Advances in Bioresearch
Adv. Biores., Vol 5 (1) June 2014: 93-101
©2014 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Antioxidant Activity, Textural Properties and Storage Stability of Honey Fortified Biscuits

Neeta Dave[1], Ila Patel[2], Vinayak Patel[3]
1*P.G. Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India
2P.G. Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India
3 P.G. Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India
E-mail :neeta_dave2004@yahoo.com

ABSTRACT
Honey is a supersaturated solution of sugars, which contains more than 180 other constituents like enzymes, amino acids and organic acids, carotenoids, Maillard reaction products, vitamins, minerals and polyphenols The objective of this study was to evaluate the effect of addition of honey on the sensorial attributes, total antioxidant capacity and textural properties of biscuits . The stability of the formulation during a one month storage period was also checked. To fulfill the objectives control and honey fortified biscuits were prepared in the laboratory. The biscuits were evaluated for their total phenols and total antioxidant capacity (from water and methanol extract of biscuits).Rheological properties from the biscuit dough and textural properties of control and honey fortified biscuits were also studied. Overall quality of control and 5% honey incorporated biscuits showed a better score as compared to 10 and 15% level of incorporation. The biscuits were stored for a period of one month and were analyzed for peroxide value on 15th and 30th day of storage period. The total phenolic content and total antioxidant activity of biscuits increased as the level of honey incorporation was increased. Methanolic extract showed higher total antioxidant activity as compared to water extract. The firmness of dough for control & experimental biscuits ranged from 8.68 – 19.55 kgf. Addition of 5gms honey produced significantly higher (P ≤.05) firmness as compared to control (12.16 kgf) & 15gms honey fortified biscuits (8.68 kgf). Addition of honey increased the adhesiveness from 0.12 (10gm honey biscuit) to 0.17kgf. mm (15gms honey biscuit). Honey incorporated samples showed a significant reduction in lipid peroxidation after 15th and 30th day of storage. Thus honey appears to be a good source of natural antioxidants, improves the textural properties of biscuits and can be used to prevent lipid oxidation in biscuits.

Keywords: *Honey, Total Antioxidant activity, Biscuits, Total Phenols*

Received 09/01/2014 Accepted 22/05/2014

How to cite this article:
Neeta D, Ila P, Vinayak P.Antioxidant Activity, Textural Properties and Storage Stability of Honey Fortified Biscuits**.** Adv. Biores., Vol 5 [2] June 2014: 93-101. DOI: 10.15515/abr.0976-4585.5.2.93-101

INTRODUCTION

Long shelf-life of biscuits makes large scale production & distribution possible. Biscuits present a fast growing segment of food in India because of consumer demands for convenient and nutritious food products. The consumers demand has increased for the quality food products with taste, safety, convenience and nutrition. Thus nutrition has emerged as an added dimension in the chain of food product development [1].

Oxidation is one of the most important processes occurring in food systems. It affects many interactions among food constituents, leading to both desirable and undesirable products. Food lipids are foods components that are very susceptible to oxidation processes, therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing, storage, distribution and final preparation of foods [2].Oxidative changes in foods include not only development of off-flavors, but also loss of color, nutrient value, and more importantly, the accumulation of deleterious compounds, which might be connected with higher incidence and mortality rates of numerous human illnesses, such as cancer, atherosclerosis, and heart disease. Therefore, the keeping quality of baked food such as biscuits, which often contain considerably high amounts of lipid, is of great nutrition and economic importance,

since these products are widely used and often stored for extended periods before consumption [3]. The oxidation of fats and oils can be prevented or even delayed by antioxidants [4].

Honey has been used as a food and medical product since the earliest times. It is a natural substance produced by honeybees, *Apis mellifera*, from the nectar of blossoms or from exudates of trees and plants giving nectar honeys or honeydews, respectively. As the only available natural sweetener, honey was an important food for Homo sapiens from his very beginnings. Indeed, the relationship between bees and man started as early as the Stone Age [5]. On an estimate, about 80% of honey is used directly in medicines and 10% is used in Ayurvedic and pharmaceutical production. Five species of honey bees are found all over the world, namely *Apis florea*, *A. cerana*, *A. dorsata*, *A. mellifera* and *Trigona iridipennis*. However, *A. cerana* and *A. mellifera* are reared in hives in India (Singh, 2007). Historically, honey was a source of sugar for thousands of years, it has the advantage of a natural origin, and confers a particular flavor to the foods in which it is included [6].

Antioxidant activity, or simply antioxidant capacity, is the ability and potential of honey to reduce oxidative reactions within the human health and food systems. Notably, these oxidative reactions can cause deleterious reactions in food products (e.g., lipid oxidation in meat, and enzymic browning in fruits and vegetables) and adverse health effects, such as chronic diseases and cancers [7].The antioxidants that naturally occur in honey contribute to its antioxidant capacity. These compounds are flavonoids, phenolic acids and some enzymes (e.g., glucose oxidase, catalase), ascorbic acid, carotenoid-like substances, organic acids, maillard reaction products, amino acids and proteins [8] found that while phenolic compounds contribute significantly to the antioxidant capacity of honey, they are not solely responsible for it. However, the antioxidant capacity varies greatly depending on the honey floral source, possibly due to the differences in the content of plant secondary metabolites and enzyme activity. The objective of this study was to optimize the level of honey in biscuit formulation and evaluation of total antioxidant capacity of the biscuits. The effect of addition of honey on the textural properties and the stability of biscuits during a one month storage period was also studied.

