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The Effect of Storage Conditions of Monofloral Honeys on Antimicrobial Activity

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Abstract: This study aimed to determine the change of antimicrobial effects of sunflower, astragalus, chestnut and citrus honey against four bacteria (*Listeria monocytogenes* ATCC 7944, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028) and two yeasts (*Candida albicans* ATCC 10239 and *Candida tropicalis* ATCC 750) depending on storage conditions (heat and light applications) and time. Each monofloral honey was stored in two different conditions, with and without heat treatment, at room temperature (<25°C) in the light and in the dark. The antimicrobial activities of the maintained honey were determined by the disc diffusion method. At the end of the study, it was determined that all honeys showed the highest activity against *S. typhimurium* in conditions that did not see heat and light.

Monofloral Balların Saklama Koşullarına Göre Antimikrobiyal Aktivite Üzerine Etkisi

Anahtar Kelimeler
Bal,
Antimikrobiyal aktivite
Isıl İşlem,
Ayçiçek,
Geven,
Kestane,
Narenciye

Öz: Bu çalışmada ayçiçek, geven, kestane ve narenciye ballarının 4 bakteri *(Listeria monocytogenes* ATCC 7944, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028) ve 2 mayaya (*Candida albicans* ATCC 10239 ve *Candida tropicalis* ATCC 750) karşı antimikrobiyal etkilerinin saklama koşulları (ısı ve ışık uygulamalarının) ile beraber zamana bağlı olarak değişimini belirlemek amaçlanmıştır. Her bir monofloral bal ısıl işlem yapılmış ve yapılmamış olarak, oda sıcaklığında (<25°C) ışık gören ve görmeyen şekilde iki farklı koşulda saklanmıştır. Muhafaza edilen balların antimikrobiyal aktiviteleri disk difüzyon yöntemi ile yapılmıştır. Yapılan çalışma sonunda balların en yüksek aktiviteyi *S. typhimurium* 'a karşı ısı ve ışık görmeyen koşullarda gösterdiği belirlenmiştir.

1. Introduction

Honey, which is essential for human health and used to treat many diseases, is also accepted as a primary nutrient and energy source [1].

Honey, which has been an important food source for human beings for centuries, is also widely used as a natural product in treatment [2]. Many studies have shown that honey is effective as an antimicrobial agent in gastrointestinal disorders [3, 4], healing of wounds and burns [5-8, 4], and proves to provide gastric protection against acute and chronic gastric lesions [8-10], Laboratory studies show that honey is effective against bacteria found in wounds such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica*, *Serovar typhimurium* [11].

Hundreds of years ago, Egyptians used honey in surgical dressings and in the treatment of eye inflammation due to its antimicrobial properties. The Chinese and Indians, on the other hand, wrapped the sick body with honey to prevent the spread of smallpox [12]. Today, it is used as a support in the sense of relieving physical and mental fatigue, energizing, revitalizing, and healing of asthma, stomach, circulation, respiratory, cancer, blood pressure and vascular diseases.

It has been reported that honey can be used as a natural food preservative due to its antimicrobial properties [11]. Oszmianski and Lee (1990) [13] studied that honey reduces enzymatic browning in fruits; McKibben and Engescth (2002) [14] observed that it prevents lipid oxidation in meat.

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In studies examining the antimicrobial effect, it has been reported that honey has an inhibitory effect on approximately 60 Gram-negative and Gram-positive bacterial species with aerobic/anaerobic properties [15, 16] .

In a study by Kolaylı et al. [17], they studied the antioxidant and antimicrobial activities of chestnut honey grown in our country - a total of 32 taken from 3 different regions. It was determined that it showed the highest antimicrobial activity against *S. aureus*, *E. faecalis*, Y. *pseudotuberculosis* and *E. coli*. In the results obtained, a relationship was established between the pollen content of chestnut honey and the activity.

Another study investigated the antioxidant and antimicrobial activities of honey grown in the Eastern Black Sea region [18]. Nine honey samples in this study showed high antioxidants and antimicrobial activity levels.

The antimicrobial activity of honey is related to hydrogen peroxide produced by glucose oxidase found in the nature of honey, and phenolic compounds [19-22].

Minor components, such as aromatic and phenolic compounds, may differ depending on the floral source, to which antimicrobial activity and other biological benefits are attributed. For this reason, there are different monofloral kinds of honey for various uses [21].

Our country is very rich in terms of flowering plant flora and endemic plant diversity, depending on its geographical location and climatic diversity. Monofloral honeys are preferred because of their different flavors and the biological benefits they provide. Among them, sunflower, astragalus, chestnut and citrus honey are among the kinds of honey whose production has become increasingly widespread from past to present. This study aimed to reveal the effect of heat and light applications on antimicrobial activity in storage conditions of sunflower, astragalus, chestnut and citrus monofloral kinds of honey, which are widely produced in Turkey.

