International Journal of Plant & Soil Science

Volume 35, Issue 14, Page 80-88, 2023; Article no.IJPSS.100648
ISSN: 2320-7035

Effect of Different Levels of Local Yeast in Pomegranate (*Punica granatum* L.) Cider

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i143023

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100648

Original Research Article

Received: 18/03/2023
Accepted: 26/05/2023
Published: 29/05/2023

ABSTRACT

The study was conducted in Completely Randomized Design (CRD) with 7 treatments replicated thrice. The treatments were T1 (pomegranate juice 1ltr + 0.5 g yeast + 500 g sugar), T2 (pomegranate juice 1ltr + 1g yeast + 500g sugar), T3 (pomegranate juice 1ltr + 1.5g yeast + 500g sugar), T4 (pomegranate juice 1ltr + 2g yeast + 500 g sugar), T5 (pomegranate juice 1ltr + 2.5g yeast + 500g sugar), T6 (pomegranate juice 1ltr + 3g yeast + 500g sugar), T7 (pomegranate juice 1ltr + 3.5g yeast + 500g sugar). The pomegranate juice was fermented using *Saccharomyces cerevisiae*. The cider was assessed for the physico-chemical changes that occurred throughout its 90 days of storage, as well as its sensory quality using a 9-point Hedonic scale that was put to the test on a panel of five experts. With longer fermentation times, the alcohol level, acidity, and sensory qualities increased while total soluble solids, pH, and specific gravity decreased. From the above treatments, it is concluded that treatment T6 was found superior in respect of the parameters

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like Total Soluble Solids, Acidity, pH, Alcohol content, Specific gravity, Color and Appearance, Taste, Aroma and Overall acceptability. In terms of cost benefit ratio, the highest net return, Cost Benefit Ratio was also found in the same treatment.

Keywords: Cider; pomegranate; Saccharomyces cerevisiae; sugar; fermentation.

1. INTRODUCTION

The pomegranates are highly perishable and susceptible to weight loss and improper handling of post-harvest and storage leads to decaying. Besides from external postharvest quality, symptoms of internal quality losses of browning appear in the peel and arils . India produces a good amount of pomegranate, so cider making could be a useful way to diversify the economy and avoid postharvest losses. Its scientific creation is less well-documented than that of other beverages of a similar nature Banjare, et al. [1].

Cider is an alcoholic beverage made from pressing and fermenting fruits like apples. Yeast, specifically the *Saccharomyces cerevisiae* strain of fungi, is used for alcoholic fermentation, which is characterized by the conversion of sugar into ethanol. *Saccharomyces cerevisiae* strain currently handles the majority of the wine-making process due to its dependable and quick fermentation. All of the sugar in the cider vat is consumed by the yeast, which converts it to alcohol and carbon dioxide. The yeast die for lack of nourishment after the full sugar inside the cider is transformed, which stops the fermentation process Ghosh et al. [2].

In general, yeast starter cultures that are precisely chosen for the winemaking process based on features that have been scientifically proven complement and optimize the quality of the wine's distinctive qualities Swiegers, et al. [3]. Many different strains of *Saccharomyces cerevisiae* are currently used in the majority of cider production because they enable quick and dependable fermentation lower the danger of slow or blocked fermentation, and guard against microbial contamination Romano, et al. [4].

To reduce post-harvest loss and strengthen ties between business and agriculture, food processing is crucial. By turning the excess food into goods with value additions like fermented and non-fermented beverages, the loss can be reduced. In order to create new fruit-based products with altered physio-chemical and sensory properties, particularly in terms of flavor and nutritional value, fermentation is a viable method Dudley, [5].

Pomegranate is one of the fruits which, not only has been used for its juice but also for its seeds for ages, and serves various purposes. Its juice is highly loaded with potassium, vitamins and antioxidants. As it contains a good proportion of sugar which is suitable for cider making. Any fruit with good proportion of sugar may be used in producing cider and the resultant cider is normally named after the fruit. Unlike apple cider, people are not much aware of pomegranate cider, but the presence of polyphenolic antioxidants have several health benefits as it helps in reducing cholesterol and blood pressure. Therefore, an experiment has been carried out with an aimed to study the effect of different levels of yeasts on pomegranate cider and to estimate the economics of various treatments.

