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Analysis of Chemical Constituents of Honey from Kashmir

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Abstract—*Honey is the natural sweet substance produced by honey bees, from the nectar of plants (blossoms). Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties. In jammu and kashmir, honey production has the potential to develop as a prime agro-horticultural and forest-based industry which can be a major foreign exchange earner if international standards are met. Honey is one of the most popular natural sweet substances. From a chemical point of view, it could be defined as a natural food mainly composed of sugars and water together with minor constituent such as protein, fat and ash. Each of these constituents is known to have distinctive nutritional or medicinal properties and the unique blend accounts for the varied and different applications of natural honeys Its composition is particularly variable, depending on its botanical and geographical origins. The aim of this study was to determine principal compounds present in honey and the analytical methods employed for their analysis. The results of this study indicate that the samples compare favorably with samples in many parts of the world and also fall within the limits of international standards.*

Keywords: *Honey , Chemical composition, Analysis.*

Introduction

Honey is the natural sweet substance produced by honey bees, from the nectar of plants (blossoms). Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties.. It is defined as the natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew (Codex Alimentations, 2001). Honey is one of the few virtually totally non-allergic foods that body easily assimilates. It contains nutrients especially as energy provider Rahman et al. (2010), it is a high-energy carbohydrate food (80–85%) and the honey sugars are easily digestible as those in many fruits (White and Doner, 1980). Bogdanov et al. (2004) found more than 22 sugars in honey; however, fructose and glucose are the major sugar content. Primary sugars existed in honey are fructose and glucose, and in nectar honey the fructose content should exceed that of glucose Zafar et al. (2008). Furthermore, the sum of fructose, glucose, fructose/glucose ratio and glucose/water ratio are other important factors related to honey quality Honey contains more than 180 substances, including amino acids, enzymes, protein, vitamins, minerals, ash, organic acids and phenol compounds Ouchemoukh et al. (2007), each of these minor constituents is known to have distinctive nutritional or medicinal properties and the unique blend accounts for the varied and different applications of natural honeys [3]. Moisture content of honey represents a major importance to its stability against fermentation and granulation. The low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods (AL-Naji and Hujazy, 1982; Buba et al., 2013; Akhtar et al., 2014). Moisture content of honey is a critical variable influencing product quality, granulation and texture, is significantly affected by conditions under which honey is stored following its extraction from the hive. This study is aimed to evaluate the chemical and nutritional characteristics of honey obtained from the local market of Srinagar Kashmir.

Materials and methods

Honey samples Honey was collected from the local market of Srinagar Kashmir. All samples were stored at ambient conditions (_28 ±2 _C) till further analysis to avoid the effect of laboratory conditions on the chemical composition and physical properties of honey samples (El-Metwally, 2015).

Moisture content

Moisture content of honey was determined from the refractive index of the honey. A digital refractometer (NR 101 Spain) was used for the determination of moisture content.

Crude protein (%)

Total nitrogen was determined by the Macro-Kjeldhal procedure (AACC, 2000). Two grams of samples were digested in Kjeldhal flask with digestion mixture (copper sulphate and potassium sulphate in 1:9 ratio) and concentrated H_2SO_2 (20 ml) till light green colour appeared and finally cooled. Ammonia released by distillation of digested samples with saturated NaOH (80ml) was captured in 0.1N HCl and per cent nitrogen was estimated. The percentage of nitrogen quantified was transformed into protein content by multiplying by a conversion factor of 6.25.

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Ash (%)

Standard AACC procedure (AACC, 2000) was followed. 5 g sample was put in a pre weighed silica dish, charred on the hot plate and incinerated in muffle furnace at a temperature of 550±10 °C for about 3 hours. The dish was cooled, weighed and ash content was expressed as percent ash as below:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude fibre (%)

Crude fibre was estimated by the Fibre Tech (AOAC, 1965). The sample was subjected to acid and alkali digestion, residue obtained comprised ash. The residue was ignited, organic matter was oxidised and inorganic residue or ash was left behind. The difference in weight before and after ashing was determined.

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W} \times 100$$

Where,

W = wt. of sample in grams

W_1 = wt. of residue (crude fiber + minerals) in grams

W_2 = wt. of ash remained

Carbohydrates (%)

Per cent carbohydrate was determined by the difference method as follows:

Carbohydrate (%) = 100 - (Moisture % + Fat %+ Protein % + Ash %+ Fiber %)

Sugars (reducing sugars, sucrose, glucose and fructose)

The estimation of reducing sugars was carried out using the Layne-Enyon method as described in AOAC [23]. About 2.6 g of honey was weighed and transferred to a 500 mL volumetric flask. Five milliliters (5 mL) of standardized Fehling's solutions A and B were transferred to a 250 mL Erlenmeyer flask containing 7.0 mL of water and 15.0 mL of honey solution. The Erlenmeyer flask was heated and 1.0 mL of methylene blue (0.2%) was added. Titration was carried out by adding the diluted honey solution until the indicator decolorizes.

Sucrose content was determined by inversion, adding 10 mL of dilute HCl, 50 mL of diluted honey solution and water in a 100 mL volumetric flask. The solution was then heated in a water bath, cooled and diluted to the mark. Finally, the Layne-Enyon method was applied and the sucrose content was obtained by difference.

