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Chemical and nutritional properties of 'tej', an indigenous Ethiopian honey wine: variations within and between production units.

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Abstract

A total of 200 samples of 'tej', an indigenous Ethiopian honey wine, were collected from ten production units at different production times. The pH values of samples varied between 3.07 and 4.90 and 77% of the samples had pH values <4.0. Difference in pH value among all samples was significant ($p < 0.01$). The range for titratable acidity was 0.1 g/100ml to 1.03 g/100ml and mean values for the different production units were 0.34 - 0.6 g/100ml. About 65% of the samples had titratable acidity values of 4 g/100ml and variations within samples of production units ($CV > 10\%$) or among all samples ($p < 0.01$) were significant. Mean total alcohol content for the various production units was 6.98% -10.9%. About 58% of the samples had alcohol content of 5 - 10%. Fusel oil content

of samples ranged between 0.1 g/100L and 88 g/100L. Mean values for the production units were 13.6 - 27.4 g/100L. About 50% of the samples had fusel oil content of >20 g/100L. Variation in total alcohol and fusel oil content within samples of production units was significant (CV>10%) and difference in these values were also significant ($p<0.01$). Mean values for total carbohydrate, total lipid, total protein and reducing sugars were 1.49-3.73 mg/ml, <1.00 mg/ml, 0.33-4.66 mg/ml and 0.46-2.09 mg/ml, respectively. Variations in these values within samples of production units (CV>10%) or among all samples ($p<0.01$) were significant. As '*tej*' fermentation is a spontaneous process that depends on microflora naturally present on the substrates and equipment, the different metabolic products of the randomized microflora at different stages, the physical and chemical environment, duration of fermentation and concoction practices would result in physico-chemical variations in the final product.

Introduction

In nearly all areas of the world, some type of alcoholic beverage native to its region is prepared and consumed. In Africa, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and rain making ceremonies (Zvauya *et al.*, 1997), at festivals and social gatherings, at burial ceremonies and settling disputes (Steinkraus, 1983). They are also used as medicines for fever and other ailments by adding barks or stems of certain plants (Okafor, 1972).

Indigenous fermented alcoholic beverages from different parts of the world are described by Steinkraus (1983) and some of the indigenous African fermented alcoholic beverages include Egyptian bouza, Tanzanian Wanzuki, gongo, tembo-mnazi and gara, Nigerian palm-wine, Kenyan muratina and uragua, and South African kaffir beer. Indigenous Ethiopian fermented beverages include '*tej*' (Vogel & Gobezie, 1983; Fite *et al.*, 1991), tella (Sahle & Gashe, 1991), borde and shamita (Ashenafi & Mehari, 1995; Bacha *et al.*, 1998,1999).

Alcohol in traditional beverages serves as source of calories valuable to the calorie-deficient villager. The primitive beverages provide not only calories but also B vitamins due to residues of the substrates, the fermenting yeasts and other microorganisms (Steinkraus, 1983).

Fermented beverages produced from cereals are usually referred to as beers while those produced from fruits are classified as wines (Pederson, 1979). Fermentation of a variety of foods or blends of fruits, cereals, milk sap, honey molasses and/or other foods are also wines (Pederson, 1979). Honey wines are primitive types of wines that are not crystal clear products. Instead they are cloudy, effervescent containing residues of substrates and fermenting yeasts and other microorganisms (Steinkraus, 1983).

'Tej' is a home-processed, but also commercially available honey wine. It is prepared from honey, water and leaves of Gesho (*Rhamnus prenoides*). Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite *et al.*, 1991). Some also add different concoctions such as barks or roots of some plants or secrete herbal ingredients to improve flavor or potency and to attract customers. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their composition.

According to Vogel & Gobezie (1983), during the preparation of 'tej', the fermentation pot is seasoned by smoking over smouldering *Rhamnus prenoides* stems and olive wood. One part of honey mixed with 2 to 5 (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *Rhamnus prenoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prenoides*.

Good quality 'tej' is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of 'tei' depends upon the part of the country where the bees have collected the nectar and the climate (Vogel & Gobezie, 1983).

Fermentation of 'tej', like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates, fermentation vats or equipment. As these fermentations are natural and, thus, uncontrolled, alcohol and fusel oils produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable microflora of such spontaneous fermentation, variability of the product is inevitable. The aim of this study was, therefore, to assess variations in the various nutritional and chemical components of the product and evaluate the extent of the variation between and within production units at the time 'tej' was made ready for consumption.

