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Effect of Different Level of Sugar and Yeast on Production and Quality of Sea Buckthorn (*Hippophae rhamnoides)* Cider

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A B S T R A C T

The study was conducted in Completely Randomized Design (CRD) with 8 treatments with three replication each. The treatments were T1 (Seabuckthorn juice 500ml + sugar 100g + yeast 0.8g), T2 (Seabuckthorn juice 500ml + sugar 100g + yeast 1.0g), T3 (Seabuckthorn juice 500ml + sugar 100g + yeast 1.5g), T4 (Seabuckthorn juice 500ml + sugar 150g + yeast 0.5g), T5(Seabuckthorn juice 500ml + sugar 150g + yeast 1.0g), T6 (Seabuckthorn juice 500ml + sugar 150g + yeast 1.5g), T7 (Seabuckthorn juice 500ml + sugar 200g + yeast 0.8g), T8 (Seabuckthorn juice 500ml + sugar 200g + yeast 1.5g). Total soluble solids, pH, and Specific Gravity decreased while the alcohol content, Acidity, and Sensory Qualities increased with the increasing length of fermentation. From the above treatments, it is concluded that treatment T4 was found superior in respect of the parameters like Total Soluble Solids, Acidity, pH, Alcohol content, and Specific gravity. In terms of Colour and Appearance, Taste, Aroma, and Overall acceptability also T4 was found best. In terms of the cost-benefit ratio, the highest Net Return, Cost Benefit Ratio was recorded in treatment T4. This study showed that acceptable Cider can be produced from Seabuckthorn berries by using yeast, especially *Saccharomyces cerevisiae.*

Introduction

Seabuckthorn is a spinescent, deciduous, and anemophilous shrub or a small tree with a high economic and ecological potential. It is frequently used as a pioneer species in anthropic and eroded soils due to its low pedoclimatic demands, strong rooting system, and ability to fix atmospheric nitrogen. Their usual height is ranging between 2 to 10 m (Jarvis, *et al.,*1995). The bark is rough brown, the young branches are grey and usually spiny and the buds are alternate and golden-brown. The leaves are linear or linear-lanceolate, 2-6 cm long, with short petioles and entire margin, acutish, covered on both sides with silvery scales. Seabuckthorn berries, leaves, and bark have a high content of nutritive and active substances that promote this species for use in food and medical industries. One of the most requested therapeutical products on the market is seabuckthorn oil, extracted from both pulp and seeds

(Adrian Veskan *et al.,* 2010). Thanks to the high content of vitamin C, carotenoids, flavonoids, tocopherols, and other potentially health-beneficial components the Sea buckthorn juice and pulp are often used as food or beverages. Recently, the amount of Seabuckthorn fruits used to produce health- supporting drugs (i.e., lotions, cosmetics, nutritional supplements) is rising (Lin *et al.,* 2012).

Cider is considered to be a pleasant, refreshing, thirst-quenching, and hygienic beverage. It is also highly nutritive and health-giving among all fermented drinks. Besides, it is said to possess remarkable therapeutic properties like the prevention of stone formation in the bladder on account of its diuretic properties. Cider is produced all over the world and consumed throughout European countries.

Cider making process includes various steps such as harvesting fruits, washing, grinding, pressing, blending, testing, fermentation, racking off, filtering, bottling, and storage (Ansari *et al.,* 2022).

Seabuckthorn berries have been used for the preparation of various products such as juices, jams, wine, herbal tea, Pickles, Antioxidant herbal supplements, and UV protective oil. Nutritional and bioactive substances in Seabuckthorn berries and leaves have attracted researchers' interest in developing products for preventive and curative purposes.

Numerous products are made from Seabuckthorn fruit and leaves. A wide array of products is possible from Seabuckthorn for nutraceutical, cosmetic and medicinal purposes (Singh and Peter, 2018). Seabuckthorn contains good sugar proportion which makes it suitable for cider and wine making.

Any fruit with a good proportion of sugar may be used in producing cider and wine and the resultant cider/wine is normally named after the fruit. Ready-to-serve Seabuckthorn beverages are getting quite popular nowadays in the market. However, unlike apples and other fruits, its cider is not yet available. Besides apples, there are reports of cider preparation from pear, peach, and pineapple also. However, no such drink has been prepared from Seabuckthorn commercially except for juices, which has got low alcohol content and high nutritional value. Since Seabuckthorn berries are widely grown in India, the Ladakh region remains the major site for its natural resource with over 70% of the total area (13,000 ha) under Sea buckthorn in the country development of mild fermented Seabuckthorn cider would be a profitable strategy and will contribute to boost our Economy (Stobdan *et al.,* 2013).

