PROPOLIS EXTRACT AS AN OXIDATIVE STABILIZER OF RAW BEEF AND PORK PATTIES DURING CHILLED STORAGE

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Abstract – The present study was undertaken to examine the utilization of propolis extract (PE, 2%) as a source of natural antioxidants to reduce lipid (Lox) and protein oxidation (Pox) and color deterioration, acting as an oxidative stabilizer in beef and pork patties during chilled storage (2°C/9 days/under darkness). Propolis extract was characterized by its *in vitro* antioxidant activity (AOX). Meat samples were evaluated for Lox (thiobarbituric acid reactive substances, TBARS), Pox (carbonyls), color (a*), metmyoglobin formation (MetMb %), total phenolic content (TPC) and free-radical scavenging activity (DPPH%). Results indicated that PE is rich in TPC, and its incorporation in bovine and porcine patties significantly reduced (P<0.05) Lox and Pox (80 and 88.7 % inhibition; 47.3 and 30.6 % inhibition, respectively), as well as color loss, thereby increasing the oxidative stability of patties during chilled storage.

Key Words – Lipid oxidation, Protein oxidation, Natural antioxidants.

I. INTRODUCTION

Beef and pork meat is a major source of high quality dietary nutrients for human metabolic processes, providing fat (polyunsaturated fatty acids) and protein content (essential amino acids). However, oxidative degradation of polyunsaturated fatty acids (Lox) and proteins (Pox) may result in negative physiological effects on the texture, color and flavor of meat, including a loss of quality [1]. With regards to Pox, the nature of the oxidation by-products formed is highly dependent on the amino acids involved and how the oxidation process initiated, potentially decreasing the quality of meat for consumption by reducing tenderness and juiciness and causing flavor deterioration and discoloration, which all affect meat quality and ultimately consumer acceptance of meat [2].

Although synthetic antioxidants have been widely used in the meat industry to inhibit the processes of both Lox and Pox, the use of synthetic antioxidants has been also shown to have potential health risks. As a consequence, strict regulations have been promoted for their use in foods [3]. Natural antioxidants, such as honey, royal jelly and propolis have been shown to decrease oxidation as effectively as synthetic antioxidants. Therefore, replacing synthetic antioxidants with natural ingredients can be both effective and beneficial to health [4].

Propolis is a substance with a complex composition and viscous consistency, which bees make from the resinous material obtained from different plants. Many studies have demonstrated the excellent biological properties of PE, containing antimicrobial, antifungal, anticancer and antioxidant characteristics, among others, which is associated with its phenolic composition and ability to capture free radicals [5]. Nevertheless, the effect of PE as an oxidative stabilizer of raw beef and pork meat was previously unknown.

The objective of this work was to determine the effectiveness of propolis as an inhibitor of Lox, Pox and color changes in beef and pork meat subjected to chilled storage.

II. MATERIALS AND METHODS

Propolis samples used in this work were collected in an apiary, located in Ures, Sonora, Mexico (29.1476 N, -110.1239 O; 632 m). Phenolic

compounds were extracted with ethanol at room temperature (25 °C), concentrated under reduced pressure and lyophilized. Afterwards, AOX content of PE was assessed by the determination of total phenolic content (TPC) using the Folin-Ciocalteu method and a DPPH radical scavenging assay [6]. The chemical composition of PE was determined with a HPLC-DAD system [7]. The beef and pork patties were composed of meat obtained from a local processor (18 h *postmortem*) and homogenized with 1.5% salt (NaCl, w/w) and 10% fat (w/w, in final formulation). Polystyrene trays containing the patties were wrapped with polyvinyl chloride film (17,400 cm^3 O_2/m^2/24 h at 23 °C). For each replication (two), beef and pork patties were assessed with four treatments: 1) B (negative control, beef patties without additives); 2) B+PE (beef patties with 2% w/w propolis extract); 3) P (negative control, pork patties without additives); 4) P+PE (pork patties with 2% w/w propolis extract) [7]. The patties were subjected to refrigerated storage at 2 °C in the dark for 0, 3, 6 and 9 d, and 2 packs were opened for subsequent analysis of the following characteristics: TBARS, carbonyls, index a*, TPC, and DPPH (%). Data were processed using the SPSS21 statistical package by ANOVA analysis followed by a Tukey post-hoc test, and a principal component analysis (PCA) was applied in order to study the correlation between all the variables ($P<0.05$).