2. MATERIALS AND METHODS

The study was conducted in Completely Randomized Design (CRD) with 7 treatments and replicated thrice. The treatments were T1 (pomegranate juice 1ltr + 0.5g yeast + 500 g sugar), T2 (pomegranate juice 1ltr + 1g yeast + 500g sugar), T3 (pomegranate juice 1ltr + 1.5g yeast + 500 g sugar), T4 (pomegranate juice 1ltr + 2g yeast + 500 g sugar), T5 (pomegranate juice 1ltr + 2.5g yeast + 500g sugar), T6 (pomegranate juice 1ltr + 3g yeast + 500 g sugar), T7 (pomegranate juice 1ltr + 3.5g yeast + 500 g sugar).

2.1 Raw Material and Extraction of Juice

Healthy and uniformed sized pomegranate (*Punica granatum* L.) variety Mridula free from diseases, pest and cracked were selected and brought from a local market. Completely rotten fruits were discarded and a rotten part of the fruits was removed. After washing, the fruits were cored and the arils was thoroughly washed again to remove the adhering dirt and the clean arils or fruits were pulverized using sterile Philip electric blender. The slurry was further diluted in a ratio of 1:1 (water and pulp) and sieved with a muslin cloth of pore size 0.8 mm to obtain the filtrate "must". The methods of Amerine and Kunkee as used by Robinson were used.

2.2 Yeast and Inoculum Preparation

Saccharomyces cerevisiae was obtained from local market. The inoculum was prepared by inoculating 0.5g, 1g, 1.5g, 2g, 2.5g, 3g and 3.5g brewer's yeast was added to 10ml of lukewarm water in separate beakers according to treatments and stirred gently. The activated Saccharomyces cerevisiae was added to the pulp according to the treatments respectively.

2.3 Preparation of Cider

Cider was prepared with different concentrations of *Saccharomyces cerevisiae*. Steps of preparation are given below:

