Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 03 (May–Jun 2024); pp 837–842

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

A Chemometric Study on the Antioxidant Activity of Honey and Poultry Eggs in Haryana, India

Namrata[1], Shardulya Shukla[2] and Arup Giri*[3]

[1-3] Department of Zoology, Baba Mastnath University, Rohtak - 124 001, Haryana, India

Received: 10 Mar 2024; Revised accepted: 27 May 2024

Abstract

Nutritionally enriched food products such as eggs and honey are known to be beneficial for human health. In addition, they also have vital biological functions, such as antibacterial and antioxidant properties. Dietary antioxidants are known to be beneficial for reducing oxidative damage and promoting human health. The objective of the present study was to assess the antioxidant activity and radical scavenging activity of honey and poultry eggs. The antioxidant potential of all the samples was measured by DPPH and FRAP assays. The FRAP values of the egg samples were reported to be significantly greater in the summer season (25.80 mg GAE/g) than in the winter season (22.88 mg GAE/g). The DPPH radical scavenging activity of poultry eggs exhibited a greater trend in winter (26.86%) than in summer (24.53%). In contrast, the FRAP values of honey samples were reported to be highest for Panipat (279.52 µM Fe(II)), followed by Gurgaon (141.19 µM Fe(II)), Rohtak (87.41 µM Fe(II)) and Hisar (87.19 µM Fe(II)) (the lowest). DPPH radical scavenging in honey samples was greatest in Panipat (43.92%) and was similar in samples from Rohtak (17.79%), Gurgaon (17.63%) and Hisar (17.02%). To date, little research has been conducted on this topic involving eggs and honey. For that reason, more studies are required to determine the antioxidant properties of these food products and their impact on human health. This study provides insight into the antioxidant potential of egg and honey samples collected from different districts of Haryana.

***Key words*:** Antioxidant, DPPH, FRAP, Honey, Poultry, ROS

Over the past few decades, the prevalence of chronic diseases such as diabetes mellitus, cancer, atherosclerosis, cancer, heart disease, hypertension and Alzheimer's disease has increased globally. These diseases have now become a major concern due to increasing deaths worldwide [1-2]. Some recent evidence indicates the role of oxidative stress in the occurrence and severity of these diseases [3-4]. Oxidative stress can be defined as the imbalance of antioxidants and oxidants, which potentially results in damage to cells [5]. This is due to an increase in production or a decrease in the removal of reactive species through antioxidant defences. Oxidative stress results in oxidative damage. It is the damage to biomolecules that occurs when reactive species attack the components of living organisms [6].

To counteract the damage caused by oxidants such as superoxide, O_2, lipid peroxy radicals and OH^-, antioxidants are needed. Aging, cancer, atherosclerosis, degenerative lingering diseases and the synthesis of mutagens are prone to oxidative stress [7]. However, cells have a defense system in response to oxidative damage. This defensive system is composed of free radicals and some protective agents, such as peroxidase, catalase, tocopherol, superoxide dismutase, ascorbic acid and polyphenols, which protect against oxidation [8]. These antioxidants fuel biomolecules such as proteins, nucleic acids, carbohydrates and lipids. This stimulation alters the cells and eventually provokes the antioxidant response [9]. The ingestion of antioxidants along with dietary sources is considered to be beneficial for reducing oxidative stress [10-12]. Antioxidants maintain homeostasis and protect cellular functions [13].

Honey is known to possess high antioxidant activity [14]. This antioxidant capacity is beneficial for the prevention of some acute and chronic diseases, such as diabetes, cancer, inflammation, thrombosis, cardiovascular risks and allergies. The antioxidant activity of honey can be accessed via ferric reducing antioxidant power (FRAP) assays and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays [15]. Poultry eggs also exhibit antioxidant activity. There are various compounds in egg yolk and albumen that display antioxidant properties. Several egg proteins, such as phosvitin and ovalbumin, and egg lipids, such as phospholipids, ovotransferrin, some micronutrients, vitamin A, selenium, carotenoids and vitamin A, are known to exhibit antioxidant properties. Additionally, eggs can be made rich in antioxidants such as carotenoids, selenium, iodine and vitamin E by manipulating poultry feed [16]. Ovalbumin is a naturally occurring antioxidant found in eggs. It is composed of 385 amino acids and accounts for approximately 54% of the total egg protein [17-18]. Ovotranferrin constitutes 12 to 13% of the total egg protein and belongs to the transferring family. It is also called conalbumin [18-19].

