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Bovine Collagen Isinglass

Collagen is suitable for use in the clarification of fermented liquids such as beer and wine. The term 'fining' is used to describe the process in which the suspended matter in the wine or beer is brought to the bottom of the treatment vessel. The standard fining agent used in the brewing industry is piscine isinglass, a collagen-based product derived from the swim bladder of certain fish. Brewers regard the use of isinglass as a highly cost-effective pre-treatment aid prior to filtration.

The collagen from the swim bladder tissue is extracted with dilute acid solutions resulting in solubilisation of the collagen through the breaking of intermolecular bonds. However, the process must be carefully controlled to maximise both the yield and molecular size of the soluble collagen. Disruption of bonds within the collagen molecules results in denaturation of collagen to form gelatine. This denaturation is accompanied by the dissolution of the collagen molecule into randomly coiled polypeptides. The conformations of the collagen molecule must determine the molecular functionality since the transition to the randomly coiled conformation of gelatine results in significant loss in its performance as a fining agent.

The molecular weight of the isinglass is also of importance. The larger the molecule, the greater the number of charged sites available for combination with other particles—thereby giving larger aggregates that will sediment more quickly. The larger molecules are able to protrude further from the surface of a particle, such as a yeast cell, enabling bridges to be more readily formed with other particles, thereby assisting flocculation.

Piscine isinglass has traditionally been considered to be the best fining agent for the brewing industry; however, it does have some disadvantages.

- The fining performance of an isinglass preparation is related to the source of the fish bladder raw material, ie fish species.
- It has low thermal stability which is attributed to the low hydroxy proline content of fish collagen.
- Its batch-to-batch performance has been seen to be inconsistent.
- It is expensive.
- Its availability is limited.



Due to the varying quality of piscine fining material, there is a market opportunity in the brewing industry for an improved alternative to piscine fining agents. Supply of a more thermally stable fining agent would be of significant importance to the brewer in the commercial environment.

As early as the 1930s, cattle hides were recognised as having significant potential to replace piscine isinglass for fining purposes. In particular, the thermal stability of hide collagen made it an attractive alternative to fish collagen.

Manufacture of bovine isinglass

De-hairing

In order to utilise bovine hide as a raw material for collagen extraction, it must first be de-haired and de-fleshed. This results in the isolation of the collagen-rich corium layer as the base material for the collagen extraction.

Cattle hair is composed primarily of a single structural protein, keratin. Individual keratin molecules are held together to form hair fibres by covalent disulphide bridges between cysteine amino acid residues. These bonds can be easily broken by chemicals with reducing ability, such as sodium sulphide.

Lime sulphide process

Prior to the development of an acidic treatment to remove hair, the hides were treated with alkali and sulphide to open up the skin. A major part of this opening-up process consisted of removing protoglycans which help to stabilise the structure of the collagenous proteins. The alkaline process modified the protein by partly removing amine and amide groups, with swelling and hydrolysis of the amide groups occurring during the early stages of the process, and a noticeable evolution of ammonia. This amidolysis of the collagen is undesirable as is the considerable occupational health and safety problems associated with handling lime-sulphide.



Acid process

The acid de-hairing process avoids these disadvantages and has the advantage of being less time consuming. In acid de-hairing the bovine hides are soaked in 3% acetic acid solution (pH 2.3) at 25°C for 48 hours. The acid bath is held at this temperature, with occasional agitation applied, until the pH of the solution and hide 'splits' equilibrates. After soaking, the hides are removed and scraped on both sides to remove hair, epidermis and any residual flesh and fat. This leaves a collagen-rich corium layer.

The corium layer is then comminuted to enable collagen to be extracted in an efficient manner. Heat generated during comminution will damage the collagen (converting it to gelatine), so every effort must be made to reduce the possibility of heat generation. The following factors will assist in reducing heat generation in the acid-swollen collagen.

- The choice of equipment—a cutter, such as a Comitrol flaker, is less likely to generate heat than a mincer.
- Equipment set-up—if possible cool the equipment. A chilled water-cooling coil can be wound around a mincer throat to reduce heat build-up in the throat casting.
- Equipment maintenance—all blades must be sharpened regularly. Blunt blades generate more heat.
- Raw material temperature—pre-cool the hide pieces (to -20°C is recommended) after acid treatment prior to comminuting.
- Ice inclusion—ice can be added directly into the throat of the comminution equipment with the hide pieces.

