Inhibitory Effect of Propolis (Bee Gum) Against *Staphylococcus aureus* Bacteria Isolated From Instant Soups[1]

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In this study, microbiological quality of instant soups sold in markets and the antibiotic susceptibility of *Staphylococcus aureus* isolated from instant soups were examined. Total aerobic mesophilic bacteria and *S. aureus* counts of 6 different types of instant soups were analyzed. Microbiological analysis was carried out in a total of 72 packages of soups, including three replicates. Total aerobic mesophilic bacteria counts and *S. aureus* counts were determined to be 3.51–4.53 log cfu/g and 0.93–1.71 log cfu/g, respectively. Coliform bacteria were not detected in any of the tomato soups analyzed. The highest number of coliform bacteria (2.10 log cfu/g) was detected in Ezogelin soups. *Escherichia coli* was not detected in any of the samples analyzed. In addition, the inhibitory effects of five different antibiotics and three different propolis extracts supplied from three different regions of Turkey (Corlu, Kirklareli and Ordu) were examined against *S. aureus* isolated from the instant soups. Using *S. aureus* bacteria isolated from tripe soup, a zone diameter of 36.62±0.17 mm was observed with Cefixime. The smallest zone diameter was obtained with Streptomycin (14.74±0.4mm). Zone diameters with propolis samples from Ordu, Corlu and Kirklareli were 10.18±0.04 mm, 7.07±0.45 mm and 6.21±0.14mm, respectively. All of the *S. aureus* bacteria isolated were sensitive to the Amoxicillin. Propolis extracts were inhibitory towards *S. aureus* isolated from instant soups, but propolis samples obtained from different geographical regions showed varying antimicrobial effects.

Key Words: instant soup, Propolis, Antimicrobial effect, Antibiotics

Hazır Çorbalardan İzole Edilen *Staphylococcus aureus* Bakterisine Karşı Propolisin İntibitör Etkisi

Bu çalışmada, marketlerden alınan hazır çorbaların mikrobiyolojik kalitesi ve hazır çorbalardan izole edilen *Staphylococcus aureus'un* antibiyotiğe duyarlılığı incelenmiştir. Hazır çorbaların 6 çeşidinde 72 pakette 3 tekrar olmak üzere toplam mezofil bakteri ve *Staphylococcus aureus* sayısı belirlenmiştir. Toplam mezofil bakteri sayısı ve *S.aureus* sayısı sırasıyla 3,51–4,53 log kob/g and 0,93–1,71 kob/g bulunmuştur. Domates çorbasında koliform bakteri bulunmazken, en yüksek koliform bakteri ezogelin çorbasında tespit edilmiştir (2,10 log kob/g). *Escherichia coli* bakterisi hiçbir örnekte belirlenmemiştir. İlave olarak, Çorlu, Kırklareli ve Ordu'dan alınmış 3 farklı propolis ektraktları ve 5 farklı antibiyotik hazır çorbadan izole edilmiş *S.aureus* üzerine inhibitor etkisi incelenmiştir. Üçlü çorbadan izole edilen *S. aureus* bakterileri kullanılarak, Cefixime ile 36.62 ± 0.17 mm, en küçük zon çapı, Streptomisin (14.74 ± 0.4mm) tespit edildiştir. Ordu, Çorlu ve Kırklareli'den alınan propolis örneklerin zon çapları sırasıyla 10.18 ± 0.04mm, 7.07 ± 0.45 mm ve 6.21 ± 0.14 mm olarak belirlenmiştir. Izole edilen *S.aureus* bakterilerinin tamamının Amoxicillin'e karşı duyarlı olduğu, propolis örneklerinin ise toplandığı bölgelere göre farklı antimikrobiyal etki gösterdiği tespit edilmiştir.

Anahtar Kelimeler: Hazır çorba, Propolis, Antimikrobiyal etki, Antibiyotik

[1]Hakan Apaydın'ın Yüksek Lisans tezinden hazırlanmıştır.