Both natural and artificial antioxidants have the ability to neutralize free radicals and eliminate overproduced reactive

***Correspondence to:** Arup Giri, E-mail: arupsatadal@gmail.com; Tel: +91 9501059476

Citation: Namrata, Shukla S, Giri A. 2024. A chemometric study on the antioxidant activity of honey and poultry eggs in Haryana, India. *Res. Jr. Agril. Sci.* 15(3): 837-842.

oxygen species (ROS) by inhibiting oxidative diffusion [20]. Artificial antioxidants such as butylated hydroxytoulene (BHT) and butylated hydroxyanisole are less expensive and possess powerful antioxidant properties. However, these synthetic antioxidants can cause toxicity, possible carcinogenic effects and other side effects if consumed over a longer time period; hence, their use is legally restricted [21-23]. Therefore, the research interest of the scientific community has shifted towards the use of natural antioxidants in food and for therapeutic applications. However, investigations have explored substances from natural dietary sources that are economical and have excellent antioxidant properties [24-28]. Some natural antioxidants are anthocyanin, peptides obtained from soybean, isoflavone, whey, eggs and bioactive protein [29-31]. According to the available literature, few investigations have been conducted in this field. In comparison to plant-derived antioxidants, available data on antioxidants from animal sources are limited. Therefore, this study aimed to assess the antioxidant potential of honey and poultry eggs in different districts of Haryana.

MATERIALS AND METHODS

Study site

This study was carried out in four districts of Haryana, namely, Rohtak, Gurgaon, Hisar and Panipat. Haryana is a state that lies in the northern part of India. It is located between 29.0588° N and 76.0856° E and lies at an altitude varying from 700 to 3600 ft above sea level. The study area lies between Rohtak, 28.8955° N and 76.6066° E; Gurgaon, 28.4595° N and 77.0266° E; Hisar, 29.1492° N and 75.7217° E; and Panipat, 29.3909° N and 76.9635° E (Fig 1).

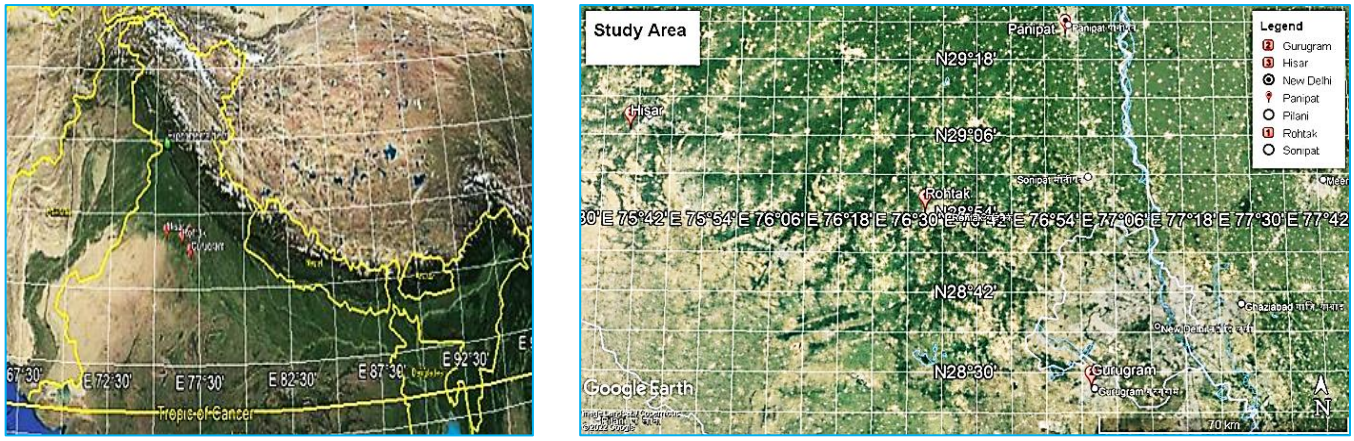

Fig 1 All egg and honey samples were collected from four districts of Haryana viz. Rohtak, Hisar, Gurgaon and Panipat

