Studies on the Use of *Sesuvium Portulacastrum* - Part II: Preservation of Skins

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Abstract

Sesuvium portulacastrum (*S. portulacastrum*) a perennial halophyte has been used as a replacement for salt in the curing process of Goatskins. The quality of phyto-preserved skins has been assessed with respect to hair slip, putrefaction odor, bacterial count, moisture content, shrinkage temperature and total extractable nitrogen. Phyto-preserved skins have been processed into finished leathers and assessed for organoleptic properties and physical characteristics. The product for phyto-preservation made from *S. portulacastrum* has been found to be as effective as conventional salt based curing process of goat skins. The quality of the preserved skins has been found to be on par with that of salt cured skins. The quality of resultant leathers of the experiment has been found to be comparable with control. The preservation efficacy of phyto-preserve could be due to the synergistic action of its antimicrobial metabolite present in its essential oils and the salt present in the plant.

Resumen

El halófilo perene *Sesuvium portulacastrum* (*S. portulacastrum*) ha sido empleado como sustituto de la sal en el proceso de preservación de pieles caprinas. La calidad de las pieles así fito-preservadas se ha evaluado con respecto a caída de pelo, olor putrefacto, conteo bacterial, contenido de humedad, temperatura de contracción, nitrógeno total extraíble. Pieles fito-preservadas han sido procesadas hasta cuero terminado y evaluados en cuanto a propiedades físicas y características de toque. El producto resultante por medio de Fito preservación en base a *S. portulacastrum* se ha encontrado ser tan efectivo como la preservación convencional de pieles caprinas por medio de sal. La calidad de las pieles preservadas se encuentra a la par con aquellas preservadas con sal. La calidad de los resultantes cueros del experimento, se ha encontrado ser comparable con la del control. La eficacia de la preservación por Fito preservación podría ser por la acción sinérgica del metabolito antimicrobiano presente en los aceites esenciales, como la de la sal presente en la planta.

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INTRODUCTION

Discharge of saline wastewater reduces soil fertility and contaminates the ground water. The conventional method of preservation of raw hides and skin demands the use of more than 40% of common salt (sodium chloride). Hence, the resultant soaking wastewater is rich in TDS and chlorides.[1,2] The dehydrating nature and the bacteriostatic property of the salt accounts for its high efficiency in the preservation process.[3] Predominantly, the soaking process wastewater is evaporated in open pans and the salt is recovered. Apart from the requirement of large area, the salt recovered is unsuitable for reuse as it is contaminated with organic load and halophilic bacteria. The advanced desalination techniques such as reverse osmosis, electro dialysis and membrane techniques may not be viable for the treatment of soak water as these techniques are associated with substantial capital and working cost.

Various physical and chemical preservation techniques either with low salt or without salt have been explored. The chemicals that are reported as curing agents include boric acid[1,4], potassium chloride[1,5], soda ash[1,6], biocides like benzalkonium chloride[1,7], antibiotics like aureomycin, terramycin and tetracylin[1,8], sodium metabisulphite with acetic acid[1] and silica gel.[3] Physical treatment methods like controlled drying using drying chamber[1,9], irradiation with gamma rays[1,10] chilling[1] and electron beam[10] have been explored. However, high capital investment, substantial operating cost and inadequate preservation efficiency in some cases, difficulties in processing are the major short comings.[3]

The present study explored the possibility of using the halophyte *S. portulacastrum* as an alternate to salt for the curing process. *S. portulacastrum* is a perennial halophyte belonging to the Aizoaceae family and is distributed through out the world. The thick fleshy leaves are borne on succulent reddish green stems that branch regularly forming dense stands close to the ground. It produces decorative branches with pink purplish and white flowers.[11,12] *S. portulacastrum* has a long history of use in folk medicine as a remedy for fever, kidney disorders and scurvy.[11,13] This plant is known to have anti-bacterial, anti-fungal and anti-oxidant activity due to presence of wide range of essential oils.[11,14-27] In addition, it has been known to contain a polysaccharide, which showed positive activity against human immunodeficiency virus.[28,29] It is a rich source of an array of amino acids. The free amino acids that are found in the succulent stems and leaves are proline, aspartic acid, glutamic acid, alanine, serine and glycine.[30] Accumulation of these free amino acids can be ascribed to a disturbed nitrogen metabolism due to high salinity, wherein the amino acids are not further used for the synthesis of proteins.[30]

