

Research Paper

Influence of Addition of Apple Insoluble Solids, Different Wine Yeast Strains and Pectinolytic Enzymes on the Flavour Profile of Apple Wine

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Abstract

Different levels of insoluble solids (ISS) from apple juice were added to the apple juice must along with wine yeast strains of *Saccharomyces cerevisiae* and pectinolytic enzymes to study their effect on sensory quality of wine. The flavour profile of the apple wine of different treatments by quantitative descriptive analysis (QDA) showed that the wine without ISS was rated better in most of the flavour characteristics studied than those with ISS. Addition of insoluble solids imparted flavour quality characteristics of the wine which are not considered desirable. Out of various wine yeast strains, W and USD 595 performed better than UCD 505 and UCD 522. The wines without ISS were perceived having highest apple aroma, least cooked vegetables aroma, lower sweetness, lesser bitterness and lower higher alcoholic flavour. Both the addition of wine yeast strains and enzymes improved the flavour profile of the wine and from this point of view, wine made from presettled juice possessed improved flavour characteristics of the wine. Application of PCA weekly separated the wines fermented with or without insoluble solids. The flavour profile did show some interactions between insoluble solids, yeast strains and the enzymes which modified the flavour attributes of the resultant wine.

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Keywords: Apple, Insoluble solids (ISS), Pectinolytic enzymes, Flavour profile, Wine, *Saccharomyces cerevisiae*, yeast

Introduction

Quality of the product cannot be compromised. Clarity of wines is an indispensable quality parameter, which is achieved by racking/ filtration or use of pectolytic enzymes in grape juice for wine preparation (Ough and Crowell, 1979). However, Schanderl (1959) reported that the musts which were cloudy, naturally or artificially, fermented much more rapidly than those musts which had been clarified.

Clarification of red and white wines with pectic enzymes had reportedly slowed down fermentation (Ough *et al.*, 1975) and thus, increasing the chances of a stuck wines (unfinished fermentation). Though pectolytic enzymes have been used for clarification of grape juice and wines (Ough and Berg, 1974; Williams *et al.*, 1975; Ough and Crowell, 1979) it is believed that it removed/lowered the sensory characteristics (Ough and Berg, 1974). Bardiya *et al.* (1974)

reported improvement in quality of wine prepared from guava juice without pectinase. Liu *et al.*, (1987) concluded that wines from clarified musts were generally preferred to those from non-clarified juices and the wines made from unclarified juices tended to be higher in fusel oil content but low in methanol content. Servili and Montedoro (1988) reported that among the factors examined, the must clarification effected the quality of the resulting wine to the highest extent.

Studies of Bobkova (1968) and Singleton *et al.*, (1975) have indicated that the less turbid musts produced wines of better quality. In some countries like West Germany, France and South Africa, bentonite is commonly added to the white grape musts before fermentation which markedly improved the quality and stability of the wine (Keshkovskii *et al.*, 1976). Addition of different levels of ISS to the clarified grape juice caused fermentation to finish faster than in controls but generally increased volatile esters and fusel oils also without affecting the wine quality (Groat and Ough, 1978). The must containing lower levels of grape solids produced wines with correspondingly higher levels of residual sugar, lower fusel alcohol and higher esters. Similarly, under anaerobic conditions, the level of fusel oil increased sharply when grape pulp was present in fermenting medium (Crowell and Guymon, 1963). Wine made without ISS attained higher levels of decanoic acid during alcoholic fermentation than treatment with ISS, yeast ghosts, grape solids and yeast solids (Edward *et al.*, 1990). But, all of these studies are on the grape wine (Houtman *et al.*, 1980, Houtman and Dulessis, 1981) and no report on sensory quality of apple wine is available.

To profile flavour analytically, the technique of descriptive analysis (DA) has been applied to a variety of beverages, including wines (Noble, 1978; Schmidt, 1981) and cider (Williams, 1975). Wu *et al.* (1977) evaluated 86 descriptors used to characterize the wine and found 33 having the greatest meanings. Apple juice odour (aroma) has been described as multidirectional sensation while that of apple juice essence had been described by a panel in Switzerland using 18 terms, including pungent, grass-like, pomace like, solvent like, floral, etherish, almond like, cooked apple like, alcoholic, fusel oil like, heavy, rancid, oily, soapy, stuffy and rotten (Durr, 1979). Noble and Shannon (1986) characterized the Zinfandel wines by flavour profiling techniques. Canal-Llauberes (1990) developed a pectinases preparation which contained α -glucosidase, x-

arabinosidases and x-rhamnosidase activities and its use made the wine more aromatic and with better flavour as revealed by the sensory evaluations.

