FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

New Opportunities for Seafood Processing Waste

Appendix 12: Barramundi Swim Bladders: Optimisation of Sanitising, Cleaning And Drying Of Air Bladders For Human Consumption

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WAFIC
WESTERN AUSTRALIAN FISHING INDUSTRY COUNCIL INC

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1. Objective

The objective of the project is to develop a cost-effective method to sanitise and dry air bladders (fish maw) to provide a suitable and safe product for the Chinese and Hong Kong export markets.

2. Background

2.1 Introduction

Swim bladder or air bladder is an internal gas-filled organ found in most fish, widely used as raw material for making fish maw (Sinthusamran, Benjakul, and Kishimura 2013). Fish maw is also known as fa kau (花胶) in the Chinese dialect, Cantonese. Fish Maw is actually extracted fish air-bladder that is cleaned and sun dried. In the Chinese cuisine, fish maw is a delicacy. When cooked, it has a marshmallow texture and tastes bland. To add taste, it is often braised or double boiled in soups.

Due to its soft texture and unique taste coupled with therapeutic values, fish maw is considered as a luxury food in China and South-East Asia (Sinthusamran, Benjakul, and Kishimura 2013). Chinese consumers place fish maw in soup. It has a texture of a sponge and soaks up the soup. It does not have a fishy taste.

A large number of fish are used as sources for the production of fish maw. These include Brown croaker (*Protonibea diacanthus*), Seabass/barramundi (*Lates calcarifer*), Croaker (*Otolithes* spp.), Giant catfish (*Arius thalassinus*), Bigeye snapper (*Priacanthus tayenus*), Solder croaker (*Boesmania microlepis*), Eels, Ling, Congor pike, Cod (Sinthusamran, Benjakul, and Kishimura 2013) yellow tail tuna, big head carp (*Hypophthalmichthys nobilis*) (Kaewdang et al., 2014), Sturgeon (Koochekian, Ghorban and Yousefi, 2006).

In other countries, air bladders can be salted on board and dried before being further processed into “isinglass” which is commercially widely used in the clarification of beer and wine (Koochekian, Ghorban, and Yousefi 2006).

The project partners, Dried Seafood Corporation, have demonstrated an Asian market interest in dried Barramundi air bladders. According to the Australian Fisheries Research and Development Corporation (1994), Barramundi is preferred for its premium grade fish maw

and thus, the fish maw from Australian Barramundi can become a valuable export commodity.

2.2 Proximate Composition of swim bladders

The swim bladder weight in sturgeons accounted for 0.5 to 1.0 % of the entire fish while volume wise, the swim bladder could reach up to 4 to 11 % of the total fish volume (Koochekian, Ghorban, and Yousefi 2006).

Results from a study on the proximate composition, amino acid and fatty acid composition of fish maws of *Cynoscion acoupa (*croaker sp)*, Congresox talabonoides* (eel sp) and *Sciades proops* (catfish sp) indicated high protein and low fat content (Wen et al. 2016). The protein was particularly rich in functional amino acids where the ratio of functional amino acids to total amino acids (FAA:TAA) ranged from 0.68 to 0.69 (Wen et al. 2016). Glycine was the most abundant amino acid found in all the three species while arachinodonic acid was the primary PUFA with values higher than those reported in fish muscles (Wen et al. 2016). Croaker *C. acoupa* showed the most desirable result with the lowest value of index of atherogenicity (IA) and index of thrombogenicity (IT) while it showed the highest value of hypocholesterolmic to hypercholesterolmic ratio (Wen et al. 2016). Partially for this reason, the maws from the croakers are the most highly priced (Wen et al. 2016).

Other result from analysis of Fish maw are shown below.

Table 1 Swim bladder proximate compositions – most likely raw swim bladder

	Yellowtail tuna	**Sturgeon**	**Big head carp *Hypophthalmichthys nobilis)***	**Barramundi**
Moisture content	83.33%	65%	75.2%	70.11%
Aw				0.99
Ash	0.29%	4.5%		
Fat	1.44%	2.5%		2.9%
Protein	12.09%	32%		
% weight of whole fish	3.5-5.5%	0.5-1.0%		
pH		6.1		6
Reference	Kaewdang et al. (2014)	Koochekian, Ghorban, and Yousefi (2006)	Kaewdang et al. (2014)	Fisheries Research Development Corporation (1994)

2.3 Quality parameters in fish maw

Parameters that are considered when judging quality of fish maw are described below.

a. Size: The bigger the fish is, the bigger the stomach is thus the higher the price is commanded. Retail customers buy large fish maw and smaller fish maw is purchased by restaurant trade. Size was important, with thicker and larger maws more premium.
b. Source Species: The quality of fish maw also depends on the type of fish. Fish such as sturgeon, hake, conger-pike, croaker and carp get high price for their fish maws. The reason is that these fishes have large and developed air bladders
c. Thickness (related to gender): Male fish's air bladder is thicker and thus commands higher price. Fish maw from males has a thicker body and is soft and smooth and does not easily dissolve in mouth. The female fish maw is thinner and tender to bite
d. Colour: Good fish maw, has to be yellow. The richer the colors are, the more it is able to maintain texture through an extended period of cooking.
e. Age: the older the fish maw is, the better it is. Reason being, it has less oil and less fishy taste
f. Aroma: Aroma is not an influencial factor with dried fish maw, in comparison to other dried seafood.
g. Texture: Cooked fish maw should be soft and thick.

Fish maw grades can be divided into king fish maw, premium fish maw, grass seabass fish maw and fried fish maw in general. King fish maw and premium fish maw are the best quality and most valuable amongst the four types. Grass Seabass fish maw and fried fish maw are however more popular due to their lower pricing. Fish maw is also graded by their gender. Male fish bladder has thicker body and is soft and smooth, not easily dissolved in the mouth while female bladder has a thinner body and tender to bite.

Fish maw substitution does occur and can be distinguished as below.

Colour: Real fish maw has golden yellow colour whereas fake fish maw has light and whitish yellow unnatural colour.
Grain: Real fish maw has natural grain whereas fake fish maw does not have obvious and orderly grain.
Smell: Real fish maw has light sea water smell whereas fake fish maw has none and may carry chemical or plastic smell.

An Australian fish maw study was funded by the Fisheries Research and Development Corporation (1994). Large sized fish maws fetch a higher market price as compared to the smaller ones and therefore, the difference in pricing due to size has a great impact on the viability of drying fish maw (Fisheries Research Development Corporation 1994). Further, the size of the fish maw is an important parameter for the retailer with premium prices

awarded to thicker and larger maws. Retail customers prefer large fish maws while the restaurant trade prefer smaller ones.

The desirable quality attributes of dried fish maw includes,

i. Odour – no fishy odour
ii. Golden brown colour without any stain
iii. Non sticky – indication of collagen being not degraded
iv. Translucent
v. Thick
vi. No staining present
vii. The cooked ready to eat final product must be both soft and thick (FRDC 1994).