Introduction

Instant soup is widely consumed by people in Turkey and in the world. Various types of instant soups are available in Turkey including tarhana, ezogelin and yogurt soup with rice. Furthermore, in recent years ready-to-eat soups prepared with hot water have become popular in many countries including Turkey (Erkekoğlu et al., 2009). However, such soups may be dangerous because harmful bacteria may grow and produce toxins that are fairly resistant to heat, pH and NaCl (Balaban and Rasooly, 2000), unless stored under proper conditions and prepared with sufficiently hot water. If the temperature of water used for the preparation of instant soups does not exceed 100°C, it may exacerbate the microbiological risk for consumers. Additionally, some spore forming bacteria may become resistant to high temperatures and propagate if the soups are stored under improper conditions (Oomes et al., 2007).

Total mesophilic aerobic bacteria (TMAB) count is a criterion used to determine the general microbial safety of instant soup products. The acceptable value for TMAB varies between

countries. TMAB values may show variations between different instant soup products; therefore the predictive value of TMAB for microbiological quality has been called into question. Therefore, in addition to TMAB, analyses on the presence of pathogenic bacteria, yeast and mold need to be carried out.

Propolis, also known as bee glue and bee propolis, is a resinous product that is collected by honeybees from buds, leaves, bark and exudates of several trees and plants (Nedji et al., 2014; Mirzoeva et al., 1997). Propolis is extensively used in folk medicine, and a number of investigations have shown that propolis possesses antibacterial, antiviral, antifungal immunostimulatory and anti-carcinogenic activities (Kujumgiev et al., 1999; Park et al., 1998). The precise composition of raw propolis varies according to its source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Cirasino et al., 1987). The wax and organic debris are removed during processing, creating propolis tincture (Burdock, 1998). Bees produce propolis to protect the hive from harmful bacteria, viruses and fungi. Pharmacologically active constituents in propolis are the flavones, flavonols and flavonones, and various phenolics and aromatics. Flavonoids play a major role in plant pigmentation and are thought to account for much of the biological activity of propolis. The active components of propolis that show antibacterial effects include pinocembrin, galangin, caffeic acid and ferulic acid (Bauer et al.,1966).

The antibacterial activity of propolis is of great interest because of its possible wide clinical applicability. However, the mechanism behind the antibiotic effect of propolis is not clear, and the details of its effects on the different aspects of microbial physiology have not been investigated (Mirzoeva et al., 1997).

In this study, the microbiological quality and the antibiotic susceptibility of pathogenic bacteria from instant soups sold in markets were examined. Additionally, the antibacterial activity of propolis supplied from different geographical regions of Turkey (Corlu, Kirklareli and Ordu) against *Staphylococcus aureus* isolated from instant soups was examined. This was compared with the antimicrobial effect of the antibiotics Tetracycline, Cefixim, Amoxicillin, Ampicillin and Streptomycin against *S.aureus*

Materials and Methods

Collection of Propolis

Propolis samples were collected by beekeepers from two locations in the Thrace region Turkey: Corlu (41° 10’ N, 27° 48’ E) and Kirklareli (41° 24’ N, 27° 21’ E) and one location in Ordu (40° 58’ N, 38° 4’ E) in the Black sea region lying in the north of Turkey. Climate is the biggest difference between the Black Sea and Thrace locations; while the Black Sea region gets more rainfall during the year, the Thrace region is relatively more sunny. Each sample was collected by using plastic nets and was stored at +4°C in dry glass jars in the dark until use. Finally, It was purchased Tripe, Ezogelin, Yoghurt with rice, cream of chicken, tarhana and tomato of six different instant soup samples.

Extraction of propolis

The propolis samples were ground into a fine powder, and 30% (w/v) extract was prepared in 100% methanol. The mixture was incubated in a rotary shaker incubator at 60 ^{0}C, 150 rpm for 24 hours. The extracts were then centrifuged at 4000 rpm for 10 min and the supernatant was filtered with Whatman 4 paper and concentrated in a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany) set at 50^{o}C (Uğur and Arslan, 2004).

Microbiological Analysis

In a sterile Stomacher bag, 10 g of the soup samples were each mixed with 90 mL of sterilized peptone-water (10^{-1} dilution) and serially diluted in the same solvent. Total mesophilic aerobic bacteria (TMAB) counts were determined by plating appropriate dilutions on Plate Count Agar using the pour plate technique (Lambert et al., 1992).