2. Material and Method

2.1. Honey samples

Among the monofloral honeys used in the study, Chestnut honey was obtained from Kastamonu, Astragalus honey from Niğde, Sunflower honey from Tokat, and Citrus honey from Hatay.

2.2. Storage conditions

After the monofloral honeys were homogenized and divided into two parts, some of them were exposed to storage and shelf conditions in their natural state. The other part was exposed to the storage conditions after being exposed to the temperature (<60°C) and time applied during packaging. Each monofloral honey, with and without heat treatment, was stored at room temperature (<25°C) in two different conditions, in the light and in the dark (Table 1).

Table 1. Storage conditions of honey samples

Honey samples	Light environment		Dark environment	
	Not Heat Treated	Heat Treated	Not Heat Treated	Heat Treated
Sunflower	AT1	AT2	AT3	AT4
Astragalus	GN1	GN2	GN3	GN4
Chestnut	KK1	KK2	KK3	KK4
Citrus	NH1	NH2	NH3	NH4

2.3. Microbiological tests

2.3.1. Microorganisms and culture medium

In antimicrobial activity studies, 6 microorganisms taken from Muğla Sıtkı Koçman University Culture Collection (MUKK) were used. These are: *Listeria monocytogenes* ATCC 7944, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10239 and *Candida tropicalis* ATCC 750.

L. monocytogenes, *S. aureus*, and *S. typhimurium* were grown by incubation in Nutrient Broth (NB) medium at 37±0.1°C for 24 hours. *E. coli* was grown by incubating in LB broth at 30±0.1°C for 24 hours, and in *C.albicans* and *C. tropicalis* TSB for 48 hours at 30±0.1°C.

In the study, 2 different applications, 1:2 and 1:1 concentration were made from each of the honey samples and the results were examined.

2.3.2. Determination of antimicrobial activities

Agar well diffusion method was used for the antimicrobial activities of honey samples [23]. The honeys were subjected to 1:1 and 1:2 dilutions with water. A hundred µL of each microorganism was taken and spread on Muller-Hinton agar petri dishes. After spreading, wells of 6 mm in size were drilled into the petri dishes. A hundred µL honey samples prepared beforehand were put into these wells. It was then placed in an incubator at 30±0.1°C / 37±0.1°C for *L. monocytogenes*, *E. coli*, *S. aureus*, *C. albicans* and *C. tropicalis* growth. After an incubation period of 24 hours for bacteria and 48 hours for yeasts, inhibition zones formed around the well were measured. This process was repeated every 6 months to determine the time-dependent change in antimicrobial activity.

3. Results

Antimicrobial activity of sunflower honey is given in Table 2. It showed the highest antimicrobial activity against *S. aureus* (33-38 mm zone) and *S. Typhimurium* (26-31 mm zone) in sunflower honey samples. In the

first measurements, honeys with 1:1 and 1:2 concentrations showed close inhibition effects. It has been observed that the inhibition effect disappears over time and only AT1 honey has an effect against *S. aureus* (11 mm zone). A moderate effect was observed in the first measurements of honey with a 1:1 concentration against *L. monocytogenes.* It was determined that all sunflower honeys did not have an antimicrobial effect against Gram (-) bacteria *E. coli*. Therefore, the effect of antimicrobial activity according to storage conditions could not be determined. It was determined that *C. albicans* and *C. tropicalis* strains were effective only in honey itself at a low level. It has been observed that the sunflower honey that has been heat treated has less antimicrobial effect than the ones that have not been heat treated. It was determined that the activity observed in all microorganisms at the beginning decreased with time.

When the antimicrobial activity in Astragalus honey was examined, it was determined that there was a moderate effect in general (table 3). The antimicrobial activities of Gram (+) bacteria are moderately effective. Honeys showed high activity against Gram (-) *S. typhimurium* (24-31 mm). The effects decreased with time and concentration. In the last measurements, the antimicrobial effect against *S. typhimurium* was seen only at 1:1 concentration of GN3 honey. It showed no effect against *E. coli*. There was no effect after the first measurement in honey in activity against *C. tropicalis*. It was determined that the samples with 1:1 concentration showed activity against *C. albicans*. In time, less inhibition zone measurements were made in the heat-treated samples compared to the non heat-treated samples. In the dark and without heat treatment, an inhibition effect was observed even in the last measurements.

The antimicrobial activity results of chestnut honey are given in Table 4. In the first measurements, the highest inhibition zone values were observed in *S. typhimurium* (22-28 mm zone). While an effect was observed in the 2nd measurement in honey stored in the dark, no effect was observed in the last measurements. Honey samples with 1:1 concentration showed inhibition effect against *C. albicans* until the last measurement. It was determined that chestnut honey, which was not heat treated and kept in the dark, had the highest inhibitory effect (6 mm zone) against *S. aureus* until the last measurement.

