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Development of Protocol for Mead Preparation Using Different Sources of Honey

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ABSTRACT: *Honey being a good source of sugars, proteins, organic acids, minerals and flavouring compounds is more amicable for fermentation. So, an attempt was made to standardize the protocol for mead preparation using yeast culture Saccharomyces ellipsoideus No. 101, and also to develop the synbiotic mead by inoculating a probiotic yeast culture (Saccharomyces boulardii) in the Department of Agricultural Microbiology, University of Agricultural Sciences, G. K. V. K., Bengaluru during 2009-2011. The results of the biochemical analysis indicated that mead prepared from Saccharomyces ellipsoideus No. 101 was found superior to other wines with respect to alcohol content. Among the meads prepared from different honey sources, polyflower mead inoculated with Saccharomyces ellipsoideus No. 101 yielded the highest alcohol content (11.07%) followed by sunflower mead (10.97%). Mead produced from Saccharomyces ellipsoideus No. 101 recorded the highest score (17.1 out of 20) for overall acceptability during organolaptic evaluation.*

INTRODUCTION

Mead is a traditional alcoholic beverage prepared from honey, containing 8-18 per cent alcohol. Mead fermentation is a time consuming process, often taking several months and fermentation rate depends on several factors, such as honey variety, yeast strain, yeast nutrition, control of pH *etc.*, (Navratil *et al*., 2001). Mead may be flavored and display taste, aroma and characteristics of the source of honey. Mead is composed of water, alcohol, pigments, esters, vitamins, carbohydrates, minerals, tannins *etc*. with medicinal and therapeutic values. Virtues of mead were about the same as those of honey. Mead as " wine most agreeable to the stomach, as it restores appetite, softens the bellies, good for those who have coughs, quartan ague and cachexia. Mead helps to guard against diseases of the brain for which wine is pernicious. Its principal medicinal value was in kidney ailments. Mead ranked not only as a curative but also as a preventive medicine for gut rheumatism and helps in digestion.

Honey is a natural sweet substance produced by honey bees from nectar of blossom. Honey bees produce honey by regurgitation of nectar. Plant nectar contains carbohydrates and pollens contain protein. It is a super saturated sugar solution with approximately 17 per cent water, vitamins, minerals, lipids and organic acids besides carbohydrates (Monica *et al*., 2007). Many health promoting and curative properties attributed to it are the sole basis for including in some traditional folk medicine throughout the world.

Honey is a prebiotic. It contains Fructo oligosaccharides (FOS) compounds which act as prebiotics for promoting probiotics (Slavin, 1997).

Wine yeasts with high alcohol tolerance are generally recommended for fermentation. Fermentations with probiotic yeast cultures may still enhance the quality of mead.

MATERIAL AND METHODS

An investigation was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, G. K. V. K., Bengaluru. Yeast strains were screened for the production of mead from three honey samples *viz.* sunflower (*Helianthus annuus*), eucalyptus (*Eucalyptus lanceolatus*) and polyflower (multiflower nectar) along with reference yeast strain (*Saccharomyces ellipsoideus* No.101), *S. boulardii* (*S b*) and yeast isolated from toddy (*S* t).

The yeast cultures were maintained in yeast extract peptone dextrose (YEPD) broth. Starter culture of inoculum was prepared in test tubes containing 12.5 ml of diluted honey (1honey: 2.5 water). The tubes

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were incubated overnight at 25° C for growth. These yeast cultures were added to 250 ml diluted honey in 500 ml flasks.

Honey samples were diluted with water (Honey: water = 1:2.5) and blended. Honey must (300 ml) was transferred to conical flask and inoculated at the rate of 10 per cent.

Must was incubated under aerobic conditions for two days followed by anaerobic condition up to 40 days. They were sealed with rubber cork fitted with glass tube and other end of the tube was air locked in water and molten wax was poured near cork and glass junction. The flasks were incubated at room temperature (28 °C). Alcohol per cent was recorded at regular intervals. Yeast cells settled at the bottom of the flask were discarded by decanting the supernatant. The clear wine after fermentation was pasteurized and siphoned out into bottles and stored for aging. Ethanol was estimated by colorimetric method (Caputi *et al.,* 1968).

RESULTS AND DISCUSSION

Alcohol Production

The experimental results of standardization of the protocol for mead preparation from honey samples collected from different sources of nectar using reference strain *Saccharomyces ellipsoideus* No. 101 are presented in this chapter.

The results of alcohol content of mead prepared from sunflower honey showed the highest alcohol content. Honey fermented with *Saccharomyces ellipsoideus* (10.77%) and the lowest alcohol (9.87%) was recorded with toddy isolate. *Saccharomyces ellipsoideus* inoculated mead yielded the highest alcohol content (10.43%) in eucalyptus mead compared to all other treatments on 40th day. However, toddy isolate yielded only 9.33 per cent alcohol which is the lowest of all. Similarly in polyflower mead, *Saccharomyces ellipsoideus* inoculated treatment recorded the highest alcohol content (11.07%). The lowest alcohol content (10.4%) was observed with toddy isolate.

