Draft PROGRESS REPORT:

PRODUCT DEVELOPMENT OF CHIILED SEA URCHIN FOR HUMAN CONSUMPTION

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Executive Summary

The widely distributed edible sea urchin (cawaki) species that grows wild in Fiji is the *Tripneustes gratilla* species. This species is mainly harvested near intertidal zones by women and are sold in the urban markets fresh either with shells or as the gonads packed in plastic containers. However it has not been commercially processed for sale in supermarkets or for export. Sea urchin gonads are rich in proteins and a good source of vitamin A and Bs, and are also believed to enhance virility. There has been no published research on the wild populations and catch data of sea urchins in Fiji and hence further research is warranted to establish these. This project aimed at developing fresh-chilled sea urchin gonad products and other high quality gonad products suitable for export.

Three separate processing trials were conducted during November 2014 to March 2015 on sea urchin species *Tripneustes gratilla* that were harvested by Ministry of Fisheries officers from different locations in the Kalokolevu district to the west of Suva harbour. Harvesting was done on the day of processing and the whole urchins stored in a commercial chiller prior to processing. Samples were processed, preserved using selected preservatives, packed and stored in selected temperatures for shelf life determinations which included standard plate count, total coliforms, psychrophiles, pH, salinity and physical characteristics such as colour, texture, flavour and taste. The preservatives used were dry salt, salt solution or brine, alcohol and a solution containing dextrin, salt and sodium alginate. Storage temperature was varied between -5°C to +5°C or ambient (approximately 25-30°C) and of up to 62 days. Microbial, organoleptic and chemical analyses were conducted in determining the shelf life of each product formulation and storage condition. The desirable organoleptic characteristics aimed at are bright mango-orange or yellow colour, whole firm texture, fresh seaweed odour, fresh seaweed-sweet taste and free of leaking fluids.

Results showed that the most desirable and acceptable organoleptic characteristics were the gonads preserved in 5% dry salt stored at -5.4^{0}C with the shelf life of 23 days and the 8% alcohol mixed with 5% dry salt stored at ambient temperature with the shelf life of 34 days. These recommendations were obtained after evaluating the three trials of different processing procedures and formulation. It is interesting to note that brining appeared to be unacceptable due to the oozing and leaking of yellow and orange fluid into the brine, contributing to unacceptable milky-turbid solution. Soaking in sodium phosphate did not stop the oozing, instead aggravated milkiness and turbidity of brined samples hence, the adoption of dry salting and alcohol based preservation formulations respectively.

Further work is required to test for consumer acceptance and for further inter-laboratory analyses to confirm the in-house and acceptability tests. Further research is also needed to establish the ideal time and protocol for harvesting including temperature and storage conditions prior to processing

1.0 Introduction

Sea urchins are marine benthic invertebrates that have calcareous shells and have moveable spines. These animals are generally found in the mild to low intertidal zone; at depth up to about 50 meters (Reynolds and Wilen, 2000). There are many species of edible sea urchins, however some common ones that are readily available and consumed in some parts of the world include red sea urchins; *Strongylocentrotus franciscanus,* green sea urchins; *Strongylocentrotus droebachien,* purple sea urchins; *Strongylocentrotus purpuratus, Evechinus chloroticus* (endemic in New Zealand), *Psammechinus miliaris, Paracentrotus lividus* (purple sea urchin) and *Echinus esculentus* (West-coast of Scotland) (Suckling *et al*., 2011). In Fiji the widely distributed edible sea urchin is the *Tripneustes gratilla* species which grow wild and are harvested by women. Some of these sea urchins are sold in the markets either in shells or as the gonads packed in plastic containers. Even though sea urchins are naturally known to be important in reducing algal biomass by grazing on them and improving the health of the marine ecosystem, limited information is available on its wild population and the catch data in Fiji.

In Japan and other European countries, sea urchin is regarded as one of the most valuable fishery products and highly prized commodity due its unique flavour (Chen *et al*., 2013). Sea urchin gonad of both male and female are delicacies in many countries such as Japan, Korea, Greece, France and New Zealand which are consumed in various ways such as eating raw in sushi or with lemon, onion, and olive oil, as flavours in omelettes, scrambled eggs, fish soup and mayonnaise. Japan is the largest market of gonad in the world, as it is sold in sushi bars as delicacy (Sonu, 2003). In Fiji and its neighbouring Pacific Island countries, sea urchin is not highly regarded compared to fin fish and sea cucumber, hence the development of this product for the overseas market.

The price for sea urchin gonad is determined by its colour, quality, appearance and nutritional value and these factors are affected by season, temperature, photoperiod and food intake as major factors (Chen *et al*., 2013; James *et al*., 2007; Schlosser *et al*., 2005). The colour of gonad is an important criterion for marketability and obtaining high price in which bright mango orange or yellow colours being the most desirable. These colours are derived primarily from various types of carotenoids present in them. Studies have shown that increasing the concentration levels of carotenoids in the diets of sea urchin enhanced gonad quality and provided its preferred mango-orange colour (Shpigel et al., 2006). This means that the diet of sea urchin (aquaculture) or what the sea urchins feed on (wild) determined the colour of gonads.

Furthermore, studies have identified the major carotenoids naturally found in sea urchin gonads. These include β-carotene, α-carotene, β-echinenone, zeaxanthin, canthaxanxin, lutein, astaxanthin, diatoxanthin, fucoxanthin and alloxanthin (Shpigel et al., 2006). Of these, β-echinenone had been discovered to develop and accumulate the bright yellow-orange or mango-orange coloured gonad. Apart from richness in

carotenoids, gonads of sea urchins are also shown to be a good source of energy, omega 3 fatty acids, polyunsaturated fatty acids, protein, minerals (such as zinc) and vitamins (Suckling *et al.,* 2011).

There are various ways of processing and preservation of sea urchins. These include fresh (chilled), salted, steamed, baked and frozen depending on the market, distance and the mode of transportation (Kato and Schroeter, 1985).

This project aimed at developing fresh-chilled sea urchin products and other sea urchin gonad products suitable for export in collaboration with Sai Yee Food Industries Ltd located in Wailada, Lami. Sai Yee Food Industries processes and export quite a number of fresh and vacuum packed local root crops such as taro, cassava, breadfruit, yams, plantain; fruits and vegetables such as jackfruit, okra and duruka; and reef fish which it purchases from villages and landowners that plant and grow them. The project was identified and prioritised by the Fiji Market Access Working Group (MAWG) of the Australian Aid-funded Pacific Horticultural & Agricultural Market Access program (PHAMA).

2.0 Desirable Attributes for Sea Urchin Gonads

The most desirable attributes for sea urchin gonads that had been developed for export markets include; bright mango-orange or yellow colour, whole firm texture, fresh seaweed odour, fresh seaweed-sweet taste, free of leaking fluids and high nutritional value (Shpigel et al., 2006). The best size for individual pieces of gonad for packing ranges from 40-50mm in length (Kato, 1972; Kato and Schroeter, 1985).

Studies have revealed that orange gonads are obtained from male sea urchins while yellow gonads are from the female sea urchins. Dark brown coloured gonads are thought to be degenerated gonads mainly due to starvation (Kato and Schroeter, 1985). Sea urchin gonad colour and flavour quality correlated with the types of food consumed by sea urchins (Kato, 1972).

3.0 Summary of Processing and Preservation of Sea Urchin

Based on the gonads quality attributes discussed above the processing of sea urchins adopted the following flow chart procedures. Four preservation methods were conducted as outlined in the flow chart below. Note the critical control points will be identified in the HACCP plan that will proposed for each processing line for each product in the final report.

