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EFFECT OF PULP RATIO, NITROGEN CONCENTRATION AND INOCULUMS CONCENTRATION ON PUMPKIN BASED FUNCTIONAL WINE

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ABSTRACT

Pumpkin (*Cucurbita moschata*) is widely grown for commercial use and both in food and recreation. Preparation of alcoholic beverages using pumpkin pulp as a base is another outlet for its economic utilization. Pomegranate (*Punica granatum*) is also one of the commercially important fruit which is extensively cultivated in many tropical and subtropical regions. However, the wild apricot (*Prunus armeniaca L.*) is a wild crop found in the Western Himalayan region. In the present studies pulp of pumpkin, pomegranate and wild apricot in different concentrations (T_1 60:20:20, T_2 50:25:25 and T_3 40:30:30) were blended and used to prepare a pumpkin based functional wine. The musts were prepared using sucrose (24^{o}B) and SO_2 (100 ppm) constant in all the treatments. Further, the effect of addition of different concentrations of Diammonium hydrogen phosphate (0.1%, 0.2% and 0.3%) as nitrogen source and yeast inoculum (2.5%, 5% and 7.5%) was studied. In all the treatments fermentation was carried out at 27^{o}C. Among, different treatments 8 best treatments were selected on the basis of sensory scores, phenols, antioxidant activity, higher alcohols, carotenoids, ascorbic acid. Among these, the treatment containing pulp ratio (60:20:20) with 0.1% Diammonium hydrogen phosphate and inoculum concentration 5% v/v was adjudged as best as the treatment contains higher amount of phenols 291 mg/l and alcohol (v/v) 10.30 % and lower volatile acidity content (0.024 % as per acetic acid). Further, the results on

antioxidant activity showed that the treatment had also higher DPPH Scavenging activity 66.44 %, Ferric Reducing Antioxidant power 549 (mmol Fe (II)/g) and ABTS activity 51%.

Keywords: Pumpkin (*Cucurbita moschata*), phenols, alcohol, DPPH and Ferric Reducing Antioxidant power

INTRODUCTION

Pumpkin (*Cucurbita moschata*) from the family *Cucurbitaceae* and genus *Cucurbita* is one of the important tropical vegetable grown all around the world. The estimated production of pumpkins in the world during 2014 was 6500 thousand metric tonnes (1). The production of pumpkin in the India during 2015 was 1084000.05 tonnes (2). Pumpkin is also popular in traditional medicine for several ailments (antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, intestinal antiparasitia, antiinflammation, antalgic (3). It contains many biologically active components including high levels of α- and β-carotene, β-cryptoxanthin, lutein and zeaxanthin, polysaccharides, phytosterols, unsaturated fatty acids, proteins and peptides (4). Pomegranate (*Punica granatum*) belongs to the *Punicacea* family and is one of the commercially important fruit which is extensively cultivated in many tropical and subtropical regions (5) such as Iran. The fruit is rich source of anthocynins, vitamin C and antioxidant and is either consumed directly or processed into various products such as juice, jams, syrup, and sauce (6). Wild Apricot (*Prunus armeniaca L.*) is one of the most popular stone fruit in the temperate region and classified under the *Prunus* species of the *Rosaceae* family. The fruit is similar to cultivated apricot but is usually found wild and known with name Zerdali (wild apricot) (7). It is thought that wild apricot is a rich food in terms of antioxidants because it contains the flavonoids and carotenoids (8, 9). Hence, wine preparation using pumpkin as a base along with pomegranate and wild apricot can be one of the alternatives to utilize these fruit plants. There is scanty information on preparation of pumpkin based functional wine. However, since the pumpkin is very low in acid content and therefore needs amelioration with different high acid fruits. Further, the higher pulp content in the musts also needs addition of enzyme like pectin esterase. Therefore, in the present investigations, an effort has been made to study the effect of different pulp concentrations, DAHP and inoculums on the quality of pumpkin based functional wine.

