Abstract

Honey is a natural sweet substance that bees produce by transforming flower nectar or other sweet secretions of plants. Depending on the raw material used by the bees, honey may be classified as nectar, honeydew or mixed nectar–honeydew. Honey is used as a food, sweetener and medicinal agent since long back. It is also used as a food preservative, preventing deteriorative oxidation reactions in foods, such as lipid oxidation in meat and the enzymatic browning of fruits and vegetables. Although in India honey is produced and consumed in large scale, there is a lack of information on the comparative biochemical properties of commercial and forest honey samples from different geographical regions with respect to their antioxidant levels, use in food product formulations etc. Hence the present study was planned to evaluate the nutraceutical properties of honey for which six experiments were planned. The material and methods used and the results obtained are presented hereunder.

Commonly consumed five brands of the marketed honey samples were purchased from the local market and four honey samples were procured from four different regions/forest of Gujarat viz., Dang, Banaskantha, Panchmahal and Saurashtra. Each sample was diluted with distilled water in the ratio of 1:25 (w/v) and the percentage antioxidant activity (by FRAP, DPPH and ABTS assays) as well as the total phenolic contents and flavonoids were analyzed. Marketed and forest samples were also analyzed for colour, moisture and total sugar content. Over all, the antioxidant capacity measured by three different methods was found higher in forest samples compared to marketed samples. The forest and marketed honey samples were found to have a strong correlation between FRAP, DPPH and ABTS assays using the three standards. The total phenolic content (mg GAE/100gm) of forest honey samples was found in the range of 34.41 to 511.77 which was determined using gallic acid as standard. The flavonoid content of fresh forest honey samples ranged between 5.61 (sample A) to 141.29 (Sample B) mg Rutin equivalent /100 gms of honey (mg RE equivalent/100gm). The total phenolic content of marketed honey ranged from 59.17 (sample A₁) to 97.25 mg GAE / 100 gm (sample B₁). The flavonoid content of fresh marketed honey varied between 7.15 (sample A₁) and 21.53 (sample D₁) mg RE equivalent/100gm. Regression analysis of total phenols of forest honey samples with FRAP, DPPH and ABTS assay as well as flavonoid content indicated a positive and high significant relationship. The flavonoid content also showed a positive and high
significant relationship with FRAP, DPPH and ABTS assay. The total phenolic content of forest honey samples showed a strong correlation with FRAP values equivalent to trolox ($r = 0.987$, $P \leq 0.01$), ascorbic acid ($r = 0.990$, $P \leq 0.05$) gallic acid ($r = 0.988$, $P \leq 0.01$) as well as with DPPH RSA equivalent to trolox ($r = 0.993$, $P \leq 0.01$), ascorbic acid ($r = 0.885$, $P \leq 0.01$) and gallic acid ($r=0.993$, $P \leq 0.01$). The flavonoid content of forest honey samples had significant correlation with the FRAP values equivalent to trolox ($r = 0.990$, $P \leq 0.01$), ascorbic acid ($r= 0.992$, $P \leq 0.01$) gallic acid ($r = 0.90$, $P \leq 0.01$). Honey samples having an L* value >50 are lighter honeys whereas samples having an L value ≤50 are dark honeys. Therefore based on this classification, the studied forest and marketed honey can be regarded as dark honeys because the L* values ranged from 25.24 to 38.2. The moisture content (%) in the investigated samples ranged from 16.54 to 22.23. Except one honey sample of forest and market, all other tested samples had moisture content below 20% which is the maximum prescribed limit for the moisture content as per the codex standard for honey (Codex Alimentarius, 2001). The total sugar content of different samples of forest and marketed samples ranged between 42.83 to 64.83 gm% and 54.78 and 68.41gm%, respectively. A positive and significant relationship of L* and a* values of forest honey samples with TAC (using FRAP, DPPH and ABTS assay) as well as total phenolics and flavonoids was observed. A positive and significant relationship between L* values and FRAP assay while a positive and non significant relationship was observed between L* values and DPPH assay, ABTS assay, total phenolics as well as flavonoids of marketed honey samples.

All honey samples were stored in triplicate for a period of six months. Each honey sample was analyzed for total antioxidant capacity (by FRAP, DPPH & ABTS assay), total phenols and flavonoids. All honey samples showed a significant reduction in the total antioxidant activity. Except a few samples all other samples showed a decrease in the phenolic and flavonoid content after six months of storage. Percentage decrease in total antioxidant capacity, total phenols and flavonoids varied in each honey sample.

The interaction between mixtures containing vitamin E, β carotene and vitamin C in pure honey using the response surface methodology statistical technique and to identify the proportion of the three compounds that would maximize the antioxidant capacity of honey in order to elaborate a nutraceutical property of honey was also
studied. The experimental results showed that 50.0 mg of vitamin E, 12.0 mg of β carotene and 320.0 mg of vitamin C in 100 gm of honey was found to be optimum to have highest antioxidant capacity by the three different methods.