MATERIAL AND METHODS

Procurement of raw material

All the raw materials for the preparation of control and experimental biscuits mentioned in table 1 were purchased from the local market of Vallabh Vidyanagar. Control biscuits were prepared by the method given in AACC [9]. Experimental biscuits were prepared by addition of honey at a level of 5, 10 and 15%.

Preparation of biscuits

The control biscuits were prepared basically using the rubbing method. The refined wheat flour, glucose powder and skimmed milk powder were mixed and sieved twice to ensure uniform homogenous blending as 'Dry mix'. The vanaspati ghee was creamed till light and fluffy. To this sugar powder was gradually added, continuing the creaming process till the mixture became light. Salt, sodium bicarbonate and ammonium bicarbonate were dissolved in small quantity of water and mixed into ghee-sugar mixture, followed by addition of essence. This mixture is termed as 'paste'. The 'Dry mix' was added to 'paste' gradually, mixed gently and kneaded into smooth dough. The dough was sheeted on the platform to a thickness of 0.5 cm using a wooden rolling pin. The dough-sheet was cut into circular shape using a metallic cutter of 5 cm diameter. Each raw biscuits was transferred and systematically arranged on a baking sheet about 10 mm apart from each other. The tray was transferred, as quickly as possible, to a preheated baking oven (Bajaj Ltd) at 180º c and baked for 7 minutes. The biscuits were immediately transferred on a container having holes on the surface for cooling. The cooled biscuits were packed in aluminum foil. The experimental biscuits were also prepared by similar method except that varying levels of honey was incorporated in it. The composition of control and experimental biscuits are presented in Table.1.

Rheological analyses of biscuit dough and textural properties of control and honey fortified biscuits

The rheological analyses of dough of control and experimental biscuits as well as the textural properties of control and honey fortified biscuits were performed using a food texture analysis System "Lloyd LF plus and Nexygen Software" (Lloyd Instruments Ltd., U.K.) model TA500.

Sensory evaluation

Sensory evaluation of the control and experimental biscuits was carried out by a team of six panel member using composite scoring test [10]. The control and experimental biscuits were evaluated for various visual and sensory attributes like color, surface character, crumb color, crumb texture, taste, mouth feel and overall quality.

Determination of total phenols

The total phenols were analyzed by the method described by Singleton et al [11].
1 gm powder of control and experimental biscuits were taken in separate beakers. To all of these 25 ml of 0.3 N HCL was added and shaken for about 1 hour followed by centrifugation at 8000 rpm for about 10 minutes. The supernatant obtained was evaporated to dryness in a vacuum dryer at 40° C. The residue was dissolved in hot water in a volumetric flask to a known concentration. 0.05 ml from the above sample was taken as an aliquot. To this 0.5 ml of Folin-Ciocalteu reagent (diluted 1:1) was added. The content was cyclomixed for 4 min and 10 ml sodium carbonate was added. Content was made upto 12 ml with distilled water and allowed to incubate for 1 hour at room temperature. Standard series of known concentration of gallic acid (5-20µg) were prepared and to this 0.5 ml of Folin-Ciocalteu reagent (diluted 1:1) was added and there after treated in same way as sample. The colour intensity was measured at 750 nm on a UV spectrophotometer.

Determination of Total Antioxidant Capacity

It was determined using Ferric Reducing Antioxidant Power Assay as per the method described given by Benzie and Strain [12]. 1gm of sample was taken and volume was made to 40 ml with distilled water/methanol and mixed well. It was then filtered through whatman no. 1 filter paper. 100 µl of above aliquot was taken in a test tube and volume was made up to 300 µl with distilled water/methanol. 1.8 ml of Ferric Reducing Antioxidant Power (FRAP) working reagent was added and the contents were incubated at 37º C for 10 minutes. Standard series of known concentration of trolox (1-4µg), gallic acid (0.2-0.8 µg) ascorbic acid (1-4µg) were taken and the volume was made up to 300µl with distilled water. There after all three tubes were treated in the same way as sample.The absorbance was read at 593 nm using the double beam UV Spectrophotometer (Hitachi, Japan model 2205).

