

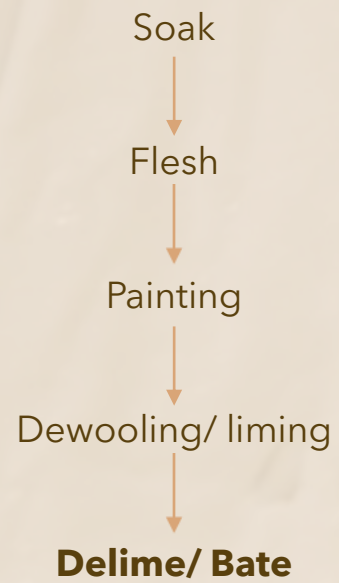
# Eco-friendly skin preservation and depilation

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# Flow Chart

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## Historical context

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- Schlosser et al. (1986) using an undisclosed *Lactobacillus* culture to preserve hides, based on the fact many lactic acid bacteria were used in the preservation of food.
    - Hides were incubated in this culture,
    - The hides were preserved, but also depilated over time.
    - No further research was carried out to understand the mechanisms.
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## Historical context

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- Dettmer et al. (2013) used a *Bacillus* enzyme preparation to depilate hides and reduce COD, BOD and the total nitrogen and sulphides in the wastewater. However, the grain was damaged and sulphide was needed to increase the depilatory activity. Others found the same result (De Souza & Guterres, Wahuntari & Hendrawati, Lopéz, et al)
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## Eco-friendly process

- Removes wool from sheepskins using **whey** or **whey permeate**, omitting the need for sulphide, lime, enzymes.
- Prevents microbial degradation of skins for up to a week at ambient temperatures,
  - Potentially avoids the need for salt and biocides for temporary preservation.





## Chemical options in Eco-friendly Dewooling trial

### **Bovine - milk based**

Milk, UHT milk, milk powder, whey, sterilized whey, whey powder, permeate solution

### **Controls**

Water, Acetic and Lactic (acid solutions adjusted to pH 4.5)

### **Normal**

Sodium sulphide (125g/l), lime, Solvitose™.



# Results

Depilation trials with milk products and by-products.

	Initial pH of solution	Final pH	Days to depilate	Smell	Depilated skin condition
Milk	6.8	4.5	3	Fermented/ sour milk	Pink, shiny, plump, and very smooth
UHT milk	6.7	4.5	3		
12.5% (w/v) milk powder solution	6.8	4.5	3		
Whey	6.2	4.5	3	Putrefied and rotten	Grey, easily broken into pieces, and rigid
Sterilised whey	6.0	4.5	3		
Sterilised permeate	6.0	4.5	3		
6.25% (w/v) whey protein solution	6.5	6.0	5		
H <sub>2</sub> O	7.0	7.5	5		
H <sub>2</sub> O with acetic acid	4.5	7.5	3		
H <sub>2</sub> O with lactic acid	4.5	8.0	3		

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PCR primer sets for bacteria 16S and fungal 18S ribosomal ribonucleic acid (rRNA) encoding gene amplification.

Primer name	Sequence (5' → 3')	Target gene	Amplicon length (bp)
27f	AGAGTTTGATCCTGGCTCAG	Full length	1400
1492r	GGTTACCTTGTACGACTT	16S rRNA	
Eub338	ACTCCTACGGGAGGCAGGAG	V3 region of	200
Eub518	ATTACCGCGGCTGCTGCTGG	16S rRNA	
nu-SSU-0817-5'	TTAGCATGGAATAATRRAATAGGA	Nuclear small	420
nu-SSU-1196-3'	TCTGGACCTGGTGAGTTTCC	subunit of	
		18S rRNA	

PCR primers for bacterial and fungal amplification



## Identification of viable microorganisms

TSB, LB, MRS, malt, and Wilson's media were used to culture microorganisms

Microbial genomic DNA (gDNA) was extracted from cells

Sequences were analysed using the BLAST algorithm against the NCBI nucleotide collection



