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Sea Cucumber (*Isostichopus* sp. aff *badionotus*) Dry-Salting Protocol Design

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Abstract

Sea cucumbers are common foods in Asian culture. They are commonly eaten as dry-salted. Thus, a dry-salting protocol for cucumbers includes the processes of: gutting, cooking, salting, drying and packing. Drying trials were conducted at 50˚C and 60˚C in an oven and air-dried, with no difference ($P > 0.05$) in yield observed between them. Protein content varied depending on the type of drying, while fat content did not change significantly ($P > 0.05$). Final yield ranged between 5% to 7% of initial mass, with the main losses in mass (due to water loss) registered in the gutting and cooking stages. The sea cucumbers dried in an oven at 60°C reached the humidity goal faster and there were statistically significant differences ($P < 0.05$) between the times tested. The slope of each drying curve for each temperature showed evidence of rapid loss of mass. Microbiological analysis demonstrated that the consumption of dried sea cucumbers does not represent risks to the human health. As a native species, dry-salted *Isostichopus* sp. aff *badionotus* is for consumers a viable alternative source of protein.

Keywords

Sea Cucumber, Drying Curves, Protein, Protocol, Temperature

1. Introduction

Sea cucumbers are one of the most important marine animals used as a food source for humans, especially in some Asian and European countries [1]. Processed sea cucumber is sold both dry-salted (*bêche-de-mer*), and dry-smoked. Asians also consume sea cucumbers cooked, cooked and frozen, salted, boiled in coconut milk, and canned as preserves [2] [3] [4] [5] [6]. The main importers for *bêche-de-mer* are Chinese Mainland, Taiwan, Malaysia, Japan and Korea [7] [8];

while México, the USA, Papua New Guinea, Ecuador, Perú, and the Philippines, are the largest exporters [9] [10] [11] [12] [13].

Technology and commercialization play an important role in sea cucumber profitability [14]. The methods of drying and salt-drying them are characterized by the dehydration of raw biomass. The first achieved this through the interaction of temperature and air flow and the second, by using salt as dessicant. In salt-drying, the sea cucumber is exposed to the drying action of salt to eliminate moisture, providing good product stability over time since low moisture levels prevent microbial growth [15] [16] [17].

Sea cucumber muscle walls are the main component consumed in cucumbers preserved by these processes. They are composed of collagen, polysaccharides, essential amino acids, vitamins and omegas, while having a low fat content and low cholesterol levels [18]. Sea cucumbers have been exploited worldwide, with growing global demand due to their valuable nutrients, therapeutic and aphrodisiac properties, and medicinal benefits [13] [19] [20]. In Colombia, there is little known about sea cucumber biology, or of *bêche-de-mer* preservation techniques. However, some authors state that Asian fishing fleets have marketed native Colombian cucumber species [21] [22] [23]. The purpose of this study was to design a salt-drying protocol for the sea cucumber *Isostichopus* sp. aff *badionotus*, and to evaluate it based on its yield, chemical composition and microbiological features. This study utilized drying curves to evaluate the efficiency of this process in terms of processing time, performance, and final product appearance. In addition, the drying temperatures used on sea cucumbers processed in the oven and with air-drying were compared. The goal of this study was to have a benchmark for salt-drying protocols used on *Isostichopus* sp. aff *badionotus*, and to compare this with processing characteristics of commonly exploited sea cucumber species in Asian food markets.

2. Materials and Methods

2.1. Methodological Design

Sea cucumbers were captured in Rodadero Bay (11˚12'00"N-74˚13'00"W) and quickly transported live in plastic containers filled with refrigerated sea water at 18˚C and 37 Practical Salinity Units (PSU) of salinity to the Centro de Desarrollo Pesquero y Acuícola (CDPA) at the Universidad del Magdalena (Santa Marta, Colombia) for processing.

2.2. Preliminary Tests

Gutted sea cucumbers were exposed to cooking tests in which they were cooked in boiling water for the following amounts of time: t_1, 40 min; t_2, 50 min; and t_3, 80 min in order to determine which trial would provide the cooking process with the smallest loss of mass.

To test dry-salting, two trials were tested with drying times of 24 and 48 hours and 1:1 and 1:2 sea cucumber: kg salt ratios. The control variables were mass and moisture content. These trials were undertaken to find the optimal sea cucumber: kg salt relationship, and salting time.

2.3. Drying Procedure

1) Sea cucumbers were washed and weighed to determine the amount of salt that should be added.

2) Gutting: Small cuts (2 to 4 cm) were done near the mouth. Then, the sea cucumber body was pressed with the fingers to remove the viscera, gonads, water, and sand.