Determination of Peroxide value

Peroxide value was estimated from the fat that was extracted from control and experimental biscuits at 0, 15th, and 30th day of storage by gravimetric solvent extraction procedure according to soxhlet method. Rancidity tests were carried out by following method. The method adopted for estimation of peroxide value was the one prescribed by Sadasivam [13]. 5 gm oil sample was weighed in a 250ml glass stopper conical flask. 30 ml of acetic acid:chloroform (3:2 by volume) mixture was added followed by addition of potassium iodide solution. The solution was swirled for exactly one minute and then 30ml distilled water was added. Finally the content of flask was titrated with standard 0.01 N sodium thiosulphate solution with constant and vigorous shaking using freshly prepared starch solution used as an indicator. Titration was continued until the yellow color almost disappeared. Then 2 ml starch solution was added. The titration was again continued by drop wise addition of standard 0.01 N sodium thiosulphate solution till it showed a change in color from blue violet to colorless.

Statistical analysis

Three replicates of each sample were used of statistical analysis and the values were reported as mean ± SD. Pearson's correlation and One-way ANOVA with Duncan's multi-variant analysis were carried out using SPSS, version 16.0 software.

RESULTS AND DISCUSSION

The mean values of rheological properties of dough for control & experimental biscuits are presented in table 2. The firmness of dough for control & experimental biscuits ranged from 8.68 – 19.55 kgf. Addition of 5gms honey produced significantly higher ($P \leq 0.05$) firmness as compared to control (12.16 kgf) & 15gms honey fortified biscuits (8.68 kgf). The firmness of 10gms honey fortified biscuits was 10.27 kgf which was found to be non significantly lower ($P \leq 0.05$) than the control biscuits & significantly higher ($P \leq 0.05$) than 15gms honey fortified biscuits. Frank & Mathew (1999) also found a non significant decrease in firmness of muffins as the level of honey was increased. A significant difference ($P \leq 0.05$) was found in firmness between dough prepared with water, honey, lemon juice or honey / lemon juice [14]. The adhesiveness of dough of control & experimental biscuits ranged from 0.10 (control) to 0.17 kgf. mm (15gms honey biscuit). Addition of honey increased the adhesiveness from 0.12 (10gm honey biscuit) to 0.17kgf. mm (15gms honey biscuit). 5gms honey fortification in biscuit dough had 0.15 kgf. mm adhesiveness. The results are in agreement with few researches (Frank & Mathew, 1999) in which they had demonstrated an increase in adhesiveness of the product with the addition of honey. The cohesiveness of dough of control & experimental biscuits ranged from 0.01 (10gm honey biscuit) to 0.53 (15gms honey biscuit). Addition of 15gms honey to the dough showed a significantly higher ($P \leq 0.05$) value compared to all other samples. At 10gms level honey produced the least cohesiveness. Control biscuits showed a value of 0.06 which was near to the value obtained by 5gms honey incorporated biscuits. No specific trend was observed upon increasing the level of honey in the dough. Frank & Mathew [15] also reported an increase in cohesiveness of muffins by incorporating honey in muffins. The

springiness ranged between 0.59 and 0.83 mm. Dough containing 5 (0.62mm) & 15gms (0.62mm) honey exhibited similar springiness. Maximum springiness was observed in the dough having 10gms of honey while 5gms honey fortified dough had the least springiness among the honey fortified dough. The results are in agreement with the results reported by Qunyi et al [16]. These researchers found an increase in the springiness of bread as honey powder was increased up to 10% honey powder / flour level.

Table 1: Composition of control and experimental biscuits

Ingredients	Product			
	A	B	C	D
Refined wheat flour (gm)	100	100	100	100
Sugar powder (gm)	30	25	20	15
Fat (gm)	25	25	25	25
Essence (ml)	0.2	0.2	0.2	0.2
Skimmed milk powder (gm)	2.0	2.0	2.0	2.0
Glucose (gm)	2.0	2.0	2.0	2.0
Salt (gm)	1.0	1.0	1.0	1.0
Ammonium bicarbonate (gm)	1.0	1.0	1.0	1.0
Sodium bicarbonate (gm)	0.5	0.5	0.5	0.5
water (ml)	20	20	23	26
Honey (gm)	-	5	10	15

A – Control biscuits B – 5 gm honey fortification C – 10 gm honey fortification D – 15 gm honey fortification

Table 2: Rheological properties of dough prepared for control and experimental biscuits

Product	Firmness (kgf)	Adhesiveness (kgf.mm)	Cohesiveness (kgf.mm)	Springiness (mm)
A	12.16^{b}±0.06	0.10^{a} ±0.05	0.06^{b}±0.013	0.59^{a}±0.13
B	19.55^{c}±0.29	0.15^{ab} ± 0.01	0.08^{b}±0.00	0.62^{a}±0.01
C	10.27^{bc}±1.09	0.12^{ab}±0.00	0.01^{a}±0.00	0.83^{b}±0.16
D	8.68^{a}±0.38	0.17^{b}±0.03	0.53^{c}±0.46	0.62^{a}±0.02
F-value	64.37*	3.31	294.93*	3.43

Values are Mean ± SD of three determinations
Means bearing the same superscripts within the column do not differ significantly ($P \leq 0.05$)
*Indicates significant difference at ($P \leq 0.05$)
A – Control biscuits, B – 5 gm honey fortified biscuits, C – 10 gm honey fortified biscuits D – 15 gm honey fortified biscuits

