As. J. Food Ag-Ind. **2013**, 6(05), 281-289

OPEN ACCESS

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Research Article

Production of fermented coconut water beverages

Neela Satheesh[1] and NBL Prasad[2]

[1]Dept. of postharvest Management, College of Agriculture and veterinary Medicine, Jimma University, Jimma, Ethiopia.

[2]Jawaharlal Nehru Technological University Anantapur, Oil Technological Research Institute, Anantapur-515001 A. P. India.

Email: Neela.micro2005@gmail.com

Abstract

Coconut water (liquid endosperm) with its ample applications has become one of the world's most popular natural products. This refreshing beverage is consumed worldwide for its nutritional and medicinal benefits. Coconut water remains as the waste product in many coconut-based industries viz., desiccated coconut powder, coconut milk and virgin coconut oil manufacturing, which creates a lot of disposal problems. Coconut water spoils within short span of time after exposure to the environment due to enzymes and microbial activity. In this study, for better utilization of coconut water, a waste product (coconut water) in virgin coconut oil production was collected and fermented beverages were produced by using exclusively coconut water and a blend (1:1) of grape juice and coconut water. Further, these two fermented beverages were also found suitable for vinegar production. Physico-chemical parameters were determined for both the fermented beverages and vinegars. All quality parameters for both coconut water beverages and vinegars were well within the standards.

Keywords: grape juice, vinegar, value added, yeast extract peptone dextrose, alcohol.

Introduction

Coconut water is technically liquid endosperm, forming small quantities in the third month of coconut maturation and reaching a maximum in eight months, declining as the nut ripens [1]. It is a faintly turbid to clear liquid, colourless, sweet, naturally flavoured and slightly acidic with reported pH ranging from 4.2 to 6.0 [2]. Coconut water obtained from mature nuts, when harvested for the production of copra and coconut oil, is wasted on a large scale in several tropical countries [3].

Compositionally, coconut water contains carbohydrates (glucose, fructose, sucrose and sorbitol), essential amino acids (lysine, histidine, tyrosine and tryptophan) and organic acids (tartaric, citric and malic acids) in minor fraction [4]. The carbohydrates occur in variable ratios and may have a total concentration of up to 8% (w/v). Further, coconut water contains most of the nutrients (growth promoting factors) required for plant and microbial cell growth [5].

Coconut water is largely consumed all over the world, not only as a refreshing drink, but also because of its numerous therapeutic qualities. It can be applied to gastric disturbances, inhibition of vomit caused by cholera, treatment of dysentery, for infant feeding and other patients [6]. Medical resources routinely use coconut water for intravenous hydration and resuscitation of critically ill patients [7]. Mandal, [8] determined the presence of antimicrobial proteins in coconut water and reported their effective use against both gram positive and gram negative bacteria. It was reported that coconut water and cashew apple juice with caffeine was used for the production of fermented beverage [9]. It has also been reported that coconut water was fermented with *Lactobacillus sp.* to produce an edible product known as "coconut kefir" [10]. Coconut water is a perishable product, after nut opening on contact with the atmospheric oxygen, coconut water leads to reduction in enzymes and external microbial contamination. This finally effects and modifies coconut water's typical properties like nutritive value, taste and colour [11].

The grapevine plant (*Vitis vinifera*) is indigenous to the Northern Hemisphere and grows mostly in temperate regions, though with restrictions based upon soil and actual meso-climate (climate of the vineyard) [12]. Grapes contain sugars such as sucrose, fructose, glucose, lactose, maltose, galactose, starch and vitamins A, B,C, E, K and minerals Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn, etc. Wine is produced from both red and white grapes [13].

Fermented beverages from fruit and vegetable sources have immense importance in human food around the world. Reports proved [14] that limited consumption of fermented beverages from fruit sources are always beneficial for human health. Vinegar is used as flavouring agent in different food preparations and it acts as a good food preservative.

Production of fermented beverages from coconut water by using yeast was scarce in literature which instigated the present work and the main objectives are to develop a process by utilizing a waste product such as coconut water for the production of fermented coconut water beverage and vinegar (value added products).

Materials and Methods

Coconut water collection

In wet processing of virgin coconut oil, mature coconuts were processed by dehusking and coconut water was collected through the pore. Immediately after collection of coconut water it was filtered through the double layered muslin cloth into Erlen -Mayer flask and allowed for further processing.

