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EFFECT OF DIFFERENT LEVELS OF YEAST ON THE PRODUCTION OF ORANGE WINE (*CITRUS SINENSIS*)

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Abstract– The present research work had six treatments which are T1 Orange juice (750 ml) + Sugar (50%) + Wine yeast (1g), T2 Orange juice (750ml) + Sugar (50%) + Wine yeast (2g), T3 Orange juice (750 ml) + Sugar (50%) + Wine yeast (3g), T4 Orange juice (750ml) + Sugar (50%) + Wine yeast (4g), T5 Orange juice (750 ml) + Sugar (50%) + Wine yeast (5g) and T6 Orange juice (750 ml) + Sugar (50%) + Wine yeast (6g). The experiment was laid out in CRD (Complete Randomized Design) with six treatments replicated four times each. Here attempt of preparation of wine were done and its physical and sensory quality was evaluated to determine its suitability. Analysis revealed that the sensory quality was increased when the treatment T3 Orange juice (750ml) + Sugar (50%) + Wine yeast (3g) was used for the wine preparation. The treatment T3 Orange juice (750ml) + Sugar (50%) + Wine yeast (3g) was found superior in respect of the parameters like Total Soluble Solids, Acidity, pH, Alcohol content. From economics point of view treatment T3 had maximum cost benefit ratio observed.

INTRODUCTION

Oranges are one of the most popular fruits around the world. While they are enjoyable as a snack or as a recipe ingredient, its juice is highly associated with good health which acts as an integral part of a healthy breakfast. Oranges are round citrus fruits with finely- textured skins that are orange in color just like their pulpy flesh. The size of the fruit ranges from about three inches in diameter. Oranges are classified into two general categories sweet and bitter. The word orange is derived from the Sanskrit 'naranga" which means orange tree. Usually, ripe oranges consist of 40 % - 55 % juice by weight, depending on their variety. Like other citrus fruits, its rind contains essential oils which are used in cooking and perfumery. Sweet oranges are divided into five or six main categories. Common sweet oranges, blood, navel, acidless, bitter and mandarin, are available at different times of year (lglesias *et al.* 2007). The orange types basically belong to two different species and are classified according to the acid concentration, color of pulp and presence of reproductive orange. One species, the Citrus sinesis, produces sweet oranges. The ripe fruits contain high percentage of water (85-90%) and many constituents; carbohydrates, organic acids, vitamin C, minerals and small amount of lipids, proteins, carotenoids, flavonoids and volatile compounds (Okafor, 2007). The consumption of citrus fruits like orange and lemon singly and especially when combined offer significant protection against various cancers, diabetes, Parkinsons disease and inflammatory bowel disease. The fruit of Citrus sinensis is called sweet orange to distinguish it from *Citrus urantium*, the bitter orange. Yeast species are used in many industrial fermentation processes including alcoholic beverages production (Kunkee, 1984). Yeast fermentation of orange juice shows at once, which has been no destructive effect on vitamin C result in harmony with the observations, the contrary, the activity of vitamin C persisted for a very long time-being retained for 51 days (Lepkovsky *et al.*, 1925). Wine has been enormous health benefits similar to those of fruits from which they are derived (Jacob, 2001). Wine making is an ancient practice in many countries and considerable work has been done on various aspects of wine making from different fruits. The character and quality depend mainly on the variety, composition

and yield of the fruits. To determine the effect of different levels of yeast on the physio-chemical properties, quality and acceptability of orange wine and to estimate the economics of various treatments.

MATERIALS AND METHODS

The Preparation of wine from oranges prepared with 6 treatments and four replicates were stored for 90 days under ambient room temperature. Which were T1 Orange juice (750ml) + Sugar (50%) + Wine yeast (1g), T2 Orange juice (750ml) + Sugar (50%) + Wine yeast (2g), T3 Orange juice (750ml) + Sugar (50%) + Wine yeast (3g), T4 Orange juice (750ml) + Sugar (50%) + Wine yeast (4g), T5 Orange juice (750ml) + Sugar (50%) + Wine yeast (5g) and T6 Orange juice (750ml) + Sugar (50%) + Wine yeast (6g). and four replications. After the preparation of wine that were stored for 90 days under ambient room temperature. The procedure of making orange wine is given below:

