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Preservation of microbiological and sensory quality of sheep meat using pomegranate bark extracts

K. Elfazazi[1,*], L. Chafki[1,2], M. Benbati[1], S. Fakhour[1], A. Haddioui[2] and M. Elhansali[2]

[1]INRA Maroc, CRRA Tadla. Km 19 Route Béni Mellal Marrakech, PB 567, 23040, Beni Mellal (Morocco)
[2]FST Béni Mellal, Science de la vie. Mghilat, PB 523, 40000, Beni Mellal (Morocco)

Abstract. Fresh meat is a sensitive and unstable product over time due to its composition and physicochemical characteristics (pH and aw). The length of storage time depends on temperature, atmospheric oxygen, endogenous enzymes, humidity, light and, above all, the microorganisms initially present. The challenge of preserving meat dates back to antiquity and has now intensified with the industrialization of meat products. Thus, in this work, we studied the conservation of meat by pomegranate bark extract to extend meat storage time, which may be a promising way to promote this coproduct of pomegranate in meat industry. We evaluated the effect of pomegranate bark extract on the growth of microorganisms naturally present in sheep meat. We carried out the microbial germ count by the decimal dilution method. We performed successive dilutions of the sample in sterile physiological water. Then, we evaluated total aerobic mesophilic flora (TAMF) and total coliforms (TC) The treated meat with three concentration of extracts (50-25-0.12 mg /ml) were stored at 4°C. Then, we monitored the physicochemical and microbiological modification during 12 days of conservation. The results show that the microbial kinetics of the strains were significantly affected by pomegranate bark extract especially with high concentration (C1 = 50 mg/ml). A significant increase in UFC (colony-forming unit) over time was observed in the untreated meat. In conclusion, pomegranate bark extract has a preservative effect against microbial alteration of raw sheep meat.

Keywords. Pomegranate bark – Sheep meat – Conservation – Microbial alteration.

Préservation de la qualité microbiologique et sensorielle de la viande de mouton par l'utilisation des extraits d'écorces de grenade

Résumé. *La viande fraîche est un produit sensible et instable dans le temps en raison de sa composition et de ses caractéristiques physico-chimiques (pH et aw). La durée de conservation dépend de la température, de l'oxygène atmosphérique, des enzymes endogènes, de l'humidité, de la lumière et, surtout, des micro-organismes présents au départ. Le défi de la conservation de la viande remonte à l'Antiquité et s'est intensifié avec l'industrialisation des produits carnés. Ainsi, dans ce travail, nous avons étudié la conservation de la viande par l'extrait d'écorce de grenade afin de prolonger la durée de conservation de la viande, ce qui pourrait être un moyen prometteur de promouvoir ce coproduit de grenade dans l'industrie de la viande. L'effet de l'extrait d'écorce de grenade sur la croissance de micro-organismes naturellement présents dans la viande de mouton a été évalué. Les caractéristiques des écorces de grenadier ainsi que la charge microbienne notamment, la flore mésophile aérobie totale (TAMF) et les coliformes totaux (CT) ont été évalués. La viande traitée avec trois concentrations d'extraits (50-25-0,12 mg / ml) a été stockée à 4 ° C. Le suivi des échantillons a été effectué pendant 12 jours de conservation. Les résultats montrent que l'extrait d'écorce de grenade a un effet important sur la cinétique microbienne des souches, en particulier à forte concentration (C1 = 50 mg / ml). Une augmentation significative de l'UFC (unité formant des colonies) au fil du temps est observée en l'absence de l'extrait. En conclusion, l'extrait d'écorce de grenade a un effet retardateur d'altération microbienne de la viande de mouton crue.*

Mots-clés. *Ecorce de grenade – Viande de mouton – Conservation – Altération microbienne.*

I – Introduction

Fresh meat is a highly perishable product due to its biological composition. Many interrelated factors influence the shelf life and freshness of meat such as holding temperature, atmospheric oxygen, endogenous enzymes, moisture, light and most importantly, microorganisms. With the increased demand for high quality, convenience, safety, fresh appearance and an extended shelf life in fresh meat products, alternative non-thermal preservation technologies such as natural biopreservatives are highly demanded.

Furthermore, several works carried out on pomegranate bark has proven its antimicrobial, antioxidant, anti-carcinogenic (Al-Saeed *et al*., 2015), antiseptic and antifungal activities (Kanoun, 2004). However, the exploitation of this antioxidant and antibacterial potential in the agri-food sector remains unknown and very limited. Thus, we have been interested in studying the conservation of meat by pomegranate bark extract to extend meat storage time, which may be a promising way to promote this coproduct in meat industry.

II – Materials and methods

The pomegranate fruit is the Sefri variety. This fruit was collected from the region of Ouled Abdellah of Beni Mellal. The barks were dried in an oven at 60°C for 24 hours. Subsequently, we crushed them with a food grinder. The particle size is less than 0.25 mm.