Glucose content of the honey samples was determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., UK). Twenty microlitres (20 µL) of the sample or standard was allowed to react with 2.0 mL of the reagent, mixed well and incubated for 10 min at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against a reagent blank within 60 min. Glucose concentration was calculated as follows:

$$\textit{Glucose content (mg/dL)} = (A_{sample}/A_{standard}) \times \textit{Conc. of standard}$$

$$= (A_{sample}/A_{standard}) \times 100 \textit{ (mg/dL)}$$

Fructose content was determined using the resorcinol reagent method [24]. To a solution of the honey sample, 1.0 mL resorcinol reagent was added and mixed thoroughly, and then 1.0 mL of dilute HCl was added. Standard solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL and made up to 2 mL with distilled water was also treated with 1.0 mL of the resorcinol reagent and 1.0 mL of diluted HCl as above. A blank solution was also prepared along with the standard and treated in the same manner. The test solution, the standard and blank were then heated in a water bath at 80°C for about 10min, the solution was then removed from the water bath, cooled by immersing in tap water for 5min and then the absorbance of both the test and standard solution were read against the blank solution at 520 nm within 30 min. The fructose contents of the honey samples were then extrapolated from a standard curve prepared using the absorbance of the standard.

Calorific value(Kcal/100g)

Calorific value was estimated by the formula given below :

Energy (Kcal) = 9 × Fat + 4 × Protein + 4 × Carbohydrate

Results and discussion.

Moisture content.

In the present study, the moisture content of the examined honey was 15.78 %(Table 1). Moisture content of honey is a critical factor in Determining the quality, stability and spoilage resistance against yeast fermentation. Lower moisture prolongs the shelf life of honey. The relatively low moisture content of the honey is of advantage to this product, low moisture not only ensures longer shelf life , but is also usually associated with higher protein content.

Crude protein.

The protein content of honey was 1.58 ± 0.017 mg/g (Table 1). It is well known that honey contains only trace amount of protein usually originated from pollens which is a natural and protein-rich food source Scha¨ fer et al. (2006), and some enzymes such as glucose oxidase invertase and diastase (Anklam, 1998; Subramanian et al., 2007).

Ash content.

In the present study, ash content of honey was recorded as 2.21 g/100g. The ash contents of honey obtained in this study were all within the limits of<0.6 g/100 g specified by international norms (codex-Almentarious, (2001a,b.). These results referred to the rich content of pollen source surrounding the apiary yard during honey production.

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Crude fat

The fat contents of the honey investigated in this study was of 0.32 g/100 g. Reports indicating that honey contains little or no fat are available in the literature (Tab et al.,1988, Singh and Kaur, 1997) but the presence of free fatty acids like palmitic, oleic and linolenic acids have been reported in white clover honey.

Carbohydrates

The amount of carbohydrates present in honey was calculated as 82.6 g/100g. The monosaccharides, fructose and glucose, are the main sugars found in honey; these hexoses are products of the hydrolysis of sucrose. In addition to these sugars, 25 others have been detected in honey samples (Doner, 1977, Siddiqui, 1970).

Fiber content

The fiber content of honey was in the range of 0.16- 0.23 g/100g, therefore honey cannot be considered as a fiber rich food.

Calorific value

The energy value of the honey was 336 Kcal/100 g. Honey is primarily a high energy carbohydrate food and the honey sugars are easily digestible sugars similar to those found in many fruits (White and Doner, 1980), therefore honey is considered as a high energy good food for infants as well as for adults.

Table 1: Chemical composition of honey.

Parameter	Honey
Moisture (%)	15.78
Crude protein mg/g	1.58 ± 0.017
Crude fat g/100 g	0.32
Crude fiber g/100g	0.16- 0.23
Ash g/100g	2.21
Carbohydrate g/100g	82.6
Reducing sugar g/100g	73
Fructose g/100g	38.8
Glucose g/100g	29.6
Calorific value (Kcal/100g)	336

Sugars (reducing sugars, sucrose, glucose and fructose)

The reducing sugar contents of the samples used in this study had average value of 73 g/100 g, the fructose and glucose contents of honey were 38.8g/100g and 29.6 g/100g. The results show that fructose and glucose are the dominant sugar types in honeys. The sum of fructose and glucose for the honey , used in this study, indicates that samples have their values corresponding to the limit required by the international norms; i.e., 60g/100 g and above. According to White and Doner (1980) the dominance of fructose over glucose is one way in which honey differs from commercial invert sugar. Fructose/ glucose ratio also indicates the ability of honey to crystallize. If honey has less glucose than fructose, it is the glucose that crystallizes when honey granulates because it is less soluble in water than fructose (white and Doner, 1980). When the fructose/glucose ratio is high, honey remains liquid. Honey crystallization is slower when the fructose/glucose ratio is more than 1.3 and it is faster when the ratio is below 1.0 .

Conclusion

The values of quality parameters for all the honey samples studied coincide with those specified by the international honey regulations. This study reveals that honey possesses some nutritional quality that can be used as supplement for the need of human

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