Receive raw fresh sea urchin.

Store in the chiller (3-5^{0}C)
Wash shell – with Cool (0-2^{0}C) sea water with 3.5% w/v) (S1)

Shell split lengthwise to ensure gonad is not damaged and remove and discard digestive organs; sterilize tools and tables (S2)

Sort gonad by colour and remove left over intestinal lining with sterilized tools (S3)

Take out gonads without damage and wash with 3.5% salt solution (3.5% w/v) - Don't touch by hands. Use sterilized tools (S4)

Wash gonads in 0-2.0^{o}C salt solution (3.5% w/v). Change salt water regularly and maintain at 0-2.0^{o}C

Wash gonads repeatedly until no turbidity observed in salt water (3.5% at 0-2.0^{o}C) (S5)

Drain well - Keep its temperature at 0 -2.0^{o}C with the use of ice

(S6)

- Dry Salt
  - Add salt - 10% drained gonads salt. Sprinkle salt on the chopping board first. Then lay out gonads on the board and sprinkle remaining salt on the gonads.
- Brine
  - Pack with salt water in containers
- Alcohol
  - Add salt - 8% drained gonads salt. Sprinkle salt on the chopping board first. Then lay out gonads on the board and sprinkle remaining salt on the gonads.
- Dextrin & Alginate
  - Add 5% branched dextrin into 2.8% (w/v) sodium chloride and 0.3% sodium alginate
  - Soak the gonads in the solution for 10sec

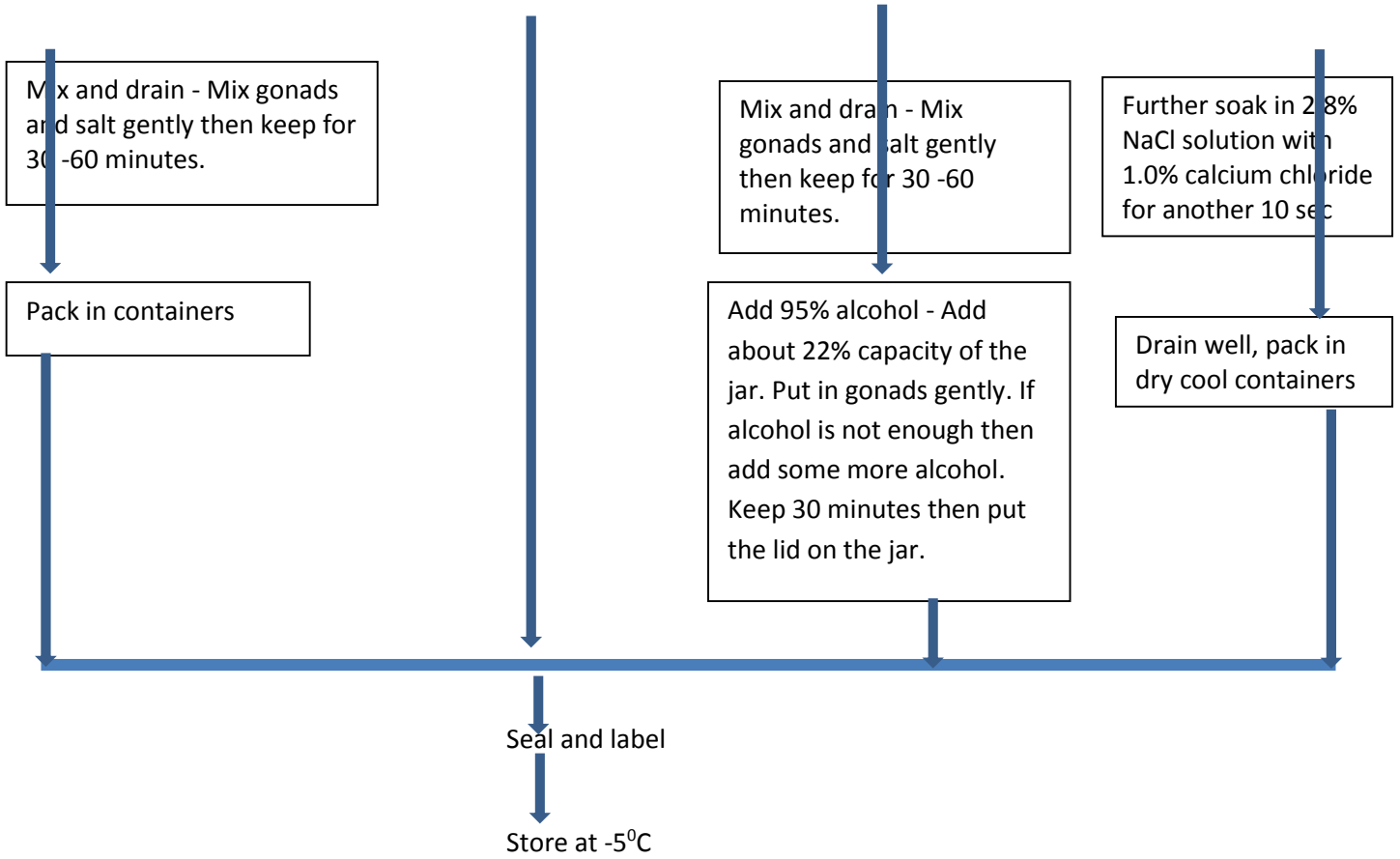

Figure 1: Summary of Processing Flow Diagram of Sea Urchin

4.0 Gonad Yield

Gonad yield and its quality vary according to seasons. Studies on the lunar cycles showed that higher gonad yield is obtained during full moon compared to last quarter moon (Manuel *et al.,* 2013). At the last quarter moon, sea urchins are found to have been already spawned or were in their spent conditions which contributed to low yields. The ideal test diameter of around 6-7 cm at 15 months are assumed to be sexually matured in which spawning begins (Manuel *et al.,* 2013*;* McManus *et al.,* undated*)*.

In Fiji limited research related to the spawning of sea urchin is available. It is therefore important to note that unless the spawning time in Fiji is fully established harvesting time would then be appropriately determined to ensure that high yield of gonads are obtained. However, the scope of this research was beyond the study of sea urchin spawning. Nonetheless, based on the three different processing occasions conducted in Dec 17th 2014, Jan 14th 2015 and Feb 11th 2015, the highest yield was obtained in the January 14th processing. This may indicate that the spawning time of sea urchin was around January 14th which was evident in the acceptable size of 8-10mm x 40-50mm and high quality gonads. Studies in the Philippines revealed the peak spawning of *T. gratilla* species is from December to January in the Philippines (McManus *et al.,* undated).

5.0 Quality Assurance

Processing of sea urchins for each preservation technique complied with the food safety regulation (Fiji Ministry of Health, 2010). This included compliance of the good manufacturing practices (GMP) which included the cleaning protocol of the processing room and all contact surfaces and utensils with 100ppm chlorine and sanitized with 75% alcohol before and after processing.

Processing of sea urchin was maintained below 5^{0}C of the 3.5% salt solution. Ice was continuously used at every station to retain the cold chain below 5^{0}C as the operational limit. A temperature data logger was used in recording the temperature.

Shell split was done lengthwise to avoid and reduce damage to gonads due to oozing if the tissue was physically damaged. Sorting of gonad size and colour was conducted after shell splitting to ensure reasonable gonad size with acceptable yellow and orange as shown in Figure 2 below were retained while dark brown was rejected. Only full size of at least 8-10mm thickness (any length) gonads were accepted.

a. Lengthwise split showing yellow gonads

b. Crosswise split showing orange gonads

Figure 2: Yellow and Orange gonads with two types of shell splits a and b

6.0 Processing Trials, Preservations and Shelf Life Tests

The processed sea urchin gonads discussed in section 3.0 above were treated with various preservatives and stored at selected temperatures;

-5^0C, 0^0C, 5^0C and ambient temperature for a certain duration of time prior to microbial analyses, chemical and organoleptic tests in order to determine shelf life or period of storage until the gonads are unfit for consumption.