TABLE I
Evaluation of phyto-preserved and salt cured goat skins

Concentration of phyto-preserve (w/w)	Hair slip	Odor	Putrefaction
5	At the Edges	Slight	Yes
10	No	No	No
20	No	No	No
40	No	No	No
60	No	No	No
40 (Control-salt)	No	No	No

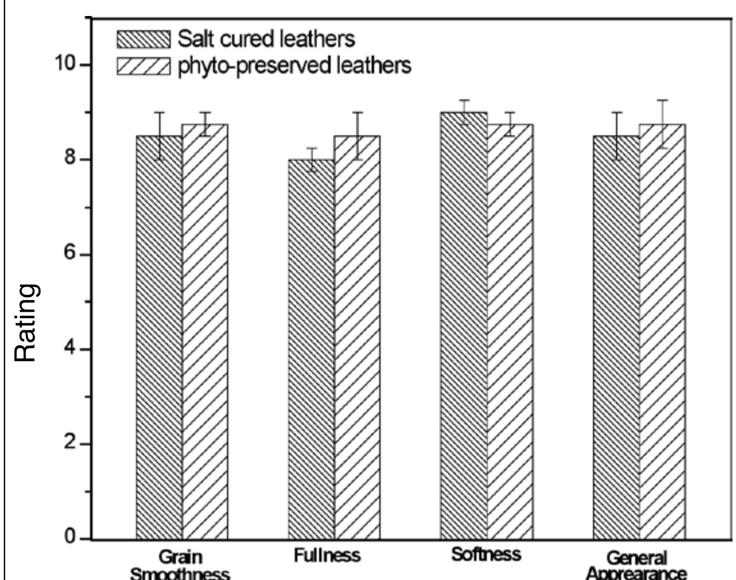

Figure 1: Graphical representation of organoleptic properties of salt and phyto-preserved matched pair leathers

S. portulacastrum, contains salt stored in its vacuoles. The leaves of this plant contain around 18% (dry weight) of sodium and 15% of chloride content.[30] It has been shown that major phyto-constituents of *S. portulacastrum* are trans-4-hydroxyprolinebetaine, proline and 3,5,4'-trihydroxy-6,7-dimethoxyflavone 3-glucoside.[25,26] The occurrence of these compounds is associated with a possible role in osmo-regulation.[26,27] Hence, the synergistic action of essential oils as antifungal and antibacterial agents and the presence of salt in the plant is thought to bring about adequate preservation effect.

Materials and Methods

Materials

Fresh Indian goat skins of average weight 1 kg per skin have been taken for the study. *S. portulacastrum*, a halophyte, found in salt marsh creeks lands of Ennore, Chennai, Tamil Nadu has been collected and used for the study. The plant has been washed by soaking in tap water. The dried plant material has been extracted as fine powder (Phyto-preserve) and and used for preservation experiments as a paste prepared with 20% water. [31]

Methods

Freshly flayed goat skins obtained from a nearby slaughterhouse have been cut into two halves. The right halves have been taken for experimental trials and the left halves for conventional salt curing system that is referred to as control. Various concentration of phyto-preserve at 5, 10, 20 and 40% (dry weight) on the weight of the skins has been applied on flesh side of the right halves of the skins. Three skins have been used for each experiment. The corresponding left halves of the skins have been treated with 40% salt. The skins have been piled and stored at ambient temperature of 35±3°C. The skins have been monitored periodically for physical changes like odor and hair slip that have been considered as indications of putrefaction.[32,33] The efficacy of the preservation system has been assessed by estimating the moisture content, total extractable nitrogen and bacterial count.