The PCA was successfully employed to determine the extent and significance of differences detected giving the pictorial representation. The data from flavour profile techniques could be subjected to the analysis by multivariate analysis such as Principal Component Analysis (PCA) and the wines could easily be grouped. Effect of addition of insoluble solids, pectin esterase enzyme and yeast strains on sensory quality was investigated in apple wine and the results obtained are described in this communication.

Material and Methods

Preparation of apple wine with different treatments

To study the effect of insoluble solids on the fermentation behavior, physico-chemical and sensory characteristics of apple wine, different combinations of yeast strains and insoluble solids were tried. The level of Insoluble solids (ISS) viz., 2.5 per cent, 5.0 per cent, 10.0 per cent along with control (no addition of ISS), four yeast strains i.e. W, UCD 505, UCD 522 and UCD 595 with and without enzyme was employed and the fermentation was conducted with the respective yeast as described earlier. Sediment in bottles of processed apple juice was taken out and used as the natural source of insoluble solids. All the treatments were replicated twice. The final total soluble solids (TSS) of the musts were adjusted to TSS of 24°B with cane-sugar syrup. The other details of fermentations were the same as outlined by Joshi and Bhutani. 1991. After the completion of fermentation, the wines were racked, filtered and filled in 200 ml bottles keeping 2.5 cms head-space, followed by crown corking and mild pasteurization. After aging for a year, the flavour characteristics of the wines were estimated by the quantitative descriptive analysis profile technique.

Flavour profiling

One year matured wines were used for sensory analysis by descriptive analysis as detailed earlier (Joshi, 2006). The judges were got familiarized in the training session with different wines and the standards prior to flavour profiling session. The various descriptors used were chosen as described (McClellan *et al.*, 1983., Sharma and Joshi, 2004). Discussion was allowed only in the familiarizing session. Five judges participated in the sensory evaluation session. The judges evaluated the wines in triplicate at different times. No wine was presented to the

judges in the combination of more than once. The judges were asked to evaluate the wines for different terms called as descriptors, and to award score (1-9) depending upon the intensity of flavour tested, in comparison to the standard whose intensity was rated as the highest (9 score). Evaluations were carried out in the isolated booths at the room temperature. During the session, the panelist used plain water to rinse their mouth in-between the wine tasting session(Clapperton and Piggot 1978). The wines were served in tulip shaped wine glasses, covered with glass dishes.

Principal component analysis

The data of flavour profiling were first assessed by the analysis of variance using three factors Randomized Block Design (RBD) for significance of differences between the treatments, performance of judges and the significance of attributes. Names of only significant terms were used for principal component analysis (PCA). The statistical computer program was run on a personnel computer in the computer centre of Dr. Y S Parmar UHF Nauni, Solan (HP) India using PCA, BAS computer package (Ludwig and Reynolds, 1988). Various descriptors, treatments and the scores constituted the data. The output was obtained in the form of principal component (First three) for the treatments (Species) and attributes (Sampling units), correlation coefficient matrix and eigen vectors. The analysis was performed without rotation. The values for principal components (PC) for attributes as vectors and principal component values for the wines of different treatments obtained by fermentation by different sources of fermentation were plotted simultaneously. The interpretation of data from PCA was made accordingly. The means of different descriptors were plotted as spider web diagrams with respect to the insoluble solids enzymes or yeasts.