Preparation of yeast starter culture

The yeast starter culture was prepared from a known quantity of the must for fermentation, small quantity of sugar, yeast and a known volume of water. The mixture of all these were treated and allowed to stand for 24 h. Approximately 200 ml of water was boiled for fermentation. This was allowed until completion of fermentation as was evidenced by lack of appearance of bubbles in the container usually within 3 weeks. Secondary fermentation was done for 21 days. When fermentation stopped, the wine was promptly racked off ensuring minimum exposure to oxygen, the upper liquid was transfered to the other clean container in order to remove impurities. Then the mixture continued to ferment at 20 °C for more days. After that, it was allowed to attain 37 °C and 200 ml of the mixture of Orange must respectively treated with sugar. Exactly 3.7 ml representing approximately 108cfu/ml (measured using McFarland standard) of the yeast (*S. cerevisiae*) after centrifugation was added to the mixture, stirred properly and allowed to stand for 24 h before use. The following parameters were monitored before and during fermentation process are; Specific gravity, pH, Titratable acidity, Total soluble solid and Alcohol content

Fermentation of must

The primary fermentation was initiated by the addition of the starter culture. The must was stirred every 12 h with subsequent reading of the specic gravity, pH, temperature and alcohol content for 4 days. After 4 days, the wine was racked into the secondary fermenter. The secondary fermentation was done in an air tight container in which a tube was passed into a clean bottle containing clean water. The essence was to monitor the course of under the storage conditions of 20 °C aged 3 months.

Clarification of wine

After completion of fermentation, the obtained wine was siphoned off and filtered through a clean sterilized muslin cloth, Whatman No.1 filter paper, sieve and syphon tubes sterilized by 70 % alcohol and collected in sterile glass jars. The wine was racked for a period of 3 weeks to clear the wine. The residues were removed and the filtrates were allowed to mature before other chemical analysis was carried out. Clarification is an important procedure in wine production as the fermented wine contains sediments.

Maturation of wine

After clarification, the wine was kept in the refrigerator for maturation (2 weeks) and then packaged for further analysis.

Aging

Wine ageing and its ability to potentially improve wine quality for its consumption is most important step after wine production. After maturation, the supernatant was taken off and transferred into fresh sterile bottles, corked and subjected to pasteurization at 82 ^{o}C for 20 minutes. After cooling, further allowed to age in long neck 750 ml bottles for 17 days at 22-25 ^{o}C before analysis (Chowdhury and Ray, 2007). The wine was analyzed for physio-chemical properties at 30 days interval after 30 days from fermentation i.e., 30, 60 and 90 days

Packaging and preservation

The mature wine was packed in clean containers for storage and marketing.

Storage

Storage is an important consideration for wine that is being kept for long-term ageing and, fresh wine should be aged till it is drinkable and marketable, thus the evolution of the product in the bottle before its consumption is very important. It is usually aged for an extensive period for the maturation of

flavours, and wine is one of the few commodities that can improve with age but it can also rapidly deteriorate if kept in unfavourable conditions. The composition of wine is subjected to continuous changes during storage and these changes are a result of a function of parameters such as temperature, illumination, position of bottles, oxygen content and storage time.

RESULTS AND DISCUSSION

The present investigation entitled "Effect of different levels of yeast on the production from orange wine (*Citrus sinensis*)" was carried out under the horticulture post-harvest laboratory in the department of Horticulture, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during 2021 – 2022. The main objectives of the present investigation were to use different levels of sugar concentration on the production of best quality wine from orange and to find out its acceptability during storage.

Total Soluble Solids (°Brix)

In terms of Total Soluble Solids, the lowest score of TSS (12.66, 12.13, 10.69 and 10.05 °Brix) at Initial, 30, 60 and 90 days after storage was observed in treatment T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g), followed by treatment T4 (Orange juice (750ml) + Sugar (50%) + Wine yeast 4g), with (13.35, 12.80, 11.77and 10.45 °Brix) at Initial, 30, 60 and 90 days after storage, whereas the maximum score was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g), with (14.50, 14.17, 13.28 and 12.40 ° Brix) during 90 days storage. The decrease in TSS content of wine indicates the utilization of the sugar present in the must during fermentation. Similarity has been seen in Sonar *et al.* (2004) in jamun wine, Idise *et al.* (2010) in pomegranate wine, Isitua *et al.* (2010) in banana wine.

