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Studies on Value-added Fermentation of *Madhucalatifolia* Flower and Itspotential as a Nutrabeverage

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Abstract

For the past centuries, mahua flowers have been known for the production of distilled liquor by the tribal and rural people of India, which is hazardous to health. Production of wine from mahua flowers is uncommon. The main aim was to improve the traditional brewing process to produce a fermented product with low alcohol content enriched with nutraceuticals. The mahua must was blended with guava and fermentation was set at 18ºC, 25ºC and 30ºC. The most favourable ratio of ^{o}Brix :titratable acidity (^{o}6:0.56 %) was found in mahua-guava product fermented at 25^{o}C. The alcohol content of the product was 8.0 - 9.0 % with higher alcohol content of 0.03, <0.01 and <0.01 % w/w for C3, C4 and C5 respectively. The potential of mahua as a nutrabeverage was evaluated for the first time on the basis of total phenolic content (TPC) and antioxidant value. The TPC of the blended product was 171.83 ± 5.21 mg GAE/L and showed the highest antioxidant activity of 96.5 % and 89.6 % with ABTS and DPPH assays respectively. In our study, the mahua-guava product showed higher degree of protection against lipid peroxidation. Thus, these blending approaches could be adopted for the improvement of the antioxidant potential of mahua-based fortified products which could solve the problem of low quality traditional beverages by enhancing the nutritional value of the final product, mask the unpleasant flavour of mahua product and improve the texture of the product. Currently, several beverages and food products are marketed based on their antioxidant capacity; mahua guava blended product has the potential to be presented in the lucrative market for their high antioxidant activity. These studies would facilitate the development of a value added

product which could serve as a new brand of potential cardio-protective mahua based products.

Keywords: Madhucaindica, Psidiumguajava, fermented product, ABTS, DPPH, Lipid peroxidation.

1. Introduction

The new dietary habits and rising trend in production and consumption of designer foods have an environmental, health and social impact. At present, life style diseases such as obesity, cancer, diabetes, aging problems and degenerative diseases have been on the rise. Antioxidants play a significant role in control of these diseases as they prevent or delay the oxidation of easily oxidizable substrates. This has led to an interest in antioxidant rich natural beverages like tea, coffee and wine. Studies have reported that certain types of wines reduce the incidence of heart attacks and high cholesterol level (Katalinic et al, 2004). Also, fruits wines like cranberry, elderberry and blueberry are also good source of flavonoids (Dey et al, 2009; Negi and Dey, 2009). Blackberry wine phenolics have also been reported to stimulate endothelial nitric oxide dependent vasorelaxation (Mudnic et al, 2012).These fact findings have given new dimension and orientation towards non grape wines or fruit wines.

Non-Timber Forest Products (NTFPs) are by far, one of the biggest resources which can be utilized for development of such nutraceutically enhanced products (Arnold and Ruiz Perez, 1998; Wills and Lipsey, 1999). Among the NTFPs, mahua (*Madhucaindica*) tree contributes significantly to the socio-economic status of the ethnic tribes of India.Currently, the only practised post-harvest processing of mahua involves the production of illicit distilled liquor. The process of alcoholic brewing is a house-based economic industry using indigenous knowledge (Tamang, 2010). The present paper describes the standardisation of a value-added alcoholic fermentation of mahua flower must. Additionally, it also evaluates the effects of fermentation on physico-chemical parameters and the antioxidant activities.

2. Material and Methods

2.1 Collection of mahua flower & guava fruits

The mahua flower buds were collected from Rewa, Madhya Pradesh, India in the month of April-May and were air-dried. The samples were further sorted, cleaned and stored. The ripened guava fruits were procured from the local market of Solan, Himachal Pradesh, India and were also sorted, cleaned and stored for further use.

2.2 Extraction & preparation of mahua flower must

For the extraction of must, 500g of mahua flowers were thoroughly washed, dried, grounded and then diluted with water in 1:2 (% w/v) ratio. The extract was left for sedimentation, filtered and then centrifuged to get the juice. Finally it was thermally

treated at 90^{o}C for 10 minutes and stored at 4^{o}C for further analysis.500g of fresh guavas fruits were mashed manually. The fruit pulp was diluted with water in 1:2 (% v/v) ratio and treated thermally at 90^{o}C for 10 min.