MATERIALS AND METHODS

Pumpkin and pomegranate were procured locally and the wild apricot was collected from Janjehali area of district Mandi and Jubal area of Shimla district. The locally obtained sucrose was used to ameliorate the must. The culture of *Saccharomyces cerevisiae* var. *ellipsoideus* strain UCD 595 used in the study was obtained from the Department of Food Science and Technology, UHF Nauni, Solan (H.P.). The pectinestrase enzyme used was manufactured by M/S Triton Chemical, Mysore, India, under the brand name of 'Pectinol'.

Method of Wine Preparation:

The wine was prepared using pumpkin as base, along with wild apricot and pomegranate as acidulant. The pumpkin and wild apricot was made by boiling pumpkin with 10% water. However, pomegranate juice was extracted through fruit juicer. The must was prepared by blending pumpkin pulp, wild apricot and pomegranate in different ratios T_1 (60:20:20), T_2 (50:25:25) and T_3 (40:30:30). In all the must initial total soluble solids (TSS) were raised to 24^0B with sucrose and acidity was adjusted to 0.50% (as citric acid). Further, the Diammonium hydrogen phosphate (DAHP) was added in different concentrations N_0 (without nitrogen), N_1 (0.1%), N_2 (0.2%) and N_3 (0.3%) respectively. To each treatment, 100 ppm SO_2 in the form of KMS (potassium metabisulphite) was added. The fermentation of all the musts were carried out by using yeast inoculums in different proportions IC_0 (control), IC_1 (2.5%), IC_2 (2.5%) and IC_3 (7.5%). Each fermentation was carried out at 27^0C and monitored for reduction in sugars and alcohol production. On completion of fermentation the different wines were siphoned twice and filtered. To different wines 50 ppm potassium metabisulphite was added and filled in clean sterilized glass bottles. Finally, the filled bottles were pasteurized at 62-65 ^{0}C for 20 min.

Physico-chemical characteristics

The prepared wines were analyzed for TSS, titratable acidity, pH, rate of fermentation, ascorbic acid, carotenoids, ethanol, total phenols, total esters, higher alcohol, total sugar, reducing sugar, volatile acidity and antioxidant activity by DPPH, FRAP and ABTS methods. Various physicochemical characteristics such as TSS was measured using Erma Hand refractometer (0 to 32^0B). Titratable acidity as percent citric acid was estimated by titrating a known volume or aliquot of sample against N/10 NaOH solution (10). Rate of fermentation was calculated by determining the decrease in total soluble solids in number of days of complete fermentation. The pH was measured with Delux pH meter after calibrating it at 4, 7 and 9 pH. The total and reducing sugar were estimated by Lane and Eynon's volumetric method (10). The ascorbic acid content determined by titration method given in (11). The *β*-carotene was determined as per the method given by (10). Ethyl alcohol in the finished wines was determined by colorimetric method (12). Volatile acidity as percent acetic acid was determined by titrating the distilled aliquot with 0.025N NaOH (13). The total phenols in different wines were determined by the Folin-Ciocalteu procedure given by (14). The esters in wines were also estimated using a calorimetric method (15). Higher alcohol content in different wines was estimated as per the method described by (16). The antioxidant activity of different wines was evaluated by DPPH method according to the procedure described by (17). The ferric reducing power of wines was determined according to the method described by (18) and ABTS activity by the method of (19).

Sensory analysis

The sensory analysis of different wines using composite scoring procedure was conducted by a panel of judges. Coded chilled samples were given to judge and asked to rinse their mouth before or in between tasting the given sample. Each sample was evaluated for various quality attributes viz. colour and appearance, aroma, volatile acidity, total acidity, sweetness, body, bitterness, astringency and overall impression (13) on a prescribed Performa.

Statistical analysis of data

The data of quantitative estimation of various Physico-chemical characteristics of different wines was analysed by Completed Randomized Design (CRD), while that of sensory evaluation was analysed by the Randomized Block Design (RBD) as described by (20).

