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Estimation of protein in *Apis cerana indica* honey sample from Annamalai Nagar, Tamil Nadu, India

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Abstract
In south India *Apis Cerana Indica* is used for commercial beekeeping and honey production. It provides nutritional, economic and ecological security to rural communities, especially in the developing countries like India. Honey is a natural, viscous, sweet product used as an ingredient flavour, colour, sweetness and caramelisation. Honey is composed mainly of fructose and glucose, water with low protein levels. Protein are minor, but important components of honey originating from nectar, protein. There are some protein compounds in honey in addition to sugar, lipids and mineral compounds. The quantitative of proteins in honey is considered as good index. In this research honey samples was collected from Annamalai Nagar apiary and the studies were carry out to determine the protein quality.

Keywords: Viscosity, protein, caramelisation, minerals, sugar colour, sweetness, commercial, ecological security

Introduction

Honey bees are social insect and nature's gift to mankind. They are industrious pollinators because they have co-evolved with following plants over millions of years. Bees play a remarkable role in maintaining ecological balance and biodiversity that can exploit virtually all habitats in the world (Ghisalbeeti, 1979). Honey bee exhibits a large range of advantages to public though its remarkable products like honeybee – pollen, bee bread and royal propolis and bee venom (Cornara *et al*., 2017) [6].

Worldwide, honey the most valuable product with reference to medicinal as well as economic point of view. It has been widely adopted as food and medicine by both ancient and modern generations. Honey is a rare viscous, palatable and sweet product with high nutritional value and assay of health benefits (Bogdonov *et al*., 2008). Honey comprises of water and sugars along with vitamins, aminoacids, proteins, polyphenols, minerals, micronutrients and aroma compound (White and Donee, 1980, Bogdanov *et al*., 2008). Honey also comprises range of vitamins such as ascorbic acid, pantothenic acid, niacin and riboflavin. The mineral composition of honey includes calcium, iron, magnesium, manganese, copper, phosphorus, pottasium, zinc along with traces of selenium and chromium.

Honey, a natural sweet product prepared by honeybees using flower nectar (Dashora *et al*., 2017), has been used for its nutritional and medicinal properties for over 5000 years (Adebolu, 2005, Samarghandian *et al*., 2017) [10]. Honey is the sole natural product derived from insects and is valued for its nutritional, cosmetic and therapeutic uses. It is considered a balanced diet suitable for people of all ages and genders due to its high nutritional value (James, 1906; Bansal *et al*., 2005) [3, 9]. Honey is rich in fructose and glucose, along with the other nutrient such as proteins, vitamins, amino acid, minerals, organic acids, enzymes (Borel *et al*., 2020) [5].

Proteins are arge biomolecules and macromolecules with long chains residues. It is made up of smaller components called amino acids. Some proteins are having all of the essential amino acid. Other proteins are incomplete, these proteins are missing some of the essential amino acids. The trace amount of protein in honey is also incomplete. In general the honey is classified by means of origin source of the nectar from the plants. Honey carbohydrates have the caping to rotate linear polarized light. The specific rotation power is based on the quantity and quality of the sugar present in honey. The protein and carbohydrate content in the honey may warry depend on the floral source and environmental factor.

Material and methods

Honey samples were collected from Annamalai Nagar apiary at regular intervals by means of normal rotary extractor. Protein estimation was done by Bradford's method 1976. It is based on the observation of a shift in wavelength from 465nm to 595nm for coomassive brilliant blue G-250 dye in an acidic solution as it binds to a protein. The dye interacts with both hydrophobic and basic amino acid of the protein. With increasing protein concentration, the dye changes colour brown to blue to darker shades of blue. The assay is mainly based on the colour changes achieved with basic amino acids combined with coomassive dye, it is under acidic conditions, changes the colour of the sample from brown to blue.

Types of honeybees

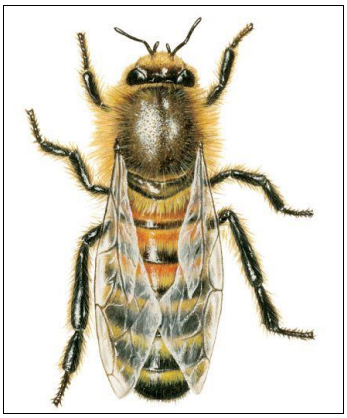

Fig 1: Worker

Fig 2: Queen

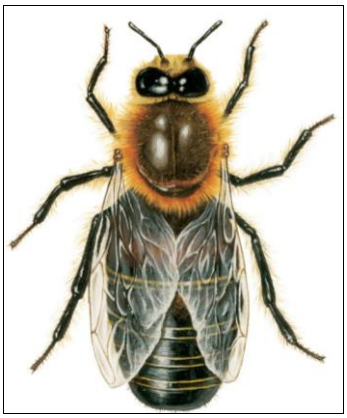

Fig 3: Drone

Preparation of BSA Standard stock:

Standards	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Dist. H2O	900µl	500µl	500µl	500µl	500µl	500µl	500µl	500µl
Serial dilution of BSA	100µl from the stock	500µl	500µl	500µl	500µl	500µl	500µl	500µl
Conc. of standards	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	0.312 mg/ml	0.156 mg/ml	0.078 mg/ml	0.039mg/ml

Procedure:

1. Honey sample was mixed with 10 µl of each standard solution and test samples to the Elisa titter plate then it was mixed with 100µl of Bradford's reagent for 10 minutes the plate in incubated. The value was measured at 595nm a microplate reader. The unknown concentration value was collected.

