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Effect of Feeding Honey on the Serum Antioxidant Status of Young Females.

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ABSTRACT

Honey naturally contains a number of components known as antioxidants including vitamin C, catalase and pinocembrin and phenolic compound. In the present study the impact of honey consumption on serum antioxidant status of healthy human subjects was assessed. Twenty five normal young females (age 20-24 yrs) were selected. The experimental group (n = 15) was fed a known quantity of honey for four weeks and control group (n = 10) was fed with equal quantity of water and treated as placebo. The total phenolic content of honey was found to be 138.20 mg % while the flavonoid content was 39 mg %. The total antioxidant capacity of honey as measured by Ferric reducing antioxidant power (FRAP) was 424.95 mg% equivalent to trolox and the ability of honey (40 mg) to scavenge 1, 1, diphenyl-2, picrylhydrazyl radicals (DPPH) was 22.42%. Supplementation of honey significantly ($p \leq 0.05$) increased the whole blood glutathione indicating a percent rise of 206% while whole blood ascorbic acid increased by 33.33%. Following consumption of honey serum Vitamin E significantly increased ($p \leq 0.05$) by 38.46mg%. The serum total antioxidant capacity significantly increased ($p \leq 0.05$) indicating a percentage rise of 23.12%. This study suggests that consumption of honey enhances the antioxidant defence system of body.

Keywords: Honey, Antioxidant, Free radical, total phenols

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INTRODUCTION

Antioxidants specifically retard deterioration, rancidity or discoloration due to oxidation caused by light, heat and some metals. Nevertheless, the composition of honey and its antioxidant activity vary greatly depending on the floral source and external factors such as the season and environment [1]. Honey contains at least 181 substances and is considered as part of traditional medicine [2]. It is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes, and vitamins [3, 4]. It was found that honey intake caused a higher antioxidative effect in blood than the intake of black tea, although it's *in vitro* effect measured as Oxygen Radical Scavenging Activity (ORAC) was five times smaller than that of black tea [5]. Honey been used as sweetening agent from ancient times. It also possesses of natural antioxidants that are effective in preventing oxidative deterioration in foods [6] such as inhibiting browning reaction in fruits and vegetables [7,8] and lipid oxidation in cooked ground poultry [9]. The antioxidant activity of honey is mainly due to presence of ascorbic acid, phenolic compounds, Maillard reaction products, enzymes like peroxidase and catalase etc [6. However, the antioxidant activity of honey varies depending on the honey floral source and positively correlates with the color of the honey [10]. Therefore, in view of the above findings the study aimed at investigating the effect of feeding honey on the serum antioxidant status of young females.

MATERIALS AND METHODOLOGY

25 young females in the age group of 20-24 years were enrolled from P.G. Department of Home science, Sardar Patel University. All the subjects were divided into control (N=10) and experimental (N=15) groups. The subjects were chosen on the basis of their willingness to co-operate and were free from any apparent complications. Approximately 5ml of venous fasting blood sample was drawn from each subject and collected in a clean dry centrifuge tube containing dried Ethylene Diamine Tetra Acetic Acid (EDTA) before and after honey supplementation. The samples were then spun at 3,000 rpm for 10 minutes and serum was separated. The serum was stored at -20º C temperature until analyzed. 25ml of honey was diluted in 50ml of water and fed to the subjects in the morning (9 to 9:30 a.m.) in front of the investigator for 30 days. 5ml of water was fed to the control subjects for a period of one month.
A written consent was taken from all the subjects.

Honey analysis

Total Phenols

Total phenols were determined by using Folin - Ciocalteu method given by Singleton and Rossi, 1965 [11]. 1gm of honey was taken and dissolved in 25 ml of distilled water. 0.05 ml from diluted sample of honey 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 minutes and 10 ml sodium carbonate was added. Content was made up to 12 ml with distilled water and allowed to incubate for 1 hour at room temperature. The colour intensity was measured at 750 nm on a UV spectrophotometer. For blank 0.5 ml of Folin Ciocalteu reagent (1:1) was taken. The content was cyclomixed for 3 minutes followed by addition of 10 ml of sodium carbonate and 1.5 ml of distilled water and then treated same as sample. 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.