Alcohol content (%)

In terms of Alcohol content, the highest score of Alcohol content (10.21, 11.228 and 11.83) at 30, 60 and 90 days after storage was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g). followed by treatment T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g). with (9.26, 11.22 and 11.47) at 30, 60 and 90 days after storage, whereas the minimum score was observed in treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g). with (9.34, 10.22 and 10.34) during 90 days storage. The increase in Alcohol content of orange wine with different levels of sugar concentration during storage may possibly be due to the variation in performance of the yeast to utilize the fermentable sugars affecting the fermentability, hence the varied alcohol product. The above results are similar with the findings of Chowdhury and Ray

Table 1. Studies on different level of yeast of colour and appearance, taste, and specific gravity in orange wine during storage.

Treatment	Treatment Combination	Colour and appearance			Taste			Specific Gravity (Orange wine)		
		30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
T1	Orange juice (750ml)+ Sugar50%+ wine yeast 1g	6.24	6.53	6.95	5.74	6.19	6.53	1.46	1.30	1.13
T2	Orange juice (750ml)+ Sugar50%+ wine yeast 2g	5.89	6.24	7.15	5.47	6.47	6.88	1.43	1.24	1.17
T3	Orange juice (750ml)+ Sugar50%+ wine yeast 3g	7.37	7.52	7.99	7.48	7.85	7.94	1.21	1.16	1.03
T4	Orange juice (750ml)+ Sugar50%+ wine yeast 4g	6.31	6.44	7.39	6.19	6.36	6.79	1.50	1.22	1.11
T5	Orange juice (750ml)+ Sugar50%+ wine yeast 5g	5.74	5.95	7.25	6.12	6.56	6.89	1.54	1.34	1.07
T6	Orange juice (750ml)+ Sugar50%+ wine yeast 6g	5.71	6.08	7.12	6.21	6.61	7.04	1.75	1.48	1.24
	F test	S	S	S	S	S	S	S	S	S
	C.D. @ 0.5	0.48	0.15	0.32	0.26	0.25	0.23	0.29	0.08	0.08
	S.Ed.	0.23	0.07	0.15	0.12	0.12	0.11	0.14	0.04	0.04

Table 2. Studies on different level of yeast of pH, overall acceptability, and TSS in orange wine during storage.

Treat-ment	Treatment Combination	pH				Overall acceptability			TSS			
		Initial	30 days	60 days	90 days	30 days	60 days	90 days	Initial	30 days	60 days	90 days
T1	Orange juice (750ml) +Sugar 50%+ wine yeast 1g	4.13	4.00	3.83	3.36	6.06	6.38	6.76	13.85	13.39	12.55	11.29
T2	Orange juice (750ml) +Sugar 50%+ wine yeast 2g	4.10	3.89	3.59	3.20	6.07	6.54	7.06	13.44	13.15	12.55	11.43
T3	Orange juice (750ml)+Sugar 50%+ wine yeast 3g	3.80	3.63	3.25	3.07	7.34	7.57	7.96	14.50	14.17	13.28	12.40
T4	Orange juice (750ml)+Sugar 50%+ wine yeast 4g	4.20	4.11	3.79	3.31	6.52	6.64	7.18	13.35	12.80	11.77	10.45
T5	Orange juice (750ml)+Sugar 50%+ wine yeast 5g	4.38	4.11	3.46	3.24	6.10	6.46	7.07	13.07	12.40	11.15	10.58
T6	Orange juice (750ml)+Sugar 50%+ wine yeast 6g	4.37	4.14	3.33	3.21	6.10	6.51	7.07	12.66	12.13	10.69	10.05
	F test	S	S	S	S	S	S	S	S	S	S	S
	C.D. @ 0.5	0.19	0.10	0.23	0.17	0.15	0.13	0.16	0.32	0.21	0.42	0.52
	S.Ed.	4.13	4.00	3.83	3.36	0.07	0.06	0.07	0.15	0.10	0.20	0.25

Table 3. Studies on different level of yeast of Alcohol, Acidity and Aroma in orange wine during storage.

