World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:3, No:6, 2009

Comparative Analysis of Total Phenolic Content in Sea Buckthorn Wine and Other Selected Fruit Wines

Bharti Negi and Gargi Dey

***Abstract*—**This is the first report from India on a beverage resulting from alcoholic fermentation of the juice of sea buckthorn (*Hippophae rhamnoides L*) using lab isolated yeast strain. The health promoting potential of the product was evaluated based on its total phenolic content. The most important finding was that under the present fermentation condition, the total phenolic content of the wine product was 689 mg GAE/L. Investigation of influence of bottle ageing on the sea buckthorn wine showed a slight decrease in the phenolic content (534 m mg GAE/L). This study also includes the comparative analysis of the phenolic content of wines from other selected fruit juices like grape, apple and black currant.

***Keywords*—**Alcoholic fermentation, *Hippophae*, Total phenolic content, Wine

I. Introduction

THE rich nutritional and bioactive substances in *Hippophae rhamnoides L.* commonly known as sea buckthorn have attracted many people who are interested in the production or utilization of nutritional and medicinal sea buckthorn products for preventive or curative purposes. There is strong recent or re-emerging interest in such products in Europe, North America and Asia [1]. The berries of sea buckthorn are among the most nutritious and vitamin-rich fruit known. They contain large amounts of essential oils and vitamins C. Sea buckthorn is also rich in protein, especially globulins and albumins, carotene, fatty acids (saturated and unsaturated), free amino acids, flavonoids and vitamins E [2], [3], [4],[5].

Beneficial effects of the sea buckthorn berries and berries fractions on human health have been extensively investigated and substantiated by studies, suggesting a great potential of the berries for maintaining and promoting human health [4], [6], [7].

A. Bharti Negi is with the Dept of Biotechnology and Bioinformatics,Jaypee University of Information Technology, Waknaghat, HP,INDIA. (e-mail: negibharti@gmail.com).

B. Gargi Dey is with the Dept of Biotechnology and Bioinformatics,Jaypee University of Information Technology, Waknaghat, HP,INDIA. (Corresponding Author, Phone; (91)1792-239351; Fax: (91)1792-245362 (e-mail: gargi.dey@juit.ac.in, drgargi.dey@gmail.com).

Oral administration of sea buckthorn berry juice increased the plasma HDL-cholesterol concentration and decreased the susceptibility of LDL to oxidation in human. Flavonoid extract from sea buckthorn berries showed in vivo antithrombotic properties in an animal model, probably due to inhibition of platelet aggregation. Moreover, in vitro assays evidenced distinct cytoprotective properties of sea buckthorn berry extracts, which could be attributed to their anti-oxidant activity [8].

Based on established reports of nutritional and medicinal properties of the berries, we thought it is worthwhile to develop a product which can serve as "functional foods" which are specialized foods capable of providing additional physiological benefits such as preventing or delaying onset of chronic diseases, as well as meeting basic nutritional requirement.

As the fermentation process has many advantages like increasing the shelf life of product, improving the aroma and texture of the product and in some cases enhancing the nutritional value of the final product, an attempt was made to produce sea buckthorn wine using alcoholic fermentation. To the best of our knowledge, this is the first report on production of sea buckthorn wine, using lab isolated yeast strain. So far only malolactic fermentation of sea buckthorn juice has been reported by [9].

In the present study we report a comparative analysis of total phenolic content of the sea buckthorn wine and other selected fruit wines from grape, apple and blackcurrant produced with same strain under similar conditions. As this is the first report on development of alcoholic fermented product from sea buckthorn juice, the influence of bottle aging was also investigated on the phenolic content of the sea buckthorn wine in order to investigate the effect of storage condition on the total phenolic content.

II. Materials and Methods

A. Strains

The strains of *S.cerevisiae*, used in this study were of two type i.e. lab isolated yeast strain (S_4) and two industrial strains from two breweries, viz, Vintage and Minchy's Pvt Ltd.

B. Sources of juice samples

Commercially available fruit juice of apple, grape, black currant and sea buckthorn were purchased from *Real* and *Leh Berry*, respectively. To avoid variation between batches, we obtained a large consignment of the same batch from a single vendor.