Results and Discussion

Descriptive analysis profiling of apple wine

The means of scores of flavour awarded to the wine of different treatments were plotted in form of spider web. Figure 1 shows that the wine without insoluble solids had the highest apple aroma, least cooked vegetable odour, lesser sweetness, more astringency less sourness, comparable vinegary, sharpness, ethanolic flavour, less bitterness and low higher alcoholic flavour. Thus, the addition of the insoluble solids to the must deteriorated the

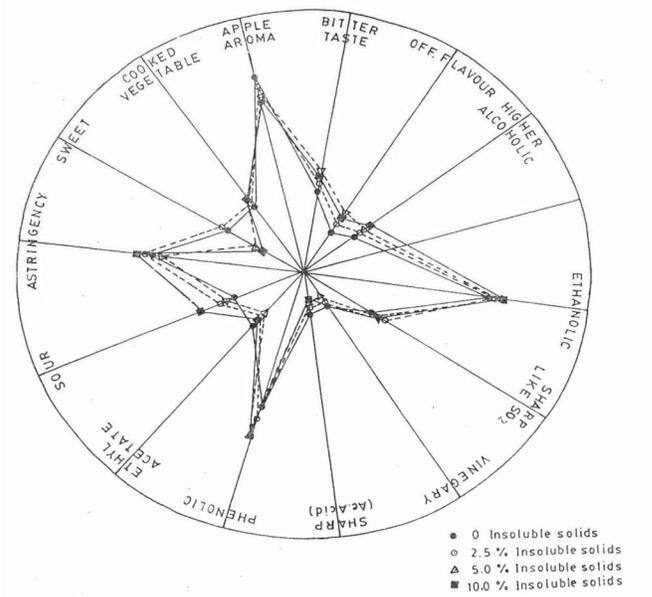


Fig. 1: Quantitative Descriptive Analysis Profile of apple wine as effected by the addition of insoluble solids

quality of the wine and presettled juice is preferable to the unclarified or unsettled apple juice for wine preparation.

The type of yeast employed did effect the flavour profile of wines to greater extent (Figure 2). Further, the results showed that yeast strain UCD 595 and W gave comparable but better profile than UCD 505 and UCD522. The most effected descriptors were astringency, ethyl acetate, higher alcohol, cooked vegetable, ethanolic and phenolic and the least effected descriptors were sweet, sour and vinegary sharpness. Thus, the yeast strains have effected the flavour profiling appreciably and could be used to improve upon the quality of apple wine.

Figure 3 shows that the addition of enzyme also effected the various flavour descriptors. The enzyme addition clearly improved the apple aroma, astringency, ethyl acetate and reduced that off flavor, bitter taste, higher alcoholic, ethanolic, cooked vegetable and sweetness which was also desirable. Flavour descriptors like vinegary, sharp, phenolic, virtually remained unaffected by the addition of enzyme and proved quite beneficial, irrespective of insoluble solid addition or the types of the yeast strain used.

Principal components analysis: Principal components analysis was applied to the data from flavour profiling of

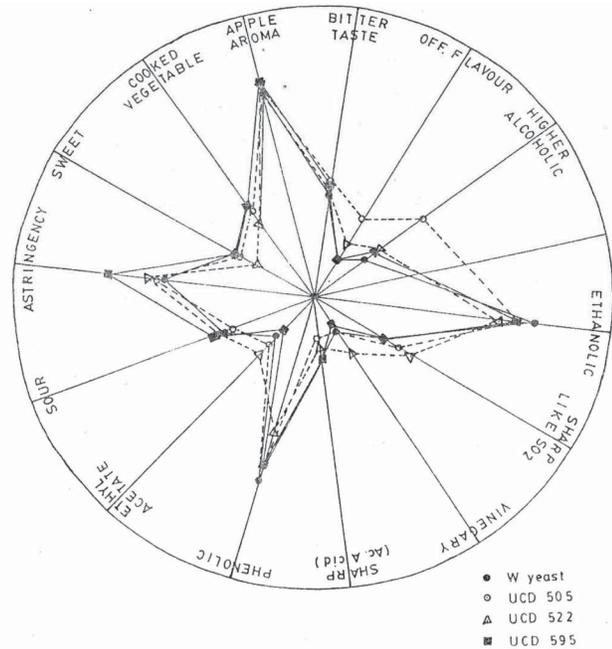


Fig. 2: Quantitative Descriptive Analysis Profile of apple wine as effected by the yeast types