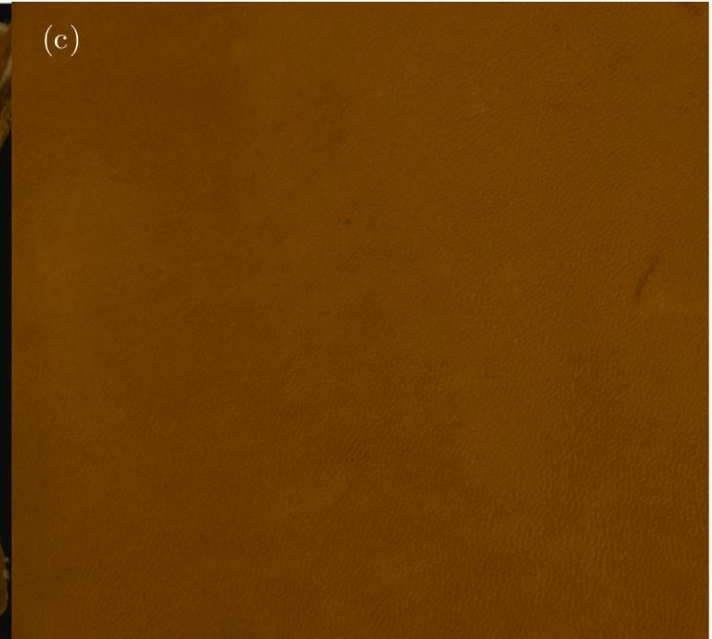
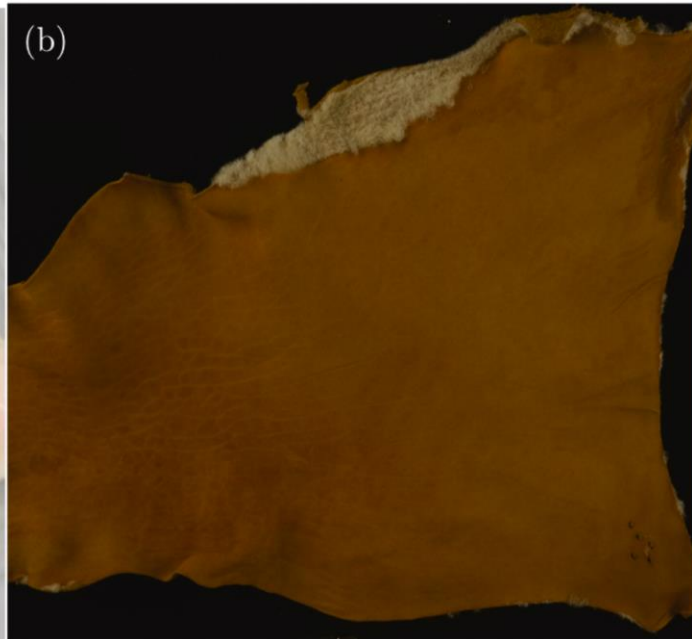
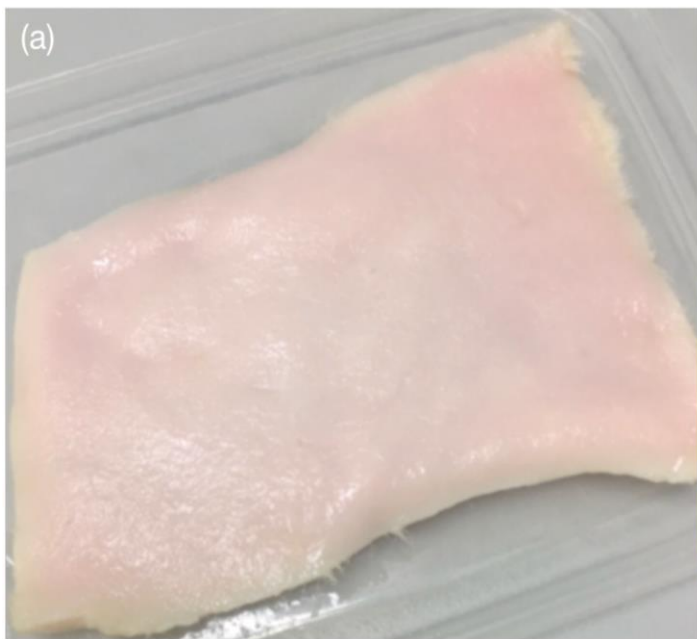
NCBI BLAST identification	Accession	Primer used	% Identity	Culturing media	Depilation media
<i>Bacteroides xylanolyticus</i>	MT192666.1	27f/1492r	98.6%	Malt	Sterilised permeate
	MT459291.1	Eub338/Eub518	100.0%		
<i>Citrobacter europaeus</i>	NR_156052.1	Eub338/Eub518	98.8%	Malt	Whey
<i>Enterobacter</i> sp.	MH477686.1	Eub338/Eub518	99.4%	Wilson's media	Sterilised permeate
<i>Enterococcus faecalis</i>	MT158867.1	Eub338/Eub518	100.0%	Wilson's media	Whey
<i>Empedobacter falsenii</i>	MN198120.1	Eub338/Eub518	98.7%	MRS	Sterilised permeate
<i>Escherichia fergusonii</i>	NR_074902.1	27f/1492r	99.1%	LB	Whey
	MT645516.1		100.0%	MRS	Sterilised permeate
<i>Escherichia coli</i>	CP066366.1	27f/1492r	100.0%	LB	Whey
	MW846276.1	Eub338/Eub518	100.0%	MRS	Sterilised permeate
<i>Hafnia alvei</i>	LR699008.1	27f/1492r	98.9%	TSB	Whey
	KC210872.1		99.1%	Wilson's media	Sterilised permeate
			98.3%		Raw milk
	KX674363.1		99.4%		Milk
<i>Hafnia paralvei</i>	MT470952.1	27f/1492r	99.2%	TSB	Whey
	NR_116898.1		99.7%	MRS	Sterilised permeate
			98.8%	Wilson's media	Milk
	NR_025334.1		99.2%		Milk powder
	MT470952.1	Eub338/Eub518	99.4%		Milk
	MN868256.1		99.3%	Malt	Sterilised permeate
<i>Klebsiella aerogenes</i>	MW784626.1	Eub338/Eub518	99.3%	Wilson's media	Sterilised permeate
<i>Kurthia gibsonii</i>	MN966854.1	27f/1492r	99.5%	Malt	Sterilised whey