S. aureus was determined as follows: 10 g of each of the soup samples was homogenized in 90 ml peptone-water using a Stomacher. The samples were serially diluted, seeded onto Baird Parker Agar and incubated at 35-37 °C for 24 and 48 h. The samples producing typical colonies (grey-black, surrounded by a dull halo) were counted (Anon, 1998).

Yeast and Molds were determined as follows: the samples (0.1 mL of each dilution) were inoculated in acidified potato dextrose agar medium followed by incubation at 25 °C for 3–5 days, according to the method described by Downes and Ito (2001).

Coliform bacterial count was determined by incubating the samples in Violet Red Bile Agar (VRBA,Merck) at 37 °C for 48h as described in the Bacteriological Analytical Manual (AOAC, 1998).

E.coli counts was determined the samples in Tryptone Bile X-glucuronide agar (TBX, Merck) and incubated at 44°C for 24 h (AOAC, 1998).

Antibiotic discs

Antibiotic discs containing the antibiotics Tetracycline (10 µg/disc), Cefixime (5 µg/disc), Amoxicillin (10 µg/disc), Ampicillin (10 µg/disc) and Streptomycin (10 µg/disc), were purchased from Oxoid Inc. and stored at +4 ^{0}C in the original packaging.

Disc Diffusion Method

To determine the antibacterial effects of propolis, the disc diffusion method ?for antimicrobial susceptibility was carried out as described previously (Khalmeter et al., 2006). A bacterial culture adjusted to 0.5 McFarland standard was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test as described below.

Holes of 6 mm diameter (same diameter as the commercially sourced antibiotic discs) were opened on the plate surface. The three different propolis extracts (100µl from each) were applied to these holes and the plates were incubated at 37°C for 24 hours. The inhibition zone diameters were then measured using calipers and recorded. The measurements were taken on days 1, 3, 5 and 7 were used to determine daily changes in the zone diameter for each of the propolis samples. Commercially obtained antibiotic discs containing different known antibiotics were also applied to the bacterial lawn on the Muller Hinton agar plates and used as controls.

The antimicrobial effect of propolis samples and known antibiotics were calculated by using the formula $\% \, inhibition = \frac{(a-p)}{a} * 100$

Where “a” and “p” refer to the zone diameter (mm) formed by the known antibiotics and propolis extracts respectively.

Sensory Evaluation

The effects of propolis on color, taste and odor of instant soups was evaluated by 10 selected panelist using a sensory evaluation test. The samples were prepared by mixing 0.2 ml of propolis to 200 ml instant soup (0.1 vol%). Six different soup samples either supplemented or not with propolis were provided to panelists and evaluated for color, taste and odor (Onoğur and Elmaci, 2014). The panelists were asked to evaluate the differences over a scale of 0-5 points. The differences between the control and test samples were determined with a paired comparison test method.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Science (SPSS) software (SPSS 18.0 for Windows, 2007) using one-way analysis of variance (ANOVA). Duncan’s multiple range test (DMRT) was applied to calculate the significant difference between samples. Results were expressed as the average ± standard deviation (Anonymous, 1999).

Results and Discussion

Total mesophilic aerobic bacteria (TMAB), coliform bacteria, yeast and mold, *S. aureus* and *E.coli* counts of the six different instant soup samples are shown in Table 1.

Twelve replicates from each of the six soup samples were analyzed for each microbial species. TMAB counts were in the range of 3.51 ± 0.35 to 4.53 ± 0.61 log cfu/g, similar to the data reported by Çoksaygılı and Başoğlu (2011). Coliform bacteria were detected in yoghurt soup with rice (2.86 log cfu/g) but not in the tomato soup. *S. aureus* count was in the range of 0.93±0.26-1.71±0.29 log cfu/g. Korkmaz (2012) have previously reported a higher *S. aureus* count of 2.83±1.49 log cfu/g.