Table 3: Sensory scores of control and experimental biscuits

Product	Color	Surface character	Crumb color	Crumb texture	Taste	Mouth feel	Overall quality
A	6.42^{a} ±0.23	6.17^{ab}±0.27	6.29^{c}±0.28	6.46^{c}±0.23	6.67^{d}±0.19	6.42^{b}±0.29	6.67^{c}±0.19
B	6.54^{a}±0.19	6.58^{b}±0.15	6.13^{bc}±0.20	5.75^{c}±0.24	5.88^{c}±0.30	5.58^{b}±0.31	6.21^{c}±0.16
C	6.04^{b}±0.22	5.88^{a}±0.21	5.24^{b}±0.25	4.42^{b}±0.31	5.00^{b}±0.30	4.17^{a}±0.47	4.79^{b}±0.33
D	5.29^{a}±0.34	4.88^{a}±0.26	4.58^{a}±0.31	3.38^{a}±0.36	3.96^{a}±0.29	3.21^{a}±0.41	3.46^{a}±0.27
F-value	5.058*	10.58*	8.9*	22.20*	17.91*	14.30*	34.50*

Values are Mean ± SD scores of a composite scoring test by a panel of 6 members × 2 replications

Table 4: Texture analysis of control and experimental biscuits

Product	Maximum Load (N)	Maximum Deflection (mm)	Fracture Stress (N/m^2)	Fracture strain (%)
A	14.01^{d}±0.76	1.37^{a}±0.89	430793.33^{b}±73013.83	0.03^{a}±0.00
B	10.16^{c}±0.64	1.40^{a}±0.02	400746.67^{b}±18607.31	0.02^{a}±0.00
C	5.58^{b}±0.66	1.71^{b}±0.03	230046.67^{a}±16076.15	0.03^{a}±0.00
D	3.56^{a}±0.26	3.05^{c}±0.06	139286.67^{a}±10997.43	0.05^{b}±0.00
F-value	176.90*	199.17*	12.76*	53.77*

Values are Mean ± SD of three determinations
Means bearing the same superscripts within the column do not differ significantly (P ≤ 0.05)
*Indicates significant difference at (P ≤ 0.05)
A – Control biscuits, B – 5 gm honey fortified biscuits, C – 10 gm honey fortified biscuits D – 15 gm honey fortified biscuits

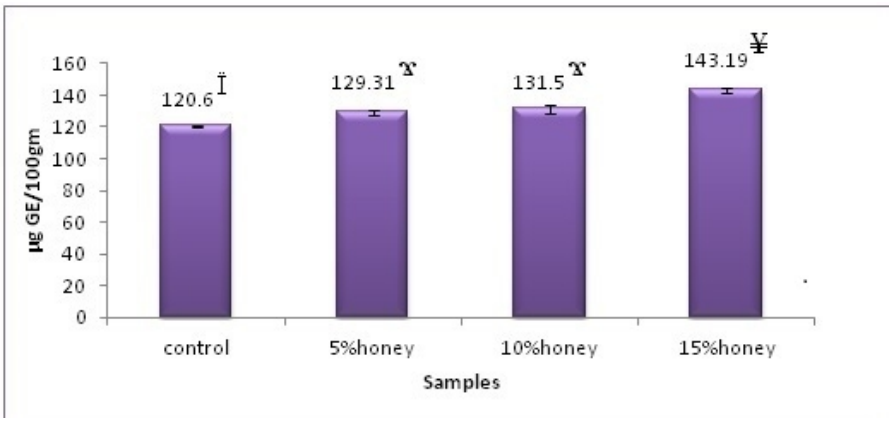

Fig. 1 Total phenolic content of control and honey fortified biscuits expressed as Gallic acid Equivalent (GAE)
Bars not sharing the same superscripts are significantly different (P ≤ 0.05)

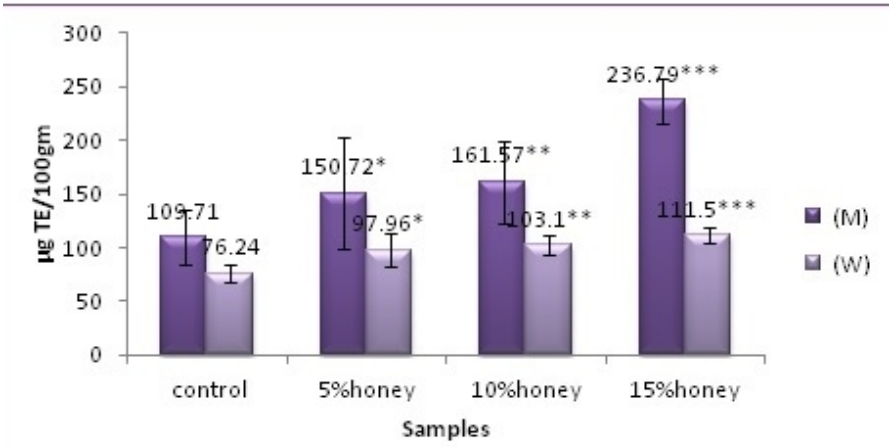

Fig.2: TAC (equivalent to Trolox) of Methanol (M) and Water (W) extract of control and honey fortified biscuits
Bars sharing the same superscripts are significantly different

