GIDA (2008) 33 (3) : 123-127 *Research Article (Araştırma Makalesi)*

ANTIMICROBIAL ACTIVITY OF PINE BARK EXTRACT AND ASSESSMENT OF POTENTIAL APPLICATION IN COOKED RED MEAT

ÇAM KABUĞU EKSTRESİNİN ANTİMİKROBİYAL AKTİVİTESİ VE PİŞMİŞ ETDEKİ UYGULAMASININ DEĞERLENDİRİLMESİ

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ABSTRACT: The present study was conducted to evaluate the antimicrobial activities of the pine bark extract *(Pinus pinea)* which grows at Western parts of Turkey (Çine, Aydın) and Pycnogenol® in cooked red meat against *Staphylococcus aureus.* For this purpose, *P. pinea* and Pycnogenol® were added at 1% concentration to raw meat. *S. aureus* was inoculated at 10^3 cfu g^{-1} concentration to cooked meat. After that, meat was stored at 4˚C for 9 days. *P. pinea* reduced the numbers of *S. aureus* detected, which were $7.9x10^2$ cfu/g after 6 days and $7.1x10^2$ cfu/g after 9 days; whereas, the values for Pycnogenol® were $8.6x10^2$ cfu/g and $9.8x10^2$ cfu/g, respectively. On the other hand, values of $13.7x10^2$, $17.2x10^2$ cfu/g were obtained for the control after 6 and 9 days of storage. Therefore, *P. pinea* and Pycnogenol® tested on cooked red meat reduced the numbers of *S. aureus* during storage when compared with the control. Results presented here may suggest that the use of pine bark extracts may provide protection against *S. aureus*, and thus, they present a potential to be used as a natural preservative in food industry.
Keywords: *Pinus pinea*, Pycnogenol®, *Staphylococcus aureus*, antimicrobial activity, meat application

ÖZET: Bu çalışmada, *Pinus pinea*'nın kabuk ekstresinin ve ticari bir çam kabuğu ekstresi olan Pycnogenol®'ün pişmiş kırmızı etteki *Staphylococcus aureus*'a karşı antimikrobiyal etkileri araştırılmıştır. Bu amaçla, *P. pinea* ve Pycnogenol® ete %1 oranında ilave edilmiştir. *S. aureus* ise pişmiş ete 10^3 kob/g olacak şekilde inokule edilerek 4˚C'de 9 gün depolanmıştır. *P. pinea*, *S. aureus* sayısını 6. günden sonra $7.9x10^2$ kob/g ve 9. günden sonra $7.1x10^2$ kob/g'a düşürürken, Pycnogenol® için elde edilen değerler $8.6x10^2$ kob/g ve $9.8x10^2$ kob/g'dır. Diğer yandan, kontrol için 6. ve 9. günlerden sonra elde edilen değerler $13.7x10^2$, $17.2x10^2$ kob/g'dır. Dolayısıyla, *P. pinea* ve Pycnogenol®'ün, kontrolle kıyaslandığında 6. ve 9. günlerde *S. aureus* sayısını düşürdüğü tespit edilmiştir. Sonuç olarak, çam kabuğu ekstresinin *S. aureus*'a karşı koruyucu etkisi olduğu ve gıdalarda doğal koruyucu olarak kullanım potansiyeline sahip olduğu belirlenmiştir.

Anahtar kelimeler: *Pinus pinea*, Pycnogenol®, *Staphylococcus aureus*, antimikrobiyal aktivite, et uygulaması

INTRODUCTION

Recently, there has been an increasing interest in discovering new natural antimicrobial agents. Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial properties to serve as antimicrobial agents against food borne pathogens (1, 2), and have been preferably employed in foods because of their potential health benefits. Furthermore, there is an increasing concern from consumers' side over the safety of foods containing synthetic chemicals (3) and this has recently put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning microbial safety (4).

Pine bark extracts are phenolic compounds containing catechin, epicatechion, taxifolin, phenolic acids (5), and have received considerable attention because of their antimutagenic, anticarcinogenic, and high antioxidant

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activity. The most common commercially available pine bark extract is Pycnogenol®, a standardized extract of French maritime pine bark that contains naturally occurring chemicals called proanthocyanidins, as well as other compounds. Pycnogenol® and pine bark extracts are used as nutritional supplements and phytochemicals for various diseases (6).