Total Flavonoids

The total flavonoid content was measured by using colorimetric assay, used by Singleton et al, 1999 [12]. 0.1ml of diluted honey sample was taken and volume was made up to 5 ml with distilled water. At 0 minute, 0.3 ml of sodium nitrite, at 5 minutes 0.6 ml of 10% aluminum chloride and at 6 minutes 2 ml of 1N sodium hydroxide were added to the mixture. This was followed by the addition of 2.1 ml of distilled water to it. The solution was mixed well and the intensity of pink colour was measured at 510 nm in a UV visible spectrophotometer (Hitachi 220s Japan) against blank. For blank 5 ml of distilled water was taken and treated same way as sample. Standard series of known concentration of Rutin (20-80 µg) was prepared and final volume was made up to 5 ml with distilled water and there after treated in same way as sample.

Total Antioxidant Capacity

Ferric Reducing Antioxidant Power (FRAP)

The procedure described by Benzie and strain, 1996 [13] was used to evaluate the Total Antioxidant Capacity (TAC) of honey. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to its ferrous colored form (Fe2+-TPTZ) in the presence of antioxidants.

1gm of honey was taken and dissolved in 25 ml of distilled water. 0.1ml of diluted honey sample was taken and volume was made up to 300µl with distilled water. 1.8 ml of FRAP reagent was added and allowed to incubate at 37^0c for 10 minutes. The coloured complex was measured at 593nm using double beam U.V. spectrophotometer (Hitachi # 220s, Japan). For blank to 300µl of distilled water, 1.8 ml of FRAP reagent was added. Standard series of known concentration of trolox (1-4µg) was taken and the volume was made up to 300µl with distilled water. There after all tubes were treated in the same way as sample.

Determination of antioxidant activity in the reaction with DPPH (1,1-diphenyl-2-picrylhydrazyl radical)

The antioxidant activity was determined by the ability of extract to scavenge DPPH radicals. This method was described by Brand-Williams et al, 1995 [14]. The assessment of antioxidant activity is a free radical colorimetry that relies on the reaction of specific antioxidant with a stable free radical DPPH dissolved in methanol. As a result of reduction of DPPH by antioxidant, the optical absorbance at 517nm of this purple colored solution of DPPH in methanol decreases. This change is detected by UV spectrophotometer. 1gm of honey was taken and dissolved in 25 ml of distilled water. 1ml of diluted honey sample was taken and volume was made up to 1ml with methanol. 3ml of DPPH reagent was added followed by vigorous shaking. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. To 1ml of methanol 3ml DPPH was added and used as control. Methanol was used as blank.

Whole blood analysis

Glutathione (GSH)

Blood glutathione was estimated by the method given by Ellman, 1959 [15]. 0.5 ml. of blood sample was mixed with 1 ml of 5% TCA. The mixture was mixed by using cyclomixer followed by centrifugation at 5000 rpm for 10 minutes to obtain a protein free supernatant. After centrifugation, 0.1 ml of supernatant was taken in a plasma tube and added 3.9 ml of phosphate buffer (pH-8) and 0.2 ml of DTNB solution. Mixture was mixed and allowed to stand for 10 minutes at room temperature. The color developed was read at 412 nm in a double beam UV visible spectrophotometer (Hitachi 220S,Japan).The standard series of 4 to 16 µg was prepared. The volume was made up to 4 ml with phosphate buffer (pH-8.0) and treated in the same way as sample. Blank was prepared by using 4 ml of phosphate buffer (pH-8.0) and treated in the same way as sample.