The maximum yeast and mold count was detected in Tarhana soup, most likely because it is a fermented product. E. coli was not detected in any of the samples analyzed. Demirci and Sezer (1995) have previously identified E.coli in 33% of the soup samples examined. These authors also reported that S.aureus was one of the most important pathogenic bacteria isolated from the analyzed soup samples. The high number of S. aureus is of great significance as it is an indicator of poor sanitary conditions and risk of production of enterotoxin. Detection of low counts of S. aureus also does not reduce the risk of enterotoxins since these toxins cannot be inactivated by thermal treatment, inhibitors or dehydration.

Table 1. Microbiological quality of different instant soups

Soup samples		T.M.A.B. Counts (log cfu/g)	Coliform Counts (log cfu/g)	*S. aureus* Counts (log cfu/g)	Mold & Yeast Counts (log cfu/g)	*E.coli* Counts (cfu/g)
Tarhana	n=12	4.53±0.61^{a}	0.74±0.45^{ab}	1.21±0.47^{a}	4.12±0.4^{a}	0^{a}
Tripe	n=12	3.92±0.64^{a}	0.29±0.29^{b}	0.93±0.26^{a}	3.45±0.49^{a}	0^{a}
Yoghurt with rice	n=12	3.89±0.43^{a}	2.10±0.80^{a}	1.64±0.29^{a}	3.42±0.38^{a}	0^{a}
Chicken with cream	n=12	3.51±0.35^{a}	1.14±0.45^{ab}	1.15±0.31^{a}	3.14±0.23^{a}	0^{a}
Ezogelin	n=12	4.21±0.79^{a}	1.43±0.82^{ab}	1.71±0.29^{a}	3.01±0.43^{a}	0^{a}
Tomato	n=12	4.21±0.87^{a}	-^{b}	1.15±0.27^{a}	3.21±0.43^{a}	0^{a}

a,b Means within the same column with a different superscript letter are statistically different (P<0.05)

Table 2.Inhibitory effects of propolis samples (100 L) against *S. aureus* isolated from instant soups (data indicative of inhibitory zones measured as mm)

Soups	Days	Propolis (Corlu)	Propolis (Kirklareli)	Propolis (Ordu)
Tripe	1st day	7.22±0.52^{aB}	7.52±0.41^{aB}	10.55±0.27^{abA}
	3rd day	7.13±0.44^{abB}	6.92±0.17^{abcB}	10.14±0.03^{abA}
	5th day	7.08±0.43^{abB}	6.34±0.13^{abcdB}	10.17±0.03^{abA}
	7th day	7.07±0.45^{abB}	6.21±0.14^{abcdC}	10.18±0.04^{abA}
Ezogelin	1st day	7.20±0.26^{aAB}	6.14±0.14^{abcdB}	8.14±0.90^{cdeA}
	3rd day	6.06±0.04^{cdeB}	6.56±0.25^{abcAB}	7.79±0.80^{deA}
	5th day	6.06±0.01^{cdeB}	6.59±0.24^{abcAB}	7.75±0.77^{deA}
	7th day	6.06±0.02^{cdeB}	6.73±0.17^{abcAB}	7.78±0.74^{deA}
Yoghurt with Rice	1st day	6.55±0.23^{abcdB}	6.68±1.14^{abcB}	9.59±0.14^{abcA}
	3rd day	6.60±0.05^{abcdB}	6.14±0.94^{abcdB}	9.22±0.32^{bcdA}
	5th day	6.31±0.11^{bcdB}	6.12±0.90^{abcdB}	9.17±0.43^{bcdA}
	7th day	6.32±0.09^{bcdB}	6.07±0.92^{abcdB}	9.00±0.29^{bcdA}
Cream of Chicken	1st day	6.74±0.04^{abcB}	5.58±0.24^{cdC}	10.89±0.56^{abA}
	3rd day	6.59±0.19^{abcdB}	5.08±0.03^{dC}	10.01±0.17^{abA}
	5th day	6.55±0.22^{abcdB}	5.06±0.02^{dC}	10.62±0.52^{abA}
	7th day	6.58±0.21^{abcdB}	5.02±0.05^{dC}	10.61±0.47^{abA}
Tarhana	1st day	5.37±0.11^{efA}	6.29±0.04^{abcdA}	6.58±1.15^{efA}
	3rd day	4.89±0.34^{fA}	5.99±0.04^{bcdA}	5.94±0.82^{fA}
	5th day	4.92±0.18^{fA}	5.97±0.03^{bcdA}	5.96±0.81^{fA}
	7th day	4.93±0.22^{fA}	5.99±0.04^{bcdA}	5.86±0.81^{fA}
Tomato	1st day	7.30±0.23^{aB}	7.37±0.18^{abB}	11.21±0.50^{aA}
	3rd day	5.85±0.35^{deB}	6.20±0.10^{abcdB}	10.58±0.05^{abA}
	5th day	6.10±0.27^{cdeB}	6.24±0.17^{abcdB}	10.57±0.06^{abA}
	7th day	6.30±0.15^{bcdB}	6.25±0.13^{abcdB}	10.14±0.05^{abA}

