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RESEARCH ARTICLE

PREPARATION AND EVALUATION OF MEAD

Hookman Jimi Rai and Prakash Manandhar
Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal.

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Abstract

Mead is an alcoholic beverage which is obtained by fermentation of honey must, with the possible addition of various spices and hops having about 9% to 18% alcohol by volume. Honey itself contains many phenolic and flavonoids which are great source of antioxidants the content of which increases upon further fermentation of honey. Therefore, consumption of honey or mead has huge positive health impact. Honey consists of more than 70% sugar therefore the fermentation is carried out by dilution of honey by adding appropriate water. The fermentation of honey to produce mead was done using different honey and the quality of the mead was determined by alcohol content, antioxidant activity, total soluble solid and organoleptic quality of all the produced mead was analyzed. Four different honeys were collected out of which two were commercial honey and two were honey from local market. Twelve different blend of honey musts were prepared. The fermentation process was carried out by Saccharomyces cerevisiae standard culture for the production of alcohol. Standard wine yeast and baker's yeast were used as starter culture. The fermentation was carried out until constant total soluble solid was obtained. The alcohol content of the mead was in the range of 9% to 12%. The fermented mead also showed antioxidant activity, and had acceptable organoleptic characteristics.

Introduction:-

Alcoholic beverages are part of different culture and tradition throughout the world, with different names in different communities respectively. Mead is an ancient beverage, which has been brewed for more than 3000 years, whose origin can be traced back to African countries. It was believed to possess magical and healing powers like increasing strength, virility and fertility and referred as drink of nobles and gods which provides knowledge and immortality [1].

In most of the traditional technique mead fermentation is often carried out by the use of indigenous microorganisms naturally present in honey itself. Often the microorganisms survive on the equipment used on the fermentation due to the present of residual substrates [2]. In these cases, the mead fermentation is often more unpredictable and most of the times, mead is spoiled by contaminating yeasts and bacteria which degrades the overall quality of the mead or even make it undrinkable. The fermentative process is complicated by several problems which are delayed or arrested fermentation, low mead quality and generation of unpleasant smell during the fermentation process.

Corresponding Author:- Hookman Jimi Rai
Address:- Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal.

Generally, all these spoilages rise due to the unfavorable and stressful growth conditions to which the employed yeasts are not favorably adapted [3].

Honey consists of various carbohydrates, minerals etc. Carbohydrates are the major constituents of honey, which make up about 90% to 95% of the dry matter [4]. Water is the second major component of honey. It depends not only on environmental factors, such as the weather and humidity inside the hive, but also on the treatments applied during nectar and honey collection and storage. It is an important quality parameter because it predicts the shelf life of the product and the capacity of the honey to remain stable and free from fermentation. Higher water content increases the probability that the honey will commence fermentation during storage, because part of the water is bound to sugars, it is unavailable to microorganisms. The available water (free water content) that determines water activity (a_w) is one factor that influences the microbial stability of honey. The water activity value of any honey can vary between 0.55 and 0.75. Honeys with a water activity < 0.60 are microbiologically stable [4]. Regardless, the simple and fast measurement of the water content has proven sufficient for assaying the fermentation risk of honey.

Mead fermentation is generally slow fermentation as there is less amount of nitrogen source for the growth of yeast. Traditionally, fermentation is usually arrested as there was no way to measure the sugar content of the honey water wort. Often inorganic salts are added for better fermentation, salts like ammonium sulphate and ammonium hydrogen phosphate are generally used in 0.2 g/l and 0.02 g/l concentration respectively [5]. Fermentation of mead is generally carried out by yeast specially by S. cerevisiae During fermentation, the fermentable sugar that are present in the substrate are fermented to pyruvate by the process of glycolysis. Consequently, the pyruvate is decarboxylated to carbon dioxide and acetaldehyde by enzyme pyruvate decarboxylase. S. cerevisaeconverts the acetaldehyde to ethanol and carbon dioxide by reduction with enzyme alcohol dehydrogenase [6]. Bacteriostatic or bactericidal activity, low pH and high content of reducing sugars present in honey can prevent growth of many microbes thus can lead to delayed or arrested fermentation. Due to these, honey is also virtually unspoiled for extended period of time if it is packed properly. These are also the reason for long period of fermentation of mead [7].