The dried swim bladder, particularly smaller sizes, is often transformed into puffed fish maw by frying (Sinthusamran and Benjakul 2015) (Figure 1) and the desirable attributes of a fried fish maw includes,

i. Expanded raw material with desirable cell structure and porous protein network
ii. Mouth feel
iii. Low oil uptake
iv. High expansion ratio

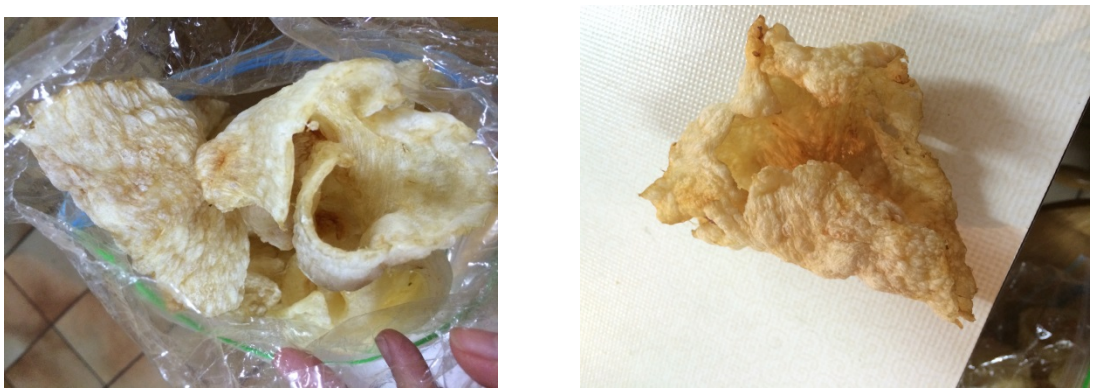


Figure 1: Examples of fried fish maw

Market feedback on previous trials on barramundi air bladder: SEAFOOD AUSTRLIA Centre of food tech REF 1994- pdf)

2.4 Practices to increase quality.

The FRDC study determined that the quality of fish maw is affected by the handling of fish on board. In order to prevent staining and textural damage, it was crucial to ensure the effective removal of the blood vessels and the fatty tissue present on the surface of the raw material before freezing.

Decreasing/preventing gall belly stain or gall burn is a critical quality parameters for fish maw quality (see Figures 2 and 3). A study on the handling and value addition of farmed Barramundi observed belly stain at all times of the year and in fish from all farms (Seafood Services Australia Ltd. 1997). The study noted the high frequency of severe or medium belly burn in fish grown in indoor tanks and starved during the usual purging practice. On the other hand, fish from farm dams and cages where fish were not starved for more than a day prior to harvesting were less affected.

The root cause of severe stain was found to be the increase in the volume of gall stored in the bladder with the deprivation of food prior to harvesting and killing in slurry (Seafood Services Australia Ltd. 1997).

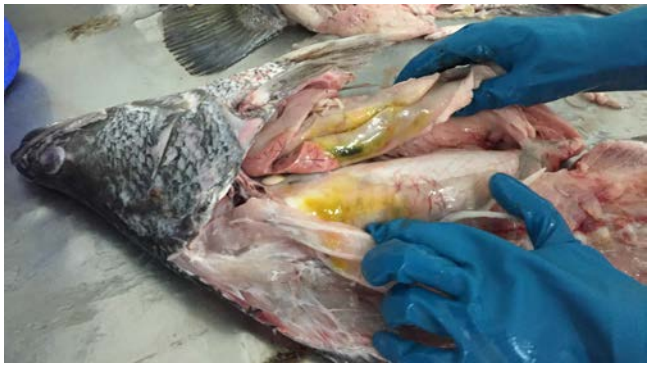


Figure 2 Belly stain in Barramundi swim bladder. Source: Sealanes.

Figure 31 Dried maw from Barramundi with gall belly stain

Belly stain in dried maw from Barramundi is a quality assessment parameter; the severe its prevalence, the poor is its quality and hence, poor consumer acceptability. Therefore, the following recommendations have been provided (Seafood Services Australia Ltd. 1997) with the aim to minimize the incidence of belly stain.

i. Fish farmers should handle live or ungutted fish with care in order to minimize damage to the fish.
ii. Care must be taken during the harvest and killing of fish to ensure there is no unduly stress or physical injury.
iii. Care must be taken to feed any unsold fish after purging to minimize the incidence of severe belly stain.
iv. With the awareness of the nature and cause of belly stain, the fish merchants should trim away any severely stained swim bladder when producing fish maws.

2.6 Market Research on currently available fish maw products

Table 3 summarises market aspects of fish maw.

Table 3: market aspects of Fish Maw.

Appearance	**Notes**
Format sold	Unprocessed: dried only flat, raw Processed : dried and deep fried. Puffy, light On the bone: air bladder still attached to the fish frame
Uses	Soups, Can be stuffed and refried, Dim sum Ssekitoleko said besides being edible, fish maws are used in the manufacture of plane and space shuttle body parts, car parts, surgical stitching threads and anesthetic drugs. Fish maw is also used in the preparation of isinglass which is widely used commercially in the clarification of beer and wine (Koochekian, Ghorban, and Yousefi 2006).
Grading	Gender: Male: thicker body, soft and smooth, not easily dissolved in the mouth Female: thinner body, tender to bite Quality: Premium King Grass seabass Fried
Benefits	Excellent source of collagen High in calcium and phosphorus Low in fat
Colour	Dark or light- no reflection on quality Golden yellow
Smell	Very mild aroma. If the aroma is strong, it is not fresh. No fishy odour. The fishy odor can be removed by salting the swim bladder (Koochekian, Ghorban, and Yousefi 2006).

Examples of commercially available fish maw are shown in Figures 4-6.

Figure 4: Dried Ling fish maw produced by Oceanwild

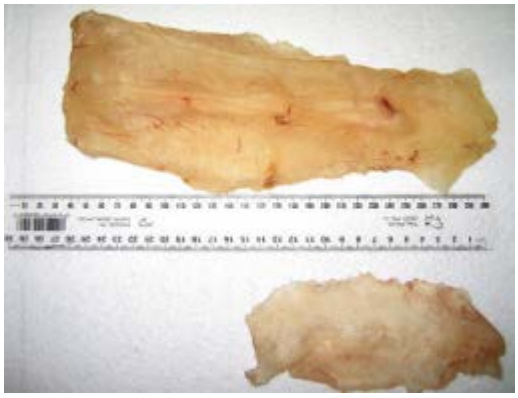


Figure 5: Dried Ling fish maw- Wildfish NZ

Figure 6 On the bone Ling fish maw produced by Wildfish NZ

The results of a survey on market prices for various types of fish maw was conducted by Curtin University. Results are shown in Table 3.

Table 3 The market price of air bladders from different species.

