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Optimization of mixed culture fermentation conditions for the development Orange (*Citrus reticulate* Blanco) wine

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Abstract
The present investigation was carried out for optimization of mixed culture fermentation conditions for the development of Orange Wine from orange juice. Optimization conditions for fermentation of orange wine were at the optimal fermentation of orange Juice was recorded at 27ºC temperature, pH 4.5 and total soluble solids of 24°Brix with an inoculums level of 10% (v/v). The fermentation of the fruit juice was completed within 7 days. A maximum ethanol content of 8.1% (v/v) was detected in the orange wine under optimized conditions. In present investigation attempts were made to optimize the fermentation conditions of mixed culture wine from orange juice and with the following aims and objectives, To prepare orange wine from mixed culture fermentation of orange juice, To optimize the conditions of mixed culture fermentation and To develop an unstructured mode for orange wine from mono and mixed culture.

Keywords: *Citrus reticulate Blanco*, mixed culture fermentation, orange juice, alcoholic fermentation

Introduction
Orange (*Citrus reticulate* Blanco) is also known under several other names of which is "mandarin" denoting its origin in the Far East. Some have attempted to discriminate between tangerine and mandarins have different varities, the former having deeper colored skins than the latter, but the nomenclature has become so confused that they are practically used as synonyms. There are a very large number of varities of tangerines in India, known by the names "santra", "kamala", "kinnow" and others. Tangirines are the most valued commercial oranges in India. The fruit has the the shape of a slightly flattened sweet orange. It has a thin, lose rind which can be easily detached from the fruit. The pulp of fruit is sweet, juicy and has a characteristic and attractive flavor. The fruits are the mostly eaten as fresh dessert fruit and are used in the production of orange juice. The fruit is cultivated in the North-Eastern parts of India, Sikkim, Punjab, in Nagpur area of Maharashtra and the Coorg (kodagu) district of Karnataka. (Shakuntala Mane; 2008) [32].

Tropical citrus fruits have been used as substrates for the production of wines and the alcohol profiles, types and quantities were not stated in the majority of these reports. Various factors influence the fermentation process and determine the end products obtained. These include substrate related factors such as cultivar types, cultivation conditions, conditions at harvest and post harvest handling. Yeast species are used in many industrial fermentation processes including alcoholic beverages production. The quality of wine produced greatly depends on the yeast strain. Development of improved starter organisms for fermentation of citrus juice may offer a relative simple avenue for reducing post harvest wastage of citrus fruits in low utilization environment **(**Nikhil Gupta; 2009) [20]

In some cases, wine produced with pure yeast monocultures lacks the complexity of taste and other desirable characters that originate from the indigenous yeasts (Naoufel Cheraiti, 2005) [1]. The incorporation of several wine yeast strains with different technological capabilities into the same active dry yeast ADY starter may help overcome these shortcomings. In one study of mixed strain cultures for enological purposes, an exchange of metabolites between strains was observed. The impact of fermentation of *S. cerevisiae* and *S. cerevisiae var. ellipsoideus* on the final organoleptic balance of the wine also has been studied. In all of these studies, the analysis focused on the fermentation products and not the fermentation kinetics.

Interactions between *Saccharomyces* strains may occur in mixed cultures (Bisson 1991 and Grossmann 1996) [4, 9], but the underlying mechanisms have not been investigated in detail. The objective of the present study is to optimize the mixed culture fermentation conditions and to understand the effect of mixed culture fermentation.

This work describes the first trial of a research project aimed at studying the multistarter inoculation in making fruit wines. The results enhance our understanding of the behaviour mixed culture fermentation. The use of multi-starter inoculation should be an alternative to control the extent of the fermentation with the goal of enhancing the flavor complexity of the wine. (Ciani *et al*. 1998) [5] have recently confirmed the unacceptable increase in ethyl acetate content in a mixed culture of *H. uvarum*/*S. cerevisiae*. *H. uvarum* strains also possess enzymatic characteristics of interest to winemaking because of their technological effects and their contribution to aroma formation.

