**Final Report** 

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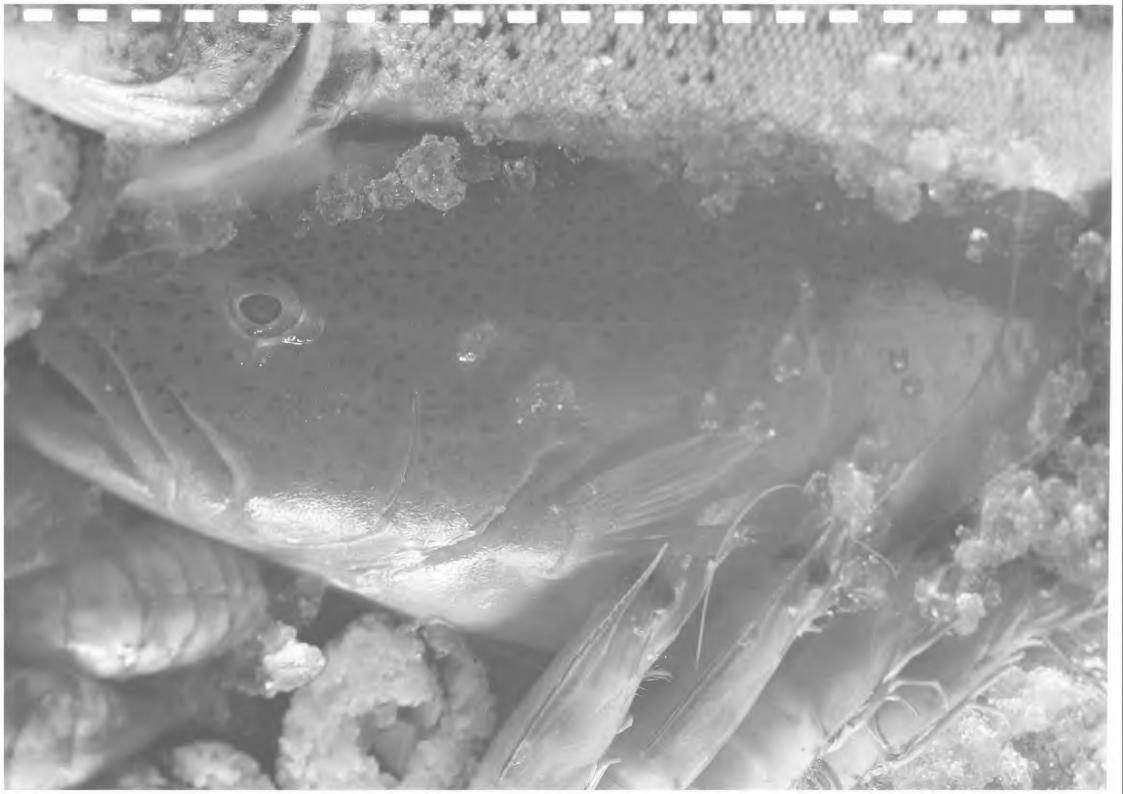
## The Manufacture of a Liquefied Fish Collagen Product for use as a Finings Agent in the Brewing Industry

by

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### SUMMARY

This project was undertaken in collaboration with Pacific Export Services Queensland Pty Ltd and had the primary objective of developing a liquefied fish collagen product (known as Isinglass) on a pilot commercial scale for the domestic brewing market.

The investigations undertaken in this project required several discrete development steps:

- sourcing of the raw material,
- removal of excess fat and mucous from the bladders,
- development of processing protocols,
- establishment of suitable quality manufacturing and testing procedures, and
- identification of the markets and market requirements.

Considerable time and effort was committed to the development of the appropriate techniques and equipment necessary for the production of a quality beer fining product produced from fish swim bladder. The procedure, which is currently employed for Isinglass manufacture, begins with sun-dried swim bladders (or maws) which are sourced mainly from developing countries in the tropics (eg Asia, Africa and South America). The maws are then subjected to a sequence of dry milling steps to make the product amenable to incorporation into the beer process stream. The final product has low moisture content and is generally packaged to ensure that it arrives at the ultimate destination in an active form. In this state, it does not require refrigeration, although any heat applied to this collagen powder is likely to affect the ultimate quality of the final product. Before this dried product is used, it is shear mixed with a cutting agent (usually citric or tartaric acid). The swollen collagen fibres are then introduced into the beer to enhance the fining process. Although the ultimate aim of this project was to produce a beer fining aid, the main research was directed toward producing a concentrated and stable liquid emulsion of the fish maw that was ready for direct injection into the beer.