Fish species	Company	Form at	Price	Country	Reference
Cod (*Gadus Morhua*)	Gingason (Iceland)	salted swimming sold (cleaned and uncleaned)		Iceland	http://gingason.is/portfolio-items/cod-swim-bladder-cleaned-gadus-morhua
Ling (*Genypterus blacodes*	Wildfish (NZ)	On the bone Off the bone (fig) Dried (Fig)			
	Oceanwild (NZ)	Dried (Fig)			
	Crystalnest	Dried (NZ)	$298/200g		http://crystalnest.com/new-zealand-fish-maw-200g.html
Jew Fish	On hing dried seafood	Dried and rare (Raw?) Dried –top Dried extra large	AUD $6000/kg AUD $3000/kg AUD $ 2400.kg		http://en.onhing.com.au/goods.php?id=118
Australian fish maw	On hing dried seafood	Large-dried Medium- dried Small- dried	AUD $ 1800/kg AUD $1300/kg AUD $ 800/kg		
Aus King salmon	On hing dried seafood	Dried rare Dried- top Dried- extra large	AUD $1000/kg AUD $700/kg AUD $ 550/kg		
Australian Salmon		Large-dried Medium- dried Small- dried	AUD $450 AUD $350.kg AUD $250/kg		
		Dried	AUD $81/300 g	Taiwan	
		Fried	AUD $11/300 g	Taiwan	

Fish species	Company	Form at	Price	Country	Reference
		Fish maw processed in sauce and vacuum packed	AUD $41/600 g	Taiwan	
		Dried	AUD $112/kg	Australia	
		Dried (large)	AUD $50/kg	Australia	
		Dried (small)	AUD $17/kg	Australia	
Barramundi		Dried-broken pieces	AUD $159/kg	Australia	
		Dried maws (whole small)	AUD $250/kg	Australia	
		Cooked	AUD $1.10/60g	Australia	(Fisheries Research Development Corporation 1994).
Spider maw			US >223-638 /kg for retail or wholesale	NA	(Clarke 2004)
Croakers (Man To)			US >170/kg wholesale	Venezuela	(Clarke 2004)
Fried eel maw			US >21/kg (wholesale)	NA	(Clarke 2004)
Man To (On nam)			US 153/kg (w)	Vietnam	(Clarke 2004)
Pak Fa Kau (white croaker)			US 319/kg	India, Vietnam, Bangladesh	(Clarke 2004)

3. Optimising Sanitation of Harvested Swim Bladders.

3.1 Aim

The aim of this section was to develop methods to reduce microbial load on harvested bladders prior to drying.

3.2 Methods

3.2.1 Samples

Bladders (2-4 mm thickness) were removed from wild caught Barramundi fish on board immediately after harvest, halved, scraped to remove fat and blood and chilled in an ice slurry. They were snap frozen in single layers, separated by plastic sheets and transported frozen to Curtin University.

3.2.2 Sanitising Treatments

Air bladders were thawed the day before use at 4˚C.There was an untreated control.

Sanitizing with Chlorine Dioxide Solution

Three solutions with differing concentrations of Chlorine Dioxide were made in 3.5% salt in cold (<5˚C) filtered water, using Clean oxide tablets.

- 10ppm
- 25ppm˚
- 50ppm

Air bladders were placed in the appropriate solution for 30 minutes.

Sanitizing with Silver Ion Solution

The solution was prepared by adding 10mL/L silver ion solution (17mg/L stock solution provided) into cold (<5 C) water. Air bladders were soaked in solution for 30 minutes.

3.2.3 Microbiological Analysis

Untreated frozen air bladder was analysed for Total Plate Count (TPC), *E.coli* and *Salmonella.* Treated airbladders were analysed for TPC.

3.3 Results

The microbial load of the air bladders before treatment is detailed in Table 4 with high TPC in the untreated control indicating that sanitizing treatments are necessary.

Table 4 Untreated air bladder microbial results

Analysis	**Result**
Total plate count	4 400 000/g
E. coli	<10/g
Salmonella	ND/25g

Microbiological results for the sanitized treatments are shown in Table 5.

Table 5 Air bladder TPC results for different sanitising treatments

Treatment	**TPC/g**	**Comments**
Nil	1 800 000	
10ppm Chlorine dioxide	300 000	Colourless solution
25ppm Chlorine dioxide	40 000	Solution pale yellow
50ppm Chlorine dioxide	22 000	Solution yellow and quite strong odour.
Silver ion solution	580 000	Colourless

The different sanitation methods were effective at decreasing the TPC of the air bladders; 50ppm chlorine dioxide solution was the most effective. The silver ion solution was the least effect sanitizer. Although the 50ppm chlorine dioxide solution did significantly decrease the TPC and there is no specified limit in the Food Standards Code, the levels are still quite high and alternative methods to further decrease the microbial load at harvest prior to sanitation could be explored. This could include:

- Optimising on board processing techniques
- Keeping air bladder attached to frame and processing on shore

There was no observable difference in texture and colour between the different sanitising treatments. After sanitising the air bladders, there was a bright yellow stain that appeared on most of the air bladders (Figure 6). The stained mark is from part of the stomach lining.

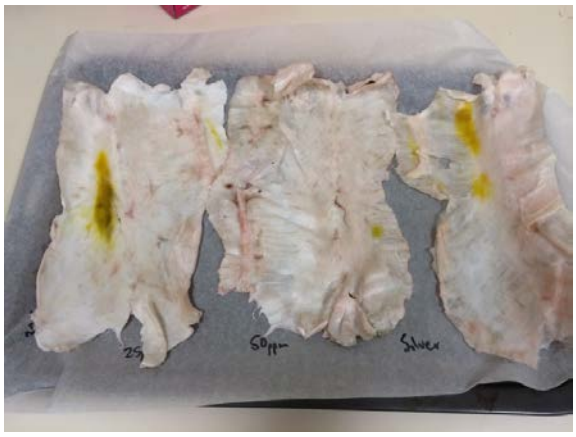


Figure 7: Air bladder after being treated in different solutions

4. Optimising Drying of Air Bladders.

4.1: Aim

Trial drying sanitized air bladders, with an aim to mimic the sun dried product as much as possible

4.2 Methods

4.2.1 Drying Treatments

In the first trial, sanitised air bladder from part 3 were dried were dried in convection ovens at 2 different temperatures:

- 30C for 18 hours
- 60C for 24 hours

In the second trial three airbladders were sanitised with 50ppm Chlorine dioxide and then dried at 30˚C for 23 hours.

In the third trial three airbladders were sanitised with 50ppm chlorine dioxide and dried at 30˚C for 23 hours. Results were compared to commercially available ling and cod air bladders.

4.2.2 Analysis

Moisture Loss

Three air bladders were dried in a convection oven at 30°C for 23 hours. The weight of the air bladders was recorded at different stages of the drying process to determine the moisture loss and rate of drying.

Water Activity

The dried air bladder water activity was measured in the Aqua Lab Water Activity Meter was measured in 3 different formats: whole, cut into small pieces and grated.

Microbiology

Some airbladders were analysed for TPC

Proximate Analysis

Proximate Analysis was conducted by NMI**.**

4.3 Results

4.3.1 Trial 1

After 18 hours of drying, both samples were checked. The air bladders in the drying at 60C still contained moisture and was kept in for a further 6 hours. The air bladders drying at 30C were dry after 18 hours.

Figure 8: Air bladder after drying (Left: at 60C. Right: at 30C)

The dried air bladders were very thin and strong and had shrunk in size. The air bladders dried at 30C had curled along the edges and were a light pink colour. The air bladders dried at 60C only slightly curled at the sides, which could be due to a slower rate of moisture removal. The colour was a transparent golden due to the higher drying temperature.

The stain on the air bladder called the gall belly stain or gall burn (Seafood Services Australia Ltd. 1997) was a dull yellow colour after drying at 30C but had turned green when dried at 60C. The stain is an undesirable characteristic of the air bladder. Commercially dried products have a similar thickness but the colour is a golden yellow colour with no visible stains.

4.3.2 Trial 2

The second drying trial was conducted at 30˚C as this had previously produced a better quality product in Trial 1. Figures 9-11 show the airbladders through the drying procedure.