Acid-swollen collagen is very heat sensitive and the need to avoid heat generation during comminution cannot be overemphasised.

Extraction of collagen with acid

A number of methods have been investigated for the extraction of collagen isinglass from the comminuted corium layer. The methods used a variety of extraction times and temperatures, acid types and concentrations, and enzymes to assist extraction. The combinations trialed are summarised in Table 1.

Table 1. Extraction conditions for bovine hide

Isinglass	Solvent	Enzyme	pH	Temp.°C	Duration(h)
Sample 1	3% Acetic Acid		2.3	25	24
Sample 2	3% Acetic Acid	pepsin	2.3	25	24
Sample 3	3% Acetic Acid (extracted twice)	pepsin	2.3	25	48
Sample 4	5% Acetic Acid	pepsin	2.3	25	24
Sample 5	3% Acetic Acid	aspergillus saitoi	2.3	25	24
Sample 6	3% Acetic Acid	aspergillus oryzae	4.8	25	24
Sample 7	3% Acetic Acid		4.8	25	24
Sample 8	5% Acetic Acid		2.3	25	24
Sample 9	3% Acetic Acid	papain	2.3	25	48
Sample 10	3% Acetic Acid	pronase	2.3	25	48
Sample 11	3% Acetic Acid	pepsin	2.3	25	72
Sample 12	0.1M Hydro- chloric Acid	pepsin	2.3	25	48

The hide-to-solvent ratio used was 1:70; with the hide-to-enzyme ratio from 100:1 for aspergillus oryzae and aspergillus saitoi, to 125:1 for papain and pronase.

Due to the viscous nature of the solubilised collagen the isinglass is ideally produced at a concentration of 4% by weight. It can be stored and transported to users at that strength or it can be concentrated by separation of water though the addition of certain salts. The paste formed requires reconstitution before use. Alternatively it can be freeze dried for later reconstitution.

Bovine isinglass performance

This method for processing isinglass from bovine hide has proven simple and efficient. The freeze-dried bovine isinglass was completely re-solubilised in 5 to 10 minutes, requiring less time than commercial piscine isinglass to be solubilised under the same conditions. This is an important advantage for bovine isinglass as it is essential that the isinglass is completely solubilised to be effective as a pre-filtration aid.

Product from all processing variations assessed have been analysed for collagen content, molecular weight, total solids and amino-acid profile. All products have also been trialed for fining ability and stability against known commercial piscine isinglass.

The determination of collagen contents showed the highest concentrations (75–85%) occurred in isinglass prepared by extraction with pepsin, aspergillus oryzae and aspergillus saitoi enzymes. The collagen contents in these samples were significantly higher than in the other samples, demonstrating the effectiveness of these enzymes in the extraction process.

The fining test showed that the bovine isinglass, produced by this simple method exhibited relatively superior fining to commercially available piscine isinglass—clarifying beer within 8 hours. The floc in the beer was quick to settle to a compact cake in the bottom of the settling vessel. Following freeze drying and reconstitution the fining efficiency was found to be greater than when used as a paste. A similar effect has been noted with piscine isinglass.

Estimated market value for isinglass

1997 figures indicate Australian beer production as some 1,800 million litres and Australian wine production at some 400 million litres. At a usage rate of 20 milligrams per litre, these industries would use approximately 37 tonnes and 8 tonnes of isinglass respectively per annum. The value of this market is estimated at \$3.6 million based on an isinglass price of \$80 per kilogram.

Estimated enzyme costs for production

The major processing cost would be the cost of the enzyme. For every tonne of beef hide processed, approximately \$900 worth of pepsin would be required for a single extraction—or approximately \$675 worth of pepsin if extracted twice,

recycling the pepsin. 800 tonnes of hide would be required to produce the 45 tonne of isinglass required for the Australian market. The total enzyme cost for isinglass production for the Australian market would be some \$600,000.