Determination of bacterial count

The control and experimental skins of known weight (~5 g) have been cut into small pieces and shaked well for 30 minutes at 35 rpm in 250 mL sterile distilled water. 1 mL of this skin extract has been serially diluted and inoculated in nutrient agar medium plates. The inoculated plates have been incubated at 37°C for 24 h. Subsequently after incubation, the numbers of colonies have been enumerated.[32,34]

Determination of moisture content and extractable nitrogen

The preserved skin samples of known weight (~5 g) have been washed with ten changes of water at 30 -35 rpm in a shaker bottle for 3 h. The resultant wash liquor has been filtered using Whatman 1 filter paper and digested with 10% acid mixture containing 1:1 HNO_3 and H_2SO_4. The amount of nitrogen present in the wastewater has been determined using Kjeldahl method.[35] The moisture content of skins of known weight (~2 g) has been determined using standard method.[35]

Determination of structural destabilization of the skin matrix

The structural destabilization of the skin matrix that is likely to occur due to inadequate preservation has been assessed by testing the shrinkage temperature using a Theis shrinkage meter.[36] The values reported are an average of four measurements for each experiment.

Comparision of optimized experimental and control leathers

Matched pair comparison of experimental and control processing has been carried out using four fresh goat skins. Right half of goat skins have been preserved using phyto-preserve (10% phyto-preserve; temperature 35±3°C; duration of preservation 15 days). Corresponding left halves of goat skins have been preserved using 40% sodium chloride. Both experimental and salted skins have been chrome tanned and processed into upper leathers.[36]

Physical Testing Analysis

The leathers made from matched pair control and experimental processes have been tested for physical characteristics, cut from the official sampling position (IUP 2[37] method), and conditioned at 80±4°F and 65±4% R.H. for 48 hours. The tensile strength, elongation at break, tear strength and grain crack strength have been tested as per IUP 6,[38] IUP 8,[39] and IUP 9[40] methods respectively. Four samples have been used for each test.

Organoleptic Properties of Tanned Leathers

The matched pair control and experimental crust leathers have been assessed for grain smoothness, fullness, softness and general appearance by hand evaluation technique. The functional properties of the leathers in a scale of 0 -10 points have been rated by three experienced tanners and the average values are reported. Higher values indicate better property

Results and Discussion

Putrefaction is a processs initiated by autolysis and followed by bacterial degradation of the skin. Reducing the moisture below 30% would hamper the growth of the bacteria and hence putrefaction can be prevented. Hair slip is the first indication of putrefaction as the proteins present in the hair bulb are degraded by the bacteria during the onset of putrefaction. Bacterial population is the direct measure of bacterial growth and degree of skin putrefaction is directly proportional to bacterial growth. Bacterial degradation results in the generation of extractable nitrogen. The level of total extractable nitrogen is one of the important indicators of the extent of putrefaction. The phyto-preserve has been found to contain 12% salt, 22% essentials oils and 12% moisture. The indicators of putrefaction at various concentrations of the phyto-preserve and salt based preservation are given in Table I. As given in Table I, hair slip and odor have been observed indicating putrefaction at 48 hrs of preservation using 5% phyto-preserve. However, at higher concentrations no hair slip and odor has been observed. Rehydration of the phyto-preserve skins, which is one of the important indicator of a curing agent has been comparable to that of control samples. Rehydration property of phyto-preserve skins could possibly be due to the presence of salt in phyto-preserve. Hence, from these observations it is seen that phyto-preserved skin requires a minimum of 10% of the dry plant product for efficient preservation as compared to conventional salt based curing process.