The level of honey was optimized in biscuit formulation. Evaluation of total antioxidant capacity of the biscuits as well as the stability of biscuits during a one month storage period was also checked. 5gms honey incorporated biscuits dough possessed the highest firmness. At 15gms of honey incorporation the dough produced the maximum adhesiveness, and cohesiveness compared to the other samples. Thickness of biscuits non significantly decreased with the increase in honey fortification. Highest spread ratio (9.56mm) was observed at 10gm honey fortification level while the lowest (6.57mm) was observed in 5gms honey fortified biscuits. The textural properties of biscuits indicated that the control biscuits had significantly higher (P ≤ 0.05) “maximum load “compared to the experimental biscuits. “fracture stress” of biscuits was inversely related with the level of honey added, that is, at maximum level of honey incorporation the “fracture stress” of biscuits was decreased. Conversely the “fracture stress” of biscuits decreased with increasing the level of honey.

Overall quality of control and 5% honey incorporated biscuits showed a better sensory score as compared to 10 and 15% level of incorporation. It appeared that with the increase in the level of honey there was increased in the total antioxidant capacity of biscuits. The methanolic extract of control & experimental biscuits exhibited higher total antioxidant capacity compared to water extract of their counterparts. 15% level of honey incorporation was the most effective in preventing lipid oxidation.

A synbiotic yoghurt was prepared using three different strains of bacteria viz. L. bulgaricus & S. thermophilus as well as two probiotic bacteria namely L.acidophilus & L. casei with varying levels of honey. The samples were stored for 7 and 14 day at refrigerated temperature. Physicochemical properties as well as antioxidant capacity were studied from the fresh and stored samples. Fresh yoghurt samples were analyzed for microbial count and sensorial characteristics. Addition of 4 % honey in yoghurt samples with L.acidophilus & L. casei decreased the titratable acidity values as compared to 2 % level of honey. L.acidophilus & L. casei yoghurt samples showed a decrease in pH value with an increase in the titratable acidity in honey incorporated samples. Honey incorporated yoghurt samples had lower syneresis and higher total
solids as compared to the samples without honey. Total antioxidant capacity using DPPH RSA was found to be higher in the yoghurt sample with 4 % honey compared to 2 % level of incorporation. Addition of honey at 4% level showed a non significant lower value of ABTS RSA of yoghurt compared to 2 % level of incorporation. The DPPH RSA increased significantly (P ≤ 0.05) in the honey added samples with \textit{L.acidophilus} and \textit{L. casie} (except 2% honey incorporated sample that showed a non significant increase) as the storage period was increased from 7 to 14 days. On the other hand samples with \textit{bulgaricus} and \textit{S. thermophilus} had an increase of DPPH RSA on 7th day of storage followed by a non significant decline among honey treated samples.

Effect of honey feeding on the plasma antioxidant markers and lipid profile of young females was investigated. Supplementation of honey for thirty days significantly (P ≤ 0.05) increased the whole blood glutathione in the experimental group (N=15) by 206% compared to the control group (N=10). Whole blood ascorbic acid increased by 33.33% in the experimental group compared to the control group. Plasma vitamin E significantly increased (P ≤ 0.05) in honey supplemented group. The plasma TAC (mg trolox equivalent per 100gm) significantly increased (P ≤ 0.05) by 23.12%. Percentage reduction in the serum total cholesterol in the control subjects was 5.05 while a reduction of 13.81 was observed in the experimental subjects. Among honey supplemented group the serum triglyceride level at baseline was 110.17mg/dl which significantly (P ≤ 0.05) decreased to 52.61mg/dl. Feeding honey for one month significantly increased (P ≤ 0.05) the serum HDL level from 50.35 to 57.22 mg/dl depicting a percentage rise of 13.64. A decrease of 23.41% and 52.2% in the LDL and VLDL levels were observed (P ≤ 0.05) among honey supplemented group.

In conclusion the distribution of phenols and flavonoids is affected by the floral origin of honey. The present study shows that honey contains copious amounts of phenolics and flavonoids, which may function as effective natural antioxidants. Although decrease in antioxidant capacity of honey upon storage was observed, the antioxidant levels observed in different samples of honey are sufficient to protect against oxidative deterioration in food systems and against the deleterious effects of oxidation in human health. Addition of vitamin E, β carotene and vitamin C improves the total antioxidant capacity of honey. Moreover this will also act as a dietary supplement which supplies 50% recommended daily allowance of vitamin E, β carotene and
vitamin C. 5% addition of honey could be recommended in biscuit making as a substitute of sugar and enhance oxidative stability. Honey as an antioxidant source may be an effective replacement for sucrose in individuals who are high risk for coronary heart diseases. From all these points, we concluded that honey can be used as a nutraceutical ingredient to improve the overall health as well as to enhance the food quality.