Sample collection

The honey and egg samples were collected from four districts (Rohtak, Gurgaon, Hisar and Panipat). A total of 10 eggs were collected from each district during the summer (May-June) and winter (Dec-Jan) seasons. Eggs were cleaned using distilled water, and then, drying was performed using a towel to decontaminate the shell. The eggs were then broken, and the contents were poured into sterilized Falcon tubes. These were then stored at 4°C for further use. A total of 5 honey samples were collected from each district. Samples were collected from local apiaries and beehives. All the samples were collected in prelabelled Falcon tubes and were ensured to be free from contamination. All the samples collected from different locations were heated at 400°C for 30 min and then cooled for 24 hours. It was then filtered through a cotton filter mesh and stored in Falcon tubes at room temperature (25 to 35°C) for further use.

Methods used to determine antioxidant activity in honey samples

Ferric reducing antioxidant potential (FRAP) assay

For determination of FRAP, the Benzie and Strain [32] method was used with slight modifications. O.2 ml of 50% (w/v) honey solution was dissolved in 2.8 ml of FRAP reagent containing 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$, and 25 ml of 0.3 M acetate buffer at pH 3.6. The mixture was then incubated for 15 min at 37°C. The absorbance of the reaction mixture was measured spectrophotometrically at 593 nm. For the calibration curve, an aqueous standard solution of $FeSO_4.7H_2O$ (100-1000 µM) was used, and the results are expressed as the FRAP value (µM Fe(II)) of a 50% honey solution [33].

DPPH assay for honey and egg samples

The radical scavenging activity of honey was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH), a synthetic free radical. This assay was previously described by Blois [34]. This method was used for the present study with slight modifications. For this purpose, 2 g of honey sample was mixed in 10 mL of distilled water to make a solution. Then, 0.2 ml of this honey solution was dissolved in 1.8 ml of 0.1 mM DPPH solution in methanol and kept at room temperature in the dark for 60 min. A UV–VIS spectrophotometer at 517 nm was used to measure the decrease in absorbance. Trolox and quercetin at concentrations ranging from 0.1-100 µg/mL in methanol were used as positive controls. For analysis of the egg samples, 100 µL of each egg sample was dissolved in 1000 µL of methanol [35-36]. All calculations were performed in triplicate. The radical scavenging activity was determined using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Methods used to determine antioxidant activity in egg samples

Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing property of the egg samples was determined using the assay described in [37-38] with some modifications. An aqueous solution of egg samples was prepared. Egg yolk extract (0.15 ml) was dissolved in 2.4 ml of distilled water, 0.75 ml of HCl, 0.75 ml of 1% potassium ferricyanide ($C_6N_6FeK_3$), 0.45 ml of ethanol, 0.25 ml of 1% sodium dodecyl sulfate ($NaC_{12}H_{25}SO_4$) and 0.25 ml of 0.2% ferric chloride ($FeCl_3$). The tubes with samples were then capped and incubated for 20 min at 50°C. The mixture was then

allowed to cool at room temperature. The absorbance was measured at 750 nm. The antioxidant activity was expressed as mg equivalents of gallic acid (standard) per g of sample [39].

Quality control and assurance for controlled methods

Quality control during analysis was the primary concern. The environmental conditions of the experimental areas were maintained according to the ISO standards. The glassware used during the experiments was kept in 20% HNO_3 (6 M) for one day and then washed carefully with deionized water. All the samples were analyzed in triplicate. In every analysis, quality control (QC) was carried out at regular intervals. A control chart was constructed at the time of analysis to check for deviations from the QC standard [40].