A total of three processing trials of sea urchins were carried out on separate occasions as indicated below;

- trial 1-Dec 17th 2014,

- trial 2 – Jan 14th 2015 and
- trial 3 – Feb 11th 2015.

6.1 Trial 1 Processing

6.1.1. Processing Design and Protocol

Trial 1 processing involved the processing of 3 prototypes: preserved in 5.3% brine stored at 5^0C and 0^0C as well as in 21% alcohol stored at ambient temperature as shown in the experimental flow diagram in Figure 3 below.

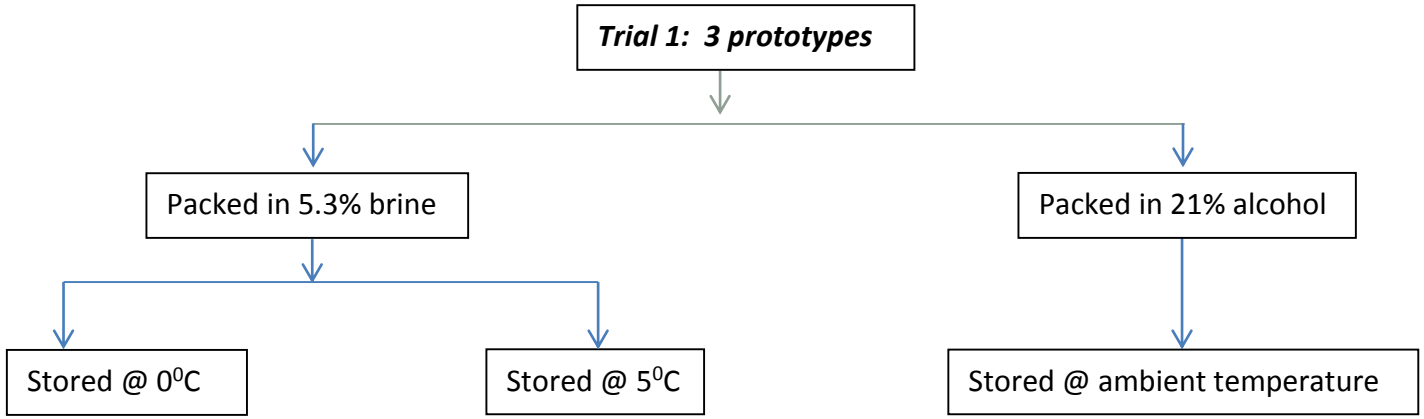

Figure 3: Trial 1 prototypes and storage temperatures

6.1.2 Shelf Life Prediction Test

The three prototypes processes in Figure 3 above were stored at the selected temperatures as indicated and tested for shelf life over a period of 15 days using the following indicators; microbial determinations, organoleptic assessments and chemical analyses. These tests were carried out on all the preserved samples stored at -0.4^{0}C, 0.7^{0}C and ambient temperature of 28.1^{0}C as indicated in Table 1 below. It is important to note

these temperatures are only average values and that there had been high variations in the range of temperature recorded. The details of tests with results are discussed below.

6.1.2.1 Microbial Determination

Three major microbial analyses; standard plate count, psychrophilic bacterial count and coliforms were employed in the shelf life prediction of gonads preserved in 5.3% brine stored at 0.7^{0}C and -0.4^{0}C and the 21% alcohol stored at ambient temperature over a period of 15 days for the trial 1 processing of gonads. *E.coli* was to be further analysed only when coliform levels were found high. Due to insignifant levels of coliform (<3MPN/g) obtained in day 1, the test was discontinued at days 7 and 15 which indicated good quality control and safe processing conducted.

Table 1: Microbial Levels of Gonads from Processing Trial 1

Type of test	**5.3% brine**						**21% alcohol stored @ ambient tempt (28.1^{0}C)**		
	Stored @ -0.4^{0}C			**Stored @ 0.7^{0}C**					
	Day 1	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**
SPC (Aerobic Plate Count) (CFU/g) or EAPC/g-<300)	1×10^2 (eapc)	2.1×10^2 (eapc)	5.1×10^2	2.1×10^2 (eapc)	1.8×10^2 (eapc)	7.7×10^2	2.2×10^3	2.0×10^3	2.9×10^2 (eapc)
Psychrophilic (CFU/g or EAPC/g-<2500)	2.6×10^4	1.5×10^4	2.1×10^3 (eapc)	9.7×10^3	9.8×10^3	3.0×10^3	6.6×10^3	1.5×10^2 (eapc)	1.0×10^2 (eapc)
Coliforms (MPN/g)	<3			<3			<3		

EPAC – estimated plate aerobic count is referred to <300 counts for Standard Plate Count (SPC) and <2500 counts for psychrophilic bacteria.

The microbial results shown in Table 1 revealed that even though the aerobic plate count increased over time, the levels were significantly lower as evident in the count of <300, while the psychrophilic bacteria were reduced in count over the 15 day shelf life duration for the three protocols. Results also showed that 5.3% brine stored at -0.4^{0}C had lower levels of microbes compared to 0.7^{0}C and much lower in 21% alcohol on day 15. Furthermore, the day 1 microbial levels indicated the effectiveness in the compliance of the Good Manufacturing Practices (GMP) and the safe processing methods in maintaining of <5^{0}C chilled of 3.5% brine solution which effectively controlled the growth of bacteria below 300 count of

SPC and retained the firmness of gonads as shown in 6.1.2.2 below. This may indicate that even though the microbial levels are low until day 15, the organoleptic assessments discussed below appeared to be the major determining factor in the acceptance of the gonad products hence, the importance of employing multiple indicators in the shelf life prediction protocol.

6.1.2.2 Organoleptic Assessments

The organoleptic assessments employed two techniques; the descriptive profiling and hedonic scaling of each protocol stored at various temperatures indicated in Tables 2 and 3 below. Table 2 showed that preservation in 5.3% brine resulted in milky- turbid brine solution, however -0.4^{0}C storage revealed the following descriptive profile of gonads; lighter turbidity fluid, bright firm gonads, retained strong seaweed odour and flavour with some sweet taste up to day 7 compared to 0.7^{0}C storage with the following descriptive profile; intense milky –turbid fluid, formation of film like on the surface of brine, soft gonads with weak seaweed odour and flavour and have lost the sweet taste. On day 15, the 0.7^{0}C stored sample became dull in colour, softer texture with neutral odour and weak seaweed flavour compared to -0.4^{0}C sample had retained seaweed flavour and sweet taste and just begun to get soft in texture and a bit taint in colour. Given these characteristics, the -0.4^{0}C storage is estimated to have a shelf life of 10 days calculated as 70% of day 15 while 0.7^{0}C storage sample is estimated to have the shelf life of 5 days calculated as 70% of day 7. The 21% alcohol sample stored at room temperature had a shelf life of more than 15 days even though had strong alcohol flavour and bitter aftertaste, the texture was more firmer, dark brighter in colour with sweet-alcohol odour.