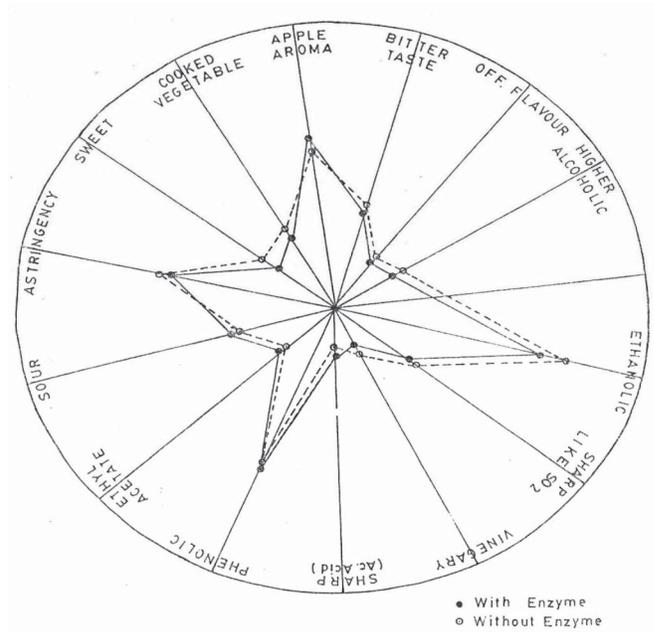


Fig. 3: Quantitative Descriptive Analysis Profile of apple wine as effected by enzymes

apple wine as effected by (yeast, insoluble solids, enzyme addition). Different attributes were plotted as vectors and along with wines simultaneously for characterization, using the treatment and different descriptors.

When PCA was applied to the correlation matrix obtained from means of flavour profiling data, comprising 14 terms, the eigenanalysis (Table 1) showed that first 2 PCs were more important, though the values of third was also near 1. According to the Kaiser’s criterion applied to determine the importance of Principal components. The eigenanalysis showed that 3 PC could account for 84.90 per cent of variations. It is clear from Fig 4 using 32 wines and 14 attributes that first PC was related greatly with phenolic, apple aroma, ethanolic and sharp (SO₂), sweet, amyl alcoholic, acetic and estery flavour attributes and with astringency and sour to a small extent. The position of the wines on the 2nd component was defined largely by bitterness, cabbage and fusel alcohol flavour. However, no clear grouping or separation took place.

Table 1: Principal component analysis output (Summary of Eigen analysis) of apple wine flavour profiling data as affected by insoluble

solids

Sr. No.	Eigen values*	Per cent of trace	Accumulated per cent of trace
1.	24.991	77.80	77.80
2.	1.263	3.90	81.80
3.	0.997	3.10	84.90
4.	0.938	2.90	87.80
5.	0.845	2.60	90.50
6.	0.790	2.50	92.90
7.	0.584	1.80	94.80
8.	0.491	1.50	96.30
9.	0.476	1.50	97.80
10.	0.332	1.00	98.80
11.	0.243	0.80	99.60
12.	0.118	0.40	100.00
13.	0.013	0.00	100.00
14.	0.00	0.00	100.00

* Only first 14 Eigen values have been shown

The flavour profile clearly depicts that there is a strong interaction between flavour attributes of wines fermented with different yeast strains, with insoluble solids and the

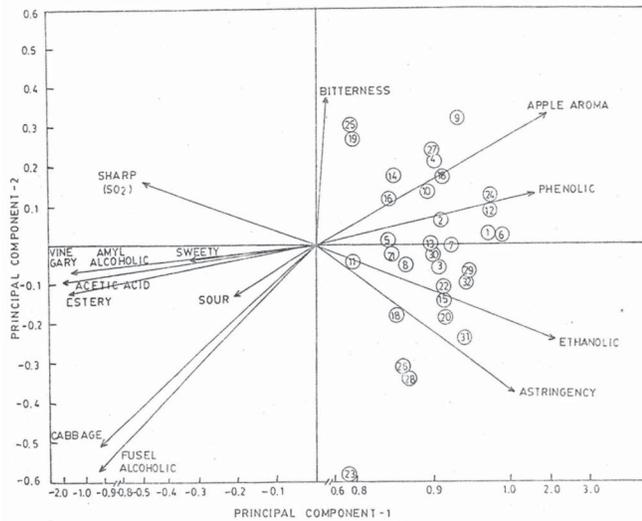


Fig. 4: Projection of flavour profiling data of apple wines fermented with different levels of insoluble solids in planes defined by principal components 1 and 2 (Numbers in circles represent the treatment code)