Treatment	Treatment Combination	Alcohol			Acidity				Aroma		
		30 days	60 days	90 days	Initial	30 days	60 days	90 days	30 days	60 days	90 days
T1	Orange juice (750ml) + Sugar 50%+ wine yeast 1g	9.34	10.22	10.34	0.83	0.85	1.02	1.05	6.20	6.43	6.80
T2	Orange juice (750ml) + Sugar 50%+ wine yeast 2g	9.56	10.09	11.08	0.86	0.91	1.07	1.09	6.84	6.92	7.15
T3	Orange juice (750ml) + Sugar 50%+ wine yeast 3g	10.21	11.28	11.83	0.79	0.84	0.95	0.98	7.17	7.33	7.94
T4	Orange juice (750ml) + Sugar 50%+ wine yeast 4g	9.22	10.11	11.33	0.82	0.94	1.05	1.11	7.06	7.12	7.38
T5	Orange juice (750ml) + Sugar 50%+ wine yeast 5g	9.65	10.87	11.19	0.76	0.90	1.09	1.13	6.45	6.87	7.07
T6	Orange juice (750ml) + Sugar 50%+ wine yeast 6g	9.26	11.22	11.47	0.81	0.85	1.03	1.08	6.36	6.84	7.06
	F test	S	S	S	S	S	S	S	S	S	S
	C.D. @ 0.5	0.25	0.33	0.16	0.08	0.07	0.04	0.05	0.18	0.14	0.36
	S.Ed.	0.12	0.15	0.08	0.04	0.03	0.02	0.02	0.09	0.07	0.17

(2007) in jamun wine and Yadav *et al.* (2009) in Mahua wine.

Titratable acidity (%)

In terms of Acidity the lowest score of Acidity (0.55, 0.57 and 0.59) at 30, 60 and 90 days after storage was observed in treatment T5 (Orange juice 500ml + Sugar 40 0brix + Wine yeast 0.133%), followed by treatment T4 (Orange juice 500ml + Sugar 35 0brix + Wine yeast 0.133%) with (0.58, 0.62 and 0.64) at 30, 60 and 90 days after storage, whereas the maximum score was observed in treatment T1 (Orange juice 500ml + Sugar 20 0brix + Wine yeast 0.133%) with (0.69, 0.76 and 0.80) during 90 days storage. The increase in acidity may be due to the increased alcohol production from the high initial sugar concentration reported that organic acids such as citric, malic, lactic, tartaric, oxalic and succinic acids were produced during fermentation in cocoa beans by *S. cerevisiae*. The increment of titratable acidity during fermentation is attributed to the production of different organic acids as observed in kiwi wine (Akubor *et al.*, 2003) in banana wine, (Pratima *et al.*, 2006), who reported that level of inoculums had no effect on the TA of fermenting juice.

pH

In terms of pH, the lowest score of pH (3.80, 3.63, 3.25 and 3.07) at initial, 30, 60 and 90 days after storage was observed in treatment T3 (Orange juice (750 ml) + Sugar (50%) + Wine yeast 3g) followed by treatment T2 (Orange juice (750ml) + Sugar (50%) + Wine yeast 2g) with (4.10, 3.89, 3.59 and 3.20) at

initial, 30, 60 and 90 days after storage, whereas the maximum score was observed in treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) with (4.13, 4.00, 3.83 and 3.36) during 90 days storage. The decrease in pH with increase in acidity of wine observed may be due to dissociation of parental acids and formation of hydrogen ions. The above results are similar with the findings of (Reddy and Reddy, 2005) in mango fruit wine, (Panda *et al.*, 2014) in sapota wine. The pH of the wine depends on composition of the must, number of organic acids and sugars present in the wine.

