Comparative Study on Physio-chemical and Organoleptic Attributes of Apple Wine Fermented from Local and Imported Yeasts

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ABSTRACT

With home micro-brewing on the rise, use of local indigenous yeast in the fermentation process is a prevalent practice in the country. The indigenous strain found in locally processed yeast can impart certain desirable flavours and aromas to the fermented wine. Furthermore, the use of antioxidant-rich wild herbs in local yeasts is reported to enhance the nutritional value of the wine. However, local yeasts are home-made under dubious processing conditions and cannot afford the advantage of being in a pure uncontaminated microbial form, unlike the commercial strains. This might affect the fermentative capability and compromise the end quality of the wine. Thus, this study was conducted to compare the organoleptic acceptability and physio-chemical properties (total soluble solid, alcohol by volume, transmittance value, pH and titratable acidity) of apple wine fermented by two local yeasts from Trashi Yangtse and Samtse districts in Bhutan and one commercial yeast manufactured in India. Both the local yeast strains seem to yield a wine with better appearance (clarity) and taste (sweetness) based on the sensory evaluation result. In terms of fermentative capability, the commercial strain had shorter fermentation time, but the local strains were able to yield wine with similar alcohol strength to the commercial strain. For the physio-chemical parameters, only pH and titratable acidity results were significantly different with the wine from local yeast from Samtse having a significantly higher malic acid content, while the wine from commercial yeast had lower pH. However, the results deduced from the sensory evaluation does not compliment the values obtained for Total Soluble Solute and clarity measurement. Besides, the difference in pH did not resonate with the taste perception (i.e., lowest pH wine considered sweeter). Thus, other flavour components might have a role to play in the sweet taste perception. Overall, the local strains showed promising results in terms of fermentation capability (ability to convert sugar to alcohol) and organoleptic attributes of the end wine in comparison to the commercial strain.

Keywords: *Yeast, Apple wine; Physio-chemical; Organoleptic attributes*

1. Introduction

Fermentation of cereals such as wheat, barley, maize, and rice to process into alcoholic beverages has been an age-old practice in Bhutan. Some of these alcoholic beverages are *Chankey* – fermented cooked rice, *Bangchang* and *Singchang* - local beer, and *Ara*- distilled liquor Lhendup (2008). In the last few years, the National Post Harvest Center has observed a growing number of

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entrepreneurs interested in availing training and technical guidance on alcoholic fermentation from fruit juice particularly alcoholic beverages made from apple, peach, plum and apricot. Currently, the Army Welfare Project Limited, a state-owned enterprise, and Bhutan Brewery Private Limited (a unit of Tashi Group of Companies) are the two major producers and distributors of alcoholic beverages in the country.

Despite the availability of commercial alcoholic beverages in the country, home brewing of traditional beverages is still prevalent and preferred in many parts of the country (Dorji, 2012). The increasing number of people availing training on processing alcohol from surplus fruits and cereals for self-consumption is a testament to the popularity of the home microbrewery. Homebrewing of fermented liquor in the country is usually done with starter culture from the previous fermented batch or with a circular disc of local yeast starter. These yeast starter discs are processed locally in certain regions of the country. The locally cultured yeasts prevalently known as *pham* or *phaw* in the local dialect are reported to be integral to the quality of fermented beverages in Bhutan (Namgyel, 2009; Yudon, 2015).

The *Pham or Phaw* is usually made from cereals powder and grits (maize and wheat), rice husks, and dried wild herbs. The mix is inoculated with a mother starter culture by adding yeast made from a previous batch. The dried mixture is hydrated with water and kneaded into a dough; the dough is then shaped by hand into a flattened circular disc. The circular disc is either strung from a string or left on shelves above traditional mud oven to provide a warm environment for the culture to thrive and multiply. This is essentially the incubation phase followed by air drying phase. During the incubation phase, the nutrient-rich disc is exposed to the probable microflora of wild yeast (indigenous non-Saccharomyces yeast) present in the air, this exposure could lead to the contamination of the disc. Due to lack of microbiological analysis to identify the strain of yeast; at this juncture, the *pham* can contain either wild yeast or Saccharomyces or both. Due to this uncertainty, the yeast in the *pham* will be referred to as indigenous yeast strain in this paper.