Figure 2 Air bladders prior to drying

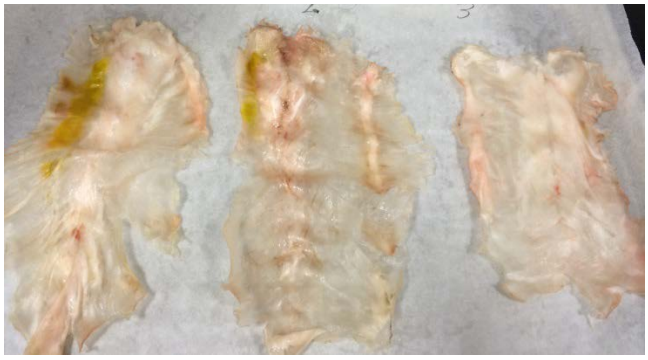


Figure 10: Air bladders after drying for 6 hours at 30C

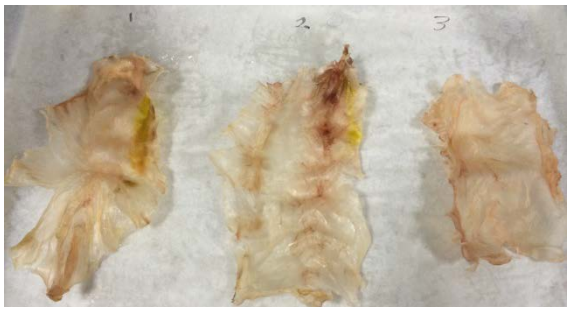


Figure 11: Air bladder after drying for 23 hours at 30C

The dried bladders after 23 hours appeared similar to the commercial air bladders produced in Norway and supplied by the industry partners for comparison (Figure 12)

Figure 3 Dried air bladders sourced from Norway

Moisture Loss

As well as moisture being removed from the air bladder during drying, oil was also leeching from the air bladder onto the baking paper. One piece of fish maw that had a higher percentage of weight loss had less fat on, which may account for the increased weight loss. The average moisture loss of the air bladders is displayed in Figure 13. Moisture loss is

greater at the start of drying and plateaus over time, with an average total moisture loss of 55.42%.

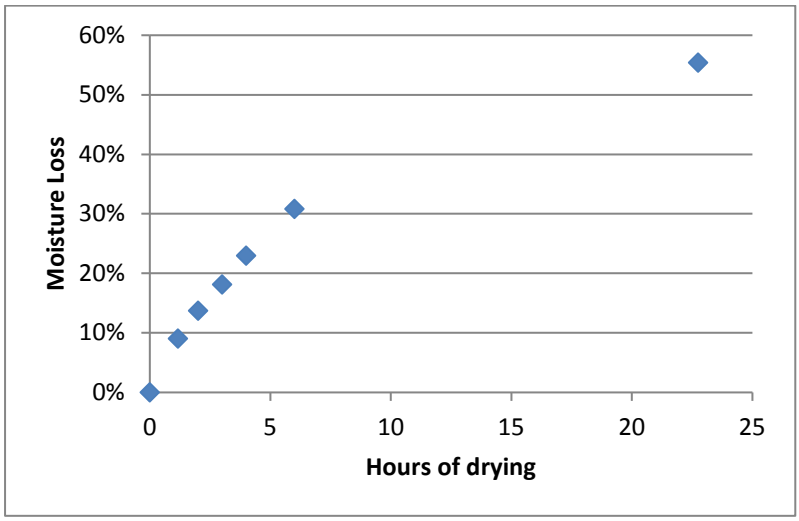


Figure 13: Moisture loss with drying time.

Water Activity

The air bladder has a cross pattern structure Figure 14, making it extremely difficult to cut or grate the air bladder into smaller pieces. It was impossible to grate the air bladder and only one sample analysis was conducted for the whole and cut up air bladder with a water activity of 0.643 and 0.547, respectively. The water activity is low enough to inhibit the growth of pathogenic bacteria, spoilage molds and microorganisms. A water activity of 0.90 limits growth of pathogenic bacteria, 0.70 Aw limits growth of spoilage molds and 0.60 Aw is the minimum for all microorganisms (AquaLab 2015).

Figure 14 cross section of dried air bladder

Microbiological Analysis

The dried air bladders from Barramundi produced in July 2015 was tested for total viable aerobic count in February 2016. Even upon storage at room temperature for approximately 7 months, the total viable aerobic count was 30 estimated CFU/g.

Table 6: Microbiological analysis of Barramundi fish maw stored for approximately 7 months

Microbiological analysis	Analysis Result
Total viable aerobic count	30 st. CFU/g

4.3.3 Trial 3

Based on the optimized condition from the earlier trials, the frozen air bladders from Barramundi were thawed the day before use at 4 degree Celsius and sanitized by soaking in 50 ppm chlorine dioxide solution for 30 minutes. The sanitized air bladders were then dried in a hot air oven at 30 degree Celsius for 18 hours. Stages of the drying are shown in Figures 15-17.

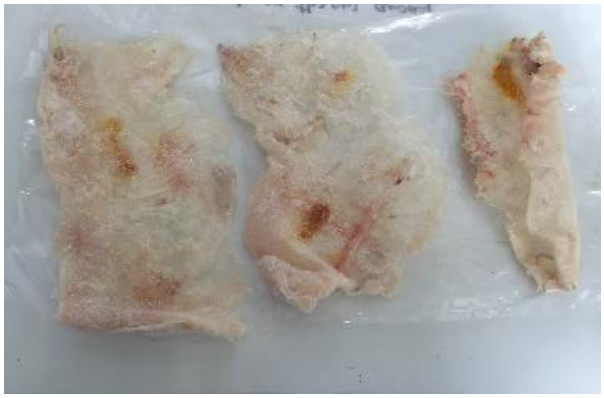


Figure 15: Frozen Barramundi swim bladders

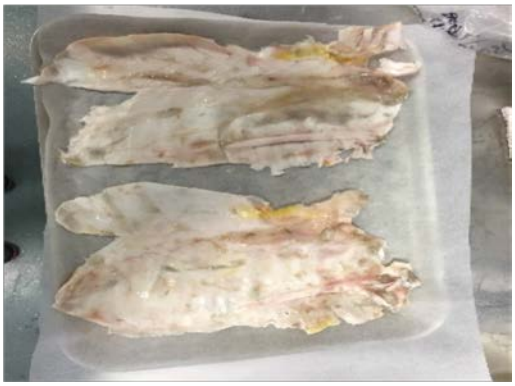


Figure 16: Thawed barramundi swim bladders

Figure 17: Barramundi swim bladders dried at 30 degrees Celsius for 18 hours

It was observed that the air bladders were adequately dried. The dried fish maw was translucent with light silvery white color. As compared to the commercially available dried fish maw, it was thinner. Nevertheless, it resembled the Norwegian dried air bladders in terms of its slightly curled edges and red marks.

Amounts of adipose tissue, connective tissue and blood clots were adhered to the surface of the maw and there was fat exudation on the maw surface after drying, attributing to poor visual appearance with oil induced staining present. The greenish gall stain was also present.

Moisture and Water activity

Table 7 shows the results of the moisture and water activity of the dried barramundi bladders when compared to commercially available ling and cod maw.

*Table 7: M*oisture and Water activity analysis

Proximate Composition	Analysis Result
Barramundi fish maw	
Moisture	11.45 %
Ash	0.031 %
Water activity (cut into small pieces)	0.346
Water activity (whole)	0.339
Commercially available Ling maw	
Moisture	10.65 %
Commercially available Cod maw	
Moisture	12.44 %
Water activity	0.418

The moisture content of the dried fish maw from Barramundi of 11.45 % was close to that of the commercially available dried maws from Ling (10.65 % moisture). The ash content of dried maw from Barramundi was found to be 0.031 %.

