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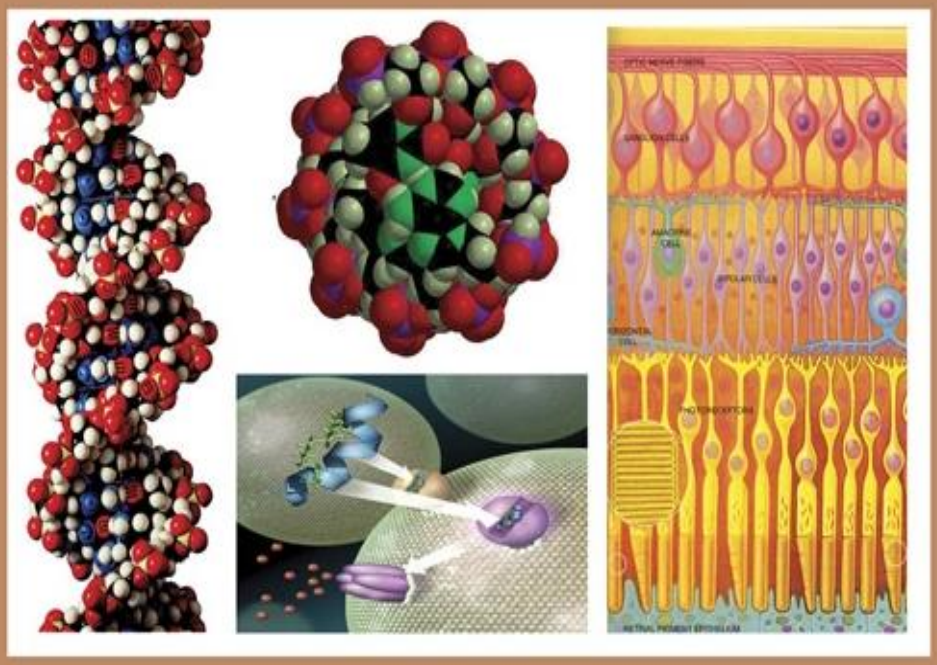

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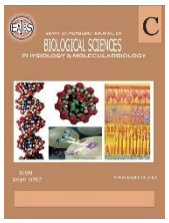

Study of the Preservation of Beef by The Aqueous Extract of *Opuntia ficus indica* (microbiological and sensory study)

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ABSTRACT

The objective of this work is to demonstrate the importance of the aqueous extract of *Opuntia ficus indica* species in the preservation of beef and to maintain the microbiological quality of beef by slowing the rate of proliferation of microorganisms of intrinsic and extrinsic alteration (*Staphylococcus aureus*, *Pseudomonas aeorogenosa*, *Bacilus substilis*, *Listeria monocytogenes*, *Eschirichia coli*, *Enteroccocus fecalis* and *Selmonela thyphi*). For this, we applied the extract directly to the beef and then performed a series of sensory analyses. The aqueous extract of *Opuntia ficus indica L.* leaf showed better antibacterial activity with an inhibition zone ranging from 16–21 mm at a concentration of 100%.

The study's findings indicate good bacterial and sensory quality. The majority of the tasters preferred the meat preserved with the aqueous extract.

INTRODUCTION

Human traditions have developed the knowledge and use of medicinal plants to improve human health (Abdelkader, A., 2022). The medicinal properties of plants are due to products synthesized by the plants themselves called secondary metabolites. Many secondary metabolites, mainly polyphenols, are antibiotics in the broadest sense, as they protect plants against fungi, bacteria, animals, and even other plants (Bastow *et al.*, 2014).

Algeria, given its privileged biogeographic position and its extension between the Mediterranean and sub-Saharan Africa, is considered among the countries known for their floristic diversity (Messai, 2011), to which is added a secular tradition of use of plants. There are about 3000 species of plants, of which 15% are endemic. This potential of medicinal plants includes thousands of species with various interests and constitutes a particular axis of scientific research (Belaoura, 2013).

In this context, we are interested in the study of the plant *Opuntia ficus indica* L. It contains bioactive molecules called betacyanins (a class of reddish pigments) that have antioxidant and anticancer properties and can therefore be used in natural treatments.

The flowers and extract of *Opuntia ficus* body. (Perreault, M, 2017).

The aim of our study is to carry out a test of beef preservation by the use of an aqueous extract of *Opuntia ficus indica* L. For this purpose, microbiological and sensory studies have been carried out.