Wild herbs are considered one of the most vital ingredients in the *pham.* Often wild herbs are collected from the forest, dried and added to the wheat and maize powder. These wild herbs are locally known as *bainang yangrim*, *chong yang rim* (Yudon, 2015) and *khagai numba/ru yangrim* (Bhutan Cultural Atlas, 2020). *Bainang yangrim* is identified as *Hedyotis pinifolia, chong yangrim* as *Buddleja asiatica* (Grierson & Long, 1983, 1984) and *khagai numba/ru yangrim* as *Hedyotis scandens* (Thinley, 2010*)*. Wangchuk, Yeshi, and Jamphel (2017) have also documented the use of *Buddleja bhutanica* (*Scrophulariaceae*) leaves also known in traditional Tibetan medicine (*Sowa Rigpa*) as *Chang-rtsi* in yeast making. For this study, *Hedyotis scandens* was added to the local yeast from Trash Yangtse (Jigme, personal communication, January 5, 2020) and *Buddleja asiatica* in the local yeast from Samtse (K. Norbu, personal communication, January, 6, 2020). As per Arjun, Verma, and Prasad (2014) extracts from both the plants are used in a similar manner as growth supplements for yeast prepared for fermenting *judima:* a local alcoholic beverage prepared

by the *Dimasa* tribes of Assam in India. As per the study, the plant extracts were found to be a good source of antioxidants namely polyphenols, alkaloid and flavonoid.

Yeast is integral to the fermentation of alcoholic beverages because of its ability to convert sugar into alcohol (ethanol), carbon dioxide, and minor metabolites (Boulton, Singleton, Bisson, & Kunkee, 1999). These minor volatile metabolites are derived from the fruit as well as from the metabolic activity of the yeast. The yeast-derived metabolites are mainly organic acids, esters, alcohols, carbonyl compounds and fatty acids. These metabolites are sensorially significant and play an important role in defining the flavour and aroma of the wine (Dubourdieu, Tominaga, Masneuf, des Gachons, & Murat, 2006). Indigenous yeast contains a diverse group of yeast strain and these diverse microflora produces a plethora of secondary metabolites which makes up a complex bouquet of flavour and aroma (Lambrechts & Pretorius, 2000). The diversity of the microflora is dependent on the geographical location and climatic conditions, so wine fermented with indigenous yeast often imparts a unique flavour and aroma attributes characteristic of a particular region (Eder, María, Reynoso, Lauret, & Rosa, 2017). The floral flavour and an aroma characteristic of the wine produced in Muscat or the volatile fruity wine of the Sauvignon are all examples of how the diverse indigenous microflora native to the afore-mentioned regions impart a unique taste and aroma profile to the wine (Dubourdieu et al., 2006).

Despite the desirable complexity in the flavour and aroma produced by the indigenous yeasts, it is also imperative to understand that indigenous yeast could be unpredictable resulting in the brewer having lesser control over the fermentation. This unpredictability of the indigenous yeast will lead to inconsistent quality of the final product. Moreover, unhygienic conditions during the local yeast making process and unprotected condition of storage lead to the presence of contaminants such as dirt, dust, insect and hair particles in the *pham* proving detrimental to the safety and quality of the end fermented product. Thus, starter culture of selected commercial strains of Saccharomyces is used to ensure consistency and predictability in the wine quality. *Saccharomyces cerevisiae* is the most commonly used yeast strains commercially available for alcoholic fermentation (Andorrà et al., 2019). This particular strain is preferred for brewing because of its ability to tolerate high alcohol content, anaerobic condition and ability to convert sugar into alcohol. However, widespread commercial use of this strain often leads to a monotonous repetition of flavour and aroma, stripping off the uniqueness that is desirable in wine. Winemakers still argue that commercial strain does not add to or enhance the regional character of the wine and some still prefer spontaneous fermentation with indigenous yeast over the use of commercial strain (Rainieri & Pretorius, 2000).

Physiochemical parameters such as TSS (Total Soluble Solids), pH (hydrogen ion concentration), titratable acidity and ABV% (Alcohol by Volume %) can give important insights into the organoleptic attributes and fermentative capability of the yeast. These parameters may differ with different yeast strains because of the specific metabolic pathway unique to the individual strain

(Sharma, Singh, & Sawant, 2012). Organoleptic acceptability can be assessed through sensory evaluation of the end product by a panellist.

