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Research Article

SYNTHETIC HONEY PREPARATION BY YEAST CELLS AND IMMOBILIZED YEAST CELLS FROM SALIVARY GLANDS OF HONEY BEES

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ABSTRACT
Yeast from the salivary glands of honey bees *Apis mellifera* and *Apis cerana* was isolated and used for preparation of synthetic honey. Free as well as immobilized yeast cells of these two species were studied for efficiency in preparation of synthetic honey from sucrose solution. The solutions exhibited lowered pH and increased level of fructose and total amino acids. The content of synthetic honey increased with increase in the time of incubation till 48 hours and decreased thereafter. The obtained solution possessed properties similar to the natural honey.

Keywords: *Synthetic Honey, Apis Mellifera, Apis Cerana, Immobilized Yeast*

INTRODUCTION
Honey is a natural sugary substance synthesized from the nectar by honey bees. The bees collect honey and transform it by combining it with their specific substances that is dehydrated and stored in the honey comb to ripen and mature. Honey consists of D-fructose and D-glucose and compounds like organic acids, enzymes, and solid particles collected by bees. The color of honey varies from colorless to dark brown. It may be fluid, viscous, or solid. Honey flavor and aroma may vary depending on the plant origin. Varieties of honey can be identified by their color, taste, flavor, and manner of crystallization. Under some circumstances, the honey sediment is also analyzed for the content of pollen grains. In honeydew different varieties of honey and other components which are characteristic of them, like algae, fragments of mycelium, spores, or leaf fragments, are determined. Some other characteristics are helpful in identifying the honey type like specific conductivity, variety-specific flavor compounds, and sugar content. Honey, obtained from the sealed comb cells, is a naturally converted form of sugary food from the nectar of flower and other plants exudation, systematically collected and stored by honey bees (Anonymous, 1988).
Nectar, the raw material of honey is produced by the flower of many plants. To change the nectar to honey, the bees convert the complex sugar sucrose to simple sugar fructose and glucose using the enzyme produced by special salivary glands. Invertase is an enzyme present in the nectar and more is added the bees from salivary gland. Ripening of the honey takes place by the action of this enzyme and by the evaporation of water by fanning (vibration) of wings (Srivastava, 1993).
Sucrose $\xrightarrow{\text{invertase}}$ Levulose (fructose) and Dextrose (glucose).
Therefore, attempt were made to study production of “Synthetic Honey” by free as well as immobilized yeast cells, isolated from the salivary glands of *Apis cerana* and *A. mellifera* (Kathiresan and Srinivas, 2005).

MATERIALS AND METHODS
Bees: The honey bees *Apis cerena* and *A. mellifera* were obtained from Entomology Unit, Department of Zoology, Science College Nanded. Bees were dissected and the salivary glands were isolated under dissecting microscopes.
Media and Culture Conditions: The yeast cells were isolated by streaking the salivary glands of bees aseptically on the Sabouraud maltose agar medium of pH 5.6. After 3-5 days yeast cells appeared as opaque, pasty. Sabouraud Maltose agar was used for the isolation and culture maintenance of yeasts.

Temperature of incubation was 28°C for yeast. Yeasts were maintained in stabs of Sabouraud Maltose agar slants and subculturing was done with transfers every 15 days. Freeze drying was used for longer conservation periods.

Isolation and Taxonomical Study of Fermentation Yeast:

Fermentation yeast was isolated on plates of an agar-solidified YPD medium (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; tap water, 1,000 ml) containing 50 μg chloramphenicol/ml. The isolated strain of yeast was maintained on YPD slopes. A taxonomic study of the yeast strain was carried out.

Immobilization and Inoculation of Yeast Cells: The yeast cells were immobilized by using sodium alginate (3%) and $CaCl_2$ (0.2M). The yeast cells and immobilized yeast cells were inoculated in sterile sucrose solution containing sucrose (8%), peptone (1%) and pH 6.5. The control was prepared by without inoculating these cells. The flaks were incubated on a shaker. The sucrose solution was observed every 24hrs for 6 days and analyzed for sweetness, pH, total amino acids and fructose concentration (Plumer, 2004).

Inoculation of Salivary Glands: Salivary glands from dissected bees were used for the inoculation. Ten glands were inoculated into 250 ml conical flask, containing 100 ml of 8% sucrose and 1% peptone (pH 6.5). The flasks were incubated in a shaker. Sensory evaluation for sweetness was made at every 24 hr for 6 days with the help of three persons of 25 ± 1 year old. The intensity of sweetness increased at each time was recorded qualitatively in '+' marks by the persons separately and average of the marks is given in Table 1.

RESUTLS AND DISCUSSION

Results

Identification of Yeast Cells: The yeast cells were appeared as opaque, pasty and appeared as spherical and budding under microscope after staining with lactophenol cotton blue.

