AECOM
KALANG

Pacific Horticultural and Agricultural Market Access (PHAMA) Program
Department of Foreign Affairs and Trade
15-Mar-2017

Product development of chilled and alcohol preserved sea urchin gonads for human consumption

Technical Report 80

Development of chilled and alcohol preserved sea urchin gonads for human consumption

Technical Report 80

Client: Department of Foreign Affairs and Trade

ABN: 47 065 634 525

Prepared by

AECOM Services Pty Ltd
Level 28, 91 King William Street, Adelaide SA 5000, Australia
T +61 8 7223 5400 F +61 8 7223 5499 www.aecom.com
ABN 46 000 691 690

15-Mar-2017

Job No.: 42444251

AECOM in Australia and New Zealand is certified to ISO9001, ISO14001 AS/NZS4801 and OHSAS18001.

Quality Information

Document	Product development of chilled and alcohol preserved sea urchin gonads for human consumption
Ref	42444251
Date	March 2016
Prepared by	Dr Jimaima Lako
Reviewed by	Bronwyn Wiseman

Revision History

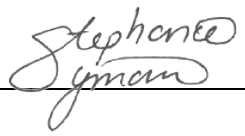
Rev	Revision Date	Details	Authorised	
			Name/Position	Signature
1	15 March 2017	FINAL	Stephanie Symon AECOM Project Manager	

Table of Contents

Executive Summary

The widely distributed edible sea urchin (cawaki) species that grows wild in Fiji is the *Tripneustes gratilla*. 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 B, 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.

Four separate processing trials were conducted during November 2014 to June 2015 on sea urchin species *Tripneustes gratilla* that were harvested by Ministry of Fisheries officers from different locations along the Kalokolevu district harbour to the west of Suva harbour. Harvesting was done in the morning of the processing day and these whole sea urchins were then 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. Preservatives used were dry salt, salt solution or brine, alcohol and a solution containing dextrin, salt and sodium alginate. Storage temperature varied between -5°C to +5°C or ambient (approximately 25-30°C) and stored up to 42 days for the salted samples while 62 days for the alcohol preserved samples. Microbial (standard plate count, total coliforms and psychrophiles) and chemical (pH, salinity) analyses and organoleptic characteristics (colour, texture, flavour and taste) assessments were conducted in determining the shelf life of each product in terms of formulation and the storage condition. The desirable organoleptic characteristics aimed at 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 after the third trial were the gonads preserved in 5% dry salt stored at -5.4^{0}C that had the shelf life of 23 days and the 8% alcohol mixed with 5% dry salt stored at ambient temperature that had a shelf life of 34 days. These recommendations were further confirmed in a fourth trial where slight modification was made to the alcohol based samples. Detailed results revealed that brined samples appeared to be unacceptable and rejected by the taste panellists due to the oozing and leaking of yellow and orange fluid into the brine, contributing to unacceptable milky-turbid solution even though gonad samples *per se* were acceptable. This led to soaking in sodium phosphate to stop the oozing which however was not successful. Instead it aggravated milkiness and turbidity of brined samples hence, the adoption of dry salt and alcohol based preservation formulations. Inter-laboratory tests were conducted on the fourth trial samples which confirmed the in-house microbial tests.

Based on the data gathered, even though sea urchin gonads were successfully processed using 5% dry salt and stored at -5.0^{0}C and the 8% alcohol mixed with 10% dry salt stored at ambient temperature achieved longer shelf lives of 23-26 days and 34-40 days respectively, further research on the spawning time of sea urchins in Fiji and the best sites that produce the bright yellow and orange gonads have yet to be confirmed. This will help determine the ideal harvesting time and the sites that produce high gonad yields especially if positive cost-benefit outcome of reasonable profit is to be achieved.

1.0 Terms of Reference

Coordinate and facilitate the research and development work between relevant stakeholders (farmers, potential exporter, research assistants, Fisheries staff) to:

(i) Agree on areas, volumes, timing of sea urchins to be harvested from the sea by Fisheries staff with resource owners.

ii) Agree and implement the research plan at the pack house and in USP laboratories by Food Technologist, research assistants and pack house staff.

(iii) Develop the process from harvesting to packaging and shelf life testing by Food Technologist.

(iv) Undertake preliminary consumer testing to determine the market acceptability of the products.

(v) Undertake testing of ingredients to ensure compliance with international food standards, including via the IAS laboratory.

(vi) Document the process including HACCP Plan.

2.0 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 value-added products 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) determine 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 in 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).