Inhibition zone results of citrus honey measured against microorganisms are given in Table 5. The highest antimicrobial effect was seen against *S. typhimurium* only in the first measurements (23-31 mm zone). In general, it has been determined that the antimicrobial effect of citrus honey is low and completely lost over time.

Table 2. Time dependent variation of antimicrobial activity of sunflower honey against test bacteria.

Microorganisms		*C. albicans*			*C. tropicalis*			*E. coli*			*S. typhimurium*			*L. monocytogenes*			*S. aureus*		
Honey samples	Cons	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
AT1	1:1	9	8	7	19	6	5	-	-	-	31	14	9	15	-	-	38	17	11
	1:2	-	-	-	-	-	-	-	-	-	26	-	-	10	-	-	36	-	-
AT2	1:1	16	13	11	9	-	-	-	-	-	29	16	8	12	-	-	35	7	-
	1:2	-	-	-	-	-	-	-	-	-	27	-	-	10	-	-	33	-	-
AT3	1:1	8	7	6	14	7	-	-	-	-	30	18	13	12	-	-	36	16	-
	1:2	-	-	-	-	-	-	-	-	-	28	-	-	9	-	-	-	-	-
AT4	1:1	7	6	5	17	-	-	-	-	-	28	7	-	18	-	-	38	-	-
	1:2	-	-	-	-	-	-	-	-	-	26	-	-	10	-	-	34	-	-

-: no effect
AT1: Sunflower honey- that no heat-treated in the light environment
AT2: Sunflower honey- that heat-treated in the light environment
AT3: Sunflower honey -that no heat-treated in the dark environment
AT4: Sunflower honey -that heat-treated in the dark environment

Table 3. Time dependent variation of antimicrobial activity of Astragalus honey against test bacteria

Microorganisms		*C. albicans*			*C. tropicalis*			*E. coli*			*S. typhimurium*			*L. monocytogenes*			*S. aureus*		
Honey samples	Cons	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
GN1	1:1	6	5	4	12	-	-	-	-	-	30	13	-	8	7	-	28	-	-
	1:2	-	-	-	6	-	-	-	-	-	27	-	-	5	-	-	12	-	-
GN2	1:1	8	7	6	14	-	-	-	-	-	28	12	-	6	5	-	10	-	-
	1:2	6	-	-	-	-	-	-	-	-	25	-	-	8	-	-	6	-	-
GN3	1:1	8	7	5	9	-	-	-	-	-	28	26	8	7	5	-	8	4	-
	1:2	6	-	-	7	-	-	-	-	-	24	-	-	9	-	-	-	-	-
GN4	1:1	13	6	-	6	-	-	-	-	-	31	-	-	8	5	-	16	-	-
	1:2	9	-	-	-	-	-	-	-	-	20	-	-	9	-	-	9	-	-

-: no effect
GN1: Astragalus honey -that no heat treated - light environment
GN2: Astragalus honey -that heat-treated in the light environment
GN3: Astragalus honey -that no heat-treated in the dark environment
GN4: Astragalus honey- that heat-treated in the dark environment

Table 4. Time dependent variation of antimicrobial activity of Chestnut honey against test bacteria

Microorganisms		*C. albicans*			*C. tropicalis*			*E. coli*			*S. typhimurium*			*L. monocytogenes*			*S. aureus*		
Honey samples	Cons	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
KK1	1:1	8	6	5	7	6	5	-	-	-	27	-	-	10	8	-	14	10	-
	1:2	7	-	-	-	-	-	-	-	-	25	-	-	8	-	-	7	-	-
KK2	1:1	7	6	5	-	-	-	-	-	-	24	-	-	12	6	-	13	-	-
	1:2	-	-	-	-	-	-	-	-	-	22	-	-	13	-	-	6	-	-
KK3	1:1	8	8	6	-	-	-	-	-	-	27	16	-	8	7	-	14	10	6
	1:2	7	7	-	-	-	-	-	-	-	24	-	-	6	-	-	8	-	-
KK4	1:1	8	8	5	8	-	-	-	-	-	28	13	-	-	-	-	14	12	-
	1:2	8	-	-	8	-	-	-	-	-	28	-	-	-	-	-	14	7	-

-: no effect
KK1: Chestnut honey -that no heat treated in the light environment
KK2: Chestnut honey -that heat treated in the light environment
KK3: Chestnut honey -that no heat treated in the dark environment
KK4: Chestnut honey- that heat treated in the dark environment

Table 5. Time dependent variation of antimicrobial activity of Citrus honey against test bacteria