TABLE II
Moisture content of salt and phyto-preserved goatskins

Duration	Control - %Salt	Moisture Content at different % offer of phyto-preserve (%)			
	(%) 40	5	10	20	40
0 hrs	70±28	70±3.1	70±2.9	70±3.2	70±3.2
1 day	44±1.6	54±2.2	52±2.3	50±2.5	50±2.4
2 days	39±1.7	50±1.6	48±2.0	44±2.0	43±2.1
7 days	34±1.2	-	40±1.8	38±1.8	36±1.8
14 days	33±1.4	-	32±1.6	33±1.5	32±1.6
21 days	30±1.1	-	30±1.4	31±1.4	30±1.4
30 days	28±1.1	-	28±0.9	28±1.0	28±1.1

Note: The values are mean ± standard deviation of three values

TABLE III
Total extractable nitrogen (mg/g) of salt and phyto-preserved goatskins at different concentrations

Duration	Control - %Salt	Amount of extractable nitrogen at different % offer of phyto-preserve (mg/g)			
	(mg/g) 40	5	10	20	40
0 hrs	2.13±0.02	2.16±0.04	2.14±0.06	2.12±0.01	2.14±0.02
1 day	2.71±0.03	4.9±0.06	2.98±0.05	2.96±0.04	2.84±0.06
2 days	3.58±0.02	12.6±0.12	2.42±0.03	2.56±0.02	2.62±0.05
7 days	3.69±0.04	-	2.36±0.08	2.31±0.03	2.36±0.03
14 days	3.76±0.06	-	2.32±0.06	2.28±0.03	2.24±0.02
21 days	3.91±0.05	-	2.54±0.05	2.10±0.01	2.22±0.03
30 days	3.96±0.07	-	2.58±0.04	2.32±0.02	2.28±0.02

Note: The values are mean ± standard deviation of three values

Effect of preservation on moisture content

Moisture content is one of the important factors that can be used to assess the ability of preservation for phyto-preserve. Reduction in moisture has been observed for a period of 30 days as given in Table II. It can also be observed that the moisture content of the preserved skin employing 10% of phyto-preserve for preservation is 48% against 39% for the corresponding salt cured skin after 48 h. After 7 days of preservation, the moisture content of the phyto-preserved skin has been 40% compared to 34% for the corresponding salt cured skin. As seen in Table II, there is a steady decrease in the moisture content of 10%, 20% and 40% phyto-preserved skins. In spite of higher levels of moisture content in phyto-preserved skins, the bacterial population has been found to be lower during initial stages of preservation. This could be due to the potential antimicrobial property of *S. portulacastrum* against the degrading microorganisms of raw hides and skins. However, after 30 days, the moisture content of the phyto-preserved skin has been comparable to the moisture content of control, which has been found to be 28%. The moisture content of the skins for other concentrations of phyto-preserve followed a similar trend.

Effect of preservation on extractable nitrogen

The amount of extractable nitrogen in the process liquor is an important parameter to be considered to assess the effectiveness of the preservative. The data given in Table III, is an index of the degree of microbial attack and the degradation of the skin during preservation of hides and skins by phyto-preserve. As given in Table III, the total extractable nitrogen of the skins preserved using phyto-preserve showed a decreasing trend with an increase in the preservative used. It can be observed that the phyto-preserve used at a concentration of 5% based on the weight of skin resulted in high levels of extractable nitrogen indicating putrefaction at 24 and 48 hrs of preservation. The extractable nitrogen of cured skins employing 10% phyto-preserve has been found to be 2.42 mg/g at 48 hrs of preservation. The extractable nitrogen of control skin preserved with 40% offer of salt has been 3.58 mg/g at 48 h. However, the release of extractable nitrogen at 40, 20 and 10% offer of phyto-preserve has been lower compared to the corresponding salt cured skins. It has been observed that the total extractable nitrogen increased during the first 24 hours of incubation. However major variations in the nitrogen levels have not been observed in 10, 20 and 40% phyto-preserved skins. On the other hand, the salt cured skins showed a gradual increase in the soluble nitrogen level. The decrease in extractable nitrogen content in the experimental trials is probably due to the anti microbial property of the halophyte. This anti-microbial character is an additional advantage of the phyto-preserve compared to the salt. The above observations indicate that effective preservatio can be attained at 10% of phyto-preserve.

