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Microbial Production of Vinegar (Sour wine) by using Various Fruits

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ABSTRACT *The production of vinegar was carried out by both seeded and non-seeded fruits. In this study Mango, Pineapple, Banana, Sapota and Coconut water fruits were used. Vinegar is a fermented food product and produced by fermentation technique. Some biochemical tests were done like sugar test, titrable acidity, pH, Phenol, Tannin and Flavonoid etc. Vinegar has several medicinal importants; it controls blood glucose level and diabetic management, cervical cancer screening tool etc. The microbial isolates of acetic acid bacteria were detected during Vinegar production.*

Introduction:-

Vinegar is produced in a way much similar to wine. Vinegar is a product of alcoholic and acetic fermentation by yeast and acetic acid bacteria. Vinegar is a product of alcoholic and acetic fermentation by yeast and acetic acid bacteria Vinegar is defined as a condiment made from sugary or starchy materials by an alcoholic fermentation followed by an acetous fermentation (Okafor, 1987). It must contain not less than 4% acetic acid (Beheshti Maal *et al.*, 2010). Vinegar from fruits and malt liquor carry flavor characteristics of these matrerials. It is interesting that different countries have preference for certain raw materials for vinegar production for ex- in Bratain, malt wort is used, whereas in France grape must is used etc. (Hawker,1974).

Naturally the production of vinegar depends on a mixed fermentation involving both yeast and acetic acid bacteria (Food and Agriculture Organization of the United Nations, Rome, 1998). Among the most important acetic acid bacteria, the strains of genus *Acetobacter* are mainly involved in vinegar production (Kadere *et al.*, 2008). It is considered as condiment composed of water, calories, carbohydrates, sugar, calcium, iron, magnesium, phosphorous, potassium, sodium, acetic acid, tannins, polyphenols with medicinal and therapeutic values. Vinegar has been safe and healthful beverage.

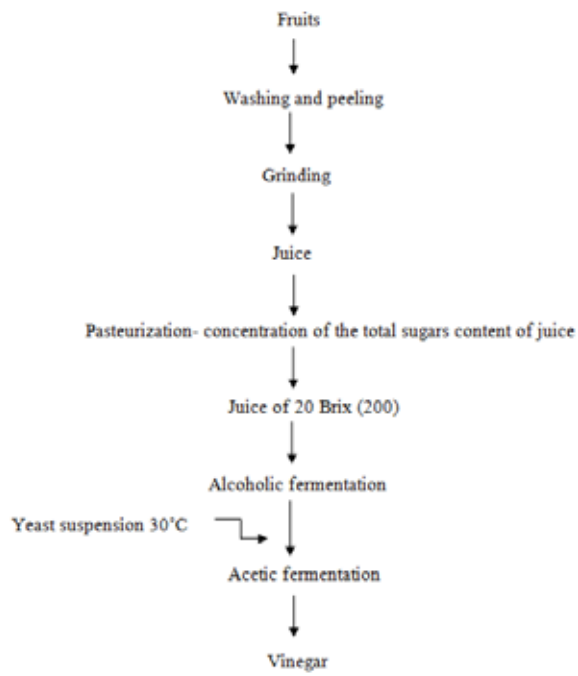
Natural vinegar is a superior food additive over synthetic vinegar as it carries essential amino acids from its fruit sources and is reported to act as medicine for aches and gastric troubles. (Yamagishi *et al.*,1998). However it is ignored by both the consumer and the producer. In rural areas the population resorts to traditional fermentation methods without the use of proper cultures/cultural conditions. Entrepreneurs hesitate to found vinegar production ventures due to the low efficiency of the process and the long fermentation times. Moreover, there is also a lack of awareness of the properties of natural vinegar, besides the problem of the high cost of investment.

Fruit vinegars are made from fruit wines, usually without any additional flavouring agent. Vinegar from fruits and malt liquor carry characteristics of these materials. Fruits are prime crop in all over the world and its juice is a substrate of choice for natural vinegar because of its high sugar content and availability. (Tewari *et al.*, 1991). The fruits are rich in vitamins and minerals etc. so, the vinegar produced from fruits is indirectly rich in vitamins and minerals etc.

According to Ethiraj and Suresh (1990) Mangoes are particularly biodegradable and highly perishable fruits, because of their high water content and the presence of carbohydrates. The sugars adhering to the mango fruits are ideal for fermentations. Several reports suggested the possibilities of alcohol and vinegar production from juice.

Material and Methods:-

Vinegar is a fermented food product, for its production fermentation technique is used. The production of vinegar was assayed according to following protocol.



For the production of vinegar from fruits first off all all fruits are washed to eliminate the dirt, afterwards the fruits are peeled off. Juice was obtained from the peeled fruits by grinding. The juice was heated at 65°C for 30 mins. in order to prevent microbial contaminations and to concentrate sugar unitl 20 brix. After cooling at room temperature the juices are distributed in 200 ml sterile bottles. Yeast suspension (2.5% inoculum) is added into it and bottles are kept in incubation for 4-5 days at 30°C.

Bottles are being examined every 24 hrs. according to alcoholic fermentation and sour smelling appearance. The production of acetic acid is determined by titration of sample with NaOH using phenolphthalein indicator. The acidity of

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vinegar is expressed in degrees of acetic acid was defined as the mass in a gram of acetic acid in 100 gm pure vinegar. Lotong *et al*., (1989).