	MK898830.1	Eub338/Eub518	100.0%		
<i>Lactobacillus brevis</i>	NR_116238.1	27f/1492r	99.5%	MRS	Whey
	MG722900.1	Eub338/Eub518	99.4%		
			100.0%	Malt	Sterilised permeate
<i>Lactobacillus curvatus</i>	MT645312.1	Eub338/Eub518	99.4%	MRS	Sterilised permeate
<i>Lactobacillus graminis</i>	MN640858.1	Eub338/Eub518	99.3%	MRS	Milk
<i>Lactobacillus plantarum</i>	MF623219.1	27f/1492r	97.9%	MRS	Raw milk
	MK652787.1	Eub338/Eub518	100.0%		
	EU931245.1		99.4%		Milk
	KT626385.1		100.0%		Sterilised permeate
			99.4%	Malt	Sterilised whey
<i>Lactococcus lactis</i>	NR_113960.1	27f/1492r	100.0%	TSB	Whey
			99.2%	Malt	Milk
	GQ337875.1		98.5%		Sterilised whey
			98.2%		Sterilised permeate
	MT545096.1		99.5%	MRS	
	MT597705.1		99.9%		Raw milk
			99.9%	Wilson's media	Sterilised whey
	MH666046.1	Eub338/Eub518	100.0%	MRS	Raw milk
			98.6%		Sterilised permeate
			99.3%	Wilson's media	
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	NR_040954.1	27f/1492r	99.3%	Wilson's media	Milk powder
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	MF108188.1	27f/1492r	99.7%	Malt	Sterilised permeate
<i>Leuconostoc holzapfelii</i>	NR_042620.1	Eub338/Eub518	98.8%	Malt	Milk
<i>Lysinibacillus macroides</i>	NR_114920.1	27f/1492r	97.9%	MRS	UHT milk
<i>Pseudomonas</i> sp.	KJ496054.1	27f/1492r	100.0%	Malt	Sterilised permeate
	MW844014.1	Eub338/Eub518	100.0%		
<i>Proteus vulgaris</i>	KX867797.1	27f/1492r	98.1%	MRS	Sterilised whey