2.3 Pectinase treatment

Mahua and mahua-guava must were both treated separately with pectinase at the rate of 1g / 100ml for 24 hours.

2.4 Strain

Saccharomyces cerevisae(MTCC 10420) was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India and maintained as glycerol stocks.

2.5 Fermentation process

The alcoholic fermentation was setup by using mahua flower must and mahua-guava must 1:1 ratio (% v/v) separately. For the fermentation, 1 liter of mahua and mahua-guava must were used. Preliminary studies were performed to standardise the inoculum size and ratio of the starter culture. 0.2 %, w/v yeast was used to inoculate the must for each. Ammonium dihydrogen orthophosphate (0.2 %, w/v) was added as the nitrogen source. The fermentation was set up at different temperatures (18^{o}C, 25^{o}C and 30^{o}C). The process was monitored by the daily measurement of ^{o}Brix level of the product and it was terminated when the product reached a constant ^{o}Brix. For each sample and each temperature, fermentation was setup in triplicates.

2.6 Physico-chemical analysis

The residual sugar content was estimated by the reported method of Miller (1959). Total soluble sugar (TSS) was measured as ^{o}Brix by Hand-held refractometer (ERMA, Japan). The organic acid (acetic acid (%), lactic acid (%), malic acid (%) and citric acid (%)) estimation was done as per the method of Joshi (2011). The ascorbic acid content in the product was estimated using the method of Sawhney and Singh (2001). The alcohol content of the product was measured by the method of Beutler (1988).Determination of higher alcohols was done by GC-MS.

2.7 Determination of Total Phenolic Content

The Folin-Ciocalteu method was used for the determination of total phenolic content against a gallic acid standard (Singleton et al, 1999).

2.8 Evaluation of Anti-oxidant Activity

The scavenging activity was estimated according to the modified procedure of Pellergini et al, (1999) known as the ABTS method and was also done according to the procedure described by Shimada et al, (1992) known as the DPPH method.

2.9 Lipid peroxidation assay

Lipid peroxidation was measured by determining the malondialdehyde (MDA) content in the samples according to the method of Wills (1966).

3. Results and Discussion

The distilled mahua liquor has negative impact on health due to lack of standardized methodology. Another disadvantage of the product is that the non-volatile components of the fermenting substrate are destroyed during the process (Yadav et al, 2009). Additionally, development of nutrabeverages from mahua flowers could form a good matrix for the therapeutic and nutritionally active constituents. Thus, this study was undertaken up to standardize a method for fermented product development as it would be a measure of sustainable NTFP management for tribal development.

3.1 Standardisation of fermentation

The main aim was to improve the traditional brewing process to produce a fermented product with low alcohol content, enriched with nutraceutical components and with a potential in the market. The fermentation process was set up at three different temperatures (18, 25 and 30ºC, respectively) as shown in figure 1.

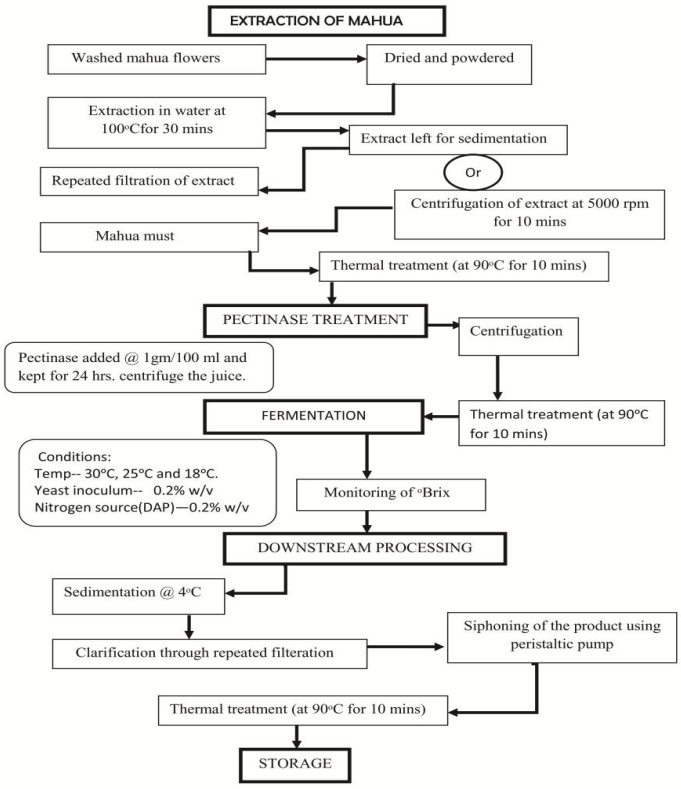

Figure 1: Standardized fermentation process.