Materials and Methods

Raw materials

Oranges

Fresh oranges were obtained from the local market. 'Nagpur Santra' (*Citrus Reticulata, Blanco*) orange variety used in the study was purchased from the local market in Nagpur. Sound ripened oranges were washed with water, peeled and juice was extracted using the screw press juice extractor.

Pretreatment of orange juice

The degree brix of the orange juice was raised as required by adding sugar. PH was adjusted by adding a saturated solution of Sodium bicarbonate. Then it was pasteurized and allowed to cool to room temperature

Cultures

For the fermentation process, yeast strain *Sacchromyces cerevisiae CFTRI* (102) *and (Saccharomyces cerevisiaevar. ellipsoideus) ATCC* (4921) were obtained from National Chemical laboratory, Pune.

Preparation of broth

The broth media was prepared in 250 distilled water consisting of 0.75 g malt extract, 2.5 g glucose, 0.75 g yeast extract and 1.25 g of peptone. This media was sterilized and divided into different flasks to maintain a volume of 30ml then and inoculated with the yeast strain and kept for incubation at 27 ^{0}C for a day and then stored at low temperature. (Garcia *et al.*, 2006) [7].

Experimental setup and procedure

Batch fermentation set up: 500ml of pasteurized orange juice of 24^0 Brix and all nutrients addition was taken in 1000ml conical flask and broth media is added under sterile conditions. Fermentation is carried out at 27 ^{0}C under anaerobic condition with arrangement for CO_2 to escape. After completion of fermentation the wine was filtered, centrifuged and filled in glass bottles. (Robert, 2006) [28]

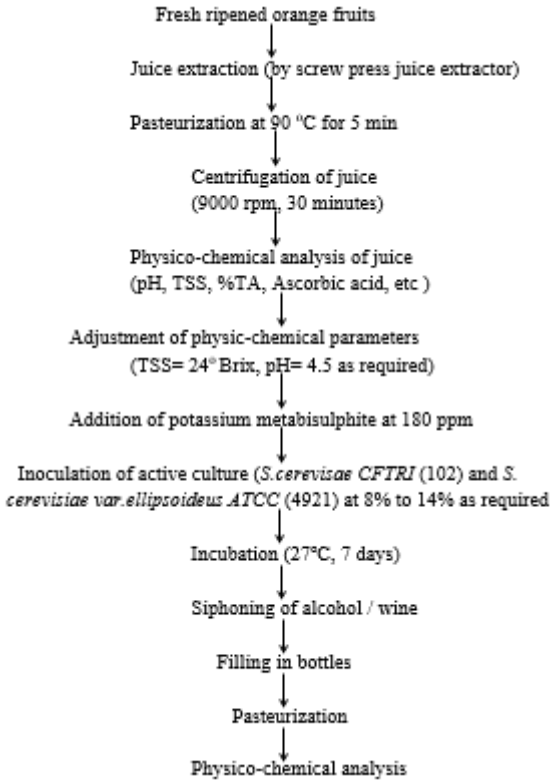

Fig 1: Flow sheet: flow diagram for the preparation orange wine

Results and Discussion

Physico-chemical characterization of orange juice

Physico-chemical characteristics of the juice are as follows: total solids, *p*H, acidity, Ascorbic acid, juice yield, clarity and specific gravity were examined as 11.4, 3.4, 0.63, 35 mg, 45 percent, 0.278 percent and 1.014 respectively in orange juice (Ranganna; 1986) [27] Total acid content, titratable acidity (% citric acid, v/v) Total sugars and total acids are the two main factors which are considered for the production of wine from any fruit juice. The amount of fermentable sugars and acid content adjusted in the orange juice made suitable for its use in making wine. It has been reported that orange juice contains very high amount of vitamin C as compared to other fruit juices. Oranges are less expensive as compared to other fruits. Moreover, Ascorbic acid present in juice as natural antioxidant aid to increase shelf-life of juice.