The water activity of the dried maw from Barramundi was 0.346 for sample cut into small pieces and 0.339 for the whole sample. The low water activity established was adequate to inhibit the growth of pathogenic bacteria, spoilage molds and microorganisms. It is also comparable to the water activity of the commercially available dried maw from Cod (0.418).

5. Optimising Post-Harvest Processing Options to Improve Product Quality.

5.1 Aim

Determine the most effective enzyme/chemical to clean the air bladder post-harvest to minimize handling and processing and also ensure a higher quality product.

5.2 Literature Review

After drying in the previous trial it was noted that amounts of adipose tissue, connective tissue and blood clots were adhered to the surface of the maw and there was fat exudation on the maw surface after drying, attributing to poor visual appearance with oil induced staining present. The greenish gall stain was also present. Such issues were decreasing the quality of the product. It was therefore decided to investigate post-harvest processing options to improve the quality of the air bladder prior to drying.

Currently the air bladders are scraped down with a knife to clean. The blood vessels and fatty tissues present in the raw material must be adequately removed before freezing to prevent staining and textural damage. The retention of connective and adipose tissue on the surface of air bladder is an indication of poor handling as it causes fat exudation on the surface later during the production of dried maw (Fisheries Research Development Corporation 1994). This was also found in the trial for dried Barramundi maw sanitized using 50 ppm chlorine dioxide solution.

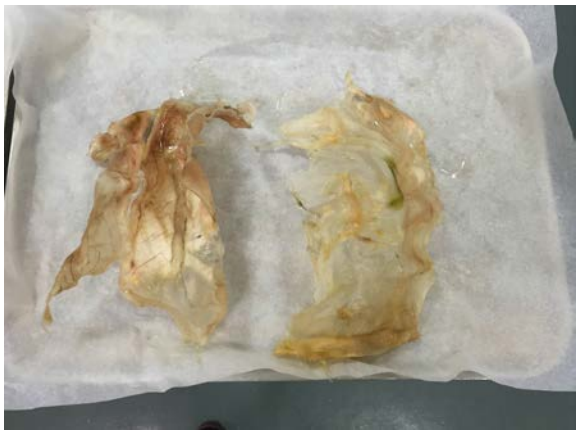


Figure 18: Dried maw from Barramundi with surface fat exudation.

Attempts are to be made to remove the surface layer of fatty tissue in air bladders so that the resulting dried maw is as close to the commercially available ones in terms of textural and other quality attributes. Two possible treatments were envisaged: acid or enzymes.

5.2.1 Organic acid treatment

Studies on the application of organic acids (Hamby et al. 1987) to beef carcasses or cuts have demonstrated significant reduction of microbial loads. Different organic acids at varying concentrations have been used as a dip for inoculated meat in order to study its

antimicrobial activity; 1.2% acetic acid, 1.25% lactic acid, and a combination of acetic and propionic acids (Hamby et al. 1987).

Solutions of organic acids (up to 1-3%) such as acetic acid and lactic acid have been reported to be widely used chemical interventions in the meat industry for commercial dressing of beef and lamb. Organic acids have been evaluated as a sanitizing method for beef carcasses during spray chilling wherein, a significant reduction in the total aerobic count and pathogen populations was observed. Further, organic acids have demonstrated to be the most effective when applied as a warm (50-55 degree Celsius) carcass rinse (Food Science Australia 2006).

Organic acids upto 2.5% of a solution are applied as a part of a carcass wash pre-chill in the US. Although the use of organic acids is not permissible under EU regulations, it has been approved in Australia and USA. The USDA has specifically approved the use of acetic acid, citric acid and lactic acid to livestock carcasses as antimicrobial agents (Food Science Australia 2006).

5.2.2 Enzymes

Enzymes have also been suggested as being a post-harvest processing option to increase the quality of the air bladders.

Indeed for the enzymatic cleaning of cod swim bladders in Iceland, industrial equipment has been constructed (Gildberg 1993a). 20 minutes of enzymatic hydrolysis of cod swim bladder removes the tightly bound thin black membrane encasing it (Gildberg 1993b; Haard and Simpson 2000). The enzyme used for this process (whilst not described) may originate from a marine organism; such as pepsin or collagenase from cod and crab hepatopancreas, respectively (Haard and Simpson 2000).

To understand the most suitable enzyme to clean up the air bladder on board, the tissues that are attached to the air bladder must be noted. Parts that are the hardest to remove, for example, membranes, blood, connective tissue, fat will be targeted. Removal of membranes on swim bladder requires selective tissue degradation (Shahidi, Han, and Synowiecki 2006).

Seitz (1974) reported the removal of excessive fat from menhaden fish by using lipolytic fungi, *Candida lipolytica* and *Geotrichum candidum*. These microorganisms reduced the lipid content of the fatty fish and significantly improved the protein content of the final product.

Lipase is also applied in the process for the preparation of smoked carp where the carp is first steeped in brine solution for 2 weeks and then in a solution of lipase for 4-5 hours before the fish is smoked (Seitz 1974).

The enzyme treatment of air bladders will be done by the use of Lipolase which has an optimal temperature of 40-60°C, however it is not food grade.

5.3 Methods and Results

5.3.1 Trial 1: Acetic acid and citric acid dipping treatments.

Methods

Frozen Barramundi swim bladders were thawed at 4˚C overnight.

Organic Acid solutions were prepared in distilled water. Treatments are shown in Table 8.

Table 8: Dipping Treatments

Treatment	Organic acid treatment	Dip time
1	Nil	10 mins
2	1% acetic acid solution	10 mins
3	1% citric acid solution	10 mins
4	3% acetic acid solution	10 mins
5	3% citric acid solution	10 mins
6	5% acetic acid solution	10 mins
7	5% acetic acid solution	10 mins

The swim bladder/acid solution ratio = 1:2 (w/w)

The treated air bladders were dried in a conventional oven at 30C for 18 hours.

Evaluation of textural attributes was conducted including the presence or absence of tissues adhering on the surface of the dried maw, fat exudation, colour, odour, transparency and curling.

Results

The results are shown in Figure 19 from left to right acid concentrations are 1%, 3% and 5%).