TSS gives a measure of sugar content and thus can be used as an indicator for sweet taste in wine. The alcohol content will contribute to the mouthfeel and after taste of the wine. Acid content and strength also have an important bearing on the flavour perception in wine (Chidi, Bauer, & Rossouw, 2018). The acidity in wine is due to the presence of dissociated and un-dissociated organic acid in fruit. Malic acid is the predominant organic acid found in apple followed by other organic acids such as citric, quinic glycolic, succinic, lactic, galacturonic and citramalic (Dharmadhikari, 1996). Titratable acidity and pH are the two important variables that express acidity in the wine. The pH scale is used to measure the alkalinity or acidity of a solution and is calculated as the negative of the base-ten logarithm of the molar concentration of hydrogen ion released by the dissociation of organic acids. It is measured on a 1-14 scale with 1 being highly acidic, 7 neutral and 14 being highly alkaline. The scale is logarithmic, meaning there is a difference of 10 times in 1 pH unit. On the other hand, titratable acidity measures the potential hydrogen ion than can be dissociated. It is measured by titration with a strong base (usually sodium hydroxide) to a neutral endpoint pH of 7.00 and is expressed as mg of organic acid per litre of sample. Thus, pH measures the strength of the acid (hydrogen ion concentration) and titratable acidity measures the concentration of acid (Comuzzo & Battistutta, 2019).

With the prevalent use of *pham* to ferment alcohol in the country, it is important to study the fermentative capability of the local *pham* and the end quality of the fermented alcohol in comparison to that of commercial strains of *Saccharomyces cerevisiae.*

Thus, the objectives of this study were:

a) to compare the physio-chemical properties of wine fermented by two different local yeast made in Bhutan and a commercial yeast from India.
b) to study if the yeast has attributes that contribute to the difference in sensory acceptability of the wines.

2. Materials and Methods

The experiment was conducted in the food analysis laboratory at the National Post Harvest Center (NPHC) of the Department of Agriculture. Apples stored in the cold store at 4 ^{0}C were washed in 0.1 % water solution of potassium metabisulphite. All the utensils and equipment used were rinsed with potassium metabisulphite solution to reduce the possibility of contamination by other microbes. The juice extraction was done in two stages: first in the juice pulper to extract juice from raw apples. Crushed apples and juice were obtained from the pulper. Further, the crushed apple flesh was squeezed in the hydraulic juice press to extract the remaining juice. The TSS of the juice was adjusted to 22 ^{o}Brix by addition of sugar. Pectinase and amylase were added at 0.03 % to clarify the juice. The material preparation method was adapted from Kanwar and Keshani (2016). The local yeast from Trashi Yangtse (LYT) was obtained from a shop in Trashi Yangtse, local

Yeast from Samtse (LYS) from a shop in Baangdey, Paro, and the Indian commercial yeast (CYI) from an Indian supplier. Both the LYT and LYS were crushed and sieved to remove unwanted dirt and dust particles. The CYI was in powdered form and was used directly. The yeasts were inoculated at 0.3 % in the juice. Potassium metabisulphite at 0.01 % was added to prevent contamination by bacteria and moulds.

After one week, the wine was racked to separate sediments (lees) and flocculation from co-precipitated tannins and protein to prevent off flavour development (Jackson, 2008). Each treatment was replicated three times and each replication weighed 10 litres. The TSS and pH were measured each day until the values for the measured parameters remained unchanged for 48 hours. This marked the end of fermentation whereby there is no further conversion of sugar to ethanol (Nout, 2014). The TSS for the samples were measured by a digital handheld refractometer (Atago PAL 3, 0-93% range with temperature compensation) and the TSS is expressed as degree Brix. The refractometer was calibrated before use with distilled water as blank to give a measurement of zero-degree Brix. The pH was measured by a handheld digital pH meter (EcoTestr pH1, Range – 0.00- 14.00). Since the predominant organic acid in apple is malic acid, the total titratable acidity was measured and expressed in terms of malic acid with titration done against 0.1N NaOH using phenolphthalein as an indicator (AOAC, n.d.).

The alcohol percentage was measured in ABV%, i.e., the volume of ethanol in litres per 100 litres of wine measured at 20 °C and expressed as % vol. The OIV-MA-AS312-01A method was used to obtain the distillate from the sample and an alcoholmeter was used to obtain the reading. The actual % ABV was obtained from the standard alcohol temperature correction table. An advanced microprocessor UV-VIS Single Beam Spectrophotometer (LI-295) was used to measure the transmittance value of the fermented wine at 660 nm with distilled water as blank. The blank was used to calibrate the spectrophotometer to give a value of 100 % transmittance or 0 % absorbance (Berutu, Fahrurrozi, & Meryandini, 2017).