RESULTS AND DISCUSSION

Fermentation behaviour of must

The comparison of fermentation behaviour (Fig. 1) among the different treatments clearly revealed that at the initial stage (24 hour), the musts of all the treatments have witnessed fast reduction in TSS. Further, the same trend was observed in treatment $T_1N_1IC_1$ (Pulp ratio T_1 60:20:20, N_1 0.1% and inoculums concentration 2.5%) and $T_2N_1IC_1$ (Pulp ratio T_2 50:25:25, N_1 0.1% and inoculums concentration 2.5%) up to 48 hours. After that there was steady decrease in TSS and the pattern remained the same in all the treatments. After 96 hours, the trend changed clearly with the bending of curve followed by stabilization at 144 hours in wines prepared. The highest (9.2^0B) TSS was recorded in wine treatment ($T_1N_1IC_1$) prepared with pulp concentration in the ratio 60:20:20 (T_1), without nitrogen source (N_0) and 2.5% inoculum concentration (IC_1) and lowest (6.9^0B) in wine treatment ($T_1N_1IC_1$) prepared with pulp concentration in the ratio 60:20:20 (T_1), with nitrogen source (N_1) 0.1 per cent and 2.5% inoculum concentration (IC_1). Hence, it can be concluded that the treatment $T_1N_1IC_1$ has better fermentablity as compare to other treatments.

The comparison of fermentation behaviour (Fig. 2) among the different treatments clearly revealed that at the initial stage (24 hour), the musts of all the treatments have witnessed fast reduction in TSS. However, the trend remains same in treatment $T_2N_2IC_3$ till the 48 hours of fermentation. After that there was steady decrease in TSS and the pattern remained the same in all the treatments. After 96 hours, the trend changed clearly with the bending of curve followed by stabilization at 144 hours in wines prepared. The highest (8.7^0B) TSS was recorded in wine ($T_3N_0IC_3$) prepared with pulp concentration in the ratio of 40:30:30 (T_3), without nitrogen source (N_0) and 7.5% inoculum concentration (IC_3) and lowest (7.7^0B) in wine ($T_2N_2IC_3$) prepared with pulp ratio (T_2) 50:25:25, 0.2 per cent nitrogen source (N_2) and 7.5 per cent inoculum concentration (IC_3).

Rate of fermentation of pumpkin based functional wines

In (Fig. 3) the highest rate of fermentation (2.39) was recorded in wine ($T_1N_1IC_1$) prepared with pulp concentration in the ratio 60:20:20 (T_1), with 0.2 per cent nitrogen source (N_2) and 2.5% inoculum concentration (IC_1) and lowest (2.20) in wine prepared with pulp concentration in the ratio 60:20:20, without nitrogen source (N_0) and 2.5% inoculum concentration (IC_1). Temperature is responsible for growth rate of microorganism (21) and another significant variable is the concentration of fermentable sugar ranging between 125 and 250 g/l (22). The initial higher decrease in total soluble solid content is attributed to the higher fermentability of must of different treatments because of more availability of sugar and lesser ethyl alcohol in the medium. With increase in time, however, the ethanol content increased exerting inhibitory effect on the fermentability (23). Further, the behaviour of fermentation rate within the same fermentation condition is due to their individual characteristics of different treatments and the imbalance in assimilable nitrogen and sugar content of must (13, 24).