C. Chemicals

Chemicals used in this study were Yeast Extract, Malt Extract, Peptone, Glucose (Dextrose), Diammonium ortho phosphate (DAP), Gallic acid, Folin & Ciocalteu's Phenol Reagent (F-C), Sodium Carbonate. All reagents used for the study were of analytical grade. Gallic acid and F-C reagent were purchased from Sigma–Aldrich.

D. Wine production

Different fermentation was set up using different strains. The pasteurized juices (100ml) were inoculated with 0.2g (wet weight) of the yeast strains. To the juice 0.2g of DAP and 9g of dextrose was also added prior to fermentation. The wine fermentation was set at 30^{o}C at stationary phase for 7days.

E. Total phenolic content

TPC was determined using the Folin-Ciocalteu reaction [10], with gallic acid as the standard.

III. RESULTS AND DISCUSSION

As sea buckthorn juice has many therapeutically useful compounds, an attempt was made for the first time to develop a fermented product using a lab isolated strain. For the fermentation, 14 strains of yeast were collected from different locations of state of Himachal Pradesh (India). Of them, one strain S_4, was found to have highest ethanol tolerance (13% v/v) and hence was used for the development of the final product. The wine was made using the juice of the berry and the S_4 strain. Earlier studies performed in our lab showed that the final wine product was of semi-dry style with a pH of 3.02 [11].

A. Total Phenolic Content

Phenolic acids have been associated with color, sensory qualities, and nutritional and antioxidant properties of foods [12]. Phenolics behave as antioxidants, due to the reactivity of the phenol moiety [13]. Current thought links the high antioxidant content of fruits and foods with the inhibition of oxidative damage diseases such as coronary heart disease, stroke and cancer [14]. With respect to wines, it is a well established fact that red wines gets its antioxidant and anti-inflammatory properties due to the phenolic constituents [15], [16], [17]. Wines are unique because they contain both antioxidant and alcohol. Its antioxidants are protected once they go through the body by the same process that our body uses to detoxify ingested ethanol. In the liver there are two NAD-dependent enzymes, alcohol dehydrogenase converts ethanol to acetaldehyde and aldehyde dehydrogenase converts acetaldehyde to acetate, these enzymes produce NADH in each step. This NADH is then capable of recycling used up antioxidants by reducing them and in the process of regenerates NAD^{+}, which in turn is required to detoxify more ethanol [18]. In this regard, studies based on phenolic content of berry wines have shown that they are more effective than red and white wines. By the study of [19], on the basis of specific phenolic content, summer cherry, blackberry and blue berry wines were 30-40% more efficient in superoxide radical scavenging than red grape wines. More recently [20] evaluated the antioxidant activities of fruit wines and arranged them in the following order bilberry > blackberry > black mulberry > sour cherry > strawberry > raspberry > apple > quince > apricot > melon. So far the most detailed study reported on fruit wine phenolics and antioxidant activity is by [21] the amount of total phenolics in the berry and fruit wines and liquors ranged from 91 to 1820 mg GAE/L. Therefore certain fruit wines can be classified as functional food, owing to their established healthful protective effects, as functional foods are dietary components that may provide a health benefit beyond basic nutrition. In this backdrop we found that sea buckthorn which is also another berry has not been explode for oenological studies. The result in our lab showed that total phenolic content of sea buckthorn juice was 933 mg GAE/L.

Based on these result, we used sea buckthorn juice to develop a wine product and investigate whether it could be consider as a functional beverage on the basis of phenolic content.

For the development of wine, the more essential factor was yeast strain and the fermentation time. Recently there has been report of reduction of catechin, rutin and quercetin levels by interaction with wine-related micro-organisms like *Pedicoccus pentosaceus* ATCC 25745, *Saccharomyces cerevisiae* ATCC 36026, and a probiotic strain, *Bifidobacterium longum* DJO10A [22]. This would have important implications for the overall bioavailability of phenolics in the sea buckthorn functional beverage. Therefore, in this study, three strains of *S cerevisiae* were examined for their ability to absorb and/or transform the phenolics.