Descriptive test and sensory evaluation were done on taste, colour, aroma, clarity, mouthfeel, and alcohol strength and after taste on the fermented wines. The 5-point hedonic rating scale (Table 1) adapted and modified from Jones, Peryam, and Thurstone (1955) was used to evaluate and score the samples.

Table 1. Five-Point Hedonic Scale.

Hedonic rating	Score
Like extremely (LE)	5
Like moderately (LM)	4
Neither like nor dislike (N)	3
Dislike moderately (DM)	2
Dislike extremely (DE)	1

A total of 36 panelists comprising 21 females and 15 males performed the sensory evaluation and the descriptive test. For the descriptive test, subjective descriptors such as for taste (sweet, neither sweet nor sour or sour), colour (pale yellow, darkish yellow or brown), aroma (weak or strong), clarity (clear to hazy), mouth feel (thin to full), alcohol strength (low to high) and after taste (mild to harsh) were used.

One-way Analysis of Variance (ANOVA) and cross-tabulation were done to study if the yeast type had a significant effect on the sensory evaluation scores and physio-chemical parameter of the fermented wine. The analysis was carried out using the Statistical Package for Social Science software (SPSS). P values ≤ 0.05 were considered significant in all the analyses. The mode of the scores for sensory evaluation was taken as the representative score for each attribute.

3. Results and Discussion

3.1. Total soluble solids

Total soluble solids (TSS) is the measure of the total amount of solids soluble in a sample (Garner, Crisosto, Wiley, & Crisosto, 2008); thus TSS was used to express the total amount of sugar in a solution expressed as degree Brix. The concentration of TSS was determined by passing a light through the sample and measuring the refractive indices (a measure of the bending of light rays in a medium) with a refractometer. During the fermentation process, the TSS value is expected to drop as the yeast converts sugar into alcohol and carbon dioxide. Figure 1 shows a sharp drop in TSS at the beginning of the fermentation until the 10th day after which the TSS dropped at a slower pace, and after the 16th day, there was not much change in the TSS.

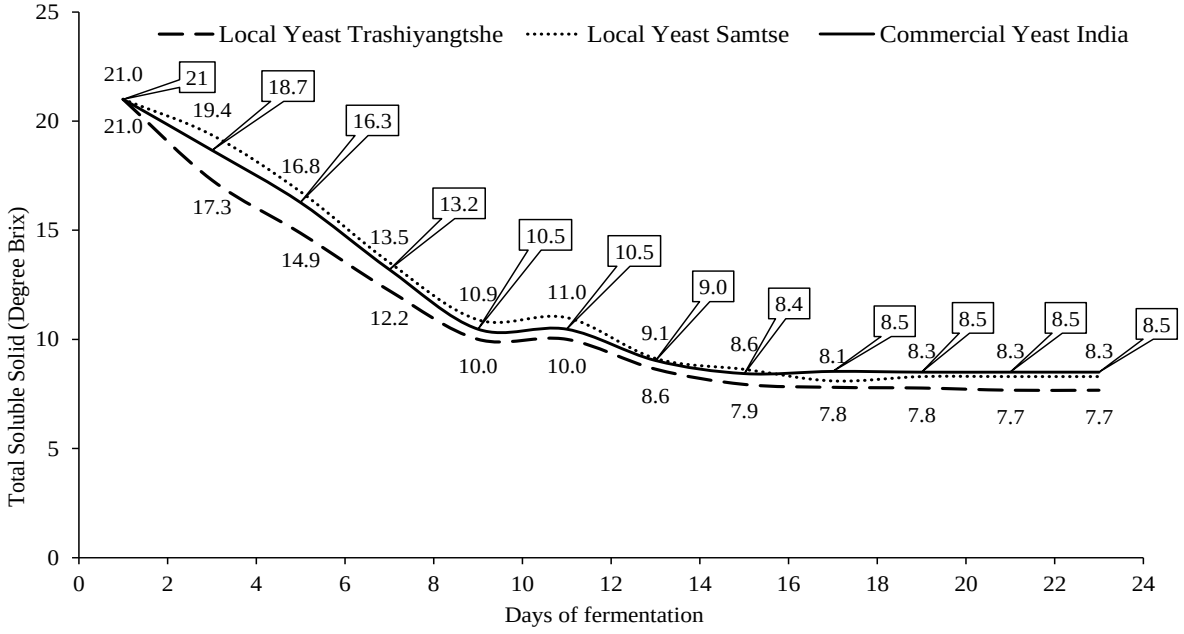

Figure 1. Average TSS in wine fermented by the three yeast strains over 23 days.