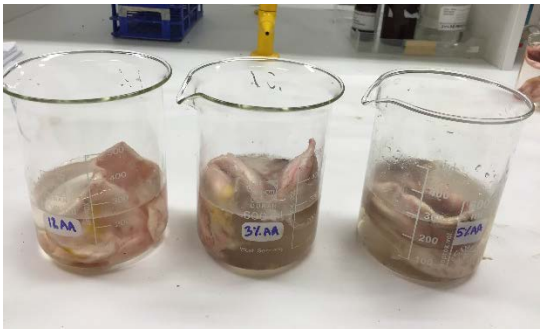
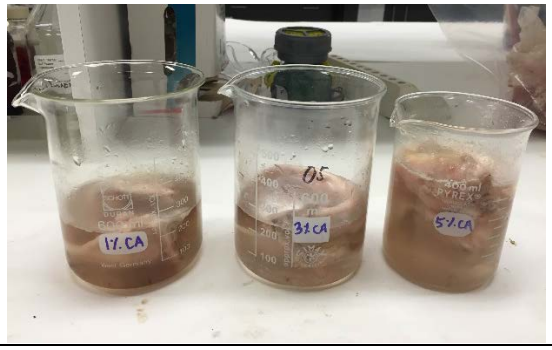
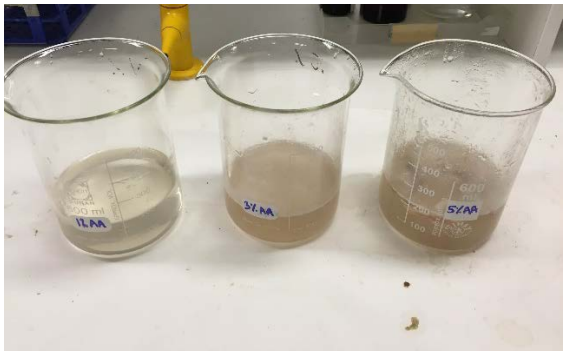
Acetic acid treatment	Citric acid treatment
Acetic Acid Treatment 	**Citric Acid treatment** 
Solutions remaining in liquid after treatment with acetic acid 	***Solutions remaining in liquid after treatment with citric acid*** 
Adhering material removed in 5% solution 	***Adhering material removed in 5% solution*** 
Treated air bladders 	

Figure 19: Results of acetic and citric acid dipping trials.

It was observed that there was only slight loosening of the adhering membrane from the swim bladders treated in 1% citric acid solution while the loosening of the adhering membrane was slightly more with 3% citric acid treatment. 5% citric acid treatment resulted in the removal of the adhering membrane from the swim bladders as evident from the debris left in the acid solution. Similar observations were made with acetic acid treatment at concentration of 1%, 3% and 5%. Further, the degree of removal of the membrane from the surface of air bladders was notably higher with 5% acetic acid solution. However, the treatment did not result in the complete removal of the membrane (see final photos of treated air bladders).

Summary

The most effective method to remove the tissues and membrane attached to the air bladder was acetic acid at 5%. However the bladders were still not suitable for drying therefore acetic acid will be used with variations on concentrations and time of soaking to seek improvement.

5.3.2 Trial Acid and Enzyme treatments to Clean Air Bladders

Based on the Trial 1 results. Acetic acid was tried with a longer dipping time and also lipolase enzyme was a further treatment.

Method

Clean and uncleaned frozen barramundi swim bladders were thawed at 4°C overnight.

Table 9 lists the dipping treatments and preparation protocols are described below.

Table 9 Swim bladder dipping treatments

Sample	Cleaned/uncleaned	Treatment	Treatment time
CONT1	Cleaned	NIL	10 mins
CONT2	Cleaned	NIL	10 mins
AA10	Uncleaned	5% acetic acid	10 mins
AA30	Uncleaned	5% acetic acid	30 mins
LIP1	Uncleaned	3% lipolase (w/w) in 40C waterbath	30 mins

Control

Cleaned and split barramundi air bladders only required the sanitations and drying process.

Acetic Acid

1. Prepare 5% acetic acid solution with distilled water.
2. Record the weight of each uncleaned air bladder.
3. Calculate the weight of the water required in the beaker for each air bladder at a1:2 ratio bladder: solution and pour into beaker.
4. Place air bladder in the beaker and leave in solution for the allocated time.
5. Remove the air bladder and allow to drain.

Enzyme treatment

1. Record the weight of each uncleaned air bladder.
2. Calculate the amount of enzyme is required for each air bladder based on the weight.
3. Heat distilled water to 40C using a water bath and place enzymes in (1:2 ratio airbladder: water)
4. Place air bladder in the beaker and leave in solution for up to 30 minutes, monitoring the process. cover with lid and insulate if possible.
5. Remove the air bladder and allow to drain.

Sanitation and Drying

The treatment air bladders were cut in half. The acid or enzyme treated air bladders, along with the control were placed into 50ppm chlorine dioxide solution for 30 minutes. The air

bladders were patted dry with paper towels before laying them flat on a tray and dried at 30C for 18 hours or until dry in a convection oven. The weight of the air bladders before and after drying was recorded.

Visual Observations

Visual observations on the appearance of the treated air bladders to determine the effectiveness of the treatments, including colour and the absence or presence of tissue and membrane attached to the air bladder before and after treatment.

The dried air bladders were observed for fat exudation, colour, odour and texture.

Results

Before drying

Results are summarised in Table 10 and photos are shown in Figures .

Acetic acid treatment

Most of the excess material attached to the air bladder was slightly opaque and grey in colour. The gall stain was noted. There were no blood vessels attached. Immediately after the air bladder was placed into the solution, fat had dissolved. Soaking for 30 minutes was too long. The inside of the air bladder still had the membrane attached when opened. The air bladder had become observably tougher, with the edges crunchy like cartilage to touch. It was a straw yellow colour and opaque.

Lipolase treatment

The swim bladder treated with lipolase initially had a large amount of excess fatty tissue, blood vessels and membrane attached to the inner and outer layer. There were light grey translucent parts and white opaque parts still attached to the swim bladder. Heating the solution to 40C would have liquefied some lipids with lower melting points. Some of the membrane inside the air bladder had disappeared. The temperature had affected the colour of the air bladder after treatment. It was opaque white, similar to the appearance of the cleaned air bladder supplied. However most of the excess membrane was still undigested, which may be attributed to one or a number of the following factors:

- Enzyme concentration
- Treatment time
- Temperature of the solution
- The enzymes is not correct for breaking down the particular membrane

Table 10: Summary of dipping trials.

Sample	Before treatment	During treatment	After treatment
AA10	Slight gall bladder stain. No		Not much change in

	blood vessels. Mostly transparent fat, quite a large amount		appearance.
AA30	Mainly transparent light grey fat attached. Least amount of all air bladders, gall stain noted. No blood	Immediate dissolving of fat notes	Long exposure time has turned some parts of the air bladder transparent and hard. Membrane inside air bladder
Lip1	Blood vessel present and a large amount of dark grey fat adipose tissue. Also large membrane with opaque cloudy white fat. Largest amount of membrane attached	At 10 mins, there was still opaque membrane attached	Heat has cooked the air bladder and turned it a white in colour- more similar to the control air bladder. Some fat has dissolved. Air bladder has membrane that has dissolved to a certain degree.

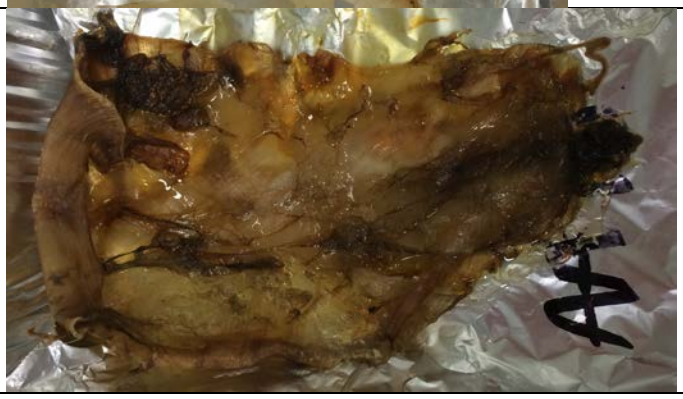
Figure 20 after treatment but before sanitation. whole air bladders