Screening of yeast strains for fermentation orange juice for wine

The amount of ethanol produced from orange juice by different strains of *S. cerevisiae* was quite variable. *S. cerevisiae CFTRI* (102) screened *S. cerevisiaevar. ellipsoideus ATCC* (4921) for alcoholic fermentation of orange juice, *S. cerevisiae* showed the maximum (8.5%, v/v) determined by oxidation (Okunowo *et al.*, 2007) [23] and specific gravity method, of monoculture and *S. cerevisiae var. ellipsoideus* and *S. cerevisiae* (8.1%, v/v) of mixed culture production of ethanol for wine. These strains were, therefore, selected for further studies. The fermentation potential of each organism varies from strain to strain and also depends upon the type of the substrate used for fermentation.

Optimization of orange wine with total soluble solids as fermentation condition

The total soluble solids of the orange juice juice were varied from 20-26 (°Bx), (hand Refractometer) to study its influence on fermentation of juice to develop wine. An increase in ethanol production was recorded with the increase in total soluble solids up to 24°Bx and thereafter, no change was recorded for this parameter (Fig. 1). Therefore, 24°Bx was considered optimized condition for mixed culture fermentation. The maximum ethanol production has been reported in juice with 24°Bx after 7 days. Earlier also *p*H 4.5 has been reported optimal for alcoholic fermentation of orange juice to develop Orange wine.

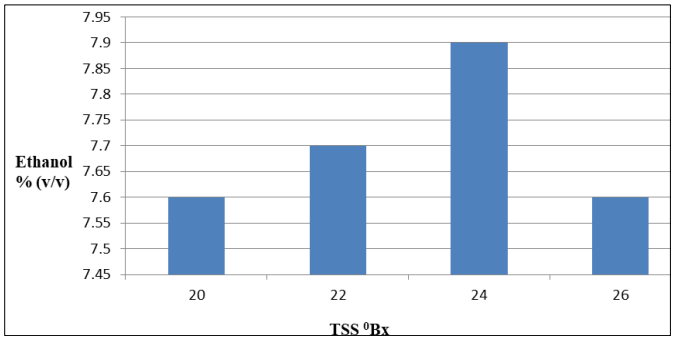

Fig 2: Optimization of orange wine with total soluble solids as fermentation condition (Fermentation conditions: Initial *p*H: 4.5; Inoculum level: 10% (v/v); Temperature: 27 °C; Fermentation time: 7 days)

Optimization of orange wine with *p*H as fermentation condition

The pH of the orange juice was varied from 4 to 5.5, to study its influence on mixed culture fermentation of juice to develop wine. An increase in ethanol production was recorded with the increase in *p*H and thereafter, no change was recorded for this parameter (Fig. 3). Therefore, 4.5 pH considered optimized conditions of mixed culture fermentation. The maximum ethanol production has been reported in juice with 4.5 pH after 7 days of fermentation

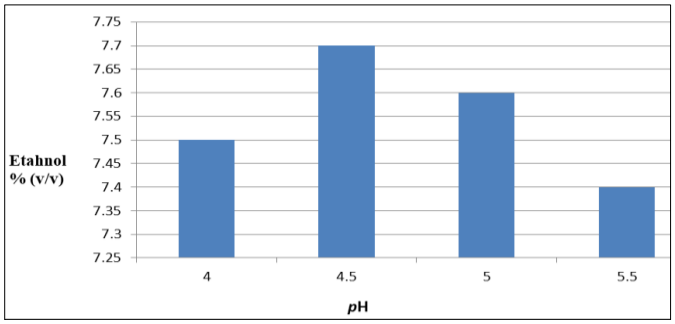

Fig 3: Optimization of orange wine with pH as fermentation condition (Fermentation conditions: Total soluble solids: 24 °B; Inoculum size: 10% (v/v); Temperature: 27 °C; Fermentation time: 7 days)

Optimization of orange wine with percent inoculum as fermentation condition

The results presented in Fig.4 depict the effect of inoculum level of *S. cerevisiae* and *S. cerevisiae var. ellipsoideus* on ethanol production in orange juice. Ethanol production was increased with the increase in inoculums concentration up to 10% for a fermentation period of 7 days. A slight decrease in ethanol production was recorded beyond inoculum level of 10%. An inoculum level of 10% has been optimized condition of fermentation.