a,b Means within the same column with a different superscript letter are statistically different (P<0.05; n=6)
A,B Means within the same line with a different superscript letter are statistically different (P<0.05; n=6)

Therefore, the incorrect classification of the food as 'clean' when they have low counts of S. aureus may still cause serious toxicity when consumed (Unluturk and Turantas, 2003). In the current study, antimicrobial effects of known antibiotics and propolis supplied from different geographical regions of Turkey were investigated on S. aureus isolated from instant soup samples. The results of the antibiotic susceptibility test carried out with the disc diffusion method for propolis samples and known antibiotics are shown in Table 2.

A zone diameter of 7.30 mm was obtained from S. aureus isolated from tomato soup on the 1st day. At the end of 7th day, S. aureus from tripe soup had the largest zone diameter of 7.07 mm. The zone diameter generated after inoculation of the plate with propolis sample from Corlu was in the range of 4.89–7.30mm. The minimum inhibitory effect of this propolis sample was observed in tarhana soup with a zone diameter 4.89 mm on the 3rd day. The zone diameter generated after inoculation with the propolis sample from Kirklareli was in the range of 5.02-7.52 mm. The same propolis sample showed the highest inhibitory effect against S. aureus isolated from tripe soup with a zone diameter of 7.52 mm on the 1st day. The zone diameter of the propolis supplied from Ordu was detected in the range of 5.86–11.21 mm. The largest zone diameter of 11.21 mm was obtained with this propolis sample against S. aureus isolated from tomato soup on the 1st day.

Kujumgiev et al. (1999) investigated the antimicrobial (S. aureus and E. coli), antifungal (Candida albicans) and antiviral (Avian influenza virus) effect of propolis supplied from different geographical regions. All of the propolis samples tested showed antimicrobial, antiviral and antifungal effects. These authors reported variations in the chemical composition of propolis in relation to the geographical location of their collection. Popova et al., (2005) reported variations in the antimicrobial efficacy of propolis collected from different geographical regions of Turkey (Bursa, Iznik, Kayseri, Sivas, Yozgat, Erzurum, Hatay and Artvin) against S. aureus and E.coli. These two studies corroborate the data obtained in the current study and support the variations observed in the antimicrobial effects of propolis according to geographical location.

Antimicrobial activity of antibiotics

The inhibitory effect of antibiotics such as Tetracycline, Cefixime, Amoxicillin, Ampicillin and Streptomycin on *S.aureus* was determined with the disc diffusion method. The zone diameter (mm) obtained with antibiotic discs were compared to the data from propolis samples on the 7th day after incubation with *S. aureus* isolated from instant soups (Table 3).

Using S. aureus bacteria isolated from tripe soup, a zone diameter of 36.62±0.17 mm was observed with Cefixime. The smallest zone diameter was obtained with Streptomycin (14.74±0.4mm). Zone diameters with propolis samples from Ordu, Corlu and Kirklareli were 10.18±0.04 mm, 7.07±0.45 mm and 6.21±0.14mm, respectively.

Using S. aureus bacteria isolated from Ezogelin soup, the highest zone diameter was observed with Tetracycline (35.98±0.7mm) whereas the smallest zone diameter was observed with Streptomycin (16.04±0.7mm). Propolis from Ordu was the most effective against these bacteria with a zone diameter of 7.78±0.74mm.