This project has developed ideas also in the area of greater utilisation of the Australian fish resource, particularly the maw of the Barramundi and other tropical fish. The project has successfully developed a method for the manufacture of a finely emulsified white paste derived from the swim bladder of the barramundi. The commercial partner was satisfied with the results of the project, although the cost of production did not make it sufficiently competitive in the marketplace against imported products, and hence is not currently in production.

### INTRODUCTION

Isinglass powder has been used for many years for the removal of yeast (fining) in beer production. This project was undertaken to develop a product to fit into a perceived niche market – specifically beer fining using a liquid Isinglass product from Australian resources.

Information regarding the actual methods for the production of Isinglass powder was difficult to obtain, possibly because it was proprietary knowledge. Although a number of patents have been granted in this area, the process outlined in this report was developed for this particular product and manufacturer using the limited information available from these sources. It was a requirement of the process for the participating commercial partner that the capital outlay not be too prohibitive in the first instance.

The project developed from discussions between Dr Davis of the Centre for Food Technology and the commercial partner (Mr Trevor Jordan of Pacific Export Services Queensland) with the support and encouragement of Manager of the National Seafood Centre. A significant market demand exists in Australia and there is also potential to supply product into export markets. The product has the potential to import replace and to utilise an otherwise wasted resource. Although the size of the market and the value of the finished product are not freely available, it was decided that the potential financial rewards warranted the risks involved in the venture. Although small by comparison with the current market producers of the powdered Isinglass product, this liquid product would occupy a niche market in the domestic market and would be likely to be able to be supplied at a competitive price. The primary purpose of this project was to develop a liquid collagen product for an existing and growing market. The aim of this project was to develop and optimise the processes for the production of a liquid fish collagen product for domestic use from local raw materials. The project focused primarily on developing a process to manufacture a liquid Isinglass product with comparable or superior characteristics to the imported material.

These product characteristics are:

- a) a fine particle size (<40  $\mu$ m)
- b) minimal fat contamination (reduces the fishy smell and taste)
- c) a white colour
- d) a high protein content (especially collagen)
- e) low microbiological levels
- f) food standard-acceptable proximate values (heavy metals, etc).

Additionally, relevant proximate and microbiological analyses were developed for the product to compete in the marketplace. A pilot scale preparation was trialed in a domestic brewery and the likely costs of full-scale production were determined.

Further value-adding initiatives (e.g. packaging, characterisation of fining capacity, etc.) were investigated and experiments to develop assay procedures for the determination and characterisation of fining capacity were undertaken.

### AIM

### MATERIALS AND METHODS

The commercial partner expressed a preference for Australian (and Queensland) fish maws, which had been collected from fish, caught for meat either from the wild fisheries or from aquaculture. This decision was based on the utilisation of an existing waste stream and on the likely price and quality of the local raw material. Much of the imported material is sourced dried because it comes from countries with low labour costs and poor access to hygiene and freezing technologies. Financial considerations placed important restrictions on the level of size reduction of the fresh maws that could be performed before delivery to the manufacturer. These considerations, along with the final product requirements, were important contributors to the process that was developed for the manufacture of Isinglass for the commercial partner. Each of these steps required some level of process development that will be outlined in as much detail as possible. The flow diagram below outlines to major steps in the production of such a product.

Raw material (clean frozen Barramundi swimbladders)

 $\downarrow$ 

Coarse chopping (to open the swim bladder)

 $\downarrow$ 

Cleaning and Sanitation (removal of fat and mucous and reduction of microbiological count)

 $\downarrow$ 

Wet Milling (to produce a fine white paste)

 $\downarrow$ 

Packaging (to meet market requirements)

# Figure 1. A flow diagram representing the process developed for the preparation of Isinglass paste.

### (i) Raw material

The commercial partner expressed a desire that (wherever possible) the raw material be derived from local (Queensland or Australian) fisheries and that (if practical) this be a by-product or waste stream of an existing industry. This desire was partly driven by financial concerns, but also by an interest in the conservation of our marine resources and the more total utilisation of the catch. For the purposes of cleanliness and sterility of the final product, the cleaned maws were preferably sourced from export registered fish processing premises, although this was difficult to arrange when many of the Gulf fishermen processed their fish on the boat. To facilitate the later steps in the processing, the maws were purchased in a "knife-clean" form (i.e. slit and free of mucus and adherent fat). Storage of this material in a frozen state from collection until processing is important for the production of a quality final product. Material should be as free as possible of blood, flesh and other debris, and packed into clean poly bags or boxes (see below). These procedures are necessary to minimise the potential for enzymatic or bacterial spoilage of the maws. Comparisons between Isinglass products derived from Barramundi and Ling suggest that the product derived from tropical species have some superior characteristics (proximate composition, yield and fining capacity). Availability, accessibility and price were also contributing factors in the decision to use maws from local Barramundi fishermen.