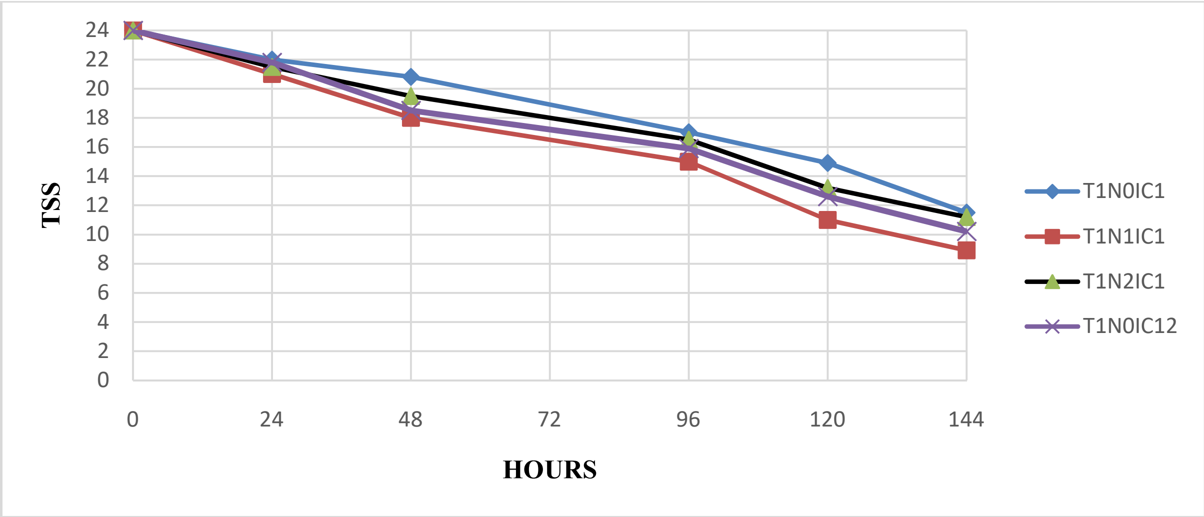

Fig 1: Comparison of fermentation behaviour of pumpkin based functional wines

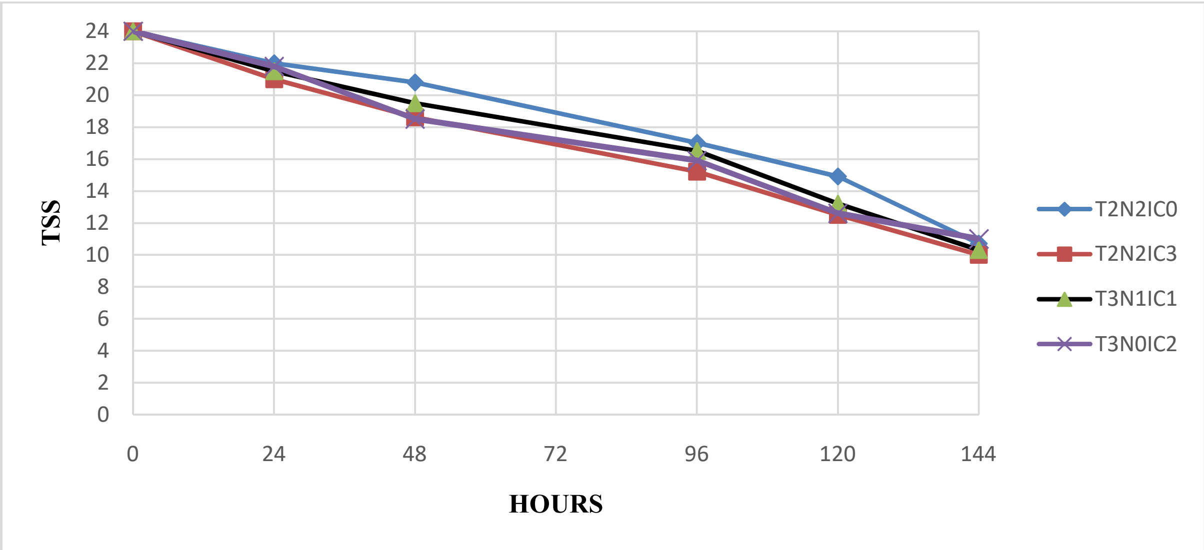

Fig 2: Comparison of fermentation behaviour of pumpkin based functional wines

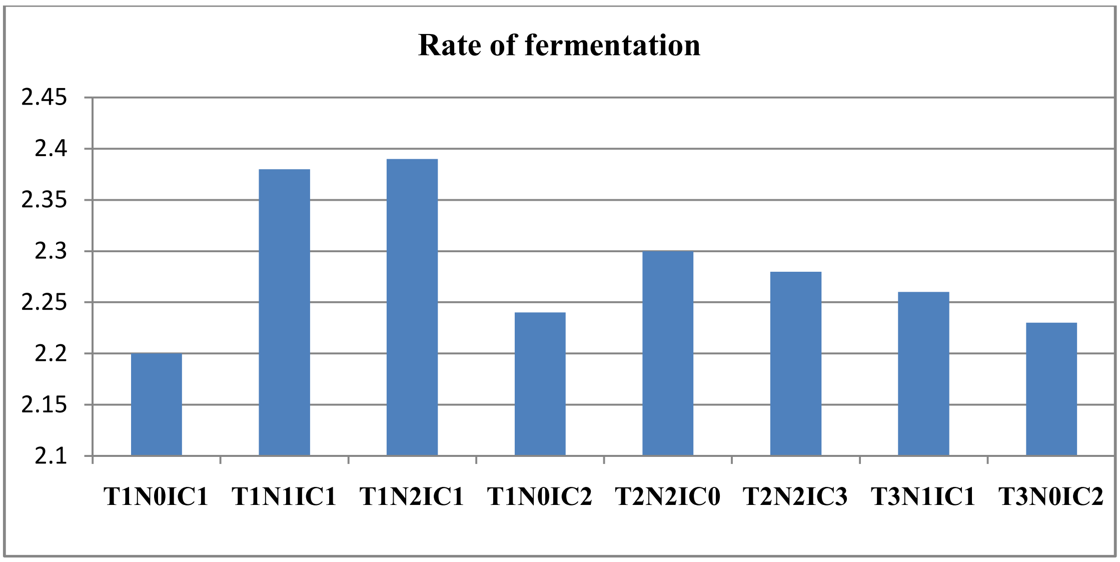

Fig 3: Rate of Fermentation of must of different treatments

Physico-chemical characteristics of pumpkin based functional wine

The perusal of (Table 1) clearly revealed that there were significant differences among the wines prepared with different concentrations of pulps, nitrogen source and inoculums concentration. Among the various treatments the TSS content ranged between 6.7 to 9.2^{0}B. It conclude that highest rate of fermentation had lowest total soluble solids and vice-versa. This is because of utilization of sugar content and production of ethanol so that higher the fermentation and lower is the TSS (25, 24, 26). The titratable acidity as per citric acid were ranged between 0.42 to 0.58 percent. The titratable acidity is an important characteristic of fruits due to its effect on taste (27). Further, the results of titratable acidity were in corroboration with pH. Higher the titratable acidity lowers the pH and lowers the titratable acidity higher the pH. The pH ranges from 2.43 to 3.10 percent. Further, the