Among the different mead samples, polyflower mead showed the highest alcohol content compared to sunflower and eucalyptus mead. It might be due to the acidity and antimicrobial compounds in eucalyptus honey. Such compounds may be interfering with yeast growth. The yeast cultures were differing in their ability to produce alcohol content. Among the isolates of yeasts, the highest alcohol (11.06, 10.97 and 10.43%) was observed with reference strain *Saccharomyces ellipsoideus* 101.in poly flower, sunflower and eucalyptus honey mead respectively) Chaudhari and Chincholkar (1996) reported that among the 30 yeast strains, Y11 was able to ferment 15 per cent total sugars in molasses yield 51 g / l ethanol.

Table 1
Alcohol Content of Meads Prepared from Different Honey Sources

Yeast strains	*Treatments*			*Mean*
	Sunflower honey	*Eucalyptus honey*	*Polyflower honey*	
T_1	10.97[a]	10.43[a]	11.07[a]	10.82
T_2	10.70[a]	9.97[b]	10.70[a]	10.46
T_3	9.87[b]	9.33[c]	10.43[a]	9.88
T_4	10.63[a]	10.07[ab]	10.77[a]	10.49
T_5	10.00[b]	9.47[c]	10.53[a]	10.00

Note: T_1- honey + *Saccharomyces ellipsoidies* (*S e*), T_2- honey + *S boulardii* (*S b*), T_3- honey + toddy isolate (S t), T_4- honey + *S b*+ *S e,* T_5- honey + *S b*+ S t.

Organoleptic Evaluation

Sensory evaluation was done by selected panel of members through organoleptic procedures. The experimental results showed that the mead prepared by *Saccharomyces ellipsoideus* scored the highest marks in all the evaluated parameters such as appearance, colour, aroma, bouquet, flovour, astringency, vinegar, total acidity, sweetness, body and general quality whereas toddy isolate used mead scored the lowest marks. Score obtained by all the treatments are presented in Table 2.

The organoleptic evaluation showed that the highest score was recorded in mead produced by poly flower honey inoculated with reference yeast strain.

Treatments with same letter indicate do not differ significantly (on par with each other) at 1% level of significance.

CONCLUSION

Honey being prebiotic acts as a good substrate for the growth and activity of probiotic organisms and the product so formed is synbiotic. A protocol has been developed and standardized for preparation of synbiotic mead based on the biochemical parameters.

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Table 2
Organoleptic Evaluation of Mead Prepared from Different Floral Sources of Honey
(Mean Average of Five Judges)

Treatments	*Appearance (2)*	*Color (2)*	*Aroma (2)*	*Bouquet (2)*	*Vinegar (2)*	*Total acidity (2)*	*Sweetness (1)*	*Body (1)*	*Flavour (2)*	*Astringency (2)*	*General quality (2)*	*Overall accepta-bility (20)*
						Sunflower Mead						
T_1	1.7	1.7	1.7	1.6	1.6	1.7	0.6	0.7	1.7	1.7	1.7	16.4
T_2	1.5	1.5	1.4	1.5	1.5	1.5	0.6	0.5	1.5	1.5	1.5	14.5
T_3	1.4	1.4	1.3	1.3	1.4	1.4	0.7	0.5	1.4	1.4	1.3	13.5
T_4	1.5	1.5	1.4	1.5	1.5	1.5	0.6	0.5	1.6	1.5	1.6	14.7
T_5	1.5	1.5	1.4	1.5	1.5	1.4	0.6	0.6	1.5	1.4	1.4	14.3
						Eucalyptus mead						
T_1	1.7	1.7	1.6	1.5	1.6	1.7	0.5	0.7	1.6	1.6	1.6	15.8
T_2	1.5	1.5	1.4	1.4	1.4	1.5	0.5	0.5	1.4	1.5	1.5	14.1
T_3	1.4	1.4	1.2	1.3	1.4	1.4	0.5	0.5	1.3	1.4	1.3	13.1
T_4	1.5	1.4	1.4	1.4	1.5	1.5	0.6	0.5	1.5	1.4	1.6	14.3
T_5	1.4	1.4	1.3	1.4	1.4	1.4	0.5	0.6	1.4	1.4	1.4	13.6
						Poly flower mead						
T_1	1.8	1.7	1.8	1.7	1.6	1.7	0.7	0.7	1.8	1.7	1.8	17
T_2	1.6	1.6	1.5	1.5	1.5	1.5	0.6	0.6	1.6	1.5	1.6	15.1
T_3	1.5	1.5	1.4	1.3	1.4	1.5	0.6	0.5	1.5	1.4	1.4	14
T_4	1.6	1.5	1.5	1.5	1.5	1.5	0.6	0.5	1.6	1.5	1.6	14.9
T_5	1.5	1.5	1.4	1.5	1.5	1.4	0.6	0.6	1.6	1.5	1.4	14.5

Note: T_1- honey + *Saccharomyces ellipsoidies* (*S e*), T_2- honey + *S boulardii* (*S b*), T_3- honey + toddy isolate (S t), T_4- honey + *S b*+ *S e*, T_5- honey + *S b*+ S t.

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