Materials and Methods

The study was conducted in Completely Randomized Design (CRD) with 8 treatments with three replication each. The treatments were T1 (Seabuckthorn juice 500ml + sugar 100g + yeast 0.8g), T2 (Seabuckthorn juice 500ml + sugar 100g + yeast 1.0g), T3 (Seabuckthorn juice 500ml + sugar 100g + yeast 1.5g), T4 (Seabuckthorn juice 500ml + sugar 150g + yeast 0.5g), T5 (Seabuckthorn juice 500ml + sugar 150g + yeast 1.0g), T6 (Seabuckthorn juice 500ml + sugar 150g + yeast 1.5g), T7 (Seabuckthorn juice 500ml + sugar 200g + yeast 0.8g), T8 (Seabuckthorn juice 500ml + sugar 200g + yeast 1.5g).

Procurement of raw material and fruit selection

Freshly ripened Seabuckthorn berries are harvested by hand using the 'beat the bush' method in September. Polythene sheet is placed on the ground just below the canopy, and the berries are collected by beating the branches with a stick. The collected berries are then used to extract juice, as they are highly perishable. Only the juice is transported for research purposes, ensuring its freshness and preserving its quality.

Extraction of juice

The juice was extracted using a pulper and the resulting must was filtered through muslin cloth and a siphoning tube. After inoculation, the must was stored in glass bottles and sealed with balloons. The balloons had tiny holes made with needles to allow the release of gas during fermentation and minimize the risk of the bottles exploding. This method helps facilitate the fermentation process while ensuring safety and preventing any potential accidents.

Yeast inoculum

Saccharomyces cerevisiae was used for cider making and was obtained from the market. 0.5g, 0.8g, 1.0g and 1.5 g quantity of yeast powder were added to lukewarm water in separate beakers according to the treatments and stirred gently with the wooden spoon. The activated *Saccharomyces cerevisiae* was added to the pulp according to the treatments, respectively.

Preparation of Cider

Cider was prepared with different concentrations of sugar and yeast (*Saccharomyces cerevisiae*). Flow chart of the preparation cider is given below

Physico-chemical and Organoleptic Characteristics

During the storage of the cider under room conditions, various physico-chemical changes were monitored. The pH of the product was measured using a digital pH meter, Total Soluble Solids (TSS) were determined with a hand refractometer, Titrable acidity was assessed using the titrimetric method, and the alcohol content and specific gravity were measured using a hydrometer.

Furthermore, the cider underwent sensory evaluation by a panel of judges. They assessed the product's color, appearance, flavour, aroma, and overall acceptability. The evaluation was conducted using a 9-point Hedonic scale, where the judges rated each attribute based on their preference and liking.

By monitoring these parameters and conducting sensory evaluations, the study aimed to assess the changes in the physico-chemical properties of the cider during storage and after the completion of the fermentation process. This comprehensive analysis provides valuable insights into the quality and acceptability of the cider.

Statistical Tools

Microsoft Excel 2019 was used for statistical analysis at 95% confidence level.

Results and Discussion

TSS (°Brix)

The TSS content of Seabuckthorn Cider showed decreasing order in all Treatments during storage. The TSS showed that there were significant differences among all the treatments during storage. It is possible due to the fermentation of sugars into alcohol by the action of the yeast.

The above results are similar to the findings of Adesokan, 2019 From table 1 it is observable that the lowest score of Total Soluble Solids ranging from 19.13 to 5.20° Brix in T1 (Seabuckthorn juice 500ml + sugar 100g + yeast 0.8g), followed by T2 (Seabuckthorn juice 500ml + sugar 100g + yeast 1.0g) ranging from 18.13 to 7.07°Brix, whereas maximum score was observed in Treatment T8 (Seabuckthorn juice 500ml + sugar 200g + yeast 1.5g) with 29.30 to 11.20°Brix at initial,30,60 and 90 days of storage.

Alcohol content

The yeast's ability to convert sugar into alcohol during fermentation can vary, leading to differences in the alcohol content among the treatments.

Factors such as yeast strain, fermentation conditions, and nutrient availability can all influence the yeast's performance and its ability to utilize the sugar effectively. As a result, these variations in the yeast's sugar consumption during fermentation can contribute to the differences observed in alcohol content among the different treatments.