Effect of preservation on bacterial growth

The extent of preservation by any substance depends mainly on the inhibition of the growth of collagenolytic and proteolytic bacterial species. Hence, bacterial population is a direct indicator of the degree of putrefaction. Table IV shows the bacterial population of control and phyto-preserve skins at different time intervals. Skins preserved using 10% phyto-preserve, exhibited a bacterial count of $5X10^7$/g after 48 h of preservation. Bacterial population had been reduced with time and increase in the amount of phyto-preserve in experimental skins. However, salt cured sample resulted in a bacterial count of $9X10^{10}$/g for 24 h preservation period, $5X10^7$/g for 14 day preservation and reduced to $4X10^6$/g in 30 days preservation. The decrease in bacterial population in the salt cured skins is probably due to the fact that salt acts only as a bacteriostatic agent. However, from the data presented in Table IV, the bacterial count of 10%, 20% and 40% phyto-preserved skins is lower than the salt preserved skins, which could be due to the synergistic action of the essential oils that provide antimicrobial effect and the salt present in the halophyte.

Effect of preservation on structural stability

The shrinkage temperature of control and experimental skins preserved with phyto-preserve are given in Table V. Putrefaction eventually leads to destabilization of skin structure. Hydrothermal stability is one of the direct indicators of structural stability. The shrinkage temperatures of phyto-preserve skins show marginal difference in comparison with salt cured skin. The shrinkage temperature of skins preserved using 10% phyto-preserve has been found to increase by 6^{o}C in 30 days of preservation. The increase in shrinkage temperature of salt cured skins in 30 days of preservation has been 4^{o}C. However, there has been no significant increase in shrinkage temperature at higher concentrations of phyto-preserve even after 30 days of preservation. Hence, it can be concluded that phyto-preserve does not bring about any adverse structural modifications in the skin matrix.

Effect of preservation on the visual and physical properties of crust leathers

The changes brought about by any curing system will reflect on the quality of hide and skins and in turn on finished leathers. Hence, determination of various visual and physical properties of crust leather is also important to assess the efficacy of the curing system. The organoleptic and physical properties of matched pair comparison are given in Figure 1 and Table VI. The organoleptic properties indicate that the properties of phyto-preserved leathers are comparable to that of salt preserved leather.

Understanding the function of *S. portulacastrum* in preservation of skins

The major chemical compounds present in the essential oils are α-pinene, camphene, β-pinene, terpinene, bornyl acetate, tridecane, trans-carryoohyllene and α-humulene. [11] These chemical components are toxic to microorganisms as they disrupt the membrane integrity of bacteria or fungi.[11,14-16] α-pinene and β-pinene destroy cellular integrity and inhibit respiration and ion transport processes in bacteria.[11] They also increase the membrane permeability in yeast cells and isolated mitochondria in Gram-negative bacteria.[11,14,15,18] Michael L. Magwa et al.,[11] showed that the presence of the essential oil in *S. portulacastrum* containing 12 major phyto-constituents, exhibited antibacterial activity against *Acetobacter calcoacetica, Bacillus subtillis, Clostridium sporogenes, Clostridium perfringens, Escherichia coli, Salmonella typhii, Staphylococcus aureus and Yersinia enterocolitica*. The essential oil components such as trans-caryophyllene, limonene, camphene, (−)-bornylacetate, tridecane, O-Cymene and α-humulene exhibited antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus flavus and Penicillium notatum*. O-Cymene and limonene demonstrate strong antifungal properties.[11,17] *S. portulacastrum* also produces resin-like materials, which contain monoterpenes to defend themselves against the penetration of the attacking pathogens.[19] Volatile compounds, such as trans-caryophyllene, (−) -bornylacetate, tridecane and α-humulene, are likely to be the precursors of the complex menthols or resins, which have been claimed to

TABLE IV
Total bacterial count (expressed as CFU/g of skin) of salt and phyto-preserved goatskins at different concentrations