Statistical analysis

The data presented in this study were analyzed for the mean and standard error (SE). The significance level among different districts (Rohtak, Gurgaon, Panipat and Hisar) was calculated by one-way ANOVA and t tests using SPSS statistical software.

RESULTS AND DISCUSSION

Antioxidant content in honey samples collected from different districts of Haryana

The DPPH radical scavenging assay was used to determine the radical scavenging activity of the collected honey samples. DPPH is known to be a stable radical with nitrogen in the center. It has been widely utilized to measure the free radical scavenging potential of different samples. DPPH is used for this evaluation because the antioxidant potential of honey is known to be directly linked to the concentration of phenols and flavanoids present in it [41]. In the present study, the radical scavenging activity of honey was expressed in terms of % DPPH inhibition. A higher DPPH scavenging activity indicates a high antioxidant potential of the sample. The samples from Panipat exhibited the highest inhibition percentage (43.92%), indicating their high antioxidant activity. In contrast, the DPPH scavenging activities of the samples from Rohtak, Gurgaon and Hisar were 17.79%, 17.63% and 17.02%, respectively (Table 1). Honey samples from Hisar exhibited the lowest DPPH scavenging activity.

The antioxidant capacity of the honey samples was determined via the FRAP assay. It is an uncomplicated test that is generally carried out to analyze the antioxidant activity of various samples. Among all the samples, the honey samples from Panipat had the highest antioxidant capacity (279.52 µM Fe(II)), which indicates that they have greater antioxidant properties. Higher FRAP values suggest increased reduction of ferric ions to ferrous ions [42]. The antioxidant capacities of the Rohtak, Gurgaon and Hisar samples were reported to be 87.41 µM Fe(II), 141.19 µM Fe(II) and 87.19 µM Fe(II), respectively. Honey from Rohtak and Hisar had similar antioxidant capacities.

The percentages of DPPH inhibition in the analyzed honey samples were similar to those recorded in previous studies conducted by Khalil and others and Dżugan *et al*. [43]. Other studies demonstrating the radical scavenging activity and antioxidant capacity of honey are listed in Table. The values obtained for antioxidant capacity in honey were greater than those reported by Khalil and others in 2012 and Bundit and others in 2016. The antioxidant properties possessed by honey are usually contributed by pollen, nectar and substances that consist of enzymes, organic acids and vitamins [44]. Variation in these properties also depends on the floral source, method of collection [45], processing and handling, method of storage, season and other environmental factors [46].

Table 1 Antioxidant activity of honey samples collected from different locations

Parameters	Rohtak	Gurgaon	Hisar	Panipat
FRAP (µM Fe(II))	87.41±0.73^{a}	141.19±1.06^{b}	87.19±1.60^{a}	279.52±3.29^{c}
Range	85.97-88.30	139.30-142.97	84.97-90.30	272.97-283.30
DPPH (%)	17.79±0.50^{a}	17.63±0.53^{a}	17.02±0.62^{a}	43.92±1.47^{b}
Range	17.13-18.78	16.80-18.62	15.97-18.12	41.99-46.80

The mean ± standard error bearing superscripts ($^{a-d}$) indicates a significant difference ($p< 0.05$)

Antioxidant content in egg samples collected from different districts of Haryana

During the summer season, DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity was highest in honey samples from Gurgaon (24.86%), followed by those from Rohtak (24.78%) and Hisar (24.53%), and lowest in those from Panipat (23.93%). However, there was no significant difference between the values obtained after analysis of egg samples from all four districts (Table 2). The antioxidant capacity analyzed using the FRAP assay was the highest in Panipat (31.64 mg GAE/g), followed by Gurgaon (26.19 mg GAE/g) and Hisar (23.30 mg GAE/g), and was the lowest in Rohtak (22.08 mg GAE/g). In winter, the samples collected from Hisar had the highest radical scavenging activity (27.94%), followed by those from Panipat (27.05%) and Rohtak (26.89%), and the lowest radical scavenging activity was from Gurgaon (25.58%). The average concentration of FRAP was the highest in Panipat (26.30 mg GAE/g), followed by Hisar (25.97 mg GAE/g) and Gurgaon (20.08 mg GAE/g), and the lowest was in Rohtak (19.19 mg GAE/g). Overall, the FRAP values of the analyzed honey samples were reported to be greater during the summer than during the winter. Similarly, the DPPH radical scavenging activity was greater in the winter season than in the summer (Table 3).

