
# The Effect of Replacing Honey with Sugar on Oxidative Stability Properties of Pistachio Butter

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## Information

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## Abstract

**Introduction:** Pistachio butter is one of the products of pistachio processing industry that has a high nutritional value as well as high added value.

**Materials and Methods:** In this original study, pistachio butter produced under different conditions of temperature, grinding time, and sugar-to-honey ratio was evaluated in terms of oxidation (acidity, peroxide value and anisidine value). Pistachio butter samples were stored in a refrigerator (4 °C) for 6 months. Sampling time was in the time period of zero to six and with an interval of two months.

**Results:** In investigating the optimal points, the acid value of four treatments (containing 10% honey) confirmed the mutual intensification property existing between temperature and grinding time and sugar-to-honey ratio. The use of low temperatures and grinding times as well as the usage of honey instead of sugar in the composition of pistachio butter reduced the acid value in the treatments. With increasing the time and temperature of grinding, the peroxide value increased and the highest peroxide value was observed in 6 hours and a temperature of 45 °C. The anisidine value increased with increasing temperature and grinding time in all samples of pistachio butter. Moreover, the anisidine value increased significantly with increasing the sugar-to-honey ratio in all samples.

**Conclusion:** Pistachio butter containing honey has less oxidation than pistachio butter containing sugar.

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## 1. Introduction

Nuts are a main part of people's daily diet. Pistachio is a valuable source of various nutrients including protein, fiber, fat, minerals, and vitamins. Pistachios are commonly eaten raw or roasted, and they are used as a nut product. The high nutritional value, special green color, and suitable aroma of pistachio have resulted in its being used as a part of other food products such as breakfast cereals, chocolates, etc. Pistachio butter is one of the products of pistachio processing industry that has nutritional properties and good taste. This pasty product is obtained by mixing pistachio paste, sweetener, emulsifier and flavoring compounds (1).

Pistachio is one of the tree nuts with high nutritional value in the world. The stability of pistachio kernels and products obtained from pistachio kernels depends, to a large extent, on the stability of its oil. Oil stability is one of the factors determining the shelf life of a product. Oxidized oil has an unpleasant taste and, its quality and shelf life are thus reduced, and it may even affect the texture, color, and appearance of the product (2, 3).

Given their high levels of unsaturated fatty acids, tree nuts and the products obtained from the are sensitive to oxidation. Unsaturated fatty acids such as oleic, linoleic, and long-chain polyunsaturated fatty acids enter the oxidation process (4). Free radicals play a the most significant role in oxidation. They form hydroperoxides, and as a result of further decomposition, the products arising from secondary oxidation such as ketones and aldehydes are formed. The severity of an undesirable taste or saltiness of the product is owing to improper storage of nuts or their long-term storage (5). The production of secondary oxidation products is associated with changes in the smell and flavor of the product, as a result of which one will observe a high level of saltiness. Oxidation of fats negatively affects the flavor, odor, color, and nutritional value of the product during storage and this is likely to affect the use of tree kernels in processed and fortified foods as well as dietary supplements (6). The oxidative stability of different tree nuts and their oils under different conditions has been investigated by some researchers (5- 7). The evaluation of oxidation status is often combined with a sensory evaluation. For example, in roasted peanuts, the formation of secondary oxidation products including pentanal, hexanal, and octanal reduces the aroma of peanuts (8). Several machine methods have been applied to evaluate the oxidation of fats in tree nuts and their oils, one of which is measuring peroxide value as well as anisidine value (7).

Raw pistachios contain 44% fat, 21% protein, 28% carbohydrates, 3% ash and 4% moisture. However, some reports have mentioned the amount of fat to be between 45 to 72%, depending on the type of variety and harvesting time (9). The predominant fatty acid in pistachio oil is oleic acid, it accounts for 56 to 64%. Linoleic acid (23 to 31%) and palmitic acid (9 to 13%) constitute the other fatty acids in pistachio oil (9). The oxidation rate of fatty acids increases with the increasing degree of unsaturation and decreases with the presence of fat-soluble antioxidants such as tocopherol. It has been reported that in 100 g of pistachios, depending on different cultivars and regional conditions, there is about 0.1 to 1560 mg of alpha tocopherol (10- 15). Any increases in free fatty acids, peroxide value, and anisidine value during storage period have been reported by other researchers (16- 19). Oil stability analysis of some tree nuts under accelerated oxidation conditions (60 °C) indicated that among the studied samples (pecans, pistachios, hazelnuts, almonds, Brazil nuts, and walnuts), pistachio oil has shown the lowest value of anisidine after 12 days of being stored (7).