Vitamin C

Blood vitamin C was estimated by the method given by Roe and Kuether, 1943 [16] and Bessey et al, 1947 [17]. Exactly 0.5 ml of blood was mixed with 1 ml of chilled 5% TCA, centrifuged at 5000 rpm for 10 minutes. 0.4 ml of the supernatant was taken and volume was made up to 1 ml with 5% TCA and added 0.2 ml of 2,4- Dinitrophenylhydrazine / Thiourea / Copper sulphate (DTC) solution. The mixture was incubated at 37°c for 3hrs, 1.5 ml of 65% H_2SO_4 was added, mixed and was allowed to stand at room temperature for an additional 30 minutes. Absorbance was determined at 520 nm using blank. The standard series of 2 to 8 µg was prepared in 1.0 ml 0f 5% TCA and treated in the same way as the sample. Blank consisted of 1 ml 5% TCA and 0.2 ml DTC solution which was incubated for 3hrs. 1.5 ml of 65% H_2SO_4 was added and mixed.

Serum analysis

Total Antioxidant Capacity using FRAP method: The procedure described by Benzie and strain, 1996 [13] was used to evaluate the TAC. 0.02 ml of serum was taken in a test tube and volume was made up to 300 µl with the distilled water. 1.8 ml of FRAP reagent was added and allowed to incubate at 37°C for 10 minutes.

The coloured complex was measured at 593 nm using double beam U.V spectrophotometer (Hitachi220S, Japan). Blank was prepared using 300 µl distilled water and 1.8 ml FRAP reagent and treated in the same way as sample. The standard series of 1 to 4 µg of trolox was prepared the volume was made up to 300 µl with distilled water thereafter all these tubes were treated in the same way sample.

Vitamin E

Plasma vitamin-E was estimated by the method given by Desai, 1986 [18]. Exactly 0.2 ml of plasma was pipetted out into a clean and dry test tube. To the tube, the same amount of 1% pyrogallol in ethanol was added. After equilibration for 2 minutes at 70°C, 0.05 ml of saturated KOH was added and the resultant mixture was incubated for further 30 minutes. After cooling in ice 0.5 ml distilled water was added, followed by 2 ml of Hexane. After 2 minutes of vigorous mixing centrifugation was done at 1500 rpm for 5 minutes. The upper layer was carefully removed and a known amount was evaporated to dryness under vacuum at 40°C. The residue was dissolved in 1.0 ml ethanol. The standard series of 100 to 400 µg were also prepared in ethanol having 1.0 ml final volume. To each tube 0.2 ml of 0.02% bathophenanthroline reagent was added and the contents of the tubes were thoroughly mixed. The assay was processed very rapidly from this point onwards and unnecessary exposure to direct light was minimized. 0.2 ml of $FeCl_3$ reagent was added and vortex mixed. After 1 minute 0.2 ml of orthophosphoric acid reagent was added, thoroughly mixed and color developed was read at 536 nm against. Blank containing 1 ml of ethanol was treated same as sample.

Statistical analysis

The results are expressed as Mean ± SD. The data was analyzed by student's paired T test. A value of P ≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 depicts the mean values of total phenolic content, flavonoid and total antioxidant capacity of the honey sample.

Table 1: Total Phenols, Flavonoid and Total Antioxidant Capacity of honey

Total Phenol (mg GAE/100gm	Flavonoid (mg RE/100gm)	Total antioxidant capacity	
		DPPH (% inhibition)	FRAP (mg TE/100gm)
138.19 ±2.02	13.39 ±0.46	22.42 ±0.10	424.95 ±0.61

Values are Mean ±S.D. of three observations

Total Phenols and Flavonoids

The concentration and type of phenolic substances depend on the floral origin of honey and those are the major factors responsible for biological activities of honey (19). The total phenolic content, expressed as equivalent to gallic acid, was found to be 138.20 mg%. These values were higher than those of natural honeys [19, 20, 21]. Honey flavonoids can originate from nectar, pollen or propolis. Propolis, being a natural constituent of honeycombs, has components that are probably distinguished between the relatively lipophilic beeswax and the more hydrophilic honey. Compounds in honey that have been identified include flavones such as Apigenin, flavonols such as kaempferol, flavanones such as hesperetin and phenolic acids [22]. The total flavonoid content, expressed as equivalent to rutin was 13.39 mg%. In our study, the value of the flavonoid content was higher than the value (13.5 to 44.5 µg/g) reported by Kaskoniene et al, 2009 [23] and lower than the flavonoid content of raspberry herbhoney (28.5 mg/100 g) expressed as quercetin equivalent [24].