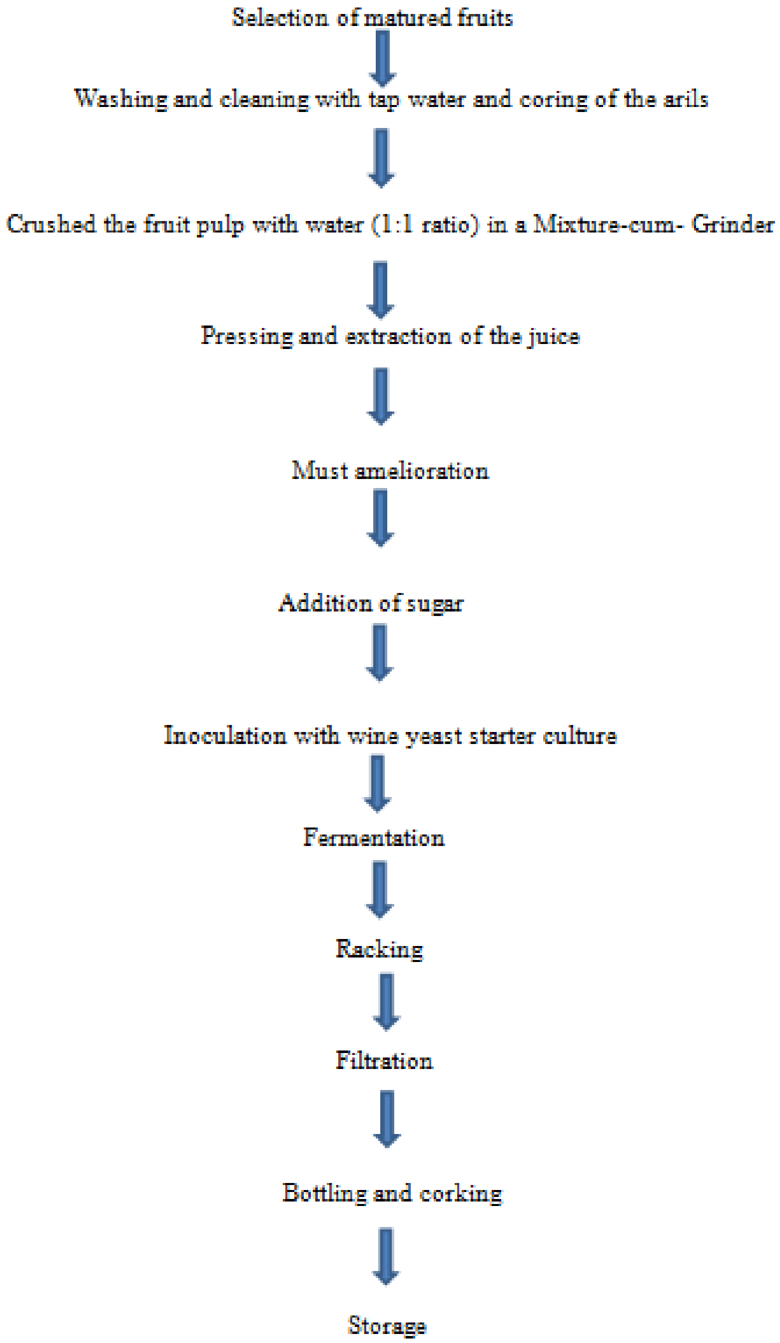

Fig. 1. Flow chart of pomegranate cider preparation

Table 1. Meteorological data during experimental period (October 2022-December 2022)

Months	Time span	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
		Max.	Min.	Max.	Min.	
October	1st week	36.2	25	97	53	11.8
	2nd week	33.2	22.2	97	56	38.6
	3rd week	32.8	20	94	55	0
	4th week	32.6	17.6	94	55	0
November	1st week	32.6	17	96	55	0
	2nd week	32.6	15	94	53	0
	3rd week	30.8	11.8	85	50	0
	4th week	28.6	11.6	95	52	0
December	1st week	27.2	10.2	95	55	0
	2nd week	29.4	9.4	97	48	0
	3rd week	28.6	8.8	97	60	0
	4th week	26.6	7	97	59	0

Source: Agro-meteorological observatory unit, College of Forestry and Environment, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj- 211007 (U.P.)

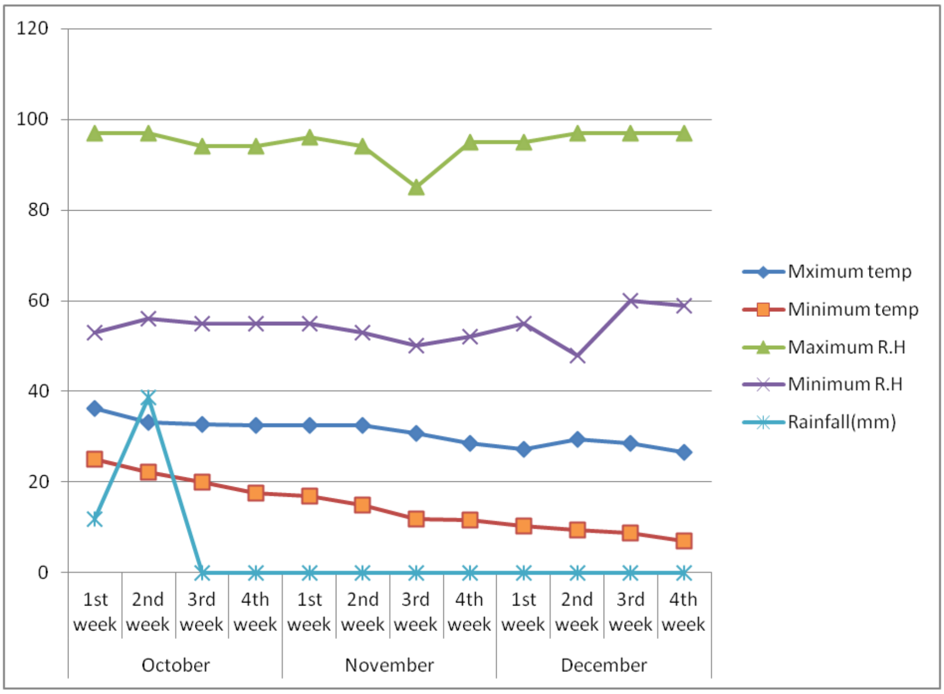

Fig. 2. Depicting Agro-meteorological data of SHUATS, Prayagraj (October 2022 - December 2022)

Source: - Agro-meteorological observatory unit, College of Forestry and Environment, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj- 211007 (U.P.)