Numerous studies have been conducted with the purpose of mixing pistachio butter or similar products such as Tahini (Tahina) with other ingredients, including sweeteners. A set of variables are involved in the production of expected products. One of the important features is the oxidative stability of the product.

Al-Mamary et al (2002) investigated the antioxidant activity and phenolic content of different types of honey. They have confirmed that the honey they investigated had a high antioxidant content (2). Given the presence of substances such as apigenin, pinocembrin and kaempferol in its structure, honey enjoys high antioxidant properties (21).

In their study, Vinson and Cai (2012) indicated that nuts have high levels of polyphenolic antioxidants that bind to lipoproteins to inhibit the oxidative process (22).

Shakerardekani (2008) indicated that Tahini and sunflower paste can be used in pistachio butter formulation. The results of the aforementioned study showed that treatments with 10% sunflower paste and 10% Tahini had the best efficiency possible and were therefore selected as the best treatments (23).

In part of their research, Ling et al (2015) studied the physicochemical properties and oxidative stability of fresh and roasted pistachio kernel oil. They concluded that given the nature and high chlorophyll content, cold-pressed pistachio kernel oil is highly susceptible to degradation via optical oxidation; it should be stored in containers with anti-light properties. In addition, natural antioxidants are recommended to be added to the product (24).

In order to increase the shelf life, Shakerardekani et al (2009) investigated the effect of a type of industrial antioxidant (Butylated hydroxytoluene, (BHT) at levels of zero, 0.1 and 0.2% (in three replications) on peroxide value of pistachio paste and pistachio butter after four months of being stored at 25 °C. This test indicated that the effect of adding BHT antioxidant on the shelf life of pistachio butter was significant (25).

Shahidi Noghabi et al (2019) investigated the effect of different concentrations of BHT (at three levels of 100, 150 and 200 mg/l) on changes in the peroxide value and acid content of walnut butter during 75 days of storage at room temperature. The results of their study indicated that by adding BHT at 200 mg/l, the peroxide value significantly reduced in comparison to the control sample. They have indicated that the acid number increased after 75 days of storage (26).

Shakerardekani and Shayegh (2019) produced pistachio butter by applying date powder instead of using sugar. The final formula of pistachio butter containing 10% of date powder (selected from Ghasab cultivar) was selected as the best treatment (27). The purpose of the present study is to investigate the oxidative stability of pistachio butter containing honey (as a food-medicine with a natural sweetener).

## 2. Materials and Methods

In this original study, Fandoghi cultivar pistachio was prepared from Rafsanjan in Kerman province. After removing impurities and wastes, the hard shell of the pistachio was manually separated from the kernel and the kernel was roasted semi-industrially. The intended natural and healthy honey was prepared from the Animal Science Research Institute of IRAN. Pistachio butter was prepared according to the method proposed by Shakerardekani et al (27, 28). The prepared samples were filled into containers that had been previously sterilized by ultraviolet (UV) radiation. Containers were stored and tested in a refrigerator (4 °C) for 6 months. In this study, samples of pistachio butter sweetened with honey were tested in terms of acidity, peroxide value, and anisidine value.