Saccharomyces cerevisiae is a unicellular, oval yeast which are usually present in fruits and sugar. It is both fermentative and oxidative. Therefore, it can ferment sugar and produce alcohol in anaerobic condition. It also produces carbon dioxide and give a high yield of biomass under anaerobic and aerobic condition respectively. It is also used as single cell protein (SCP). S. cerevisiae is on the most commonly used microorganism because it supports high ethanol yield from hexose sugars. It can produce high ethanol concentration in its growth medium and can tolerate the high ethanol concentration and other inhibitory compounds [8].

Methodology:-

The study was done at St. Xavier’s College Maitighar, Kathmandu in 2021.

For quality evaluation of honey

Moisture, vitamin C, pH and antioxidant content was done along with sugar test. For sugar test Molisch’s test, Benedict’s test, Felhing’s test, and iodine test were performed.

Preparation of starter culture

A loopful of isolated and confirmed strains of wine yeast and baker’s yeast were cultured in the honey wort in which TSS was kept at 6°Brix. The mixture was then kept in a shaker incubator at 30°C for an overnight period at 60 rpm.

Preparation of substrate

Four different types of honey were used. Among them, one was wild rock honey, one was local honey and two was commercial honey. 3 more substrates were made by mixing wild honey with local, and two commercial honeys in 1:1 ratio. Two types of commercial culture yeasts were used, which were lyophilized wine and baker’s yeasts.

Fermentation of mead

205 g of honey was taken and mixed with 500ml of water to prepare honey must. Then, Total soluble solid was maintained at 21° Brix. The honey must was then pasteurized by gently heating and cooled at room temperature. Then 5% of starter culture was added and left to ferment at room temperature under anaerobic condition. The TSS of the must was measured and recorded daily, until constant reading for consecutive 3 days was observed. The mead was then siphoned, pasteurized and bottled.

For Physicochemical analysis of mead
Total soluble solids, pH, acidity, vitamin C content, antioxidant activity,reducing sugars and alcohol % of the mead was evaluated.

Sensory evaluation test
The test was conducted using a 9-point hedonic scale and one commercial wine was used as control.

Collection of honey
Quality evaluation of honey
(Moisture, vitamin C and antioxidant activity)
Blending of honey was done by mixing commercial honey with local honey in 1:1 ratio
Dilution of honey by adding drinking water and TSS maintained at 21°Brix

Culture of yeast on PDA
Preparation of starter culture

Fermentation of honey
Bottling and pasteurization of mead
Physiochemical analysis of mead
(pH, acidity, alcohol%, antioxidant activity, turbidity)
Sensory evaluation

Flg1:- Diagrammatic representation of mead production and analysis of produced mead.

Result:-

Characteristics of honey
Moisture, pH, vitamin C and antioxidant activities of 4 different honey was done and shown on Table 1. Where, moisture, vitamin C content and antioxidant activity was found to be highest in sample R which is wild honey. The range of the pH, moisture and vitamin C is on accordance to Bogdonov et al (2009).

Table 1:- Characteristics of honey.

Honey sample	pH	Range	Moisture percentage	Range	Vitamin C (mg/1000g)	Range(mg/100g)	Antioxidant activity/%
R	4.30		20.45		61.51		65
L	4.25	3.5-4.5	18.30	15-20	40.20	0.34-75.9	60
C1	4.40		17.20		45.36		51
C2	3.50		16.85		42.52		54

Note: R Wild rock honey
L Local honey
C1 Commercial honey type 1
C2 Commercial honey type 2

Sugar tests of honey
Various sugar tests were performed on all the sample of the honey. Molish's, Barfoed's Benedict's, and iodine test were performed and the result is shown in Table 2. In which all the test showed positive results expect iodine test which conclude absence of starch and presence of sugar, monosaccharides and reducing sugar by Molish's test, Barfoed's test and Benedict's test respectively.

Table 2:- Sugar tests of honey.

Honey sample	Molish's test	Barfoed's test	Benedict's test	Iodine test
R	+	+	+	-

L	+	+	+	-
C1	+	+	+	-
C2	+	+	+	-

Note: + indicates positive test result
– indicates negative test result

R Wild rock honey
L Local honey
C1 Commercial honey type 1
C2 Commercial honey type 2

Change of Total Soluble Solid during fermentation of mead

The change of TSS was noted daily during fermentation until constant TSS was obtained for three consecutive days.

