Making artificial honey using yeast cells from salivary glands of honey bees

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The salivary glands of a honey bee, *Apis cerana* and the yeast cells isolated from these glands were studied for their effects on sucrose solution. This solution exhibited lowered pH and increased levels of fructose and total amino acids as the time of incubation proceeded. The solution thus made was similar to the natural honey.

**Keywords:** *Apis cerana*, Artificial honey, Honey bees, Yeast cells

The traditional Indian systems of medicine, Ayurveda and Siddha, rely heavily on honey as a carrier of drugs. The honey is composed of 80% sugars and 20% water and the sweetness is mainly due to the presence of fructose. The honey bees collect nectar from the flowers and mix it vigorously with their saliva. During this process the level of fructose in the plant nectar is increased and honey is produced and is deposited in the honey combs. The process of honey-making is due to the enzymes that are produced either by salivary glands or by yeast cells present in the glands. Therefore, an attempt has been made under laboratory condition to produce 'artificial honey' by converting sucrose into fructose with the help of saliva and/or yeast cells, isolated from the salivary glands of the honey bee, *Apis cerana* Fabr.

**Isolation and inoculation of salivary gland**—Salivary glands were dissected from the honey bee species, collected from Pichavaram mangrove forests, Tamil Nadu. Ten glands were inoculated into 250 ml conical flask, containing 100 ml of 8% sucrose and 1% peptone (pH 6.6). The flasks were incubated in a shaker (Piscines Instruments, India), under a dust-free environment. 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.

**Isolation of yeast cells**—The yeast cells from the salivary glands were isolated by streaking the glands aseptically on the Petri dishes containing with Sabouraud agar medium consisting of neo-peptone (5 g l⁻¹), glucose (10 g l⁻¹), agar (20 g l⁻¹) at pH 6.5. After 4-7 days, yeast cells developed on the plate as white colonies initially and became yellow later. The yeast cells appeared as pasty, opaque and usually pale coloured with sweet smell reminiscent of ripen apple. The cells were picked from the Petri plates and observed under a microscope. After staining with lacto-phenol cotton blue, the cells were observed spherical and budding.

The yeast cells were inoculated in sterile sucrose solution in the 250 ml conical flasks, containing of sucrose (8 g l⁻¹) and supplemented with a nitrogen sources of peptone (1 g l⁻¹) at pH 6.5. The flasks were incubated in a shaker in a clean and dust-free room. The sucrose solution was observed every 24 hr for 6 days and analysed for sweetness, pH (using pH pen), levels of total amino acids (using ninhydrin as reagent) and fructose (using dinitrosalicylic acid as reagent).

**Salivary gland-induced changes**—The salivary glands increased sweetness of the sucrose solution, as the time of incubation proceeded up to 144 hr especially between 24 and 48 hr; however, there was no change in the control solution without salivary gland (Table 1).

**Yeast-induced pH change**—There was no change in pH in sucrose solution that was not inoculated with

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<th>Time (hr)</th>
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<th>With salivary gland</th>
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Table 1—Changes in sweetness of sucrose solution inoculated with or without salivary glands of the honey bee *A. cerana*

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Yeast cells. However, when the yeast cells were inoculated, the sucrose solution showed a decline in pH over the culture period up to 144 hr. The pH was drastically reduced from 6.5 to 6.0 between 24 and 48 hr and then there was no change (Fig. 1a).

Yeast-induced change in level of fructose—There was no formation of fructose in the control solution untreated with yeast. However, when yeast cells were inoculated, a substantial increase in the level of fructose was noticed in sucrose solution. This increase was drastic within 24 to 72 hr (Fig. 1b).

Yeast-induced change in level of total amino acids—The level of total amino acids was consistently higher in yeast-treated sucrose solution throughout the culture period than the control; the level increased at 24 hr of culture and then showed a declining trend in the yeast-treated solutions, whereas the total amino acids showed a declining trend in control throughout the days of experiment. The level was higher by 92.9% in sucrose solution after 144 hr of incubation (Fig. 1c).

Salivary glands of honey bees are important in honey making, as is evident in the present study that the salivary glands increased the sweetness of sucrose solution (Table 1). Therefore, some factors that are present in the salivary glands are responsible for honey making. One 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 honey, responsible for its sweet taste. The present experiment has shown that the yeast cells convert sucrose solution into honey-like solution, with enhancement in the levels of fructose, total amino acids and sweetness as revealed by sensory evaluation. The honey-like solution is similar to the natural honey in terms of fructose, glucose, sucrose and pH (Table 2). Therefore, honey can be produced artificially using yeast cells and there is a great scope for developing this honey making as an industrial venture.

References

3 Wakhle D M, Beekeeping technology—production characteristics and uses of honey and other products, in Perspectives in Indian apiculture edited by R C Mishra & Garg R (Agro Botanica, Vyas Nagar, Bikaner, India) 1998, 150.
4 Varadharajan M, Foraging strategies of the Indian honey bee, Apis cerana indica, with reference to pollen preferences and nectar collection under central-place foraging and risk-


