

Development and quality evaluation of small rock oyster sauce from *Saccostrea* spp.

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Abstract

Small rock oyster (*Saccostrea* spp.) are abundant in the Province of Samar and presently there is no oyster sauce produced from this species available in the Philippine markets. Hence, this study aimed to produce small rock oyster sauce at different concentrations (*i.e.* 70, 80 and 90%; treatments 1–3) and investigated their microbial and sensorial qualities. Based on the result of the overall acceptability of sensory evaluation, treatment 1 containing 70% of small rock oyster extract showed no significant difference with the control commercial oyster sauce suggesting that the treatment 1 is the most acceptable product. There is no microbiological standard limit for fermented fishery products in the Philippines. The total plate counts of the treatments ranged from 3.64 to 4.62 log CFU ml⁻¹. Halophilic bacteria were present in all treatments, ranging from 3.49 – 4.67 log CFU ml⁻¹. Also, lactic acid bacteria were detected in all treatments, ranged from 1.75 – 3.07 CFU ml⁻¹. This study concludes that the small rock oyster sauce produced locally can compete with the available commercial oyster sauces in the market.

Keywords: Oyster sauce; product development; quality evaluation.

1 | INTRODUCTION

Fermentation is one of the oldest techniques in food preservation that extends the shelf-life of food and enhances its flavour and nutritional value (Visessanguan *et al.* 2004). Fermented fish products are produced in different parts of the world and the method of processing varies according to the availability of raw materials, consumer's preference and the climatic conditions of the region (Al-Jedah *et al.* 2000). In Southeast Asia, fermented fish sauce is prepared from various types of fish, from both freshwater and marine fish species by various methods (Dagadkhair *et al.* 2016).

Fish sauce is a fermented product commonly used a liquid seasoning in Asian countries such as Philippines (known as patis), Japan (shotturu), Malaysia (budu), Thailand (nam-pla), Vietnam (nouc-nam), Indonesia (ketjapikan or bakasang), China (yu lu) and Myanmar (ngapi) (Sanceda *et al.* 2003). It contains a mixture of amino acids and other protein degradation products (Fukami *et al.* 2002; Ichimura *et al.* 2003). Fish sauce is basically produced from a mixture of fish and salt, allowed to ferment for not less than 6 months (CODEX Alimentarius 2013). It is the water or juice in the flesh of fish that is extracted during the process of prolonged salting and fermentation (Lo-

petcharat *et al.* 2001). Small sardines, shrimps, shellfish, squid and fish eggs are among the top species utilised as resource for fermented seafood (Steinkraus *et al.* 1993).

The biochemical characteristics of fish sauce depends on several factors such as quality of raw materials, salt concentration, method of processing and the fermentation period, as well as the fish species (Mueda 2015). Fermentation of fish is brought about by autolytic enzymes and microorganisms present in the fish (Gram and Huss 2000). It preserves food and promotes food safety through the production of compounds such as lactic acid, acetic acid, bacteriocins, together with lowering the water activity of food, resulting in an inhibition of spoilage and pathogenic bacteria (Adams and Nicolaides 2008; Gaggia *et al.* 2011).

Small rock oyster (*Saccostrea* spp.) is abundant in Samar, Philippines, locally known as “sisi” and is being processed by salt-fermentation and sold to wet markets and even transported to other places. Presently, there is no product of oyster sauce, made from *Saccostrea* spp., is available in the markets. Therefore, in order to expand the markets for this species, it is essential to study and develop different products from this species. Hence, in this study, attempts have been made to produce oyster sauce from small rock oyster with special reference to the assessment of the sensorial and microbial characteristics of salt-fermented rock oyster sauce.

2 | METHODOLOGY

2.1 Sample collection

Approximately 3 kilograms of small rock oyster were purchased at the wet market of Catbalogan City, Samar, Philippines. It was then transported to the fish processing laboratory in Samar State University Mercedes Campus and washed immediately with potable water. After cleaning, the product was fermented for three months (March – May 2019) by mixing the small rock oyster with 20% table salt in a sterile glass jar. It was covered and stored in a cool dry place until further processing.

2.2 Sample preparation

The fermented rock oyster was boiled for three minutes in a casserole at a temperature of 100°C. The liquid extract was collected and filtered using a filter paper. After filtering, the liquid extract was mixed with other ingredients, following the formulation shown in Table 1 to produce small rock oyster sauce. The mixture was boiled for two minutes and then packed in sterile bottles.

A commercial oyster sauce was used as control sample. There are actually three leading brands of oyster sauce in the local markets. But we used the one that has almost similar composition with our developed products. Its ingredients include oyster flavour, modified starch, spices, seasoning, caramel colour, soy sauce extract, MSG, water

and sodium benzoate.

TABLE 1 Formulation of small rock oyster sauce used in the present study.