Studies on the antimicrobial activity of different plant extracts have revealed that most of them possess broad-spectrum antimicrobial activities against both Gram-negative and Gram-positive food borne pathogens (7, 8); thus enabling them to be regarded as 'natural' alternatives to chemical preservatives (8, 9) for the food industry. Food borne diseases are very common worldwide and sometimes cause life-threatening problems for millions of people around the world. *S. aureus* is one of the most common causes of food borne diseases and foods that are susceptible to staphylococcal food poisoning include meat and meat products, poultry and egg products, salads and bakery products. Food poisoning caused by *S. aureus* is estimated to be around 185,060 in USA each year (10). Staphylococci can cause diseases due to their ability to multiply and spread widely in tissues, and produce many extracellular toxic substances like extoxin, leenkocidin and enterotoxin. Thus, microbiological safety of food products and in particular, meat products has been a major focus because of their susceptibility to contamination during slaughtering, processing and storage (11).

Potential plant extracts investigated by researchers as antimicrobial agents in meat products are mostly from rosemary, grape seed extracts and oregano (3, 8, 12). However, there is little information on the antimicrobial activity of pine bark extracts and very rare studies regarding their utilization in food systems (3).

The aim of this study was to investigate antimicrobial effects of a pine bark extract and Pycnogenol® (extracted from *P. maritima*) on cooked red meat during storage at 4°C.

MATERIALS AND METHODS

Pine Bark Samples

P. pinea bark specimens were collected from Aydın-Çine (N: 37°32' 30.1", E: 28° 08' 35.6", altitude: 520 m) between June and August 2006. The specimens were left overnight to dry at room temperature, ground by using a conventional grinder, and stored at +4°C.

Preparation of Pine Bark Extract

Pine bark extract was obtained by the method developed by Masquelier (1987). A 100 g of pine bark was further ground for 1 min, at a speed setting of 2 using a mixer (Waring, USA) to obtain coarse powder and extracted with 600 ml of boiling water, then cooled down to 20°C. 250 ml of liquid was collected de-watering the solid portion by filtration and sodium chloride was added up to saturation and the precipitate formed was removed by filtration. Subsequently, the filtrate was extracted three times with ethyl acetate (10 ml filtrate/1 ml ethyl acetate (v/v)). The ethyl acetate phase was collected, dried using anhydrous sodium sulphate, and reduced to 1/5 of its volume by rotary vacuum evaporator. The extract was then poured into three volumes of chloroform, while stirring mechanically. The proanthocyadins were precipitated and collected by filtration. The light beige colour powder extract obtained was stored at -20°C. All chemicals used were analytical grade and obtained from Merck.

Microbial Analysis on Cooked Red Meat

Sample Preparation

S. aureus ATCC 6538P was cultivated at 37°C in trypticase soy agar (TSA, Oxoid) for 18 h prior to use. The culture was suspended according to Mc Farland turbidity standards with sterile saline water (0.85% NaCl), and serially diluted to 10^4 cfu/mL for inoculation.

Experimental Design

Freshly slaughtered beef muscles were obtained from a local retailer and transported to the laboratory within 1 h. Excess fat in the meat was combed out with a knife and meat was homogenized for 2 min, at a speed setting of 2 using a mixer (Waring, USA). Mixed red meat divided into 10 g pieces were placed in sterile jars and pine bark extracts from *P. pinea* and commercial pine bark extract (Pycnogenol, from *P. maritima*) were added into meat to make a 1.0% final concentration. Following adequate mixing again, all samples were cooked in water bath until the internal temperature reached to 75°C. The cooked meat samples were cooled down in the refrigerator, inoculated with *S. aureus* at a final concentration of 10^3 cfu/g, and the cooled red meat samples were stored at 4°C. Subsequently, samples were plated and incubated at 37°C for 24 h to determine the populations of bacteria on Tryptic soy agar (TSA) after 0, 3, 6 and 9 days of storage. Parallel tests were performed simultaneously for growth (positive control) and sterility controls. Analyses were conducted in triplicates.

