ANTIOXIDANT CAPACITY OF MESQUITE (*Prosopis velutina*) LEAF EXTRACT FOR QUALITY PRESERVATION OF RAW PORK PATTIES

Margarita I. Ramírez-Rojo [1], Livier Zavala-Cárdenas[1], Rey D. Vargas-Sánchez[1], Gastón R. Torrescano-Urrutia[1] and Sánchez-Escalante Armida

[1]Centro de Investigación en Alimentación y Desarrollo, A.C. Laboratory of Meat and Meat Products Research, Carretera a La Victoria km 0.6, 83304 Hermosillo Sonora, México.

Abstract – Mesquite leaf extracts (MLE) (*Prosopis velutina*) were performed and their antioxidant *in vitro* activity determined by measuring total phenolic content (TPC), total flavonoids (TF), radical scavenging activity of DPPH (DPPH), antiradical activity ABTS$^+$ (ABTS) and ferric reducing ability power (FRAP). The objective of this study was to obtain extracts with antioxidant activity and to use these to preserve the quality of raw pork patties during their refrigerated shelf life. For assessment of raw pork patties, the following were considered as treatments: control (no addition), vitamin C (VC) (0.02%), butylated hydroxytoluene (BHT) (0.02% fat basis) and 0.05 and 0.1 % MLE. For the evaluation of oxidative conditions, conjugated dienes (CD), thiobarbituric acid reactive substances (TBARS) and percentage of metmyoglobin formation (Met%) were considered during the refrigerated storage (2 ^{0}C) of meat for 10 days. Results obtained for the antioxidant capacity of MLE measured TPC (282.90 GAE/g), TF (110.71 RE/g), DPPH (87.10% at 100 µg/ml), ABTS (118.7 mg TE/g) and FRAP (0.95 OD) values. For the assessment of the stability of meat oxidation, results showed that CD, TBARS and Met% values were lower for treatments with 0.05 and 0.1% MLE than those of other antioxidants. We conclude that the MLE exerted an antioxidant effect on raw pork patties during refrigerated storage.

Key Words – Antioxidant activity, mesquite leaf extract, quality pork patties.

I. INTRODUCTION

The loss of meat quality in meat products is mainly due to lipid oxidation and the presence of substances that create unpleasant odor, appearance and taste for humans [1]. To prevent this, synthetic food additives such as BHA and BHT are commonly used, although these are falling into disuse due to potential adverse effects on consumer health [2]. The search for new alternatives leads us to the use of naturally occurring additives in order to preserve meat, such as phenolic compounds from plants [3]. Such are present in mesquite leaves and have demonstrated antioxidant activity [4]. In the state of Sonora, Mexico, there is abundance of mesquite (*Prosopis velutina*), a plant that has long been used as a source of medicinal remedies, including the use of the seed as a food source [5]. The objective of this study was to obtain extracts from mesquite leaves (*Prosopis velutina*) in order to attain phenolic compounds with antioxidant activity, thereby assessing their effect on the oxidation of pork patties, which leads to a deterioration of quality. During the evaluation, mesquite extract was applied to refrigerated pork patties that were assessed over the course of a period of 10 days.

II. MATERIALS AND METHODS

Mesquite (*Prosopis velutina*) leaves were collected in Rancho Viejo, Ures, Sonora, Mexico (29.1476 N, -110.1239 O; 632 m), then rinsed and dried at ambient temperature in the shade, and finally ground to obtain a mesquite leaf powder. Mesquite leaf extract was made by mixing the mesquite leaf powder with solvent (ethanol) in a 1:20 ratio, afterwards sonicated for 30 minutes at 42 Hz and at a controlled temperature (25 °C). Subsequently, the extracts were centrifuged at 4200xg for 10 minutes to obtain the supernatant, followed by a second extraction period. The supernatants were dried in a rotary evaporator in order to be lyophilized, and the yield of extract was obtained. In order to characterize the antioxidant activity, the mesquite leaf extract (MLE) was assessed by measuring total phenolic content (TPC) [6], total flavonoids (TF) [7], ferric reducing ability power (FRAP) [7], scavenging radical activity to DPPH (DPPH) [2] and radical inhibition ABTS$^+$

(ABTS) [2]. Subsequently, the addition of MLE in raw pork patties was carried out at 0.05 and 0.1%, in addition to treatment with vitamin C (VC) (0.02%), butylated hydroxytoluene (BHT) (0.02% fat basis) and the inclusion of a control group (no addition). Changes were measured in the oxidative stability of pork patties over the course of 10 days of refrigeration (2 °C), and during storage, the following parameters were considered: CD [8], TBARS [9] and Met% [10]. The results were statistically analyzed by ANOVA, considering Tukey-Kramer comparisons and a confidence level of $P<0.05$, using the statistical software NCSS 2007.