The fermentation of honey must by wine yeast and drop of TSS in must is shown on Figure 1. The fermentation took about 3 weeks to complete but in case of WR (wild honey) the fermentation halted 7 days prior and TSS of WR, WL and WC1 was at 10°Brix whereas WC2 was at 11°Brix at the end of fermentation.

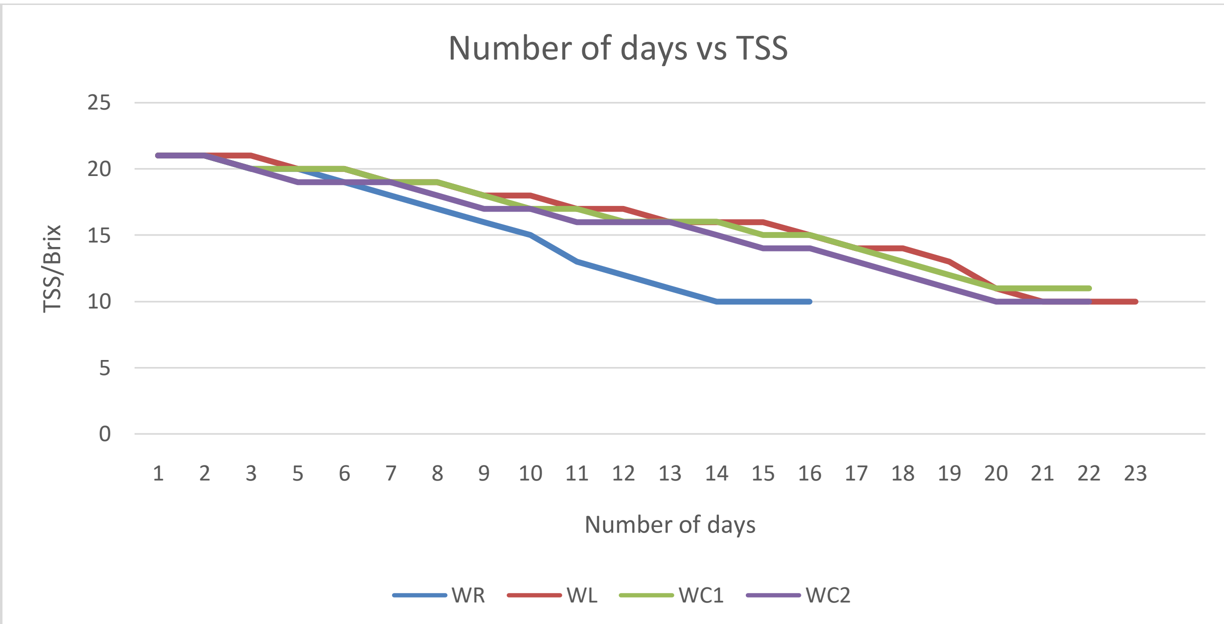


Fig1:- Change of TSS during fermentation of honey must by wine yeast.

Note: WR Mead fermented from wild rock honey with wine yeast
WL Mead fermented from local honey with wine yeast
WC1 Mead fermented from commercial honey type 1 with wine yeast
WC2 Mead fermented from commercial honey type 2 with wine yeast

The TSS of the honey must during fermentation of mead by Baker's yeast is demonstrated in Figure 2. Nearly all the fermentation rate is constant and is ceased at 19th day of fermentation. The fermentation was terminated at 9°Brix for BR in 16th day, 10° Brix for BL and BC1 in 17th and 19th day respectively and 12°Brix for BC2.

