PROPOLIS ETHANOLIC EXTRACT AGAINST *S. AUREUS* GROWTH IN BEEF PATTIES

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I. INTRODUCTION

Staphylococcus aureus is considered the second causative organism responsible for food intoxications, and it has isolated from several foods, such as milk and dairy products, salads, meat and meat products. The presence of this bacteria or its enterotoxins in processed food is an indication of poor sanitation [1]. In recent years, it has evaluated by different researchers the application of natural antimicrobial agents such as extracts obtained from vegetables, plants, honeybee products to extend the shelf life of meat and meat products. Propolis is a resinous and complex apicultural product (CAS 9009-62-5), which possesses high levels of bioactive compounds which exhibit some biological properties such as antifungal, anticancer, antitumor, antiradical, antidiabetic, antihypertensive and antimicrobial [2,3]. The present work was conducted to determine the antimicrobial effect of the propolis ethanolic extract (PEE) against *S. aureus* growth in beef patties.

II. MATERIALS AND METHODS

Commercial propolis ethanolic extract (PEE1) obtained from a local market except for the non-commercial propolis (PEE2), which was prepared by maceration method. The total phenolic content (TPC) for all the PPE determined by the Folin-Ciocalteu method. The inhibitory effect of PEE against gram foodborne pathogens such as *Staphylococcus aureus* and *Escherichia coli* was evaluated using the agar well diffusion test [3]. Briefly, to assess the effect of PEE against *S. aureus* growth in a meat product, minced meat 48 h *postmortem* was homogenized with 1.5% of salt (w/w) and 10% of fat (w/w) in final formulation [2], and inoculated with *S. aureus* 1.0 McFarland. The meat divided into three different treatments: control (without extract), PEE1 2% (commercial propolis) and PEE2 2% (non-commercial propolis). All samples were storage (2 °C, under darkness, 16 days) and each sampling day two packs were opened for subsequently pH and *S. aureus* evaluation. The results reported as a mean ± standard deviation. Data submitted to ANOVA, as well as a Tukey-Kramer comparison test ($P<0.05$).

III. RESULTS AND DISCUSSION

Ethanolic extracts obtained from propolis have used as part of traditional natural medicine. The major components that make propolis extracts biologically active include terpenes and polyphenols (phenolic acids and flavonoids) [4]. Among the extracts, PEE2 had the highest TPC ($P<0.05$), >84.9% when compared with PEE1. The Mexican normative indicates that the presence of phenolic compounds is a quality index of the production and processing of propolis [5]. The results showed that no samples showed an antimicrobial effect on *E. coli* ($P>0.05$), while the order of *S. aureus* inhibition of propolis extracts was PEE2 > PEE1 ($P<0.05$). These results agree with Kalogeropoulos et al. [3] who found a positive antimicrobial effect against Gram-positive than Gram-negative bacteria.

The growth of pathogenic microorganisms is considered one of the most critical challenges facing the meat industry, due to this could lead to a series of problems in human health, as well as economic losses for producers [1,2]. In this work, the results samples showed that at day 0 PEE2 (12.1%) > PEE1 (4.8%) reduced the *S. aureus* population in meat samples when compared with the control group ($P<0.05$). While at 16 days of storage, it observed that the PEE2 addition reduced *S. aureus* population significantly by 1.0 log CFU/g

(20.2% of inhibition). The results are in agreement with Casquete et al. [4], who found that PEE reduced the pathogen counts in meat products.

Table 1 Total phenolic content and inhibition zone diameters of EEP against tested organisms.

Treatments	TPC (mg GAE/g)	Inhibition zone diameters (mm)	
		S. aureus	*E. coli*
Control	-	-	-
PEE1	8.6±1.2^{a}	7.3±0.6^{a}	-
PEE2	56.8±2.0^{b}	22.0±0.1^{b}	-

Ethanol was used as control; PEE1, commercial propolis 1; PEE2, non-commercial propolis.
Different literal (a-b) indicate significant differences between treatments (P<0.05).

Table 2 Effect of storage time and EEP addition on pH and *S. aureus* counts of beef patties.

Item	Day	Treatments		
		Control	EEP1	EEP2
pH values	0	5.8±0.02^{aB}	5.7±0.03^{aB}	5.7±0.01^{aB}
	16	5.1±0.01^{aA}	5.4±0.02^{bA}	5.4±0.02^{bA}
S. aureus counts (Log_{10} CFU/g)	0	5.6±0.03^{cA}	5.5±0.13^{bB}	5.0±0.11^{aB}
	16	5.4±0.25^{cA}	4.9±0.03^{bA}	4.3±0.03^{aA}

EEP1, commercial propolis 1; P2, noncommercial propolis.
Different literal (A-B) and (a-c) indicate significant differences between treatments (*P*<0.05).

IV. CONCLUSION

In this work, the results indicate that propolis had adequate content of polyphenolic compounds and reduced *S. aureus* growth in beef patties, thereby extending meat sample shelf life. Propolis should use as an alternative to commercially available antimicrobials.

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