



## Application of Honey to Reduce Oxidation in Soybean Oil

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### HIGHLIGHTS

- Total phenolic content of honey samples was 74.8±0.3 mg gallic acid equivalents/100 g.
- Radical scavenging activity was 23.4±0.2 mg/ml as IC50 value.
- Samples treated by 2.5 and 5% honey showed higher antioxidant capacity than control and other treatment groups.
- Honey could be applied as an alternative for synthetic antioxidants in oil-rich foods.

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### Acronyms and abbreviations

BHT=Butylated Hydroxyl Toluene

PV=Peroxide Value

TBARS=ThioBarbituric Acid-Reactive Substances

TAC=Total Antioxidant Capacity

DPPH=2, 2-diphenyl-1-picrylhydrazyl

MDA=Malonaldehyde

### ABSTRACT

**Background:** The oxidation of unsaturated lipids by free radicals is one of the main causes of food deterioration. The major purpose of the present study was to determine effectiveness of application of honey in order to reduce oxidation in soybean oil.

**Methods:** Six groups were designed, including control (soybean oil emulsion without preservative), positive control (butylated hydroxyl toluene 200 ppm), and soybean oil treatment groups (containing 1, 2.5, 5, and 7.5% honey). Each group was sampled in order to measure peroxide value, thiobarbituric acid-reactive substances and total antioxidant capacity parameters during 5 intervals (0, 1, 3, 5, and 7 days). Data were analyzed by ANOVA using SPSS statistical software.

**Results:** Total phenolic content and radical scavenging activity (IC50 in mg/ml) were estimated to be 74.8±0.3 mg gallic acid equivalents/100 g and 23.4±0.2 mg/ml, respectively. Totally, the soybean oil samples treated by 2.5 and 5% honey showed higher ( $p<0.05$ ) antioxidant capacity than control and other treatment groups.

**Conclusion:** The present study demonstrated considerable antioxidant potency of honey in oil emulsion. Owing to economical reasons, it is recommended that 2.5% honey could be applied as an alternative for synthetic antioxidants in oil-rich foods.

### Introduction

The oxidation of unsaturated lipids by free radicals is one of the main causes of food deterioration (Waraho et al., 2011). In addition, the presence of large amounts of unsaturated fatty acids such as linoleic and linolenic acids in lipids and oils increases their sensitivity to oxidation. Some factors such as oxygen, light, high tempera-

ture, and iron or copper metals induce oxidation of oil-rich foods (Sikwese and Duodu, 2007). Antioxidants as the most important combinations for postponing oxidation preserve the quality of oils and lipids (Abdalla et al., 2007). Till now, synthetic antioxidants such as Butylated Hydroxyl Toluene (BHT), butylatedhydroxyl anisole, and

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tertiary butyl hydroquinone have been useful in protecting oils and lipids and increasing the shelf life of fatty foods. The applicability of synthetic antioxidants is still a controversial issue due to the some carcinogenic and toxic reports of such materials; so, some attempts have been carried out in order to application of natural antioxidants as suitable alternatives (Aminzare et al., 2015; Padmashree et al., 2007).

Honey has always been used as a common traditional drug which includes appropriate nutritional and also treatment properties. Furthermore, the antibacterial, anti-inflammatory, and wound healing effects of honey have been reported (Al-Waili et al., 2011; Das et al., 2015; Liu et al., 2013; Zainol et al., 2013). In addition to nutritional advantages, the antioxidant effects of honey have been precisely determined (Hussein et al., 2011; Khalil et al., 2011; Kuš et al., 2014). It has been proved that the antioxidant effects of honey are mainly related to its phenolic and flavonoid substances (Beretta et al., 2005; Kishore et al., 2011; Van den Berg et al., 2008). With regards to the diverse antioxidant effect of honey, the major purpose of the present study was to determine effectiveness of application of honey in order to reduce oxidation in soybean oil, retarding edible oil. Also, antioxidant capacity of Iranian honey has been investigated *in vitro*.

## Materials and methods

### Sample preparation

In this experimental study, the soybean oil without antioxidant substance and any preservative was prepared from Ghoo Company (Tehran, Iran) and transferred to laboratory and kept at  $-75^{\circ}\text{C}$  until use. The honey was prepared from Semnan University Research Center and stored at  $4^{\circ}\text{C}$ . Six groups were designed, including control (soybean oil emulsion without preservative), positive control (BHT 200 ppm), and soybean oil treatment groups (containing 1, 2.5, 5, and 7.5% honey). Each group was sampled in order to measure Peroxide Value (PV), ThioBarbituric Acid-Reactive Substances (TBARS) as well as Total Antioxidant Capacity (TAC) parameters during 5 intervals (0, 1, 3, 5, and 7 days).