With all three yeast strains, there is a decline in the TSS reading with time, this decline is more pronounced at the beginning of the fermentation as is evident in Figure 1. This stage is called the primary fermentation stage. This decline in TSS is due to the alcoholic fermentation where the yeast feeds on the sugar and releases ethanol, carbon dioxide, energy and other minor metabolites. The amount of sugar present in the juice thus decreases and the ethanol content increases with time. As fermentation continues, increase in alcohol content and the decrease in pH suppresses the activity of yeast to convert sugar to alcohol and carbon dioxide. As a result, the decline in TSS is at a slower rate and eventually stops and this signals the end of the fermentation period (Nout, 2014).

The CYI have the shortest fermentation duration of 17 days, followed by 19 days for LYS and 21 days for LYT. All the three yeasts exhibit a similar fermentation pattern as is indicated in Table 2, where the TSS of these wines from all the threes yeasts is not significantly different.

Table 2. One-way ANOVA and post hoc Tuckey test (values expressed as Mean ± Standard error).

	Total Soluble Solute (ºBrix)	**Alcohol by Volume (ABV%)**	**Transmittance value**	**Titratable acidity (malic acid gm/L)**	**pH**
Local Yeast Samtse	7.66±0.71	11±0.57	90.76±0.95	3.7±0.00[a]	3.8±0.00 [b]
Local yeast Trashi Yangtse	8.30±1.00	10.66±0.88	89.50±2.79	3.2±0.01[b]	3.7±0.03[b]
Commercial Yeast India	8.50±0.30	10.00±0	93.5±0.72	3.1±0.00[b]	3.9±0.00[a]

Means within a column with different superscripts differ significantly ($P \leq 0.05$)

3.2. pH and titratable acidity

The Post Hoc Tuckey test revealed a statistically significant difference in the measurement for titratable acid content ($F(2,6) = 7.341$, $P = 0.024$). The test showed that the wine fermented from LYS have significantly higher titratable malic acid content as compared to wine fermented from LYT and CYI (Table 1).

In terms of pH, the wine from CYI had significantly higher pH (F (2, 6) = 13.00, P = 0.007) as compared to the other two wines meaning the wine from LYS and LYT are comparatively more acidic than the CYI wine. A difference of 0.1 (=pH CYI – pH LYT) and 0.2 (=pH CYI – pH LYS) As per Murrell (2011) a difference of 0.1 pH and 0.2 pH unit translates to a solution being 1.3 and 1.6 times more acidic; thus, the wine from LYS and LYT are 1.3 and 1.6 times more acidic than CYI.