Ingredient	Amount (%)		
	Treatment 1	Treatment 2	Treatment 3
Rock oyster extract	70	80	90
Soy sauce	15	10	5
Sugar	10	6	2
Corn starch	2	2	2
MSG	2	1.50	0.75
Salt	1	0.50	0.25
Total	100	100	100

2.3 Microbial analysis

Total Plate Count (TPC): TPC of samples was analysed by spread plating the serially diluted samples (up to 106) into plate count agar medium. After incubation at 37°C for 24 hours, colonies were counted and recorded as log CFU ml⁻¹ (APHA 1992). Analysis was done in triplicate.

Halophilic bacteria count: Halophilic bacteria count was determined by spread plating the serially diluted samples (up to 106) into plate count agar medium added with 10% sodium chloride. Agar plates were incubated for three days at 37°C (Gassem 2019). Colonies were counted and recorded as log CFU ml⁻¹. Analysis was done in triplicate.

Lactic acid bacteria count: Lactic acid bacteria count was determined by spread plating of the serially diluted (up to 106) samples onto MRS (de Mannne Rogosa Sharpe) Agar, a selective culture media for lactic acid bacteria. The plates were incubated at 37°C for 48 to 72 hours, and the colonies with clear zones were counted and recorded as log CFU ml⁻¹. Analysis was done in triplicate.

Salmonella spp. detection: Twenty five grams of the sample was mixed in a 225-ml of pre-enrichment broth and incubated at 35°C for 24 hours. One ml of the pre-enrichment broth was inoculated to tetrathionate broth (TTB) and incubated for 24 hours at 35°C. The selective enrichment cultures were streaked on xylose lysine deoxycholate (XLD) agar and plates were incubated at 35°C for 24 hours. Typical *Salmonella* spp. colonies were determined. Analysis was done in triplicate.

2.4 Sensory evaluation

Sensory evaluation was done to determine the product acceptability in terms of colour, odour, flavour, texture / consistency and overall acceptance. A total of 10 semi-trained panellists assessed the sensorial quality of the product. The panellists consisted of faculty and students of the university, between 20 and 30 years, and with prior knowledge of conducting sensory evaluation. A 9-point hedonic scale score card (Table 2) was used in the test. Analysis was done in triplicate.

TABLE 2 The 9-point hedonic scale used for sensory evaluation of the products.

Score	Comments
8.5 – 9.0	Like, extremely
7.5 – 8.4	Like, very much
6.5 – 7.4	Like, moderately
5.5 – 6.4	Like, slightly
4.5 – 5.4	Neither like nor dislike
3.5 – 4.4	Dislike, slightly
2.5 – 3.4	Dislike, moderately
1.5 – 2.4	Dislike, very much
0.5 – 1.4	Dislike, extremely

2.5 Statistical analysis

Data on microbial analysis and sensory evaluation was analysed through one-way ANOVA and a post-hoc analysis, Holm-Sidak test, to determine significant difference ($P < 0.05$) in mean values. All statistical analyses were performed using the statistical software, Sigma Plot 11.0.

3 | RESULTS AND DISCUSSION

3.1 Sensory evaluation

The mean scores of sensory attributes including colour, odour, flavour, texture / consistency and overall acceptance are presented in Table 3. In terms of colour attribute, the control sample (commercial oyster sauce) obtained the highest score of 8.20 with an adjectival rating of “like, very much”, but shows no significant difference with Treatment 1 oyster sauce and Treatment 2 oyster sauce. Sensory panels commented that the colour of the Treatment 1 and Treatment 2 samples were very similar to that of the commercial oyster sauce. Results revealed that all samples were acceptable. The acceptable limit was set to 6.50 with adjectival rating of ‘like, moderately’.

TABLE 3 Mean scores (mean \pm SD) of sensory attributes in all treatments in triplicate.

Sensorial attribute	Control	Treatment 1	Treatment 2	Treatment 3
Colour	8.20 \pm 1.03 ^a	7.70 \pm 0.82 ^{ab}	7.40 \pm 0.84 ^{ab}	6.70 \pm 1.25 ^b
Odour	8.10 \pm 0.99 ^a	8.00 \pm 0.82 ^a	7.70 \pm 0.95 ^a	7.70 \pm 1.06 ^a
Flavour	8.40 \pm 0.69 ^a	7.30 \pm 0.95 ^{ab}	6.70 \pm 1.64 ^b	6.10 \pm 1.52 ^b
Texture / consistency	8.50 \pm 0.71 ^a	8.00 \pm 0.67 ^{ab}	7.40 \pm 1.08 ^b	7.10 \pm 0.88 ^b
Overall acceptance	8.20 \pm 0.92 ^a	8.00 \pm 0.94 ^{ab}	6.40 \pm 1.08 ^b	6.90 \pm 1.10 ^b

Distinct letters in the same row differ significantly ($P < 0.05$)

The colour of fermented oyster and fish sauce varies depending on the raw material, ingredients, and method used during the fermentation process. Fish sauce usually has a colour of yellowish to brownish. The development of brown colour in fermented fish is brought about by the reaction of the primary and secondary lipid oxidation products (Aubourg 1998). The colour of all the samples was identified as dark brown, same as the colour of the control sample or the commercial oyster sauce. Based on the result of the sensory test, all samples were found acceptable.