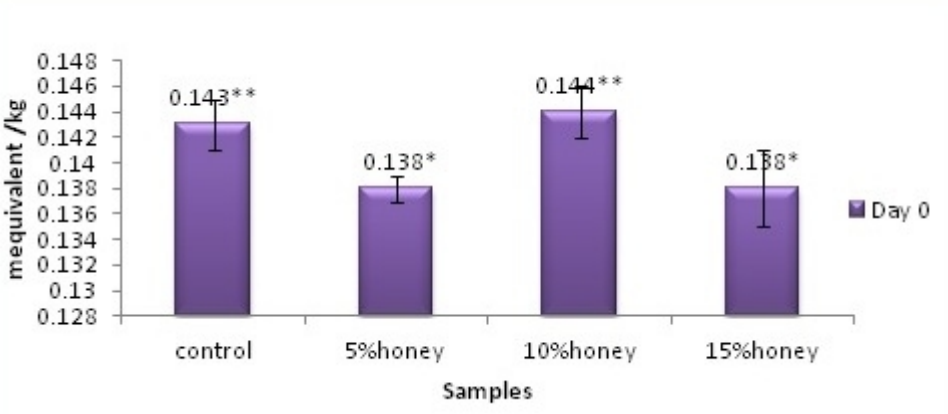

Fig 3: Peroxide value of control and honey fortified biscuits on day 0
Bars not sharing the same superscripts are significantly different (P ≤ 0.05)

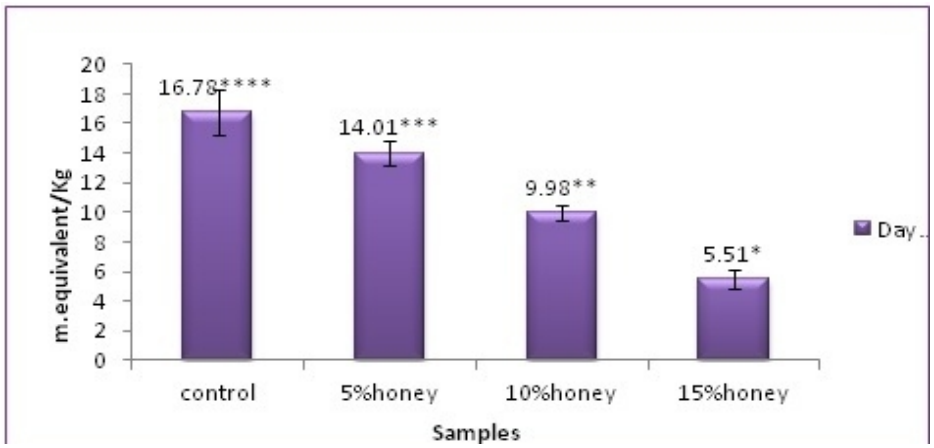

Fig 4: Peroxide value of control and honey fortified biscuits on day 15
Bars not sharing the same superscripts are significantly different (P ≤ 0.05)

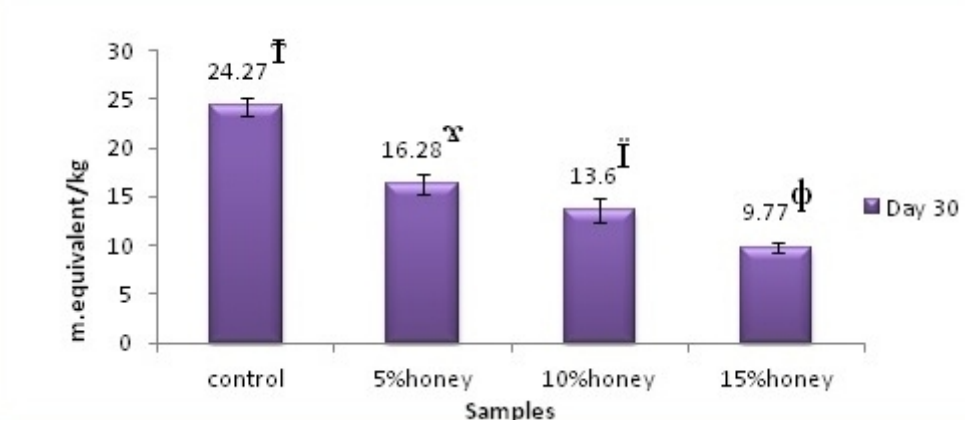

Fig 5: Peroxide value of control and honey fortified biscuits on day 30
Bars not sharing the same superscripts are significantly different (P ≤ 0.05)