Details of treatments :

1.	W	+ EN + 0	ISS
2.	W	- EN + 0	ISS
3.	W	- EN + 2.5%	ISS
4.	W	- EN + 2.5%	ISS
5.	W	- EN + 5.0%	ISS
6.	W	- EN + 5.0%	ISS
7.	W	- EN + 10.0%	ISS
8.	W	- EN + 10.0%	ISS
9.	S05	+ EN + 0	ISS
10.	S05	- EN + 0	ISS
11.	S05	+ EN + 2.5%	ISS
12.	S05	- EN + 2.5%	ISS
13.	S05	+ EN + 5.0%	ISS
14.	S05	- EN + 5.0%	ISS
15.	S05	+ EN + 10.0%	ISS
16.	S05	- EN + 10.0%	ISS
17.	S22	- EN + 0	ISS
18.	S22	- EN + 0	ISS
19.	S22	+ EN + 2.5%	ISS
20.	S22	- EN + 2.5%	ISS
21.	S22	+ EN + 5.0%	ISS
22.	S22	- EN + 5.0%	ISS
23.	S22	+ EN + 10.0%	ISS
24.	S22	- EN + 10.0%	ISS
25.	S95	+ EN + 0	ISS
26.	S95	- EN + 0	ISS
27.	S95	+ EN + 2.5%	ISS
28.	S95	- EN + 2.5%	ISS
29.	S95	+ EN + 5.0%	ISS
30.	S95	- EN + 5.0%	ISS
31.	S95	+ EN + 10.0%	ISS
32.	S95	- EN + 10.0%	ISS

pectinolytic enzymes. The analysis of flavour profiling data (irrespective of yeast and enzyme) indicated that when PC 2 was plotted against PC-3 separation of the wines fermented with or without insoluble took place to some extent i.e. the wines with insoluble solids clustered towards the centre while those without insoluble solids formed the periphery (Fig. 5).

Wines with ISS have been characterized as higher alcoholic,

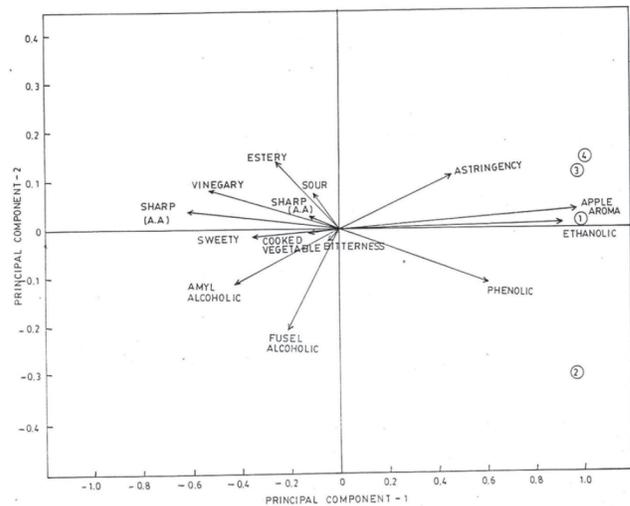


Fig. 5: Projection of flavour profiling data of apple wines with different insoluble solid (ISS) levels in planes defined by principal component 1 and 2[(1) = 0 ISS (2) = 2.5% (3) 5.0% ISS (4) = 10.0 ISS]

astringent, bitter, ethanolic, sour, cabbage, while without it were high in apple aroma and estery flavour tones. The plot of PC-2 versus PC-3 of flavour profiling data (irrespective of ISS or enzyme) did not show any specific pattern although a week separation of ‘W’ and UCD 522 occurred along the PC-2 (Figure 6) Interaction between various aroma compounds especially the higher alcohols may have led to confusion in characterizing the wines fermented by different yeasts very clearly.