results of pH were in corroboration with the earlier result of titratable acidity. The reducing sugar ranged between 0.25 to 0.36 percent. The highest reducing sugar 0.36 percent was observed in wine ($T_3N_0IC_3$) prepared using pulp concentration in 40:30:30 (T_3) ratio, without nitrogen source (N_0) and 7.5% inoculum concentration (IC_3) and lowest 0.25 percent was observed in wine ($T_1N_1IC_1$) prepared using pulp concentration 60:20:20 (T_3) ratio, 0.1% nitrogen source (N_1) and 2.5% inoculum concentration (IC_1). The total sugar content in different wines ranged between 2.22 to 2.58 percent. The highest total sugar 2.58 percent was recorded in wine ($T_3N_0IC_3$) prepared using pulp concentration 40:30:30 ratio (T_3), without nitrogen source (N_0) and 7.5% inoculum concentration (IC_3) and lowest 0.25 percent in wine ($T_1N_1IC_1$) prepared using pulp concentration 60:20:20 ratio (T_1), 0.1% nitrogen source (N_1) and 2.5% inoculum concentration (IC_1). The low values for sugar indicates better fermentability of pumpkin based wine. This is because of utilization of sugar content and production of ethanol so higher the fermentation, lower the TSS and so reducing sugar and total sugar also depends upon TSS (25, 26). The ascorbic acid content in different wines ranged between 18.05 to 21.70 mg/100g.