The mean scores of different sensory attributes obtained are presented in table 2**.** It was observed that 5gm honey fortified biscuits showed a higher score of color (6.54) compared to the control biscuits (6.42). 15gms honey fortified biscuits had significantly lower (P ≤ 0.05) score for color (5.29) as compared to 5 & 10gms (6.04) honey supplementation. Addition of 5gms honey significantly (P ≤ 0.05) improved the score for surface character (6.58) as compared to control (6.17) as well as 10gms (5.88) & 15gms (4.88) honey fortified biscuits. A non significant difference was observed between the crumb color scores of the honey incorporated biscuits. At 5, 10 & 15% level of incorporation the scores obtained are 6.13, 5.24 & 4.58, respectively. All these scores were significantly lower (P ≤ 0.05) than the score obtained for control biscuits (6.29). Highest score for crumb texture was exhibited by the control biscuits (6.46) followed by 5gms (5.75), 10gms (4.42) & 15gms (3.38) honey incorporated biscuits. Similar trend was observed for taste scores of control & experimental biscuits. Control biscuits had significantly higher (P ≤ 0.05) score (6.67) for taste than the experiment biscuits. A non significant difference in taste score was observed when honey was incorporated at a level of 10gm (5.00) & 15gm (3.96). 5gms honey supplementation improved the taste score (5.88) of biscuits as compared all the other samples.

The mean values of textural properties of control and honey incorporated biscuits is depicted in table 3.Control biscuits had significantly higher (P ≤ 0.05) "maximum load" (14.01 N) as compared to the experimental biscuits. Increasing the level of honey decreased the maximum load. 5gms honey fortified biscuits had significantly higher (P ≤ 0.05) maximum load (10.14 N) as compared to 10gm (5.58 N) & 15gms (3.56 N) honey fortified biscuits. Decrease in "maximum load" on increasing the level of honey may be attributed to increased water content of honey fortified biscuits. "Maximum deflection" was found to be significantly higher (P ≤ 0.05) in 15gms honey fortified biscuits (3.05mm) as compared to the other biscuits. Increasing the level of honey significantly increased (P ≤ 0.05) the "maximum deflection. A significant difference (P ≤ 0.05) was observed in the "maximum deflection" values among different

experimental biscuits. 5gms and 10gms honey fortified biscuits showed a value of 1.40mm and 1.71mm, respectively. A non significant difference was observed in the "maximum deflection" values of control (1.37mm) & 5gms honey fortified biscuits (1.40 mm). Fracture stress for control & experimental biscuits ranged from 139286.67 to 430793.3 N/m^2. Control biscuits (430793.3 N/m^2) and 5gms honey fortified biscuits (400746.67 N/m^2) were not significantly different ($P \leq 0.05$). Thus these biscuits were considered similar when the effect of 5gms honey fortification in biscuits was analyzed. Addition of 10 (230046.67 N/m^2) & 15gms (139286.67 N/m^2) honey decreased the fracture stress of biscuits. Control (0.03%) as well as 5gm (0.02%) and 10gm (0.03%) honey fortified biscuits did not show significant difference ($P \leq 0.05$) between each other in the fracture strain property while 15gms honey fortified biscuits showed a significantly higher ($P \leq 0.05$) value (0.05%) as compared to the other biscuits. Therefore the effect of addition of honey in biscuits at 5 & 10gms level was similar. Addition of 15gms honey significantly ($P \leq 0.05$) increased the fracture strain of biscuits compared to all the biscuits tested. Biscuits containing 5gms honey had the lowest fracture strain, being more brittle & friable than the control biscuits and biscuits containing 10 & 15gms honey (Table 2). Paula & Lupano [17] reported a non significant difference ($P \leq 0.05$) in the fracture stress for biscuits with water, honey, lemon juice or lemon juice honey. The same author has reported a significant difference in the fracture strain of biscuits prepared with water, honey, lemon juice or honey / lemon juice.

The mean values of total phenolic content of control and honey fortified biscuits is depicted in Figure 1. The highest value (significant difference at $P \leq 0.05$ level) was observed in 10gms honey fortified biscuits (143.19 mg GAE/100gm) followed by 15gms honey fortified biscuits (131.50 mg GAE/100gm), 5gms honey fortified biscuits (129.31 mg GAE/100gm) and control biscuits (120.60 mg GAE/100gm). 5gms and 15gms honey fortified biscuits did not differ significantly ($P \leq 0.05$) in their total phenol values. Honey shows great potential to serve as an antioxidant in an emulsion system. Honey has been incorporated into meat matrices to inhibit lipid oxidation [18] as well as to prevent browning reactions in fruits & vegetables. Polyphenols, including flavonoids and phenolic acids, are found in honey, and these compounds act as free radical scavengers, peroxy radical scavengers, and as metal chelators [19, 20]. The characteristic polyphenols present in honeys that are able to perform the role of biomarkers are flavonoids such as hesperetin, kaempferol, quercetin and chrysin, and phenolic acids: abscisic, ellagic, p-coumaric, gallic and ferulic [21,22].