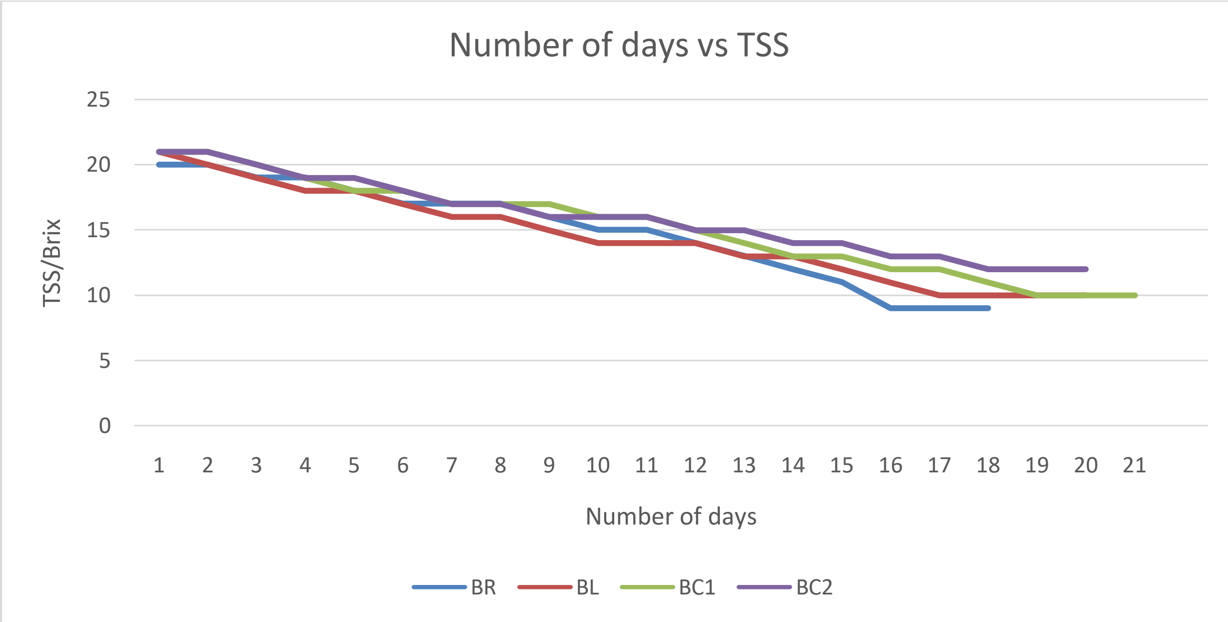


Fig2:- Change of TSS during fermentation of honey must by baker's yeast.

Note: BR Mead fermented from wild rock honey with Baker's yeast
BL Mead fermented from local honey with Baker's yeast
BC1 Mead fermented from commercial honey type 1 with Baker's yeast
BC2 Mead fermented from commercial honey type 2 with Baker's yeast

The drop of TSS of blended honeys by wine yeast during the mead fermentation is shown in Figure 3. The rate of decrease in TSS of all the must is nearly constant in all the honey must. The fermentation was completed in 19th day at 10°Brix for WRC2 and WLC1 and 9° Brix for WRC1. The fermentation was arrested at 20th day with 10°Brix for WLC2.