III. RESULTS AND DISCUSSION

Phenolic compounds are one of the major groups found in plants and bee products and have been reported to possess antioxidant properties [6]. Propolis extract (PE) was tested *in vitro* to determine its AOX by assessing TPC and DPPH. Results showed that PE presented high TPC (475.8 mg GAE/g extract) and DPPH (69.1%) at 500 µg/ml. Significant correlations were obtained between TPC and DPPH activity ($R^2= 0.997$). Our results indicated that PE is rich in phenolic compounds; previous research on the AOX of PE has suggested that its biological properties could be due to the presence of diverse phytochemicals, including phenolic compounds as flavonoids, phenolic acids and their esters [5].

Phenolic compounds identified in PE are shown in Table 1. Results indicated that the predominant flavonoids identified were pinocembrin (130.7±1.8 mg/g), naringenin (50.2±5.9 mg/g) and galangin (37.0±2.1 mg/g), while cinnamic and p-coumaric acid (<3 mg/g) were the most representative phenolic acids ($P<0.05$). These compounds could be correlated with the AOX of PE. The incorporation of natural extracts, rich in phenolic compounds, to meat and meat products is an important, novel strategy for the development of healthier meat products. In this regard, several studies utilizing fruits, spices and vegetable extracts have also shown that the addition of such extracts to raw and cooked meat products decreases Lox and Pox, improving color stability and acting overall as antioxidant agents [8]. Therefore, in this study the efficacy of PE as an ingredient to inhibit Lox, Pox and MetMb formation and to prevent color degradation and improve the total antioxidant capacity in beef and pork patties was successfully evaluated (Table 2). Lox and Pox increased over the course of storage time, although after 9 days of storage, TBARS and carbonyl production were significantly reduced in beef (B+PE) and pork (P+PE) patties treated with PE (80 and 88.7% inhibition; 47.3 and 30.6% inhibition, respectively) in comparison with the control samples ($P<0.05$). All treatments demonstrated TBARS values of less 1 mg MDA/kg per sample and carbonyl values of less 2 nM carbonyl/mg protein, which indicated that patties did not exhibit rancid flavor [2,9]. A positive correlation was found between Pox and Lox in meat samples in the present study ($r^2= 0.829$), indicating that Pox and Lox are accompanying processes [2].

In meat and meat products, color influences its acceptability and plays a great role in the decision of the purchaser [10]. Over the course of a storage time, a steady decrease in red color (a*) occurred ($P<0.05$). At day 9, B+PE and P+PE showed slight decrease of a* (15 and 15.7 %, respectively), indicating a slight loss of red color. The observed discoloration in the surface of raw patties may due to metmyoglobin formation (MetMb%), since in fresh meat Mb is commonly found in three forms (oxymyoglobin, oxy(Fe^{2+})Mb; deoxymioglobin, deoxy(Fe^{2+})Mb; and metmyoglobin, met(Fe^{3+})Mb) and is associated with Lox by-products [4]. B+PE and P+PE showed the lowest values of MetMb (%) over the course of the storage time,

demonstrating values less than the unacceptable rate of 40 %. These results confirmed that patties treated with PE, rich in phenolic compounds, experienced a reduction in Lox, Pox and color changes over the course of 9 days of storage when compared with the control samples. Commonly, *in vitro* AOX of meat extracts has been investigated by measuring lipid and protein stability [1,2]. In this context, during the present study the AOX of raw patties was determined in terms of TPC and DPPH, measured over the course of the refrigerated storage time of beef and pork patties. A significant decrease ($P<0.05$) in TPC and DPPH was observed. At day 9, B+PE and P+PE showed the highest values of TPC and DPPH (75.18 and 67.34 mg GAE/ g extract; 44.65 and 47.12 %, respectively). Meat and meat products are generally not considered as a dietary source of antioxidants [11]. However, this study demonstrated a significantly TPC and DPPH content in meat samples (>50 mg GAE/g and >20%, respectively), and PE incorporation increased both parameters ($P<0.05$).