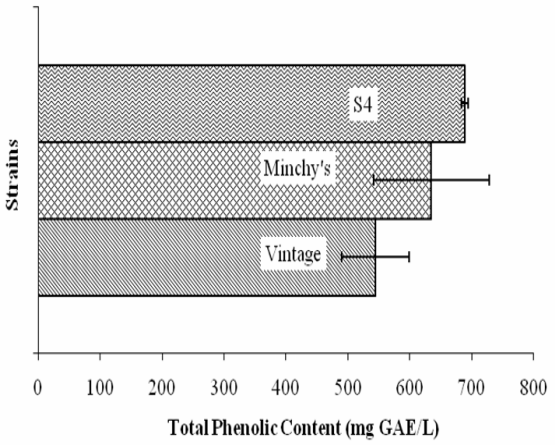

Fig. 1 Study of effect of different strains in total phenolic content of sea buckthorn wine

According to Fig. 1, the highest amount of total phenolic content which was retained in the wine was obtained by fermentation with lab strain S_4 (689 mg GAE/L) in comparison to strains from Vintage and Minchy's Pvt. Ltd. (634 and 544mg GAE/L), respectively. These observations point to the fact that the strain (S_4) has lesser absorption potential for the phenolic as compared to the other strains and hence was used for the future experiments.

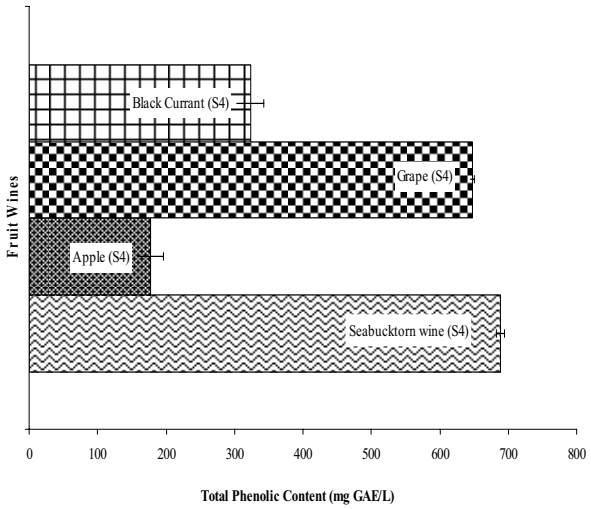

Fig. 2 Comparative study of total phenolic content of sea buckthorn wine with other selected fruit wines

We also thought it would be interesting to perform comparative analysis of the total phenolic content of sea buckthorn wine and other selected fruit wines. For this individual fruit juices were used to set up wine fermentation using the same lab isolated strain. The most remarkable finding of present study was that total phenolic content of sea buckthorn wine (689 mg GAE/L) was comparable to grape wine (647 mg GAE/L), Fig. 2. Based on the total phenolic of fruit wine, it can be arranged in the following order sea buckthorn > grape > black currant > apple. Our present result corroborates earlier reports. Rupasinghe & Clegg [23] reported that total phenolic content was highest in red (cabernet) wine followed by black currant wine and lowest in apple wine. In another study, [21] reported highest amount of total phenolic content in black currant and grape as compared to other fruit wines like apple. From the present data, it can be concluded that under the given fermentation condition and with the present lab strain (S_4), the sea buckthorn wine retains a significantly high phenolic content.

At present, there is no recommended daily intake for phenolic compounds, but it is obvious that the intake amount depends on the serving size of the food. As FDA defines the term serving or serving size as an amount of food customarily consumed per eating occasion by younger or older expressed in a common household measure that is appropriate to other food. Based on this, the serving size of red and white wine can be given as 100ml [23]. We calculated the total phenols of some of the selected fruit wines on the basis of their serving size and compared with the sea buckthorn wine. According to Fig 3, total phenols (mg/serving) of fruit wines were found to be in the following order: young sea buckthorn wine > grape wine > bottle aged sea buckthorn wine > black currant wine > apple wine.

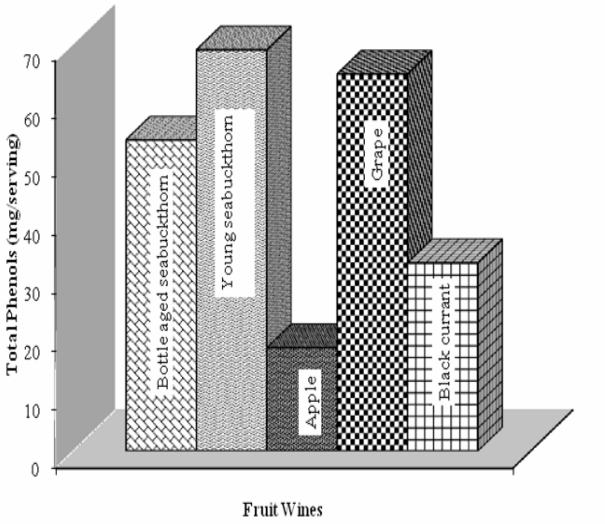

Fig. 3 Total phenols per serving for selected fruit wines.