Preparation of inoculum (Yeast Culture)

YEPD (Yeast Extract Peptone Dextrose) broth medium was used to prepare the yeast inoculum. It consists of yeast extract, Peptone and Dextrose of 1, 2 and 2% respectively. Lyophilized *Saccharomyces cereviciae* was collected from National Center for Dairy Collections - National Dairy Research Institute (NCDC - NDRI), Karnal, Haryana, India. Samples were processed according to NDRI catalogue.

100 mL of YEPD broth was prepared in the Erlen- Mayer flask of 250 mL capacity and sterilized in autoclave. Loopful of yeast culture was added into the YEPD broth after sterilization and incubated at 28 ± 1^0C in a shaking incubator at 100 rpm and incubated for 10 days. The incubated YEPD broth was centrifuged in the cooling centrifuge at 10,000 rpm for 10 min. at 27^0C. Yeast cell suspension was collected into a separate sterilized container after centrifugation. The entire process was maintained under aseptical conditions.

Production of alcoholic beverage exclusively with coconut water

Immediately after collection and filtration of coconut water, sugar concentration was determined with hand refractometer as brix Total Soluble Sugars (TSS %) and final sugar concentration was adjusted to 15 brix by using sucrose and subjected to pasteurization by closing with cotton plug. Coconut water pasteurization was carried out at 71.7^0C temperature for 20 Sec.

After proper pasteurization, the coconut water was allowed to cool and 2% yeast inoculum (culture suspension) was added. The flask was closed with cotton plug and allowed to ferment at room temperature for 10 days.

After successful completion of fermentation, the fermented coconut water beverage was filtered using Whatman No.1 filter paper, filtrate was pasteurized. Fermented beverage was preserved in sterilized bottle under aseptical conditions for further studies.

Production of Fermented Coconut Beverage Blended with Grape Juice

Freshly extracted, filtered (through muslin cloth), black grape juice was added to the filtered coconut water in 1:1 ratio and the sugar concentration was adjusted to 15 brix by adding sucrose. The mixture was pasteurized at 71.7^0C temperature for 20 Sec.

2% yeast inoculum was then added to the cool 1:1 blend of coconut water and grape juice. The flask was incubated at room temperature for 10 days. After successful completion of fermentation, fermented beverage was filtered through Whatman filter paper and the filtrate was pasteurized and stored in a sterilized bottle for quality analysis.

Vinegar production from coconut water

The coconut water fermented beverage was used as the substrate for the production of vinegar. 2% acetic acid bacteria *Acetobacter aceti* NDRI strain was used as inoculum in the submerged batch fermentation process.

Production of Acetobacter aceti inoculum

Mannitol broth medium (Mannitol - 15g, Magnesium sulphate - 0.2g, Di-potassium hydrogen phosphate - 0.5gm, Calcium Sulphate - 0.1gm, Calcium Carbonate - 5.0 gm, Sodium Chloride - 0.2 gm, and distilled water -1000 mL) was prepared and sterilized. Sterilized medium was inoculated with loopful culture of *Acetobacter aceti* NDRI strain and the flasks were incubated at 30^0C in a shaking incubator at 100 rpm for 2 days. The cultures were then subjected to centrifugation under aseptical conditions at 18,000 rpm for 10 min. Temperature of the centrifuge was maintained at 27^0C during the entire process. The culture suspension (*Acetobacter aceti*) was collected and immediately transferred to the flasks containing small quantities of fermented coconut water beverage.

Production of vinegar from fermented coconut water beverage

Both fermented beverages were pasteurized and 2% inoculum of *Acetobacter aceti* was added and the flasks were incubated at 100 rpm in a shaking incubator at 28^0C for 3 days. After incubation, vinegar was filtered through Whatman No1 filter paper and stored in a glass bottle for further analysis.

Determination of quality parameters

Different physico-chemical parameters viz., ethanol content, reducing sugars, pH, total acidity, volatile acidity, esters, higher alcohols, aldehydes, extracts and tannins for coconut water fermented beverages and total acidity, residual alcohol, total solids, ash percentage and soluble solids for vinegars produced from coconut water fermented beverages were determined according to BIS

procedures [15, 16]. Metals like Fe and Cu for both beverages and vinegars and As, Pb and Zn exclusively for vinegars were determined by using ICPMS [17].