Table 2: Descriptive Profile of Gonads from Processing Trial 1

Type of test	**5.3% brine**						**21% alcohol @ stored ambient tempt (28.1^{0}C)**		
	Stored @ -0.4^{0}C			**Stored @ 0.7^{0}C**					
	Day 1	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**
Colour	Milky–turbid brine but bright gonads	Milky–turbid brine but bright gonads	yellow-milky brine with a bit dull gonads	Intense milky-turbidity	Milky-turbid brine with film like on surface of brine	Milky brine with light brown dull gonads	Clear transparent liquid with bright color gonads	Light milky liquid with bright color	Less milky liquid with dark color

Texture	Firm	Firm	Softer than day 7	Soft–melting out	softer	Soft	Firm	Firm	Firm denatured gonads
Odor	Strong fresh seaweed	Strong fresh seaweed	Fresh seaweed	Fresh seaweed	Weak seaweed	No odour	Strong alcohol	Alcohol with sweet odour	Strong alcohol with sweet odour
Taste & Flavor	Strong seaweed flavor with sweet taste	Strong seaweed flavor with sweet taste	Seaweed flavor with sweet taste	Strong seaweed flavor with sweet taste	Weak seaweed salty flavor	Weak seaweed only	Strong alcohol	Alcohol	Sweet-alcohol flavour
Aftertaste	Sweet-seaweed	Sweet-seaweed	Sweet-seaweed	Sweet-seaweed	salty	salty	Bitter - alcohol	Bitter - alcohol	Bitter - alcohol

n=3-6

The hedonic scale of the three protocols shown in Table 3 revealed that on day 1, 5.3% brine stored at 0.7^{0}C was ranked 1st, 5.3% brine stored at -0.4^{0}C ranked 2nd while 18% alcohol stored at 28.1^{0}C ranked 3rd. On day 7, 5.3% brine stored at -0.4^{0}C ranked 1st, while 18% stored at 28.1^{0}C ranked 2nd and the 5.3% brine stored at 0.7^{0}C ranked 3rd. On day 15, the 18% alcohol stored at 28.1^{0}C ranked 1st, 5.3% brine stored at -0.4^{0}C ranked 2nd while 5.3% brine stored at 0.7^{0}C ranked 3rd.

Table 3: Hedonic Scaling of Gonads from Processing Trial 1

Type of test	**5.3% brine**						**21% alcohol @ stored ambient tempt (28.1^{0}C)**		
	Stored @ -0.4^{0}C			**Stored @ 0.7^{0}C**					
	Day 1	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**
Colour	4	2	3.5	3	2	2.5	5	4	4
Odor	4	3	3.5	3.8	3	2	3.3	3	4
Texture	3.8	2	2.5	3	2	2.5	4.3	5	4.5
Taste	4.5	5	4	4.3	2	2	2	4	4
Flavour	4.8	5	4.5	4.8	2	2.5	2	3	3.5
Overall Ranking*	2nd	1st	2nd	1st	3rd	3rd	3rd	2nd	1st

n=3-6, Overall ranking* was based on individual ranking from 1st -3rd of the 3 prototypes; 1st ranking, 2nd ranking and 3rd ranking

Based on the hedonic scale and the organoleptic assessments, the 0.7^{0}C storage condition and the use of brining were eliminated. However the following recommendations were made for trial 2 processing protocols; the use of Sodium Phosphate (alum) to stop the oozing and bleeding of gonads, both for the 5.3% brine and 10% dry salt and to be both stored at 0^{0}C and -5^{0}C respectively as well a reduction of alcohol concentration to 14% and stored at 5^{0}C and ambient temperature respectively.

6.1.2.3 Chemical Tests

Further insights into the reactions taking place during storage, three chemical analyses were measured; water activity, salinity and pH as shown in Table 4 below. Results showed that slight changes in pH, salinity and water activity that occurred. The 5.3% brine stored at -0.4^{0}C revealed a slight reduction in water activity, a slight increase in pH and salinity over the 15 days of storage while the 5.3% brine stored at 0.7^{0}C slight reduction in water activity, no change in salinity and an increase in pH. The 21% alcohol had much lower pH even though a slight increase in pH was observed over the 15 day period.

Table 4: Chemical Analyses of Gonads from Processing Trial 1

Type of test	**5.3% brine**						**18% alcohol @ ambient tempt (28.1^{0}C)**		
	Stored @ -0.4^{0}C			**Stored @ 0.7^{0}C**					
	Day 1	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**	**Day 1**	**Day 7**	**Day 15**
Water Activity	0.968 @24.5^{0}C	0.963 @25^{0}C	0.964 @24.8^{0}C	0.966 @23.9^{0}C	0.960 @25.1^{0}C	0.962 @24.3^{0}C	0.949 @25.6^{0}C	0.950 @25.8^{0}C	0.952 @25.6^{0}C
Salinity (%w/w)	7.7	7.7	7.9	8.3	7.7	8.3	>10.5	>10.5	>10.5
pH@25^{0}C	6.86	6.98	6.94	6.75	6.92	6.93	6.28	6.42	6.5

n=3

Based on the combined results of microbial, descriptive profile, hedonic scale and chemical analyses the following suggestions were made to improve the quality of the tested products;

(a) The oozing and continuous bleeding of gonads that contributed to milkiness of the brine solution needed to be inhibited by the use of Sodium Phosphate
(b) Confirmation temperatures for 5.3% brine stored at 0^{0}C and -5^{0}C respectively
(c) 10% dry salt to be developed for storage at 0^{0}C and -5^{0}C respectively.

(d) A reduction in alcohol concentration to 14% and then stored at two temperatures; 5^0C and ambient temperature respectively were to be adopted.

The suggestions were carried as part of trial 2 processing as indicated below.

6.2 Trial 2 Processing

6.2.1 Trial 2 Processing Design

Trial 2 processing involved the comparison of 6 protoptypes: soaked in 0.7% Sodium Phosphate (alum) for 1 min and then preserved in 5.3% brine and 10% dry salt prior to storage at 0^0C and -5^0C respectively; 14% alcohol then stored at 5^0C or ambient temperature respectively and then all stored for up to a total of 62 days as shown in the experimental flow diagram in Figure 4 below.