For odour attribute, the control sample obtained the highest score of 8.10 with an adjectival rating “like, very much”, but shows no significant difference with other treatments. Sensory panels commented that Treatment 3 has the strongest oyster odour and could be a good condiment. Treatment 1 obtained a score of 8.00, also with an adjectival rating of “like, very much”, while treatment 2 and Treatment 3 scored 7.70 with an adjectival rating of “like, very much”. Fermented fish sauce have distinct aroma, either ammoniacal, meaty or cheesy (Shimoda *et al.* 1996). The development of aroma in fermented fish sauce is contributed by several factors and this aroma production could be attributed by microorganisms (Adams 1986).

The control sample had the highest score of 8.40 for flavour with an adjectival rating of “like, very much” but shows no significant difference with Treatment 1 with a score of 7.30 (like, moderately). Based on the comments of the panellists, the flavour of the control and Treatment 1 samples were highly similar, both having a sweet flavour. Treatment 3 sauce scored the lowest (6.10) with an adjectival rating of “like, slightly”. The panellists commented that it was very salty and unacceptable as a condiment.

In terms of texture or the consistency of the product, the control sample revealed to have the highest score of 8.50 with an adjectival rating of “like, extremely” but shows no significant difference with Treatment 1 (8.00, “like, very much”). Panellists commented that the sticky texture of the control and Treatment 1 samples is very much acceptable as a condiment.

The overall acceptability result shows that control sample had the highest score of 8.20, with an adjectival rating of “like, very much”, but has no significant difference to Treatment 1, with a score of 8.00 (also like, very much). Overall, Treatment 1 containing 70% rock oyster extract is the most promising product compared to other sauces since it significantly resembled similarity with the commercial oyster sauce in all sensory attributes.

3.2 Microbiological analysis

Figure 1 shows the total plate count (TPC) of the small

rock oyster sauce treatments including the control sample. The control sample had the lowest microbial count of $2.12 \log \text{CFU ml}^{-1}$ and differed significantly with other treatment groups. Treatment 1 revealed to have a microbial count of $3.64 \log \text{CFU ml}^{-1}$, showed no significant difference with Treatment 2 ($4.10 \log \text{CFU ml}^{-1}$).

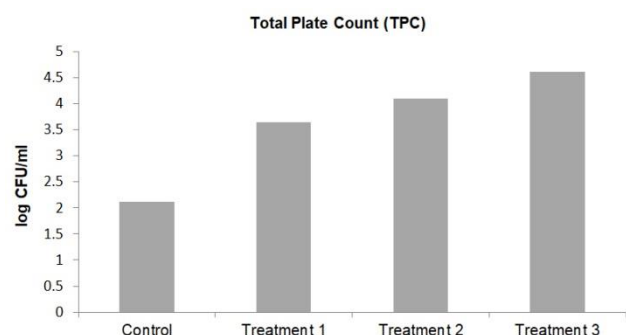


Figure 1 Total plate count in small rock oyster sauce groups.

Based on the result of the TPC analysis, as the percentage of the small rock oyster extract increases, the microbial count also increases. This clearly emphasise that the source of microorganisms in the product was from its raw material. Also, spices added with the small rock oyster extract may also have an effect on the bacterial load of the samples. According to Achinewhu and Oboh (2002), the use of available spice for fish fermentation showed antibacterial effect on the product.

Presently, there is no microbiological limit standard set in the Philippine for fermented fish and fishery products. But several studies conducted to determine the TPC of fermented fish products. Gassem (2019) determined the TPC of salted-fermented fish (Hout-Kasef) product and obtained a result of $3.77 \log \text{CFU g}^{-1}$. A bacterial load of 1.25×10^7 to $1.41 \times 10^7 \text{CFU ml}^{-1}$ were detected in fish sauce from small freshwater fishes after nine months fermentation (Faisal *et al.* 2013). Ibrahim (2010) reported a microbial load of $2.0 \log \text{CFU ml}^{-1}$ in fish sauce from *Gambusia (Affinis affinis)*.