Materials and methods Sampling

A total of ten 'tej' producing and vending houses from different parts of Addis Ababa were considered in this study. Twenty samples (one liter each) were separately collected from each sampling site in twenty different production days. Samples were collected in sterile screw cap bottles and were immediately brought to the laboratory for microbiological and biochemical analyses.

Biochemical analyses

The amount of total available protein was determined colorimetrically according to the methods of Schacterle & Pollack (1973). Total amount of carbohydrates was determined colorimetrically by the phenol-sulphuric acid method (Dubois *et al*, 1956). The reducing sugars were determined colorimetrically (Somogy, 1951). The total lipid content was determined gravimetrically (Jayaraman, 1992).

The total alcohol content of samples was determined by specific gravity method of AOAC 19.004 (Williams, 1984). The fusel oil content of samples was determined by spectrophotometric method of AOAC 9.084 (Williams, 1984).

The pH of samples was measured by dipping the electrode of a digital pH meter in the samples. The titratable acidity of samples was determined according to Zvaunya *et al*. (1997).

Statistical analysis

Significance in variation in biochemical properties of samples between and within production units was analyzed using a one-way repeated measures ANOVA method (SAS Institute Inc., 1990).

Results and Discussion

The pH values of our '*tej*' samples varied between 3.02 and 4.90 ([Table 1](#)). The variation in pH values within samples of the same production unit was significant in production units A, B, C D and E (CV>10%). There was also a significant difference in pH values among all samples considered in this study ($p<0.01$). At least a fifth of the samples from every production unit had pH values <3.5 and 77% of all '*tej*' samples had pH values of ≤ 4.0 ([table 2](#)).

Titratable acidity of '*tej*' samples varied from values as low as 0.1 g/100ml to values as high as 1.03 g/100ml ([Table 1](#)). Mean values of samples from production units ranged between 0.34 and 0.6 g/100ml. The variation in titratable acidity values within samples of the same production unit was, however, very significant in all production units (CV>10%). Differences in titratable acidity values of all '*tej*' samples were also significant ($p<0.01$). About 65% of

the total '*tel*' samples had titratable acidity values of 4 g/100ml and only 9% had values <3 g/100ml ([Table 2](#)). Mean values of pH and titratable acidity were not significantly different among production units ($p>0.01$).

Considering the pH and titratable acidity values, our '*tej*' samples may generally be considered acidic products. However, due to the spontaneous nature of the fermentation, variations in chemical properties of the final product are eminent. The mean pH of '*tej*' in our study was much higher than that of Korean honey wine (Rhim *et al.*, 1997) and slightly higher than African mango juice wine (Akubor, 1996), Nigerian oil-palm wine (Eze & Uzoechi, 1988) and Korean fruit-honey wine (Rhim *et al.*, 1997). Over 25% of our '*tej*' samples had higher pH values than various commercial honey wines (Steinkraus & Morse, 1973). But this value was much lower than that for Tanzanian honey wine and pine apple wine (Tiisekwa *et al.*, 2000).

Most of our '*tej*' samples had titratable acidity values similar to Korean honey wine (Rhim *et al.*, 1997) and these values fall within the range for different commercial honey wines (Steinkraus & Morse, 1973). They were, however, slightly less acidic than Korean fruit-honey wine (Rhim *et al.*, 1997) and African mango juice wine (Akubor, 1996) and much less acidic than cashew juice wine (Akinwale, 1999).

The total alcohol content of our '*tej*' samples varied between values as low as 2.7% to values as high as 21.7% ([Table 3](#)). The mean value for the various production units was between 6.98% and 10.9%. In every production unit, the alcohol content between samples varied significantly ($CV >10\%$) and differences in alcohol content were significantly high among all samples and samples within production units ($p<0.01$). Difference among production units was, however, not significant ($p>0.01$). About 58% of the '*tej*' samples had alcohol-content between 5.1% and 10% ([Table 4](#)). '*tej*' samples with alcohol content of <5% or >15% made up <10% and 2%, respectively of the total samples.