From the present work it can be inferred that the intake of phenolic with 100 ml of sea buckthorn wine is at least 68.6 mg GAE which falls in the range of intakes of phenolic compounds from some other well known beverages like black tea 57-357 mg, linden flower 0.27-24mg, sage, 1-105 mg, grape molasses (1-42 mg), violet carrot juice (6-110 mg) and tarhana (5-149mg) per day, respectively [24]. Hence, sea buckthorn wine can also serve as a good supply of phenolic compounds.

Apart from the source of the fruit and the fermentation conditions another important factor which affects the phenolic content of fruit wine is the reactions that take place during the process of aging. A comparison of phenolic content in young and bottle aged wine (4 months) showed a slight decrease in total phenolic from 68.9 to 53.4 mg GAE/serving (Fig. 3). Similar results showing a decrease in phenolics, have been also reported by [25] in red wines. Another study on the changes of phenolic content in blended and aged wine by [26], registered an increase in total phenolic concentration after 9 months of aging followed by a decrease at 23 months of aging in the bottle. These changes are possibly due to the transformation of phenolic compounds into condensed forms that possess slightly different chemical properties and reactivities towards the Folin-Ciocalteau reagent [27].

Based on the results indicated in the present study, the fact emerges that sea buckthorn wine can be a potential health promoting product. However the antioxidant activity of wine has to be evaluated before drawing any further conclusion. The phenolic content of wine further can be improved by

blending. Application of technology for improvement of sensory qualities of wine need to be evaluated for acceptance in the wider section of the population.

IV. Conclusion

Considering the phenolic potential of this sea buckthorn wine, more effort should be devoted to increase its production and to optimize its phenolic availability by alternative processes like blending.

V. Acknowledgement

We would like to thank Prof. S. S. Kanwar of Department of Microbiology, CSK HPKV, Palmpur, H.P, INDIA for giving us the yeast strains. Dr. G. Dey would also like to acknowledge DST, Minisry of Science & Technology, Govt of India awarding the BOYSCAST fellowship for acquiring training in Industrial Microbiology.

References

[1] D. Rosch, M. Bergmann, D. Knorr and L.W. Kroh, "Structure-antioxidant efficiency relationship of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice," *J. Agric. Food Chem.,* vol. 51, pp. 4233-4239, 2003.

[2] T. Beveridge, T. Li, B. Oamah and A. Smith, "Sea buckthorn products: Manufacture and composition," *J. Agric. Food Chem.,* vol. 47, pp. 3480-3488, 1999.

[3] V. B. Guliyer, M. Gul and A. Yildirim, "*Hippophae rhamnoides* L: Chromatographic methods to determine composition and use in traditional medicine and pharmacological effects," *Journal of Chromatography B,* vol. 812, pp. 291-307, 2004.

[4] B. Yang and K. Kallio, "Lipophilic components in seeds and berries of sea buckthorn and physiological effects of sea buckthorn oils," *Trends in food science and technology,* vol. 13, pp. 160-167, 2002.

[5] A. Zeb, "Important therapeutic uses of sea buckthorn *(Hippopae):*A review,"*Journal of Biological Science*, vol. 4 (5), pp. 687-693, 2004.

[6] J. Y. Cheng, K. Konodo, Y. Suzuki, Y. Ikeda, X. Meng and K. Umemura, " Inhibitory effects of total flavones of *Hippophae rhamnoides* L on thrombosis in mouse femoral artery and in vitro platelet aggregation," *Life science,* vol. 72, pp. 2263-2271, 2007.

[7] X. Gao, M. Ohlander, N. Jeppsson, L. Bjork and V. Trajkovski, "Cjanges in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L) during maturation," *J. Agric. Food Chem.,* vol. 48, 1485-1490, 2000.