According to the results shown in Table 1, there was an observable trend where the concentration of alcohol increased with an increase in fermentation time. In terms of Alcohol content, the highest score of Alcohol content (4.74,5.27and 12.23) was observed in treatment T5 (Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.0g) whereas the minimum score was observed in T2 (Seabuckthorn juice (500ml) + sugar (100g) + yeast (1.0g) with (4.75,4.90 and 8.05) at 30,60 and 90 days of storage.

There was a statistically significant decrease in ethanol production beyond the inoculum level of 9% (v/v) as more sugar was consumed by the biomass production at higher inoculum levels Joshi and Sharma (2009).

Titratable Acidity

Acidity plays a vital role in determining cider quality by aiding the fermentation process and enhancing the overall characteristics and balance of the cider. Lack of acidity means a poor fermentation. Acidity is also an important factor since it contributes both directly and indirectly to the quality of cider.

There was a subsequent increase in Acidity at different periods of storage. In terms of Acidity, the lowest score of Acidity (0.42,0.60 and 0.63) was observed in treatment T1(Seabuckthorn juice (500ml) + sugar (100g) + yeast (0.8g), whereas the maximum score was observed in treatment T8(Seabuckthorn juice (500ml) + sugar (200g) + yeast (1.5g) with (0.60,0.81 and 0.96) at 30,60 and 90 days of storage.

During storage yeast produced certain organic acid, which might be the reason for the increasing acidity in Seabuckthorn cider. A similar finding was registered by Kithan Thungbeni *et al.*, (2020), they found that the titratable acidity of pineapple wine increased prominently within fermentation during storage which could be due to the production of certain organic acids by yeast.

pH

The pH decreased gradually as the fermentation time increases. The variation observed was due to the effect of different yeast strains and fermentation periods. There was a subsequent decrease in pH at different periods of storage.

In terms of pH, the lowest score of pH (4.52,4.47 and 3.15) was observed in treatment T4 (Seabuckthorn juice (500ml) + sugar (150g) + yeast (0.5g), whereas the maximum was observed in treatment T1(Seabuckthorn juice (500ml) + sugar (100g) + yeast (0.8g) with (4.70,4.52 and 3.25) at 30,60 and 90 days of storage.

Low pH is inhibitory to the growth of spoilage organisms but creates a conducive environment for the growth of desirable organisms. Also, low pH and high acidity are known to give fermentation yeast a comparative advantage in natural environments Joshi and Sandhu (2000).

Specific gravity

The specific gravity of the Seabuckthorn cider produced in this study reduces as the fermentation days of the cider increase. The decrease in the Specific gravity of Seabuckthorn

Cider with different levels of yeast and sugar during storage may possibly be due to the type of yeast used in the cider production. *Saccharomyces cerevisiae* has been reported to reduce the specific quality of fruit wines during fermentation. The above results are similar to the findings of Gupta *et al.,* (2005).

There was a subsequent decrease in Specific gravity at different periods of storage. The lowest score of (1.073,1.063 and 1.005) was observed in treatment T4 (Seabuckthorn juice (500ml) + sugar (150g), whereas the maximum score was observed in treatment T6 (Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.5g) with (1.057,1.054 and 1.01) at 30,60 and 90 days of storage.

Table.1 Physico-chemical properties of different levels of concentration of sugar and yeast concentrate on the production of seabuckthorn cider

Treatments	Treatments details	T.S.S(°BRIX)				Alcohol			Acidity				pH				Specific Gravity			
		Initial	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days	Initial	30 Days	60 Days	90 Days	Initial	30 Days	60 Days	90 Days	Initial	30 Days	60 Days	90 Days
T1	Seabuckthorn juice (500ml) + sugar (100g) + yeast (0.8g)	19.13	8.10	7.03	5.20	5.91	6.37	8.27	0.33	0.42	0.60	0.63	5.85	4.7	4.52	3.25	1.076	1.033	1.029	**1.013**
T2	Seabuckthorn juice (500ml) + sugar (100g) + yeast (1.0g)	18.13	9.03	8.60	7.07	4.75	4.90	8.05	0.36	0.44	0.64	0.66	5.92	4.62	4.6	3.23	1.073	1.037	1.035	**1.012**
T3	Seabuckthorn juice (500ml) + sugar (100g) + yeast (1.5g)	19.20	12.17	9.10	8.40	3.81	5.34	8.55	0.37	0.43	0.66	0.68	5.64	4.54	4.66	3.17	1.078	1.049	1.037	**1.013**
T4	Seabuckthorn juice (500ml) + sugar (150g) + yeast (0.5g)	23.07	18.10	15.40	7.10	2.65	3.97	11.02	0.39	0.48	0.67	0.73	5.41	4.52	4.47	3.15	1.093	1.073	1.063	**1.005**
T5	Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.0g)	25.17	16.07	14.10	9.17	4.74	5.27	12.23	0.41	0.52	0.69	0.78	5.51	4.53	4.42	3.23	1.101	1.065	1.061	**1.008**
T6	Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.5g)	24.07	14.23	12.10	9.90	5.26	4.75	11.41	0.42	0.57	0.75	0.85	5.71	4.62	4.52	3.21	1.097	1.057	1.054	**1.01**