Further reading

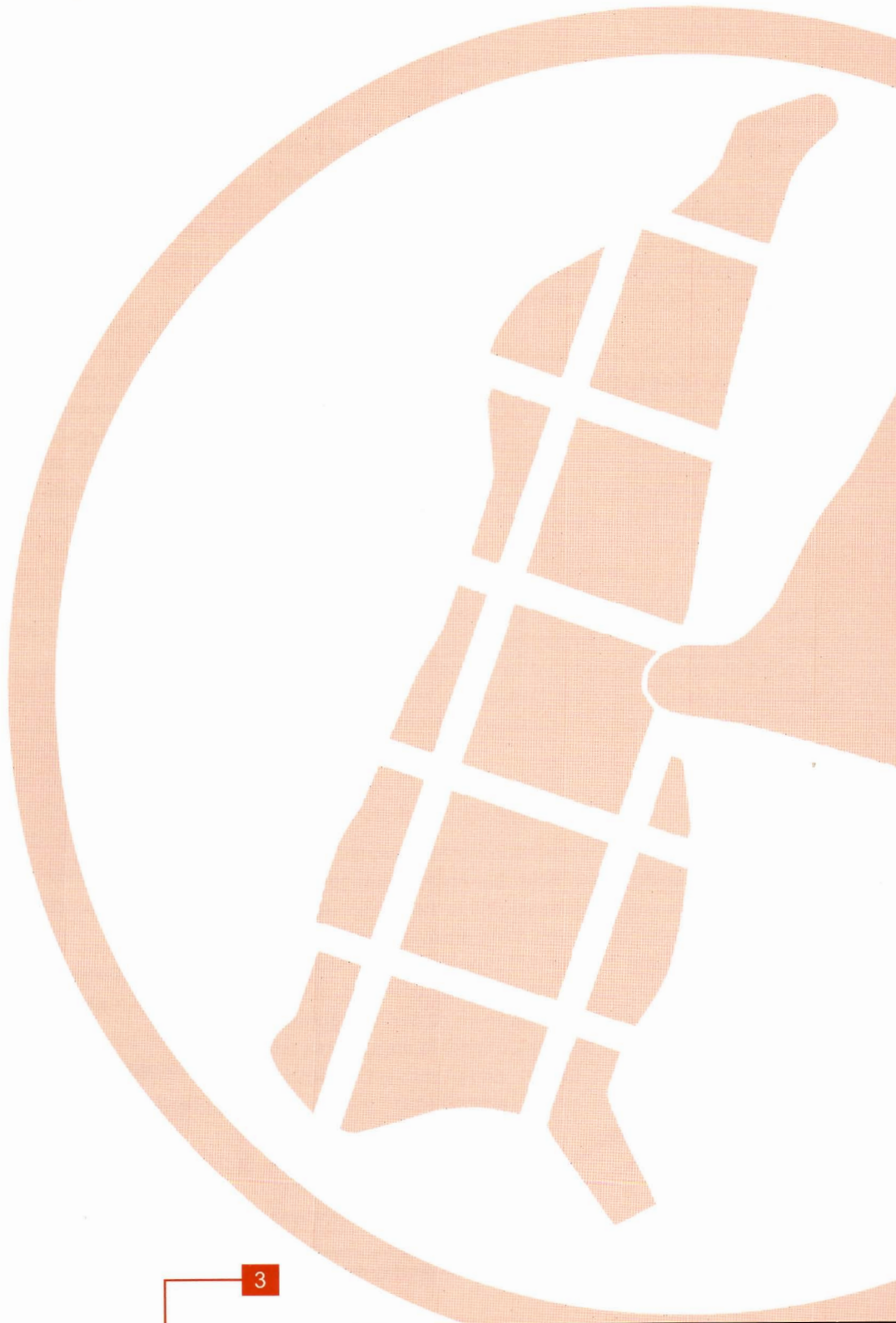
This information is a summary of information from the following project funded by the Meat Research Corporation.

- Project M218: Collagen Utilisation

Further detail is available from the final project report of Module B—Part 1 for this project, which is available from Meat and Livestock Australia.

Related information is given in the following MLA Co-products brochure.

- Edible Collagen Coatings



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