3.2 Physico-chemical characteristics

We observed a variation in the physio-chemical characteristics of the mahua product fermented at different temperatures as shown in Table 1. The alcohol content of the product was relatively low as was the aim of the present study. The major problem of the present mahua product was the bitter taste and unpleasant aroma, necessitating that appropriate improvisation was required for its removaland to increase the nutraceutical properties of the product. Thus, we decided to incorporate an additional step before fermentation for adequate value-addition of the mahua must with a suitable fruit juice which would lead to improved aroma, taste, nutritive value and palatability.

Table 1: The physico-chemical charcteristics of mahua product.

Characteristics	18°C	25°C	30°C
TSS (ºBrix)	20 ± 0.4	11 ± 0.2	17 ± 0.1
Titratable acidity (%)	0.21 ± 0.004	0.43 ± 0.008	0.35 ± 0.007
pH	5	5.1	4.1
% Alcohol	7.5 ± 0.15	9.10 ± 0.18	8.0 ± 0.16

Values are means ± standard deviation (n=3), TSS= Total soluble sugars

3.3 Value addition of Mahua must

The guava (*Psidiumguajava*) was chosen for the value addition as it exhibits antioxidant and free radical scavenging capacity (Hui-Yin Chen et al, 2007). Also, its therapeutic effects against prostate cancer and in reducing the cholesterol level due to presence of lycopene (potent antioxidant) and pectins (Mittal et al, 2010) have been reported. Moreover, the guava fruit has a major aromatic compound; Quercetin-3-O-alpha-1-arabinopyranoside (guaijaverin) which would mask the unpleasant flavour of mahua and improve the sensory qualities of the fermented product (Joseph et al, 2011).

We observed a variation in the physio-chemical characteristics of the mahua-guava must fermented at different temperatures as shown in Table 2.

Table 2: The physico-chemical characteristics of mahua - guava product.

Characteristics	18°C	25°C	30°C
TSS (ºBrix)	19 ±0.2	6 ± 0.1	17 ±0.1
Titratable acidity (%)	0.50 ± 0.005	0.56 ± 0.005	0.50 ± 0.007
pH	4.45	4.5	5
% Alcohol	8.6 ± 0.17	9.00 ± 0.19	8.9 ± 0.18

Values are means ± standard deviation (n=3), TSS= Total soluble sugars

The ethyl alcohol content varied from 8.60 % up to 9.00 % as compared to mahua product (7.5 to 9.10 %). The other higher alcohol content in the product (25 ºC) was 0.03, <0.01 and <0.01 % w/w for C3, C4 and C5 respectively as measured by GC.

According to Wills et al, (1986), the ºBrix: Titratable acid ratio is more closely related to the palatability of fruit products rather than either sugar or titratable acids alone. In our case, the most favourable ratio of ºBrix :titratable acidity was found in mahua-guava product at 25ºC (º6 : 0.56) which reflects a better quality of product. Yadav et al, (2009) reported a ratio of º11 : 0.5 for a mahua based product.

3.4 Total phenolic content and Ascorbic acid content

Phenolic components greatly contribute to the organoleptic characteristics of fermented products like wine such as color, astringency and aroma (Lopez-Velez et al, 2003). And have also been associated with beneficial physiological effects like protective effects against oxidative stress and hypercholesterolaemia. (Negi et al, 2013). In the present study, TPC of the mahua must was 431 mgGAE/L and of mahua-guava must was 250 mg GAE/L. After fermentation, there was depletion in total phenolic content in all the fermented products as shown in Table 3.

Table 3: Total phenolic content of mahuwa and mahuwa-guava products.