Determination of metals in coconut water fermented beverages and vinegar

Sample digestion

Open-acid digestion technique was employed in Teflon beakers. Clean and dried Teflon Beaker with cover was taken and 10 mL of each fermented sample and acid mixture in a ratio of 7:3:1 (HF: HNO_3: $HClO_4$) was added. Beaker was covered with the lid and allowed overnight for digestion followed by dry heat on hot plate at 150^0C. After 1 h Teflon beaker was opened, again 5mL of acid mixture was added and dried on hot plate, 20 mL of a mixture of supra pure HNO_3 and double distilled water was added in 1:1 ratio and kept at 70^0C to dissolve the precipitate. 1 mL of internal Rh (Rhodium) standard was added and volume was made up to 250 mL with double distilled water and stored in 60 mL HDPE bottles with sample identity.

Perkin-Elmer SCIEX ICP-MS (Model ELANs DRC II, Toronto) was employed to carry out the elemental analysis. The sample introduction system consists of a standard Meinhard nebulizer with a cyclonic spray chamber. All the measurements were performed using instrumental software. Several well-known isobaric interferences were programmed and the corrections were automatically applied [18].

Statistical analysis

The results obtained were presented in mean average value ± SD of three observations except the metals concentration. The significant difference between observations was analyzed with Duncan's multiple range tests [19] where values $P \leq 0.05$ were considered as significant.

Results and Discussion

Coconut water fermented products (with *Lactobacillus sp*.) are readily available in the market, but they are non-alcoholic, whereas in the present study, coconut water fermented alcoholic beverage was produced purely with the help of yeast and they are not yet commercialized. The data regarding quality control parameters of coconut water fermented alcoholic beverage is presented in Table 1.

Ethanol content is one of the major parameters for grouping the fermented alcoholic beverages into different types. In the present study, 15% sugars were taken for the fermentation process. In fermented alcoholic beverage, the ethanol content of 6.02 and 6.89% was obtained in coconut water and a blend of black grape juice with coconut water respectively. According to the stoichiometric equation of alcohol (ethanol production), one gm of carbohydrate produces 0.51 gms of ethanol [20]. As per the equation, the ethanol content should be around 7.65% but practically achieved was 6.02 and 6.89 % only in both cases which were coincides [21] with the reported values of fruit wines. This may be due to the not utilization of total carbohydrates for converting in to alcohol by the yeast.

Utilization and production of carbohydrates and ethanol respectively depends solely on the fermentation efficiency of the yeast. According to the Indian standards [22, 23] the ethanol content of 8-15% is the maximum limit for different types of red and white wines which were produced exclusively from grapes. The ethanol content in beer is also less than 10% [24], source of production clearly varies for both beer and wine, beer is purely from grain based. According to USDA [25], in distilled beverages viz., gin, rum, vodka, whiskey etc, the maximum standard limit for the alcohol is 42.5% which were also grain based.

Table 1. Quality control parameters of fermented beverages produced exclusively from coconut water and a blend of black grape juice and coconut water.

S.No.	Quality parameter	Exclusively coconut water fermented beverage	Blend (1:1) of black grape juice and coconut water fermented beverage
1	Ethanol content (%)	6.02±0.23[a]	6.89±0.93[b]
2	Reducing sugars (g/L)	2.7±0.093[a]	1.3±0.123[a]
3	pH	3.56±0.04[b]	3.72±0.05[b]
4	Total acidity as Tartaric acid (g/L)	4.2±0.016[a]	7.8±0.036[b]
5	Volatile acidity (g/L)	0.43±0.04[a]	0.5±0.19[a]
6	Esters as ethyl acetate (g/L)	1.2±0.09[b]	3.0±0.18[a]
7	Higher alcohols as amyl alcohol(g/L)	1.8±0.101[ac]	2.6±0.125[a]
8	Aldehydes as acetaldehyde(mg/L)	0.1±0.01[b]	0.9±0.02[b]
11	Amount of Iron (mg/L)	0.2[a]	0.3[a]
12	Amount of copper (mg/L)	0.03[a]	0.5[a]
13	Extracts (g/L)	10.12[b]	90.92[a]
14	Tannins (g/L)	ND	1.3±0.12[a]
15	Methyl alcohol content (g/L)	0.012±0.01[a]	0.239±0.23[a]

Note: Values followed by different letters (a, b, c) in row differ significantly from each other at P≤0.05, based on SAS software.