3.0 Desirable attributes for sea urchin gonads

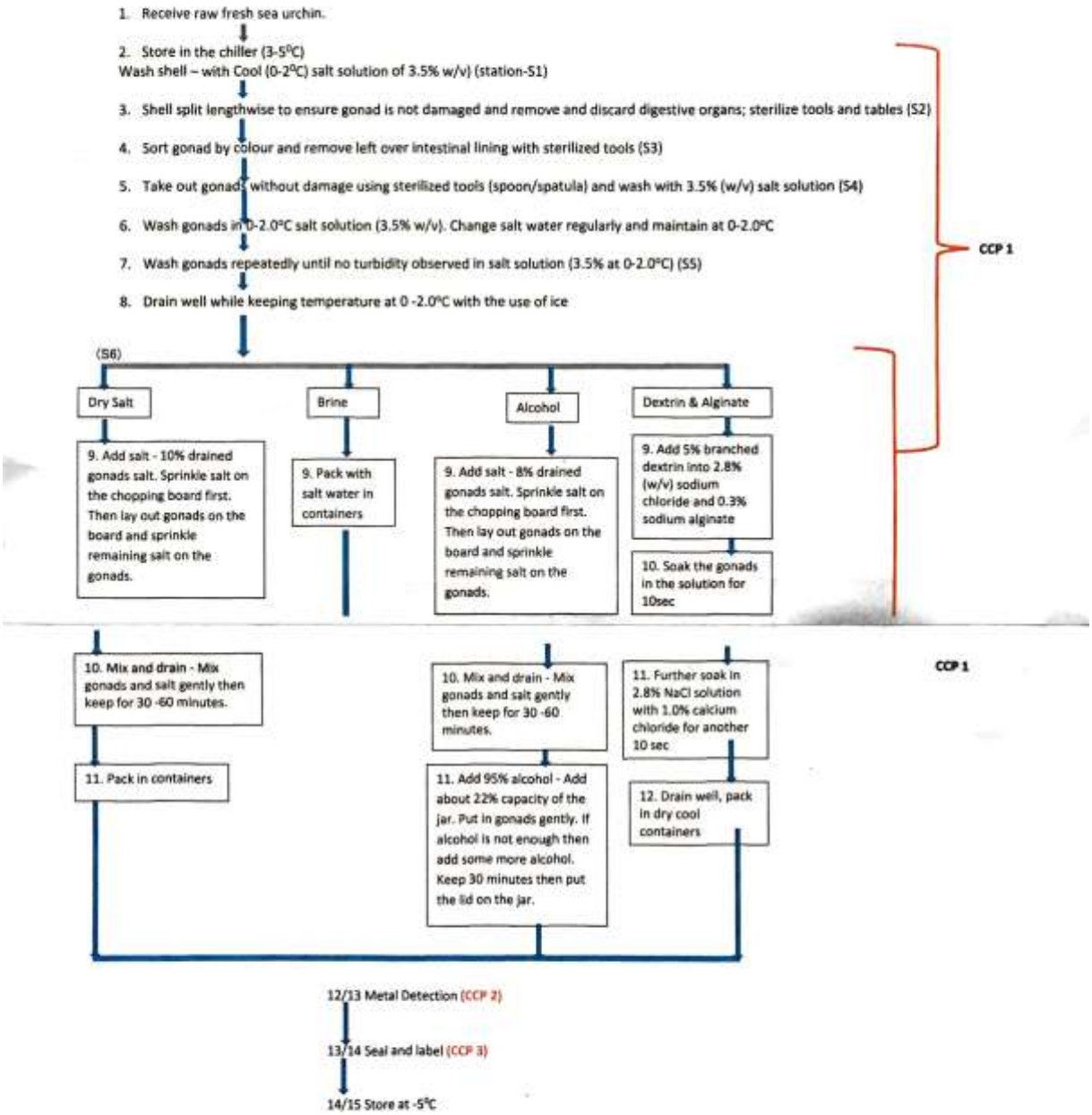
The most desirable attributes for sea urchin gonads that had been developed for export markets include; bright mango-orange or yellow colour, whole firm texture, fresh seaweed odour, fresh seaweed-sweet taste, free of leaking fluids and high nutritional value (Shpigel et al., 2006). The best size for 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). Hence this research focused in the processing of yellow and orange coloured gonads.

4.0 Summary of processing and preservation of sea urchins

Based on the gonads quality attributes highlighted in section 2.0 above the processing of sea urchins adopted the following flow chart procedures. Four preservation methods were conducted as outlined in the flow chart Figure 1 below. Three critical control points (CCP) had been identified as part of the detailed HACCP plan shown in section 8.0 and in Table 1 below. Six stations (S1-S6) were established along the processing line for sea urchin gonads as shown in Figure 1 below.

Figure 1: Process Flow Diagram of the Four Preservation Techniques of Sea Urchin Gonads



5.0 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 is obtained. However, note that this research was beyond the scope of studying sea urchin spawning. Nonetheless, based on the four different processing occasions conducted in Dec 17th 2014, Jan 14th 2015, Feb 11th 2015 and June 9th 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 good sizes of some gonads around 2.5cm x 9cm and high quality gonads weighing around 15-40g of gonads per sea urchin as shown in Figure 1 below. Sizes and weights of sea urchin tests vary from 7-11cm in diameter and 217-420g respectively as shown in Figure 2 below. Studies in the Philippines revealed the peak spawning of T. gratilla species is from December to January (McManus et al., undated) which may be similar to Fiji but this has yet to be confirmed.

Figure 1 Gonads

Figure 2 Sea urchin test sizes

6.0 Quality assurance

The 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 the use of 100ppm chlorine and then sanitized with 75% alcohol before and after processing.

Processing of sea urchin was maintained below 50C of the 3.5% salt solution. Ice was continuously used at every station to retain the cold chain below 50C as the operational limit. A temperature data logger was used in recording and the confirmation of temperature.

Table 1: Critical Control Points with Associated Hazards and Control for Commercial Processing

CCP	Hazards	Controls
CCP 1	*Vibrio parahaemolyticus*	The product is held at internal temperatures below 10^{o}C
CCP 2	Metal	Metal detection or equipment checks

CCP 3	Fish (sea urchin) is a food allergen	Label

Shell split was done lengthwise to avoid and reduce damage to gonads due to oozing especially when the tissue is physically damaged. Sorting of gonad size and colour was conducted after shell splitting to ensure reasonable gonad size with acceptable yellow and orange colour as shown in Figure 3 below were retained while dark brown was rejected. Only full sizes of at least 8-10mm thickness (any length) gonads were accepted.

Figure 3. Yellow and orange gonads with two types of splits a and b

7.0 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;

-50C, 00C, 50C 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 four processing trials of sea urchins were carried out on separate occasions as indicated below;

- trial 1-Dec 17th 2014,
- trial 2 – Jan 14th 2015
- trial 3 – Feb 11th 2015 and
- trial 4 – June 9th 2015

7.1 Processing trial 1

7.1.1 Processing design and protocol

Trial 1 processing involved the processing of 3 prototypes: preserved in 5.3% brine stored at 50C and 00C as well as in 21% alcohol stored at ambient temperature as shown in the experimental flow diagram in Figure 4 below.