In order to evaluating honey antioxidant potency, the water-based soybean oil emulsion (50:50; O:W) was applied. For this, oil (50 g) was poured in water (50 ml), then tween 20 (two ml) was added as an emulsifier and the sonicator was used for emulsion fixation (10 min/high speed). Subsequently, the emulsion was undergone vortex for 15 min and manual homogenizator was applied for emulsion integrity maintenance. The prepared emulsion was placed in tubes (4000  $\mu\text{l}$ /tube) and copper

sulfate (55  $\mu\text{mol}/\text{kg}$ ; Merck-Darmstadt, Germany) was added as a catalyzer for oxidation speed increase.

### Adding honey to oil-based emulsion

The tubes containing emulsion were separately prepared for each treatment group (1, 2.5, 5, and 7.5%) and the honey added to emulsions at different concentrations. The BHT (200 ppm) and the non-antioxidant emulsions were used as positive and negative control groups, respectively. The emulsion tubes transferred to  $60^{\circ}\text{C}$  incubator in darkness; and the PV, TBARS as well as TAC parameters were determined in different periods of time.

### Lipid extraction

Lipid extraction was done according to the method conducted by Bligh and Dyer (1959) with some modifications. For this, the samples were transferred to laboratory tubes (500  $\mu\text{l}$ ) and then two ml chloroform/methanol (2:1) was added. Subsequently, the tubes were shaken for 15 min, one ml chloroform was added to each tube and undergone vortex for one min. After that, one ml hydrochloric acid (0.1 N) was poured in tubes and again the tubes were undergone vortex. For aqueous layer extraction, the tubes were centrifuged (3000 rpm) for five min. Following upper phase (aqueous layer) extraction, one ml chloroform was added again to access purified aqueous layer and centrifuged for five min after vortex. Eventually, the upper phase was achieved to TBARS test.

### Estimation of total phenolic contents of honey

The Folin-Ciocalteu assay was applied to measure total phenolic content of the samples (Meda et al., 2005). Each honey sample (5 g) was diluted with distilled water and after filtration was mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich, USA) for five min and two ml of 75 g/l sodium carbonate (Merck-Darmstadt, Germany) was then added. After two h incubation at room temperature, the absorbance of the mixture was measured at 760 nm against methanol blank. To design the calibration curve, gallic acid was used. Finally, the total phenolic content was calculated in mg of gallic acid equivalents/100 g of honey sample.

### Radical scavenging activity

The scavenging activity of honey sample was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Meda et al. (2005). The mean  $\text{IC}_{50}$  (the half maximal inhibitory concentration) values in mg/ml was determined graphically.

### TBARS assay

To determination of TBARS, two ml thiobarbituric reagent and one ml distilled water were added to one ml extracted water phase. All tubes were placed in boiling bath (100 °C/15 min), then the tubes were cooled in laboratory temperature and centrifuged for 15 min. The absorbance of surface layer was measured at 535 nm compared with corresponding blank. The data were expressed as  $\mu\text{mol Malonaldehyde (MDA)}/\text{g}$  of emulsion.

### PV test

For PV determination, one g emulsion was combined with 30 g chloroform and acetic acid (3:2 ratio); then 500  $\mu\text{l}$  saturated potassium iodide was added. In addition, 30 ml distilled water added following one min keeping at darkness, and the solution was titrated by sodium thiosulfate (0.02 N) with 1% starch solution until blue color disappearance. The PV was determined through following formula (meq/Kg):

$$\text{PV} = \frac{1000 (S_1 - S_2) \times N}{W}$$

For above equation,  $S_1$  for sodium thiosulfate (ml) for titration,  $S_2$  (sodium thiosulfate; ml) for blank titration, N (sodium thiosulfate normality), and W (emulsion weight) were used.

### TAC test

The Ferric Reducing Ability of Plasma (FRAP) method described by Benzie and Strain (1996) was followed for determination of TAC. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in presence of antioxidants.

### Statistical analysis

All experiments were carried out in triplicate. Data were analyzed by ANOVA using SPSS statistical software (version 16.0, SPSS Inc., Chicago, IL, USA).

## Results

Total phenolic content and DPPH radical scavenging activity of honey samples were  $74.8 \pm 0.3$  mg GAE/100 g and  $23.4 \pm 0.2$  mg/ml, respectively. Totally, the soybean oil samples treated by 2.5 and 5% honey showed higher ( $p < 0.05$ ) antioxidant capacity than control and other treatment groups according to PV, TBARS, and TAC tests.