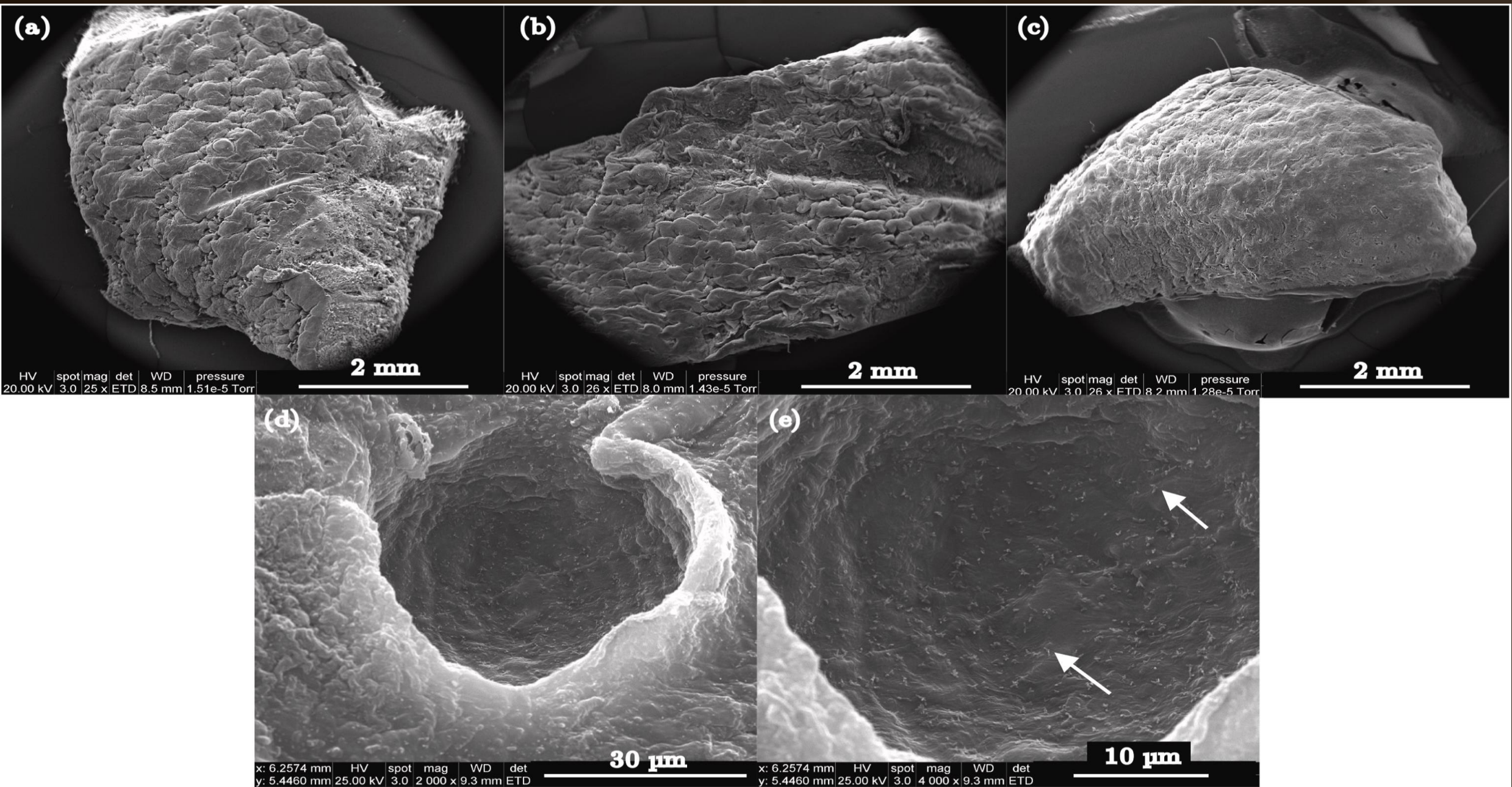
Fungal species identified in the sheepskin depilation trials using various milk products and by-products.