3) Cooking: According to previous results, 40 minutes was the optimal cooking time. Sea cucumber were cooked for 40 minutes then cooled to room temperature for salting.

4) Salting: Previous tests showed the optimal relationship of sea cucumber: kg salt was 1:1 with a 48 hour drying interval. Salting was thus performed in this manner.

5) Drying was carried out at 50˚C and 60˚C in an oven dryer (model V111 Venticell), and under natural conditions. Processing was stopped when humidity reached 10% - 12%, because this is the standard humidity value in the international market.

6) Packing and storage: The final product was packed in high-density polyethylene (PE) bags and stored in a cool, dry room.

2.4. Proximate Chemical Analysis

In order to analyze the chemical composition of the samples, 2 g of fresh muscle was used. A determining analysis (in triplicate) was performed according to Official Methods of Analysis (AOAC) [24]. The content of crude protein (N x 6.25) was determined by the Kjeldahl method [24]. In the process, a digester system with neutralizing gases, an integrated recirculation pump, a distiller (UDK 132, Velp Scrubber ®SMS, Italy), titration with hydrochloric acid, and a digital burette (50 ml) were used. Total lipids were extracted with diethyl ether extraction in a Foss tech analyzer after HCl hydrolysis and evaluated gravimetrically in an analytical balance (Adventurer-OHAUS™, China) [24]. Inorganic content (ash weight) was analyzed by incinerating the samples in a muffle furnace at 550˚C (Vulcan 3-550™, USA). The mass of pure dry ashes was calculated by subtracting dry mass from wet mass. Moisture values were determined using a moisture extractor digital balance (Ohaus MB45™, USA), and were expressed as percentages.

2.5. Microbiological Analysis

The analysis of fresh samples was carried as follows:

Escherichia coli and *Staphylococcus coagulase* positive were evaluated through the sowing on surface-direct count in plate (cfu/g or cfu/mL) method.

Salmonella sp: The "enrichment-selective isolation" method was used and results were recorded as absent or present, positive or negative.

2.6. Statistical Analysis

All treatments were replicated three times and results were reported as means ± standard deviation. An analysis of variance (ANOVA) was performed. A Tukey test was used to compare the average moisture, ash, fat, and protein content between protocol steps. Comparison among means with a 95% confidence level was used.

3. Results

3.1. Preliminary Test

In the cooking tests, performance was similar for each process and there were no significant differences between trials (P = 0.8991). Average masses of 70.40%, 14.49%, 11.71% and 5.95% were obtained in the gutting, cooking, salting and drying (60 hours) stages, respectively. The biggest losses were observed after cooking, with average reduction of sea cucumber fresh mass around 85%. Smaller losses were observed between cooking and salting (mean of 4%), when the desired humidity of 12% was reached (Table 1).

Yield showed no statistically significant differences between the cooking times used, with an average of 6.0% recorded at the end of the process. Large changes in yield were observed during the transition from fresh to gutted, cooking to salting, and salting to drying. From cooked to drying, yield was between 14% to 16% of fresh mass.

There was no significant statistical difference in yield by mass of specimens (P = 0.2098). At the end of the processing cycle, the final percentage of each sample was similar, and ranged between 5.6% and 7% on average (Table 2 and Table 3). The lowest yields were observed in specimens that were cooked for 80 minutes; however, this value did not represent a significant difference when compared with specimens cooked for 40 minutes. Yield percentages in the drying stage in the oven, exposed for 15, 30, 45 and 60 hours (Table 3) varied in very close ranges: 7.5% - 9.3%, 7.3% - 8.5%, 6.0% - 7.0%, and 5.6% - 7.0%; respectively. Thus, performance for specimens with varying weights (574 to ±740 g to 67 g) was the same. The yield in the drying step (Table 3) varied in very close

Table 1. Mass during salt-drying of sea cucumber, *Isostichopus* sp. aff *badionotus* (at 40, 60 and 80 minutes and 60 hours of drying).

	Time (min)		
	40	60	80
Processing step (g)	Average (g) ± SD		
Fresh	3172 ± 55	3189 ± 99	2989 ± 154
Gutted	2243 ± 158^{a}	2251 ± 207^{ab}	2141 ± 74^{b}
Cooked	507 ± 14^{a}	474 ± 42^{a}	417 ± 28^{c}
Salted	382 ± 17^{a}	366 ± 20^{a}	346 ± 26^{a}
Dried	194 ± 10^{a}	182 ± 19^{a}	184 ± 4^{a}

^{abc}Values in the same column with different superscripts are significantly different (P < 0.05).