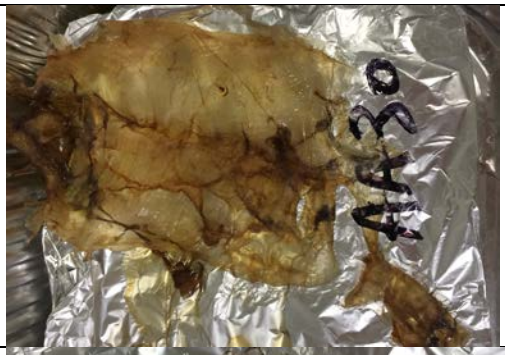
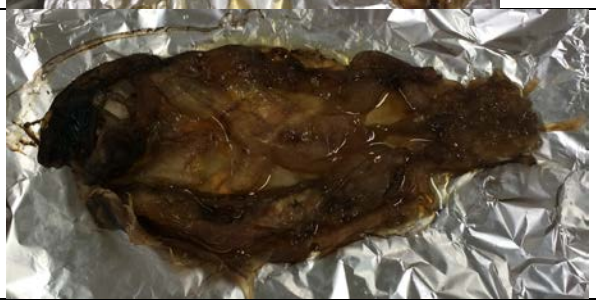
Figure 21 Sanitised air bladders cut in half

After Drying

The treated air bladders were dried at 30°C for 18 hours, with the results displayed in Table 11.

Table 11: Results of Trial 2 Dipping and Drying Trials.

Sample	Comments	Picture
Control	Slight gall stone stain visible. Minimal fat present. Relatively flat. Light straw colour. Evenly dried.	
Acetic Acid 5% 10 minutes	Lipids in membrane present. Fishy aroma, caramel brown transparent colour. Pools of fat all over. Underside of the air bladder was oily	

Acetic Acid 5% 30 minutes	Evenly dried on most parts of the air bladder. No visual bile stain. Membrane dried and slightly visible. Membrane with fat still present. Caramel brown colour.	
Lipolase 3% 40C for 30 minutes	Rancid fishy odour. Fast in membrane. The blood vessel dehydrated and dark. Colour of bladder was a caramel brown colour. Underside of the air bladder still oily	

Moisture loss results are shown in Table 12.

Table 12 Moisture Loss Results

Treatment		**Moisture Loss**
CONT1	NIL	58.87%
CONT2	NIL	60.60%
AA10	5% acetic acid	56.10%
AA30	5% acetic acid	75.86%
LIP1	3% lipolase in 40C water	40.51%

5.4 Summary

The citric acid, acetic acid and lipolase enzyme treatments were unsuccessful in cleaning the air bladders.

A next step is to analyse the components of the air bladder membrane and then try and concoct an enzyme mix that can specifically target those components.

6 Frying of Fish Maw

6.1 Aim

Develop a method for producing fried fish maw.

6.2 Methods

Dried sanitised barramundi air bladders were placed in a saucepan with ambient canola oil with the heat on high. Air bladders were cooked in oil until they had puffed up, then removed from the heat and drained.

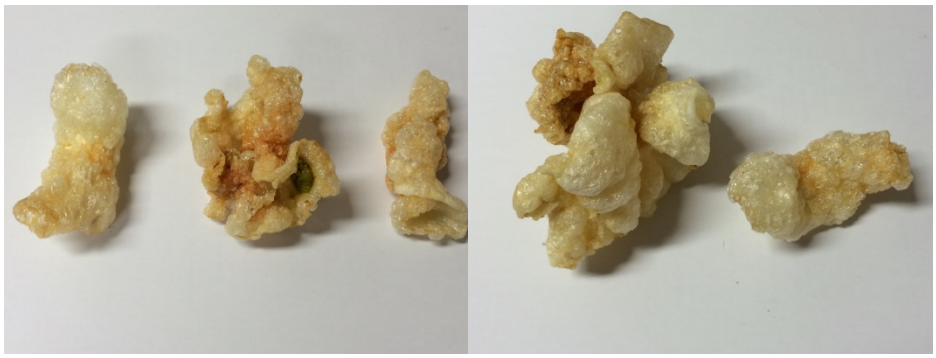


Figure 22: Fried barramundi fish maw

6.3 Results

The end product was a fried fish maw similar to the commercially available samples (Figure 22). There was no difference in thickness, texture and colour. The fish maw was a light golden colour and parts of the air bladder had curled during cooking. The yellow stain on the dried air bladder had turned a darker grey/green colour after frying. The stain is not present on any of the commercial products, which lowers the overall appearance of the end product.

7. Testing of New Dryer with Commercial Applicability.

7.1 Aim

A new dehydrator with characteristics of commercial dryers was purchased for ongoing trials. A comparative trial was completed to assess the drying impacts of the new equipment.

The aim of this section was therefore to determine if the new dehydrator works the same as the convection oven used previously to dry fish air bladders.

7.2 Methods

- Sanitise airbladders in 50ppm chlorine dioxide solution
- Each air bladder was weighed before placing two into the laboratory convection oven, and another 2 into the Sunbeam Dehydrator at 35°C for 17.5 hours, or until dry.
- Samples were removed from oven and weighed.
- Temperature loggers were used to monitor the drying temperature.

7.3 Results

After 17.5 hours drying, the air bladders in both treatments were completely dry, with a hard texture. The drying temperature for both machines was set at 35°C; however the data from the loggers indicated the dryers operated at different temperatures (Figure 23). The convection oven used in previous trials located in the 611 laboratory was operating between 33-34.5°C, whereas the newly purchased Sunbeam Dehydrator operated between 36-38°C. The air bladder moisture loss was comparable between both dryers after 17.5 hours, between 52.8-54.6% as shown in Table 13.