T7	Seabuckthorn juice (500ml) + sugar (200g) + yeast (0.8g)	28.10	22.10	13.13	10.13	3.69	3.69	9.06	0.45	0.58	0.79	0.92	5.8	4.56	4.53	3.24	1.117	1.089	1.089	**1.007**
T8	Seabuckthorn juice (500ml) + sugar (200g) + yeast (1.5g)	29.30	19.17	14.07	11.20	5.24	5.79	9.06	0.48	0.60	0.81	0.96	5.71	4.59	4.56	3.23	1.115	1.077	1.073	**1.008**
F-test		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	**S**
SE.(d)		0.128	0.103	0.071	0.104	0.011	0.03	0.02	0.007	0.008	0.006	0.009	0.009	0.03	0.034	0.008	0.008	0.001	0.001	**0.002**
CD at 0.5%		0.274	0.22	0.151	0.223	0.023	0.064	0.044	0.015	0.017	0.013	0.02	0.02	0.064	0.073	0.017	0.017	0.002	0.002	**0.003**
CV		**0.674**	**0.846**	**0.741**	**1.496**	**0.291**	**0.734**	**0.258**	**2.104**	**1.98**	**1.09**	**1.441**	**1.441**	**0.798**	**0.92**	**0.31**	**0.31**	**0.097**	**0.089**	**0.184**

Table.2 Variation in Overall Acceptability due to different levels of yeast and sugar on production and quality of Seabuckthorn cider

Treatments	**Treatments details**	**Overall Acceptability**		
		30 Days	**60 Days**	**90 Days**
T1	Seabuckthorn juice (500ml) + sugar (100g) + yeast (0.8g)	3.21	3.41	**3.40**
T2	Seabuckthorn juice (500ml) + sugar (100g) + yeast (1.0g)	4.08	4.18	**4.23**
T3	Seabuckthorn juice (500ml) + sugar (100g) + yeast (1.5g)	4.51	4.86	**5.06**
T4	Seabuckthorn juice (500ml) + sugar (150g) + yeast (0.5g)	8.13	8.18	**8.38**
T5	Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.0g)	6.08	6.08	**6.39**
T6	Seabuckthorn juice (500ml) + sugar (150g) + yeast (1.5g)	6.97	6.94	**7.13**
T7	Seabuckthorn juice (500ml) + sugar (200g) + yeast (0.8g)	7.69	7.63	**7.87**
T8	Seabuckthorn juice (500ml) + sugar (200g) + yeast (1.5g)	6.94	6.86	**7.02**
F-test		S	S	**S**
SE.(d)		0.22	0.20	**0.25**
CD at 0.5%		**0.46**	**0.44**	**0.53**