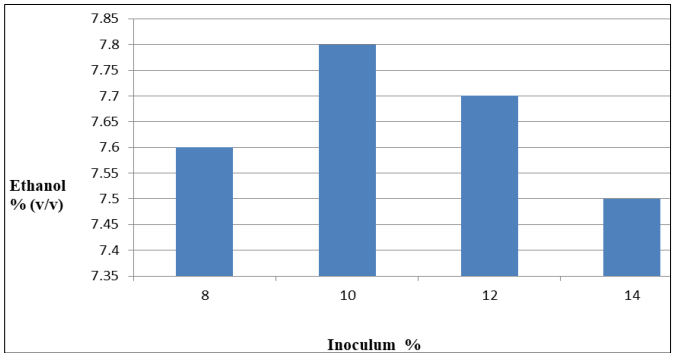

Fig 4: Optimization of orange wine with percent inoculum as fermentation condition (Fermentation conditions: Total soluble solids: 24 °Bx; *p*H 4.5; Temperature: 27 °C; Fermentation time: 7 days)

Optimization of orange wine with temperature as fermentation condition

Alcoholic fermentation of orange juice by mixed culture was carried out for 7 days at a temperature range of 24-33 °C. The maximum ethanol production was recorded at a temperature of 27 °C (Fig. 5). Comparatively, ethanol production was lesser at 33 °C than at other temperature tried. Therefore, a temperature of 27 °C has been considered optimized condition of mixed culture fermentation. Maximum ethanol production from orange juice has been reported at 27 °C.

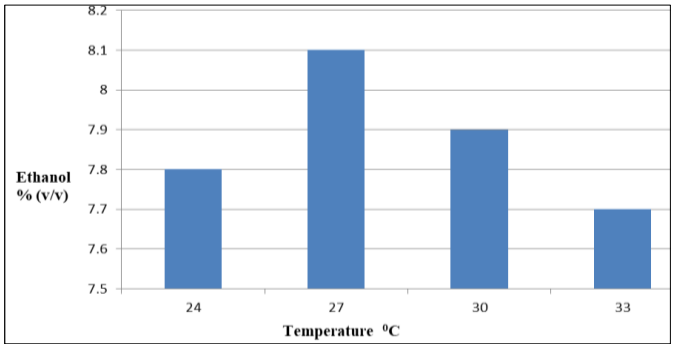

Fig 5: Optimization of orange wine with temperature as fermentation condition (Fermentation conditions: Total soluble solids: 24 °Bx; *p*H 4.5, Inoculums 10%; Fermentation time: 7 days)

Sensory evaluation

The sensory evaluation report reveals that the Orange wine was clean and of light yellow color. It was having a good aroma of natural Orange fruit. As compare to both the monoculture and mixed culture fermentation, mixed culture fermentation wine has distinct aroma with that of monoculture wines. In terms of flavors of orange wine prepared by mixed culture fermentation was more acceptable.

Conclusion

As compare to both the monoculture and mixed culture fermentation, all optimized conditions of wines have distinct aroma with that of monoculture wines. All samples of mixed cultures fermentations were superior in terms of flavor as compare to monoculture wine. The high quality wines should have a characteristic bouquet and taste which depends on the cultivar, maturity and phytosanitary conditions of the fruit, climatic conditions and most importantly, on yeast

fermentation physiology. All these factors cause the differences of aroma, fragrance, composition and quality among all kinds of fruit wines.

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