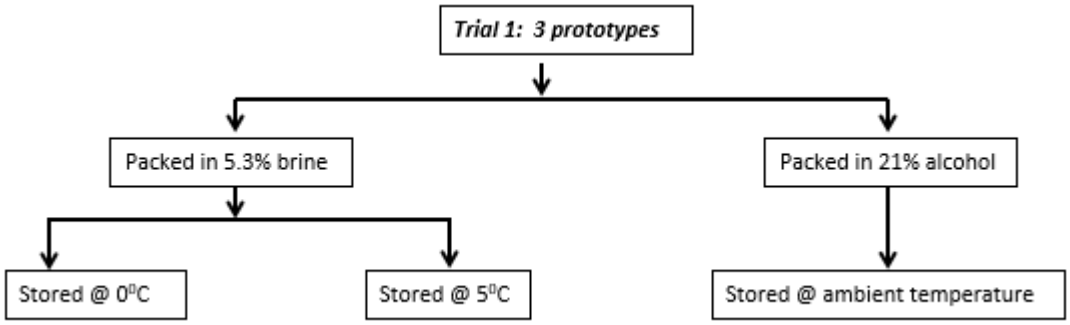


Figure 4. Trial 1 prototypes and storage temperatures

7.1.2 Shelf life prediction test

The three prototypes processes in Figure 4 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 -0.40C, 0.70C and ambient temperature of 28.10C 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 due to opening and closing of the fridge by other students who were also using the fridge at the time of the experiment. The details of tests with results are discussed below.

7.1.2.1 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 0.70C and -0.40C 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 detected high. This is because *E.coli* is a subset of coliform which coliform is an indicator of poor sanitation and hygienic practices. Hence due to the insignificant 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 protocol employed.

Table 2. 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 2 above revealed that even though the aerobic plate count or standard plate count (SPC) 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 -0.40C had lower levels of microbes compared to 0.70C 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 <50C chilled of 3.5% brine solution which effectively controlled the growth of bacteria below 300 count of SPC and that retained the firmness of gonads as shown in 6.1.2.2 below. This may indicate that even though the microbial levels were 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.

7.1.2.2 Organoleptic assessments

The organoleptic assessments employed two techniques; the descriptive profiling and hedonic scaling of each protocol stored at various temperatures as indicated in Tables 3 and 4 below. Table 3 showed that preservation in 5.3% brine resulted in milky- turbid brine solution, however -0.40C 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 0.70C 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 had lost the sweet taste. On day 15, the 0.70C stored sample became dull in colour, softer texture with neutral odour and weak seaweed flavour compared to -0.40C sample that retained seaweed flavour and sweet taste and had just begun to get soft in texture and a bit taint in colour. Given these characteristics, the -0.40C storage is estimated to have a shelf life of 10 days calculated as 70% of day 15 while 0.70C 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, firmer texture, dark brighter in colour with sweet-alcohol odour.

Table 3. 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 trained panellists

The hedonic scale of the three protocols shown in Table 4 below revealed that on day 1; the 5.3% brine stored at 0.70C was ranked 1st, 5.3% brine stored at -0.40C ranked 2nd while 18% alcohol stored at 28.10C ranked 3rd. On day 7, 5.3% brine stored at -0.40C ranked 1st, while 18% stored at 28.10C ranked 2nd and the 5.3% brine stored at 0.70C ranked 3rd. On day 15, the 18% alcohol stored at 28.10C ranked 1st, 5.3% brine stored at -0.40C ranked 2nd while 5.3% brine stored at 0.70C ranked 3rd.

Table 4. 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 trained panellists, 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 0.70C 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 00C and -50C respectively as well a reduction of alcohol concentration to 14% and stored at 50C and ambient temperature respectively.

7.1.2.3 Chemical tests

Further insights into the reactions taking place during storage were detected by the measurements of three chemical characteristics of the products. These were water activity, salinity and pH as shown in Table 5 below. Results showed that slight changes in pH, salinity and water activity had occurred. The 5.3% brine stored at -0.40C 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 0.70C 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.

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 recommendations 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 0^0C and -5^0C respectively
(c) 10% dry salt to be developed for storage at 0^0C and -5^0C 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 recommendations above were carried out as part of trial 2 processing as indicated below.

7.2 Processing trial 2

7.2.1 Processing design and protocol

Trial 2 processing involved the development of 6 prototypes: 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 00C and -50C respectively; 14% alcohol then stored at 50C 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 5 below.

7.2.2 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 -50C and 00C for both brined and dry salted samples while the 14% alcohol samples were stored at 50C 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 -1.50C, 1.70C and ambient temperature of 300C 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.