Figure 2. Knife-cleaned barramundi swimbladders.

### (ii) Cleaning and Sanitation

The cleaning and sanitation steps are undertaken as quickly as possible. Frozen swimbladders were removed from frozen storage defrosted under running water. The thawed maws are chopped coarsely and any fat and mucous is removed by an extended rolling of the product in a chlorine solution. This ensures that any undesirable odour and flavour characteristics of the product are minimised. Additionally, the potential for bacterial contamination will be severely reduced in a subsequent peroxide treatment. The protein content of the final product will be represented almost entirely by collagen, and the presence of fat in the final product should be minimised.



Figure 3. Rolled barramundi swimbladders.

### (iii) Wet Milling

The use of heat in any of the processing steps for the production of a fining agent derived from fish maws is likely to compromise the final quality of the product. This is one of the reasons that the project was directed toward developing a wet paste without the requirement for a drying step that is always associated with protein denaturation.



Figure 4. Milled barramundi swimbladder paste.

### (iv) Product specifications

Tests to substantiate the quality of this Isinglass product in the marketplace include indicative levels for the more important elements of these analyses - general proximate analyses and microbiological analyses. General proximate analyses include determinations of the protein, fat, moisture, carbohydrate and ash components of any given batch of Isinglass paste. These analyses are very useful to prospective purchasers of the final product as they give some useful information of the product source and method of processing, but have not been used by the existing manufacturers. Microbiological analyses are necessary to ensure that the product meets the relevant health regulations, and these are routinely provided with the products. A standard plate count (SPC) and an enterobacteriaciae count are the standard tests performed on each batch of Isinglass paste. The SPC should be less than 500 colonies/gm (see the Appendix) and the *E.coli* count should be less than 1 colony/gm. Specialist analyses are performed in a brewery laboratory in order to get an indication of the functional capacity of the final product.

### (v) Packaging

The initial requirement for packaging was for bulk transportation. This was achieved using 10 Kg plastic buckets, but would need further investigation if the product were to be commercialised. These containers would be used for the transportation of product to final destinations (locally, interstate and overseas). A selection of smaller commercial plastic bottles was sourced, and appropriate seals and lids were tested.

#### **RESULTS/OUTCOMES**

Frozen swimbladders (15 Kg) were removed from frozen storage and defrosted under running water. A Chlorine solution was then added at a concentration of 2% chlorine (600ml per 30L) and was replaced twice daily for two days. The swimbladder material was minced through a coarse plate and agitated overnight in a rotary mixer with 6%  $H_2O_2$  (1.8 L of hydrogen peroxide in 30L of water). The product became swollen, leaving little free water (the moisture content of this product was estimated to be 90%) and was finely minced before being used for the final paste preparation step.

A solution of metabisulphate (3 g) in 150g of water (final concentration of 3000ppm) was prepared. Finely minced swimbladder material was added to 400g of water and "cut" at the highest blade setting using a bench-top emulsifier. 50g of the metabisulphate solution was added, stirred and cut at low blade setting. A further 50g was added and "cut". The last 50g of the metabisulphate solution was added but not cut. It was stirred and massaged into the product. The pH was measured to be 3.5. This was the resultant paste stage of the Isinglass process. Several weeks of trialing resulted in a quality paste being produced on a reproducible basis. The results outlined below summarise the fining trials undertaken at the Castlemaine Brewery.

	Haze	Coulter	Yeast (*10 <sup>6</sup> )/ml
Control	30.0	4530	2.3
Magicol	14.8	4627	2.3
1% Paste	12.3	4030	2.0
5% Paste	12.7	4630	2.3

The haze is an in-house test that measures the suspended material in the beer, the Coulter result is a real measure of the cell number in the beer after fining, and the yeast measure is calculated from the Coulter counter reading. Control refers to the currently used product (a rehydrated powder), Magicol refers to another commercial paste product which was being trialed, and the Paste products are produced at the Centre for Food Technology. These results are typical of a number of trials and show that the paste products manufactured at the Centre for Food Technology are better at reducing the haze value and at least as good at removing the yeast from the beer.

The result of this project was the development of a finely emulsified liquid Isinglass paste derived from the swimbladder of the Barramundi. Isinglass is used in breweries throughout the World. There is a growing market in Asia, where large scale commercial brewing is developing rapidly. Four large multi-national companies dominate the Isinglass industry. Current factory capacity of the commercial partner and raw material supply would limit the market penetration of the final product. The success of this project was dependent upon the resolution of many problems at all stages of production. Ultimately, customer acceptance and the comparative chemical and microbiological analyses assess the product quality. The inability of this product to compete on the basis of price has meant that the commercialisation of this project will not occur at the present time.