In general, the addition of PE reduced Lox, Pox, MetMb formation and color changes, which can be associated with phenolic compounds and lipid and protein interaction mechanisms mainly related to the hydrophobicity of the aromatic nuclei of polyphenols and the availability of multiple phenolic hydroxyls that allow H-bonding.

Table 1 Lox, Pox, color and AOX levels in beef and pork patties under chilled storage.

#	Compound	Rt	PE (mg/g)
1	Gallic acid	1.9	(+)
2	Cinnamic acid	3.4	2.10
3	p-coumaric acid	7.8	2.90
4	Ferulic acid	8.7	(-)
5	Naringenin	27	50.20
6	Quercetin	31	6.50
7	Luteolin	36	3.70
8	Kaempferol	37	0.90
9	Apigenin	41	4.4 0
10	Pinocembrin	45	130.70
11	Pinobanksin	46	(+)
12	CAPE	49	(+)
13	Chrysin	51	12.30
14	Galangin	52	37.00
15	Acacetin	57	8.4 0
16	Pinostrobin	63	(+)

Rt.: retention time. (+) compound identified but not quantified. (-), compound not identified.

Table 2 Lox, Pox, MetMb, color, and AOX levels in beef and pork patties under chilled storage.

Analysis	d	B	B+PE	P	P+PE
TBARS	0	0.14^{cA}	0.09^{aA}	0.09^{aA}	0.07^{aA}
	3	0.20^{bB}	0.09^{aA}	0.43^{cB}	0.10^{aB}
	6	0.34^{bC}	0.11^{aB}	0.99^{cC}	0.11^{aB}
	9	1.04^{cD}	0.12^{aB}	1.05^{cD}	0.21^{bC}
Carbonyls	0	0.25^{dA}	0.17^{bA}	0.20^{cA}	0.10^{aA}
	3	0.90^{dB}	0.28^{bB}	0.50^{cB}	0.19^{aB}
	6	1.62^{dC}	0.93^{bC}	1.44^{cC}	0.67^{aC}
	9	2.01^{dD}	1.06^{aD}	1.88^{cD}	1.31^{bD}
MetMb	0	13.34^{cA}	9.68^{bA}	0.00^{aA}	0.00^{aA}
	3	25.17^{dB}	17.45^{cB}	6.75^{bB}	2.65^{aB}
	6	49.48^{dC}	17.58^{bB}	32.97^{cC}	16.67^{aC}
	9	85.80^{dD}	43.50^{bC}	81.84^{cD}	34.31^{aD}
Index a*	0	23.20^{bA}	22.10^{bA}	20.90^{aA}	20.40^{aA}
	3	20.90^{bB}	18.40^{aB}	22.00^{cA}	17.40^{aB}
	6	11.30^{aC}	16.00^{cC}	16.60^{cB}	15.80^{bC}
	9	9.20^{aD}	15.00^{cC}	11.60^{bC}	15.70^{cC}
TPC	0	49.74^{aA}	166.53^{cA}	53.43^{bA}	170.55^{dA}
	3	42.64^{aB}	113.46^{dB}	52.73^{bA}	90.38^{cB}
	6	38.53^{aC}	79.79^{cC}	47.69^{bB}	90.15^{dC}
	9	33.26^{aD}	75.18^{dD}	35.75^{bC}	67.34^{cD}
DPPH	0	18.86^{aA}	93.00^{cA}	20.28^{bA}	98.79^{dA}
	3	20.08^{aA}	91.98^{bB}	20.20^{aA}	93.66^{bB}
	6	18.74^{aA}	50.05^{bC}	18.05^{aB}	49.19^{bC}
	9	0.00^{aB}	44.65^{bD}	0.00^{aC}	47.12^{cD}

d, day of sampling; B, beef; B+PE, beef+propolis extract; P, pork: P+PE, pork+propolis extract. Different superscripts (a-d) within the same sampling day and (A-B) through storage time differ significantly ($P<0.05$).

IV. CONCLUSION

In this study, the current findings demonstrated that the application of PE as an antioxidant in raw beef and pork patties stored at 2 °C without illumination can effectively be used to reduce Lox and Pox, as well as color changes that may occur during chilled storage. This study has demonstrated the great potential of PE as a preservative for fresh meat and meat products during chilling storage.

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