2.4 Determination of Physio–chemical Parameters

The physio-chemical changes that the cider underwent during production and storage were examined. The must pH was determined using AOAC, (2004) procedure where a digital pH meter was used to determine the product's pH, while a hand-held refractometer was used to measure TSS and the results were expressed as degree brix (°B) (AOAC, 2000), titratable acidity was measured by using phenolphthalein as an indicator (AOAC, 2000) to titrate 10 ml of an aliquot against a standard solution of 0.1 N NaOH. Appearance of light pink color was taken as an end point, and a hydrometer was used to measure alcohol content and specific gravity (Triple scale Hydrometer). The product was also examined for color and appearance, taste, aroma and overall acceptability using a 9-point Hedonic scale with a panel of 5 experts.

3. RESULTS AND DISCUSSION

The result of the experiment entitled Effect of different levels of local yeast in pomegranate

(*Punica granatum* L.) cider was undertaken in the Post- Harvest Laboratory, Department of Horticulture, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during the year 2022-2023. The results of the investigation regarding production of cider from pomegranate influence by different levels of yeast and sugar have been presented in Tables 2–4, wherever required.

Completely randomized block design (CRBD) by Panse and Sukhtme's analysis of variance (ANOVA) approach was used for the statistical analysis. Using the critical difference (C. D. at 5%) threshold of significance, the overall significance of differences between the treatments was examined. A window-based computing tool called OPSTAT was used to statistically analyze the results (Sheoran, 2004).

3.1 Total Soluble Solids ($_o$ Brix)

Changes in TSS during fermentation of pomegranate juice inoculated with yeast *Saccharomyces cerevisiae* are presented in Table 2. The gradual decrease in total soluble solids during the storage period (90 days) of pomegranate cider might be due to the fermentation of sugars into alcohol by the action of yeast. The general decrease in TSS was a function of time and was undoubtedly caused by the yeast fermenting the sugar. This is typical cider fermentation behavior for any alcoholic fruit juice fermentation. The decrease in TSS content of cider indicates the conversion of sugar into alcohol by yeast during fermentation. The above results are similar with the findings of Wanapu et al. [6] in rose apple cider, Walker et al. [7], Sahu et al. [8] in tendu wine**,** Isitua et al. [9] in banana wine. The lowest score (7.3) was observed in treatment T6 (Pomegranate juice 1ltr + 3g yeast + 500g sugar) and the maximum score (12.3) was observed in treatment T1 (Pomegranate juice 1ltr + 0.5g yeast + 500g sugar).

3.2 Alcohol Content (%)

The increase in Alcohol content of pomegranate cider with different levels of yeast during storage may possibly due to the variation in performance of the yeast to utilize the fermentable sugars affecting the ferment ability, hence the varied alcohol production .The above results are similar with the findings of Jarvis, B. [10] in apple cider, Yadav et al. [11] in Mahua wine. It is apparent from the results shown in Table 2 that with increase in fermentation time, concentration of alcohol increased. The maximum score of alcohol (%) content (8.6) was observed in treatment T6 (Pomegranate juice 1ltr + 3g yeast + 500g sugar) followed by treatment T7 (Pomegranate juice 1ltr + 3.5g yeast + 500g sugar) with (8.2) and the lowest score (4.42) was observed in treatment T1 (Pomegranate juice 1ltr + 0.5g yeast + 500g sugar) during 90 days of storage.

3.3 Titratable Acidity (%)

Due to the presence of organic acids produced as a byproduct, TA of cider increased as fermentation progressed. Table 2 shows the TA variations that occur throughout fermentation. No matter which yeast strain was employed, TA considerably increased after 24 hours of fermentation. This increase in acidity is correlated with a decrease in the content of reducing sugars and an increase in the concentration of alcohol. The increase in acidity during the storage period (90days) in pomegranate cider is attributed to the production of different organic acids such as citric, malic, lactic, tartaric, oxalic and succinic acids. A similar finding was observed by Beera et al. [12] in mango wine. In Table 2 of Acidity (%), the maximum score (0.96) was observed in treatment T6 (Pomegranate juice 1ltr + 3g yeast + 500 g sugar) followed by treatment T5 (Pomegranate juice 1ltr + 2.5g yeast + 500g sugar) with (0.94) and the lowest score (0.78) was observed in treatment T1 (Pomegranate juice 1ltr + 0.5 g yeast + 500g sugar).