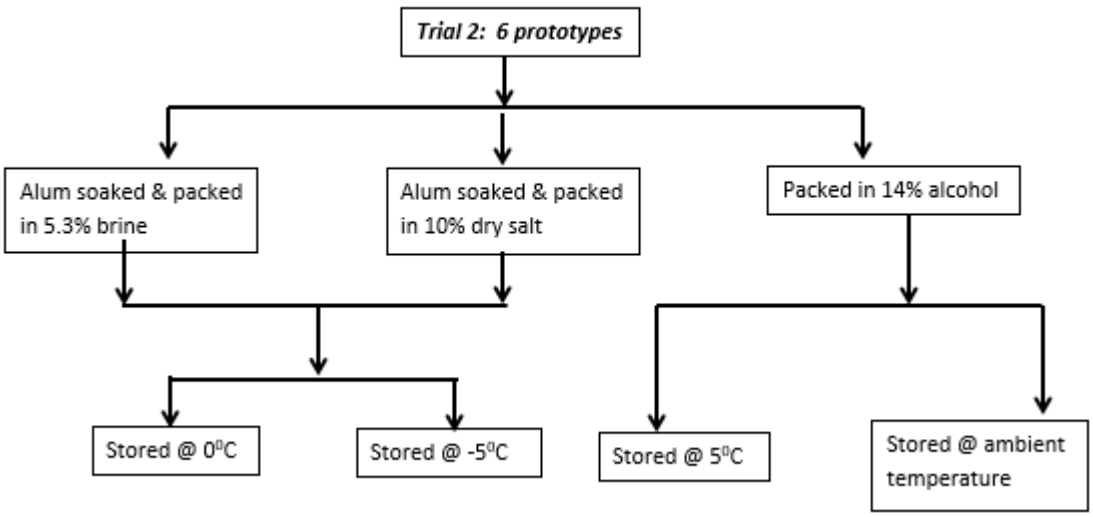


Figure 5. Trial 2 prototypes and storage temperatures

7.2.2.1 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 -1.50C and 1.70C respectively and as well as the 14% alcohol stored at 1.70C 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 insignificant 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 compliance to the HACCP plan as indicated section 8.0, we were confident that coliform levels would be also insignificant. Results as indicated in Table 6 below showed that microbial levels of gonads were generally low indicating <106 (Health Protection Agency, 2009) from day 1 to day 43 of selected samples even though fluctuations in data was observed.

It is important to note that the 5.3% brine that was soaked in sodium phosphate for 1 min prior to storage at both -1.50C and 1.70C 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 1.70C 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 in the section 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 21% alcohol, trial 2 of day 1, 14% alcohol stored at 1.70C, the microbial analysis was 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 1.70C would be sufficient to control the growth of microbes. This assumption was confirmed in data gathered.

Table 6. 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.

7.2.2.2 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 6 and 7 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 Preservation	**Alum 5.3% brine**					
Storage Temperature	**@ -1.5^{0}C**			**@ 1.7^{0}C**		
Storage Days	**D1**	**D15**	**D22**	**D1**	**D15**	**D22**
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
Texture	firm	soft	firm	firm	firm	Mealtime out
Odor	Fresh seaweed	neutral	Mild seaweed	Strong fresh seaweed	seaweed	seaweed
Taste & Flavor	Fresh seaweed& salty	Very salty	Mild seaweed sweet salty	Fresh seaweed sweet & salty	Seaweed sweet salty	Seaweed sweet
Aftertaste	Squized tongue	astringency	Squized tongue	Prawn tail and bitter	Squizzed tongue	Squizzed tongue

Type Preservation	**Alum 10% dry salt**									
Storage Temperature	**@ -1.5^{0}C**						**@ 1.7^{0}C**			
Storage Days	**D1**	**D15**	**D22**	**D29**	**D43**	**D62**	**D1**	**D15**	**D22**	**D29**
Colour	Bright yellow	Light color	Bright dark	bright	Bright	Milky bright color	Fresh ornage	Light color	Bright	Bright
Texture	Mealtime & break easily	firm	firm	firm	firm	firm	Firm but start to melt	firm	Firm	Getting soft
Odor	neutral	seaweed	Neutral	Neutral	Sea weed	Neutral	neutral	neutral	Neutral	Rotten banana

										(ester)
Taste & Flavor	Salty & sweet	Very salty	Salty	Salty	Seaweed sweet salty	Weak sea weed	Salty seaweed	salty	Salty	Salty
Aftertaste	bitter	Squizzed tongue	Squizzed tongue	Squizzed tongue	Sweet salty squizzed tongue	Slightly bitter	bitter	Squizzed tongue	Squizzed tongue	Squizzed tongue
Type Preservation	**14% alcohol**									
Storage Temperature	**@ 1.7^{0}C**					**@ ambient tempt (30^{0}C)**				
Storage Days	**D15**	**D22**	**D29**	**D43**	**D62**	**D1**	**D15**	**D22**	**D29**	**D43**	**D62**
Colour	Dark color	Dull	Bright	Bright	Bright	Bright yellow intense	Dark color	Dull	Dark	Bright	Bright
Texture	firm	firm	Firm	Firm coagulated	Firm	Very firm coagulated	firm	firm	firm	Firm coagulated	Firm
Odor	alcohol	Neutral	Alcohol	Mild seaweed strong alcohol	Alcohol	Weak alcohol	alcohol	Mild alcohol	alcohol	Strong alcohol	Strong alcohol
Taste & Flavor	alcohol	Alcohol	Alcohol	Alcohol	Alcohol	Alcohol with sweet & salty	alcohol	alcohol	Alcohol	Strong alcohol and salty	Strong alcohol umami taste
Aftertaste	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 -1.50C, the 14% alcohol stored at both 1.70C 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 1.70C 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 below which revealed that out of the six prototypes, the 5.3% brine stored at 1.70C was ranked 1st both on days 1 and 15, the 10% dry salt stored at 1.70C 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 trained panellists, 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 -1.50C and 1.70C 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, the use of sodium phosphate was eliminated. Similarly, soaked in alum 10% dry salt stored at 1.70C 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 meant that based on physical appearances and assessments, 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 with 5% concentration and to be stored at -50C, a further reduction of alcohol concentration to 8% and 11% respectively and both to be stored at ambient temperature and a new coating method in the use of dextrin and alginate.

7.2.2.3 Chemical tests

Further insights into the chemical reactions during shelf life determination continued with the three chemical analyses; water activity, salinity and pH as shown in Table 9 below. Results showed that slight changes in pH, salinity and water activity had been observed. The alum 5.3% brine stored at -1.50C and 1.70C revealed a slight reduction in pH and increased salinity over 22 days of storage while both the alum 10% dry salt stored at -1.50C and 1.70C 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 9 Chemical analyses of gonads from processing trial 2