III. RESULTS AND DISCUSSION

The results showed a yield of 6.49±2.16 % MLE from the mesquite leaf (*Prosopis velutina*) powder. This corresponds with the results of García-Andrade *et al*. [4], whom obtained a 3.62 % yield of crude extract from *Prosopis laevigata* leaves. The total phenolic content of the *Prosopis laevigata* leaf extract was 103.56±4.74 GAE/g, lower than the total phenol content of MLE (278.48±8.52 GAE/g, Table 1). Thus, a high content of TF (226.82±8.27 RE/ g), the largest group of the phenolic compounds [11], was found in MLE. The inhibition percentages of radical DPPH were 85.34±0.31, 74.76±3.88 and 68.88±6.63, at 100, 50 and 25 µg/ml, respectively, which are higher when compared to the results for the methanol extracts of *Prosopis cineraria* (60.48 %) and *Prosopis juliflora* (47.82 %) at 198 µg/ml [12]. The inhibitory activity of $ABTS^{+}$ (108.43±6.30 TE/g) and reducing power (FRAP) (1.10±0.11 OD) showed that the MLE has antioxidant potential as a hydrogen atom or electron donor.

Regarding the application of MLE to a meat product, is important to note that the formulation of raw pork patties includes 10% fat and 1.5% salt, which are ingredients that promote lipid autoxidation. The results of the application of VC, BHT and MLE (0.05% and 0.1%) showed different results in comparison to the control treatment, with statistically lower values over the course of storage on the concentration of conjugated dienes (CD) (Table 2) for both the BHT and MLE 0.1% treatments: 0.243±0.001 and 0.255±0.000 µm/mg, respectively. The formation of conjugated dienes is a by-product of the oxidation reactions of lipids in raw pork patties, mainly due to the presence of a considerable amount of unsaturated fatty acids and trace metals. A study conducted in raw ground pork with the application of onion peel extract (0.05 %) showed similar results, with CD formation at 0.46±0.006 µm/mg, with higher values at 0.54±0.003 µm/mg found for VC (0.05 %) treatment, as measured on the initial day of storage [13].

Since the formation of secondary lipid oxidation compounds leads to the presence of unacceptable odors and flavors in meat, TBARS was also measured. A concentration equal to or greater than 0.5 mg MA/kg denotes the value at which rancidity is perceived by consumers [14]. However, in this study TBARS formation during shelf life was statistically lower for treatments with 0.05 (0.056±0.001 mg MA/kg) and 0.1 % MLE (0.073±0.003 mg MA/kg) on day 10 of refrigerated storage (2 ^{0}C) (Table 3).

Metmyoglobin formation (Met %) in raw pork patties is an indicator of the oxidation of myoglobin, where values greater than 40 % [15] render a brown color and unacceptable appearance for consumers. Statistically significant differences ($P<0.05$) were obtained, with lower values for the 0.1% MLE treatment (38.27 %) (Table 4) on 10 day of refrigerated storage (2 °C).

These aforementioned results can be explained by the presence of phenolic compounds in mesquite leaves (*Prosopis juliflora*), such as apigenin, luteolin, kaempferol, quercetin and isorhamnetin [16], which exert a protective antioxidant effect on the fatty acids present in raw pork patties.

IV. CONCLUSION

Mesquite leaf extract has an antioxidant capacity proven by the radical scavenging power of DPPH and radical cation inhibition of ABTS+. This is likely explained by the considerable amount of total phenolic and flavonoid

compounds present in the MLE. An antioxidant protective effect was observed in raw pork patties at both 0.05 and 0.1 %, leading to TBARS values below the specified limit for rancidity, even on 10th day of refrigerated storage. MLE also prevented the oxidation of myoglobin until the 7th day of storage. Therefore, MLE increased the shelf life of the raw pork patties during refrigerated storage, and we have given an overview of the potential to use of MLE as an antioxidant additive in meat and meat products.