Total Antioxidant capacity

The antioxidant activity of natural honeys depends largely on their chemical composition, i.e., phenolic and flavonoid content, hence on their origin. Antioxidant activity is also affected by the processing (e.g., heating) and storage method of the material. As shown by Turkmen et al, 2006 [25], the antioxidant

activity of natural honeys rises when the temperature and time of heating are increased. In natural honeys, antioxidant activity is due to the presence of various substances such as enzymes, organic acids, amino acids, maillard reaction products, phenolic compounds, flavonoids, tocopherols, catechins, ascorbic acid, and carotenoids [26].

Ability to scavenge 1, 1, diphenyl-2, picrylhydrazyl radicals (DPPH)) assay

DPPH is stable nitrogen centered radical and has been widely used to test the free radical scavenging ability of various samples. The higher the DPPH scavenging activity, the higher the antioxidant activity. DPPH assay reflects the activity of water soluble antioxidant [10]. The percentage DPPH radical scavenging activity of the honey sample in the present study was 22.42%. The values in the present study are lower than those obtained by Liviu et al, 2008 [27] for Romanian honey (35.80-64.83%).

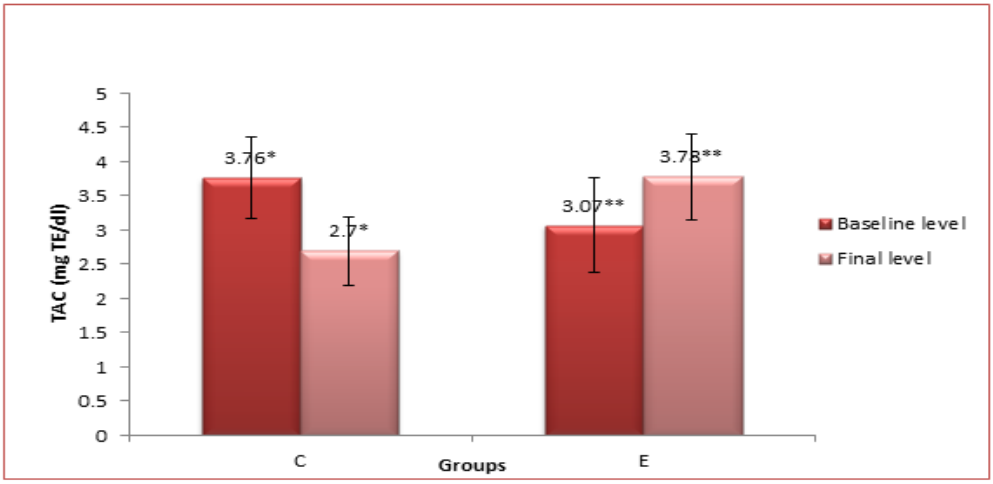
Ferric Reducing antioxidant Power Assay (FRAP)

FRAP method is used to determine the total antioxidant activity of honey. It is primarily used for determining antioxidant activity of plasma, but can be successfully applied to measure antioxidant activity of a number of biological samples and pure substances [28, 29]. The total antioxidant capacity as measured by Ferric reducing antioxidant power (FRAP) was 424.95 mg Trolox Equivalent (mg TE/100gm). Krpan et al, 2009 [30] have reported 72.87 µm Fe (II) total antioxidant capacity in Acasia honey while Ulusoyesra et al, 2010 [31] observed total antioxidant capacity of honey in the range of 33-166µmol/g equivalent to trolox. Various plants are used by honeybees to collect nectar and the bioactive compounds obtained by plants are transferred to honey [20]. The composition of active components in plants depends on various factors, mainly plant biotype, chemotype and climatic conditions. Thus one of the reasons of variations in different characteristics of honeys from different locations will be different. It was reported that the composition and antioxidant capacity of honey depend on the floral source used to collect the nectar; seasonal and environmental factors, as well as processing, affect honey composition and antioxidant activity [19, 32, 10, and 1].