Data Analysis

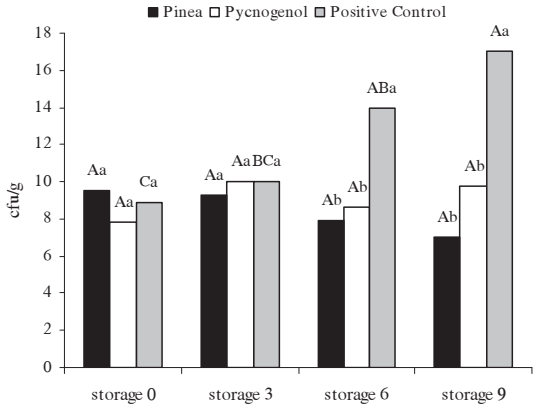
Statistical analysis of the data was carried out with Minitab for Windows (ver. 14.0). Analysis of variance was performed and data were analyzed for treatment effects, storage effects and treatment by storage interactions. When significant treatment, time or interaction effects were observed, Duncan's post hoc test was used for multiple comparisons.

RESULTS AND DISCUSSION

Meat products and beef provide a favourable environment for the growth of *S. aureus*. Bacteria found normally on the meat surface are distributed throughout the entire product during the grinding and mixing processes used in the production of ground beef (14). Naturally occurring preservatives can extend the shelf-life and improve the safety and quality of precooked meat products (3).

Therefore, *P. pinea* and Pycnogenol® were incorporated into cooked red meat at 1.0% levels. Inhibitory effects of the extracts were screened for 9 days and sampling was carried out on days 0, 3, 6 and 9. The values obtained after 0 and day 3 were $9.5x10^2$ and $9.3x10^2$ cfu/g for *P. pinea*, $7.8x10^2$ and $10.6x10^2$ cfu/g for Pycnogenol® and $8.9x10^2$ and $10.4x10^2$ cfu/g for the control. According to the statistical analysis conducted, although there were no significant differences in the number of bacteria on days 0 and 3, after these sampling days both the extract and Pycnogenol® effectively inhibited growth progress of *S. aureus* on meat after days 6 and 9, values being $7.9x10^2$ and $7.1x10^2$ cfu/g for *P. pinea* and $8.6x10^2$ and $9.8x10^2$ cfu/g for Pycnogenol® when compared with the control for which values of $13.7x10^2$ and $17.2x10^2$ cfu/g were noted for the respected storage days ($P < 0.05$). Additionally, no significant differences were observed in the number of bacteria on meat with additions of *P. pinea* and Pycnogenol® during 9 days of refrigerated storage. However, constant increase was observed with the sample of control as can be seen in Figure 1. These findings indicate that the antimicrobial activity of the extract against *S. aureus* is similar to that of the commercial product.

Although, the antimicrobial activity of pine bark extracts and essential oils of pine needles were studied by some researchers (3, 9, 16), application of pine bark extracts against a number of food spoilage and pathogenic microorganisms in meat is not documented enough. One study carried by Ahn et al (2007) regarding the antimicrobial activity of Pycnogenol® was the treatment of cooked beef with 1% Pycnogenol® and effective inhibition of the growth of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Aeromonas hydrophila* after 9 days of storage were reported. It was also presented by Shelef et al (1994) that antimicrobial effect of plant extracts increased with increasing solubility in certain foods. As pine bark extracts were



^{a,b,c,d}Bars within the same day not sharing common letter are different ($P < 0.05$).
^{A,B,C}Bars not sharing common letter are different for storage period ($P < 0.05$).

Figure 1. Inhibitory action of P. pinea and Pycnogenol® on cooked red meat.

totally soluble in cooked red meat, application of these extracts in meat products would prove to be advantageous. In conclusion, the experimental assay showed a significant effect of *P. pinea* against *S. aureus* in terms of inhibiting the progress of bacterial growth. Therefore, this could be an important potential source of new natural antimicrobial compound used as a food preserving agent especially against *S. aureus*.

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