NCBI BLAST identification	NCBI Accession	% Identity	Culturing media	Depilation media
<i>Galactomyces candidum</i>	KY457577.1	99.7	Wilson's media	Milk
		99.5		UHT milk
		99.7	TSB/LB/MRS/Malt/Wilson's media	Fresh whey
<i>Geotrichum candidum</i>	KY977411.1	99.7	MRS/Malt	Sterilised permeate
		99.7	Malt	Sterilised whey
<i>Pichia insulana</i>	NG_063091.1	99.5	Malt	Sterilised whey
<i>Rhodotorula</i> spp.	MT569975.1	99.5	Malt	UHT milk
<i>Trichosporon lactis</i>	NG_070852.1	99.7	Wilson's media	Milk powder
<i>Yarrowia lipolytica</i>	MH545931.1	99.2	Wilson's media	Sterilised permeate
		99.1	Malt	Sterilised whey
		99.7		Milk

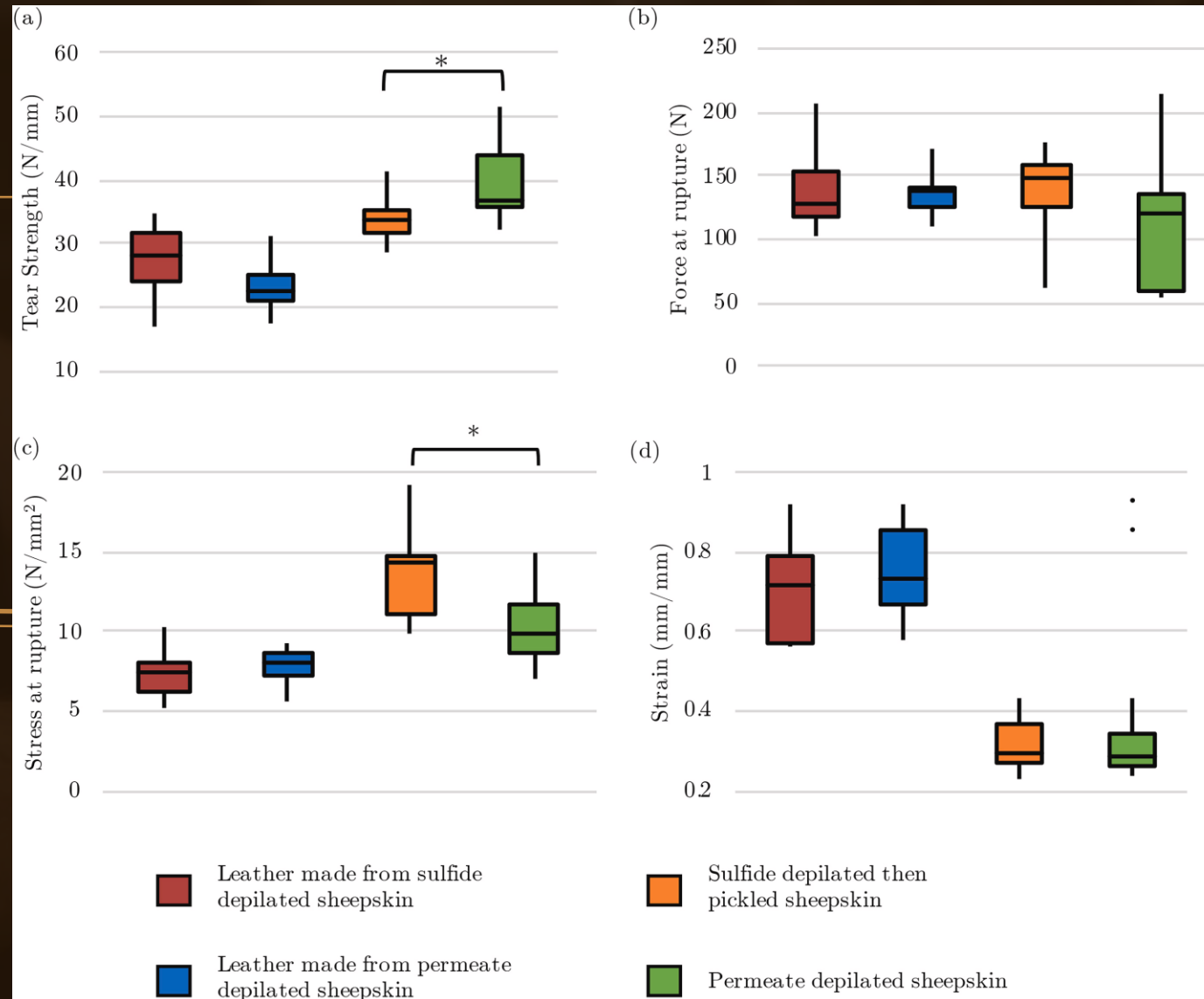


## Appearance of sheepskin









## Eco-friendly Process

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Prevention of putrefaction likely due to the high concentrations of lactose at the initial stages of the process.

- Bacterial growth on the unwashed skin and wool controlled by restricting the carbon source at the early stages of depilation in combination with a gradual reduction in pH of the depilation liquid, due to the production of lactic acid.
- Lactobacillus and Lactococcus species are known to produce many antimicrobial substances



## Eco-friendly Process

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- Bacteria and fungi are known to secrete proteases and glycosidases.
  - Those found in this environment are known to secrete enzymes that could preferentially attack the structure around the hair follicle, loosening it to allow easy removal, but without disrupting the structure of the skin.
    - Watch this space!