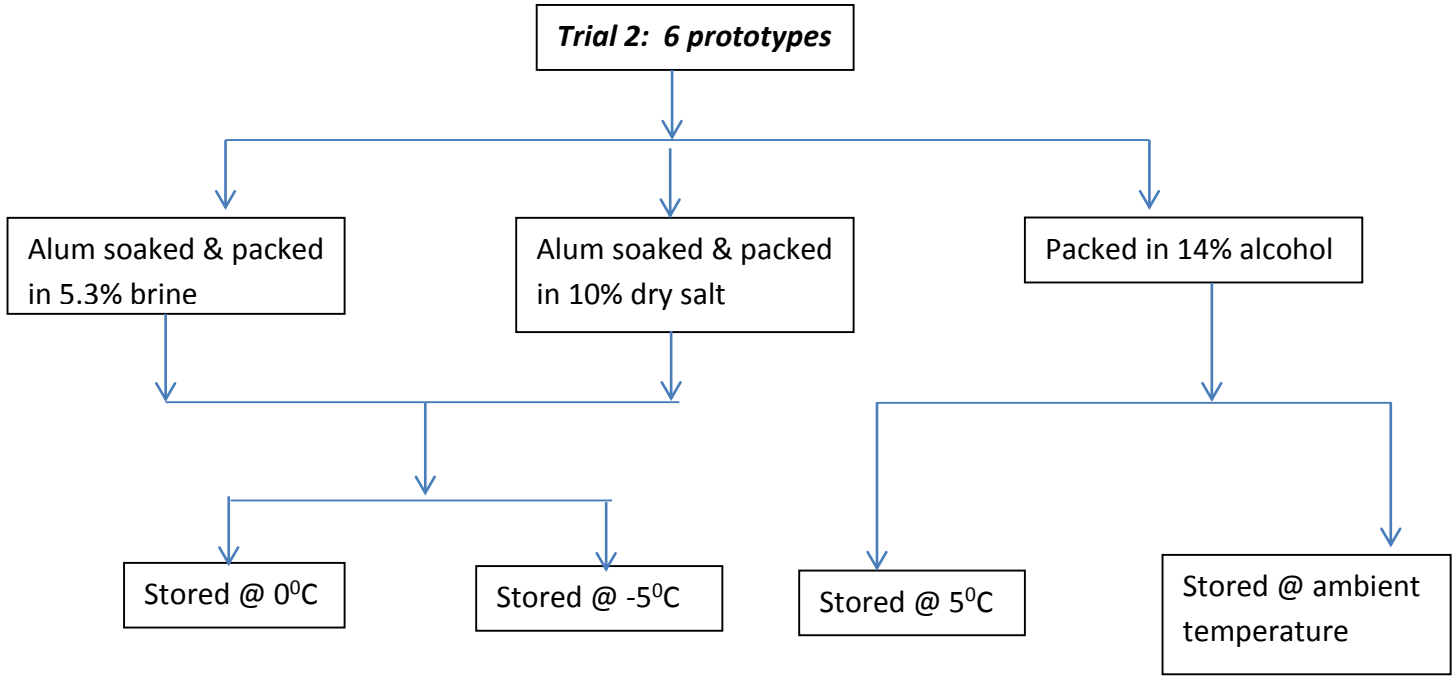

Figure 4: Trial 2 prototypes and storage temperatures

6.2.2 Shelf Life Prediction Test

The six protocols were tested for shelf life over a period of 62 days using similar indicators as in trial 1: microbial determinations (excluding coliforms), organoleptic assessments and chemical analyses. These tests were carried out on preserved samples that were planned for storage at -5^0C and 0^0C for both brined and dry salted samples while the 14% alcohol samples were stored at 5^0C and ambient temperature. However, due to temperature problems related to such facilities at the University premises as confirmed by the temperature data logger, the temperatures of the respective cool and cold storage facilities used were recorded as -1.5^0C, 1.7^0C and ambient temperature of 30^0C as indicated in Table 5 below. It is important to note these temperatures are only average values and that there had been high variations in the temperature range obtained which suggests for better control in the next round of preservation.

6.2.2.1 Microbial Determination

Only two microbial analyses; standard plate count and psychrophilic bacterial count were conducted in the shelf life prediction of gonads preserved in 5.3% brine and 10% dry salt stored at -1.5^0C and 1.7^0C respectively and as well as the 14% alcohol stored at 1.7^0C and at ambient temperature respectively for a period of up to 62 days. Total coliform test was removed for trial 2 processing based on the insignifant levels obtained in trial 1 processing. The presence of coliform in samples would signify poor hygienic practices and poor sanitation especially the removal and cleaning of digestive and intestinal organs and waste protocol or may be due to cross contamination. Given the GMP compliance and the strict observations of time and temperature control during processing, we were confident that coliform levels would be also insignificant. Results as indicated in Table 5 below showed that microbial levels of gonads were generally low indicating $<10^6$ (Health Protection Agency, 2009) from day 1 to day 43 of selected samples even though fluctuations in data was observed.

Table 5: Microbial Levels of Gonads from Processing Trial 2																									
Type of test	**Alum 5.3% brine**						**Alum 10% dry salt**										**14% alcohol**								
	@ -1.5^{0}C			**@ 1.7^{0}C**			**@ -1.5^{0}C**						**@ 1.7^{0}C**				**@ 1.7^{0}C**					**@ ambient tempt of 30^{0}C**					
	D1	**D15**	**D22**	**D1**	**D15**	**D22**	**D1**	**D15**	**D22**	**D29**	**D43**	**D62**	**D1**	**D15**	**D22**	**D29**	**D15**	**D22**	**D29**	**D43**	**D62**	**D1**	**D15**	**D22**	**D29**	**D43**	**D62**
SPC (CFU/g) or EAPC/g-<300)	3.8×10^2	3.4×10^2	1.3×10^2	4.0×10^2	2.5×10^2	2.6×10^2	2.7×10^3	2.6×10^2	1.3×10^3	2.0×10^2	5.5×10^2	2.3×10^5	6.7×10^2	2.6×10^2	6.2×10^2	1.9×10^2	5.3×10^3	8.7×10^2	1.1×10^4	4.3×10^2	4.8×10^3	2.3×10^3	4.4×10^3	1.9×10^3	2.7×10^2	3.3×10^3	6.4×10^3
Psychrophilic (CFU/g or EAPC/g-<2500)	3.5×10^3	6.0×10^2	1.0×10^2	3.3×10^3	8.0×10^2	2.0×10^2	1.8×10^3	ND	3.0×10^2	1.5×10^2	50	1.0×10^6	1.5×10^2	NC	1.5×10^2	3.0×10^2	2.2×10^3	ND	ND	3.0×10^2	7.0×10^2	2.5×10^2	3.0×10^2	ND	ND	ND	50

ND = Detection limit is 100 colonies, EPAC – estimated plate aerobic count is referred to <300 counts for Standard Plate Count (SPC) and <2500 counts for psychrophilic bacteria.

It is important to note that the 5.3% brine that was soaked in sodium phosphate for 1 min prior to storage at both -1.5^{0}C and 1.7^{0}C were both eliminated after day 22 due to the unacceptable intense milky-turbid brine solution as indicated in Table 7. Similarly, samples soaked in sodium phosphate preserved in 10% dry salt and stored at 1.7^{0}C was eliminated at day 29 due to unacceptable rotten banana odour. These may indicate that even though the microbial levels were low until certain days such as day 43 and day 62 for some samples, the organoleptic assessments discussed below appeared to be the major determining factor in the acceptance of the gonad products hence, the importance of employing multiple indicators in the shelf life prediction protocol as mentioned earlier. Based on the observations carried out in trial 1 processing of the 21% alcohol stored at ambient temperature, trial 2 of day 1 of the 14% alcohol stored at 1.7^{0}C microbial analysis were not conducted. This was due to the assumption that the combination effect of the preservatives used; 14% alcohol mixed with the 10% dry salt stored at 1.7^{0}C would be sufficient to control the growth of microbes.

6.2.2.3 Organoleptic Assessments

Similar to trial 1, organoleptic assessments employed included descriptive profiling and hedonic scaling of each prototype stored at various temperatures indicated in Tables 7 and 8 below. Table 7 showed that soaking in 0.7% sodium phosphate (alum) appeared not to be effective as it aggravated and intensified the milkiness and turbidity of the brine solution, and also contributed to bitter aftertaste and squizzed tongue. This may mean that alum is not effective in controlling the oozing in the brine solution hence did not improve the appearance of the packaged product. Even though texture and taste may have been improved to a certain degree, the appearance of the milky brine solution may not be favourable to consumers which may be assumed to be a spoilt product, hence eliminated.