Parameters	Mahua must	Mahuwa product			Mahua-guava must	Mahuwa-Guava product		
		18°C	25°C	30°C		18°C	25°C	30°C
TPC (mg GAE/L)	431± 3.2	366.8± 9.5	265.4±8.4	366.6 ±7.9	250.32± 1.2	241.50± 6.7	171.8± 2.7	202.5± 3.5
Post-fermentation Decrease (%)	--	14.89	38.2	14.94	--	3.52	31.36	19.10

Values are means ± standard deviation (n=3), TSS= Total soluble sugars

The observed variation in phenolic content after the fermentation may be correlated with several mechanisms- including adsorption of polyphenols onto yeast cell walls, condensation and polymerisation reactions and enzymatic activity (Czyzowska and Pogorzelski, 2004; Ginjom et al, 2011; Perez et al, 2011). In spite of the reduction in TPC during fermentation, the phenolic content of mahua-guava products was higher as compared to other fruit wines like apple (160 mg GAE/L), green and white currant (265 mg GAE/L) (Heinonen et al, 1998).

Another efficient antioxidant compound is ascorbic acid with reported health benefits like lowering of arterial blood pressure and improvement in arterial stiffness in patients with type 2 diabetes (Mullan et al, 2002) and it also prevents oxidative damage (Padayatty et al, 2003). By blending, we achieved a marginal increase in ascorbic acid by 28% in mahua- guava product as compared to the mahua product fermented at 25ºC.

3.5 Antioxidant Activity

Antioxidants show a protective effect against diseases associated with reactive free radicals such as coronary heart disease and cancer (Thompson, 1994; Jiang et al, 2004). In ABTS assay, the mahua product fermented at 25ºC showed the highest antioxidant potential followed by the products at 18ºC and30ºC. In the case of mahua-guava products, the values varied with no significant differences between them when fermented at different temperatures (Fig.2). Butylated hydroxyl toluene, BHT a commercial antioxidant was used as a positive control.

In DPPH assay, the mahua product fermented at 25ºC showed the highest radical scavenging activity followed by the products at 18ºC and 30ºC. The mahua-guava product fermented at 25ºC showed highest radical scavenging activity followed by the products at 18ºC and30ºC (Fig.3) indicating less significant variations.

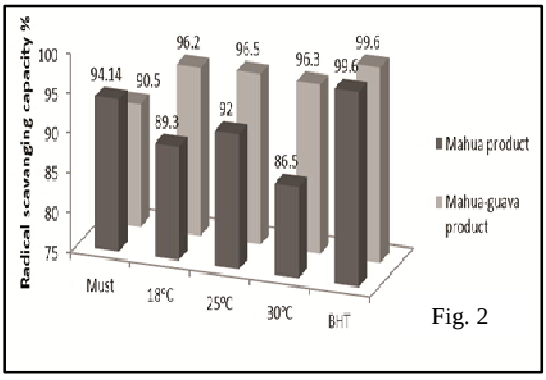

Fig. 2

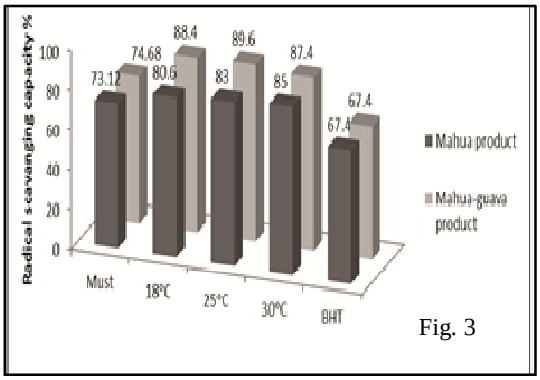

Fig. 3

Figure 2 and 3: ABTS and DPPH radical scavenging activities, where value are means ± standard deviations (n=3).

Thus, due to blending, there was an increase in the antioxidant potential of the fortified products. The free radical scavenging activities of the products were much higher as compared to BHT.