**Table 1-** Box–Behnken design for dependent variables

| Treatment | Grinding temperature (°C) | Grinding time (h) | Sugar-to-honey ratio |
|---|---|---|---|
| **1** | 35 | 6 | 0 |
| **2** | 35 | 4 | 5 |
| **3** | 35 | 2 | 0 |
| **4** | 45 | 2 | 5 |
| **5** | 35 | 6 | 10 |
| **6** | 35 | 4 | 5 |
| **7** | 45 | 4 | 0 |
| **8** | 45 | 6 | 5 |
| **9** | 25 | 4 | 10 |
| **10** | 45 | 4 | 10 |
| **11** | 25 | 6 | 5 |
| **12** | 25 | 2 | 5 |
| **13** | 35 | 4 | 5 |
| **14** | 35 | 2 | 10 |
| **15** | 25 | 4 | 0 |
| **16** | 35 | 4 | 5 |
| **17** | 35 | 4 | 5 |

## 2.1- Product acidity

As much as 5 g of the sample was completely dissolved in 55 cc of distilled water. Then, 2-3 drops of phenolphthalein solution were added to this solution and titrated with 0.1 normal sodium. The sodium applied for the emergence of a stable pink color was used to calculate the titratable acidity of the product. Acidity was measured using Equation 1 (29).

**Equation 1**

$$\frac{N \times V \times 40}{W} = \text{product acidity}$$

Where N, V, and W are normality, sodium used, and sample weight, respectively.

## 2.2- Peroxide value

As much as 40 g of the sample (in 140 cc of hexane) was mixed for 10 minutes by an ULTRA-TURRAX mixer at 2000 rpm (IKA T25, Germany). This step caused the oil to be dissolved in hexane. After passing the mixture through a filter paper, the resulting solution was vacuumed to 30 °C until the complete evaporation of hexane. Peroxide value was determined by spectrophotometry (thiocyanate method) (30). Equation 2 was used to determine the sample's peroxide value:

**Equation 2**

$$\frac{(As - Ab) \times m}{84.55 \times W \times 2} = \text{Peroxide value}$$

Where $A_s$ is the adsorption of the sample and $A_b$ is the adsorption of the control sample at a wavelength of 500 nm. m is the equation constant (equal to 40.86) and W is the weight of the oil sample.

## 2.3- Anisidine value

The anisidine value (AnV) is equal to 100 times of the optical density measured at a wavelength of 350 nm in a cell containing one gram of oil per 100 ml of iso-octane and the *p*-anisidine reagent (31). Oil samples (0.5-1± 0.01) are weighed in a 25 ml volumetric flask and is increased by volume with isooctane. Solution absorption will be measured at 350 nm. As much as 5 ml of this solution will be transferred to a test tube and add 1 ml of the anisidine reagent. This reagent contains 0.25 g of para-anisidine per 100 ml of glacial acetic acid. The adsorption of the solution will be read after 10 minutes at 350 nm. A solution containing 5 ml of iso-octane and 1 ml of para-anisidine will be used as a control. The anisidine value will be calculated based on the following formula:

**Equation 3**

$$AnV = [25 \times (1.2\, As - Ab)] / W$$

Where As refers to the absorption of the sample containing reagent after 10 minutes. Ab stands for the absorption of the control sample after 10 minutes, and W is the sample weigh in gram.

# 3. Results

## 3.1- Acid value

The investigation of the trend of acid value changes of pistachio butter samples during 6 months of storage indicated that the treatments containing 10% honey did not have a significant difference. However, the difference between the above treatments and other treatments was significant. After the abovementioned treatments, the lowest mean acid value was related to type 12 pistachio butter, which had the lowest grinding time and grinding temperature.

Comparing the mean acid value of four treatments containing 10% honey (1, 3, 7 and 15) confirmed the mutual intensification property existing between the grinding temperature and grinding time as well as the sugar to honey ratio. Because in the three treatments mentioned (having equal amounts of honey), the acid value was increasing in treatments 7> 1> 3> 15, respectively. The grinding temperature and grinding time in treatments 3, 1 and 7 were higher than that of the treatment 15. The effect of different variables on the acid value of pistachio butter is indicated in Figure 1. In general, using low grinding temperatures and times and applying honey instead of sugar in the composition of pistachio butter reduced the acid value in the treatments.