The PV of control and treatment groups is presented in Figure 1. As can be seen, up to the 3<sup>rd</sup> day, no significant

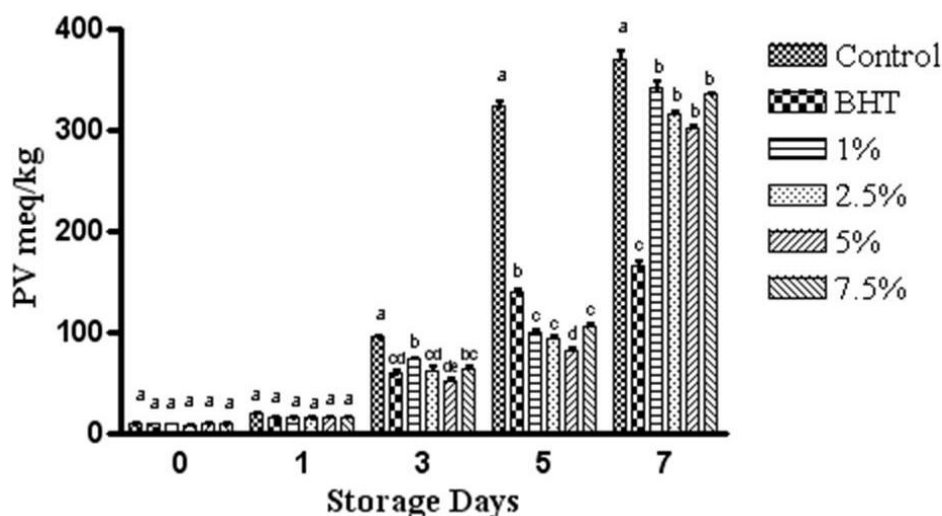
difference can be observed among control, positive control, and treatment groups ( $p > 0.05$ ). From days 3 to 7, the control group showed higher PV than BHT and treatment groups ( $p < 0.05$ ).

As presented in Figure 2, the data analysis of the first day showed that the TBARS value in control group ( $1.41 \pm 0.04$   $\mu\text{M MDA}$ ) was significantly ( $p < 0.05$ ) higher than that in BHT ( $0.77 \pm 0.05$   $\mu\text{M MDA}$ ) and treatment groups, including 1% ( $0.77 \pm 0.03$   $\mu\text{M MDA}$ ), 2.5% ( $0.65 \pm 0.02$   $\mu\text{M MDA}$ ), and 5% ( $0.56 \pm 0.05$   $\mu\text{M MDA}$ ); however, no significant difference ( $p > 0.05$ ) was observed between control group and 7.5% treatment group ( $1.26 \pm 0.09$   $\mu\text{M MDA}$ ). At last day of storage, no significant statistical difference was recorded among the treatment groups ( $p > 0.05$ ).

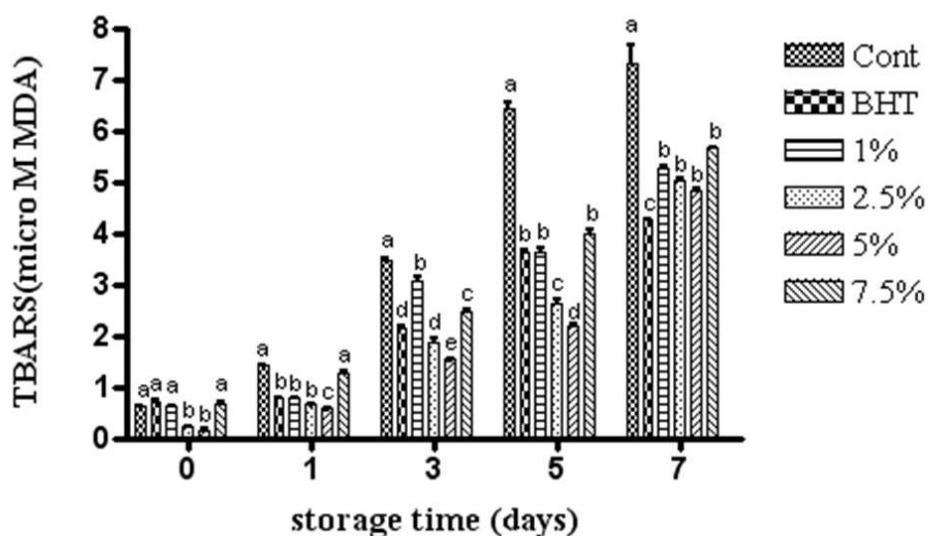
TAC values were decreased during storage time, where on the 7<sup>th</sup> day TAC could not be measured. On the first day, the mean TAC in control group (0.25 mmol/lit) was significantly ( $p < 0.05$ ) lower than that in BHT (0.65 mmol/lit) and treatment groups, including 1% (0.51 mmol/lit), 2.5% (0.57 mmol/lit), 5% (0.61 mmol/lit) and 7.5% (0.58 mmol/lit); whereas no recognizable difference was found between BHT and treatment groups ( $p > 0.05$ ). On days 1, 3, and 5, the antioxidant capacity in control group was lower than BHT and other treatment groups ( $p < 0.05$ ).