The mean alcohol content of the production units was less than the value reported for various traditional wines (Mm *et al.*, 1994; Koh *et al.*, 1994) but higher than that of Korean honey wine (Rhim *et al.*, 1997) and Tanzanian honey wine (Tiisekwa *et al.*, 2000). Over 65% of our '*tej*' samples had lower alcohol contents than reported for commercial honey wines (Steinkraus and Morse, 1973). The mean alcohol content (~f our '*tej*' samples was higher than '*tej*' obtained from different parts of Ethiopia (Fite *et al.*, 1991) but lower than the value for '*tej*' reported in another study (Desta, 1977). Differences reported by different investigators are indicative of the variability of '*tej*' samples in their physico-chemical properties.

In general it can be said that the range for alcohol content of '*tej*' is very wide and values lower than 5% could be

obtained in cases where the fermentation is far from complete. The higher titratable acidity combined with significant amount of alcohol would result in sweet-sour alcoholic flavor which would make '*tej*' preferable by consumers. This combination would also give '*tej*' the required microbiological stability, which would permit certain conservation without the use for highly specific techniques as observed in wine. Producers do not determine the end-point of the fermentation and '*tej*' is consumed while in the state of active fermentation. Aerobic conditions would result in the formation of acetic acid from alcohol making the product more and more sour, a condition termed as 'dryness' by consumers.

Fusel oil content of '*tej*' samples varied between 0.1 g/100L and 88 g/100L (**Table 3**). The mean value for the different production units ranged from 13.6 to 27.4 g/100L. Variations in fusel oil content between samples in every production unit was highly significant (CV>10%) (**Table 3**). There was also a significant difference in fusel oil content among all the samples tested in this study ($p<0.01$). A quarter of our samples had fusel oil content of <10 g/100L and about 50% had >20 g/100L. The mean fusel oil content of '*tej*' samples from half of the production units considered in this study was higher than the amount reported for '*tej*' by other workers (Fite *et al.*, 1991). About 25% of our '*tej*' samples had fusel oil content higher than the highest value reported for Zambian honey wine (Reilly *et al.*, 1979) and most of our samples had higher fusel oil content than reported for other traditional alcoholic beverages (Nikander *et al.*, 1991). Fusel oils may contribute to the flavor and odor of '*tej*'. Decrease in levels of fusel oil and acetaldehyde during aging of honey wines resulted in less intense sensory properties (Kim *et al.*, 1999). At higher levels, however, fusel oils can be toxic and, thus, hazardous to health. Producers of indigenous alcoholic beverages do not have control on products of natural fermentation processes. It is reported that home-brewed wines have higher fusel oil content than commercially brewed drinks (Greenshield, 1975)

Mean total carbohydrate content of our samples of '*tej*' from the various production units ranged between 1.49 mg/ml to 3.73 mg/ml (**Table 5**). Mean total lipid content was <1.00 mg/ml in nine of the ten production units. Mean protein content ranged between 0.33 and 4.66 mg/ml and mean values for reducing sugars ranged between 0.46 and 2.09 mg/ml for the production units. There was a significant variation in these values among samples from the same production units (CV>10%). They were also significantly different among all samples ($p<0.01$), but difference among production units was not significant ($p<0.01$).

Mean total carbohydrate content of our '*tej*' samples was much lower than that of honey wines of Eastern Europe (Steinkraus, 1983). The large number of fermentative microorganisms in '*tej*' could be responsible for the reduction of total sugars in '*tej*'. During the fermentation of Nigerian oil-palm wine (Eze & Uzoechi, 1988), total sugars decreased to values similar to those observed in our '*tej*' samples. The lipid content of '*tej*' is too low to be of any

significance. Total protein content was, however, much higher than that of different types of grape wines (Rosi *et al.*, 1987; Yokotsuka *et al.*, 1995) but much lower than traditional fermented rice wine (Kim *et al.*, 1994). 'Tej' and other traditional fermented beverages are consumed during an active state of fermentation, and dead or living microbial cells could contribute to increased protein content of the final product.

Reducing sugar content of our 'tej' samples was much lower than that of rice wine (Kim *et al.*, 1994) and other commercial honey wines which were reported to be quite sweet (Steinkraus & Morse, 1973). Reducing sugars decreased while alcohol content increased during honey wine fermentation (Rhim *et al.*, 1997). As there is no end-point 'tej' fermentation is made to stop, the high number of yeasts and lactic acid bacteria would utilize the reducing sugar even while 'tej' is ready for consumption, making it "drier" in the process.