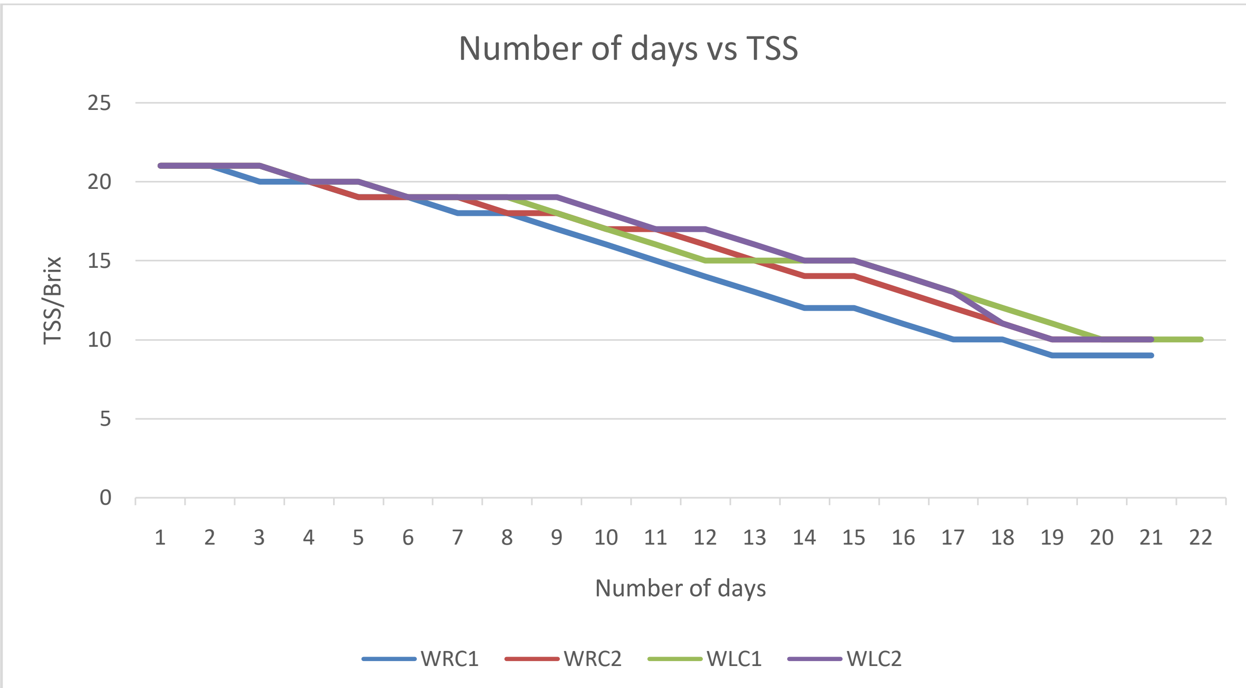


Fig 3:- Change of TSS during fermentation of blended honey by wine yeast.

Note: WRC1 Mead fermented from blended R and C1 (1:1) with wine yeast
WRC2 Mead fermented from blended R and C2 (1:1) with wine yeast

WLC1 Mead fermented from blended L and C1 (1:1) with wine yeast
WLC2 Mead fermented from blended L and C2 (1:1) with wine yeast

Physiochemical analysis of mead
TSS, pH, acidity, turbidity, alcohol percentage and reducing sugar of each mead sample was measured and Table 3 shows the values of them respectively. All the values are generally uniform. Whereas, turbidity of WR is the highest of all, reducing sugar is highest in WRC2 and BC1 and lowest in WLC1.

Table 3:- Physiochemical analysis of mead.

Mead sample	pH	TSS	Acidity%	Turbidity /A	Alcohol%	Reducing sugars (%)
WR	4.20	9	0.03	1570	9.50	3.54
WL	3.55	10	0.05	320	11.19	4.63
WC1	3.28	11	0.07	35	11.17	3.50
WC2	3.16	10	0.02	565	8.68	3.78
WRC1	3.70	9	0.05	367	10.35	4.58
WRC2	3.65	10	0.02	759	10.40	5.12
WLC1	3.38	10	0.06	71	10.63	3.48
WLC2	3.30	10	0.03	102	9.54	4.38
BR	4.18	9	0.04	950	11.09	3.96
BL	3.58	10	0.03	80	10.36	4.16
BC1	3.28	10	0.07	11	10.75	5.12
BC2	3.10	12	0.03	193	11.01	3.84

Note: WR Mead fermented from wild rock honey with wine yeast
WL Mead fermented from local honey with wine yeast
WC1 Mead fermented from commercial honey type 1 with wine yeast
WC2 Mead fermented from commercial honey type 2 with wine yeast
WRC1 Mead fermented from blended R and C1 (1:1) with wine yeast
WRC2 Mead fermented from blended R and C2 (1:1) with wine yeast
WLC1 Mead fermented from blended L and C1 (1:1) with wine yeast
WLC2 Mead fermented from blended L and C2 (1:1) with wine yeast
BR Mead fermented from wild rock honey with Baker's yeast
BL Mead fermented from local honey with Baker's yeast
BC1 Mead fermented from commercial honey type 1 with Baker's yeast
BC2 Mead fermented from commercial honey type 2 with Baker's yeast

Bioactive component analysis of mead
Vitamin C content, antioxidant activity and reducing sugar present in the mead was examined and noted as in Table 4. Vitamin C and antioxidant activity content was highest in WR and lowest in BC2 respectively.

Table 4:- Bioactive component analysis of mead.

Sample	Vitamin C (mg/100ml)	Antioxidants (% DPPH)
WR	1.25	39
WL	0.96	26
WC1	0.85	24
WC2	0.78	21
WRC1	1.10	30
WRC2	1.08	28
WLC1	0.93	27
WLC2	0.85	29
BR	1.16	42
BL	0.85	22
BC1	0.74	28
BC2	0.72	19

Note: WR Mead fermented from wild rock honey with wine yeast

WL	Mead fermented from local honey with wine yeast
WC1	Mead fermented from commercial honey type 1 with wine yeast
WC2	Mead fermented from commercial honey type 2 with wine yeast
WRC1	Mead fermented from blended R and C1 (1:1) with wine yeast
WRC2	Mead fermented from blended R and C2 (1:1) with wine yeast
WLC1	Mead fermented from blended L and C1 (1:1) with wine yeast
WLC2	Mead fermented from blended L and C2 (1:1) with wine yeast
BR	Mead fermented from wild rock honey with Baker's yeast
BL	Mead fermented from local honey with Baker's yeast
BC1	Mead fermented from commercial honey type 1 with Baker's yeast
BC2	Mead fermented from commercial honey type 2 with Baker's yeast

Sensory evaluation of mead

Seven panelists assessed the mead sensory quality. A nine-point hedonic scale was used along with a commercial wine as a control. The result is given on Table 5.