Reducing sugars are the sugars which were naturally present in the fermenting medium or added externally before fermentation. The sugar concentrations of 15% was maintained initially and after fermentation process 2.7 and1.3 % of the reducing sugars were detected in the fermented beverages of coconut water and a blend of black grape juice with coconut water respectively. According to the stoichiometric equation [20], the remaining reducing sugars must be 3.2 and 1.49% in the coconut water and a blend of black grape juice and coconut water respectively. However, it was detected as 2.7 and 1.3% respectively. This may be due to the remaining reducing sugars were more in the beverage exclusively produced from coconut water than in the beverage produced from a blend of black grape juice and coconut water which contains normal flora of different micro organisms. Further, the variation existed because of the reducing sugars were converted in to different organic acids by different micro organisms which were naturally occur in the fermentation process and some of the by-products like acetic acid and ethyl esters were also produced from the reducing sugars itself during the yeast metabolism [26]. The remaining reducing sugars may also be used in the production of acids, esters and higher alcohols. Reducing sugars present in both the samples were within the standard limits of BIS [22, 23].

pH, total acidity, volatile acidy, ethyl acetates, higher alcohols, acetaldehyde and methanol concentrations were as per the BIS standards[22, 23].

Metals (iron and copper)

Fe is one of the inorganic cations which help the metabolism of some important enzymes in yeast and certain amount of Fe was invariably present in the fermented beverages [27]. In present study, the concentration of Fe was detected as 0.2mg/L, 0.3 mg/L for coconut water and a blend of black grape juice and coconut water respectively and they are within the limits [22, 23].

Cu is also one of the cations which is useful for the metabolism of the yeast [27] and the concentrations of Cu present in the coconut water and a blend of black grape juice and coconut water beverages produced were 0.03 mg/L and 0.5mg/L respectively and they are within the accepted limits [22, 23].

Extracts concentration was determined in fermented beverages from coconut water (10.12gms / L) and a blend of black grape juice and coconut water (90.92 gms / L). Different chemical constituents (phenolic compounds and tannins) were present in the extracts which were obtained from source material (grapes) [26]. The variation may be due to the presence of high concentration of extracts in grapes. Both the samples showed extracts within the standard limit [22, 23].

Detectable amount of tannins were not found in the beverage produced exclusively from coconut water where as tannin concentration of 1.3 mg/L was detected in fermented beverage from a blend of black grape juice and coconut water which is within the standards [22, 23]. Certain amount of different tannins was present naturally in grapes [27] but not in coconut water [4] this may be one of the reasons for no detection of tannins exclusively in coconut water beverage.

Quality parameters of vinegar from the fermented beverages

Vinegar was produced exclusively from coconut water and a blend of black grape juice and coconut water fermented beverage and different quality control parameters were studied. The data is depicted in Table 2.

Total acidity determines the concentration of acetic acid present in brewed vinegar. The total acidity of 8.8gms/L and 6.7 gms/L are present in vinegar from coconut water and a blend of black grape juice and coconut water fermented beverages respectively and the total acidy found in both the samples were within the standard limits [28].

In vinegar production, the total alcohols present in the alcoholic beverage may not be converted in to acetic acid and some amount remains as such. The residual alcohol V/V% of 0.97 and 1.03 was detected in vinegar from coconut water and a blend of black grape juice and coconut water fermented beverages respectively and they were within the standard limits [28] and correlated with the reported study [29].

Total solids, soluble solids and ash percentages were determined in both the vinegars produced from coconut water and a blend of black grape juice and coconut water fermented beverages and they were well within the standard limits [28] and correlated with reported study [29].

Table 2. Quality control parameters of vinegars produced by fermented beverages exclusively from coconut water and a blend of black grape juice and coconut water.

S.No.	Quality parameter	Vinegar from coconut water fermented beverage (exclusively)	Vinegar from a blend (1:1) of black grape juice and coconut water fermented beverage
1	Total acidity as acetic acid g/100mL	8.8 ± 0.13^{a}	6.7 ± 0.014^{b}
2	Residual alcohol V/V %	0.97 ± 0.007^{b}	1.03 ± 0.032^{a}
3	Total solids percentage mg/l	0.4 ± 0.36^{a}	1.04 ± 0.24^{a}
4	Ash percentage mg/l	0.13 ± 0.12^{b}	0.15 ± 0.02^{a}
5	Soluble solids percentage g/100 mL	0.099 ± 0.19^{ab}	0.012 ± 0.22^{b}
6	Arsenic mg/kg	0.003	0.018
7	Lead mg/kg	0.009	0.029
8	Iron mg/kg	0.18	0.28
9	Zinc mg/kg	0.02	0.07
10	Copper mg/kg	0.22	0.48

Note: Values followed by different letters (a, b) in row differ significantly from each other at $P \leq 0.05$, based on SAS software.