Table 9: 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

n=3

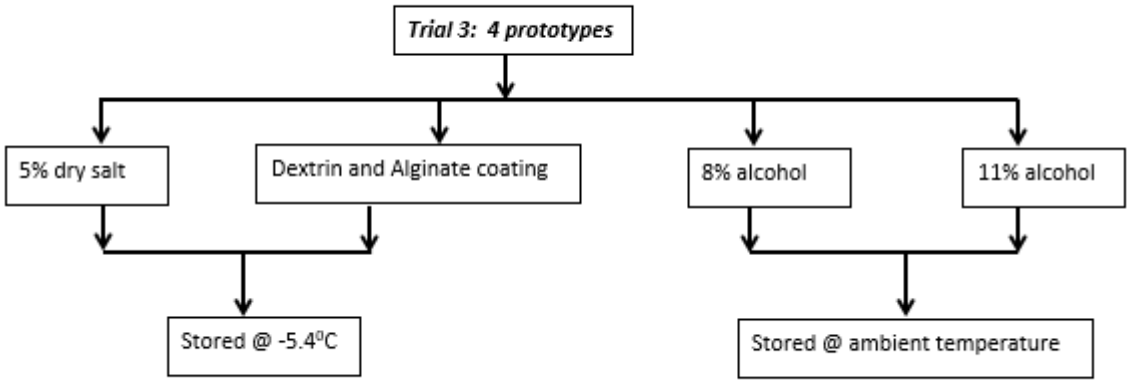
Based on combined tests results; microbial, descriptive profile, hedonic scale and chemical analyses suggestions made to further improve the quality and shelf of the gonad products were as follows; only dry salt with 5% concentration to be stored at -50C, a reduction of alcohol concentration at 8% and 11% respectively and both to be stored at ambient temperature and a new coating method in the use of dextrin and alginate. These recommendations were implemented in trial 3 processing indicated in section 7.3 below.

7.3 Processing trial 3

7.3.1 Processing design and protocol

Trial 3 processing involved the processing of 4 prototypes: preserved in 5% dry salt, dextrin and alginate coating prior to storage at -5.40C; 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



7.3.2 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 -5.40C 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 300C as indicated in Table 9 below.

7.3.2.1 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 -5.40C and the 8% and 11% alcohol stored at ambient temperature for a period of 34 days. Results as indicated in Table 10 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 usual manual 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 10. Microbial levels of gonads from processing trial 3

Type of test	**-5.4⁰C (-5⁰C)**						**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-	6.1 × 10³	1.0 × 10³	5.0 × 10²	3.6 × 10²	2.2 × 10²	5.9 × 10⁵	1.4 × 10⁴	3.8 × 10³	50	3.0 × 10³	4.2 × 10³	5.1 × 10³

<300)												
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 -5.40C 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 which may indicate the importance of employing multiple indicators in the shelf life prediction protocol especially organoleptic assessment.

7.3.2.2 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 characteristics 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 -5.40C was ranked 1st while the 5% dry salt stored at -5.40C and the 8% alcohol both ranked 2nd. On

day 15, the 5% dry salt stored at -5.40C 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.

Based on trial 3 results, trail 4 processing was recommended to reconfirm the in-house data gathered in which inter-laboratory tests and consumer acceptance tests were carried out. This mainly involved the processing of 5% dry salt stored at -5^{0}C and the 8% alcohol stored at ambient temperature.

7.3.2.3 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@23.1^{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

Based on the combined tests results from microbial, descriptive profile, hedonic scale and chemical analyses recommended the following formulation and storage conditions as final formulation;

- 5% dry salt stored at -50C and

- 8% alcohol with 10% dry salt stored at ambient temperature

7.4 Processing trial 4

7.4.1 Processing design and protocol

Trial 4 processing involved the processing of 2 final prototypes: preserved in 5% dry salt stored at -5.00C; and 8% alcohol concentrations stored at ambient temperature for a total of 42 days as shown in the experimental flow diagram in Figure 7 below.

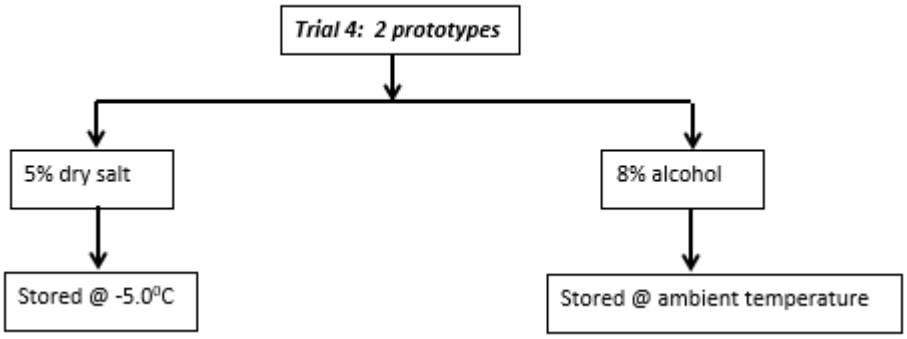


Figure 7. Trial 4 prototypes and storage temperatures

7.4.2 Inter-laboratory tests for confirmation

The two final prototypes were tested for shelf life over a period of 42 days using the following indicators; microbial determinations, organoleptic assessments and chemical analyses. These tests were carried out on preserved samples that were stored at -5.00C for dry salt and the 8% alcohol samples stored at ambient temperature of 300C as indicated in Table 14 below.

7.4.2.1 Microbial and chemical determinations

Similar to previous trials, three microbial analyses; aerobic plate count, coliforms and *E.coli* bacterial count were conducted at the Institute of Applied Sciences (IAS) for the 5% dry salt stored at -5.00C and the 8% alcohol stored at ambient temperature for a period of 42 days. Results as indicated in Table 14 below showed that day 1 microbial levels of gonads were normal and reduced to insignificant levels up to day 14 for SPC for both samples indicate and confirm the safety of products. Similarly, coliforms and *E.colis* were insignificant demonstrating compliance of the HACCP plans showed in section 8.0 that were implemented.