Table 5:- Sensory evaluation of mead.

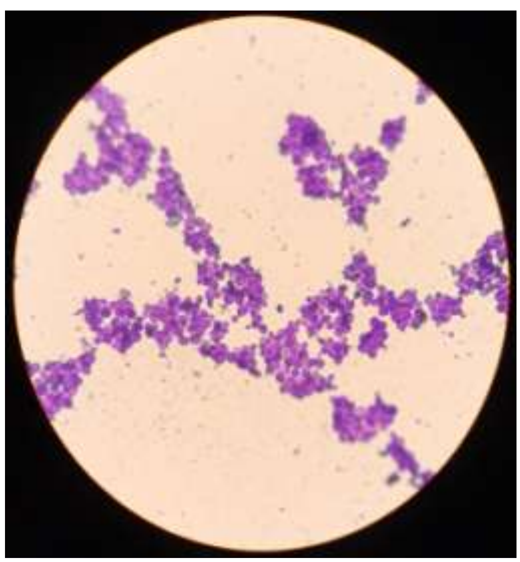
Sample	Color	Astringency	Taste	Persistence	Hardness	acceptance
WR	7	8	7	7	7	7
WL	7	8	7	6	6	6
WC1	8	7	8	7	6	7
WC2	7	7	8	8	7	7
WRC1	8	6	7	7	6	6
WRC2	7	7	8	7	7	7
WLC1	9	7	8	6	7	7
WLC2	7	7	7	7	8	7
BR	6	6	5	6	7	5
BL	8	8	8	7	6	6
BC1	8	7	8	7	6	7
BC2	9	9	8	8	7	8
Control	9	9	8	7	7	8

Note:

WR	Mead fermented from wild rock honey with wine yeast
WL	Mead fermented from local honey with wine yeast
WC1	Mead fermented from commercial honey type 1 with wine yeast
WC2	Mead fermented from commercial honey type 2 with wine yeast
WRC1	Mead fermented from blended R and C1 (1:1) with wine yeast
WRC2	Mead fermented from blended R and C2 (1:1) with wine yeast
WLC1	Mead fermented from blended L and C1 (1:1) with wine yeast
WLC2	Mead fermented from blended L and C2 (1:1) with wine yeast
BR	Mead fermented from wild rock honey with Baker's yeast
BL	Mead fermented from local honey with Baker's yeast
BC1	Mead fermented from commercial honey type 1 with Baker's yeast
BC2	Mead fermented from commercial honey type 2 with Baker's yeast

Photograph 1:- Wild honey, local honey, commercial honey 1 and commercial honey 2.

Photograph 2:- *S. cerevisiae culture on PDA.*



Photograph 3:- Microscopic examination of *S.cerevisiae*, cell with buds is pointed.

Photograph 4:- Starter culture of Baker's yeast and wine yeast.

Discussion:-

The pH of the honey sample was also within range of 3.5 to 4.5, with type 2 commercial honey being the lowest with 3.5 and highest in type 1 commercial honey with 4.4. The moisture content of honey was also within the range of 15 to 20 % [9].

The wild honey had highest vitamin C content and also had highest antioxidant activity among all of the honey sample. All other honey had relatively similar vitamin C content and comparable antioxidant activity. In a research, similar data was obtained[10]. The antioxidant activity of the honey depends on the type of the flower and also the species of honey too. It is also studied that the honey with darker color contains has more antioxidant activity as compared to light colored honey. In a study, the dark color in honey is due to the phenols present in the honey, because of which the larger the amount of phenols in honey, the darker the honey is and has higher antioxidant activity [11].