Ampicillin was the most effective antibiotic against S. aureus isolated from yoghurt with rice, cream of chicken, tarhana and tomato soups (18.69±2.36 mm; 21.48±0.15mm;12.30±0.15mm;14.76±0.18mm). Streptomycin showed the lowest efficacy against S. aureus isolated from yoghurt with rice, cream of chicken soups, while Tetracycline showed the lowest efficacy against S. aureus isolated from tarhana and tomato soups (12.30±0.75 mm, 14.76±0.18 mm).

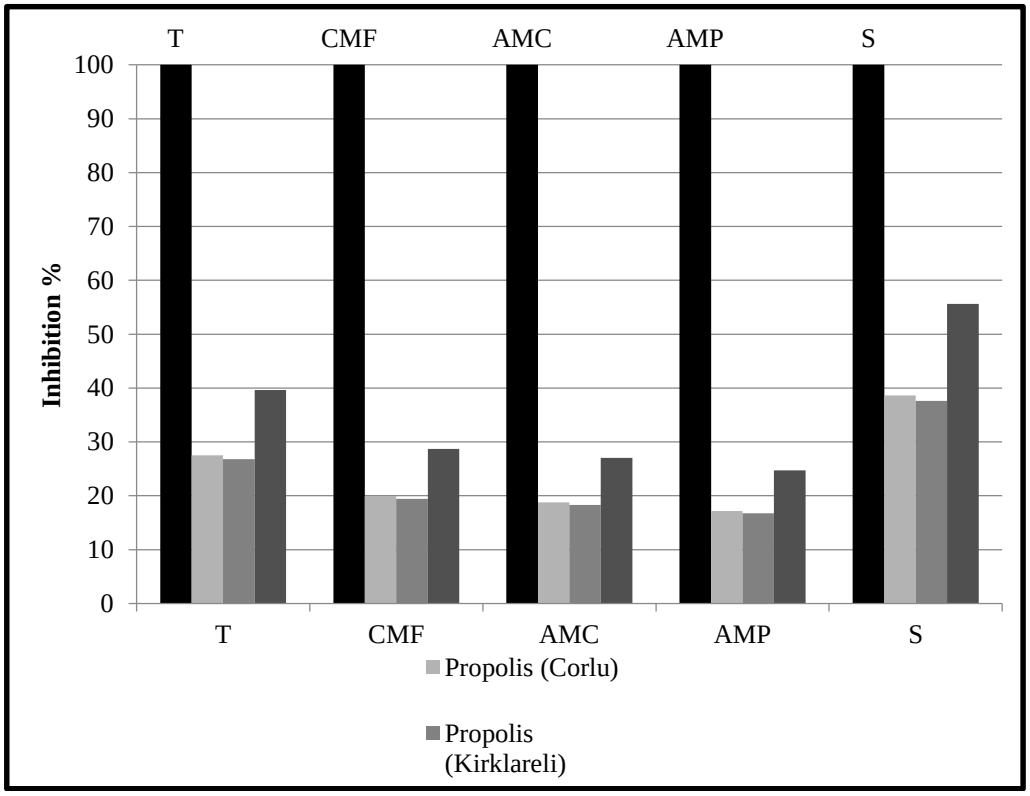
Table 3. Zone diameter of antibiotic discs and propolis samples against *S. aureus on the 7th day*

