain strength was more in the sap based vinegar. Thus it could be concluded tha partially fermented sap is a better substrate for the production of natural vinegar.

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Table 3: Overall comparison of vinegar produced from mature coconut water, coconut inflorescence sap and market available synthetic vinegar

Parameters	Mature coconut water based vinegar	Partially fermented sap based vinegar	Market available synthetic vinegar (Double Horse™)
Ingredients	Mature coconut water, sugar, yeast, mother vinegar	Coconut inflorescence sap	Water, acetic acid
Colour	Dark yellow	Light yellow	Colourless (Water clear)
Flavour	Harsh	Sweeter	No distinct flavour
Titratable acidity (% acetic acid) within 30 days	4.2	5.87	4-5
Grain strength	42	58.7	Not mentioned

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