The preparation of the aqueous extract of pomegranate bark was carried out by the infusion method. Several concentrations were prepared from the powder obtained (50-25-0.12 mg /ml) in boiling water, macerated for 2 hours under stirring, the samples were then filtered (filter porosity: 0.22 µm) and the filtrates stored at 4°C.The chemical characteristics of bark powder extracts: pH, titratable acidity, ash content and soluble solidity rate as well as colour and total polyphenols.

The sheep meat is provided by Deroua Research Station of the national institute of agronomical research (INRA–CRRAT), located in Beni Mellal, Morocco. The ram is of the boujaâd breed. Slaughter took place with in Research station and the pieces used for the test were prepared from the thighs.

After cutting the carcass, we recovered the thigh muscles. The fat around the muscles was removed. The meat was cut into pieces of about 5 g (2x2 cm). Then, we treated the pieces of meat by immersion in three-concentrations prepared from extracts of pomegranate bark. The meat pieces were drained and putted in a sterile petri dish to be deposited at 4°C (Fig. 2). The colour, water retention capacity and microbiological monitoring were evaluated over time of storage.

The meat quality was monitored using the accelerated ageing method. It is a fast method based on the provocation of a severe deterioration of the product by using high temperatures. For this test, meat samples were stored at 4°C. The evolution of the total aerobic mesophilic flora (FMAT) and total coliforms (TC) was carried out during 12 days of conservation (at 0, 3, 6, 6, 9 and 12 days of storage).

The meat was cooked in 10 g pieces without the addition of salts and in the absence of oil. Twenty tasters had been invited to analyse the products and complete the sensory evaluation sheets of meat witness and those with a dose of pomegranate bark extract (12.5-25-50 mg /ml). The preference test and triangle test were assessed to evaluate the consumer acceptability.

The analyses of variance (ANOVA) were carried out by (Statistical Package for the Social Sciences). All analyses were performed at least in duplicate. The results were presented by the mean with its standard deviation. The differences were considered significant at $p < 0.05$.

Fig. 1. Carcass cutting in the INRA Béni Mellal abattoir.

Fig. 2. Meat treatment with the three concentrations of the extract.

III – Results and discussion

The main physicochemical characteristics of pomegranate bark powder and their values are detailed in Table 1.

Table 1. Chemical characteristics of pomegranate bark powder

Criteria		Values
Ph		4.68±0.02
Titratable acidity (g/ml)		2.04±0.25
TSS (Brix°) %		3.07±0.12
Ash content		10%
Colour	L	8.71±0.01
	a	1.70±0.04
	b	1.48±0.04
Total Polyphenols (g EAG/100g)		16.48±2.32

The pomegranate bark powder has a pH value of 4.68. These results are in agreement with those reported by Mediani and Guerhli (2015) who found 4.77. The value of the titratable acidity is 2.04 g/ml, which is slightly higher than that reported by Sidoummou in 2011 (1.59). Moreover, the Brix value of the powder is 3.07%. Color is among the physico-chemical parameters that influence the acceptability of the food product. It was measured by three wavelengths L, a and b; for our pomegranate bark powder, the value of L is 8.71.The red index a is 1.70. this value is lower than that (3.9) mentioned by Mediani and Guerhli (2015). Concerning the yellow index b, the value is 1.48. The total phenolic compounds value is 16.48 (g EAG/100 g MS); this result is close to that reported (21.7 g EAG/100 g MS) by Ben Nasr *et al*. (1996).

1. Evaluation of meat treatment with pomegranate bark extract

The carcass pH measured immediately after slaughter is 6.32, and after 24 h of slaughter it decreased to 5.88. This decrease in pH is related to the accumulation of lactic acid produced by the degradation of intramuscular glycogen.

The results of monitoring the water loss of meat treated with pomegranate bark extract are presented in Table 2.

Table 2. Effect of pomegranate bark powder extract on water retention at raw sheep meat during storage at 4°C for 12 days

	After slaughter	After 3 days	After 6 days	After 9 days	After 12 days
T	32.0±1.61	29.5±3.79	29.4±1.77	27.1±1.66	23.3±4.45
C1	–	31.4±2.91	30.8±4.18	29.2±1.5	19.9±5.97
C2	–	28.7±2.73	29.2±0.62	26.8±0.77	17.7±0.27
C3	–	27.4±0.94	27.7±4.11	25.6±1.11	16.4±3.43

T: Control without pomegranate bark extract; C1:25mg/ml; C2:12,5mg/ml; C3:6,25mg/ml.The values in the same column are significantly different ($p < 0.01$). The values of the same line are significantly different ($p < 0.01$). All results are expressed as an average ± Standard deviation. All results are averages of 3 replicates.