Soups	Tetracycline (10µg)	Cefixime (5µg)	Amoxicillin 10µg	Ampicillin 10µg	Streptomycin 10µg	Propolis (Corlu) 10µL	Propolis (Kirklareli) 10µL	Propolis (Ordu) 10µL
Tripe	31.89±0.51^{bB}	36.62±0.17^{aA}	27.96±0.66^{cC}	32.51±0.02^{dB}	14.74±0.40^{aD}	7.07±0.45^{aF}	6.21±0.14^{aF}	10.18±0.04^{abE}
Ezogelin	35.98±0.70^{aA}	29.98±0.28^{cC}	30.56±0.25^{cC}	32.84±0.27^{dB}	16.04±0.70^{aD}	6.06±0.02^{bF}	6.73±0.17^{abF}	7.78±0.74^{cE}
Yoghurt with Rice	18.69±2.36^{cBC}	23.61±2.25^{dB}	30.02±2.02^{cA}	33.70±2.62^{cdA}	17.17±2.67^{aC}	6.32±0.09^{bD}	6.07±0.92^{abD}	9.00±0.29^{bcD}
Cream of Chicken	21.48±0.15^{cB}	34.51±2.21^{abA}	35.85±0.19^{bA}	36.51±0.27^{bcA}	17.28±0.48^{aC}	6.58±0.21^{abE}	5.02±0.05^{bE}	10.61±0.47^{aD}
Tarhana	12.30±0.75^{dE}	31.17±0.03^{bcC}	39.09±1.25^{aB}	43.43±0.25^{aA}	16.21±0.24^{aD}	4.93±0.22^{cF}	5.99±0.04^{abF}	5.86±0.81^{dF}
Tomato	14.76±0.18^{dD}	30.57±0.20^{cC}	34.77±0.56^{bB}	37.57±0.21^{bA}	14.95±0.17^{aD}	6.30±0.15^{bF}	6.25±0.13^{abF}	10.14±0.05^{abE}
Average	22.52±1.52	31.08±0.85	33.04±0.76	36.09±0.76	16.06±0.47	6.20±0.14	6.04±0.17	8.93±0.34

a,b Means within the same column with different superscript letters are statistically different ($p<0.05$; n=6)

A,B Means within the same line with different superscript letters are statistically different ($p<0.05$; n=6)

Considering the overall data, antibiotic samples showed higher inhibitory effect against the S.aureus than propolis (Figure 1). The Zone diameter obtained with propolis were calculated with respect to the zone diameters obtained from commercial antibiotics considered as 100%. The efficacy of propolis from Kirklareli and Corlu was 30%, while the efficacy of propolis from Ordu was 40% of Tetracycline. Similarly, the efficacy of propolis from Corlu and Kirklareli was 20% and that from Ordu was 30% of the efficacy of Cefixime, Amoxicillin and Ampicillin. Additionally, the efficacy of propolis from Ordu was 55% of the efficacy of Streptomycin. Thus, the bactericidal efficiency of the propolis samples from Corlu and Kirklareli were similar but less than that of the propolis supplied from Ordu. However, bactericidal effects of the all propolis samples are close to antibiotic effect of Streptomycine which is an inhibitor of bacterial protein synthesis.



(T : Tetracycline, CMF : Cefixime, AMC : Amoxicillin, AMP : Ampicillin, S : Streptomycin)

Figure 1. The inhibitory effect of propolis samples in comparison to commercial antibiotics

Table 4. EUCAST clinical breakpoints against *Staphylococcus* spp.

	Resistant	Intermediate Sensitivity	Sensitive	Effector Mechanism
Tetracycline (10µg)	<19	19-22	>22	Inhibition of protein synthesis
Cefixime (5µg)	<24	24-29	>29	Inhibition of protein synthesis
Amoxicillin (10µg)	<19	19-22	>22	Disruption of the cell membrane

The zone diameter obtained with Tetracycline, Cefixime and Amoxicillin after incubation for 7 days was evaluated with EUCAST standards (Table 4) (Anonymous, 2014). EUCAST standards for Ampicillin and Streptomycin against Staphylococcus spp. have not been established yet and therefore were not evaluated. *S. aureus* isolated from instant soups sold in the Turkish market showed the highest sensitivity to the antibiotics Amoxicillin (100%) and Cefixime (83.3%). The least sensitivity was observed with Tetracycline (41.7%), and 5 out of 12 samples of *S. aureus* isolated were determined to be resistant to this antibiotic. Only one sample was found to be resistant to Cefixime.

In the light of the findings obtained in the current study, propolis may restrict the growth of *S. aureus* that commonly exists in instant soups. In the sensory evaluation study, 1ml/L of propolis was added to the soups and evaluated for flavor, color and odor with respect to the control. The panelists reported little difference in terms of color and odor of the soups. However, 2.27/5 point difference was determined between the samples in terms of the flavor (Figure 2).