**Table 2-** Properties of pistachio butter sweetened with sugar and honey produced with different variables

| Treatment | Acid value (percentage of oleic acid) | Peroxide value (Milliequivalents per kilogram) | Anisidine value |
|---|---|---|---|
| **1** | 0.487 | 1.77 | 2.20 |
| **2** | 0.577 | 2.63 | 3.42 |
| **3** | 0.452 | 1.69 | 2.06 |
| **4** | 0.634 | 2.77 | 3.63 |
| **5** | 0.594 | 2.70 | 3.61 |
| **6** | 0.579 | 2.65 | 3.63 |
| **7** | 0.659 | 2.79 | 3.73 |
| **8** | 0.702 | 2.91 | 3.83 |
| **9** | 0.436 | 1.58 | 1.95 |
| **10** | 0.673 | 2.87 | 3.75 |
| **11** | 0.411 | 1.52 | 1.82 |
| **12** | 0.376 | 1.45 | 1.71 |
| **13** | 0.538 | 2.59 | 3.28 |
| **14** | 0.510 | 2.38 | 2.66 |
| **15** | 0.364 | 1.31 | 0.75 |
| **16** | 0.531 | 2.55 | 3.12 |
| **17** | 0.522 | 2.39 | 3.10 |

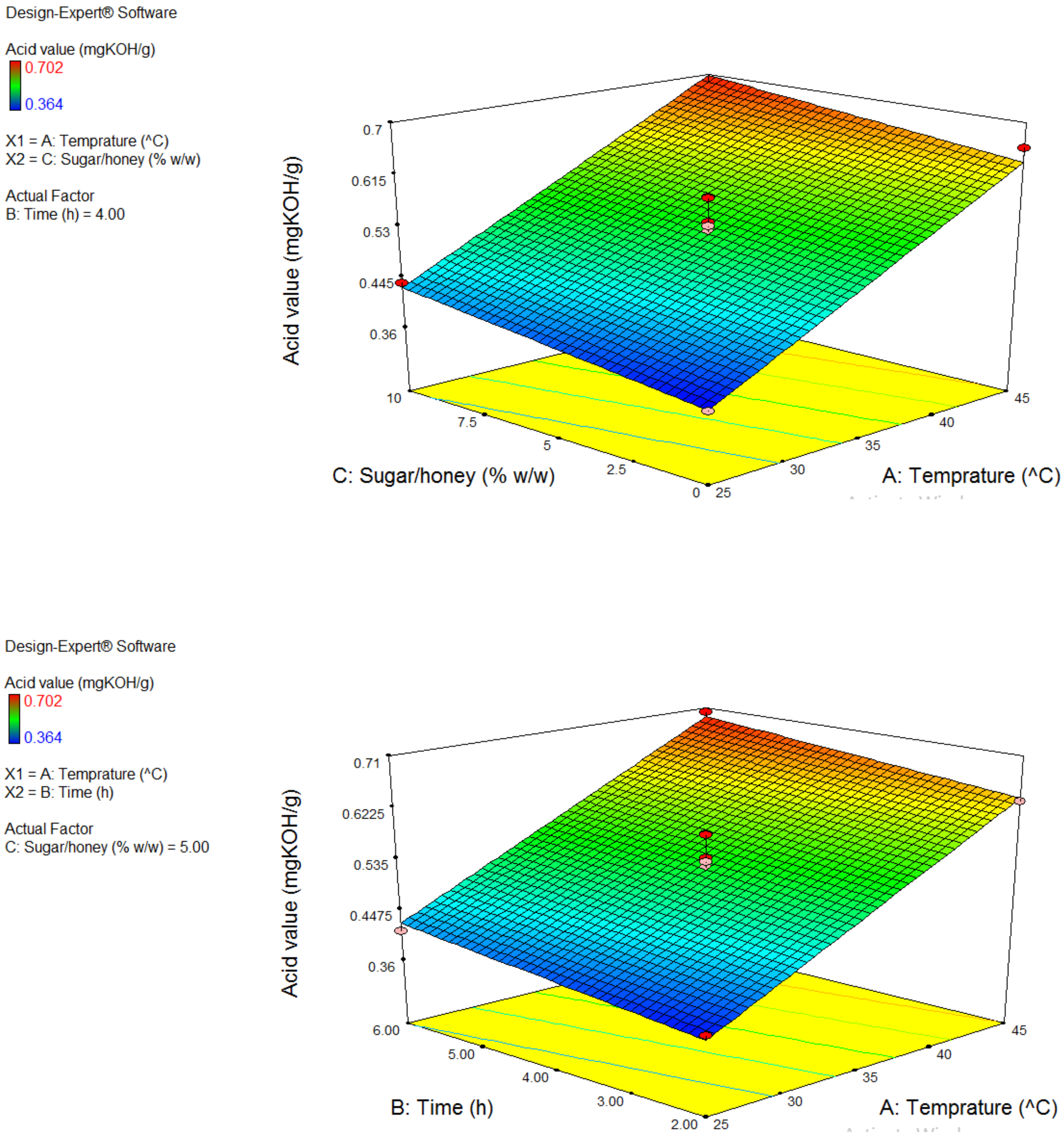



**Fig. 1-** Three-dimensional shape of acid value using three different variables: grinding temperature ($X_1$), grinding time ($X_2$) and sugar-to-honey ratio ($X_3$)

### 3.2- Peroxide value

In all treatments, sample number 8 had the highest rate of oxidation compared to the other treatments. Sample 15 showed the least oxidation in comparison to the other samples. Peroxide value is expressed in Milliequivalents peroxide in 1000 g of sample that oxidizes potassium iodide under test conditions (32).

### 3.3- Anisidine value

The anisidine value increased with increasing grinding temperature and grinding time in all samples of pistachio oil. However, after 6 months of being stored under oxidation conditions, none of the samples reached 10, which is the maximum allowable value. The presence of honey as a natural sweetener in the structure of pistachio butter can stabilize the oil of products containing pistachio oil and thus affect the anisidine value of the product. The anisidine value indicates an estimate of the amount of aldehydes being the secondary compounds arising from the oxidative degradation of the oil. Table 2 shows the anisidine index of different treatments in pistachio butter. This index attempts to investigate the interaction of grinding time and grinding temperature as well as the type of sweetener on the anisidine index. The highest anisidine index is related to sample number 8 and the lowest is related to sample number 15. In general, with increasing grinding time and grinding temperature and also the ratio of sugar to honey in the anisidine value, an upward trend was observed. This indicates that anisidine index depends on the type of treatment and time. Anisidine valude increased with increasing grinding temperature and grinding time in all samples of pistachio butter. Moreover, anisidine value increased significantly with increasing the sugar-to-honey ratio in all samples (Table 2).