## Discussion

In the present study, lower oxidation levels were found in soybean oil samples contained honey indicating considerable antioxidant capacity of honey. Honey contains antioxidant complexes, including phenolic acids and their derivatives, flavonoid aglions, carotenoids, and ascorbic acids (Hermosín et al., 2003). Beretta et al. (2007) demonstrated that all antioxidant effects of honey depend on its phenolic combinations. Most researches have been mainly focused on the structural and nutritional features of honey such as amino acids, proteins, microelements, volatile agents, carbohydrates, and pollen properties; and the effect of geographic, botanic, and climatic changes on such parameters. In some papers, the antioxidant effect of honey was considered in general terms, and the samples mainly taken from diverse geographical regions were measured by various tests. Al-Mamary et al. (2002) following the comparison of antioxidant and phenolic contents of five different types of honey in Yemen, indicated a linear significant correlation between total phenolic capacity and antioxidant potency in such honey samples. Aljadi and Kamaruddin (2004) compared the antioxidant capacities of the acetate ethyl extracts of two honey types in Malaysia reporting the significant correlation between phenolic compounds of such honey types and their reduc-



**Figure 1:** The Peroxide Value (PV) in different control and treatment groups according to days of test. Values followed by the same lowercase letter within the same days are not significantly different



**Figure 2:** ThioBarbituric Acid-Reactive Substances (TBARS) level in different control and treatment groups according to days of test. Values followed by the same lowercase letter within the same days are not significantly different

tion strength and anti-radical effects. Furthermore, Meda et al. (2005) conducted a study on 27 honey samples from diverse regions of Burkina Faso regarding to phenolic content, flavonoids, and proline rate; and compared their anti-radical effects which demonstrated that the honey proline rate is an effective factor as far as antioxidant capacity is concerned (Meda et al., 2005). Also, Buratti et al. (2007) compared 12 honey types, 12 propolis, and 14 royal-gel in Italy by a novel electro-

chemical method for antioxidant potency and analysed the results by DPPH test, proving the significant correlation between such methods. Till now, no comprehensive research has been conducted on the antioxidant potency of Iranian honey in nutritional models; accordingly, we evaluated the protective effects of honey in inhibiting catalyzed oil emulsion peroxidation. In this study, the phenolic content and radical scavenging activity of the Iranian honey samples were similar to the average values



found for some Burkina Faso (Meda et al., 2005) and Yemeni (Al-Mamary et al., 2002) honey samples.

In the current investigation, the findings of nutritional model tests indicated the efficient antioxidant potency of honey in oil emulsion, where, unlike the control group, honey could decrease the primary and secondary oxidation in PV and TBARS tests, respectively. The high antioxidant effect of honey may be related to the existing natural antioxidants, especially phenolic combinations (Beretta et al., 2005). In comparison to other honey concentrations, by raising the concentration up to 5%, the antioxidant activities of honey were significantly increased. On the 7<sup>th</sup> day, the 2.5 and 5% honey showed a significantly higher antioxidant potency in PV and TBARS tests than BHT (synthetic antioxidant); but in case of 7.5% concentration, the antioxidant potency of honey was decreased. The group with 7.5% honey showed a similar antioxidant potency with 1% concentration during the test, which may be due to confounding effects in oil emulsions at concentrations higher than 5%. White et al. (1963) indicated that hydrogen peroxide was produced due to the effect of existing glucose oxidase enzymes on carbohydrates (bee submandibular glands origin), and along with the rise in honey concentration the hydrogen peroxide increased as well. The weak antioxidant effect of 7.5% honey concentration, concerning oil emulsion inhibition, may be related to such a reason. Results of PV, TBARS, and TAC on the final day of the test indicated that the antioxidant potency significantly decreased in catalysed emulsion by copper sulfate and the treatment groups showed further reduction in comparison with BHT group. It should be noted that in this research, the samples treated by honey were kept in incubator at 60 °C in order to increase oxidation; so, the decrease in antioxidant potency on the 7<sup>th</sup> day may be pertaining to the effect of heat on honey antioxidant combinations. In this regard, Taormina et al. (2001) demonstrated the negative effect of heat on natural antioxidant potency. In contrast, Turkmen et al. (2006) claimed that heat treatment increases antioxidant effect due to maillard reaction and melanoid polymer formation in honey. However, since the honey samples of this study were assessed in an emulsion with 50% water and oil, maillard reaction does not occur noticeably at this moisture content (Gerrard, 2002).

## Conclusion

This study demonstrated considerable antioxidant potency of honey in oil emulsion. Owing to economical reasons, it is recommended that 2.5% honey could be applied as an alternative for synthetic antioxidants in oil-rich foods.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgments

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