INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

STANDARDIZATION OF FERMENTATION PROCESS FOR THE PRODUCTION OF CASHEW WINE

[1]Umashankar, N., [1]Mohan Chavan, [2]Benherlal, P.S. & [3]Maruthesh.A.M.
[1]College of Agriculture, Hassan – 573225
[2]College of Agriculture, Mandya
[3]AHRS, Katthalagere, Davanagere District

ABSTRACT
Cashew wine was prepared by fermentation of cashew apple using Yeast. The present study was done to focus on standardization of fermentation time for the preparation of cashew wine from Ullal 1, 2 & 3 varieties which is developed by University of Agricultural Sciences, Bangalore by analyzing the changes in biochemical parameters *viz.* Brix, pH, Alcohol, Total Soluble sugars, Total Reducing Sugars, Total Soluble Proteins, organic acids and antioxidant activities on fermentation of cashew apple juice. The wine had higher amount of Total Soluble Sugars at 9th day of fermentation and it is on par with 12th day wine (2.953 mg/ml). The Total Soluble Solids and Total Reducing Sugars were reduced at 15th day of Fermentation. The alcohol content increased and it was maximum at 12th day (6.42%) with slight increase at 15th day (6.61%). There was increase in both polyphenol and DPPH activity upto 12th day. The cashew wine can be fermented for 12 days and can be considered as low alcoholic wine.

KEY WORDS: Cashew apple, Antioxidant, Fermentation, Wine, Ullal 1, 2 & 3.

INTRODUCTION

The cashew tree is a tropical plant and the nut (the true fruit) has excellent nutritional and sensory properties and widely consumed by people in the world. The pseudo fruit has three to six folds increased vitamin C content than orange (Soares *et al.*, 2007). Cashew apple belongs to the family Anacardiaceae, is a pseudo-fruit formed by an enlarged peduncle, and the true fruit, a kidney-shaped (reniform) achene is about 3 cm long with a hard grey-green pericarp and presently India is currently the second largest fruit producer in the world after China (Costa *et al.*, 2009). Cashew apple juice is a rich source of vitamin (Assuncao & Mercandante, 2003) and also presents functional properties such as cancer prevention (Kubo *et al.*, 1993), and the prevention of bacteria (*Helicobacter pillory)* that causes severe gastritis (Kubo *et al.*, 1999; Carvalho *et al.*, 2006), besides preserving antioxidant properties (Wharta *et al.*, 2004; Kubo *et al.*, 2006). In recent years the economic value of cashew (*Anacardium occidentale)* apple juice has increased. Fresh cashew apple juice (CAJ) and processed juice, called cajuina, are among the most popular natural products in Brazil (Cavalcante *et al.*, 2003). A blended beverage based on coconut water, cashew apple juice and caffeine was developed (Carvalho *et al.*, 2005, 2006 & 2007). The fruit powders were produced from cashew apple and guava (*Psidium guajava* L.) residues after extracting the juice and used as a source of vitamin C and lipid respectively (Costa *et al.*, 2009).

Ullal 1 Ullal 2 Ullal 3

The cashew apples are rich in volatile compounds and its composition was analysed by many researches. Forty eight volatile compounds were identified in the cashew apple juice, the volatile compounds being predominantly esters (42% of total compounds), followed by aldehydes (14%) (Franco and Janzanti, 2005). Because of the low cost and

large availability, cashew apple juice has been used as an alternative raw material for the production of various industrially important products such as surfactants (Grio *et al.,* 2009), alcoholic beverages (Mohanty *et al.,*2006), cashew apple syrups (Lavinas *et al.,* 2008). It is also used as a substrate for fermentative processes (Honorato *et al.* 2007). Thus, the object of the present work is to look-over the min possibility of utilizing the cashew apple juice produced from the variety Ullal – 1, 2 and 3 developed by University of Agricultural Sciences, Bangalore and standardizing the fermentation process for production of wine from these verities.