Metals (Fe, Cu, Zn, Pb and As)

The amount of mineral concentrations present in the fermented alcoholic beverages directly shows the metals in vinegar samples. The amount of the metals present in the vinegar depends directly on geochemical nature of cultivating soil and farming and processing conditions.

The amount of Fe 0.18 mg/L and 0.28 mg/L was detected in vinegar produced from coconut water and a blend of grape juice and coconut water fermented beverages. Both the samples of vinegar are shown Fe concentrations within the standard limits [28] and lower than the reported study [30].

Concentrations of 0.22 and 0.48 mg/L Cu was determined in vinegar from coconut water and a blend of grape juice and coconut water fermented beverage samples respectively and are within the standard limits [28] correlated with the reported study [30].

0.02 and 0.07 mg/L concentrations of Zn were detected in the brewed vinegar from coconut water and a blend of grape juice and coconut water fermented beverage respectively. Zn concentration present in the both the vinegar samples were within the acceptable limit [28] and lower than the reported study [30].

The amounts of 0.009 mg/L and 0.029 mg/L Pb were observed in brewed vinegar from coconut water and a blend of grape juice and coconut water fermented beverages. The amounts of Pb present in both the vinegar samples are in harmless limits [28, 29] and lower than the reported study [30].

Concentration of 0.003mg/ L and 0.018mg/L of As is present in the vinegar from coconut water and a blend of black grape juice and coconut water fermented beverages respectively. The concentration of As is in harmless limits in both vinegar samples [28, 29] and correlated with the reported study [30].

Conclusion

A blend of nutritionally rich fruit materials (grape juice and coconut water) was used in the production of alcoholic beverages. Ethanol concentration is the major parameter to classify beverages into the different groups. In wine produced from fruit juices, the ethanol content is at 10 per cent. Fermented beverages exclusively produced from coconut water and a blend of black grape juice and coconut water are satisfied the quality characters of wine and they may be classified under wine beverages and named as “coconut water wine” and “blended of grape juice and coconut water wine”. Wines from both the sources (exclusively produced from coconut water and a blend of black grape juice with coconut water) may be suitable for human consumption. Vinegar from both the fermented beverages can effectively use in different food and industrial applications.

Acknowledgements

The authors greatly acknowledge the JNT University authorities for providing financial support and also permitting to work in Oil Technological Research Institute (OTRI) and Dept. of Chem. Eng., JNTUCEA. They are also thankful to the Directors of OTRI and IRP JNTUA for their constant support and encouragement.

References

1. S. Shivashankar (1991). Bio-chemical changes during Fruit maturation in coconut, ***Journal of Plantation Crops,*** 19(2):102-119.

2. U. Santoso, K. Kubo, T. Ota, T. Tadokoro and Maekawa (1996) A Nutrient composition of Kopyor coconuts, ***Food Chemistry***, 57(2): 299-304.

3. N.M. Brito, S. Navickiene, L. Polese, G. Jardim, R.B. Abakerli and M.L. Ribeiro (2002) Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high performance liquid chromatography with ultraviolet detection, ***Journal of Chromatography A***, 957(2): 201-209

4. J.W.H. Yong, G. Liya, Y. Fei Ng and S. Ngin Tan (2009) The Chemical Composition and Biological Properties of Coconut (*Cocos nucifera* L.) Water, ***Molecules,*** 14(12): 5144-5164.

5. M.E. Smith and A.T. Bull (1976). Studies of the utilization of coconut water waste for the production of the food yeast *Saccharomyces fragilis*, ***Journal of Applied Bacteriology,*** 41: 81-95.

6. T. Kuberski (1980). Appropriate technology: coconut water for the oral rehydration of childhood diarrheas, ***New Zealand Medical Journal***, 91: 390-396.

7. D.C Falck, T. Thomas, T.M. Falck, N. Tutuo and K. Clem (2000). The Intravenous Use of Coconut Water, ***American Journal of Emergency Medicine***, 18(1):108-111.