Effect of feeding honey on the antioxidant status of young females

Serum Total Antioxidant Capacity

Figure 1: Serum TAC values of Control (C) and Experimental (E) subjects



Bars sharing the same superscript within each set of two are significantly different (P ≤ 0.05)

The mean values of serum total antioxidant capacity of control and experimental subjects are displayed in Figure 1. The serum total antioxidant activity of control subjects was 3.76 mg equivalent to trolox/dl which significantly reduced (P ≤ 0.05) to 2.70. Honey supplementation for one month significantly increased (P ≤ 0.05) the serum TAC from 3.07 to 3.78 mg equivalent to trolox/dl depicting a percentage increase of 23.12. The results of this investigation revealed that an increase in serum total antioxidant capacity was observed after honey consumption for one month. The increase in the serum total antioxidant capacity is

attributed to the presence of phenol compounds, catalase, peroxidases, glucose oxidase, enzymes, flavonoids, carotenoids and vitamin C, which contribute to the total antioxidant capacity of honey [1,33,20]. Derek et al, 2003 [34] found an increase in the plasma antioxidant activity after consumption of honey. The serum antioxidant capacity determined by ORAC increased significantly ($p < 0.05$) by 7% following consumption of buckwheat honey in water [35].

Flavonoids are a large family of polyphenolic compounds synthesized by plants that have a common chemical structure. Polyphenolic compounds, or polyphenols, are polyhydroxylated phytochemicals, of which the two main classes comprise flavonoids and phenolic acids. Flavonoids may be further divided into several subclasses, i.e., flavones (and isoflavones), flavanones, flavonols, flavanols (also called catechins), and anthocyanidins [36].

Flavonoid rich chocolate, spinach, strawberries, and red wine have been observed to increase plasma antioxidant capacity in human subjects by 7-25% when consumed in quantities of around 4 g/kg body weight [37, 38]. Mauro et al, 1988 [39] demonstrated an increase in plasma total antioxidant capacity after consumption of alcohol free red wine. Coffee, tea as well as chocolate consumption [40, 41] also increased the plasma total antioxidant capacity.

Plasma total antioxidant capacity is also contributed by urate, an antioxidant. It has powerful reducing and free radical scavenging activities. For instance, urate may contribute as much as 60% to plasma FRAP, 60–90% to plasma TRAP, and about 40% to plasma ORAC-PCA [36]. Several researchers have reported that foods containing flavonoids may elevate plasma urate concentration but the mechanism by which it occurs is yet not known (38, 42). Moreover several scientists have reported that fructose increases the plasma urate levels [43, 44]. Fructose metabolism in this manner leads to a transient decrease in hepatic ATP and inorganic phosphate, which is important inhibitors of 5'-nucleotidase and AMP deaminase, respectively, and thus increased degradation of AMP to uric acid [45, 46, and 47]. Omotayo et al, 2012 [48] have reviewed various papers and reported that the fructose content of honey varies from 21.0% to 43.5%, and the ratio of fructose to glucose ranges from 0.46 to 1.62 [49-53]. In the present study consumption of honey increased the total antioxidant capacity of plasma in young females. The increase may be due to the presence of fructose in honey which might have increased the plasma urate levels thus contributing to the total antioxidant capacity.