Microorganisms		*C. albicans*			*C. tropicalis*			*E. coli*			*S. typhimurium*			*L. monocytogenes*			*S. aureus*		
Honey samples	Cons	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
NH1	1:1	9	8	-	6	-	-	-	-	-	24	-	-	15	-	-	11	-	-
	1:2	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	-
NH2	1:1	6	5	-	16	-	-	-	-	-	26	-	-	-	-	-	-	-	-
	1:2	-	-	-	-	-	-	-	-	-	24	-	-	-	-	-	-	-	-
NH3	1:1	8	6	-	17	-	-	-	-	-	31	-	-	-	-	-	-	-	-
	1:2	-	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-	-
NH4	1:1	16	11	-	18	-	-	-	-	-	29	-	-	20	-	-	-	-	-
	1:2	10	8	-	-	-	-	-	-	-	24	-	-	12	-	-	-	-	-

-: no effect
NH1: Citrus honey -that no heat treated in light environment
NH2: Citrus honey -that heat treated in light environment
NH3: Citrus honey- that no heat treated in dark environment
NH4: Citrus honey -that heat treated in dark environment

4. Discussion and Conclusion

It is known that honey has a broad spectrum antimicrobial effect against bacteria and many yeast/mold species [18, 24-26]. In a study examining the antimicrobial activity and mechanism of action of multifloral and monofloral honeys, it was determined that monofloral honeys provided inhibition at 100% (v/v) concentrations of *L. monocytogenes* ATCC 15313 strain [27].

In our study, it was determined that the antimicrobial activity of monofloral honeys at 100% (v/v) concentrations was higher than at 50% (v/v) concentrations. After a period of 6-12 months, no effect was observed in honey with 50% concentration.

It was determined that the antimicrobial properties of monofloral honeys did not make a significant difference between Gram properties of bacteria. While most of the honeys used in the study showed the best antimicrobial effect against Gram-negative bacteria, *S. typhimurium*, no effect was detected against *E. coli*, another Gram-negative bacterium. On the other hand, it has been found to have a high antimicrobial effect against another Gram-positive bacterium, S. *aureus*. In the two honeys in question, the inhibition effect was higher in the samples that did not undergo heat treatment.

It can be said that the antibacterial effects of the honey used in this study are higher than the antifungal effects. When the effects against *C. albicans* and *C. tropicalis* were compared, it was determined that all honeys were more effective against *C. albicans.* Although low inhibition zone value was observed, it was determined that the effect continued until the last measurement in other honeys except citrus honey. When the initial zone and last zone measurements were compared, it was determined that the most antimicrobial effect was in AT3, GN3, KK3 and NH 3 without heat and light application.

In the study of Lee et al. [28], antimicrobial activity of sunflower honey against *L. monocytogenes* and *S. aureus* was determined. The results of our study are compatible with this study in the literature. AT1 honey showed the highest activity against the aforementioned microorganisms.

In the study of Sağdıç et al. [29] with 50% concentration samples of astragalus honey, it was determined that honeys were not effective against *E. coli, S. typhimurium*, *L. monocytogenes* and *S. aureus* strains. The results of our study are compatible with this study in the literature. It can be said that keeping astragalus honey in conditions where heat is not applied and where there is no light is effective in its activity.

In a study by Kolaylı et al. [17], it was determined that the antimicrobial effect of chestnut honey is more effective against bacteria than fungi. The highest antimicrobial activity was reported against *S. aureus*. In our study, it was determined that chestnut honey had a better inhibition effect against *S. aureus* compared to mushrooms. It was determined that the effect lasted for 12-18 months, especially in the KK3 honey sample, where heat and light were not applied. In another study, it was determined that chestnut honey did not show any effect against *E. coli* in 50% concentration samples. In the same study, it was determined that the effect against *S. areus* and *L. monocytogenes* was measured as 0-6 mm zone [29]. The results obtained in our study also support this study.

In a study conducted on citrus honeys, an inhibition zone of 26.4 mm against *S. aureus* and 25.4 mm against *E. coli* was measured [30]. In our study, while the effect of citrus honey against *E. coli* was not observed, the inhibition effect of NH1 honey was observed against *S. aureus*. In general, it was determined that citrus honeys showed the lowest activity compared to other honeys.

In our study, antimicrobial activity properties of different monofloral honeys were investigated. The highest antimicrobial activity was detected in sunflower honey. It was determined that the highest inhibition effect of monofloral honeys was against *S. typhimurium.* It has been determined that the antimicrobial effect of honey stored in heat-treated – light-exposed areas decreases rapidly depending on time. As a result, with this study, it was determined that the effect of keeping the honey in conditions where heat was not applied and where there was no light would be high in terms of antimicrobial activity.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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