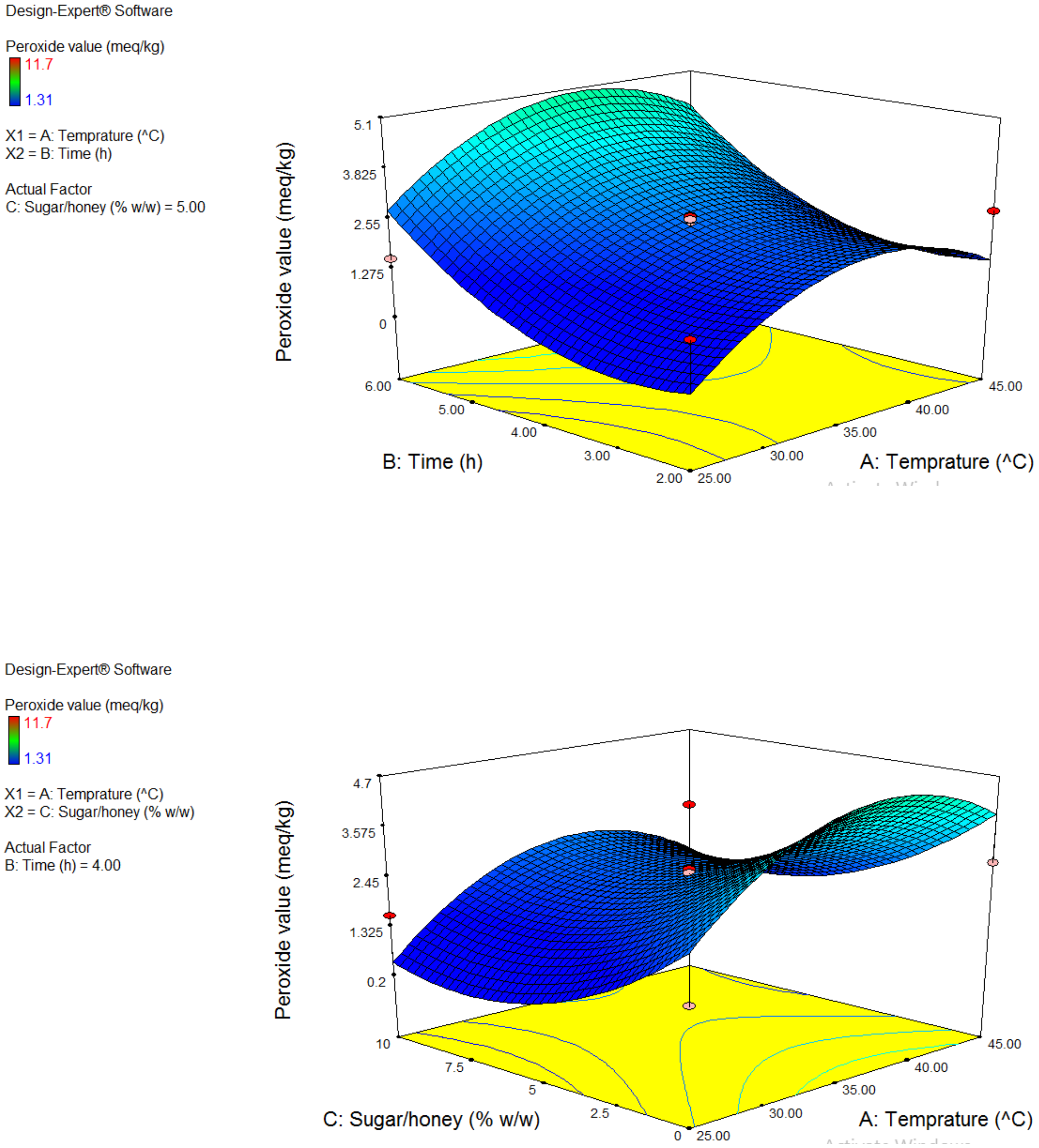
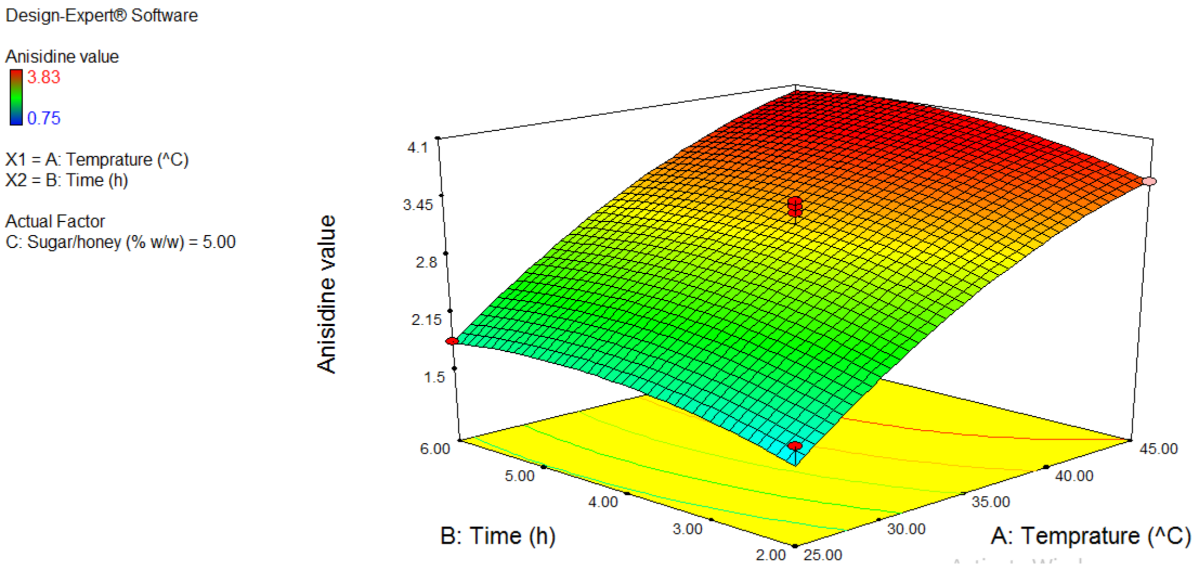