Figure 2 shows the total halophilic bacteria in small rock oyster sauce and control sample. Results showed that the control sample had the least number of halophilic bacteria count of $1.93 \log \text{CFU ml}^{-1}$. Whereas Treatment 1, 2 and 3 had total halophilic bacteria of 3.49, 4.15 and 4.67 $\log \text{CFU ml}^{-1}$ respectively. Halophilic bacteria are those that thrive in saline conditions such as in salted and fermented fish products. Several studies detected halophilic bacteria in fermented fish products. A total of $4.32 \log \text{CFU g}^{-1}$ halophilic bacteria were enumerated by Gassem (2019) in salted-fermented fish product. Anihouvi *et al.* (2007) reported an initial halophilic bacteria count of $4.74 \log \text{CFU g}^{-1}$ in fermented cassava fish (*Pseudolithus* spp.). Further, Ibrahim (2010) reported $2.0 \log \text{CFU ml}^{-1}$ of halophilic bacteria in fish sauce from *Gambusia (A. affinis)*.

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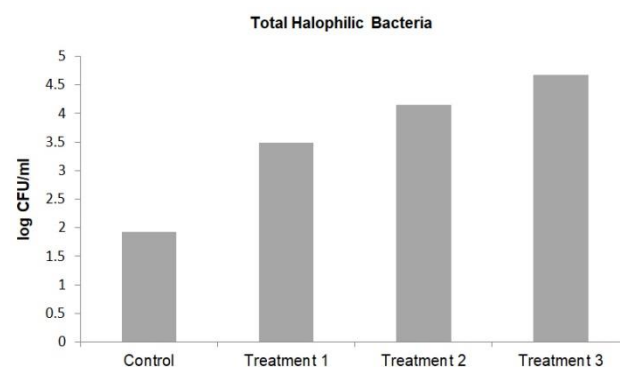


FIGURE 2 Total halophilic bacteria count in small rock oyster sauce and the control sample.

Figure 3 shows the total lactic acid bacteria (LAB) count of the small rock oyster sauce and the control sample. Findings revealed that treatment 3 containing 90% rock oyster extract had the highest count of lactic acid bacteria of $3.07 \log \text{CFU ml}^{-1}$ while the control sample had zero count. Treatment 1 and 2 had a LAB count of 1.75 and $2.55 \log \text{CFU ml}^{-1}$ respectively. It is evident that the bacterial count increases with the percentage of oyster extract in the samples increases. It is presumed that the source of LAB was the raw material, the fermented rock oyster extract. Growth of LAB were also enhanced by the addition of starch as an ingredient in the sample. LAB are naturally found in oyster and *Lactobacillus*, as the major LAB accounted for 75% of its total microflora (Shifflet *et al.* 1966). It was also reported that the gastrointestinal of shellfishes contains 4.5×10^4 cells of LAB g^{-1} wet weight (Buntin *et al.* 2008). Additionally, shellfish during harvest have LAB count of $10^3 - 10^5 \text{CFU g}^{-1}$ per shellfish (Cook 1991).

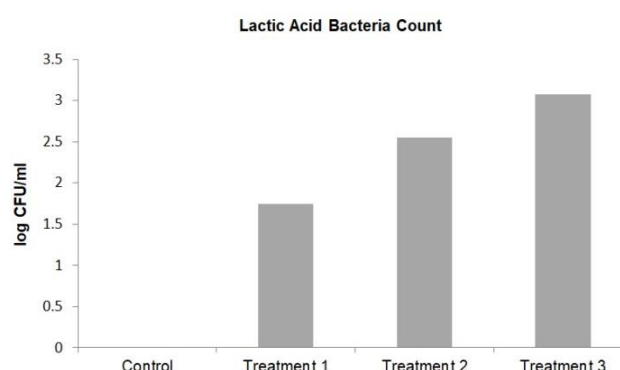


FIGURE 3 Total lactic acid bacteria count in small rock oyster sauce and the control sample.

Previous studies reported the presence of LAB in fermented fish products produced by spontaneous fermentation (Francoise 2010; Koyanagi *et al.* 2011). In the Philippines, Olympia (1992) investigated the presence of LAB in fermented fishery product “burong bangus”. In Thailand, a total LAB count of $3.2 \times 10^3 - 2.0 \times 10^5 \text{CFU g}^{-1}$ was

detected in Hoi-dong, a fermented shellfish mussel (Nanasombat *et al.* 2012). Bekasam, an Indonesian fermented fish, revealed to have an LAB count of $1.4 \times 10^8 - 9.0 \times 10^8$ CFU ml⁻¹ (Desniar *et al.* 2013).

This study revealed that small rock oysters (*Saccostrea* spp.) can be processed into an oyster sauce that may compete to the available commercial oyster sauce in the market. In terms of overall acceptability in sensory evaluation, treatment 1 containing 70% small rock oyster extract is as acceptable as the commercial oyster sauce. Further studies on the determination of chemical composition and product shelf-life are recommended.

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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CONTRIBUTION OF THE AUTHORS

Jerson CS conceptualise the research and data analysis;
Jedith CS sample preparation and sensory evaluation;
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AG sample procurement and preparation;
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