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DEVELOPMENT OF THE OPTIMUM CONDITIONS OF MICRO-ORGANISMS TO UTILIZE MOLASSES FOR THE PRODUCTION OF VINEGAR

Part-II CONVERSION OF ALCOHOL TO ACETIC ACID

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The effect of packing materials, pH, temperature, aeration and concentration of alcohol on the rate of fermentation of alcohol to acetic acid in a vinegar generator, were studied. The standard selected procedure gave the best result, however, the replacement of beechwood shavings by corncobs as packing material produced comparable results. The lower concentration of alcohol in the stock was found to give a faster rate of fermentation. Corncobs as packing material yielded a product which was judged to be better in its colour, taste and clarity. Corncobs can successfully replace the beechwood shavings in vinegar manufacture.

INTRODUCTION

The conversion of alcohol to acetic acid, an oxidative change, is brought about through the activity of Acetobacter species under aerobic conditions. The success of this conversion depends upon a number of factors such as the type of bacteria, aeration of the medium, charge of the generator, temperature, pH and concentration of alcohol in the medium.

Underkofler and Hickey (1954) believed that the use of pure culture for the conversion of alcohol to acetic acid is un-economical and impractical but Prescott and Dunn (1959) recommended *Acetobacter aceti* for slow process. The oxygen or air requirments for this process have been investigated by vonLoesecke (1929), Robatanova (1944) and Hromatha and Ebner (1950). A number of investigations have been carried out on the packing materials of vinegar generators. Cruess (1946) suggested the use of cornocobs, coarse coke, charcoal and grape stems while Allgeir *et al.* (1952) recommended rattan wood, berl saddles and beechwood shavings as packing materials. However, beechwood shavings have been more commonly used than any other packing material. The temperature requirements of acetic acid bacteria are

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different for their growth than for the acetic acid fermentation. Various temperatures ranging from 80-95°F have been suggested for the production of vinegar in generator method by various workers (Biolitti, 1912; Cruess, 1921; and Mitchell, 1926).

Acetification of vinegar stock is a common practice but with no reference to the change in pH caused by such a treatment. The extent of acidification has ranged from 10-25 per cent by volume of strong vinegar and to bring the final acidity of the stock to 1 per cent. The concentration of alcohol in the vinegar stock has been recommended at 10-13 per cent by volume (Prescott and Dunn, 1959) and 9.0-9.2 gms per 100 ml of stock (Allgier *et al.* 1952).

MATERIALS AND METHODS

The vinegar stock, prepared by employing the standard conditions determined in earlier studies on fermentation of molasses (Nadeem and Tremazi, 1967) was subjected to acetous fermentation according to the following procedure.

The vinegar stock was inoculated with vinegar bacteria using live, unpasteurized vinegar. The inoculated stock was then fermented in a vinegar generator, devised in the Department of Food Technology, University of Agriculture, Lyallpur. It consisted of a hollow plastic cylinder closed on both sides by plastic plates and strengthened with additional aluminium plates. It was equipped with two perforated plates each located about 6 inches from the top and bottom. Between these perforated plates, the packing material was packed. Just below the lower perforated plate several holes were made for the supply of air from a compressor. The air was passed through water before entering the generator in order to remove any undesirable microorganisms which might otherwise be carried by air. A tilting boatshaped spout was devised out of plastic plates and located just above the upper perforated plate. The function of this spout was to distribute the vinegar stock uniformly over the packing material. The recirculation of the stock was carried out with the help of a sigmamotor which could take the stock from the bottom of the generator and put it at the top of the generator continuously. A set of three thermometers, located at the top, bottom and middle positions, was used to record the temperature in the generator. Each batch of stock was circulated in this charged generator for 7 days and it was analysed every 24 hours for acetic acid contents

To begin with, a standard procedure for the acetous fermentation with the following conditions was adopted. Alcoholic concentration - 5.61 per

cent by weight, pH - 4.1 (without adjusting the pH of vinegar stock), temperature-84°F, aeration - maximum, packing material - beechwood shaving. In addition to the standard procedure, another concentration of alcohol i.e. 2.8 per cent, a restricted aeration treatment, a pH of 4.6, a temperature of 94°F and two packing materials viz., corncobs and sheeshamwood shaving were also tried. These variables were tried by substituting one at a time in the standard procedure.

The packing materials were prepared by soaking in water overnight, washing in water several times, drying in air, soaking in 5 per cent alcohol solution for 2 hours, drying and finally treating with 4 per cent acetic acid solution.

Determination of Acetic Acid: The acetic contents of the stock before and during fermentation was determined according to the total acid method as described in the A.O.A.C. (1).

Organoleptic Evaluation of Vinegar: The vinegar produced by using the various kinds of packing materials was evaluated organoleptically by using the scoring method with some modifications due to limited facilities available. The staff members and students of the Department of Food Technology, numbering 10 in all, were tried as judges, using samples of vinegar obtained from local market. Out of these only 6 members who were most consistent in their reaction, were finally chosen to constitute the panel of judges. The experiments were conducted under ordinary ventilation, light and temperature conditions because no control over these conditions was possible.