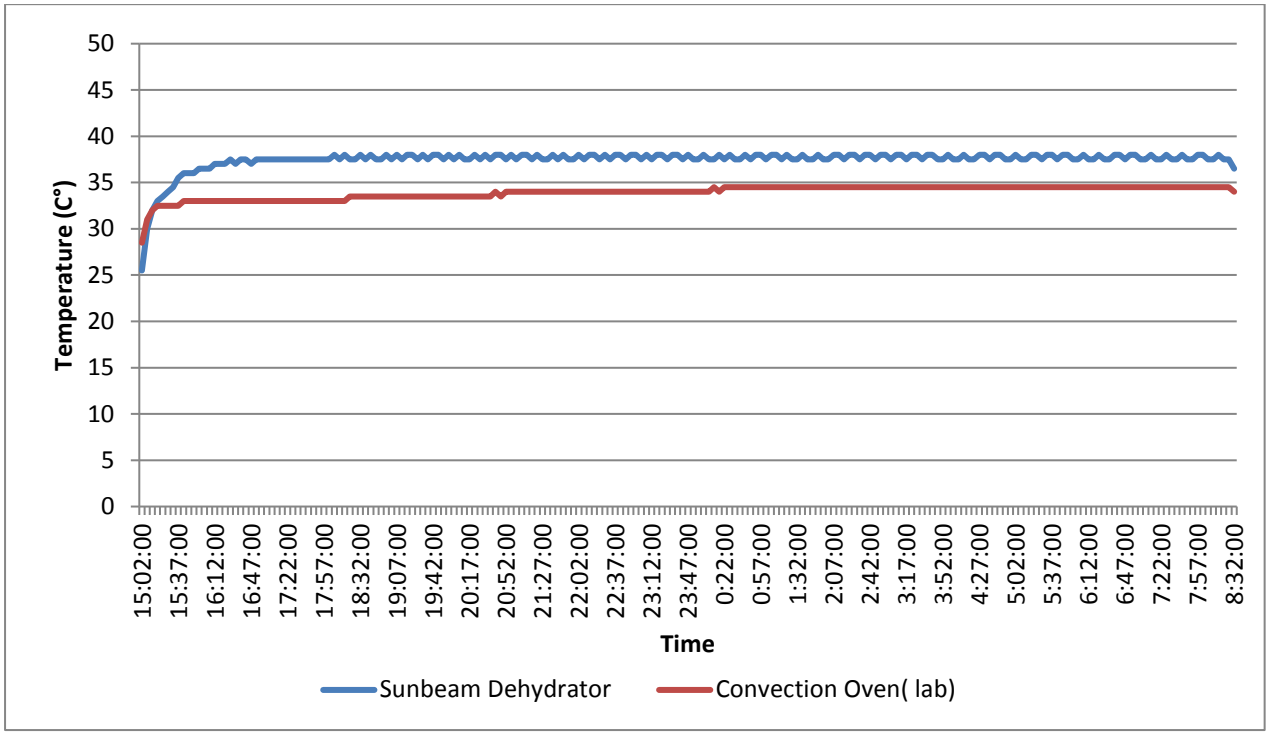


Figure 23 Temperature logging data of the different drying machines to dry air bladders

Table 13 Moisture loss and drying temperatures for the air bladders dried in the different machines.

Air bladder	Oven	Drying temperature range	Moisture loss %
1	Lab Convection	33-34.5°C	52.8%
2			54.2%
3	Sunbeam Dehydrator	36-38°C	54.0%
4			54.6%

The appearance of the air bladders after drying was similar between treatment groups. The air bladders had curled up and the colour was straw yellow (Figure 24). The dried air bladders had some fatty membrane still attached which exuded fat during drying (Figure 25). A rancid odour was noted with dried air bladders from both treatments, caused by oxidative rancidity of the fat. Removal of the fatty membrane before drying would enhance the final product appearance and aroma. The gall bladder stain was observed on all samples, which changed from yellow to green during drying.

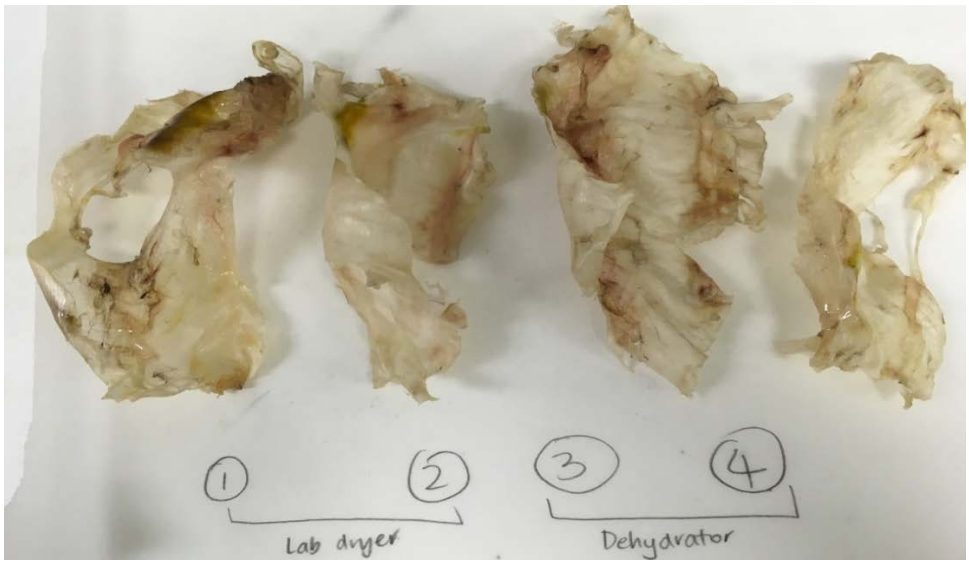


Figure 54 Air bladders dried in the convection oven (left) and Sunbeam dehydrator (right)

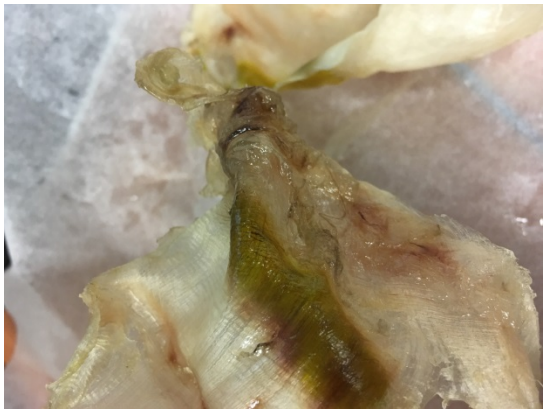


Figure 25 Fat membrane still attached to the air bladder after drying

7.4 Conclusion

The Sunbeam Dehydrator was successfully used to dry barramundi air bladders with no observable difference in appearance to air bladders dried in the convection oven at 35°C, used in previous trials. It is important to note the importance of removing the fat membrane from the air bladder before drying to maximise final dried product quality. Further air bladder drying trials will be conducted in the Sunbeam Dehydrator.

8 Conclusions and Next Steps.

The project has resulted in dried air bladders (non-fried and fried) that are similar in texture, thickness, and colour to commercially available examples from other species. The product is stable at room temperature and food safe.

However there are two issues that are barriers to commercial production.

Gall bladder staining decreasing the quality of the product

The current method of manual cleaning by the fishermen is not considered feasible on board . Attempts to clean the air bladders by alternate means (dilute organic acids and enzymes) were unsuccessful. For wild harvest fishermen the cleaning of the air bladders is not likely to occur on board, however, on shore processing can be done in a facility for aquaculture barramundi. This will also allow for controlling the last meal before harvesting thereby decreasing the gall staining.

Enzymatic cleaning of the air bladder is occurring in Norway but the nature of the enzyme mix had not been publicised. Analysis of the 'membrane" may allow targeted enzyme trials in the future. This is suggested as it has been previously shown that processing of swim bladders from cod-liver with enzymes to remove the surrounding membrane increases processing efficiencies eight-fold, in comparison to manual processing (Martin et al. 2000). Collagenase is used commercially to skin squid (Kim 2014) and may be appropriate here. Collagen often consists of proline, hydroxyproline and glycine sequenced in a triple-helix structure(Kim 2014). Type 1 collagenase was recommended as this targets fat and liver cells, epithelial layer and lung fat. To remove such connective tissues however, primarily comprised of type 1 collagen protein , the only enzyme that that can digest this is collagenase, which is very expensive (Bremner 2002).

Suggested next steps for the project include

Commercial dryer has been purchased by industry partner and trials commenced.

Using the optimised methodology developed and the new dehydrator (see Section 7), complete drying and analysis of different swim bladder products for comparison with existing commercial examples. Look for differences within and between fish species (see aligned FRDC 2013/711 report: Small Scale Dehydration of Air Bladders from Different Species of Fish to Product Fish Maw).

Further Market investigation (desktop and by interview/advice) on the samples produced including feedback on fish maw samples produced by the suggested methods (as part of above step).

Develop best practice protocols for removing fish maw by processor to reduce the amount of cross contamination from the fish stomach (and hence further reduce staining)

Trial alternate enzymes for post-harvest handling, then, if successful develop on board treatment trials by sanitisers/enzymes to decrease post-harvest handling requirements

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