A plot of PC-1 against PC-2 have almost separated the wines along PC-2 into those fermented with or without ISS and separation has occurred mainly due to sour, phenolic fusel alcoholic, amyl alcohol parameters as shown by the (Figure 7). A plot of PC-1 against PC-2 (Figure 8) of mean scores of flavour profile of wines with or without insoluble solids (ISS) irrespective of yeasts types or enzymes did separate the wines with or without ISS.

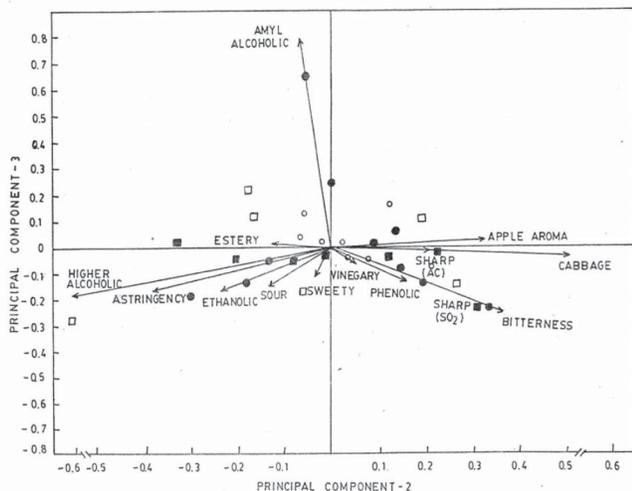


Fig. 6: Projection of flavour profiling data of apple wines as affected by the different yeasts in planes defined by principal component 2 and 3 (○ = Isolate 'W', ● = UCD 505, □ = UCD 522,

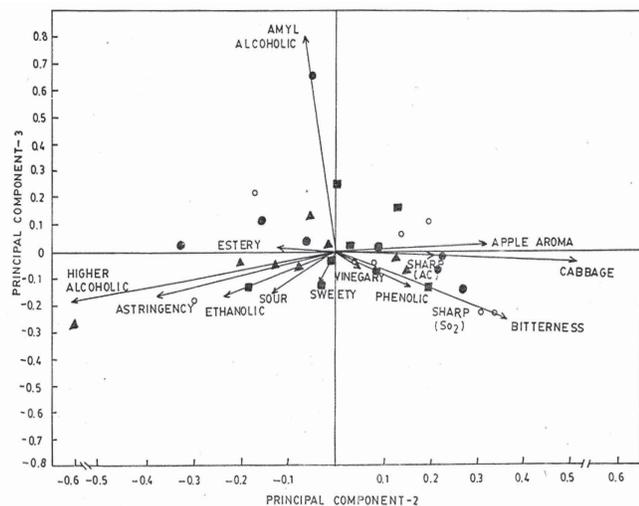


Fig. 8: Projection of flavour profiling data of apple wines fermented with or without insoluble solids in planes defined by principal component 2 and 3

Clearly the wines without ISS were characterized as rich in apple aroma and ethanolic while those with 2.5 per cent ISS were higher in phenolic and those with 5 per cent and 10 per cent ISS astringent. Similarly Figure 9 shows the separation of wines due to yeasts strains. But both the figures clearly reveal the modification of the flavour profiles due to the interactive effect of ISS, yeast types and addition of enzyme. Results are in agreement with those obtained by others on the effect of must turbidity on the wine quality (Bobkova, 1968; Singleton *et al.*, 1975) which indicated

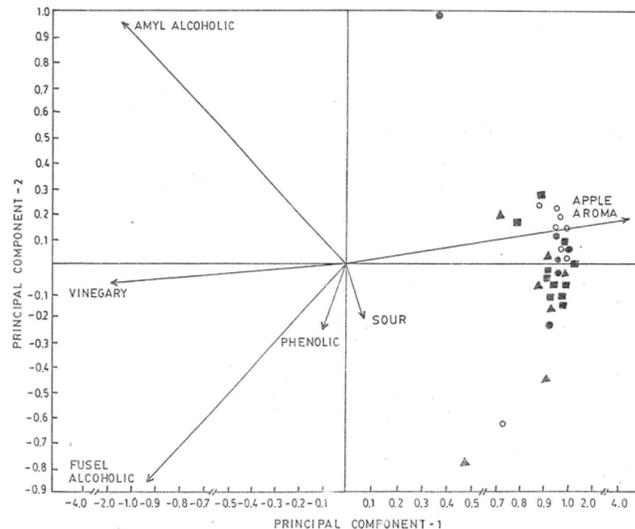


Fig. 7: Projection of flavour profiling data of apple wine fermented with different levels of insoluble solids in planes defined by principal component 1 and 2 (○ = 0.1% ISS, ● = 2.5% ISS, □ = 5% ISS, ▲ = 10.0% ISS)