MATERIALS & METHODS

The ripened cashew apples (Ullal -1, Ullal-2 and Ullal-3) were collected from the orchard of Bio fuel Park, Hassan. After sorting out and the peduncle was washed and crushed in blender and the juice was extracted manually by pressing the cashew apple in muslin cloth.

Preparation of must

The extracted cashew apple juice was heated to 85^{o}C along with 0.5% food grade gelatin to reduce the tannins. The tannins settled down as sediments were separated out. The must was kept in glass containers and Potassium metabisulphite (KMS) of 100 ppm was added to inhibit the growth of undesirable microbes (Attri, 2009).

Preparation of wine

100 ml of cashew juice was taken in twenty 250 ml conical flask and brix value was adjusted to 20^{o} using cane sugar. No nitrogen source was added. *Saccharomyces cerevisiae* inoculum was inoculated with 5% (v/v) (Attri, 2009). The experiment was conducted in completely randomized design with four replicates. Fermentation was carried out in a glass container at 23- 25 ^{o}C and the juice was stirred and samples were drawn at the interval of 3 days till day 15 for analysis. The wine was filtered, pasteurized at 75^{o}C for 20 min and clarified by centrifugation (4000 rpm for 10 min) before consumption. Samples were analyzed in four replication for various physical and chemical parameters.

Physical and chemical parameters

pH was measured by digital pH meter (Systronic Pvt. Ltd), before measurement, pH meter was calibrated with 0.05M potassium hydrogen phthalate for 4.00 pH, 0.025M potassium dihydrogen phosphate for 6.85 pH and 0.025M disodium hydrogen phosphate for 9.00 pH at 25^{0} C. fermented juice was used for checking the pH. Total soluble protein was extracted by taking 0.5ml of fermented juice sample and 5ml of 0.2M phosphate buffer of pH 7.00 consisting of 0.2mM β-mercapto-ethanol, 0.02mM PVP and kept for shaking for 10 min at the rate of 60 RPM. To the mixture 15 percent Trichloro acetic acid was added and kept for overnight for protein precipitation. Next morning mixture was centrifuged at 6000 RPM and total precipitated protein was settled which was dissolved by 5ml 0.1M sodium hydroxide and used as aliquot for protein estimation. Protein estimation was done according to Lowry *et al*.(1951) method and measured absorbance at 660nm. Percent titrable acidity was determined by taking 10ml of sample juice. From 10% volume made up sample juice and it was titrated against 0.1N sodium hydroxide solution using one or two drops of phenolphthalein indicator and total acidity was is expressed as percent citric acid (g/100ml juice sample), appearance of pink color indicated the end point (Normas Analíticas Do Instituto Adolfo Lutz, 1985). Brix analysis was done using refractro-meter, refractro-meter sample surface was cleansed twice with doubl distilled water and with the help of pasture pipet two drops of sample was smeared on refractro-meter sample surface and reading was recorded (Torres Neto *et al*., 2006)

Total reducing sugar estimation was done by dinitro-salycyclic acid method (Miller, G.L. 1972). For estimation of Total soluble sugars 5ml of sample was hydrolyzed by adding equal volume of 2.5N hydrochloric acid (2.5N HCl), later sample was neutralized with pellet of sodium carbonate till the effervescence ceased, volume was made up to 50ml and centrifuged at 6000RPM for 10min.from the supernatant 0.5 and 1.0ml aliquot in duplicate was collected and 4ml of freshly prepared anthrone reagent was added and color was developed by keeping it on water bath, cooled absorbence was read at 630nm (Hedge, J.E. and Hofriter, B.T., (1962). Ethyl alcohol estimation was done using potassium dichromate, where 1ml of sample was taken in to round bottom distillation flask connected with condenser and diluted with 30ml of distilled water. The sample was collected at 74-75 $^{\circ}$C. The distillate was collected in other end of the flask containing 0.23N potassium dichromate alcohol was collected till the total volume obtained was 45ml, similarly standard (20-100mg ethanol) were mixed with 2ml of 0.23ml potassium dichromate separately. The distillate of the sample and standard were heated under water bath at 60°C for 20min and cooled. The volume was made upto 50ml with distilled water and absorbance was read at 600nm (Caputi *et al.,* 1968) Total microbial load was measured by using turbidity method using spectrophotometer.