Table 7: Organoleptic Evaluation of Gonads from Descriptive Profile of Gonads from Processing Trial 2

Type of test	Alum 5.3% brine						Alum 10% dry salt										14% alcohol											
	@ -1.5^{0}C			@ 1.7^{0}C			@ -1.5^{0}C						@ 1.7^{0}C				@ 1.7^{0}C					@ ambient tempt (30^{0}C)						
	D1	D15	D22	D1	D15	D22	D1	D15	D22	D29	D43	D62	D1	D15	D22	D29	D15	D22	D29	D43	D62	D1	D15	D22	D29	D43	D62
Colour	Pale orange turbid brine	Milky turbid brine	Light milky turbid brine	Yellow-milky brine	Very milky brine with fresh bright gonads	Very milky turbid brine	Bright yellow	Light color	Bright dark	bright	Bright	Milky bright color	Fresh ornage	Light color	Bright	Bright	Dark color	Dull	Bright	Bright	Bright	Bright yellow intense	Dark color	Dull	Dark	Bright	Bright
Texture	firm	soft	firm	firm	firm	Mealtime out	Mealtime & break easily	firm	firm	firm	firm	firm	Firm but start to melt	firm	Firm	Getting soft	firm	firm	Firm	Firm coagulated	Firm	Very firm coagulated	firm	firm	firm	Firm coagulated	Firm

Odor	Fresh seaweed	neutral	Mild seaweed	Strong fresh seaweed	seaweed	seaweed	neutral	seaweed	Neutral	Neutral	Seaweed	Neutral	neutral	neutral	Neutral	Rotten banana (ester)	alcohol	Neutral	Alcohol	Mild seaweed strong alcohol	Alcohol	Weak alcohol	alcohol	Mild alcohol	alcohol	Strong alcohol	Strong alcohol
Taste & Flavor	Fresh seaweed & salty	Very salty	Mild seaweed sweet salty	Fresh seaweed sweet & salty	Seaweed sweet salty	Seaweed sweet	Salty & sweet	Very salty	Salty	Salty	Seaweed sweet salty	Weak seaweed	Salty seaweed	salty	Salty	Salty	alcohol	Alcohol	Alcohol	Alcohol	Alcohol	Alcohol with sweet & salty	alcohol	alcohol	Alcohol	Strong alcohol and salty	Strong alcohol umami taste
Afterta ste	Squized tongue	astringency	Squized tongue	Prawn tail and bitter	Squizzed tongue	Squizzed tongue	bitter	Squizzed tongue	Squizzed tongue	Squizzed tongue	Sweet salty squizzed tongue	Slightly bitter	bitter	Squizzed tongue	Squizzed tongue	Squizzed tongue	alcohol	Alcohol	Alcohol	Alcohol	Alcohol	bitter	alcohol	Strong alcohol	Alcohol	Alcohol	Weak alcohol

Out of the six prototypes, only three reached the 62 days of shelf life testing. These were the 10% dry salt stored at -1.5^{0}C, the 14% alcohol stored at both 1.7^{0}C and ambient temperature respectively that retained bright colour, firm texture and seaweed odour and flavour. However, the 10% dry salt was too salty, the 14% alcohol stored at 1.7^{0}C had light milky liquid which may not be favourable to consumers and the 14% alcohol stored at ambient temperature appeared to have the most favourable appearance even though with a bitter alcohol taste and strong alcohol odour and flavour. These were confirmed by the hedonic scaling as indicated in Table 8 above which revealed that out of the six prototypes, the 5.3% brine stored at 1.7^{0}C was ranked 1st both on days 1 and 15, the 10% dry salt stored at 1.7^{0}C was ranked 1st on days 22 and 29 while the 14% alcohol stored at ambient temperature ranked 1st on day 2.

Table 8: Hedonic Evaluation of Gonads from Processing Trial 2

Type of test	Alum 5.3% brine						Alum 10% dry salt										14% alcohol											
	@ -1.5^{0}C			@ 1.7^{0}C			@ -1.5^{0}C						@ 1.7^{0}C				@ 1.7^{0}C					@ ambient tempt (30^{0}C)						
	D1	D15	D22	D1	D15	D22	D1	D15	D22	D29	D43	D62	D1	D15	D22	D29	D15	D22	D29	D43	D62	D1	D15	D22	D29	D43	D62	
Colour	3.7	3.5	2.5	4.2	4	1	3.2	3.5	4	3.7	4.5	3.0	4	3.5	4	4.3	3	3.5	3.8	4.5	4.0	4.5	3.5	3.5	4	4.5	4.5	
Odor	4	3	4	4.3	3.5	3.5	2.8	2.5	3	3.5	4.5	3.5	3	2.5	3	4	2.5	3.5	3.0	3.0	3.5	3.8	3	3.5	3.2	3.0	4.0	
Texture	3.5	2.5	3	4.3	3.5	2.5	3.3	2.5	3.5	3.3	4.5	3.5	3.7	3	3.5	4.7	4	4.5	4.3	4.5	4.0	4.5	4.5	4.5	3.8	4.5	4.5	
Taste	3.8	3	3.5	4.2	3.5	3.5	3	2.5	2.5	2.3	4.5	3.5	2.2	2	2.5	4.2	2.5	2	2.7	3.0	3.0	4.2	3.5	1.5	3	2.5	4.0	
Flavor	3.3	3	3	4.7	3.5	3	2.5	1.5	2.5	2.2	4.5	4.0	2.3	1.5	2.5	4	2.5	1.5	2.5	3.5	3.0	3.7	3	1.5	2.8	2.5	3.5	
Overall ranking*	2nd	3rd	2nd	1st	1st	5th	4th	5th	3rd	4th	1st	3rd	5th	5th	1st	1st	4th	6th	2nd	2nd	2nd	3rd	2nd	4th	3rd	3rd	1st	

n=3-6, Overall ranking* was based on individual ranking from 1st -3rd of the 6 prototypes; 1st ranking, 2nd ranking and 3rd ranking, etc

Based on the hedonic scale and the organoleptic assessments, the 5.3% brine soaked in sodium phosphate (alum) for 1 min prior to storage at both -1.5^{0}C and 1.7^{0}C were both eliminated after day 22 which left the shelf life at 16 days. However due to unacceptable intense milky-turbid brine solution and the effect of squizzed tongue eliminate the use of sodium phosphate. Similarly, soaked in alum 10% dry salt stored at 1.7^{0}C was eliminated at day 29 due to unacceptable rotten banana odour which made the shelf life at 20 days, however due to tongue squizzed effect, this sample may not be acceptable. This means that based on physical appearances the only acceptable samples were the two 14% alcohol preserved samples, however were bitter in aftertaste due to strong alcohol odour and flavour. Thus the following recommendations were made for trial 3 processing prototypes; only dry salt to be used with 5% concentration and to be stored at -5^{0}C and a further reduction of alcohol concentration to 8% and 11% respectively and both to be stored at ambient temperature.

6.2.2.4 Chemical Tests

Further insights into the reactions taking place during shelf life determination continued with the three chemical analyses; water activity, salinity and pH as shown in Table 6 below. Results showed that slight changes in pH, salinity and water activity had been observed. The alum 5.3% brine stored at -1.5^{0}C and 1.7^{0}C revealed a slight reduction in pH and increased salinity over 22 days of storage while both the alum 10% dry salt stored at -1.5^{0}C and 1.7^{0}C had slight reduction in pH and increased salinity. Both the 14% alcohol samples also showed a reduction in pH over the 64 day period. Note that water activity was conducted only on day 1 to assess the effectiveness in the level of preservatives used.