The TAC values of methanolic extract equivalent to Trolox (µgTE/100gm) ranged between 109.71 (control) and 236.79 µgTE/100gm (15gm honey fortification). 15gms honey fortified biscuits had significantly higher ($P \leq 0.05$) value as compared to all other biscuits studied. Increasing the level of honey increased the TAC values of biscuits. A TAC value of 150.72 & 161.57 µgTE/100gm were observed in 5 & 10gms honey fortified biscuits, respectively. A range of 76.24 to 111.50 µgTE/100gm of TAC of water extract was observed among control & experimental biscuits. 15gms honey fortified biscuits had the maximum value (111.50 µgTE/100gm) while the least value (76.24 µgTE/100gm) was observed in the control biscuits. A little variation was observed in the TAC values of 5gm (97.96 µgTE/100gm) & 10gm (103.10 µgTE/100gm) honey fortified biscuits (Fig. 2). From the above results it appears that with the increase in the level of honey there is an increase in the total antioxidant capacity of biscuits. Further the methanolic extract of control & experimental biscuits exhibited higher total antioxidant capacity compared to water extract of their counterparts. The difference may be due to the fact that type of solvent & polarity affects the single electron transfer & the hydrogen atom transfer, which are the key aspects in the measurements of antioxidant capacity [23]. Many researchers have demonstrated the influence of solvents by FRAP assay [24, 25]. They have also reported a higher FRAP value of methanolic extract of certain pure antioxidants as compared to the water extract. Biscuits containing 15 gms honey had the highest antioxidant content & also was most effective in preventing lipid oxidation. Mckibben and Engeseth [26] have also demonstrated that honey was much more effective in preventing lipid peroxidation in ground turkey in comparison to butylated hydroxytoluene & tocopherol.

The oil extracted from different sample after 15 & 30 days of storage in polyethylene bags at room temperature was used to investigate the efficiency of various concentration of honey at reducing lipid oxidation. Initially (Day 0) the peroxide values (Fig.3) ranged from 0.138 to 0.144 m equ./kg. Sample containing 5 & 15gms honey had an initial peroxide value of 0.138 m equ./kg respectively showing a non significant difference between each other. Control sample had a peroxide value of 0.143 m equ./ kg. After 15 & 30 day of storage the peroxide value ranged from 5.51 to 16.78 m equ./kg & 9.77 to 24.27 mequ./kg respectively (Fig.4 & 5, respectively). As the total antioxidant capacity of honey was increased in the samples the lipid peroxidation also decreased. At day 15 & 30 the peroxide value of control sample had maximum lipid peroxidation (16.78 & 24.27 m equ./ kg, respectively) while on the same days 15gms honey containing biscuits had least peroxide value of 5.51 & 9.77mequ./kg, respectively. At 10gms level

of honey the peroxide value at 15 & 30 days was found to be 9.98 & 13.60 m equ./kg, respectively. Significant differences ($P \leq 0.05$) in peroxide value were observed between biscuits prepared without honey and with different levels of honey. Biscuits containing 15 gms honey had the highest antioxidant content & also was also found to be most efficient in preventing lipid oxidation. Foods can be subjected to some chemical changes during thermal treatment. One of them is the non-enzymatic browning due to maillard reaction which occurs when sugars condense with free amino acids and leads to the formation of a variety of brown pigments [27]. Antony et al [28] reported that when honey was added to turkey breast meat before heating, it had an antioxidative effect on the meat which was attributed to maillard reaction products. The final stage of maillard reaction is characterized by the formation of nitrogen containing brown pigment melanoidin [29]. Katrina [30] reported that polyphenols are involved in honey melanoidin formation and that the presence of phenols in melanoidin provided melanoidins with antioxidant activity brought by phenolics. In the presnt study the maillard browning was higher as the concentration of honey was increased in the biscuit which was evident from the colour of the biscuits. As mentioned earlier the products that are formed from maillard browning act as antioxidant. Therefore in the present study these maillard products in combination with honey antioxidants might have reduced lipid peroxidation in honey incorporated biscuits.

CONCLUSION

The textural properties of biscuits indicated that the control biscuits had significantly higher ($P<.05$) "maximum load "compared to the experimental biscuits. Increasing the level of honey decreased the "fracture stress". Incorporation of honey at 15gm% level increased the "fracture strain" of the biscuits, being more resistance to breakage than other types of biscuits. Biscuits having 5gms honey exhibited better color and surface character attributes. Incorporation of honey at 15gm% level increased the total antioxidant activity of biscuits when expressed as equivalent to trolox .Total phenolic content was found to be highest at 15 gms of honey supplementation. Addition of honey in biscuit reduced lipid peroxidation after 15th and 30th day of storage. The capacity to reduce peroxide formation in biscuit samples increased with increasing the antioxidant capacity of honey. 15% level of honey incorporation was the most effective in preventing lipid oxidation. Thus honey can be used as a nutraceutical ingredient in the preparation of biscuits.