Table 2 Antioxidant activity of egg samples collected from different locations during the summer season

Parameters	Rohtak	Gurgaon	Hisar	Panipat
FRAP (mg GAE/g)	22.08±0.45^{a}	26.19±1.79^{a}	23.30±0.67^{a}	31.64±1.45^{b}
Range	21.63-22.97	22.63-28.30	22.63-24.63	28.97-33.97
DPPH (%)	24.78±0.80^{a}	24.86±0.30^{a}	24.53±0.15^{a}	23.93±0.04^{a}
Range	23.39-26.17	24.27-25.16	24.27-24.78	23.89-24.02

The mean ± standard error bearing superscripts ($^{a-d}$) indicates a significant difference ($p< 0.05$)

Table 3 Antioxidant activity of egg samples collected from different locations during the winter season

Parameters	Rohtak	Gurgaon	Hisar	Panipat
FRAP (mg GAE/g)	19.19±0.67 [a]	20.08±1.44 [a]	25.97±1.50 [b]	26.30±0.69 [b]
Range	17.97-20.30	17.63-22.63	24.30-28.97	24.97-27.30
DPPH (%)	26.89±0.60 [b]	25.58±0.11 [a]	27.94±0.39 [b]	27.05±0.00 [b]
Range	26.17-28.07	25.41-25.79	27.43-28.70	27.05-27.05

The mean ± standard error bearing superscripts ([a-d]) indicates a significant difference ($p< 0.05$)

Table 4 Average antioxidant activity of egg samples during the summer and winter seasons

Parameters	Summer	Winter
FRAP (mg GAE/g)	25.80±1.23	22.88±1.10
DPPH (%)	24.53±0.22	26.86±0.30

There was a significant difference in the (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging and FRAP values obtained from the analysis of honey samples from four different locations. The overall antioxidant activity of honey tended to increase in the summer season. This could be most likely because of heat stress. The most favourable temperature for laying hens is 20°-25°C [47]. For that reason, if the temperature surpasses 30°C, environmental challenges linked to temperature start appearing, particularly heat stress. Increased temperature conditions severely affect health and production in the poultry sector [48]. Antioxidant activities differ significantly due to increases in heating temperature. This could be most likely due to alterations in the structure of proteins present in eggs. High temperatures can impact food by damaging its antioxidant compounds [49]. The deficiency of naturally protecting substances and increased exposure stimulate the generation of reactive oxygen metabolites [50], which results in the progression of oxidative damage to some major biological macromolecules, such as DNA, proteins and lipids. This interferes with their normal functioning and eventually results in decreased performance and several diseases [51].

CONCLUSION

The present study provides insight into the antioxidant potential of honey and poultry eggs. Moreover, it also depicts the effect of season on the antioxidant activity in eggs. The figure also shows the variations at different selected locations. The FRAP values of poultry eggs tended to increase in the summer. This could be due to the increase in temperature and heat stress conditions during summer. The FRAP values of honey from different locations varied significantly and were highest in Panipat and lowest in Hisar. This indicates that season is an important factor for antioxidant activity. However, other factors can also be considered. To date, the antioxidant potential of honey and eggs has not been explored much. Additionally, in comparison to data on antioxidants obtained from plant sources, data on animal-derived antioxidants are scarce. Therefore, further investigations and surveys must be conducted on this topic. Such studies could unveil the significant antioxidant properties and components in food products and their benefits in the promotion of human health.