The fermentation of mead usually takes longer time as compared to other alcoholic fermented beverages as honey naturally contains many antimicrobial compounds. TSS of the honey must was daily evaluated by a hand-heldrefractometer and noted. The refractometer determines the estimate of presence of free sugar in the solution as sugar has refractive property. The TSS of the honey decline as the fermentable sugars are rapidly converted to the alcohol. The fermentation of honey was completed as the TSS of the honey must was constant. The fermentation was terminated as the available sugar was depleted and the produced alcohol also inhibit the viability of the yeast. The fermentation of honey was completed in 21 days but the wild honey must fermentation was completed within 14 days. In similar fermentation, the fermentation of honey took 3 to 4 weeks to several months depending on the type of honey and the culture used for fermentation [10].

Physiochemical evaluation of the produced mead was done by analyzing pH, TSS, acidity, turbidity and alcohol content. The pH of all the fermented mead was in the range of 3-5. The TSS of the mead was found to be 10º Brix in general but the WR, WRC1 and BR had 9 TSS and BC2 TSS was 12ºBrix. The acidity of the mead was determined by titration of mead with 0.1 N NaOH. The acidity % of the mead was noted at a range of 0.03-0.07%. The turbidity of the mead was checked by digital turbidimeter, and the mead fermented using wild honey had highest turbidity as the wild honey is raw honey as filtration of the honey during processing was not done. The turbidity of the commercial honey was comparatively less as they were filtered. The blended honey mead turbidity was in between the wild and commercial honey as they were blend of wild and commercial honey.

Alcoholic beverage quality is usually determined by alcohol content. Alcohol is produced by the fermentation of the sugars present in the honey. The alcohol % of all the mead was found to be in the range of 9-12%, with mead fermented using local honey with wine yeast (WL) being having the highest percentage of alcohol (11.19%). Though, the two variants of yeast; wine yeast and baker's yeast, the alcohol produced was nearly uniform. Although wine yeast focuses mainly on alcohol production the difference was not observed in the performed study.

Vitamin C content, antioxidant activity and reducing sugar were quantitively analyzed. The vitamin C content was determined by iodometric titration and vitamin C content was found to be highest in wild honey mead (1.25mg/100ml). The antioxidant activity was also highest in mead fermented from wild honey (39%). Wild honey contains many types of compounds as honeybees collect nectar from different types of wild flowers which attributes to the high antioxidant activity. High vitamin C content in wild honey may also be the reason for high antioxidant activity as vitamins C also has high antioxidant activity.

The residual reducing sugar present in the mead was analyzed. The reducing sugar quantity was determined by using 3,5-dinitro salicylic acid. Honey contains different types of reducing sugars, which accounts for the presence of reducing sugar in the mead. In all the fermented mead the reducing sugar was found to be in the range of 3.48 to 5.12 % by weight to volume. Honey itself contains many reducing sugars which are then fermented by yeast to give alcohol as a product. Honey usually have at least 60% of reducing sugar [10]. The honey was diluted and fermented which resulted in the decrease of reducing sugar in the final product.

The sensory evaluation of mead was done by 7 panelists using hedonic scale and each panelist tasted every beverage. One commercial dry wine was also placed in the test. All the samples were assessed using both ranking and descriptive analysis method. The panelists were asked to rank their preference from “dislike extremely” to “like extremely” based on 9-point hedonic scale. The mean average values of color, mouthfeel, taste, aroma, persistence, hardness and general acceptance of 7 panelists were calculated and noted. The more distinguished character of the alcoholic beverage is aroma and taste which is the major attribute of sensory evaluation [12]. All the produced mead were of acceptable quality. Where the mead prepared from type 2 commercial honey (WC2 and BC2) and the wine used for control has high score, whereas the mead prepared from wild honey with baker’s yeast (BR) had the lowest score. All other mead had average score in between them.

Conclusion:-

In this study, mead was made by fermenting different honey. The fermentation was carried out by dilution of the honey. Four different honeys were used for fermentation and the final alcohol percentage was determined and was found to be in the range of 8.68% to 11.19%. The prepared meads had acceptable taste and satisfactory characteristics. The mead prepared from locally available honey had diverse organoleptic properties whereas, mead fermented from commercial honey were consistent. Acidity, pH, TSS, presence of phenolic compounds, flavonoids, clarity and antioxidant activity of the mead was done to assess the quality of mead and was found to be of acceptable standard.

Authors Contribution

HJR and SP has contributed to the plan of the research work, sample collection, sample processing, data analysis, intellectual content design and result interpretation. PM supervised the research project.The authors drafted the manuscript and agreed for its publication.

Competing interests

No competing interests.

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Ethical approval and consent

Not applicable.

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