**Fig. 2-** Three-dimensional shape of peroxide value using three different variables: grinding temperature (X1), grinding time (X2) and sugar-to-honey ratio (X3) given the quadratic model



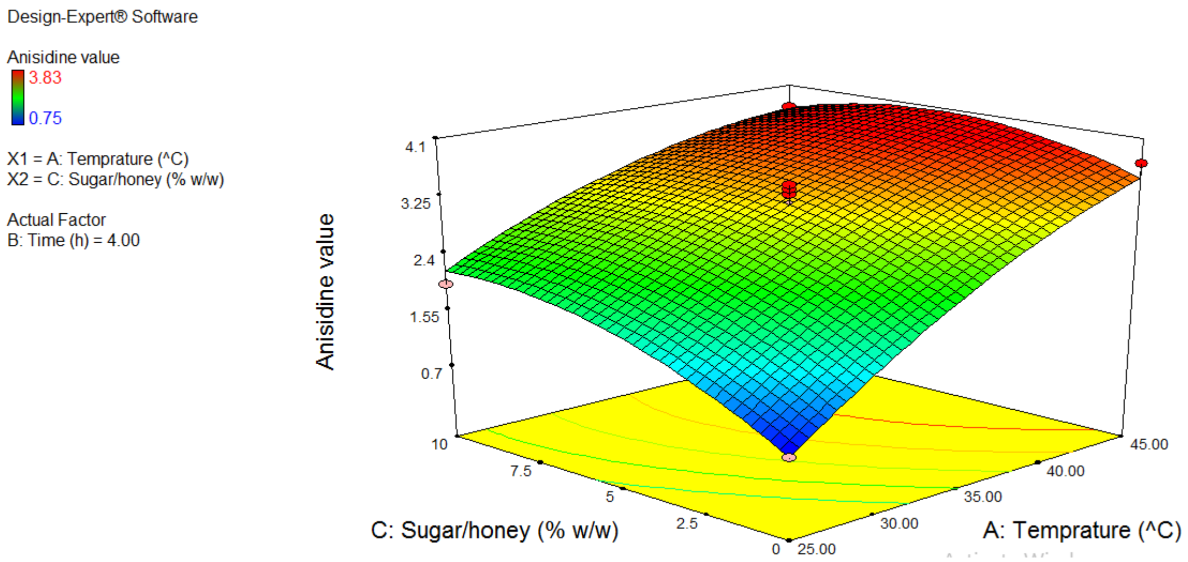




**Fig. 3-** Three-dimensional shape of anisidine value using three different variables: grinding temperature ($X_1$), grinding time ($X_2$) and sugar-to-honey ratio ($X_3$) given the quadratic model

# 4. Discussion

## 4.1- Acid value

The difference between treatments containing higher amounts of honey and other treatments in terms of acidic value can be attributed to the high antioxidant properties of honey in comparison to sugar. In fact, at higher temperatures and times of grinding, part of this antioxidant property is lost and leads to an increase in the acid value of samples with higher temperatures and times of grinding. Moreover, honey stabilizes pistachio butter as well.

Thus, the percentage of oil exposed to the air reduces, and the acid value experiences a slighter increase. After that, one can observe the treatments 14, 17, 16, 6, 5, 2, 4, 7 and 8, respectively in the order of the mean increase of acid value; the difference between the means of the treatments was significant.