The fermentation of vinegar is carried out in two successive stages alcoholic fermentation at 30°C for 4-5 days and acetic acid fermentation at 30°C for 10 days i.e. fermentation of vinegar is carried out in 15 days. The organisms concerned in vinegar production usually grow at the top of the substrate to form jelly like mass. This mass is known as "Mother of Vinegar." The mother is composed of both *Acetobacter* and yeast together.

After this fermentation, fermented solutions are inoculated on PDA agar and nutrient agar. The macroscopic traits of isolated colonies from fermented solutions are examined for morphological characteristics. Microscopic and biochemical examinations of pure individual colonies are carried out using Gram's staining, catalase and oxidase reaction. Time to time acetic acid is examined through titration assay. The biochemical tests which are performed in this study are shown as follows-

Sugar test, titrable acidity, test of tannin, flavonoids, phonolics, alcohol percentage.

Result and Discussion:-

Acetic bacterium identification

After the inoculation of fermented broth on PDA agar the plates are incubated at 30°C for 72 hrs. and after 72 hrs. pure single colonies are yielded on petriplates. The Gram staining revealed that the microorganisms were Gram-negative short rods to cocobacilli. Catalase and oxidase tests showed that they were catalase and oxidase positive. These characters are the main characters of *Acetobacter genus*. Thus, the isolated strain was affiliated to this genus and was coded *Acetobacter species*.

Table 1:- Recorded pH of vinegar protocols

Sr. No.	Sources	pH		
		Before Fermentation	After Fermentation	
			Alcoholic	Acetic
1	Mango	6	1-2	1
2	Banana	7	1-2	1
3	Pineapple	6.2	1	1
4	Sapota	7	1	1
5	Coconut water	6	5.6	2-3

Initially, pH of all fruits was basic and then after fermentation pH was acidic i.e. about 1-2. After alcoholic fermentation coconut water had pH 5.6 and after acetic fermentation was 2-3. As shown in table no.1

Ishiwu, C.N. *et al*., (2006), studies on Physico-Chemical and Sensory Properties of vinegar produced from Pineapple Peels, and they recorded pH before second stage fermentation showed an average of 5.3. In addition, the decrease in pH from 5.3 to 4.0 after the second stage fermentation. But in the present study we got pH 6.2 before second stage fermentation and after second stage fermentation was 1. According to Hufnagel and Hafmann (2008), pH is strongly dependent on organic acids such as acetic acid, malic acid or lactic acid levels.

Table 2:- Titrable acidity of vinegar protocols

Sr. No.	Sources	Titrable acidity
1	Mango	0.1
2	Banana	0.1
3	Pineapple	0.1
4	Sapota	1.5
5	Coconut water	0.2

Titrable acidity table shows that there is higher acidic content in Sapota i.e. 1.5. Mango, Banana and Pineapple had lesser acidic content than others i.e. 0.1 while Coconut water has slightly higher acidic content i.e. 0.2. It is shown in table no.2

But accordance to Ishiwu, C.N. *et al*., (2006), acidity of Pineapple peels increased from 3.12 to 4.55% after the primary and secondary fermentation respectively. They recorded the pH of pineapple peels and it was slightly lower than 6.2. This suggests that vinegar which has higher acidity value than that of alcohol occurred during the secondary fermentation of ethanol, consistant with Adams and Moss (1995).

Table 3:- Biochemical Test of vinegar protocols

Sr. No.	Sources	Phenol	Tannin	Flavonoid
1	Mango	No colour change	No white ppt	No colour change
2	Banana	Green	White ppt	No colour change
3	Pineapple	No colour change	White ppt	No colour change
4	Sapota	Black	White ppt	No colour change
5	Coconut water	No colour change	White ppt	No colour change

According to table no. 3 there was colour change in Banana and Sapota protocol to Green and Black respectively it indicates that presence of Phenolic group. Napthol or Catachol may be present in Banana protocol while Tannic acid or Gallic acid may be present in Sapota protocol. There was no formation of white ppt in Mango and formation of white ppt is observed in Banana, Pineapple, Sapota and Coconut water. No colour change in all 5 protocols during Flavonoid test indicates that there was absence of flavonoid in it. Flavonoid contain of Banana was recorded by David C. Mieman in 2012.

Table 4:-Sugar Test of vinegar protocols

Sources	Sugar Percentage
Mango	0.50%
Banana	0.50%
Pineapple	0.50%
Sapota	More than 2%
Coconut water	Absent

Mango, Banana, Pineapple protocol contains same sugar percentage i.e. 0.5% Sugar is absent in Coconut water while Sapota protocol contains more sugar than above all protocols. Ishiwu, C.N. and Iwouno, J.O. has studied on sugar content in Pineapple peels. The result showed the concentration of glucose 8.80 gm /100ml. according to table no. 4

Table 5 :-Alcohol percentage of vinegar protocols

Sr. No.	Sources	Alcohol percentage
1	Mango	0%
2	Banana	0%
3	Pineapple	0%
4	Sapota	0%
5	Coconut water	0%

For the detection of alcohol percentage take fermented broth through which got 0% alcohol, from that it is confirmed that alcohol is completely converted into acetic acid. So, it shows that the vinegar which is produced is completely in pure as per table no. 5.

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