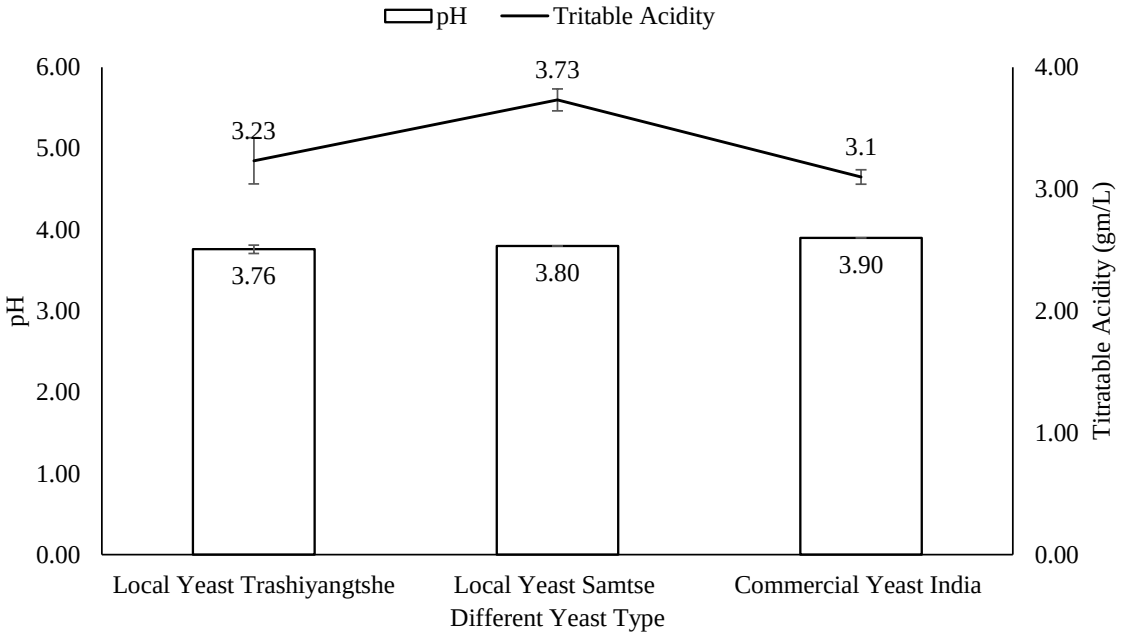

Figure 2. Average titratable acidity (gm malic acid per 100 ml of sample) and hydrogen ion concentration (pH) of the wine fermented by LYT, LYS and CYI.

3.3. Alcohol % by volume (% ABV)

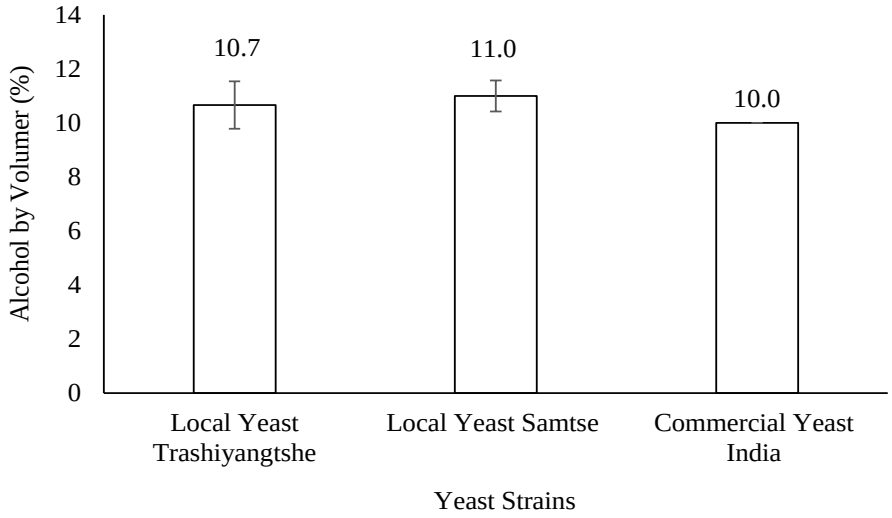

Figure 3. Average Alcohol by Volume (%) in wine fermented by LYT, LYS and CYI.

The highest average ABV% was 11 % found in the wine from LYS, followed by 10.7 % for the wine from LYT and 10 % for the CYI. However, this difference is not statistically significant as is evident from Table 2.

3.4. Clarity expressed as transmittance value

Transmittance value (expressed as percent transmittance) measured in spectrophotometer is the amount of light that passes through the sample (Garner et al., 2008) hence this value was used as a measure of clarity. The wine from CYI had the highest transmittance % followed by the wine from LYT and LYS. However, this difference is not statistically significant (Table 2).