Table 6: Chemical Analyses of Gonads from Processing Trial 2

Type of test	**Alum 5.3% brine**						**Alum 10% dry salt**										**14% alcohol**											
	@ -1.5^{0}C			**@ 1.7^{0}C**			**@ -1.5^{0}C**						**@ 1.7^{0}C**				**@ 1.7^{0}C**					**@ ambient tempt (30^{0}C)**						
	D1	**D15**	**D22**	**D1**	**D15**	**D22**	**D1**	**D15**	**D22**	**D29**	**D43**	**D64**	**D1**	**D15**	**D22**	**D29**	**D15**	**D22**	**D29**	**D43**	**D64**	**D1**	**D15**	**D22**	**D29**	**D43**	**D64**
Water Activity	0.965@23.8			0.968@23.3			0.821@24.3						0.916@24.3									0.935@24.0					
Salinity	8.3	8.6	8.6	>10.5	9.2	10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5
pH	7.16	6.53	6.75	6.99	6.72	6.28	7.71	7.65	6.46	6.26	6.22	6.10	6.7	7.53	6.40	6.13	7.66	6.32	6.27	6.32	6.51	7.51	7.35	6.43	6.19	6.35	6.43

Based on combined tests results; microbial, descriptive profile, hedonic scale and chemical analyses suggestions made to further improve the quality and shelf of the gonad products were as follows; only dry salt to be used with 5% concentration and to be stored at -5^{0}C and a reduction in alcohol concentration at 8% and 11% respectively and both to be stored at ambient temperature. These suggestions were implemented in trial 3 processing indicated below.

6.3 Trial 3 Processing

6.3.1 Trial 3 Processing Design

Trial 3 processing involved the processing of 4 prototypes: preserved in 5% dry salt, dextrin and alginate coating prior to storage at -5.4^{0}C; and 8% and 11% alcohol concentrations to be stored at ambient temperature then all to be stored for a total of 34 days as shown in the experimental flow diagram in Figure 6 below.

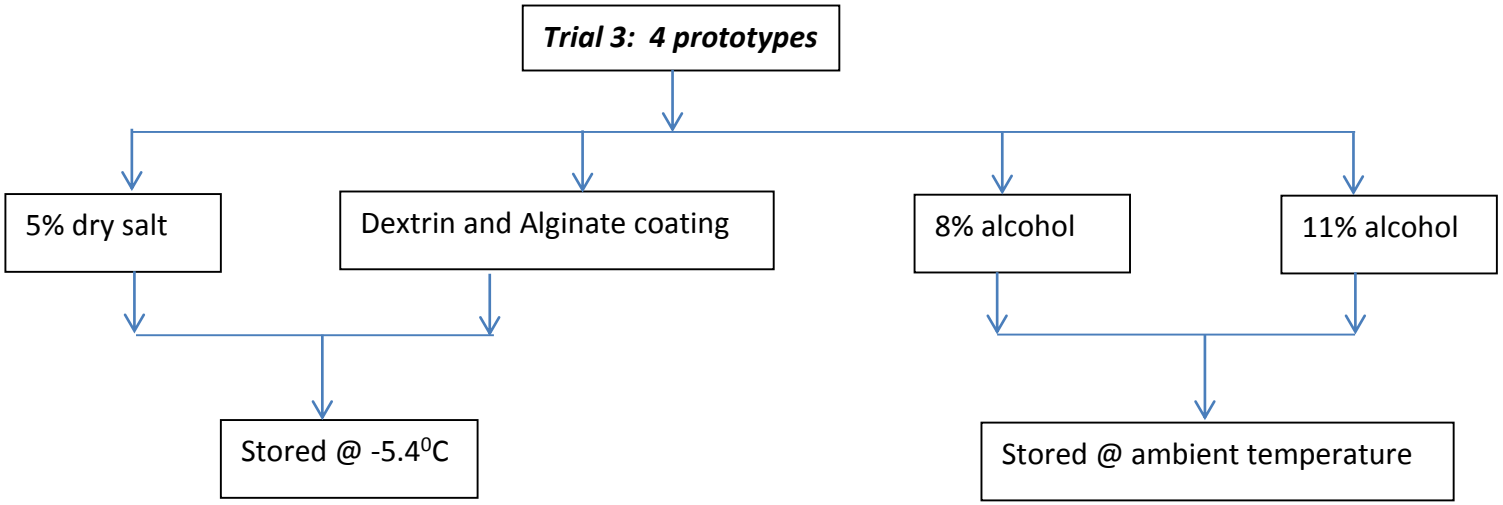

Figure 6: Trial 3 prototypes and storage temperatures

6.3.2 Shelf Life Prediction Test

The four prototypes were tested for shelf life over a period of 34 days using the following indicators; microbial determinations, organoleptic assessments and chemical analyses. These tests were carried out on preserved samples that were stored at -5.4^{0}C for dry salt and dextrin and alginate coated samples while the 8% and 11% alcohol samples were both to be stored at ambient temperature of 30^{0}C as indicated in Table 9 below.

6.3.2.1 Microbial Determination

Similar to trial 2, only two microbial analyses; aerobic plate count and psychrophilic bacterial count were conducted in the shelf life prediction of gonads preserved in 5% dry salt and dextrin-alginate coated stored at -5.4^{0}C and the 8% and 11% alcohol stored at ambient temperature for a period of 34 days. Results as indicated in Table 9 below showed that day 1 microbial levels of gonads were quite high which may be due to cross contamination from the 3.5% brine used. The 3.5% brine was this time manually fetched from a mobile cool storage parked outside the building about 30 meters away from the processing room compared to manually fetched from the cool room next to the processing room. Furthermore, on the processing day, delivery and weighing of Tahitian nuts (ivi) was made at the same entrance where the 3.5% brine was brought through hence the likelihood of cross-contamination. However, it appears that microbial levels decreased over time to acceptable levels on all the four prototypes on days 15 and 34.

Table 9: Microbial Levels of Gonads from Processing Trial 3

Type of test	**-5.4^{0}C (-5^{0}C)**						**Alcohol @ ambient tempt**					
	5% dry salt			**Dextrin & alginate**			**8%**			**11%**		
	D 1	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**
SPC (CFU/g) or EAPC/g-<300)	6.1×10^3	1.0×10^3	5.0×10^2	3.6×10^2	2.2×10^2	5.9×10^5	1.4×10^4	3.8×10^3	50	3.0×10^3	4.2×10^3	5.1×10^3
Psychrophilic (CFU/g or EAPC/g-<2500)	1.4×10^4	7.0×10^2	1.1×10^3	2.0×10^3	ND	1.0×10^2	1.0×10^2	1.7×10^3	ND	1.0×10^2	1.5×10^2	ND

ND = Detection limit is 100 colonies, EPAC – estimated plate aerobic count is referred to <300 counts for Standard Plate Count (SPC) and <2500 counts for psychrophilic bacteria.

It is important to note that the dextrin-alginate coated sample stored at -5.4^{0}C had the highest level of standard plate count on day 34. Confirmation of this unacceptable level was observed on day 34 organoleptic assessments discussed below. This may indicate the importance of employing multiple indicators in the shelf life prediction protocol especially organoleptic assessment.

6.3.2.2 Organoleptic Assessments

Similar to trials 1 and 2, organoleptic assessments used included descriptive profiling and hedonic scaling for each prototype stored in two different temperatures respectively as indicated in Tables 11 and 12 below. Table 11 shows that the dextrin-alginate coated samples lost its acceptable characteristics on day 15; hence the estimated shelf life was 10 days similar to 5.3% brine in trial 1 processing.