3.6 Lipid peroxidation (LPO)

It refers to the oxidative degradation of lipids. It causes loss of structural integrity and biochemical function, leading to its irreparable damage or cell death (Bhatia and Jain, 2004) and aging (Middleton et al, 2000)and is also responsible for several degenerative

diseases like diabetes, cardiovascular diseases and cancer (Radmark and Samuelsson, 2007). The mahua and mahua-guava products were evaluated for their potential for protection against lipid peroxidation (Fig. 4).

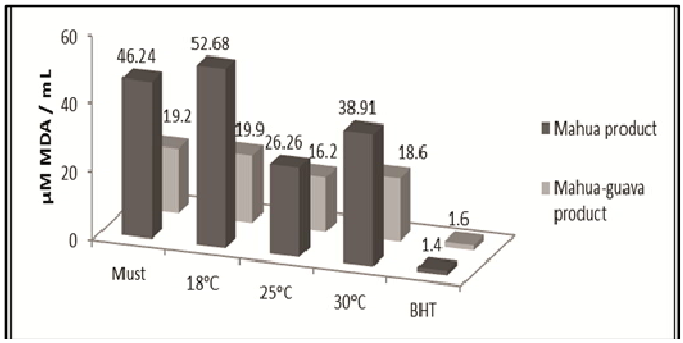

Figure 4: Inhibition of lipid peroxidation,where value are means ± standard deviations (n=3).

In our study, the mahua-guava product (25ºC) showed higher degree of protection against lipid peroxidation as compared to mahua product (25ºC). This further confirmed that the blending approaches in the present study led to an enhancement in nutraceutical value of the product.

The correlation between total phenolic content and antioxidant activity is as yet contradictory. While some studies have reported a positive correlation (Amin et al, 2006; Li et al, 2009), there are several studies which have reported that there is no strict correlation (Bajpai et al, 2005; Zhuang et al, 2011). In the present study, both the mahua and mahua-guava fermented products at 25ºC contained lower total phenolic content and higher antioxidant capacity as compared to those fermented at 18ºC and 30ºC. This may be correlated with several mechanisms like adsorption of polyphenols onto yeast cell walls, condensation and polymerisation reactions and enzymatic activity (Perez et al, 2011) such that the antioxidant capacity measured was not solely related to the phenolic constituents and could be due to the presence of other phytochemicals, pigments and tocopherol as well as the synergistic effects between them (Majo et al, 2008).

We are currently investigating the phenolic profile of pre and post-fermented extracts of mahua and mahua-guava for the purpose of correlating the antioxidant activity and inhibition in lipid peroxidation that was observed.

In today's world, externally synthetic fortified beverages are a common feature but the high content of secondary metabolites present in fruits including phenolics, minerals and vitamins which give them both therapeutic and nutritional qualities have brought the focus back on natural beverages. This has spurred the researchers to explore the potential use of other fruits with higher phenolic content for the production of nutraceuticalbeverages. The health promoting capacity of these fruit wines depends

on various factors like the environment in which fruits are grown, time of maceration and fermentation, maturation, bottling and ageing. Thus, standardised technology is required to explore the untouched potential of other raw materials like mahua and guava for the production of a nutraceutical beverage with various health benefits.

The category of low alcoholic wines is still small, but showing rapid percentage growth. The market appearance of specially formulated products like McGuigan's "9.5 Chardonnay" and "Finest Denman Vineyard Semillon" at 10.5% alcohol, "Sutter Home Fre Merlot", 1% alcohol, are indications of the rising interest in low-alcohol wine products. The production and scale of alcohol-reduced wines and the lowering of ethanol concentration in wines is an emerging trend and pose a number of technical and marketing challenges. Several strategies focus upon pre or post fermentation technologies of such low-alcohol wines like non-thermal production processes and development or selection of specific yeast strains for lower alcohol content. In the present study, the standardised methodology has relative technical merits for reducing the ethanol concentration in wine.

4. Conclusion

From the study it can be concluded that mahua flower as a substrate has potential for making good quality fermented product. A fermentation temperature of 25°C was optimum for better quality product. Addition of guava must improved the flavour of mahua product. Currently, several beverages and food products are marketed based on their antioxidant capacity; mahua guava blended product has the potential to be presented in the lucrative market for their high antioxidant activity. These studies would facilitate the development of a value added product which could serve as a new brand of potential cardio-protective mahua based products.

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