3.4 pH

With longer fermenting times, the pH gradually decreased. Different yeast concentrations and the length of the fermentation process both had an impact on the variation that was observed. According to studies, low pH during fruit fermentation inhibits the growth of microbes that cause spoiling while encouraging the growth of beneficial organisms. Additionally, according to Medina et al. (2006), fermentation yeast is recognized to have a comparative advantage in the natural environment due to its high acidity and low pH. During the storage period (90 days) as shown in Table 2, the decrease in pH may possibly be due to the acids generated by bacteria. The decrease in pH with increase in acidity of cider may be due to the formation of hydrogen ions by the action of yeast. The above results are similar with the findings of Akin et al. [13] in grape must**.** In terms of pH, the lowest

Table 2. Physio-chemical parameters of pomegranate cider during storage

Treatment symbol	Treatment details	Total Soluble Solids (◦Brix)				Alcohol content (%)			Acidity (%)				pH				Specific Gravity			
		Initial	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS
T1	Pomegranate juice (1ltr) + 0.5g(yeast) + 500g (sugar)	29.64	23.3	14.3	12.3	2.67	4.75	4.42	0.29	0.39	0.59	0.78	4.34	3.74	3.56	3.46	1.117	1.084	1.052	1.043
T2	Pomegranate juice (1ltr) + 1g(yeast) + 500g (sugar)	26.9	19.8	13.2	11.5	2.79	3.6	5.8	0.34	0.44	0.66	0.80	3.73	3.63	3.46	3.39	1.1	1.07	1.046	1.041
T3	Pomegranate juice (1ltr) + 1.5g(yeast) + 500g (sugar)	25.1	19.5	13.8	11.8	2.56	4.56	5.63	0.36	0.54	0.72	0.86	3.70	3.62	3.54	3.46	1.085	1.07	1.051	1.041
T4	Pomegranate juice (1ltr) + 2g(yeast) + 500g (sugar)	22.73	15.6	12.5	10.6	3.49	5.54	6.62	0.34	0.39	0.59	0.79	3.73	3.69	3.57	3.38	1.074	1.051	1.044	1.038
T5	Pomegranate juice (1ltr) + 2.5g(yeast) + 500g (sugar)	19.7	14.5	11.5	8.4	2.94	6.05	7.51	0.46	0.59	0.79	0.94	3.6	3.47	3.28	3.2	1.064	1.046	1.041	1.029
T6	Pomegranate juice (1ltr) + 3g(yeast) + 500g (sugar)	17.73	12.3	10.8	7.3	3.68	7.70	8.6	0.5	0.69	0.89	0.96	3.31	3.17	3.24	2.88	1.063	1.036	1.037	1.023
T7	Pomegranate juice (1ltr) + 3.5g(yeast) + 500g (sugar)	18.8	13.6	10.3	9.4	2.69	7.58	8.2	0.4	0.54	0.69	0.85	3.66	3.45	3.4	3.26	1.066	1.045	1.036	1.032
F-test		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SE.(d)		0.502	0.219	0.219	0.219	0.16	0.249	0.259	0.033	0.065	0.058	0.036	0.136	0.075	0.09	0.071	0.013	0.01	0.005	0.004
CD at 0.5%		1.086	0.474	0.474	0.474	0.347	0.539	0.56	0.072	0.14	0.125	0.079	0.295	0.162	0.196	0.153	0.029	0.022	0.01	0.009

Table 3. Organoleptic score of pomegranate cider during storage

Treatment symbol	Treatment details	Color and Appearance			Taste			Aroma			Overall Acceptability		
		30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS
T1	Pomegranate juice (1ltr) + 0.5g(yeast) + 500g (sugar)	3.33	3.6	5.5	3.55	3.5	5.2	3.5	3.37	4.6	3.46	3.5	5.1
T2	Pomegranate juice (1ltr) + 1g(yeast) + 500g (sugar)	3.5	3.4	5.4	3.35	3.7	6.1	3.7	4.4	6.3	3.51	3.82	5.93
T3	Pomegranate juice (1ltr) + 1.5g(yeast) + 500g (sugar)	3.4	3.2	5.3	3.33	3.3	5.3	3.2	3.6	4.4	3.29	3.35	5
T4	Pomegranate juice (1ltr) + 2g(yeast) + 500g (sugar)	4.7	5.14	6.4	4.52	4.7	6.13	4.3	5.3	6.6	4.6	5.05	6.4
T5	Pomegranate juice (1ltr) + 2.5g(yeast) + 500g (sugar)	5.3	6.5	7.4	7.31	7.6	7.4	5.33	7.1	7.5	6.01	7.07	7.42
T6	Pomegranate juice (1ltr) + 3g(yeast) + 500g (sugar)	7.4	7.52	8.5	8.4	8.6	8.7	6.6	7.6	8.1	7.5	7.9	8.43
T7	Pomegranate juice (1ltr) + 3.5g(yeast) + 500g (sugar)	4.4	5.3	4.2	6.32	7.2	7.1	4.2	6.8	7.1	4.10	6.43	6.13
F-test		S	S	S	S	S	S	S	S	S	S	S	S
SE.(d)		0.21	0.151	0.082	0.282	0.154	0.158	0.089	0.161	0.082	0.595	0.448	0.59
CD at 0.5%		0.454	0.328	0.177	0.611	0.334	0.341	0.193	0.348	0.177	1.29	0.97	1.279