Acknowledgements

The authors are thankful to Vice-Chancellor of Baba Mastnath University (BMU), Rohtak, Haryana, India, for all the support in this research. We are also thankful to Dr. Ravi Rana (Dean, Faculty of Sciences, BMU) and Dr. Vikash Bhardwaj (HOD, Department of Zoology, BMU) for their technical support during this work. The first author is highly thankful to all the poultry farmers for their help during sample collection.

Data availability*: All data collected, analyzed or generated during this study will be available from the corresponding author for reasonable scientific reasons.*

Declarations

Conflict of interest: *The authors declare no competing interests.*

Funding: *The author(s) received no specific funding for this work.*

Author(s) contributions

The first author collected all the samples, performed sample analysis and prepared the draft. The second author helped the first author during the sample analysis. AG was responsible for conceptualization, methodology, writing, review and editing, and supervision. All the authors have read and approved the final version of the manuscript.

LITERATURE CITED

1. Doll R. 1995. Chronic and degenerative disease: major causes of morbidity and death. *The American Journal of Clinical Nutrition* 62(6): 1301S-1305S. https://doi.org/10.1093/ajcn/62.6.1301s
2. Albright A. 2008. Biological and social exposures in youth set the stage for premature chronic diseases. *Jr. Am. Diet Association* 108(11): 1843-1845. https://doi.org/10.1016/j.jada.2008.09.017
3. Shibata N, Kobayashi M. 2008. The role for oxidative stress in neurodegenerative diseases. *Brain and Nerve Shinkei Kenkyu No Shinpo* 60(2): 157-170.
4. Kadenbach B, Ramzan R, Vogt S. 2009. Degenerative diseases, oxidative stress and cytochrome c oxidase function. *Trends in Molecular Medicine* 15(4): 139-147. https://doi.org/10.1016/j.molmed.2009.02.004
5. Sies H. 1991. Oxidative stress: Introduction. *Oxidative Stress: Oxidants and Antioxidants*. pp 15-21.
6. Halliwell B, Gutteridge JM. 2015. *Free Radicals in Biology and Medicine*. Oxford University Press, USA. http://dx.doi.org/10.1093/acprof:oso/9780198717478.001.0001
7. Halliwell B. 1989. Lipid peroxidation: A radical chain reaction. *Free Radicals in Biology and Medicine*.

8. Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N. 2001. Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry* 75(2): 237-240. https://doi.org/10.1016/S0308-8146(01)00193-5
9. Diplock AT, Rice-Evans CA, Burdon RH. 1994. Is there a significant role for lipid peroxidation in the causation of malignancy and for antioxidants in cancer prevention? *Cancer Research* 54(7_Supplement): 1952s-1956s.
10. Halliwell B. 1996. Antioxidants in human health and disease. *Annual Review of Nutrition* 16(1): 33-50. https://doi.org/10.1146/annurev.nu.16.070196.000341
11. Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration* 82(2): 291-295. https://doi.org/10.1113/expphysiol.1997.sp004024
12. Czelej M, Czernecki T, Garbacz K, Wawrzykowski J, Jamioł M, Michalak K, Waśko A. 2023. Egg yolk as a new source of peptides with antioxidant and antimicrobial properties. *Foods* 12(18): 3394. https://doi.org/10.3390/foods12183394
13. Gülcin I. 2012. Antioxidant activity of food constituents: An overview. *Archives of Toxicology* 86: 345-391. https://doi.org/10.1007/s00204-011-0774-2
14. Ahmed S, Othman NH. 2013. Honey as a potential natural anticancer agent: a review of its mechanisms. *Evidence-Based Complementary and Alternative Medicine* 2013. https://doi.org/10.1155/2013/829070
15. Erejuwa OO, Sulaiman SA, Ab Wahab MS. 2012. Honey: A novel antioxidant. *Molecules* 17(4): 4400-4423. https://doi.org/10.3390/molecules17044400
16. Nimalaratne C, Wu J. 2015. Hen egg as an antioxidant food commodity: A review. *Nutrients* 7(10): 8274-8293. https://doi.org/10.3390/nu7105394
17. Lee D, Bamdad F, Khey K, Sunwoo HH. 2017. Antioxidant and anti-inflammatory properties of chicken egg vitelline membrane hydrolysates. *Poultry Science* 96(9): 3510-3516. https://doi.org/10.3382/ps/pex125
18. Huopalahti R, Anton M, López-Fandiño R, Schade R. 2007. *Bioactive Egg Compounds*. Berlin: Springer. 5: 293-389. http://dx.doi.org/10.1007/978-3-540-37885-3
19. Superti F, Ammendolia MG, Berlutti F, Valenti P, Huopalahti R, López-Fandiño R, Schade R. 2007. Bioactive egg compounds. *Ovotransferrin* Springer. Pp 43-50.
20. He Y, Bu L, Xie H, Liang G. 2019. Antioxidant activities and protective effects of duck embryo peptides against H_2O_2-induced oxidative damage in HepG2 cells. *Poultry Science* 98(12): 7118-7128. https://doi.org/10.3382/ps/pez430
21. André C, Castanheira I, Cruz JM, Paseiro P, Sanches-Silva A. 2010. Analytical strategies to evaluate antioxidants in food: A review. *Trends in Food Science and Technology* 21(5): 229-246. https://doi.org/10.1016/j.tifs.2009.12.003
22. Vandghanooni S, Forouharmehr A, Eskandani M, Barzegari A, Kafil V, Kashanian S, Ezzati Nazhad Dolatabadi J. 2013. Cytotoxicity and DNA fragmentation properties of butylated hydroxyanisole. *DNA and Cell Biology* 32(3): 98-103. https://doi.org/10.1089/dna.2012.1946
23. Williams GM, Iatropoulos MJ, Whysner J. 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food and Chemical Toxicology* 37(9/10): 1027-1038. https://doi.org/10.1016/S0278-6915(99)00085-X
24. Lorenzo JM, Munekata PE, Gómez B, Barba FJ, Mora L, Pérez-Santaescolástica C, Toldrá F. 2018. Bioactive peptides as natural antioxidants in food products– A review. *Trends in Food Science and Technology* 79: 136-147. https://doi.org/10.1016/j.tifs.2018.07.003
25. Carocho M, Ferreira IC. 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology* 51: 15-25. https://doi.org/10.1016/j.fct.2012.09.021
26. Gül A, Pehlivan T. 2018. Antioxidant activities of some monofloral honey types produced across Turkey. *Saudi Journal of Biological Sciences* 25(6): 1056-1065. https://doi.org/10.1016/j.sjbs.2018.02.011
27. Halliwell B, Murcia MA, Chirico S, Aruoma OI. 1995. Free radicals and antioxidants in food and in vivo: what they do and how they work. *Critical Reviews in Food Science and Nutrition* 35(1/2): 7-20. https://doi.org/10.1080/10408399509527682
28. Samaranayaka AG, Li-Chan EC. 2011. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. *Journal of Functional Foods* 3(4): 229-254. https://doi.org/10.1016/j.jff.2011.05.006
29. Liberato MCTC, Morais SM, Siqueira SMC, Menezes JESA, Ramos DN, Machado LKA, Magalhães IL. 2011. Phenolic content and antioxidant and antiacetylcholinesterase properties of honeys from different floral origins. *Journal of Medicinal Food* 14: 658-663. PMid:21554131. http://dx.doi.org/10.1089/jmf.2010.0097
30. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. 2021. Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry* 209: 112891. https://doi.org/10.1016/j.ejmech.2020.112891
31. Wen C, Zhang J, Zhang H, Duan Y, Ma H. 2020. Plant protein-derived antioxidant peptides: Isolation, identification, mechanism of action and application in food systems: A review. *Trends in Food Science AND Technology* 105: 308-322. https://doi.org/10.1016/j.tifs.2020.09.019
32. Benzie I, Strain J. 1996. The ferric reducing ability of plasma (FRAP) as a Measure of "antioxidant power: The FRAP assay". *Analytical Biochemistry* 239: 70-76. http://dx.doi.org/10.1006/abio.1996.0292
33. Zarei M, Fazlara A, Alijani N. 2019. Evaluation of the changes in physicochemical and antioxidant properties of honey during storage. *Functional Foods in Health and Disease* 9(9): 593-605. http://dx.doi.org/10.31989/ffhd.v9i9.616
34. Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 181(4617): 1199-1200. doi:10.1038/1811199a0
35. Maisto M, Annunziata G, Schiano E, Piccolo V, Iannuzzo F, Santangelo R, Grieco P. 2021. Potential functional snacks: Date fruit bars supplemented by different species of *Lactobacillus* spp. *Foods* 10(8): 1760. https://doi.org/10.3390/foods10081760
36. Babbar N, Oberoi HS, Uppal DS, Patil RT. 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International* 44(1): 391-396. https://doi.org/10.1016/j.foodres.2010.10.001