MATERIALS AND METHODS

Plant Material:

The leaves of *Opuntia ficus indica L.* were collected in the wilaya of Elbayadh in the month of March 2021. The collected plant material was dried on paper at room temperature and under the sun for 7 days. The sample was weighed at 250g, but after drying, the weight became 80g. We crushed the dried leaves in a wooden Hawn and stored them in glass boxes.

Preparation of the Aqueous Extract (AE):

100g of leaves cut into small pieces are put in maceration at room temperature with 1L of distilled water, stirring, for the next 24 hours.After extraction, the extract is filtered; the recovered filtrate is dried to dryness.A sample is dried by the circulation of hot air to intensify the drying conditions or to spare the substances sensitive to heat. Drying is frequently done with a vacuum. The moisture content is obtained by differential weighing before and after drying.

Calculation of Yield:

The percentage yield was calculated by the following formula:

Rmt%=P1+P2/P3X100

Rmt%: the yield as a percentage.

P1: crystallizer weight, after-drying dry matter weight.

P2: Weight of the empty crystallizer.

P3: weight of raw materials.

Meat Selection:

Beef samples are taken directly one hour after slaughter. The samples were taken in the morning. Two pieces were placed in sterile bags and transported to the laboratory in a cooler. The samples are put in the refrigerator until the time of analysis, which is performed in the same day.

Sensory Analysis:

Sensory analysis is one of the principal criteria for the discrimination and comparison of various meat products (Drouilhet, L.,2013).

It was carried out with the help of a jury consisting of 20 members whose ages ranged from 23 to 40 years.

The following analysis is based on several parameters: aroma and juiciness. size of the piece; feel; aroma; tenderness; aftertaste; aromatic persistence. It includes of three series of experiments:

1. The first one aims to detect differences between the samples.
2. The second is descriptive of each organoleptic criterion.
3. And finally, the one showing what the consumers prefer.

According to the procedure of Solomakos *et al.* (2008).

Weighing (20) samples of 3g of meat wrapped in aluminum foil then have covered each piece of meat in. After weighing, the samples of 3 g are put in a pot with a small amount of distilled water and started cooking for 30 min.

Microbiological Analysis:

The objective of the microbiological analysis is to search for or quantify a certain number of microorganisms, indicators of one or more bacteria encountered during manufacturing processes or likely to present a risk to human health during preservation. Our microbiological analysis is based on the enumeration of germs sought in beef with the aqueous extract. We took 250 g of beef from the butcher for the experiment. We put the quantity of meat on a glass plate exposed to the air and covered it with a fine cloth to avoid any contact with insects.The next day, the weight decreased to 204 g, then after three days, the weight became 180 g. It continued to diminish until it reached 100 g.

Biological Material:

Bacteria Culture:

Mueller-Hinton Agar (MH) Preparation:

38 g per liter. Sterilization in an autoclave to prepare this medium, it is necessary to weigh 38g of powder and mix it

in 1 liter of distilled water. Stir while homogenizing and heating. Bring it to a boil for about one minute. Then sterilize the agar in an autoclave for 15 minutes at 121.1°C. This standardized agar is the agar used to test the action of antibiotics on bacteria. It can be supplemented with blood (for Streptococcus), and the extract (for Haemophilus), and it must be poured into a plate to obtain a thickness of 4 mm. There is an equivalent broth. The operation was repeated four times during the experiment. (Hattabi, H., 2020).

Experimental Protocol:

bacterial Study:

Solution Preparation:

Four solutions (stock solution, solution 1, solution 2, and solution 3) of different concentrations (100%, 75%, 50%, and 25%) were used.

As a stock solution, 1g of powder extract in 1ml of physiological water.

0.6ml stock solution + 0.4ml physiological water = solution 1.

0.4ml stock solution + 0.6ml physiological water = solution 2

solution3: 0.2ml stock solution in 0.8ml physiological water

Preparation of the Discs:

These sterile disks are immersed in the aqueous extract for 20 min at 120 °C. They are then placed in the autoclave for 20 min at 120 °C and stored at room temperature (the test tube is hermetically sealed).

Evaluation of Biological Activities:

The antibacterial activity was carried out at the central laboratory of bacteriology.

The principle of the method is based on the diffusion of the antibacterial compound in a solid medium in the Petri dishes after the contact time between the product and the target microorganisms. The effect of the antibacterial product on the target is assessed by measuring a zone of inhibition and according to the diameter of inhibition. (Hellal, 2011).