Table 14. Microbial and selected chemical analyses of gonads from processing trial 4

Type of test	5% dry salt at -5.0^{0}C (-5^{0}C)						8% alcohol with 10% salt @ ambient tempt					
	Day 1		Day 7	Day 14	Day 28	Day 42	Day 1		Day 7	Day 14	Day 28	Day 42
	IAS	In-house	IAS	IAS			IAS	In-house				
SPC (CFU/g) or EAPC/g-<300)	1.2×10^3	$7.4x10^2$	260est	120est			2.4×10^3	110est	<10est	<10est		
Total Colifom (MPN/g)	<3						<3					
E.coli (MPN/g)	<3						<3					
Salt (%)												
SPC (CFU/g) or EAPC/g-<300)	1.2×10^3	$7.4x10^2$	260est	120est			2.4×10^3	110est	<10est	<10est		
Total Colifom (MPN/g)	<3						<3					
E.coli (MPN/g)	<3						<3					
Salt (%)												

est – estimated plate aerobic count is referred to <250 counts for Standard Plate Count (SPC)

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.

7.4.3 Consumer acceptance

About 32 randomly selected consumers; 16 Fijians and 16 Japanese participated in the consumer acceptance test with the use of hedonic scale (1-5 points; 1-dislike very much, 2-dislike, 3-neither like nor dislike, 4-like, 5-like very much) and intent of purchase. Participants were selected based on their previous consumption of fresh sea urchin or frequent consumption. Two types of preserved gonads were tested; salted stored at -50C and alcohol at stored ambient temperature for a period of 30 days. Fijian participants were presented only with the salted sample while the Japanese were presented with both the samples.

7.4.3.1 Salted sea urchin gonads

Table 15 revealed that salted gonads has been well accepted and liked with an average hedonic scale of both ethnicities at 4.13, while Fijians alone at 4.45 and Japanese at 3.82. When each sensory characteristic was tested, color had the hedonic overall scale of 3.72, odour - 4.06, texture and taste - 4.31 and flavour - 4.27 with the median and mode at 4 and 5. It appears that Fijians have higher hedonic scale than the Japanese which may be interpreted as liked by Fijians and less by the Japanese. The low hedonic scale was mainly associated with the colour in which some light brown coloured gonads were used and the level of salt. In terms of intent of purchase, overall 84% responded positively from both ethnicities. When ethnicities are compared 100% Fijians and 70% Japanese indicated positive response.

Table 15 Consumer acceptance of salted sea urchin gonads

Characteristics		Data			General comments
Sample size (n)		32			
Gender		11 males (34.4%); 21 females (65.6%)			
Ethnicity		16 Fijian; 16 Japanese			
Mean age		37.0			
Age range		20-60			
		Fijians (n=16)	**Japanese (n=16)**	**Total (n=32)**	
Colour	Mean	4.19 ± 0.66	3.25 ± 0.77	3.72 ± 0.85	Still bright
	Median	4.0	3.0	4.0	
	Mode	4	3	4	
Odour	Mean	4.44 ± 0.51	3.69 ± 0.7	4.06 ± 0.72	Good seaweed odor
	Median	4.0	4.0	4.0	
	Mode	4	4	4	
Texture	Mean	4.56 ± 0.51	4.06 ± 0.85	4.31 ± 0.74	Firm
	Median	4.5	4.0	4.0	
	Mode	5	4	4	
Taste	Mean	4.50 ± 0.89	4.13 ± 0.96	4.31 ± 0.93	A bit salty
	Median	5.0	4.0	5.0	
	Mode	5	5	5	
Flavor	Mean	4.56 ± 0.63	3.97 ± 0.94	4.27 ± 0.84	Natural sea weed flavor
	Median	5.0	4.0	4.0	
	Mode	5	4	5	
Willing to buy		16 (100%)	11 (68.8%)	21 (84.4%)	

7.4.3.2 Alcohol preserved sea urchin gonads

Table 16 revealed that the alcohol preserved gonads has also been accepted and liked by the Japanese with an average hedonic scale of at 3.63. When each sensory characteristic was tested, color had the hedonic scale of 3.24, odour - 3.82, texture at 4.00, taste - 3.49 and flavour - 3.50 with the median and mode at 3 and 4. In terms of intent of purchase, 69% responded positively. The low hedonic scale was mainly associated with the alcohol flavour and salt level used. In case alcohol and salt levels were to be reduced, change of storage to 0-50C would be recommended.

Table 16 Consumer acceptance of alcohol preserved sea urchin gonads

Characteristics		Data	General comments
Sample size (n)		16	
Gender		9 males (%); 7 females (%)	
Ethnicity		Japanese	
Mean age		36.5	
Age range		20-60	
Colour	Mean	3.24 ± 0.72	Still bright
	Median	3.0	
	Mode	3	
Odour	Mean	3.82 ± 0.88	Alcohol sweet odor
	Median	4.0	
	Mode	4	
Texture	Mean	4.00 ± 0.61	Firm
	Median	4.0	
	Mode	4	
Taste	Mean	3.59 ± 0.94	A bit salty
	Median	4.0	
	Mode	4	
Flavor	Mean	3.50 ± 0.94	Alcohol taste
	Median	4.0	
	Mode	4	
Willing to buy		11 (68.8%)	

8.0 Hazard Analysis Critical Control Points Plan (HACCP)

Based on the two final prototypes identified; salted and alcohol preserved, their HACCP plans had been prepared using the HACCP plan forms which identified the 10 step procedures of the HACCP planning activities as shown in sections 8.1 and 8.2 respectively. Both the plans have the same three critical controls points

8.1 Alcohol sea urchin

HACCP PLAN FORM

FIRM NAME:

FIRM ADDRESS:

PRODUCTION DESCRIPTION: Alcohol Sea Urchin

METHOD OF DISTRIBUTION AND STORAGE: Room temperature

INTEND USE AND CONSUMER: To be RTE and consumed by the general public

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
CRITICAL CONTROL POINT	HAZARD(S)	CRITICAL LIMITS FOR EACH PREVENTIVE MEASURE	MONITORING				CORRECTIVE ACTION(S)	VERIFICATION	RECORD KEEPING
			WHAT	HOW	FREQUENCY	WHO			
CCP 1: Step 3 to Step 11 - Shell split to addition of 95% alcohol	B. *Vibrio parahaemolyticus*	Sea urchin cooled is held at internal temperatures below 10°C throughout processing	Sea urchin internal temperature	Digital time and temperature data logger in marked batches of sea urchin	*Begin the batch process *Approximately every 15 minutes record temp from step 3 to 11	Production employee	*Destroy product *Add ice	*Data logger print-out *Record or visual checks	*Check the data logger for accuracy and damage and to ensure that itis operational before putting into operation; check it daily, at the beginning of operations; and calibrate it once per year *Review monitoring, corrective action, and

									verification records within1 week of preparation

SIGNATURE OF COMPANY OFFICAL: _____ DATE: _____

PAGE: 1 of 2

HACCP PLAN FORM

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
CRITICAL CONTROL POINT	HAZARD(S)	CRITICAL LIMITS FOR EACH PREVENTIVE MEASURE	MONITORING				CORRECTIVE ACTION(S)	VERIFICATION	RECORD KEEPING
			WHAT	HOW	FREQUENCY	WHO			
CCP 2: Step 12. Metal detection	P. Metal	All of the product passes through an operating metal detector No detectable	Metal detector present and operating The product for the presence of metal fragments	Visual examination Electronic metal detector	Daily, at start of operations Continuous	Production employee Equipment itself	If the product is processed without metal detection, hold it for metal detection, hold it for metal detection *Correct operating procedures to ensure that the product is not processed	Metal detector operation log	*Conduct a validation study to determine appropriate settings for the metal detector *Develop metal detector sensitivity standards *Challenge the metal detector with sensitivity standards daily, before start-up, every 4 hours during production, whenever processing

		metal fragments are in the product passing the through the metal detector					without metal detection *Rework to remove metal fragments from any product rejected by the metal detector *Identify the source of the metal found in the product and fix the damaged equipment		factors change, and at the end of processing *Review monitoring, corrective action and verification records within 1 week of preparation
CCP 3: Step 13. Seal and label	C. Allergens	Finished product labels must declare the presence of sea urchin	Finished product labels for comparison with product formula	Visual examination of the labels on finished product packages	One label at the beginning of the production of each lot and one label every hour thereafter	Quality assurance staff	*Segregate and relabel any incorrectly labeled product *Modify labeling procedure, as appropriate	Record of review of finished product labels	Review monitoring and corrective action records within 1 week of preparation

SIGNATURE OF COMPANY OFFICAL: _____ DATE: _____

PAGE: 2 of 2

8.2 Salted sea urchin

HACCP PLAN FORM

FIRM NAME:

FIRM ADDRESS:

PRODUCTION DESCRIPTION: Salted Sea Urchin

METHOD OF DISTRIBUTION AND STORAGE: -5^{0}C

INTEND USE AND CONSUMER: To be RTE and consumed by the general public

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
CRITICAL CONTROL POINT	HAZARD(S)	CRITICAL LIMITS FOR EACH PREVENTIVE MEASURE	MONITORING				CORRECTIVE ACTION(S)	VERIFICATION	RECORD KEEPING
			WHAT	HOW	FREQUENCY	WHO			
CCP 1: Step 3 to Step 10 - Shell split to addition of 95% alcohol	B. *Vibrio parahaemolyticus*	Sea urchin cooled is held at internal temperatures below 10°C throughout processing	Sea urchin internal temperature	Digital time and temperature data logger in marked batches of sea urchin	*Begin the batch process *Approximately every 15 minutes record temp from step 3 to 11	Production employee	*Destroy product *Add ice	*Data logger print-out *Record or visual checks	*Check the data logger for accuracy and damage and to ensure that itis operational before putting into operation; check it daily, at the beginning of operations; and calibrate it once per year *Review monitoring, corrective action, and verification records within1 week of preparation

SIGNATURE OF COMPANY OFFICAL: _____ DATE: _____

PAGE: 1 of 2

HACCP PLAN FORM

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
CRITICAL CONTROL POINT	HAZARD(S)	CRITICAL LIMITS FOR EACH PREVENTIVE MEASURE	MONITORING				CORRECTIVE ACTION(S)	VERIFICATION	RECORD KEEPING
			WHAT	HOW	FREQUENCY	WHO			
CCP 2: Step 11. Metal detection	P. Metal	All of the product passes through an operating metal detector No detectable metal fragments are in the product passing the through the metal detector	Metal detector present and operating The product for the presence of metal fragments	Visual examination Electronic metal detector	Daily, at start of operations Continuous	Production employee Equipment itself	If the product is processed without metal detection, hold it for metal detection, hold it for metal detection *Correct operating procedures to ensure that the product is not processed without metal detection *Rework to remove metal fragments from any product	Metal detector operation log	*Conduct a validation study to determine appropriate settings for the metal detector *Develop metal detector sensitivity standards *Challenge the metal detector with sensitivity standards daily, before start-up, every 4 hours during production, whenever processing factors change, and at the end of processing *Review

							rejected by the metal detector *Identify the source of the metal found in the product and fix the damaged equipment		monitoring, corrective action and verification records within 1 week of preparation
CCP 3: Step 12. Seal and label	C. Allergens	Finished product labels must declare the presence of sea urchin	Finished product labels for comparison with product formula	Visual examination of the labels on finished product packages	One label at the beginning of the production of each lot and one label every hour thereafter	Quality assurance staff	*Segregate and relabel any incorrectly labeled product *Modify labeling procedure, as appropriate	Record of review of finished product labels	Review monitoring and corrective action records within 1 week of preparation

SIGNATURE OF COMPANY OFFICAL: _____ DATE: _____

PAGE: 2 of 2

9.0 Limitations of the study

The major limitation identified in the current study is the limited understanding of the spawning time of sea urchin in Fiji. 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 while trial 4 had high amounts of yellow gonads with limited orange. This perhaps suggests further investigations and research in the area to be conducted.