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

Treatment No	Treatment	Total Cost(Rs)	Pomegranate Cider output (1ltr)	Selling rate(Rs)/ bottle	Gross return (Rs)	Net return (Rs)	Benefit cost ratio
T1	Pomegranate juice 1Ltr + yeast 0.5g + sugar 500g	143.20	3.00	300.00	900.00	470.40	2.09
T2	Pomegranate juice 1Ltr + yeast 1g + sugar 500g	145.45	3.00	300.00	900.00	463.65	2.06
T3	Pomegranate juice 1Ltr + yeast 1.5g + sugar 500g	147.70	3.00	350.00	1050.00	606.90	2.37
T4	Pomegranate juice 1Ltr + yeast 2g + sugar 500g	149.95	3.00	350.00	1050.00	600.15	2.33
T5	Pomegranate juice 1Ltr + yeast 2.5g + sugar 500g	152.20	3.00	450.00	1350.00	893.40	2.96
T6	Pomegranate juice 1Ltr + yeast 3g + sugar 500g	154.45	3.00	500.00	1500.00	1036.65	3.24
T7	Pomegranate juice 1Ltr + yeast 3.5g + sugar 500g	156.70	3.00	400.00	1200.00	729.90	2.55

score (2.88) was observed in treatment T6 (Pomegranate juice 1ltr + 3 g yeast + 500 g sugar) followed by T5 (Pomegranate juice 1ltr + 2.5 g yeast + 500 g sugar) with (3.2) and the maximum score (3.46) was observed in treatment T3 (Pomegranate juice 1ltr + 1.5g yeast + 500 g sugar).

3.5 Specific Gravity

As fermentation time increases specific gravity decreases gradually as shown in Table 2. The decrease in Specific gravity of pomegranate cider with different levels of yeast during storage may possibly be due to the type of yeast used in the cider production. *Saccharomyces cerevisiae* has been reported to reduce specific quality of fruit juices during fermentation. The above results are similar with the findings of Tusekwa et al. [14] and Okafor et al. [15]. In terms of Specific gravity, the lowest score (1.023) was observed in treatment T6 (Pomegranate juice 1ltr + 3g yeast + 500 g sugar) followed by treatment T5 (Pomegranate juice 1ltr + 2.5g yeast + 500 g sugar) with (1.029) and the maximum score was observed in treatment T1 (Pomegranate juice 1ltr + 0.5 g yeast + 500g sugar) with (1.043).

3.6 Organoleptic Evaluation

In the organoleptic evaluation such as color and appearance, taste, aroma, and overall acceptability. According to Table 3, treatment T6 (pomegranate juice 1 ltr + 3 g yeast + 500 g sugar) acquired the highest sensory scores in all organoleptic characteristics measures, with scores of 8.5, 8.7, 8.1, and 8.43, indicating that the judges approved of it.

4. CONCLUSION

From the present investigation it is concluded that alcohol production increased with increasing in inoculum level of yeast strain (*Saccharomyces cerevisiae*). A fter comparing the overall result in this study, the treatment T6 was found superior in terms of physio-chemical properties i.e., total soluble solids (7.3°Brix), alcohol content (8.6%), titratable acidity (0.96%), pH (2.88), specific gravity (1.023) and overall acceptability (8.43). Similarly, the treatment T6 (Pomegranate juice 1ltr + 3g yeast + 500g sugar) showed the highest BC ratio (3.24).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/100648