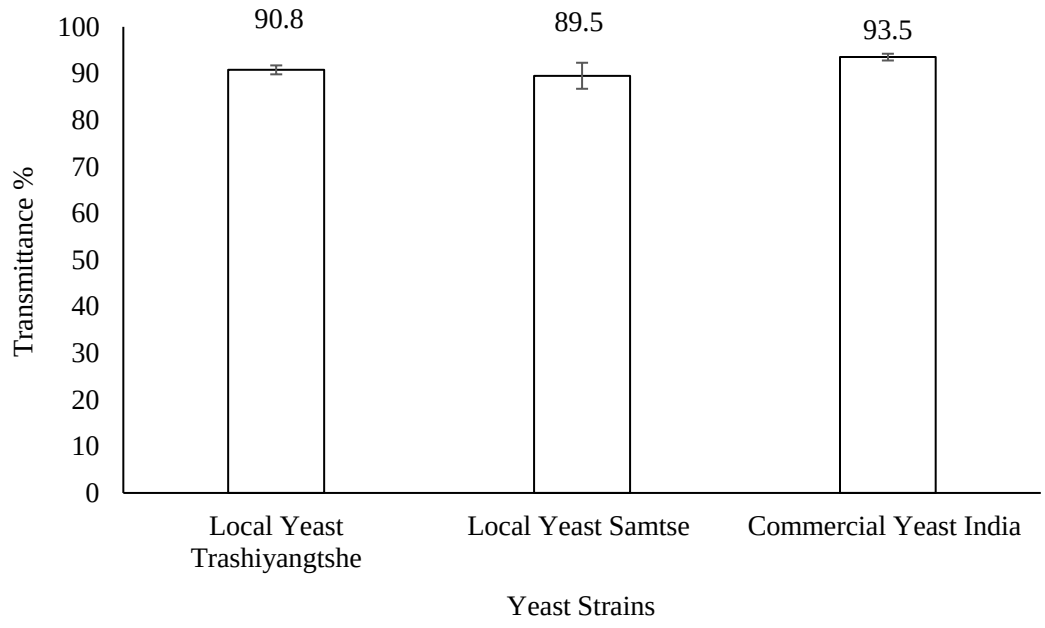

Figure 4. Transmittance% in wine fermented by LYT, LYS and CYI.

3.5. Sensory evaluation

A crosstab was done on SPSS to determine the mode to establish the most frequently occurring score as is given in Table 3. Since the mode is the most common score that majority of the panellist gave for each wine, it was used as the final scores for the attributes of different wine as presented in Figure 5. The highest percentage of panellist is highlighted in Table 3 and the score corresponding to the highest percentage is taken as the final score.

Table 3. Cross of the percentage of panellist and their hedonic scores.

	Local Yeast Trashi Yangtse					Local Yeast Samtse					Commercial Yeast India				
	DE	DM	N	LM	LE	DE	DM	N	LM	LE	DE	DM	N	LM	LE
Taste	9	15	15	29	32	6	12	24	53	6	3	9	21	50	18
Aroma	6	13	16	42	23	6	12	26	41	15	6	12	30	39	12
Clarity	3	3	28	44	22	3	9	29	26	32	3	0	39	48	9
Mouthfeel	3	16	19	38	25	3	18	21	56	3	3	9	27	48	12
Alcohol strength	3	16	16	59	6	3	6	21	59	12	3	6	18	70	3
After taste	3	13	19	45	19	6	6	27	39	21	3	9	30	42	15

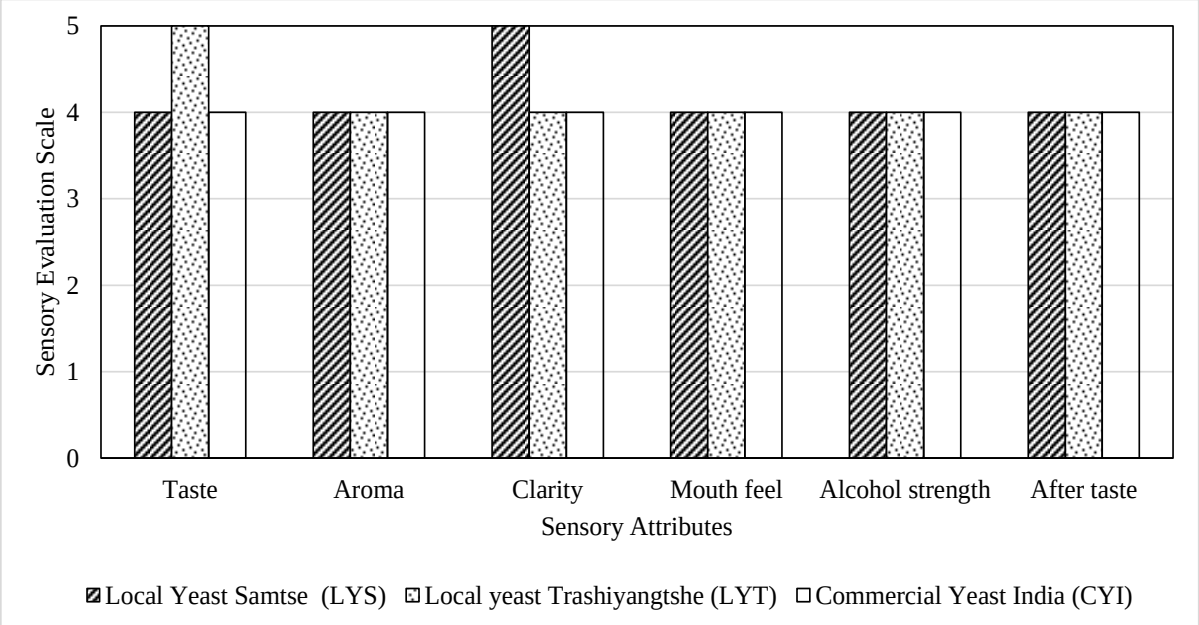

Figure 5. Horizontal column graph of the sensory evaluation score.