10.0 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 -5.00C with the shelf life of 23-25 days and the 8% alcohol mixed with 10% dry salt stored at ambient temperature with the shelf life of 34-40 days. When consumer acceptance was conducted both samples were accepted, however salted gonads appeared to be well accepted compared to the alcohol preserved one. The two final prototypes and their formulations were obtained after series of evaluations of trials 1, 2 and 3 and further confirmation at trial 4 processing. It is interesting to note that brining appeared 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.

11.0 References

Chen, Y; Chen, T; Chiou, T and Hwang, D. (2013). Seasonal variation on general composition, free amino acids and fatty acids in the gonad of Taiwan's sea urchin Tripneustes gratilla. Journal of Marine Science and Technology, 21:6; 723-732.

Fiji Ministry of Health. (2010). Food Safety Regulation 2009. Fiji Islands Government Gazette Supplement. Government Printer, Suva. Pg 246.

Health Protection Agency. (2009). Guidelines for assessing the microbiological safety of ready-to-eat foods. London. Pg 33

James, P.J; Heath, P and Unwin, M.J. (2007). The effects of season, temperature and initial gonad condition on roe enhancement of the sea urchin Evechinus chloroticus. Aquaculture, 270; 115-131.

Kato, S and Schroeter, S.C. (1985). Biology of Red Sea Urchin, Strongylocentrotus franciscanus, and its fishery in California. Marine Fisheries Review, 47(3); 1-20.

Kato.S,(1972). Sea urchins: a new fishery develops in California. Mar. Fish. Rev, 34(9-10), 23-30.

Lawrence, J. (2013). Sea Urchins: Biology and Ecology (2013). Elsevier, London. PP 270.

Manuel, J.I; Prado, V.V; Tepait, E.V; Estacio, B.M; Galvez, G.N and Rivera, R.N. (2013). Growth performance of the sea urchin, Tripneustes gratilla in cages under La Union Condition, Philipines. E-International Scientific Research Journal, V (1); 195-202.

McManus, L.T; Gomez, E.D; McManus, J.W and Juinio, A. (Undated). Sea urchin management in Bolinao, Pangasinan, Philipines: Attempts on sustainable use of communal resource.

Reynolds J.A and Wilen J.E. (2000). The sea urchin fishery: Harvesting, processing and the market. Marine Resource Economics, 15; 115-126.

Schlosser, S.C; Lupatsch, I; Lawrence, J.M; Lawence, A.L and Shpigel, M. (2005). Protein and energy digestibility and gonad development of the European sea urchin Paracentrotus lividus fed algal and prepared diets during spring and fall. Aquaculture Research, 36; 972-982.

Shpigel, M; Schlosser, S.C; Ben-Amotz, A; Lawrence, A.L and Lawrence, J.M. (2006). Effects of dietary carotenoid on the gut and the gonad of the sea urchin Paracentrotus lividus. Aquaculture, 261; 1269-1280.

Sonu, S.C. (2003). The Japanese sea urchin market: NOAA technical memorandum NMFS. U.S. Department of commerce. Southwest Region. P 34.

Suckling, C.C; Symonds, R.C; Kelly, M.S. and Young, A.J. (2011). The effect of artificial diets on gonad colour and biomass in the edible sea urchin Psammechinus miliaris. Aquaculture 318; 335-342

Appendix A

Sensory evaluation scorecard

Appendix A Sensory evaluation scorecard

Sensory Evaluation Scorecard of Sea Urchin

Name: _____Date: _____Age: _____Gender: _____

There are two sections to this sensory evaluation exercise; Sensory Profiling, Hedonic scale with Rating and you are expected evaluate your preference based on 5 sensory attributes; colour, aroma, texture and flavor.

Please follow the *instructions* given below when conducting sensory evaluation.

a. For Odor Assessment:

1. Bring the sample close your nose and take 3 short sniffs and rank your preference.
2. Wait for 2-5 seconds for olfactory senses to recover before proceeding to next sample.

b. For Texture and Flavor Assessment:

1. Rinse your mouth with water before you begin tasting and also between each sample.
2. Taste each sample and retain in mouth to detect the taste and tenderness for ranking as per your preferences.
3. Wait for at least 2-3 minutes before tasting next sample for flavor and texture.

A. Sensory Profiling

Characteristic	**Description**
Color of surface (dull - bright)	
Odor (Aroma)	
Texture (soft-firmness)	
Taste	
Flavor	

B. Hedonic Scaling

Please evaluate the sensory characteristics of the 3 sea urchin samples presented in front from you using the scale provided:

1 = Dislike Very Much, 2 = Dislike, 3 = Neither like nor dislike, 4 = Like, 5 = Like Very Much

and with reasons for your selection or comments.

1. Sample 01

Characteristic	**Scale**	**Reason for your choice and other comments**
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		
Flavor		

2. Sample 02

Characteristic	Scale	Reason for your choice and other comments
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		
Flavor		

3. Sample 03

Characteristic	Scale	Reason for your choice and other comments
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		
Flavor		

4. Sample 04

Characteristic	Scale	Reason for your choice and other comments
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		
Flavor		

5. Sample 05

Characteristic	Scale	Reason for your choice and other comments
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		

Flavor		

6. Sample 06

Characteristic	**Scale**	**Reason for your choice and other comments**
Color of surface (dull - bright)		
Odor (Aroma)		
Texture (soft-firmness)		
Taste		
Flavor		

C. Ranking Test

Please rank the presented sea urchin in the order of acceptability. Rank the most acceptable sea urchin as 1st and the least acceptable as 6rd. Do not assign the same rank to two samples. Basically, rank the samples for its overall acceptability on a scale of "1 – 6".

7. Samples

Samples	**01**	**02**	**03**	**04**	**05**	**06**
Overall Acceptability						