that less turbid wines produced wine of higher quality. An improvement in the sensory qualities of wine as a result of pre-clarification of grape juice before fermentation (Liu *et al* 1987) took place though, it is contrary to previous report on this aspect (Groat and Ough, 1978).

The decrease in the flavour quality of wine could be attributed to the increase in higher alcohol formation, phenolic compounds and the effect of insoluble solids on the growth of yeast(s) during apple wine fermentation.

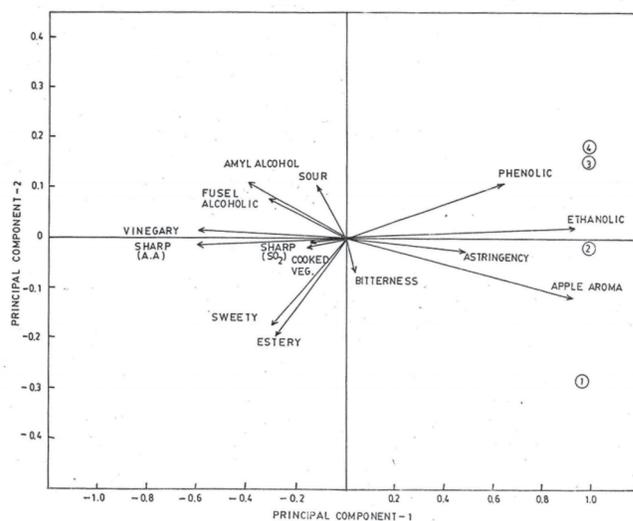


Fig. 9: Projection of flavour profiling data of apple wines with levels of insoluble solids and fermented by different yeasts in plains defined by principal component 1 and 2

The differences in the behaviour of different yeast strains are apparently due to their inherited characteristics (Joshi *et al.*, 2002) While, the addition of enzyme might have improved the flavour profile due to its effect on chemical composition of wine as well as removal of compounds producing the off flavour.

Wines made by fermentation of clarified grape juice are generally considered to be more fruity and have few off-odours (Groat and Ough, 1978; Singleton, *et al.*, 1975) although the chances of stuck fermentation is often greater when fermenting these musts. But it can be overcome by the use of massive yeast inoculation or by the aeration of yeast (Houtman *et al.*, 1980). Similar to our findings, the results of a report by Edwards *et al.*, (1990) have shown that wines without insoluble solids are more fruity and have few off odours than wines fermented with insoluble solids which is in further agreement with those obtained by Singleton *et al.*, (1975) and Groat and Ough (1978).

With regards to the sensory evaluation, the wines prepared without insoluble solids were better in most of the sensory qualities compared to those with it. Similar to these findings, wines made by fermentation of clarified grape juice have been described to be more fruity and have few off-odours (Ough and Groat, 1978; Singleton *et al.*, 1975) but the chances of stuck fermentation are increased which could be overcome by massive inoculation, aeration of juice or addition of yeasts ghosts (Edwards *et al.*, 1990). Though the effect of yeasts ghosts was inconclusive yet the quality of wine was described as comparable to the control wines. Among the yeasts, 'W' and UCD 595 were better than UCD 505 and UCD 522. Addition of enzyme improved the sensory qualities of the treated wines.

To sum up, the addition of increasing levels of insoluble solids to the apple juice resulted mostly to undesirable and sensory characteristics of wines. Three factors, viz. insoluble solids, yeasts and enzyme and their interactions are the important factors in determining the desirable sensory qualities of apple wine. Addition of insoluble solids to the wine, therefore, is unnecessary unlike the pectin esterase whose addition leads to significant improvement in the quality parameters of wine and pre-settled juice should be preferred.

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