Table 2. Individual yields of *Isostichopus* sp. aff *badionotus* cooked for 40, 60 and 80 minutes and oven-dried for 60 hours.

	Time (min)					
Processing step (g)	40		60		80	
Fresh	67	574	185	740	124	509
Gutted	50	427	137	551	93	378
Cooked	11	93	28	112	19	78
Salted	9	68	20	81	16	66
Dried 15 hours	5	53	14	63	10	50
Dried 30 hours	5	49	11	56	9	45
Dried 45 hours	4	44	10	49	8	40
Dried 60 hours	4	40	9	45	7	37

Table 3. Yields of specimens of *Isostichopus* sp. aff *badionotus* during the process of salt-drying for cooking times of 40, 60 and 80 minutes and 60 hours of drying.

	Time (min)					
Processing step (%)	40		60		80	
Fresh	100	100	100	100	100	100
Gutted	74.4	74.6	74.5	74.1	74.3	75.0
Cooking	16.2	16.4	15.1	15.1	15.3	15.3
Salted	11.8	13.4	10.9	11.4	12.8	12.9
Dried 15 h	9.2	7.5	8.5	8.9	9.3	8.1
Dried 30 h	8.5	7.5	7.6	7.9	8.2	7.3
Dried 45 h	7.7	6.0	6.6	7.0	7.7	6.5
Dried 60 h	7.0	6.0	6.1	6.4	6.8	5.6

ranges from 7.5% to 9.3%, from 7.3% to 8.5%, from 6.0% to 7.7% and 5.6% to 7.0% for the drying times: 15, 30, 45 and 60 hours, respectively. Therefore, sea cucumbers with 740 g masses showed similar yields to those with 67 g masses.

3.2. Proximate Analysis

Table 4 shows no significant difference between yield and proximate chemical composition of *Isostichopus* sp. aff *badionotus*. Forty minutes of cooking produced a salt-dried sea cucumber humidity value of 11.73%.

The proximate chemical composition of *Isostichopus* sp. aff *badionotus* after cooking was: moisture (73.45% ± 1.09%), protein (16.19% ± 0.56%), fat (1.23% ± 0.03%), and ash (3.0% ± 0.14%). The values recorded for moisture content after drying did not vary between methods because at the beginning of the study they were set at a range of between 10% and 12%.

Protein content varied according to drying method: sea cucumbers dried in the oven at 50˚C and 60˚C showed values of 44.02% and 44.65%, respectively, while those that were air-dried registered the lowest value: 38.5%. Oven-dried

Table 4. Proximal chemical composition for each cooking time evaluated in the salt-drying process of *Isostichopus* sp. aff *badionotus*.

	Isostichopus sp. aff *badionotus*		
Cooking time (min)	40	60	80
Humidity (g/100g)	73.45 ± 1.09	72.69 ± 0.33	70.99 ± 1.40
Protein (g/100g)	16.19 ± 0.56	14.54 ± 0.08	13.86 ± 0.35
Fat (g/100g)	0.62 ± 0.07	0.64 ± 0.10	0.64 ± 0.12
Ash (g/100g)	2.92 ± 0.04	3.16 ± 0.05	3.20 ± 0.05

samples at 50˚C and 60˚C did not show statistically significant differences (P > 0.05), but statistically significant differences were detected in air-dried samples (P < 0.05).

Fat content did not differ significantly between drying types, showing values that did not exceed 1.5%. The values were 1.15%, 1.1 % and 1.23% in oven-dried samples at 50˚C and 60˚C, and in air-dried samples, respectively.

Ash content showed high values typical in dry-salted products. Values in oven-dried sea cucumbers (50˚C: 38.36%, and 60˚C: 39.93%), presented statistically significant differences (P < 0.05), when compared with the air-dried sea cucumbers (44.28%).

3.3. Performance per Drying Type

Yield in dry-salted sea cucumbers was found to be within the range of 5% to 8% for each temperature trial (oven drying between 50˚C and 60˚C and air-drying). During all processing cycles, yield was similar, and there were not statistically significant differences (P > 0.05). Thus, the average yield range for each stage was: gutting 66% - 69%, cooking 16% - 17%, salting 12% - 13.5%, and, finally, drying at 5.5% - 8%. A reduction of 87% of fresh weight was found during cooking. Significant statistical differences were not apparent (P > 0.05) in drying, however, yield was better in the drying oven at 50˚C (Table 5) as compared to ovens at 60˚C and natural drying. However, to produce higher yields of salt-dried sea cucumbers, a slight increase is critical in terms of cost-benefit.