Table 11: Organoleptic Evaluation of Gonads from Descriptive Profile of Gonads from Processing Trial 3

Type of test	**--5.4^{0}C**						**Alcohol @ ambient tempt (30.6^{0}C)**					
	5% dry salt			**Dextrin & alginate**			**8%**			**11%**		
	D 1	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**
Colour	Bright	Bright	Dull	Bright	Fade dark	Dull	Bright	Bright	Bright	Bright	Fade dark	Dark
Texture	Firm	Firm	Soft and melted	Firm	Soft	Melted	Firm	Firm	Firm	Firm	Firm coagulated	Firm
Odour	Fresh seaweed	Seaweed	Strong sea weed	Mild seaweed	Neutral	Weak sea weed	Alcohol	Alcohol	Weak fermented alcohol	Alcohol	Strong alcohol	weak alcohol
Taste & Flavor	Seaweed sweet salty	Seaweed sweet & abit salty	Mild sea weed	Sweet & mild seaweed	Neutral	Neutral	Sweet & salty	Sweet salty alcohol	Umami taste (acceptable)	Strong alcohol	Strong alcohol	Weak fermented alcohol
Aftertaste	seaweed	sweet	Slightly bitter	Sweet	none	Bitter	alcohol	alcohol	alcohol	Salty & bitter	Strong alcohol	Slight alcohol

Out of the four prototypes, only alcohol preserved samples retained the acceptable until day 34. These were the 8% and 11% alcohol stored at ambient temperature that retained bright colour, firm texture, and the acceptable alcohol flavour. Given the data obtained to date, both of these alcohol preserved samples could have a much longer shelf life over 34 days. For the 5% dry salt sample, its acceptable characteristics was achieved on day 15, however these characteristics were reduced on day 34, hence its shelf life was estimated as 23 days.

These acceptable characteristics were confirmed by the hedonic scaling as indicated in Table 12 below which revealed that out of the four prototypes, the day 1 dextrin-alginate coated stored at -5.4^{0}C was ranked 1st while the 5% dry salt stored at -5.4^{0}C and the 8% alcohol both

ranked 2nd. On day 15, the 5% dry salt stored at -5.4^{0}C and the 8% alcohol stored at ambient temperature both ranked 1st while 11% alcohol stored at ambient temperature ranked 3rd. On day 34, the 8% alcohol ranked 1st, while 11% alcohol and the 5% dry salt both ranked 2nd. The dextrin-alginate coat was rejected on day 34.

Table 12: Hedonic Evaluation of Gonads from Processing Trial 3

Type of test	**-5.4^{0}C**						**Alcohol @ ambient tempt (30.6^{0}C)**					
	5% dry salt			**Dextrin & alginate**			**8%**			**11%**		
	D 1	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**	**D 1**	**D 15**	**D 34**
Colour	3.3	4.5	3.0	4.2	3.5	2.0	4.3	4.0	3.5	3.8	3.5	3.0
Texture	4.3	4.5	3.0	3.3	2.0	2.5	3.5	3.5	4.0	2.8	3.0	3.0
Odor	3.5	4.5	3.0	4.2	2.5	2.5	4.3	4.5	4.5	4.2	4.5	4.5
Taste & Flavor	3.7	4.5	4.0	4.2	2.0	3.5	3.0	5.0	5.0	2.2	3.5	4.0
Aftertaste	4.2	4.5	3.5	3.8	2.0	3.5	3.7	5.0	4.5	2.3	3.0	3.0
Overall ranking	2nd	1st	2nd	1st	4th	4th	2nd	1st	1st	4th	3rd	2nd

Furthermore, based on the collective hedonic scale and organoleptic assessments, the dextrin-alginate sample stored at -5.4^{0}C became unacceptable resulting in its shelf life to be 10 days and the 5% dry salt estimated shelf life for 23 days. Similarly, the two alcohol preserved samples increased their acceptance characteristics as the alcohol and the gonads mature over time and began to ferment producing umami flavour on day 34 for the 8% alcohol while the 11% alcohol had yet to reach the umami stage hence have a much longer shelf life beyond the 34 days.

For the purpose of finalizing the result of trial 3, a further processing is recommended. This would involve the processing of 5% dry salt to be stored at -5^{0}C and an 8% alcohol to be stored at ambient temperature. These two recommended formulations are expected to include consumer acceptance tests as well inter-laboratory analyses for the confirmation of the in-house tests been conducted to date.

6.3.2.3 Chemical Tests

Like trials 1 and 2, the three chemical analyses used were water activity, salinity and pH as shown in Table 13 below. Results showed that slight reduction in pH, salinity could not be measured further due to the maximum limit of detection in the refractometer used. Like trial 2, note that water activity was conducted only on day 1 to assess the effectiveness in the level of preservatives used.

Table 13: Chemical Analyses of Gonads from Processing Trial 3

Type of test	**-5^{0}C (-5.4^{0}C)**						**Alcohol @ ambient tempt (29^{0}C)**					
	5% dry salt			**Dextrin & alginate**			**8%**			**11%**		
	D 1	**D 15**	**D 36**	**D 1**	**D 15**	**D 36**	**D 1**	**D 15**	**D 36**	**D 1**	**D 15**	**D 36**
Water Activity	0.941@ 24.4^0 C			0.985@ 24.9^{0}C			0.945@2 3.1^{0}C			0.942@ 24.3^{0}C		
Salinity (%)	>10.5	>10.5	>10.5	8.3	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5	>10.5
pH	6.51	6.81	6.54	6.46	6.39	5.96	6.51	6.15	6.17	6.35	6.29	6.14

The combined tests results from microbial, descriptive profile, hedonic scale and chemical analyses suggest the use of the following;

- 5% dry salt to be processed and then stored at -5^{0}C and
- 8% alcohol with 5% dry salt and then stored at ambient temperature

These are to be implemented in the last round and final processing consumer acceptance tests and inter-laboratory analyses are to be conducted for the confirmation of the in-house tests carried out to date.

7.0 Limitations of the study

Two major limitations were identified to have affected the quality of the processed sea urchin gonad products for this project.

1. Spawning time of sea urchin. Unless the spawning time of sea urchin is clearly identified, harvesting and processing at the right time would provide acceptable size and colour in the production of high quality gonad products. This I think should be clearly identified and confirmed including the harvest sites that produces yellow and orange gonads. This was clearly evident in the amount of rejects made

during processing trials 1 and 3 and the gonad yields produced. This perhaps suggests further investigations and research in the area to be conducted.

2. The availability of reliable cool and supercooling storage facilities at -5^{0}C. This was clearly evident in the variation of temperature recorded by the temperature data logger. Even though effort was made in the transfer of samples to other facilities, it was later found that the same issue existed in such facilities. There had high variations in the temperature of the facilities which may have affected the estimation of the shelf life of sea urchin gonad products. This may mean that in the proposed final processing, a -5^{0}C supercooling facility is to be made available before the processing takes place which may also then determine the shelf life of the gonad products at such a stable temperature.

8.0 Conclusion

The project revealed that yellow and orange colored sea urchin gonads could be preserved to achieve the most acceptable and desirable organoleptic characteristics; bright mango-orange or yellow colour, whole firm texture, fresh seaweed odour, fresh seaweed-sweet taste and free of leaking fluids using 5% dry salt stored at -5.4^{0}C with the shelf life of 23 days and the 8% alcohol mixed with 5% dry salt stored at ambient temperature with the shelf life of 34 days. These were obtained from trial 3 processing after series of evaluations of trials 1, 2 and 3 processing procedures and formulations. It is interesting to note that brining appear to be unacceptable due to the oozing and leaking of yellow and orange fluid of *Tripneustes gratilla* species into the brine contributing to milky-turbid solution. Soaking in sodium phosphate did not stop the oozing, instead aggravated milkiness and turbidity in brined samples. Hence the dry salting and alcohol based preservations were the most suitable preservations identified at this stage. This therefore needs further confirmation by conducting further inter-laboratory analyses and consumer acceptance tests in the next and final round of processing.

9.0 References

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