The evaluation of the antibacterial activity of extracts of our plant of Opuntia ficus-indica was made on 6 bacterial strains.

Bacterial Strains:

S1: *Staphyloccus aureus,* S2: *Pseudomonas aerogenesa,* SA1: *Bacillus substilus,* SA2: *Escherichia coli,* SA3: *Salmonella thyphi,* SA4: *Listeria monocytogene,* SA5: *enteroccocus fecalis.*

Muller Hinton medium is poured into sterile Petri dishes and left for 15 minutes to solidify. Bacteria are deposited and inoculated with a sterile swab. The inoculation is done in such a way to ensure a homogeneous distribution of the bacteria.

The discs filled with extract are deposited on the surface of the contaminated gelose and the antibiogram is fixed in the middle of the petri dish. The antimicrobial activity is manifested by the appearance of a zone of inhibition of microbial growth produced around the discs after 24 hours of incubation at 37°C (Ozpolat, B., 2002).

RESULTS

Aqueous Extraction Yield of Prickly Pear:

P1 = crystallizer weight (319.48g) + dry matter weight (1.80g).

P2: empty crystallizer weight = 319.48

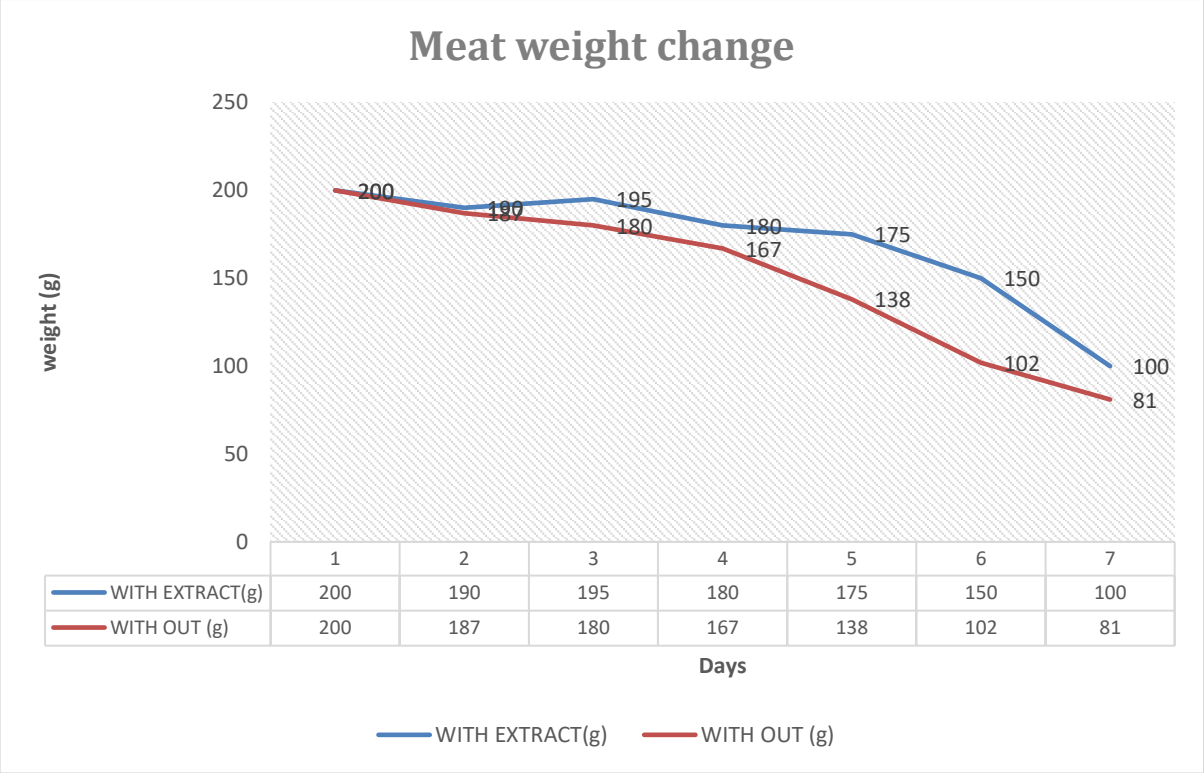
P3: raw material weight = 100 g

R: (319,48+1,80) +319,48/100x100

R = 0,0640476%

Evaluation of Microbiological Analysis:

We took two pieces of meat weighing 200g. We took the one weighing 200g wiped with a swab soaked in all the parts of meat and the other piece without the aqueous extract. The two pieces were exposed to the free air and room temperature for seven days.