Duration	Control - %Salt (CFU/g of skin)	Total Bacterial Count (CFU/g of skin) at different % offer of phyto-preserve			
	40	5	10	20	40
0 hrs	2 ± 0.10 X10^3	2 ± 0.10 X10^3	2 ± 0.13 10^3	2 ± 0.11 X10^3	2 ± 0.09 X10^3
1 day	9 ± 0.04 X10^{10}	13 ± 1.2 X10^{10}	9 ± 0.05 X10^{10}	4 ± 0.04 X10^9	2 ± 0.06 X10^9
2 days	8 ± 0.04 X10^9	-	5 ± 0.04 X10^7	6 ± 0.04 X10^6	2 ± 0.02 X10^6
7 days	6 ± 0.04 X10^8	-	7 ± 0.02 X10^6	9 ± 0.03 X10^5	5 ± 0.03 X10^5
14 days	5 ± 0.03 X10^7	-	4 ± 0.03 X10^5	3 ± 0.03 X10^5	6 ± 0.02 X10^4
21 days	8 ± 0.03 X10^6	-	5 ± 0.05 X10^4	4 ± 0.02 X10^4	2 ± 0.01 X10^3
30 days	4 ± 0.02 X10^6	-	8 ± 0.02 X10^4	5 ± 0.03 X10^3	3 ± 0.02 X10^3

Note: The values are mean ± standard deviation of three values

TABLE V
Shrinkage temperature of salt and phyto-preserved goatskin at different concentrations

Duration	Control - %Salt	Shrinkage Temperature at different % offer of phyto-preserve (°C)			
	(°C) 40	5	10	20	40
0 hrs	62 ± 0.5	62 ± 0.5	62 ± 1	62 ±1.5	62 ± 1.5
1 day	64 ± 1	58 ± 1.5	64 ± 0.5	64 ± 1	64 ± 1.5
2 days	64 ± 1	-	66 ± 1.5	65 ±1.5	65 ± 1.5
7 days	65 ± 1.5	-	67 ± 1	67 ± 1	67 ± 1.5
14 days	66 ± 1	-	68 ± 1	68 ± 1.5	68 ± 1.5
21 days	66 ± 0.5	-	68 ± 1	68 ± 1.5	68 ± 1.5
30 days	66 ± 1	-	68 ± 1	69 ± 1	69 ± 1.5

Note: The values are mean ± standard deviation of three values

also contain the antibacterial, antifungal and/or antioxidant properties.[17,20-22] The essential oils that contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have greater anti-oxidative properties.[22-24] Monoterpenes found in this essential oil act as radical scavenging agents, contribute to positive effects in the defense of the plant and exhibited an antioxidant activity threshold of 15.9 mm mean zone of color retention. Therefore, the preservation efficacy of the phyto-preserve may be attributed to the essential oils and the salt present in *S. portulacastrum*.

TABLE VI
Physical properties of salt and phyto-preserved matched pair crust leathers

Sample	Tensile Strength	Extension at break	Tear Strength	Grain Crack Resistance	
Preservation	(Kg/cm^2)	%	(Kg/cm)	Load (Kg)	Distension (mm)
Salt	248±10	61±3	60±4	41±2	9.8±0.7
Phyto-preserved	258±12	78±5	74±3	45±3	12.6±1.1

Note: The values are mean ± standard deviation of three values

CONCLUSIONS

The curing system using *S. portulacastrum* (phyto-preserve), a halophyte, provides an eco-friendly option to overcome the environmental constraints of using salt. Treatment of raw goat skins using phyto-preserve at a concentration of 10% dry weight brings about effective preservation of goat skins at ambient temperature. The present study substantiates that phyto-preserve can be used effectively as an alternative curing agent to salt. Phyto-preserve, prepared using halophyte did not bring about any structural modification in the collagenous network of the skin. Phyto-preserve can also be used at room temperatures and does not require any sophisticated instruments or new skills. In addition, phyto-preserve does not pose any health or safety problems on usage.

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