The Extraction of Collagen from Ovine Skins using Chemical and Biochemical Processes

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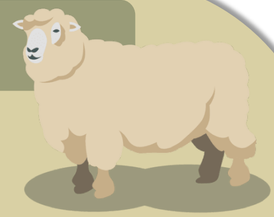
INTRODUCTION

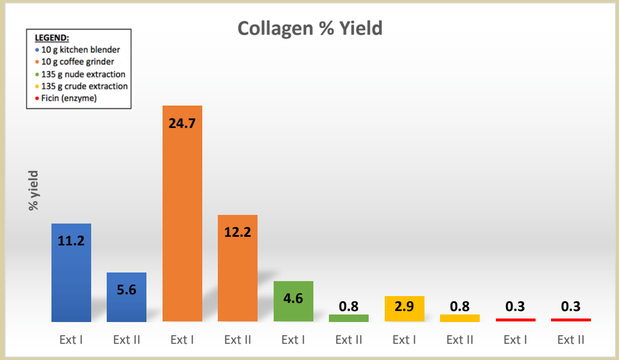
Collagen is the main structural protein of the body and makes up to 35% of total body protein content. It exists as an insoluble macromolecule due to its triple helical structure. Type I collagen is the most abundant and comes from skin, bones and tendons. It has functions in strength, elasticity and tissue development. This makes it an attractive source in medical, cosmetic & dietary industries particularly for repair and regeneration of skin, bone and joints.

Collagen is typically sourced from bovine and porcine by-products however, these animals often have disease associations as well as religious implications. There is a current search for alternative sources of collagen. Little literature exists on sheep collagen. Because lamb is a major New Zealand export, there is a potential commercial venture in the production of sheep collagen. The aim of this project was to see whether we could extract collagen from ovine skins using acidic and enzymatic methods typically carried out on porcine and bovine skins [1].

RESULTS



Yield



↑ Surface Area (SA) = ↑ % Yield. Significantly decreasing yield due to freeze-thaw which destroys the structural integrity of tissue.

FT-IR spectroscopy

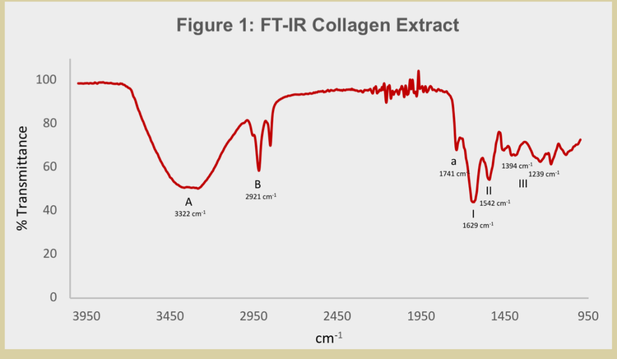


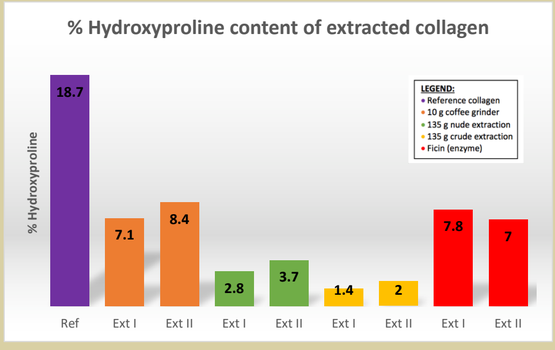
Figure 1: FT-IR Spectral Data

Chart Label	Peak Identification	Spectral Peak (cm^{-1})	Peak association
A	Amide A	3300 – 3440	NH stretching vibrations
B	Amide B	2940 – 3100	NH stretching vibrations
a	Acetic Acid	1740 - 1745	From chemical extraction
I	Amide I	1600 – 1660	Secondary structure
II	Amide II	1510 - 1580	Secondary structure
III	Amide III	1220 – 1400	Triple helical structure

Observations

The chemical extracted material was insoluble but could be hydrolysed and cooled into gelatinous product. Gelatine is the irreversibly hydrolysed form of collagen. This could be further hydrolysed into a soluble collagen. The acid-enzyme extracted material was already a more soluble product before hydrolysis.

Hydroxyproline colorimetric assay



Collagen amino acid sequence consists of repetitive Gly-Pro-Hyp motif. Hydroxyproline is a major component of collagen and plays a key role in the stability of its structure. It is found in few other proteins so is a good collagen indicator.

METHODS

PREPARATION

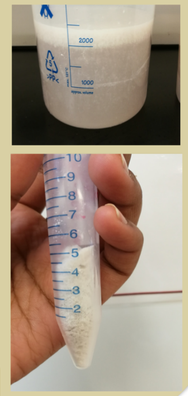
- Cut tissue into 1x1 cm pieces
- Tweeze off wool

PRETREATMENT

- Blend tissue in 0.5 M Sodium Acetate
- Incubate 12h at 4ºC
- Recover tissue by filtering on 129 µm mesh

EXTRACTION (x2)

- Blend in 0.075 M Sodium Citrate
- Incubate 12hr at 4ºC
- Filter on 129 µm mesh
- Add 0.5 M acetic acid to precipitate protein
- Centrifuge at 4ºC at 4500x rpm for 15min
- Collect pellet, freeze-dry



CONCLUSIONS

- Acidic methods successfully extracted collagen in 3.5 days
- Smaller tissue (larger SA) improves yield
- Hair removal improves yield
- Freeze-thawing of stored tissues damages structural integrity and quality of tissue resulting in significantly lower yield of collagen extracted
- Enzyme *Ficin* improves product solubility but yield improvement require optimal conditions

FUTURE OBJECTIVES

- Total Amino Acid Analysis
- SDS-PAGE (molecular weight, purity, collagen type)
- De-fatting step in pre-treatment
- Chemical treatment for wool removal
- Other chemical and hybrid methods
- Yield improvement

References: [1] Voytik-Harbin, S., Kreger, S., Bell, B., Bailey, J. (2008). Collagen preparation and method of isolation. *Patent number: US 2008/0268052 A1.* Purdue Research Foundation.
[2] Ran, X., Wang, L. (2014). Use of ultrasonic and pepsin treatment in tandem for collagen extraction from meat industry by-products. *Journal of the Science of Food and Agriculture, 94.* 585 - 590.