Tasting Test:

The organoleptic qualities of beef depend not only on the conditions of transformation of the muscle into meat but also on the composition and structure of this muscle. This composition and structure are themselves a function of many factors, such as genetics, feeding, breeding, slaughtering techniques, and also the way the meat is cooked (Warriss, 2000).

The organoleptic study of minced meat added to different concentrations of extracts shows that the size of the piece is important to achieve a better taste.

Also, the addition of the studied extracts does not influence too much the tenderness of the meat.

On the other hand, the addition of the extracts studied does influence varying degrees of flavor, taste, fragrance, scent, and aromatic persistence

Table 1: Presentation of the Tasting Sheet

Tasting steps	Description	E1 (without EA)	E2 (with EA)
Size of the pieces	Null	15	16
	Poor	18	13
	Mediocre	16	17
	Good	15	18
	Excellent	13	19
Touch	fatty	20	20
	Damp	13	17
	Dry	19	14
	Smouth	20	20
	Fibrous	19	18
	Stiff	17	19
	Soft	14	20
	Fine	15	17
Smell of the pieces	Gratinated	20	20
	Smoky	15	19
	Grilled	17	20
	Caramelize	12	20
	Sooty	15	16
	Ferric	14	16
	Leaky	15	15
Flavor	Acidic	11	0
	Dirty	12	17
	Bitter	0	0
	Sweet	0	0
	Other (please specify)		
Chewing tenderness	Dry	17	12
	Melting	15	16
	Firm	15	11
	Hard	14	13
	Tough	18	13
	Smooth	14	19
	Fat	15	19
	Soft	14	15
	Oily	14	19
	Stringy	14	15
	Floury	13	13
	Elastic	18	16
	Smooth	20	20
	Doughy	15	16
	Crumbly	16	17
	Sticky	11	16
Chewiness	Liquid	14	16
	Fluid	14	13
	Oily	14	19
	Fat	20	20
Aroma	Blood	12	11
	Ferric	13	14
	Cooked	13	13
	Melted fat	13	17
	Linseed oil	12	17
	Licorice	14	13
	Smoke	13	14
	Hazelnut	13	16
	dry grass	15	15
Aftertaste	metallic	13	12
	slightly bitter	12	11
Aromatic persistence	Short	13	14
	Medium	17	17
	Long	12	19
If you found this product on the market, would you buy it?	Definitely	14	14
	Probably	15	17
	I don't know	14	16
	probably not	15	17
	definitely not	14	14
which piece would you choose?	**E_1 (without extract)**	**13**	
	E_2 (with extract)		**20**

Evaluation of Biological Activities:
Antibacterial Activity:

The evaluation of the antibacterial activity of crude extracts of *Opuntia ficus indica* L. is estimated in terms of the diameter of the inhibition zone around the discs (Semiria, 2022) containing the extract at different concentrations of the plant to be tested against (S1: *Staphyloccus aureus*, *Pseudomonas arcogenesa* (S2) SA1: *Bacillus subtilis*, SA2: *E. coli*, SA3: *Salmonella thyphi*, and SA4: *Listeria monocytogene*, SA5*: Enteroccocus fecalis*) after 24 hours at 37 °C incubation.

Table 2 shows the values in mm of the inhibition zones reached with the studied strains.

	Eshirichia coli	*Staphylococcus aureus*	*Selmonella thyphi*	*Listeria monocytogenes*	*Pseudomonas aerogenosas*	*Bacillus substilis*	*Enteroccocus fecalis*
EA 100%	16	21	17	17	20	18	17
EA 75%	11	15	13	8	8	00	10
EA 50%	10	14	9	00	00	00	6
EA 25%	00	6	8	00	00	00	00

The study of antibacterial activity shows that aqueous extracts of *Opuntia ficus indica L*. have very low antibacterial activity against bacterial strains, where there appeared to be different inhibition zones from one bacterium to another. The diameters of the inhibition zones appear only at a concentration of 100% of *Opuntia ficus indica* L. extract.

According to the antimicrobial activity estimation scale, a bacterial strain is considered resistant to antibacterial agents when the inhibition diameter is less than 10 mm by Djenadi (2011). This leads us to deduce that the strains studied in this work are resistant to the different plant extracts.

Variations in chemical composition can probably explain the differences observed in the antimicrobial activity of extracts from the same plant species or from different plants. The optimal efficacy of an extract may not be due to one main active component but to the combined action (synergy) of different compounds in the extract (Essawi and Srour, 2000).