RESULTS AND DISCUSSION

The data on the effect of different packing material, pH, temperature, aeration and concentration of alcohol on the rate of fermentation of alcohol to acetic acid are presented in Table 1, 2, and 3.

The data on the organoleptic evaluation of vinegar are presented in Table 4. The figures represent an average of 18 judgements made by six judges in each case.

The data (Table 1) revealed that the standard selected procedure resulted in the maximum yield of 4.23 per cent acetic acid after four days of fermentation. This yield corresponded to an efficiency of 57.7 per cent. This low efficiency was probably due to the reason that the bacteria in the starter were inherently not capable of fermenting alcohol beyond this limit or that these species could not tolerate higher acidity. It was also observed that there was a gradual decrease in the acetic acid after the fourth day. This

trend was observed in all the experiments. This may probably be due to the oxidation of acetic acid by certain overoxidizing vinegar bacteria present in the starter.

The replacement of beechwood shavings by corncobs as packing material exhibited similar trend of results (Table 2). The maximum amount of acetic acid accumulated was lower than that of the standard procedure. The use of sheeshamwood shaving as packing material resulted in not only a slower rate of fermentation but also significantly lower yield of acetic acid. Increase in the pH and temperature over that of standard procedure resulted in decreased rate of fermentation and the maximum accumulation of acetic acid in this case was significantly lower than that of the standard.

In restricting the flow of air through the generator, the rate of fermentation slowed down to such an extent that it was not complete even after 7 days as compared to 4 days in case of the standard (Table 3).

The use of vinegar stock with lower concentration of alcohol than that of standard resulted in the fastest rate of fermentation. The fermentation reached its peak in 3 days (Table 3). Although such a product with only 2.01 per cent acetic acid cannot be called a vinegar yet it can profitably be used in blending with high strength vinegar.

Organoleptic Evaluation: This evaluation was limited to the vinegar produced by using different types of packing materials because the other factors studied do not influence the attributes such as colour, flavour, taste and clarity. The data (Table 4) has shown that sample B was the best product with respect to its colour, taste and clarity, followed by sample A. With respect to flavour, sample B was judged as best followed by sample A. Sample C was found to be significantly inferior product in comparison to A and B. It may, therefore, be concluded that locally available corncobs can be successfully used as packing material in place of beechwood shavings.

TABLE 1. *Rate of conversion of alcohol to acetic acid under standard conditions.*

Time in day	Standard Procedure	
	Amount of acetic acid in grams per 100 mls of the stock during fermentation	Grams of acetic acid actually produced per 100 mls of stock
0	1.80	—
1	3.09	1.29
2	4.23	2.43
3	5.28	3.48
4	6.03	4.23
5	5.70	3.90
6	5.43	3.63
7	5.25	3.45

TABLE 2. *Effect of packing material on the rate of conversion of alcohol to acetic acid.*

Time in days	Corncobs		Sheeshamwood shaving	
	Amount of acetic acid in grams per 100 mls of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock	Amount of acetic acid in grams per 100 mls of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock
0	1.80	—	1.80	—
1	2.97	1.17	2.76	0.96
2	4.08	2.28	3.42	1.62
3	5.10	3.30	4.20	2.40
4	5.94	4.14	4.86	3.06
5	5.61	3.80	5.28	3.48
6	5.34	3.54	5.55	3.75
7	5.19	3.39	5.10	3.30

TABLE 3. *Effect of pH, temperature, aeration and alcohol concentration on the rate of conversion of alcohol to acetic acid.*

Time in days	Effect of pH pH 4.6		Effect of Temperature 94±1°F		Effect of Aeration Restricted aeration		Effect of Concentration of Alcohol 2.8%	
	Amount of acetic acid in grams per 100 ml of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock	Amount of acetic acid in grams per 100 ml of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock	Amount of acetic acid in grams per 100 ml of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock	Amount of acetic acid in grams per 100 ml of the stock during fermentation	Grams of acetic acid actually produced per 100 ml of stock
0	1.44	—	1.80	—	1.77	—	1.74	—
1	2.40	0.96	2.88	1.08	2.64	0.87	2.61	0.87
2	3.57	2.13	3.60	1.80	3.42	1.65	3.33	1.59
3	4.41	2.97	4.32	2.52	3.99	2.22	3.75	2.01
4	5.10	4.66	5.04	3.24	4.44	2.67	3.51	1.77
5	5.52	4.08	5.52	3.72	4.86	3.09	3.45	1.71
6	5.31	3.87	5.34	3.54	5.34	3.57	3.36	1.62
7	5.16	3.72	5.10	3.30	5.82	4.05	3.30	1.56

TABLE 4. *Organoleptic evaluation of vinegar - Scoring method.*

Packing Material	Sample Code	ATTRIBUTES Colour	Flavour	Taste	Clarity
Beechwood Shavings	A	7.333	7.000	7.833	8.000
Corn Cobs	B	7.833	7.500	7.167	8.500
Sheeshamwood Shavings	C	3.500	2.833	7.167	2.500

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