Specific gravity

In terms of Specific gravity The lowest score of Specific gravity (1.21, 1.16 and 1.03) at 30, 60 and 90 days after storage was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) followed by treatment T2 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) with (1.43, 1.24 and 1.17) at 30, 60 and 90 days after storage, whereas the maximum score was observed in treatment T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) with (1.75, 1.48 and 1.24) during 90 days storage. The decrease in Specific gravity of orange wine with different levels of sugar concentration during storage may possibly be due to the type of yeast used in the wine production. *Saccharomyces cerevisiae* has been reported to reduce specific quality of fruit wines during fermentation. The above results are similar with the findings of (Okafor, 2018 and Idise and Odoyo, 2011).

Sensory evaluation

In terms of colour and appearance. The maximum score of colours (7.37,7.52 and 7.99) at 30, 60 and 90 days respectively was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) followed by treatment T4 (Orange juice (750ml) + Sugar (50%) + Wine yeast 4g) with (6.31, 6.44 and 7.39) whereas the minimum score was observed in the treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) with (6.24, 6.53 and 6.95) during 90 days storage.

In terms of taste, the maximum score of Taste (7.48,7.85 and 7.94) at 30, 60 and 90 days respectively was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) followed by treatment T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g) with (6.21, 6.61 and 7.04) whereas the minimum score was observed in treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) with (5.74, 6.19 and 6.53) during 90 days storage.

In terms of Aroma, the maximum score of Aroma (7.17, 7.33, and 7.94) at 30, 60 and 90 days respectively was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) followed by treatment T4 (Orange juice (750ml) + Sugar (50%) + Wine yeast 4g) with (7.06, 7.12, and 7.38) whereas the minimum score was observed in treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) with (6.20, 6.43 and 6.80) during 90 days storage.

In terms of Overall acceptability, the maximum score of Overall acceptability (7.34, 7.57 and 7.96) at 30, 60 and 90 days respectively was observed in treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) , followed by treatment T4 (Orange juice (750ml) + Sugar (50%) + Wine yeast 4g) with (6.52, 6.64, and 7.18) whereas the minimum score was observed in treatment T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) with (6.06, 6.38 and 6.76) during 90 days storage.

Economical evaluation

Among all the treatments, the highest selling rate of Rs.2000/l and Rs.1800/l was recorded in T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) and T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) while T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g) showed minimum with Rs.1100/l, and highest net return of Rs.1145/l and Rs.995/l was recorded in T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) and T1 (Orange juice (750ml) +

Table 4. Economics of different treatments and cost benefit ratio.

Treatment No.	Treatment	Total cost (Rs)	Selling rate (Rs/liter)	Net return (Rs)	Benefit cost ratio
T1	Orange juice (750ml)+Sugar50%+ wine yeast 1g	805.00	1800	995	1.23
T2	Orange juice (750ml)+Sugar50%+ wine yeast 2g	830.00	1600	770	0.92
T3	Orange juice (750ml)+Sugar50%+ wine yeast 3g	855.00	2000	1145	1.33
T4	Orange juice (750ml)+Sugar50%+ wine yeast 4g	880.00	1400	520	0.59
T5	Orange juice (750ml)+Sugar50%+ wine yeast 5g	905.00	1200	295	0.32
T6	Orange juice (750ml)+Sugar50%+ wine yeast 6g	930.00	1100	170	0.18

Sugar (50%) + Wine yeast 1g) respectively while minimum was observed in T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g) with Rs.170/l. In terms of benefit cost ratio, maximum benefit cost ratio of 1.33 was observed in T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) followed by 1.23 in T1 (Orange juice (750ml) + Sugar (50%) + Wine yeast 1g) while T6 (Orange juice (750ml) + Sugar (50%) + Wine yeast 6g) showed minimum benefit cost ratio of 0.18.

CONCLUSION

Based on findings of the present experiment it is concluded that treatment T3 (Orange juice (750ml) + Sugar (50%) + Wine yeast 3g) was found superior in terms of Total Soluble Solids, Acidity, pH, Specific gravity, Alcohol content, Colour and Appearance, Taste, Aroma and Overall acceptability. The highest net return Rs.1145/l, and Benefit Cost Ratio 1.33 was also found in T3.

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