Blood Reduced Glutathione

Table 2 depicts the whole blood glutathione levels of control and experimental subjects. The whole blood glutathione level of the control subjects at baseline was 12.09 mg/dl. After one month it significantly increased ($P \leq 0.05$) to 16.48 mg/dl. The experimental subjects also showed a significant increase ($P \leq 0.05$) from 16.88 to 51.65 mg/dl following honey treatment. A percentage rise of 36 was observed in the control subjects while honey treated groups showed an increase of 205%. Glutathione is found exclusively in its reduced form since its oxidized form glutathione disulfide (GSSG), glutathione reductase, is constitutively active and inducible only upon oxidative stress. GSSG plays a pivotal role in maintenance of balance of cellular reduction and oxidation reactions and acts as radical scavenger due to redox active sulfhydryl group directly reacting with oxidants. Lowered glutathione content has generally been considered as an indicator of increased formation of ROS and glutathione depression in mammalian cells causes cell damage by oxidative stress [54]. In the present study higher glutathione levels among experimental subjects compared to the control subjects can be attributed to the presence of honey antioxidants that might have scavenged the ROS formed in the body.

Table 2: Whole blood Glutathione levels (mg/dl) of control and experimental subjects

Groups	Initial	Final	T value
Control (N=10)	12.09 ±4.60	16.48 ±14.72	2.30
Experimental (N=15)	16.88 ±9.78	51.65 ± 19.04	2.14*
T value	2.09	2.08*	-

Values are Mean ± S.D.

* indicates significance difference ($P \leq 0.05$)

Serum vitamin C

Table 3 shows the mean values of serum vitamin C levels of control and experimental subjects**.** The initial serum vitamin C level of control subjects was 4.36 mg/dl. A non significant reduction (3.57 mg/dl) was observed after one month. Among the honey supplemented subjects a significant increase ($P \leq 0.05$) was observed from the baseline value of 3.57 to 4.76 mg/dl. Vitamin C is a water-soluble antioxidant vitamin which is found in many biological systems and foodstuffs. Vitamin C plays an important physiological role in cells as a reducing agent and antioxidant, free radical scavenger, and enzyme cofactor [55, 56].

Table 3: Serum vitamin C levels (mg/dl) of control and experimental subjects

Groups	Initial	Final	T value
Control(N= 10)	4.36 ±1.84	3.57 ±0.87	2.30
Experimental (N=15)	3.57 ±1.42	4.76 ±1.02	2.14*****
T value	2.14	2.09*****	-

Values are Mean ± S.D.
* indicates significance difference ($P \leq 0.05$)

Honey accelerates wound healing, as measured by the thickness of granulation tissue, epithelisation from the periphery of the wound, and the size of the open wound [57]. Al-Waili & Saloom, 1999 [58] reported that honey reduced scar formation and accelerated wound healing in infected surgical wounds. Honey contains appreciable amounts of Vitamin C. Buba et al, 2013 [59] have reported a range of 13.86 to 27.32 mg% of vitamin C in Nigerian honey samples. In the present study consumption of honey for one month significantly increased the serum vitamin C level by 33.33%. Al-Waili, 2003 [60] reported an increase of 47% in the serum vitamin C level after two weeks of honey supplementation.

Serum vitamin E

The mean values of serum vitamin E levels of control and experimental subjects are presented in Table 4**.** The baseline value of serum vitamin E of control subjects was 0.11 mg/dl which reduced to 0.07 mg/dl. Honey supplementation for one month significantly increased ($P \leq 0.05$) the serum vitamin E level of the experimental subjects from 0.13 to 0.18 mg/dl indicating a percent rise of 38.46.

Table 4: Plasma vitamin E levels (mg/dl) of control and experimental subjects

	Initial	Final	T value
Control	0.11 ±0.04	0.07 ±0.03	2.14
Experimental	0.13 ±0.06	0.18 ±0.07	2.30*****
T value	2.07	2.07*****	-

Values are mean ± S.D.
* indicates Significance ($P \leq 0.05$)

Vitamin E is one of the most important lipid-soluble, chain-breaking antioxidants . Vitamin E is a generic term used for several naturally occurring tocopherols and tocotrienols [61]. Adetuyi, 2009 [62] reported that the tocopherol content of honey samples varied from 17.60 µg/100g, and consumption of honey, in the present investigation, significantly increased the serum vitamin E level of experimental subjects. Busserolles et al, 2002 [63] found that honey-fed rats had a higher plasma α-tocopherol level, a higher tocopherol/triacylglycerol ratio.