37. Omri B, Alloui N, Durazzo A, Lucarini M, Aiello A, Romano R, Santini A, Abdouli H. 2019. Egg yolk antioxidants profiles: Effect of diet supplementation with linseeds and tomato-red pepper mixture before and after storage. *Foods (Basel, Switzerland)* 8(8): 320. https://doi.org/10.3390/foods8080320
38. Benzie IF, Szeto YT. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry* 47(2): 633-636. https://doi.org/10.1021/jf9807768
39. Muhammad AI, Mohamed DAA, Chwen LT, Akit H, Samsudin AA. 2021. Effect of sodium selenite, selenium yeast, and bacterial enriched protein on chicken egg yolk color, antioxidant profiles, and oxidative stability. *Foods* 10(4): 871. https://doi.org/10.3390/foods10040871
40. Giri A, Bharti VK, Kalia S, Kumar B, Chaurasia OP. 2021. Health risk assessment of heavy metals through cow milk consumption in trans-Himalayan high-altitude region. *Biological Trace Element Research* 199(12): 4572-4581. https://doi.org/10.1007/s12011-021-02593-6
41. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytical Chim. Acta* 533: 185-191. http://dx.doi.org/10.1016%2Fj.aca.2004.11.010
42. Khalil MI, Moniruzzaman M, Boukraâ L, Benhanifia M, Islam MA, Islam MN, Gan SH. 2012. Physicochemical and antioxidant properties of Algerian honey. *Molecules* 17(9): 11199-11215. https://doi.org/10.3390/molecules170911199
43. Dżugan M, Tomczyk M, Sowa P, Grabek-Lejko D. 2018. Antioxidant activity as biomarker of honey variety. *Molecules* 23(8): 2069. https://doi.org/10.3390/molecules23082069
44. Gheldof N, Wang XH, Engeseth NJ. 2002. Identification and quantification of antioxidant components of honeys from various floral sources. *Jr. Agric. Food Chemistry* 50: 5870-5877.
45. Jantakee K, Tragoolpua Y. 2015. Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. *Biol. Research* 48(1): 4. https://doi.org/10.1186/0717-6287-48-4
46. Bundit T, Anothai T, Pattaramart P, Roongpet T, Chuleeporn S. 2016. Comparison of antioxidant contents of Thai honeys to manuka honey. *Saudi Journal of Biological Science* 27(9): 2366-2372.
47. Tumova E, Gous RM. 2012. Interaction of hen production type, age, and temperature on laying pattern and egg quality. *Poultry Science* 91: 1269-1275. https://doi.org/10.3382/ps.2011-01951
48. Lara LJ, Rostagno MH. 2013. Impact of heat stress on poultry production. *Animals (Basel)* 3: 356-369. https://doi.org/10.3390%2Fani3020356
49. Nahariah N, Hikmah H. 2021. The effect of temperature levels on antioxidant activity in chicken eggs. *In*: *IOP Conference Series: Earth and Environmental Science* 788(1): 012099. http://dx.doi.org/10.1088/1755-1315/788/1/012099
50. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science (London)* 84: 407-412. https://doi.org/10.1042/cs0840407
51. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology* 39(1): 44-84. https://doi.org/10.1016/j.biocel.2006.07.001