The perusal of (Table 2) clearly revealed that there were significant differences among the wines prepared with different concentrations of pulps, nitrogen source and inoculums concentration. The carotenoids content ranged between 0.073 to 0.102 mg/100g. The total esters were found highest 163.7 mg/100g in wine ($T_1N_0IC_2$) prepared using pulp concentration 60:20:20 ratio (T_1), 0.2 per cent nitrogen source (N_2) with 2.5% inoculum concentration (IC_2) and lowest 152.0 mg/100g in wine ($T_3N_0IC_2$) prepared by using pulp concentration in ratio 40:30:30 (T_1), without nitrogen source (N_0) and 5% inoculums concentration (IC_2). Esters in general have fruity and floral impact, characteristics that are important in sensory property of wine. The amount of total esters in various wines normally ranged between 200 to 400mg/l (13). The total ester content of wines determines the fruity aroma and their amount have been linked with the type of fruit, fermentation conditions (13, 24). The volatile acidity ranged between 0.016 to 0.040 %. The volatile acidity content of more than 0.04 percent acetic acid indicated acetification of wines (13). Hence, from the volatile acidity point of view the wines can be considered to be sound showed proper methodology applied in preparation of wine. The alcohol content in different wines ranged between 10.30 to 11.80 % v/v. Further more from the earlier study on the pumpkin wine the alcohol ranged in between 8.68 to 12.20 % v/v (26). The alcohol content of wine of different treatments correlated with their respective rates of fermentation. Although the TSS (^{0}B) of all the must was kept 24^{0}B, the ethanol differed significantly among all the different treatments which could be attributed due to the differences in composition of other nutrients in respective musts (28) and conditions during fermentation (29). The higher alcohol content ranged between 142 to 150 mg/100.

Table 1: Effect of pulp blending, inoculums and nitrogen concentration on Physico-chemical characteristics of pumpkin based functional wines

Treatments	TSS^{0}B	Titratable Acidity (%)	pH	Reducing Sugar (%)	Total Sugar (%)	Ascorbic Acid (mg/100g)
$T_1N_0C_1$	9.2	0.42	3.10	0.30	2.43	16.00
$T_1N_1IC_1$	6.9	0.58	2.43	0.25	2.22	18.05
$T_1N_2IC_1$	6.7	0.48	2.92	0.34	2.20	19.70
$T_1N_0IC_2$	8.3	0.47	2.89	0.25	2.12	18.70
$T_2N_2IC_0$	8.7	0.54	2.69	0.34	2.52	21.50
$T_2N_2IC_3$	7.7	0.54	2.69	0.32	2.51	20.20
$T_3N_1IC_1$	7.8	0.53	2.67	0.26	2.49	21.70
$T_3N_0IC_2$	7.9	0.57	2.46	0.36	2.58	21.40
CD($P \geq 0.05$)	0.2	0.1	0.13	0.02	0.11	0.89

Table 2: Effect of pulp blending, inoculums and nitrogen concentration on physico-chemical characteristics of pumpkin based functional wines

Treatments	Carotenoids mg/100g	Esters mg/100g	Volatile acidity (% / AA)	Alcohol (% v/v)	Higher Alcohol mg/100g
$T_1N_0C_1$	0.101	156.0	0.040	10.60	142
$T_1N_1IC_1$	0.098	155.0	0.018	11.80	150
$T_1N_2IC_1$	0.092	162.7	0.024	10.30	146
$T_1N_0IC_2$	0.103	163.7	0.023	11.20	146
$T_2N_2IC_0$	0.089	157.0	0.016	10.50	156
$T_2N_2IC_3$	0.100	160.0	0.032	11.30	143
$T_3N_1IC_1$	0.102	162.0	0.032	11.70	146
$T_3N_0IC_2$	0.073	152.0	0.023	11.30	143
CD ($P \geq 0.05$)	0.02	1.5	0.006	0.05	0.08