Table 1. MLE antioxidant activity.

Antioxidant activity	**Mean±*SD***
TPC	278.48±8.52[a]
TF	226.82±8.27[b]
DPPH	
100 µg/ml	85.34±0.31[c]
50 µg/ml	74.76±3.88[c]
25 µg/ml	68.88±6.63[c]
$ABTS^{+}$	108.43±6.30 [d]
FRAP	1.10±0.11[e]

a: mg of gallic acid eq. per g of ext., b: mg of routine eq. per g of ext., c: radical scavenging activity of DPPH, d: mg of trolox eq. per g of ext., e: optical density at 700 nm.

Table 2. Conjugated diene concentration (CD) (µm/mg) in raw pork patties stored during 10 days under refrigeration (2 ^{0}C).

Treatment	**Storage day (2 ^{0}C)**			
	0	**3**	**7**	**10**
Control	0.397Cb	0.416BCb	0.449Ba	0.504Aa
SD	*0.002*	*0.035*	*0.015*	*0.005*
Vitamin C	0.418ABa	0.479Aa	0.417ABab	0.362Bb
SD	*0.003*	*0.000*	*0.051*	*0.005*
BHT	0.243Ae	0.172Dd	0.233Bc	0.206Ce
SD	*0.001*	*0.002*	*0.001*	*0.002*
MLE 0.05 %	0.294Bc	0.286Bc	0.356Ab	0.311Bc
SD	*0.001*	*0.001*	*0.046*	*0.002*
MLE 0.1 %	0.255Bd	0.294Bc	0.338Ab	0.261ABd
SD	*0.000*	*0.001*	*0.009*	*0.001*

All values are means and standard deviations (*SD*); Mean values with different superscripts within the same row (A-C) and column (a-d) are significantly different (p<0.05).

Table 3. Thiobarbituric acid reactive substances (TBARS) (MA mg/Kg) formed in raw pork patties during 10 days of refrigerated storage (2 ^{0}C).

Treatment	**Storage day (2 ^{0}C)**			
	0	**3**	**7**	**10**
Control	0.101Da	0.384Ca	0.945Ba	1.003Aa
SD	*0.002*	*0.006*	*0.037*	*0.001*
Vitamin C	0.016Db	0.051Cb	0.316Bb	0.531Ab
SD	*0.007*	*0.004*	*0.009*	*0.008*
BHT	0.021Db	0.041Cc	0.101Bc	0.113Ac
SD	*0.005*	*0.003*	*0.006*	*0.005*
MLE 0.05 %	0.015Db	0.031Cd	0.042Bd	0.056Ae
SD	*0.000*	*0.005*	*0.004*	*0.001*
MLE 0.1 %	0.020Db	0.026Cd	0.050Bd	0.073Ad
SD	*0.003*	*0.007*	*0.011*	*0.003*

mg MA/kg: milligram of malonaldehyde per kilogram of meat. All values are means and standard deviations (*SD*); Mean values with different superscripts within the same row (A-D) and column (a-e) are significantly different (p<0.05).

Table 4. Metmyoglobin formation (Met %) in raw pork patties during 10 days of refrigerated storage (2 ^{0}C).

Treatment	**Storage day (2 ^{0}C)**		
	3	**7**	**10**
Control	0.83Cc	69.40Bb	91.74Aa
SD	*1.12*	*4.46*	*3.62*
Vitamin C	14.77Ca	71.49Ab	60.12Bc
SD	*1.21*	*1.61*	*2.43*
BHT	8.26Cb	79.88Aa	70.30Bb
SD	*2.09*	*2.35*	*2.18*
MLE 0.05 %	6.18Cb	29.04Bc	41.69Ad
SD	*0.52*	*3.37*	*3.79*
MLE 0.1 %	8.11Cb	15.44Bd	38.27Ad
SD	*1.27*	*3.90*	*6.04*

All values are means and standard deviations (*SD*); Mean values with different superscripts within the same row (A-C) and column (a-d) are significantly different (p<0.05).

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