CONCLUSION

Using 25gms of honey per day for 30 days improved the serum total antioxidant status among young females which revealed that honey is one of the most acceptable form of food to keep balance between antioxidants and prooxidants. This balance helps to minimize the onset of many diseases.

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REFERENCES

[1] Gheldof N, Engeseth NJ. J Agri Food Chem 2002; 5 (10): 3050-3055.
[2] Chow J. J Ren Nutr 2002; 12: 76-86.
[3] Pérez RA. J Agric Food Chem 2002; 50: 2633-2637.
[4] Terrab A, Gonzále ML, González AG, Díez MF, Escudero ML, Heredia FJ. Eur Food ResTechnol 2003; 218:88-95.
[5] Gribel NV, Pashinskii VG . Voprosy Onkologii 1990; 36 (6): 704-709.
[6] Wang H, Gheldof N, Engeseth NJ. J Food Sci 2004; 69 (2): 96-101.
[7] Chen L, Mehta A, Berenbaum M, Zangerl AR, Engeseth NJ. J Agric Food Chem 2000; 48 (10): 4997-5000.
[8] Oszmianski J, Lee CY. J Agric Food Chem 1990; 38: 1892-1895.
[9] Antony SM, Han IY, Rieck JR, Dawson PL. J Food Sci 2002; 67: 1719-1724.
[10] Frankel S, Robinson GE, Berenbaum MR. J Apic Res 1998; 37: 27-31.
[11] Singleton VL, Rossi JL. Am J Enol Viticul 1965; 16: 144-158.
[12] Singleton VL, Orthofer R, Lamula Rarentos R. Enzymol 1999; 299: 152-178.
[13] Benzie IF, Strain JJ. Analyt Biochem 1996; 239: 70-6
[14] Brand Williams W, Cuvelier ME, Berset C. J Food Sci 1995; 28: 25-30.
[15] Ellmen GL. Arch Biochem Bioph 1959; 82: 70- 77
[16] Roe JH, Kuether CA. J Biol Chem 1943; 147: 399-40
[17] Bessey OA, Lowry OH, Brock MJ. J Biochem 1947; 168: 197
[18] Desai ID. Res Meth Enzymol 1986; 105: 138-142.
[19] Al-Mamary M, Al-Meeri A, Al-Habori M. Nutri Res 2002; 22: 1041-1047.
[20] Baltrusaityte V, Venskutonis PR, Ceksteryte V. Food Chem 2007; 101: 502–514.
[21] Bertoncelj J, Dobersek U, Jamnik M, Golob T. Food Chem 2007; 105: 822–828.
[22] Makawi S, Elraheed A, Saad A, Mohamed Hussein A. E-J Chem 2009; 6 (S1): S429-S437.
[23] Kaskoniene V, Maruska A, Kornysova O, Charczun N, Ligor M, Buszewski B. Chemine Technologija. 2009; 3 (52): 74-80.
[24] Socha R, Juszczak L, Pietrzyk, S, Fortuna T. Food Chem 2009; 113: 568-574.
[25] Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS. Food Chem 2006; 95 (4): 653-657.
[26] Meda A, Lamien, CE, Romito M, Millogo J, Nacoulma OG. Food Chem 2005; 91: 571-577.
[27] Liviu M, Daniel A, Adela M, Otilia BA, Laura, LA, Stefan B. Food Chem 2009; 112: 863-867
[28] Ghiselli A, Nardini M, Baldi A, Scaccini C. J Agric Food Chem 1996; 46: 361-367.
[29] Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK. J Agric Food Chem 2002; 50: 3122-3128
[30] Krpan M, Markovic K, Saric G, Skoko, B, Hruskar K, Vahcic, N. J Food Sci 2009; 27:245-247.
[31] Ulusoyesra SK.; Ali OS. J Food Biochem 2010; 34: 321-335.
[32] Gheldof N, Wang XH and Engeseth NJ. J Agric Food Chem 50:5870–5877 (2002).
[33] Aljadi AM, Kamaruddin MY. Food Chem 2004; 85: 513–518.
[34] Derek SD, Malina K., Heather R, Schrader, Roberta, RH, Marica C, Car L K. J Agric Food Chem 2003; 51 (6): 1578 -1581.
[35] Gheldof N, Wang XH, Engeseth NJ. J Agric Food Chem 2003; 51 (5): 1500-1505.
[36] Silvina LB, Balz F. Free Radical Biol Medi 2006; 41: 1727-1746.
[37] Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz, HH, Keen CL. J Nut 2000; 130: 2109S-2114S.
[38] Cao G, Russell RM, Lischner N, Prior RL. J Nutr 1998; 128: 2383-2390.
[39] Mauro S, Giuseppe M, Anna F. The J Nutr 1998 1003-1007.
[40] Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C. J Agric Food Chem 2002; 50: (21): 6211-6216.
[41] Rein D, Lotito S, Holt RR, Keen CL, Schmitz HH. Fraga CG. J Nutr 2000 130:2109S–2114S.
[42] Maxwell S, Thorpe G. Eur J Heart 2000;21:1482–1483.
[43] David W et al. J Nutr 2012; 142: 916–923
[44] Day A, Stansbie D. Clin Chem 1995; 41:1319–1320
[45] Maenpaa, PH, Raivio KO, Kekomaki MP. Science 1968; 161:1253–1254.
[46] Heuckenkamp PU, Zollner N. Lancet 1:808–809; 1971
[47] Bode JC, Zelder, O, Rumpelt HJ, Wittkamp U. Eur J Clin Invest 3:436–441; 1973.
[48] Omotayo OE, Siti AS, Mohd S Ab Wahab. Molecules 2012; 17: 1900-1915;
[49] Ischayek JI, Kern M. J Am Diet Assoc 2006; 106:1260–1262.