Polyphenolics and antioxidant activity

Poly phenols were estimated as described by Singleton *et al*. (1999). DPPH (2, 2-diphenylhydrazyl) radical Scavenging effects of the wine and the fruit were determined (Yen & Chen, 1995). Breifly 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The Scavenging effect (%) was calculated by using the formulae (Duan *et al.,* 2006). Sample blank and control samples were performed according to the method.

RESULTS &DISCUSSION

Table 1. Shows the changes in biochemical and physical characteristics of Cashew apple wine during fermentation. Total soluble solids (^{0}B) of the juice was initially adjusted to 20 ^{0}B after three days of fermentation the ^{o}B value was showed in decreased trend to 17.45 and it started decreasing as the fermentation proceeded at the end of the fermentation i.e., 15 days after inoculation it was 15.55. The decrease in brix value indicates the conversion of polysaccharides to soluble sugar form and their utilization by the microbes (Yang and Wiegand, 1949).

TABLE1: Physico – Chemical analysis of Cashew wine

	Mean								
Treatment	Alcohol (%)	TSS (mg/ml)	TRS (mg/ml	TSP (µg/ml)	Brix (^{O}B)	pH	%TA	Polyphenol (mg/ml)	DPPH
Day 0	0.195	9.1125	1.2675	0.53	20.225	3.76	0.5	0.1265	14.8525
Day 3	2.289	7.5675	3.3525	0.59	17.45	3.3775	1.075	0.16475	21.4125
Day 6	4.245	6.165	3.975	0.7075	16.275	2.8525	2.5	0.2295	26.4325
Day 9	6.2775	5.92	3.3575	0.8575	16.075	2.7	2.925	0.4115	30.4125
Day 12	6.4275	4.0675	2.9525	0.86	16	2.51	2.7	0.48425	31.66
Day 15	6.615	2.335	1.645	1.2	15.55	2.645	2.45	0.38125	33.495
F- value	*	*	*	*	*	*	*	*	*
SEM	0.2076971	0.5484	0.159343	0.01949	0.110711	0.034126	0.273988	0.00470704	0.16802
CD	0.61528395	1.624584	0.47204	0.057737	0.327972	0.101095	0.811665	0.01394419	0.497743
CV	3.97448654	20.52409	3.681974	0.192132	0.289605	0.156627	14.82853	0.02957864	0.428101

Total reducing sugars (TRS) was estimated (Miller, 1972) initially it was 1.27 mg/ml of the samples there was a gradual increase in the 3rd day of the fermentation (3.35 mg/ml of the sample) indicating conversion of complex sugar to simple sugars (monosaccharides) form and then it started further increase in the TRS the maximum was observed at 6th day of the fermentation (3.36 mg/ml of the sample) which again indicates that in the medium when there is short of sugars, microbes started breaking the non reducing sugar to reducing and later on after 6th day TRS was decreased indicating utilization of sugar by the microbes, and at 15th day it was observed 1.64 mg/ ml of sample. This results show that the yeast is utilizing the sugars present in the juice for its growth. Total soluble sugars (TSS) was high initially i,e., 9.11 mg/ml and it gradually decreased as the fermentation proceeded the lowest TSS was observed at the end of the fermentation was 2.40 mg/ml of the sample, the rapid reduction of TSS indicated that growth rate of yeast was high during these stage. Initially the alcohol percent was estimated and it was very negligible (0.19%) after three days it was increased to 2.29%, then as the days increased the alcohol percent was also increased and at the end of the experiment, at 15 days after inoculation it was 6.62%. The wine less than 7% alcohol is considered to be low alcoholic wine (Pickering, 2000), Hence the wine produced by Ullal varieties of Cashew can be considered as Low alcoholic wine, similar kind of results was also by Mohanty *et.al*, 2006. pH of the juice initially was 3.76 and it further reduced to 3.37 at 3 days after inoculation, then as the days increased the pH decreased and at 15 days after inoculation it was 2.64. The titrable acidity was 0.5 initially and it was increased as the fermentation period increased, at 9 days after inoculation the titrable acidity was maximum and it was 2.92%. After 9 days there was a slight decrease in the titrable acidity and it was not significant. This result tells us the yeast is carrying out the fermentation process and in turn alcohol production is increased and the pH and titrable acidity is increased. Total protein content was increased as the days increased initially it was 0.53 mg/ml and the maximum was observed at 15 days after fermentation (1.20 mg/ml). During the fermentation the total phenol was increased till 12th day from 0.1265 mg/ml to 0.48425 mg/ml and on 15th day slight decrease in polyphenol was observed. The significant increase of polyphenol also contributed to the antioxidants activity estimated by DPPH radical scavenging activities of compound (Duan *et al.* 2006). Even though there was decrease in total polyphenol after 12th day of fermentation occurred but there was increase in DPPH radical scavenging activity this reveals that the concentration of antioxidants was increased even though there was decrease in polyphenol.