## 4.2- Peroxide value

The peroxide value indicates the amount of primary compounds created by the oxidation of oils. Although hydroperoxides are odorless, the breakdown of these compounds, the by-products of oxidation such as aldehydes and ketones are produced that have an unpleasant odor. The formation of peroxide in the early stages is slow and this stage (depending on the type of oil, storage conditions, temperature and other factors) is likely to vary from a few weeks to several months. After that, the formation of hydroperoxides is accelerated and acts as a catalyst in acceleration of oil oxidation (33). The highest peroxide value was observed after 6 months of storage in pistachio butter sweetened with sugar. Pistachio butter number 15 showed less peroxide value after 6 months of being stored at refrigerator temperature. The reason behind this is that the honey added to pistachio butter has played an effective role in its stability. Honey can affect its stability by preventing the separation of oil in products containing pistachio oil. Moreover, the duration and temperature of grinding can delay oxidation. According to the standard, the maximum allowable peroxide value for pistachio oil is 5 milliequivalents per kilogram. Thus, after 6 months of being stored, the peroxide value of the sweetened pistachio butter is within the allowable range.

Peroxide value indicates the initial oxidation of fats and oils. It is also one of the tests used to determine the degree of oil spoilage. With increasing the grinding time and grinding temperature, the peroxide value increased and the highest peroxide value was observed in 6 hours and a temperature of 45 °C. One of the reasons for the lower peroxide value of treatment 15 was the presence of honey sweetener in the formulation of these treatments in comparison to treatments containing sugar. In fact, given its high antioxidant compounds in its structure, honey enjoys high antioxidant properties (21). The effect of different variables on the peroxide value of pistachio butter is shown in Figure 2. In general, using low grinding temperatures and short grinding times and applying honey instead of sugar in the composition of pistachio butter reduced peroxide value in the treatments.

### 4.3- Anisidine value

The anisidine value indicates an estimate of the amount of aldehydes being the secondary compounds arisin from the oxidative degradation of the oil. Table 2 indicates the anisidine index of different treatments in pistachio butter. This index attempts to investigate the interaction of grinding time and grinding temperature as well as the type of sweetener on the anisidine index. The highest anisidine index is related to sample number 8 and the lowest is related to sample number 15. Generally speaking, with increasing grinding time and grinding temperature as well as the sugar-to-honey ratio in the anisidine value, an upward trend was observed; this indicates that the anisidine index is dependent on the type of treatment and time. The anisidine value increased with increasing the grinding temperature and grinding time in all samples of pistachio butter. Moreover, the anisidine value increased significantly with increasing the sugar-to-honey ratio in all samples (Table 2).

Anisidine value is known as an indicator of by-products of oxidation. When a fatty hydroperoxide breaks down, 2 aldehyde or ketone molecules will be formed. In other words, with each unit of decrease in peroxide value, the anisidine value will increase by 2 units; increased anisidine value indicates an expansion of spontaneous oxidation reaction and the increase of secondary products of the decomposition of hydroperoxides over time (33). The aforementioned index was lower for treatments where honey was replaced with sugar in comparison to the other treatments; one of the reasons is the antioxidant properties of honey and its effect on the stability of oil in pistachio butter formulation (34). According to the results, the aforementioned index is lower for treatments with honey (rather than sugar) compared to other samples (samples with sugar and samples with equal weight of sugar and honey); one of the reasons can be the antioxidant properties of honey and its effect on the stability of the oil in the formulation, since the findings of some researchers indicate the antioxidant properties of honey and its effect on oil stability (35).

## 5. Conclusion

The difference between treatments containing higher amounts of honey and other treatments in their acid value can be attributed to the high antioxidant properties of honey compared to sugar.

Using low grinding temperatures and shorter grinding times and applying honey instead of sugar in the composition of pistachio butter reduced the peroxide value in the treatments. The behind this is the fact that the honey added to pistachio butter has played an effective role in its stability. Honey can affect its stability by preventing the separation of oil in products containing pistachio oil. Moreover, the grinding time and grinding temperature can delay oxidation as well. Generally speaking, with increasing the time and grinding temperature as well as the sugar-to-honey ration in the anisidine value, an upward trend was observed; this confirms that anisidine value depends on the type of treatment and time. The anisidine value was lower in treatments in which honey was replaced with sugar in comparison other treatments; one of the reasons is the antioxidant properties of honey and its effect on the stability of oil in pistachio butter formulation.

## Conflict of Interest

The authors declare no conflict of interest.

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