Chart.1 Preparation of Cider

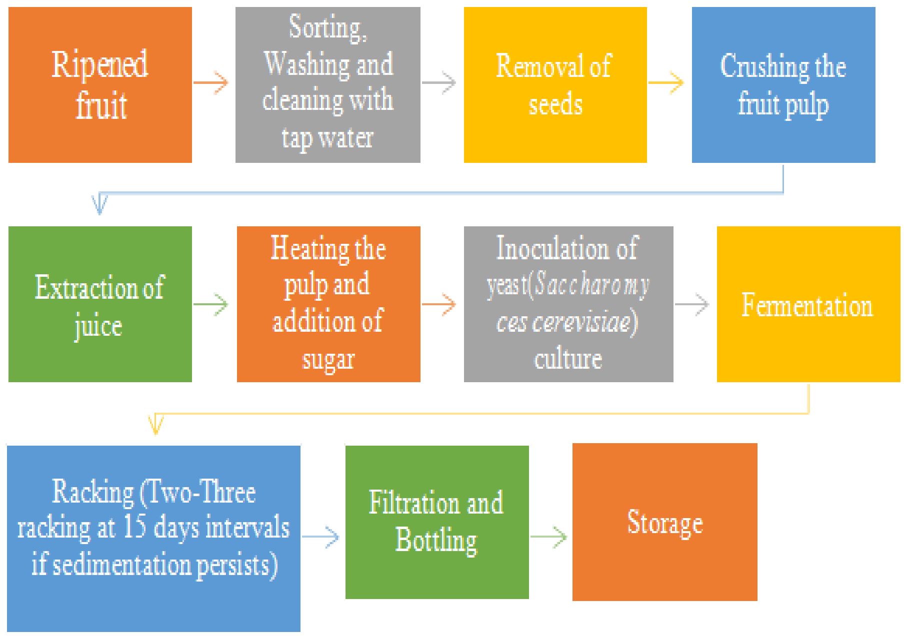

Fig.1

Fig.2 Effect of different levels of sugar and yeastt on physico-chemical properties on production and quality of Seabuckthorn cider