[8] A. Raffo, F. Paoletti and M. Antonelli, "Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three sea buckthorn *(Hippophae rhamnoides* L.) cultivars," *Eur Food Res Technol,* vol. 219, pp. 360-368, 2004.

[9] K. Tiitinen, M. Vahavaselka, M. Hakala, S. Laakso and H. Kallio, "Malolactic fermentation in sea buckthorn *(Hippophae rhamnoides* L*.)* juice processing," vol. 222, pp. 686-691, 2006.

[10] L.V. Singleton, R. Orthofer and R. M. L. Lamuela-Raventos*, Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent*. Method in Enzymology, Vol. 299, 152-178, 1999.

[11] B. Negi, P. Sharma, S. K. Sharma, G. Dey and S. S. Kanwar, "Production of sea buckthorn wine: Effect of fermentation conditions on flavonoid content of the wine," presented at the 2009 National conference on Future of Food Biotechnology, NIT, Durgapur, India.

[12] J. A. Mega, "simple phenol and phenolic compounds in food flavor," *Crit. Rev. Food Agric.,* vol. 10, pp. 323-372, 1978.

[13] F. Shahidi, P. K. Wanasundara, " Phenolic Antioxidants," *Crit. Rev. Food sci. Nutr.,* vol. 32, pp. 67, 1992.

[14] R. A. Jacob and B. J. Burri, "Oxidative damage and defense," *Am. J. Clin. Nutr.,* vol. 63, 1996.

[15] E. N. Frankel, J. Kanner, J. B. German, E. Perks and J. E. Kinsella, "Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine," *Lancet,* vol. 341, pp. 454-457, 1993.

[16] P. L. Teissedre, E.N. Frankel, A.L. Waterhouse, H. Peleg and J. B. German, " Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines," *J. Sci. Food Agric., vol.* 70, pp. 55-61, 1996.

[17] J. C. Stoclet, A. Kleschyov, E. Andriambeloson, M. Dielbolt and R. Andriantsitohaina, "Endothelial NO_3 release caused by red wine polyphenols. *J. Phhysiol. Pharmacol.,* vol. 50, pp. 535-540, 1999.

[18] C.J. Muller, *"Wine analysis and production,"* Springer Science & Business, B.W. Zoecklein, K.C. Fugelsang, B.H. Gump and F.S. Nury, Eds, 1994, pp. 14-28.

[19] R. G. Pinhero and G. Paliyath, "Antioxidant and calmodulin inhibitory activities of phenolic components in fruit wines and its biotechnological implications," *Food Biotech,* vol. 15, 179-192, 2001.

[20] H. K., Yildirim. "Evaluation of colour parameters and antioxidant activities of fruit wines", *Int. Journal of Food Sciences & Nut.,* vol. 57, pp. 47-63, 2006.

[21] M. Heinonen, P. J. Lehtonen and A. I. Hopia, "Antioxidant activity of berry and fruit wines and liquors," *J Agric Food Chem,* vol. 46, pp. 25-31, 1998.

[22] R. G. LoCasio, D. A. mills and A. l. Waterhouse, "Reduction of catechin, rutin and quercetin levels by interaction with food-related microorganisms in a resting state," *Journal of Science of Food & Agriculture,* vol. 86, pp. 2105-2112, 2006.

[23] H.P.K. Rupasinghe and S. Clegg, 'Total antioxidant capacity, total phenolic content, mineral elements and histamine concentrations in wines of different fruit sources,'*Journal of Food Composition and analysis,* vol. 20, pp. 133-137, 2007.

[24] S. Karakaya, S. N. EI and A. A. Tas, "Antioxidant activity of some foods containing phenolic compounds," *Int. Journal of Food Sci. and Nut.,* vol. 52, pp. 501-508, 2001.

[25] V. K. Joshi, "*Fruit Wine,"* Nauni, Solan (HP), Directorate of Extension Education, Dr. Y S Parmar University of Horticulture and Forestry, 1997, ch. 2.

[26] M. Monagas, V. Nunez, B. Bartolome and C. Gomez-cordoves, "Phenolic content of blends of tempranillo with graciano or cabernet sauvignon wines produced in spain," *Food Technol. Biotechnol.,* vol. 44, pp. 507-513, 2006.

[27] V.L. Singleton, J.A. Rossi, "Colourimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent," *Am. J. Enol. Vitic.,* vol. 16, pp. 144 –158, 1965.