For oven drying, rates of yield were: gutted (65.22% to 67.72%), cooked (15.0% to 16.92%), salted (12.53% to 13.18%), and dried (6.10% to 7.41%); and for air-drying, yield rates were: gutted (65.55%), cooked (16.08%), salted (12.20%), and dried (5.58%). Thus, in order to find out how many kilograms of raw material of *Isostichopus* sp. aff *badionotus*, are required to obtain a specific dry weight, raw weight must be multiplied by the mean conversion rate of the final product.

3.4. Drying Curve

Sea cucumbers dried in the oven at 60˚C reached the humidity required in the least amount of time (Figure 1), with statistically significant differences (P < 0.05) between the times tested. Oven drying at 60˚C required 54 hours, while oven drying at 50˚C required 65 hours and natural drying required 97 hours.

Table 5. Drying yields of *Isostichopus* sp. aff *badionotus* in oven-dried (50˚C and 60˚C) and air-dried trials.

Process step	50˚C	60˚C	Natural
Fresh	100	100	100
Gutted	67.72 ± 0.72	65.22 ± 0.19	65.55 ± 1.18
Cooked	16.92 ± 0.19	15.00 ± 0.42	16.08 ± 0.48
Salted	13.18 ± 0.21	12.53 ± 0.36	12.20 ± 0.24
Dried	7.41 ± 0.27	6.10 ± 0.07	5.58 ± 0.33

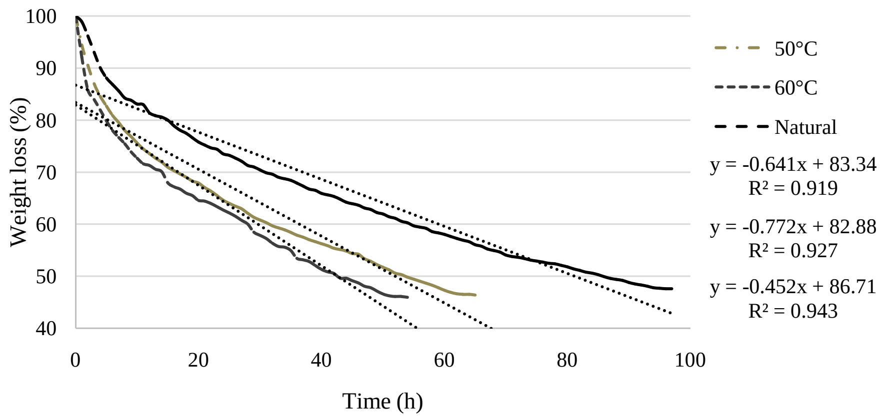

Figure 1. Drying curves in oven (50˚C and 60˚C) and air (natural) drying for *Isosticho-pus* sp. aff *badionotus*. Weight loss *vs*. time.

The slope of each drying curve for each temperature showed rapid weight loss. It was noted that oven drying at 60˚C presented a slope of 0.77, which was greater than the other trials.

3.5. Microbiological Analysis

Samples of three types of drying showed low microorganism counts (<3 MPN) and the absence of pathogens (negative for *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp.).

4. Discussion

4.1. Yield

The final yield of processed sea cucumber was independent of the sizes and masses of the samples. These were as similar for individuals with masses ranging from 574 g to 740 gas for those with masses between 67 g and 124 g. Sea cucumbers with initial masses of 67 g and 574 g showed final masses of 4 g and 40 g, respectively, representing 6% and 7% of their mass, and, therefore, did not show statistically significant differences in yield. These results are consistent with those established by Lavitra *et al*., (2009) [25], which analyzed the processing of *Holothuria scabra* and determined that there were no differences in yield between 230 g and 480 g samples.

In preliminary tests, the differences between the final masses of cooking time trials of sea cucumbers were small, showing a gradual tendency toward reduced yield as cooking time increases. In addition, the final mass percentage of the specimens tested did not depend on the time of cooking. For each sample, yield after 60 hours was within a very narrow range (5% to 7% of the initial mass); consequently, sea cucumbers of greater mass (e.g. 500 g - 740 g) did not show higher percentage yields in relation to those with lower masses (e.g. 67 g - 185 g). During the study, it was observed that individual yield did not vary significantly with regard to mass and cooking time. The percentage loss of fresh mass in sea cucumbers dried in the oven (50˚C and 60˚C) and by natural methods (air-drying) after gutting was between 31% and 34%. These results show a smaller range than that found by Purcell *et al*., (2009) [26] for *Isostichopus hermanni*, *Actinopyga spinea*, *A. palauensis*, *A. echinites*, *Holothuria lessoni*, and *H. whitmaei*. Purcell *et al*., state that most of these commercial species lost between 30% and 45% of their initial mass in the gutting stage. In addition, Purcell *et al*., found that *S. hermanni* is the species with lowest yield from the processing cycle, registering losses of 4.5%. The other species presented total losses between 88.3% and 92.7%, which was similar to those found in this study (92% and 94.5%) at the end of the process.