8. S.M. Mandal, S. Dey, M. Mandal, S. Sarkar, S. Maria-Neto and O.L. Franco (2009). Identification and structural insights of three novel antimicrobial peptides isolated from green coconut water, ***Peptides,*** 30:633–637.

9. J. Marques de Carvalho, G.A. Maia, R. Wilane de Figueiredo, E. Sousa de Brito and S. Rodrigues (2006). Development of a blended beverage consisting of coconut water and cashew apple juice containing caffeine, ***International Journal of Food Science and Technology***, 42: 1195-1200.

10. M.A. Golowczyc, J. Silva, P. Teixeira, G.L.D Antoni and A.G. Abraham (2011). Cellular injuries of spray-dried *Lactobacillus sp*. isolated from kefir and their impaction probiotic properties, ***International Journal of Food Microbiology***, 144: 556-560.

11. A.C.P. Duarte, M.A.Z Coelho and S.G.F Leite (2002). Identification of peroxidase and tryrosinase in green coconut water, ***Ciencia Technology Aliment,*** 3(5): 266-270.

12. R.J. Clarke and J. Bakker (2004). Wine Flavour Chemistry, London: Blackwell Publishing Ltd.

13. P.A. Norrie (2003). The history of wine as a medicine In, M. Sandler and R. Pinder R. Wine a Scientific Explosion, Taylor and Francis, London: 21-55,

14. United States Department of Agriculture (USDA). A National Nutrient Database for Standard Reference- Grapes composition-*http:www.ndb.nal.usda.gov/ndb/foods/show 2321;* accessed 5th December 2011

15. Indian Standards for alcoholic drinks and methods for test (IS No: 3725:2005) BIS New Delhi, India.

16. Indian Standards for Wine-methods of analysis (IS. No: 7585:1995) BIS, New Delhi.

17. R.L.S. Cerutti, J.A. Salonia, R.A. Olsina and L.D. Martinez (2005). Trace element determination of Argentine wines using ETAAS and USN-ICP-OES, ***Food and Chemical Toxicology***, 43:293-297.

18. D.M.A.M. Luykx and S.M.Van Ruth (2008). Analytical Methods. An overview of analytical methods for determining the geographical origin of food products, ***Food Chemistry,*** 107: 897-911.

19. B.B. Duncan (1955). Multiple range and multiple *f* test, ***Biometrics,*** 11: 1-5.

20. C.T. Dwarakanath (1994). Vinegar fermentation with special emphasis on possibilities of utilization of matured coconut water. In Proceedings of the National seminar on processing and marketing of coconuts (SPAMCOII), Bangalore: *Indian Coconut Development Board,* 44-50.

21. T. Tarko, A.D. Chodak, P. Sroka, P. Satora and E. Jurasz (2008). Physicochemical and antioxidant properties of selected polish grape and fruit wines. ***Acta Scientiarum Polonorum Technology Alimentaria,*** 7 (3): 35-45.

22. Indian standard specifications for table wine, IS 7058:1995, BIS, New Delhi.

23. Indian standard specifications for fortified wines, IS 14398: 2005, BIS, New Delhi.

24. Indian standard specifications for beer, IS 3865:1966, BIS, New Delhi.

25. United States Department of Agriculture (USDA)b, National Nutrient Database for Standard Reference- Alcoholic beverage, distilled, all (gin, rum, vodka, whiskey) 100 proof, *http:// www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl*, accessed 5th December 2011.

26. F. Zamora, (2009). Biochemistry of alcoholic fermentation in M.V.M Arribas and M. Carmen Polo (Eds.) Wine Chemistry and Bio-Chemistry, New York: Springer, 20-22.

27. P. Ribereau-Gayon, Y. Glories, A. Maujean and D. Dubourdieu (2006). Handbook of enology, the chemistry of wine stabilization and treatments, 2nd Edn. England: John Wiley & Sons.

28. Indian Standards for Vinegar specifications IS14703: (1999), BIS, New Delhi.

29. R.C. Chang, H.C. Lee and S. Shau-Mei (2005). Investigations of the physicochemical properties of concentrated fruit Vinegars, ***Journal of Food and Drug Analysis,***13 (4): 348-356.

30. S.G. Capar, W.R Mindak and J. Cheng (2007). Analysis of food for toxic elements, ***Analytical Biochemistry,*** 389: 159–169.