Several studies have found that Gram (+) bacteria are more sensitive than Gram (-) bacteria (Turkmen *et al.,* 2007; Falleh *et al.,* 2008), which can be attributed to differences in the outer layers of Gram (-) and Gram (+) bacteria. These findings contradict our findings, which revealed E. coli resistance to the extracts tested.

Studies have suggested that polyphenols and flavonoids are characterized by antimicrobial properties (Eyles, A.,2007). However, the antibacterial tests carried out on our EAOF extracts reveal the presence of an inhibitory effect against the growth of the studied germs (Staphylococcus sp, pseudomonas...etc).

DISCUSSION

The yield of the extracts varies a lot with the plant used, the material used for extraction, and the origin of the plant. Thus, this difference in yield can be due to two factors: the region and the period of collection (Jafarpour, M.,2012); also, the climate (rhythm of rains, temperature difference, nature of the winds) is a determining factor in everything concerning the vegetation (Béniston N. and Béniston W., 1984).

Finding a compromise between the effective dose of a flavouring agent like essential oils and sensory acceptability is a difficult task (Lambert, R. J. W., 2001).
Solomakos *et al.* (2008) found that sensory evaluation revealed that the organoleptic properties of ground beef treated with thyme essential oil were acceptable at the 0.3%

concentration level, but unacceptable at the 0.6% and 0.9% levels. Previous studies are consistent with the current findings. They also showed that high concentrations of essential oils, which are necessary to obtain antibacterial activity against foodborne pathogens, might not be applicable to foods due to their undesirable organoleptic properties (Holley & Patel, 2005).

Hulankova *et al.* (2013) found that a concentration higher than 0.2% of oregano essential oil is not acceptable to the panelists. Several other researchers report better sensory properties of ground beef treated with 0.8–1% oregano essential oil compared to the control (without essential oil) (Tsigarida *et al.*, 2000; Govaris *et al.*, 2010). Other authors found that the concentration of 1% essential oil is unacceptable (Chouliara *et al.*, 2007).

In the study conducted by Djenane *et al.* (2011), the sensory properties of minced meat treated with essential oils of Pistacia lentiscus and Satureja montana were acceptable by the panelists at all concentrations studied. However, after two days of storage, the samples not treated with essential oils gave an unsatisfactory result.

In 2012, Djenane *et al.* found that the addition of essential oils of Lavandula angustifolia and Mentha piperita practically decreased the malodors of ground meat preserved at a temperature of 9 °C.

In the work of Skandamis & Nychas (2001), the addition of 1% essential oil of oregano positively affected the odor and color of ground meat.

Abdollahzadeh *et al.* (2014), showed that the addition of Zataria multiflora Boiss, essential oil to groundfish found that the concentration of 0.4% of this essential oil was acceptable. On the other hand, the concentrations of 0.8 and 1.2% were not tolerated.

On the other hand, Ouattara *et al.* (2001) found that the addition of 0.9% of the essential oil had no effect on the sensory quality and appearance of the cooked shrimp.

The difference in sensory acceptability can be explained by the difference in aroma intensity that results from the large difference in the chemical composition of essential oils (Burt, 2004).

This composition can vary depending on the nature and geographical origin of the plant used, as well as the material and method of extraction (Del Castillo, M. R.,2004). In addition, the acceptability of the concentration of essential oil also depends on the preferences of the consumer (tasting panel) (Hulankova *et al.*, 2013).

Conclusion

Worldwide, the consumption of beef has increased more rapidly than that of other meats (Jokanović, V. R.,2021). In terms of nutritional quality, red meat can be an alternative to white meat. The caloric intake brought by the lipids of red meat has health benefits because they are unsaturated due to the lack of bio-hydrogenation, a phenomenon specific to ruminants that are sources of white meat. Also, the quantities of proteins are important and theoretically of high biological value, and finally, the sensory quality remains relative to the nature of the preparation of these meats within the transformations and methods of conservation.

The results obtained showed the good bacteriological quality of beef preserved by the aqueous extrai. On the bacteriological plan, they do not present any danger for human consumption. The product is of satisfactory quality and clean for consumption and is in conformity with the Algerian standards (JORA N° 35, 27/05/1998). As a result, it is strongly advised that increased surveillance be accompanied by rigorous and consistent control of this sensitive material throughout the year. This will preserve the quality of beef against all forms of contamination.

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