'tej' is a widespread home industry of considerable social and economic importance and mostly produced for commercial purposes. Although the basic raw materials and preparation skills may not be markedly different among 'tej' producers, inclusion of cane sugar by many as fermentable substrate and addition of various plant parts to increase potency of 'tej' would result in differences in physico-chemical properties of the final product. The fermentation is spontaneous and depends on the microflora naturally present in the substrates, on utensils and equipment used. The different metabolic products of these randomized microflora at different stages, the physical and chemical environments and duration of fermentation and concoction practices would, thus, result in physico-chemical variations in the final product. Further work on determination of appropriate substrates, selection of desirable fermenting cultures and optimization of process conditions would help to produce 'tej' with better keeping quality and acceptable levels of alcohol, fusel oils and other hazardous metabolites.

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Fig 1 a, b, c, d, e, f and g. Nitrogen fixing attributes. Nodule mass at 21 DAP, flowering and pod hardening; nodule number, nodule position, percent effective nodules and nodule density

Fig 2 a, b, c, d, e, f, g, and h: Nitrogen fixing attributes; the percentage nodulation frequency for the 3rd ad 4th growing seasons harvests 1, 2, 3, and 4.

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Figure 3. Changes in protein of cassava products on solid substrate fermentation with *Rhizomucor miehei*

Figur 4. Effect of solid substrate fermentation of cassava products with *Mucor strictus* on their protein contents

Table 1. pH and titratable acidity of ‘*tej*’ samples from different production units

Production unit	pH				Titratable acidity (g/100ml)			
	Lowest	Highest	X±SD	%CV	Lowest	Highest	X±SD	%CV
A	3.15	4.01	3.47±0.32	25.31	0.36	0.70	0.57±0.10	16.93
B	3.35	4.90	3.95±0.51	12.79	0.29	0.50	0.40±0.05	13.00
C	3.26	4.45	3.89±0.42	10.79	0.23	0.58	0.52±0.06	11.63
D	3.45	4.88	3.82±0.42	10.95	0.26	0.50	0.34±0.06	17.06
E	3.24	4.07	3.63±0.25	6.81	0.24	1.03	0.60±0.21	34.70
F	3.23	3.99	3.68±0.25	7.02	0.13	0.58	0.44±0.12	26.43
G	3.02	3.96	3.58±0.30	8.25	0.34	0.58	0.43±0.08	17.64
H	3.07	4.25	3.80±0.32	8.45	0.29	0.53	0.42±0.09	22.37
I	3.41	4.32	3.82±0.82	7.94	0.31	0.50	0.43±0.06	14.30
J	3.14	4.41	3.87±0.39	10.22	0.21	0.50	0.40±0.09	22.99

Table 2 Frequency distribution of 'tej' samples with different pH and titratable acidity values

Production unit	pH				Titratable acidity (g/100ml)				
	<3.5	3.51-4.0	4.01 -4.05	>4.5	<0.3	0.31-0.4	0.41-0.5	0.51-0.6	>0.6
A	14	5	1	0	0	2	2	9	7
B	5	6	5	4	1	9	9	1	0
C	5	7	8	0	1	0	0	19	0
D	4	12	2	2	6	11	2	1	0
E	6	10	4	0	1	0	5	9	5
F	5	15	0	0	1	5	6	8	0
G	6	14	0	0	0	7	8	5	0
H	4	10	6	0	3	8	0	9	0
I	4	10	6	0	0	6	9	5	0
J	4	8	8	0	5	5	5	5	0
Total (%)	57 (28.5)	97 (48.5)	40 (20)	6 (3)	18 (9)	53 (26.5)	46 (23)	71 (35.5)	12 (16)

Table 3. Total alcohol and fusel oil content of '*tej*' samples from different production units