Majority of the panellists scored “liked moderately” in all the attributes for all the three wines except for clarity and taste (Figure 5). LYS wine had the highest percentile i.e., 32 % of the panellist gave “extremely liked” score to the clarity attribute. Majority of the panellist i.e., 78 %

described the LYS wine to be clear. LYT wine had the same result with 32 % of panellist giving "extremely liked" score to the taste attribute. The descriptor sweet for taste attribute was used by the majority of the panellist i.e., 53 %.

3.6. Discussion

Based on the result of the one-way ANOVA and Tukey's test for post hoc analysis (Table 2), there is no significant difference in the TSS, ABV% and Transmittance %. In terms of fermentative capability, the CYI had a shorter fermentation time followed by the LYS and LYT. However, no significant difference was found in the final ABV%. Commercial yeast strains are well defined pure yeast culture selected because of their superior fermentative capacity and hence often are considered better in terms of fermentative capability (Steensels et al., 2012). In this study though the CYI had shorter fermentation time, the local strain produced wine with similar alcohol strength to the CYI. Thus, in terms of the ability to convert sugar into alcohol, the performance of all the three strains is at par with each other.

However, there is a statistically significant difference in the measurement for titratable acid content with the wine from LYS having higher titratable malic acid content as compared to wine fermented from LYT and CYI. This result agrees with the interpretation of pH measurement whereby the LYS had the highest acidity and CYI the lowest. The LYT wine was extremely liked for its sweet taste by the majority of the panellists; however, there is no significant difference in the TSS amongst all the three wines. Therefore, it can be speculated that the TSS in this case did not have any bearing on the perception of sweet taste. However, the pH value records a significantly lower value for LYT which can be interpreted as the wine being sourer than the other two. The TSS and pH measurements contradict the perceived taste of sweetness, and thus there might be factors other than TSS and acidity attributes responsible for the perception of the sweetness in the wine. The use of the wild herbs in the local yeast might be a contributing factor towards the perceived sweet taste in the wines processed from local yeast. However, as there is no prior research done on sensorially significant compounds in the aforementioned wild herbs it is difficult to establish their impact on the final sensory attribute of the wine.

Lambrechts and Pretorius (2000) have reported the effect of different yeast strains on production and accumulation of succinic acid and phenolic compound in wine which can impart a bitter and astringent taste. It can be speculated that the presence of succinic acid and phenolic compound in the other two wines had an impact on the panellists perceiving the other two wine as less sweet than the LYT. A similar finding was also reported by Bandić et al. (2018). Furthermore, Marchal, Marullo, Moine, and Dubourdieu (2011) reported on the possible contribution of yeast autolysis phenomenon and subsequent production of Hsp12p strain of protein on the sweetness on wine. Since the study of these factors was beyond the scope of this work, one can only speculate on the plausible causes. The result of the transmittance value gave no significant difference amongst the three wines, yet the panellists seemed to prefer the clarity of the LYS. Since the sensory evaluation

is subjective in nature the reason why the panellists preferred wine from LYS in terms of clarity is inconclusive.

4. Conclusion

The study found that the local yeast strains fermented apple wine with better sensory acceptance especially in terms of taste and clarity attributes. This finding is in agreement with the previous studies that have highlighted the impact of using indigenous strains on the development of desirable flavour and aroma in the wine. In terms of fermentation capability, though the local strains take longer time to reach the end fermentation point, the alcohol strength of the end wine is not significantly different from the commercial strain. The fermentation capability to convert sugar to alcohol is similar in the commercial and local strains despite the commercial strain having the advantage of being adaptively selected and cultured in pure form. It is possible to enhance the fermentation capability if the local strains are to be cultured in the pure form under controlled conditions. The literature reviewed also reveal that the use of wild herbs in the local yeast enhances the antioxidant content of the wine. Thus, it can be concluded that local yeast strains have comparable fermentative capability with the potential to possibly surpass commercial strains if it is cultured in pure form like commercial strains. In terms of organoleptic attributes, the local strains yield a wine with better acceptability and with an enhanced nutritional value from the addition of the wild herbs.

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