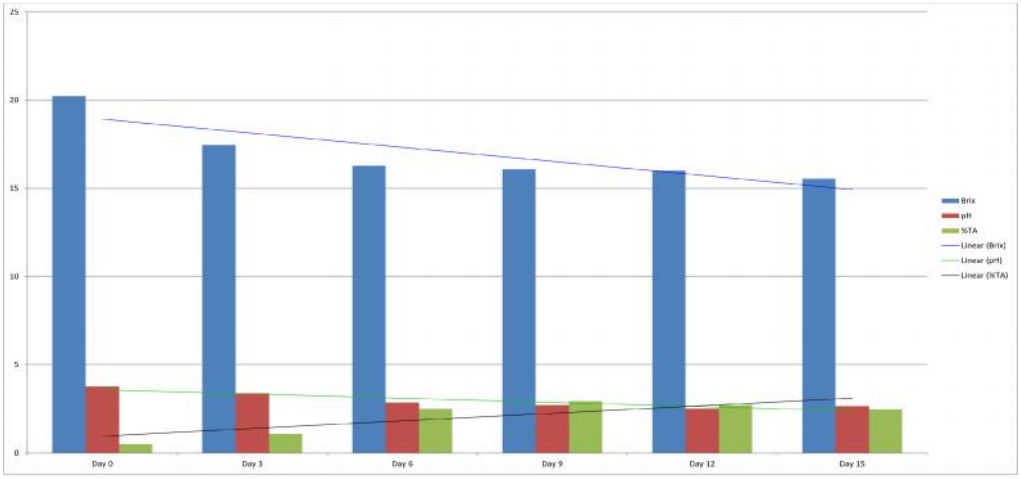

Fig 1: Correlation of Soluble sugars, reducing sugars and alcohol percent with respect to time