REFERENCES

1. Ahmad AOCS press, Champaign, Washington. Calder, P.C. (1999) Dietary fatty acids and the immune system. *Nutrition Review*., 56**:** 70-S83.
2. Erwin, W., Anna, G., Marzanna, H., Henryk, H.J., Józef, K., Maria M., Sylwia, M.S., Magdalena, R., Urszula, S., & Renata, Z.,W. (2004)*Polish Journal of Food and Nutrition Sciences.*, Vol. 13/54, SI 1, pp. 87–100
3. Reddy, V., Urooj, A., & Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry*., 90**:**317–21.
4. Magda,R.A., Awad, A.M., & Selim, K.A. (2008) *Alexandria Journal of food science and technology.*, Special volume of conference, 75-82
5. Crane, E. (1983) The *archaeology of beekeeping*. Gerald duckworth & co. Ltd. London.
6. Cardetti,M. (1998) Updated information on the use of honey in bakery products. *Technical Bulletin of the American Institute of Baking.,* 20**:** 1-6.
7. Gheldof, N., & Engeseth, N.J. (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food chemistry*., 5 **(10):** 3050-3055
8. Gheldof, N.,Wang, X.H., & Engeseth, N.J. (2003) Buckwheat honey increases serum antioxidant capacity in humans. *Journal of Agricultural and Food chemistry*., 51 **(5):** 1500-1505.
9. American Association of Cereal Chemists. (1995) *Approved methods of the American Association of Cereal Chemists*. 9th ed. Saint Paul., volume **2**.
10. Shrilaxmi, B., 2001 Evaluation of food quality. Food Science New age International Pvt., Ltd., New Delhi., 265-296.
11. Singleton, V.L.,Orthofer, R., Lamula, Rarentos, R. (1999) Analysis of total phenols and other Oxidation substances and antioxidant by means of Folin- Ciocalteu reagent method. *Enzymology*., 299**:** 152-178.
12. Benzie, I. F.F.,Strain, J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay., *Analytical Biochemistry*., 239**:** 70-6.
13. Sadasivam, S.,Manickam, A. (1996) *Biochemical Methods* .2nd edition. Publisher New Age International Ltd. 1996; 10,27.
14. Paula, A.C.,Cecilia, E.L. (2004) Functional properties of biscuits with whey protein concentrate and honey. *International Journal of Food Science and Technology*.,39**:** 745–753.
15. Frank, D.C & Matthew, S.J. (1999) The effects of liquid honey as a partial substitute for sugar on the physical and sensory qualities of a fat-reduced muffin. *Journal of Consumer Studies & Home Economics.* , 23 (4): 231-237.

16. Qunyi T., Xiaoyu Z., Fang W., Jingjing T., Pinping Z., Jing Z. (2010) Effect of honey powder on dough rheology and bread quality. *Food Research International.*, 43: 2284-2288.
17. Paula, A.C., Cecilia, E.L.(2004) Functional properties of biscuits with whey protein concentrate and honey. *International Journal of Food Science and Technology*., 39: 745–753.
18. Antony, S.M., Han, I.Y., Rieck, J.R., Dawson, P.L. (2002) Antioxidative effect of Maillard reaction products added to turkey meat during heating by addition of honey. *Journal of Food Science.*, 67: 1719-1724.
19. Chimi, H., Cillard, J., Cillard, P., Rahmani, M. (1991) Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *Journal of the American oil Chemists*., 68 (5): 307-312.
20. Shahidi, F., Wanasundara, P. (1992) Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition.*, 32: 67-103.
21. Ferreres, F., Giner, J.M., Tomas-Barberan, F.A. (1994) A comparative study of hesperetin and methyl anthranilate as markers of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture.*, 65: 371-372.
22. Kenjeric, D., Manic, M., Primorac, L., Bubalo, D., & Perl, A. (2007) Flavonoid profile of Robinia honeys produced In Croatia. *Food Chemistry*, 102, 683–690.
23. Jara, P., Fulgencio, S. (2006) Effect of solvent and certain food constituents on different antioxidant capacity assays. *Food Research International*., 39: 791-800.
24. Pulido, R., Bravo, L., Saura-Calixto, F. (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay. *Journal of Agricultural and Food Chemistry*., 48: 3396-3402.
25. Jara, P., Fulgencio, S. (2006) Effect of solvent and certain food constituents on different antioxidant capacity assays. *Food Research International*., 39: 791-800.
26. Mckibben, J., Engeseth, N.J. (2002) Honey as a protective agent against lipid oxidation in ground turkey. *Journal of Agricultural and Food Chemistry*., 50: 592-595.
27. Turkmen, N., Sari, F., Poyrazoglu, E.S., Velioglu, Y.S. (2006) Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry.*, 95(4): 653-657.
28. Antony, S.M., Han, I.Y., Rieck, J.R., Dawson, P.L. (2002) Antioxidative effect of Maillard reaction products added to turkey meat during heating by addition of honey. *Journal of Food Science.,* 67: 1719-1724.
29. Fayle, S.E & Gerrard, J.A. (2002) The maillard reaction. *Royal Scocity of chemistry.(Great Britain)*
30. B. Katrina, Honey Melanoidins: Emerging novel understanding on the mechanism of antioxidant and antibacterial action of honey, in J. Majtan (Ed). *Honey: Current Research and clinical application,* (*Nova Science Publisher, 2012)* 17-38 .