Isolation and Taxonomical Study of the Yeast: A strain of yeast was isolated from *salivary glands* was found to be a fermentation yeast with globose or subglobose cells. Pseudohyphae were not observed during cultivation. The yeast fermented sugars such as D-Glucose, maltose, D-galactose, but lactose was not fermented. Yeast was able to utilize D-glucose, D-galactose, maltose, and sucrose, but sugars such as 2-keto-D-gluconate, glycerol, D-xylose, adonitol, xylitol, inositol, D-sorbitol, N-acetyl-D-glucosamine, D-cellobiose, lactose, D-raffinose, D-trehalose, L-arabinose were not utilized. These characteristics confirmed the identity of the strain to the genus *Zygosaccharomyces* and was considered to closely resemble *S. cerevisiae* (Barnett *et al.,* 1990).

Yeast and Immobilized Yeast Induced pH Changes: There was no change in pH of control solution (sucrose). However, when the yeast cells and immobilized yeast cells were inoculated, the sucrose solution shows a decline in pH over the culture period up to 144hrs. The pH was drastically reduced from 6.5 to 5 between 24 to 48 hrs and then there was no change.

Yeast and Immobilized Yeast Induced Change in Level of Fructose: There was no formation of fructose in the control solution untreated with yeast cells and immobilized yeast cells. However, when yeast cells and immobilized yeast cells were inoculated a substantial increase in level of fructose was observed in the sucrose solution. This increase was drastic within 24 to 48 hrs.

Yeast and Immobilized Yeast Induced Changes in Level of Total of Amino Acids: The level of total amino acids was consistently higher in yeast cells and immobilized yeast cells treated sucrose solution throughout the culture period than the control. The level increased at between 24 to 48 hrs of culture and then showed a declining trend in yeast cells and immobilized yeast cells treated solutions. Where as the total amino acids showed a declining trend in control throughout the days of experiment.

Discussion

The salivary glands of honey bees are important in honey making. Therefore, some factors like enzymes are present in the salivary glands and are involved in honey making. One of such is yeast cells derived from flowers and present in the salivary glands of honey bees. The yeast cells produce the enzyme invertase. This enzyme breaks the sucrose into fructose and glucose. These two sugars are the major

constituents of the honey responsible for its sweetness (Kathiresan and Srinivas, 2005). The present experiment has shown that yeast cells and immobilized yeast cells convert sucrose solution into honey like solution with enhancement in the level of fructose, total amino acids and sweetness as revealed by sensory evaluation.

The honey like solution is similar to natural honey in terms of fructose, glucose, sucrose and pH. Therefore, honey can be produced artificially using yeast cells and immobilized yeast cells. The immobilization of yeast cells has many advantages, and there is a great scope for developing this honey making at the industry for large scale production.

Observation Tables

Table 1: Changes in Sweetness of Sucrose Solution Inoculated with or without Salivary Glands of the Honey Bee *A. Mellifera* and *A. Cerana*

Time (hr)	Without Salivary	with Salivary Glands (*A.Mellifera*)	with Salivary Glands (*A. Cerana*)
1	+	+	+
2	+	+	+
4	+	++	++
6	+	++	++
12	+	++	++
18	+	+++	+++
24	+	+++	+++
48	+	+++	+++
96	+	+++	+++
120	+	+++	+++
144	+	+++	+++

Table 2: Changes in Sweetness of Sucrose Solution Inoculated with or without Salivary Glands of the Honey Bee *A. Mellifera* and *A. Cerana*

Time (Hours)	Control	Yeast Cells (*A. Cerana*)	Yeast Cells (*A. Mellifera*)	Immobilized Yeast Cells (*A. Cerana*)	Immobilized Yeast Cells (*A. Mellifera*)
0	6.5	6.5	6.5	6.5	6.5
24	6.5	6.5	6.5	6.5	6.5
48	6.5	5	5	5	5
72	6.5	5	5	5	5
96	6.5	5	5	5	5
120	6.5	5	5	5	5
144	6.5	5	5	5	5

Table 3: Changes in Fructose Concentrations by Yeast Cells and Immobilized Yeast Cells

	Changes in Fructose Concentration in mg/ml				
Time (Hours)	**Control**	**Yeast Cells (*A. Cerena*)**	**Yeast Cells (*A. Mellifera*)**	**Immobilized Yeast Cells (*A. Cerena*)**	**Immobilized Yeast Cells (*A. Mellifera*)**
0	0	0	0	0	0
24	0	54	52	51	51
48	0	69	65	64	66
72	0	70	68	69	68
96	0	60	61	62	59
120	0	52	50	61	60
144	0	48	48	47	48

Table 4: Changes in Level of Total Amino Acids by Yeast Cells and Immobilized Yeast Cells

Level of Total Amino Acids in mg/ml					
Time (Hours)	**Control**	**Yeast Cells (*A. Cerana*)**	**Yeast Cells (*A. Mellifera*)**	**Immobilized Yeast Cells (*A. Cerana*)**	**Immobilized Yeast Cells (*A. Mellifera*)**
0	0	0.2	0.3	0.2	0.4
24	0	0.8	0.8	0.8	0.9
48	0	1.5	1.7	1.5	1.7
72	0	1.2	1.5	1.5	1.8
96	0	1	1.4	1.2	1.3
120	0	0.8	1	0.9	1
144	0	0.8	0.8	0.7	0.9

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