The average value of water loss is improved compared to the control samples. The decrease in water retention capacity is explained by the loss of myofibrillar structure caused by the attack of proteolytic enzymes. During this process, the structure at inside and between the myofibrils breaks, thus reducing the firmness of the meat. The cellular membrane becomes permeable and intracellular water moves through the extracellular fluid. Maturation itself does not change the water outlet of the myofibrils, but with time, the membrane structures disintegrate and water leaves the muscle cells much more easily, thus increasing the expressible juice (Honikel, 1989).

Table 3. Colour assessment of sheep meat during storage, treated by the different concentrations of the pomegranate extract

	After slaughter			After 3 days			After 6 days			After 9 days		
	L	a	b	L	a	b	L	a	b	L	a	b
T	31.24±0.26	60.20±0.26	27.37±1.21	33.79±1.95	71.18±2.39	54.48±1.72	28.89±0.40	68.70±0.91	40.91±0.95	20.63±0.15	48.59±0.87	20.83±0.27
C_1	–	–	–	31.13±0.69	69.69±1.99	45.90±2.05	30.41±0.45	70.37±0.73	44.66±1.06	28.95±0.08	66.75±0.19	31.36±0.12
C_2	–	–	–	38.17±1.15	77.56±1.51	62.13±2.82	27.80±0.27	63.60±0.78	37.64±0.66	31.63±0.07	70.97±0.05	34.76±0.13
C_3	–	–	–	38.65±0.51	76.71±0.28	62.09±1.39	27.62±0.25	61.75±0.64	36.18±0.54	34.05±0.71	73.39±0.80	45.04±0.27

T: Control without pomegranate bark extract; C1:25mg/ml; C2:12,5mg/ml; C3:6,25mg/ml.The values in the same column are significantly different ($p < 0.01$). The values of the same line are significantly different ($p < 0.01$). All results are expressed as an average± Standard deviation. All results are averages of 3 replicates.

Results show that clarity “L”, red index “a” and yellow index “b” of untreated meat were instable and decreases significantly after 9 days of storage at 4°C. However, these parameters increased remarkadly after 9 days of storage especially with concentration C3. Generally, treatment with all concentrations kept unchanged the color of meat even after 9 days of storage.

The microbiological monitoring (the total aerobic mesophilic flora and the total coliforms) of meat treated with pomegranate bark extracts are presented in Figures 3 and 4.

The microbial kinetics increase over the days for all treatment. However, the results indicate that the highest concentration (0.5 g / ml) acts as a delaying agent for bacterial contamination.

These results can be attributed to the fact that the pomegranate bark is rich in total polyphenols (16.48 g EAG / 100 g MS) that are characterized by their antimicrobial and antioxydant potential (Lairini *et al*., 2014). Also, Botsoglou *et al*. (2010) have shown that the addition of olive leaf extract to turkey meat improved microbiological quality of meat.

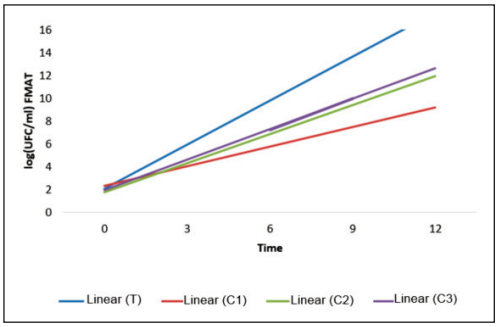

Fig. 3. Evolution of the number of FMAT total aerobic mesophilic flora expressed in log10 CFU/g, in sheep meat supplemented with different doses of pomegranate bark extract.

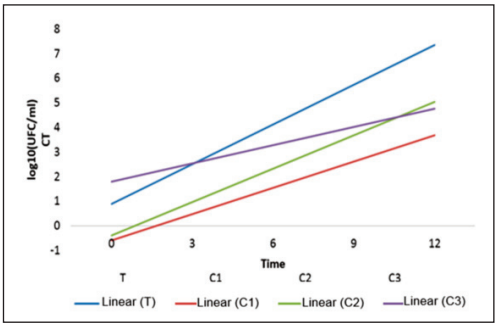

Fig. 4. Evolution of the number of total coliform CT in log10 CFU/g, in sheep meat supplemented with different doses of pomegranate bark extract.

2. Effect of pomegranate bark on the sensory properties of meat

Pomegranate bark has a significant effect on sensory quality. However, tasters accepted the new processed meat with pomegranate bark extract. In fact, they have appreciated the taste of the meat supplemented with pomegranate bark, compared to that of the control.

IV – Conclusions

In short, pomegranate fruit peel has a significant amount of total polyphenols that can be used as natural preservatives in several food applications through its antioxidant and inhibitory effect on harmful microorganisms. Our study highlighted the effect of incorporating the different concentrations of pomegranate bark extracts on microbiological and technological quality sheep meat. We showed that treating and processing meat with concentration of 25 mg/ml of pomegranate bark lead to better meat preservation in terms of water retention capacity, colour and essentially microbiological quality.

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