Production unit	pH				Titratable acidity (g/100ml)			
	Lowest	Highest	X $\pm$ SD	%CV	Lowest	Highest	X $\pm$ SD	%CV
A	7.94	12.92	10.85 $\pm$ 1.64	15.07	ND	ND	ND	ND
B	8.34	12.92	10.17 $\pm$ 1.45	14.24	0.10	35.0	22.91 $\pm$ 11.7	51.10
C	4.88	11.68	8.33 $\pm$ 2.09	25.02	1.50	33.0	13.90 $\pm$ 11.0	78.96
D	5.62	15.68	9.80 $\pm$ 3.62	29.89	1.50	33.0	13.58 $\pm$ 9.82	72.31
E	4.88	13.42	9.58 $\pm$ 2.64	27.58	1.50	67.0	23.47 $\pm$ 19.17	81.68
F	6.36	12.55	8.25 $\pm$ 3.08	33.29	1.00	50.26	19.80 $\pm$ 14.69	74.18
G	4.88	15.83	8.17 $\pm$ 4.34	53.37	1.00	33.0	15.49 $\pm$ 12.74	82.23
H	3.73	21.72	10.05 $\pm$ 3.79	37.83	2.75	88.0	27.38 $\pm$ 20.86	76.47
I	2.74	10.62	6.98 $\pm$ 2.35	33.64	1.50	50.26	25.72 $\pm$ 13.55	52.71
J	3.78	12.92	8.12 $\pm$ 2.41	29.64	1.00	50.26	23.12 $\pm$ 13.90	60.13

Table 4. Frequency distribution of '*tej*' samples with different alcohol and fusel oil content

Production unit	pH				Titratable acidity (g/100ml)				
	<5.0	5.1-10.0	10.1-15.0	>15	<10	10.0-20.0	20.1-30.0	30.1-40.0	>40
A	0	5	13	0	ND	ND	ND	ND	ND
B	0	11	7	0	2	4	4	5	0
C	1	14	3	0	7	4	2	2	0
D	0	9	8	1	6	6	2	1	0
E	2	9	7	0	2	7	1	3	2
F	1	11	6	0	5	3	3	3	1
G	4	10	3	1	7	2	3	3	0
H	1	11	5	1	1	6	2	4	2
I	5	11	2	0	2	3	4	4	2
J	3	13	2	0	2	3	6	2	2
Total (%)	17 (9.44)	104 (57.78)	56 (31.11)	3 (1.67)	34 (25.19)	38 (28.15)	27 (20.0)	27 (20.0)	9 (6.66)

*A total of 180n samples were analyzed for alcohol content

** A total of 135 samples were analyzed for fusel oil content

Table 5. Total carbohydrate, lipid, protein and reducing sugar content (mg/ml) of '*tej*' samples from different production units

Production unit	Carbohydrate		Lipid		Protein		Reducing Sugars	
	X $\pm$ SD	%CV	X $\pm$ SD	%CV	X $\pm$ SD	%CV	X $\pm$ SD	%CV
A	1.49 $\pm$ 0.42	28.45	1.34 $\pm$ 0.52	46.21	0.39 $\pm$ 0.17	43.30	0.58 $\pm$ 0.20	34.11
B	3.08 $\pm$ 0.00	28.23	0.31 $\pm$ 0.53	172.69	0.43 $\pm$ 0.26	63.31	1.18 $\pm$ 0.97	80.58
C	2.81 $\pm$ 0.80	29.37	0.56 $\pm$ 0.38	67.80	0.33 $\pm$ 0.22	55.25	2.09 $\pm$ 1.53	77.21
D	3.21 $\pm$ 1.05	32.82	0.98 $\pm$ 1.10	112.50	0.43 $\pm$ 0.18	37.14	1.69 $\pm$ 1.73	97.07
E	2.99 $\pm$ 1.51	50.49	0.64 $\pm$ 0.38	60.17	1.47 $\pm$ 1.02	69.67	1.36 $\pm$ 1.34	98.48
F	1.81 $\pm$ 0.55	30.62	0.44 $\pm$ 0.32	73.13	3.32 $\pm$ 3.56	105.01	0.46 $\pm$ 0.11	24.41
G	2.10 $\pm$ 0.61	29.68	0.36 $\pm$ 0.26	71.35	4.66 $\pm$ 2.87	55.49	0.99 $\pm$ 1.14	114.49
H	3.73 $\pm$ 1.05	28.14	0.71 $\pm$ 0.32	45.53	1.90 $\pm$ 1.68	88.29	1.72 $\pm$ 1.04	80.45
I	3.52 $\pm$ 0.96	27.33	0.87 $\pm$ 0.44	51.12	1.62 $\pm$ 1.32	81.32	1.71 $\pm$ 1.64	89.75
J	2.89 $\pm$ 1.35	46.58	0.73 $\pm$ 0.40	55.32	1.40 $\pm$ 1.28	91.35	0.92 $\pm$ 0.44	47.73