The results of this study in the phase of cooking yield were consistent with those recorded byRodriguez-Gil, *et al*., (2013) [27], where the author found that *Isostichopus badionotus* presented a yield of 16% for this stage. This can be explained because both species are of the same genus, live in similar habitats, and presented similar characteristics during cooking processes. Specimen lengths were not recorded in this study; however, sizes were reduced considerably after cooking and drying, showing an approximate 50% decrease in size after cooking and drying.

Leiva (2015), [28] showed that semi-processed *Holothuria mexicana* values differ greatly from the values found in *Isostichopus* sp. aff *badionotus*, in the gutting and cooking steps. This author found that the percentages of losses after these specimens were gutted and defrosted were 26.5% and 36.09%, respectively, as opposed to the results of this study (gutted: 29.33% and cooked: 84.0%). These differences are likely due to the fact that the specimens in question are different species, with different habitats and diets.

The final yield of *Isostichopus* sp. aff *badionotus* evaluated in the drying trials was between 5.5% and 8%. These values are within the range established by [29] for *Microhtelenobilis*, *Thelenota ananas*, and *Actinopyga echinites*, in which each species presented a final performance between 5% and 13%. Despite the differences between species, *Isostichopus* sp. aff. *badionotus* shows similar characteristics after the drying process This may be because most of the components of sea cucumbers are water, viscera and sand, which are largely lost on the first two phases of the process, resulting in smaller sea cucumber sizes and lower masses.

Moreover, [17] studied the variation in the masses of ten commercial species of sea cucumber, determining that yield in the gutting and drying stages was between 5.1% and 19.9%. The results obtained in this study are in agreement with those recorded by this author for these processing cycles. In addition, the most critical steps in terms of processing performance were gutting and cooking. In these, manipulation must be rapid, since specimens suffer autolysis due to stress and skin lacerations, causing physical damage, affecting appearance, and increasing losses of biomass.

Regarding drying time, sea cucumbers dried in the oven at 60˚C showed 54 hours on average in reaching the moisture content established in the project.

4.2. Proximate Analysis

A considerable increase was observed in protein content in cooked and dried samples of *Isostichopus* sp. aff *badionotus*. A similar increase occurred with ashes and fat, as they increased with the loss of water. This is because sea cucumber muscle is contracted by dehydration, concentrating each of these proximal components. These increases differ with those found by Pembe *et al*., [3], since increases in protein and ash for *Holothuria scabra* were proportionally lower than in *Isostichopus* sp. aff *badionotus*. This may be because fresh *H. scabra* contains higher levels of protein, lipids, and ash than *Isostichopus* sp. aff *badionotus*.

Gao *et al*., (2011) [30] and Lee *et al*., (2012) [31] evaluated the proximate chemical composition of *Apostichopus japonicus*, finding similar values to those in *Isostichopus* sp. aff *badionotus*. Nevertheless, several authors analyzing the fresh chemical composition of species of the genera found values different from those obtained in this study [30]-[37]. This shows that the chemical composition of sea cucumbers varies between species and may be linked to habitat, species biology, reproductive stage, nutritional and physiological issues, available food, geographical distribution ranges, and environmental parameters.

4.3. Microbiological Analysis

According to the results of this study, the protocol designs for sea cucumber dry-salting did not demonstrate pathogenic transfer, which indicates that these protocols contribute to the efficient use of raw materials by preventing their spoilage by pathogenic microorganisms.

5. Conclusion

In conclusion, there are no differences between *Isostichopus* sp. aff *badionotus* yields when processed utilizing with timed cooking and 60 hours of drying. The highest losses in the dry-salting process were registered in the gutting and cooking steps. At the end of these processes, the average yield was 16%. The shortest drying time was in the oven at 60˚C, at 67.5 hours. The highest protein content was presented in samples dried in the oven at 50˚C and 60˚C. The high percentage of protein and the low fat levels in dry-salted sea cucumber indicate it is a viable alternative source of protein for potential consumers worldwide.

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