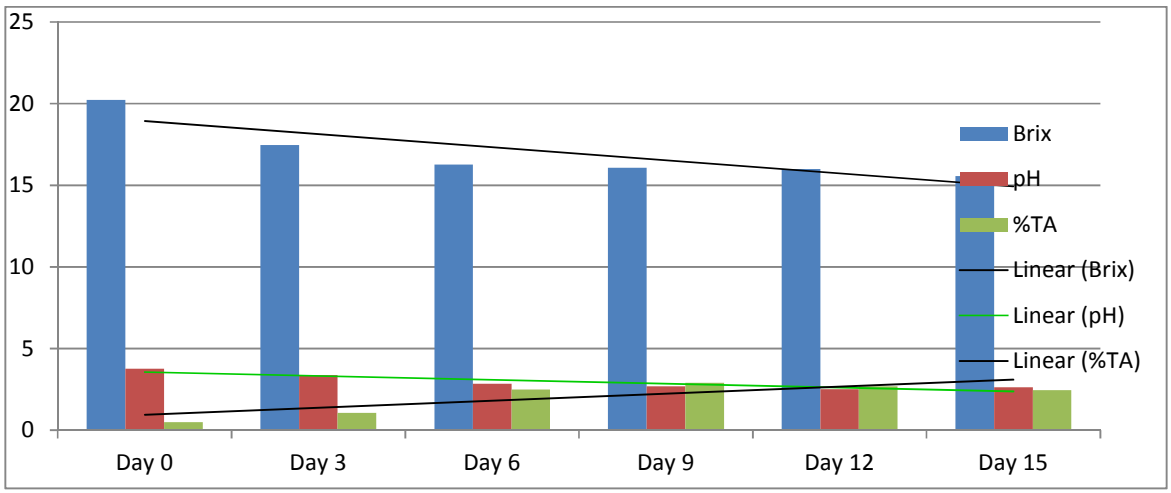

FIGURE 2: Correlation of Brix. pH and percent titrable acidity with respect to time

ACKNOWLEDGEMENT

The authors acknowledge the Abhilash, E.D., Arpitha, H.N. and Guruprasad, M.N. the B. Tech Food Science and Technology students for their help to conduct this experiment.

REFERENCES

Assuncao,R.B. and Mercandante, A.Z. (2003) Carotenoids and ascorbic acid composition from commercial products of cashew apple (*Annacardium occidentale* L.). *Journal of Food Composition and Analysis.*, 16: 647–657.

Attri, B.L., Lal, B. B. and Joshi, V. K. (1994) Technology for the preparation of san pear vermouth, *Indian Food Packer*, 48(1), 39-45.

Attri, B.L. (2009) Effect of initial sugar concentration on the physic – chemical characteristics and sensory qualities of cashew apple wine. *Natural Product Radiance*, **8**(4), 374-379.

Capauti, A., Ucda, Jr M. and Brown, T. (1968) Spectrometric determination of ethanol in wine, *Am J Enol Vitic*, 19: 160-165.

Carvalho, J.M. (2005) *Bebidas a base de agua de cocoe suco de caju: processamentoe estabilidade*. Master Dissertation, Universidade Federal do Ceara, Departamento de Tecnologia de Alimentos, Fortaleza, Brazil.

Carvalho, J.M., Maia, G.A., Figueiredo, R.W., Brito, E.S. and Rodrigues, S. (2007) Development of a blended nonalcoholic beverage composed of coconut water and cashew apple juice containing caffeine. *J. Food Quality*, 30: 664–681.

Carvalho, J.M., Maia, G.A., Figueiredo, R.W., Brito, E.S. and Rordrigues, S. (2006) Development of a blended beverage consisting of coconut water and cashew apple juice containing caffeine. *Int. J. Food Sci. Technol*., 42: 1195–1200.

Carvalho, J.M., Maia, G.A., Figueiredo, R.W., Brito, E.S. and Rordrigues, S. (2006) Development of a blended beverage consisting of coconut water and cashew apple juice containing caffeine. *Int. J. Food Sci. Technol*., 42: 1195–1200.

Cavalcante, A.A.M., Rubensam, G., Picada, J.N., Silva, E.G., Moreira, J.C.F. and Henriques, J.A.P. (2003) Mutagenicity, antioxidant potential, and antimutagenic activity against hydrogen peroxide of cashew (*Anacardium occidentale*) apple juice and cajuina. *Environ. Mol. Mutagenesis.*, 41:360–369.

Costa, J.M.C., Felipe, F.M.F., Maia, G.J., Hernandez, F.F.F. and Brasil, I.M. (2009) Production and characterization of the cashew apple (*Anacardium occidentale* L.) and guava (*Psidium guajava* l.) fruit powders. Journal of Food Processing and Preservation., 33: 299–312.

Costa, J.M.C., Felipe, F.M.F., Maia, G.J., Hernandez, F.F.F. and Brasil, I.M. (2009) Production and characterization of the cashew apple (*Anacardium occidentale* L.) and guava (*Psidium guajava* l.) fruit powders. *Journal of Food Processing and Preservation*., 33: 299–312.