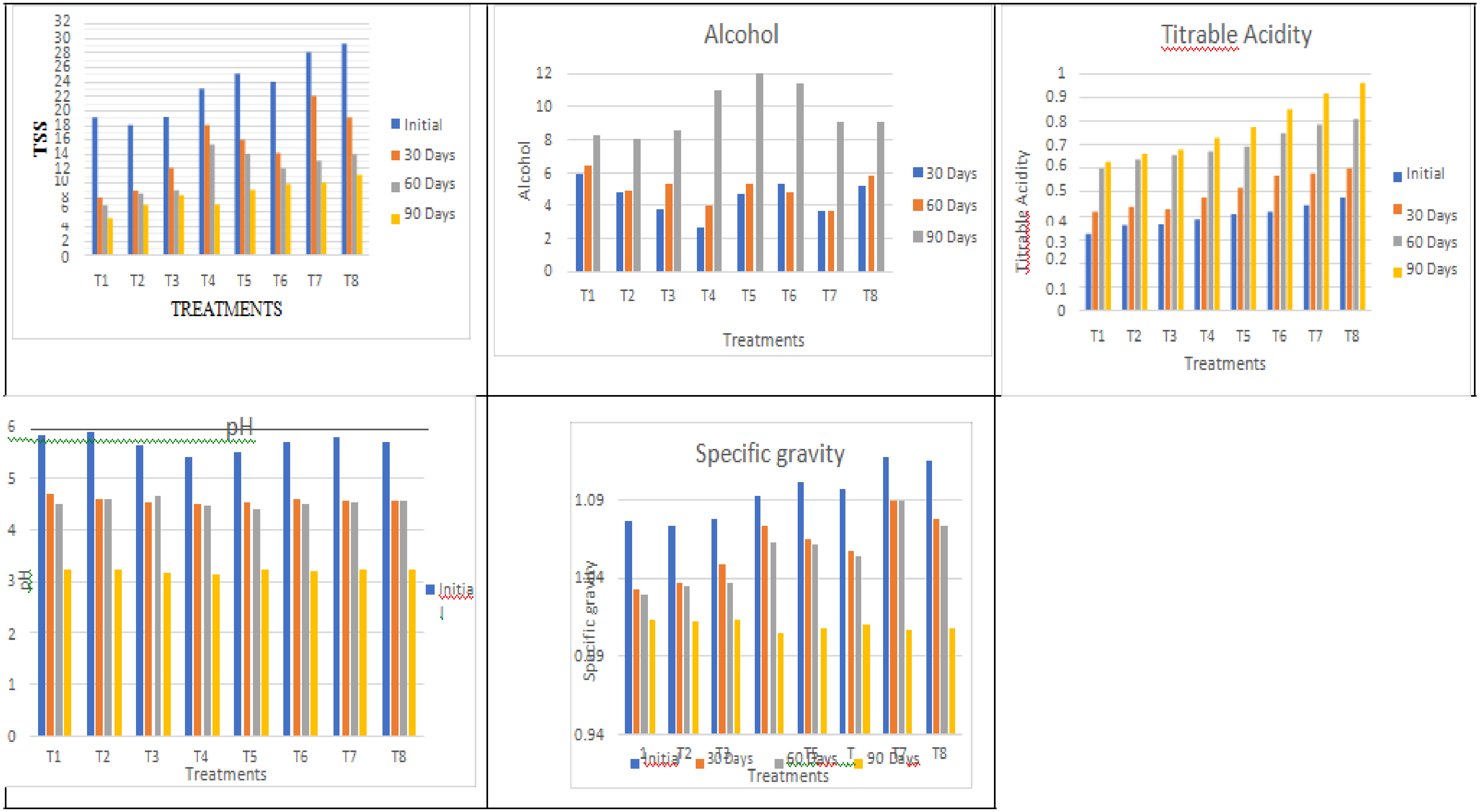

Fig.3 Organoleptic score for Overall Acceptability of Seabuckthorn cider during storage

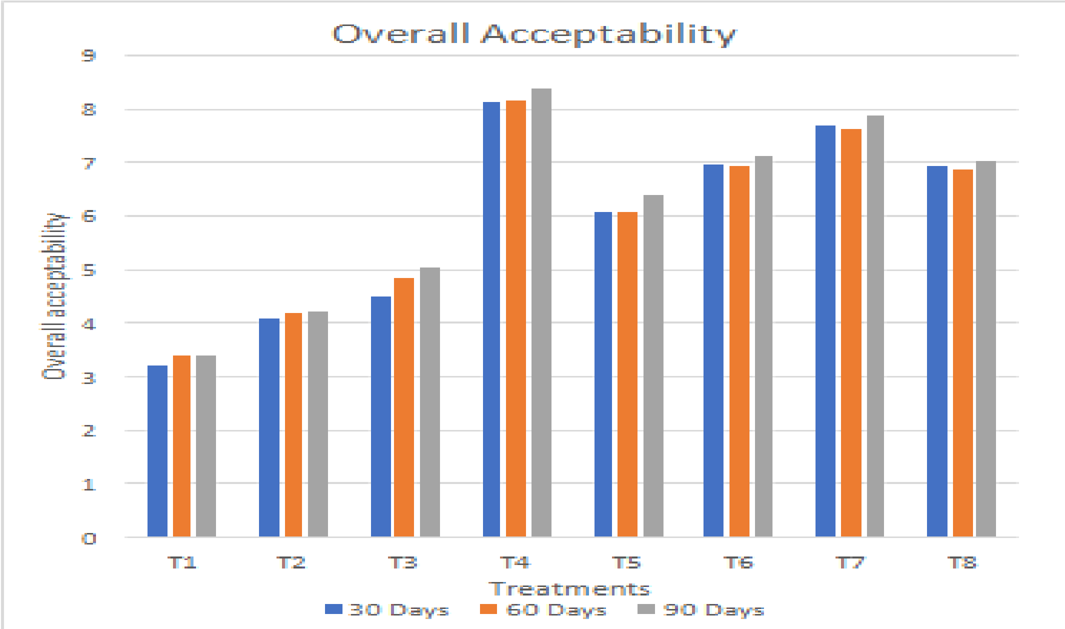

Fig.4

Organoleptic Evaluation

According to Table 2, treatment T4 (Seabuckthorn juice 500ml + sugar 150g + yeast 0.5g) received the highest sensory scores for all parameters of organoleptic evaluation, including color and appearance, taste, aroma, and overall acceptability. The highest overall acceptability score of 8.38 suggests that this treatment was well-received by the panel of judges.

The sensory scores reflect the subjective judgments of the panel of judges who evaluated the different treatments. Overall, the combination of Seabuckthorn juice, sugar, and yeast in treatment T4 seemed to have resulted in a product that was highly regarded by the judges in terms of its organoleptic attributes. This suggests that T4 may be a favorable choice in terms of taste, appearance, aroma, and overall acceptability.

The above study indicates that the strain *Saccharomyces cerevisiae* is capable of producing Seabuckthorn cider. Furthermore, it can be concluded that alcohol production increased as the inoculum level of *Saccharomyces cerevisiae*. After analyzing the overall results of the study, it was found that treatment T4 (Seabuckthorn juice 500ml + sugar 150g + yeast 0.5g) exhibited the most suitable physico-chemical properties compared to other treatments. Specifically, T4 showed favorable values for pH, total soluble solids, titrable acidity, alcohol content, specific gravity, and the organoleptic test of overall acceptability. Thus, T4 is considered the most suitable treatment for Seabuckthorn cider production.

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