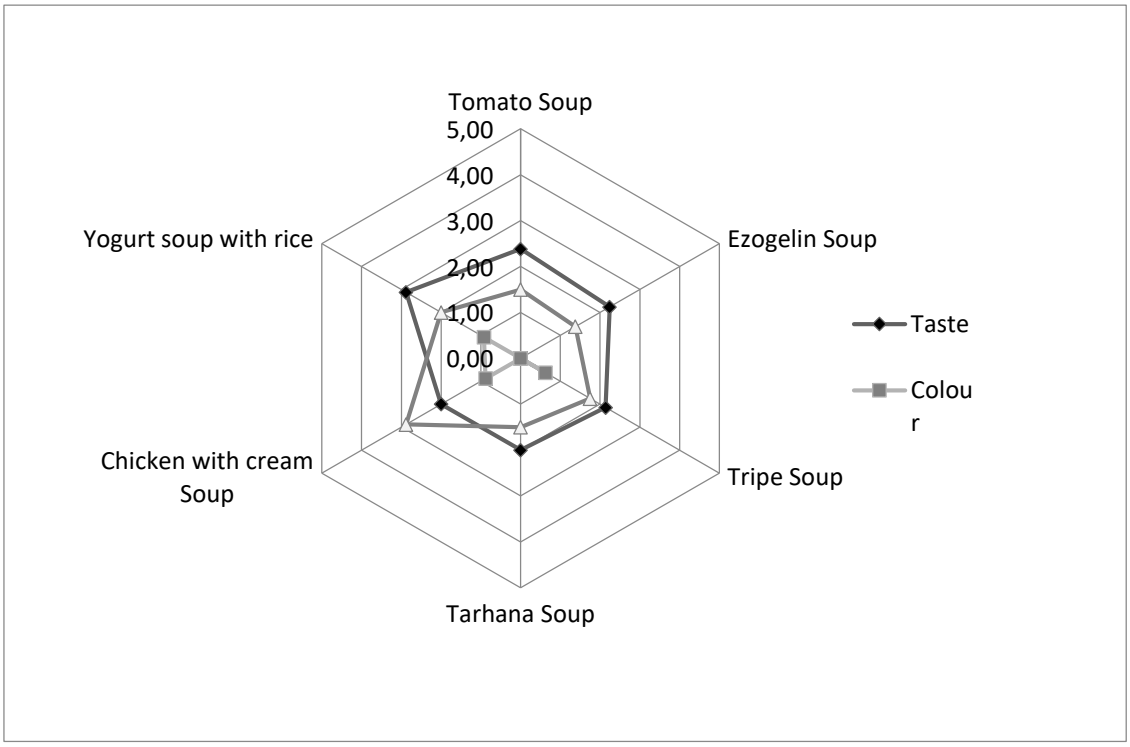


Figure 2. Sensory Evaluation of the supplementation of Propolis Extracts in Instant Soup

Therefore, the addition for propolis as a bactericidal agent in instant soups may be suggested. Propolis is a versatile natural compound that is known to act as an anticarcinogenic, antioxidant, antibacterial, and antifungal agent (Seven et al., 2007). Therefore its supplementation into easily perishable foods may bring about effects that go beyond its well established bactericidal effect.

Conclusion

Varying levels of contamination with *S. aureus* were detected in all instant soups samples (tarhana, tripe, yoghurt with rice, cream of chicken, ezogelin and tomato) analyzed in this study. *S. aureus* contamination in foods is indicative of inadequate sanitation and entails a high probability of generation of enterotoxins.

The inhibitory effects of commercial antibiotics and propolis samples obtained from different geographical locations in Turkey against *S. aureus* isolated from instant soups were compared. The propolis samples showed varying levels of antimicrobial effect on *S. aureus* which may be dependent on the flora of the regions they were obtained from. The bactericidal effect of the propolis obtained from Ordu was closest to the values obtained with commercial antibiotics. The sample from Ordu was also superior in terms of

bactericidal efficacy in comparison to the samples obtained from Corlu and Kirklareli, which are geographically closer to each other. Evaluation of instant soups supplemented with propolis showed favorable results in a sensory evaluation panel and may be suggested as an additive in instant soups prone to bacterial contamination.

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