The perusal of (Table) 3 clearly revealed that there were significant differences among the wines prepared with different concentrations of pulps, nitrogen source and inoculums concentration. The phenols content in different wines ranged between 238.0 to 291.0 mg/100. The highest phenols content 291.0 mg/100g was recorded in wine ($T_1N_2IC_1$) prepared using pulp concentration 60:20:20 ratio (T_1), 0.2% nitrogen source (N_2) and 2.5% inoculum concentration (IC_3) and lowest 238.0 mg/100g in wine ($T_1N_0IC_1$) prepared by using pulp concentration 60:20:20 ratio (T_1), without nitrogen source (N_0) and 2.5% inoculum concentration (IC_1).

The DPPH scavenging activity ranged from 36.05 to 66.47%. The highest (66.47%) antioxidant activity was recorded in wine ($T_1N_2IC_1$) prepared using pulp concentration 60:20:20 ratio (T_1), with 0.2% nitrogen source (N_2) and 2.5% inoculum concentration (IC_1) and lowest 36.05 % in wine ($T_1N_0IC_1$) prepared by using pulp concentration in ratio 60:20:20 (T_1), without nitrogen source (N_0) and 2.5% inoculums concentration (IC_1). The ABTS and FRAP value ranged between 33.60 to 51.43 % and 532 to 542 mmol (FeII/g). The results obtained were in corroboration with the earlier results of phenols as higher content of phenols also contributed for its high radical scavenging activities.

Sensory evaluation

The result on sensory analysis of pumpkin based functional wine of different treatments showed that there were significant differences for different quality attributes. The wine ($T_1N_1IC_1$) prepared with pulp concentration in 60:20:20 ratio (T_1), with 0.2 per cent nitrogen source (N_2) and 2.5% inoculum concentration (IC_1) was found superior to other treatments mainly because of better appearance, colour, total acidity, aroma, sweetness, body and overall impression, except volatile acidity, and bitterness.

Table 3: Effect of pulp blending, inoculums and nitrogen concentration on physico-chemical characteristics of pumpkin based functional wines

Treatments	Phenols mg/100g	DPPH %	ABTS %	FRAP mmol(Fe_2/g)
$T_1N_0C_1$	238.0	36.05	33.62	526
$T_1N_1IC_1$	256.0	64.12	47.90	542
$T_1N_2IC_1$	291.0	66.47	51.43	547
$T_1N_0IC_2$	270.8	46.78	38.75	529
$T_2N_2IC_0$	281.0	56.00	43.90	538
$T_2N_2IC_3$	283.9	57.20	47.43	539
$T_3N_1IC_1$	278.5	51.30	41.2	536
$T_3N_0IC_2$	273.0	48.50	39.30	532
CD ($P \geq 0.05$)	1.52	1.25	0.5	2.5

CONCLUSION

Investigations were carried out to determine the suitability of pumpkin for wine preparation. Different treatments of wines were prepared with different pulp ratio, DAHP as a nitrogen source and inoculum concentration of *Saccharomyces cerevisae*. Amongst the different treatments, the wine ($T_1N_1IC_2$) prepared with pulp concentration in 60:20:20 ratio (T_1) of pumpkin, wild apricot and pomegranate, with 0.1 per cent DAHP as nitrogen source (N_1) and 5% inoculum concentration of *Saccharomyces cerevisae* (IC_2) had many desirable characteristics like higher amount of phenols, antioxidants activity, ascorbic acid, carotenoids, alcohol, sensory scores and other comparable characteristics like acidity, pH, sugars and optimum level of volatile acidity, higher alcohol than other treatments. Therefore, for the preparation of pumpkin based functional wine pulps concentration in 60:20:20 ratio (T_1) of pumpkin, wild apricot and pomegranate, with 0.1 per cent DAHP as nitrogen source (N_1) and 5% inoculum concentration of *Saccharomyces cerevisae* (IC_2) were selected.

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