[50] Deibert P, Koni D, Kloock B, Groenefeld M, Berg A. Eur J Clin Nutr 2010; 64:762–764.
[51] Bahrami M, Ataie-Jafari A, Hosseini S, Foruzanfar MH, Rahmani M, Pajouhi, M. Int J Food Sci Nutr 2009; 60: 618–626
[52] Erejuwa OO et al. Int J Biol Sci 2011;7: 244–252.
[53] Münstedt K, Bohme M, Hauenschild A, Hrgovic I. Eur J Clin Nutr 2011;65:77–80.
[54] Medhi B, Prakash A, Avti PK, Pandhi P. Indian J Exp Biol 2008; 46: 583-590.
[55] Carr A, Frei B. Am J Clin Nutr 1999; 69:086–1107
[56] Padayatty SJ, et al. J Am Coll Nutr 2003; 22:18–35
[57] Bergman A,Yanai J, Weiss J, Bell D, Menachem PD. The Am J Surg 1983; 145 (3):374-376
[58] Al-Waili NS, Saloom KY. Eur J of Med Res 1999; 4 (3): 126-130.
[59] Buba F, Gidado A, Shugaba A. Biochem Anal Bioche 2013; 2:139.
[60] Al-Waili NS. J Med Food 2003; 6: (2).
[61] Kamal-Eldin A, Appelqvist LA. Lipids 1996; 31: 671–701.
[62] Adetuyi O, Ibrahim TA, Jude-Ojei GA, Ogundahunsi. Afr J Biotec 2009; 8 (7): 1305-1309.
[63] Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. J Nutri 2002; 132: 3379-3382.