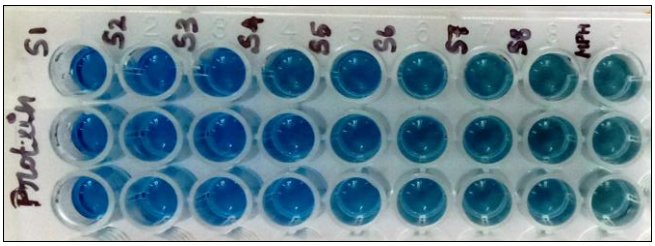

Fig 1: Protein content of the extracted honey sample (MPH)

Result and discussion

Honey is a complex natural food with many references to mythology, history and tradition of most peoples. Honey is a sweet and palatable product which consists of a complex mixture of carbohydrates, mainly glucose and fructose other sugar are present as traces, depending on the floral origin (Abrol DP 2013). The contribution and sensory characters of honey vary considerably depending on its botanical and environmental nature owing to its high nutritional and gustatory content, honey is one of the most popular food products, it is wildly used in apitherapy; (Abrol, 2013) [1].

Table 1

BSA mg/ml	OD		
5	2.991	2.993	2.974
2.5	1.004	1.054	1.026
1.25	0.808	0.867	0.878
0.625	0.7	0.779	0.798
0.312	0.56	0.578	0.599
0.156	0.342	0.381	0.37
0.078	0.11	0.126	0.148
0.039	0.033	0.091	0.091

Table 2

Name of the sample	OD value at 595 nm	Total protein content	Mean value of total protein content (mg/g)
MPH	0.521	0.61946	6.18
	0.513	0.60405	
	0.527	0.63102	

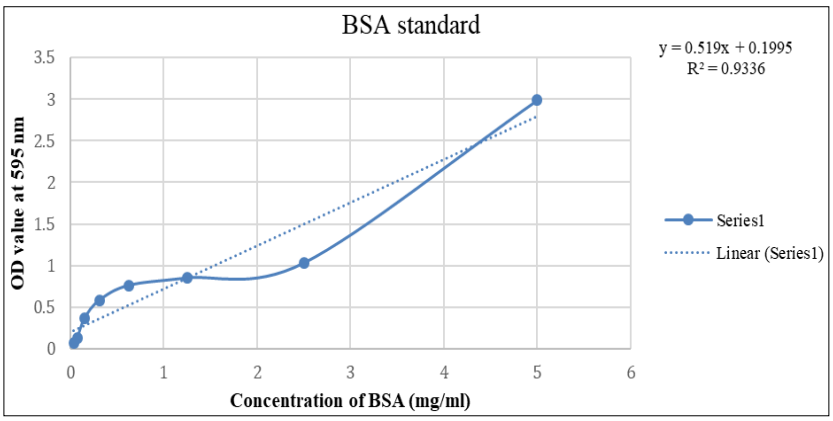

Fig 2

Proteins are polypeptide structures containing of one a more long chain of amino acid recidues. They carry out a wide variety of organism functions, transporting molecules catalysing metabolic reachons, and providing structural support to cells (Brodschneider R, Crailsheim K 2010). Proteins are needed for the body function properly. They are the basis of body structures, such as skin and hair, and of other substants like enzymes, cytokines and antibodies. Protein is an important part of a healthy diet. Proteins are made up of chemical building blocks called amino acids. The amino acids are used to build and repair muscles and bones and to make hormones and enzymes. They can also be used as an energy sources (Fraga, 2005) [7]. Proteins are of great nutritional value and are directly involved in the chemical processes essential for life. A protein molecule is very large compared with molecules of sugar or salt and consists of many amino acids joined together to form long chains. They are about 20 different amino acids that occur naturally in proteins (Arredona and Nunez 2005).

Honey has about 31 different minerals like phosphorus, potassium and magnesium. It also has several important amino acids – which are considered as building blocks of proteins. Honey is high in polyphenols and flavonoids which act as antioxidants. They help protect our body against some types of cell damage (Silva *et al*., 2009). The quality of honey depends on the season, floral abundance and environmental conditions.

Honey has been used as a wound dressing and used for the treatment of coughs due to antibacterial properties (Weston 2000) [12]. This study was carried out from honey collected from Annamalai Nagar, while compered with privacy studies honey is good in all aspects with reference to protein, carbohydrate, polyphenols and flavonoids.

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