Duan, X.J., Zhang, W. W., Li, X. M. and Wang, B.G. (2006) Evaluvation of antioxidants property of extract and fractions obtained from red algae, *Polysiphonia urceolata. Food chemistry*, **95,** 37-43.

Franco, M. R. B. and Janzantti, N. S. (2005) Aroma of minor tropical fruits. *Flavour Fragr. J*., 20: 358–371.

Giro, M.E.A., Martins, J.J.L., Rocha, M.V.P., Melo, V.M.M and Goncalves, L.R.B. (2009) Clarified cashew apple juice as alternative raw material for biosurfactant production by *Bacillus subtilis* in a batch bioreactor. *Biotechnol. J*., 4: 738–747.

Hedge, J.E. and Hofriter, B.T. (1962) Carbohydrate chemistry, 17, (Eds. Wistlers R.L and Be Miller, J.N.), Academic press, New York.).

Honorato, T.L., Rabelo, M.C., Goncalves, L.R.B., Pinto, G.A.S. and Rodrigues, S. (2007) Fermentation of cashew apple juice to produce high added value. *World J. Microbiol. Biotechnol.*, 23: 1409–1415.

Kubo, I., Masuoka, N., Ha, T.J. and Tsujimoto, K. (2006) Antioxidant activity of anacardic acids. *Food Chem.*, 99: 555– 562.

Kubo, I., Ochi, M., Vieria, P.C. and Komatsu, S. (1993) Antitumor agents from the cashew (*Anacardium occidentale*) apple juice. *J. Agri. Food Chem.*, 41:1012–1015.

Kubo, J., Lee, J.R. and Kubo, I. (1999) Anti-*Helicobacter pylori* agents from the cashew apple. *J. Agri. Food Chem.*, 47: 533–537.

Lavinas, F.C., Miguel, M.A.L., Lopes, M.L.M. and Valentemesquita, V.L. (2008) Effect of high hydrostatic pressure on cashew apple (*Anacardium occidentale* L.) juice preservation. *J. Food. Sci.*, 73: 273- 277.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Miller, G. L. (1972) Use of DNS reagent for the determination of glucose. *Anal. Chem.* 31**:** 426-42.

Miller, G. L. (1959) Use of dinitrosalicilic acid reagents for determination of reducing sugars. *Analytical Chemistry* 31., 426-428.

Mohanty, S., Ray, P., Swain, M.R. and Ray, R.C. (2006) Fermentation of Cashew (*Anacardium occidentale*. L.). "Apple" into wine. *Journal of Food Processing and Preservation*, 30 (3). 314-322.

Normas Analíticas Do Instituto Adolfo Lutz. (1985) *Métodos químicos e físicos para análises de alimentos*. 3rd ed. Editoração Débora D. Estrella Rebocho, São Paulo, Brazil.

Pickering, G.H. (2000) Low and reduced alcohol wine, a review. *Journal of Wine Research*, 11: 129-144.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 299, 152-178.

Soares, F.D., Pereira, T., Marques, M.O.M. and Monteiro, A. R. (2007) Volatile and non-volatile chemical composition of the white guava fruit (*Psidium guajava* L.) at different stages of maturity. *Food Chem.*,100: 15–21.

Torres Neto, A.B., Silva, M.E., Silva, W.B. Swarnakar, R. and Silva, F.L.H., (2006) Cinética e caracterização físico-química do fermentado do pseudofruto do caju (*Anacardium occidentale* L.). Quimica Nova, 29, 3, 489-492 .

Wharta, E.S.A., Moreira, A.V.B. and Mancini, J. (2004) Identificacaoe atividade antioxidante dos principais compostos fenolicos presents no sucoe na castanha do caju. In: *Anais do XIX CongressoBrasileiro de Cieˆncia e Tecnologia de Alimentos – Estrate´gia para o Desenvolvimento*., PE: SBCTA (CDROM).

Yang, H.Y. and Wiegand, E.H. (1949) Production of Fruit wines in the Pacific North West. *Fruit Products Journal*, 29, 8-12,27,29.

Yen, G.C. and Chen, H.Y. (1995) Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem.*, 43(1): 27-32.