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Microbial, Chemical and Sensorial Quality of Chilled Marinated Green Mussel *Pernaviridis*, (Linnaeus, 1758)

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Abstract

The study aimed to assess the physico-chemical, microbiological and sensorial quality of marinated green mussels (*Pernaviridis*) over 28 days (4 weeks) storage period at chilled temperature (2 °C). There were two treatments in the study. In treatment 1, samples were immersed in 66% vinegar and 2% salt. In treatment 2, samples were immersed in 66% vinegar, 2% salt and 1% spices such as pepper, fennel, cloves, bay leaves and paprika. Based on the physico-chemical results, the sample has a proximate composition of 13.60% protein, 1.66% lipid and 0.07% moisture. For the TVB-N value, treatment 1 decreased from 4.35 mg/100g to 1.05 mg/100g over the 4 weeks storage period. In treatment 2, TVB-N value increased from week 1 (4.14 mg/100g) to week 2 (5.38 mg/100g), but decreased further from week 2 to week 4 with a TVB-N value of 1.62 mg/100g. For pH level determination, treatment 1increased its pH level from 4.03 to 4.13 over the storage period. In treatment 2, pH has increased from 4.2 (week 1) to 4.37 (week 3), but decreased further to 4.1 (week 4). There was no significant difference ($p<0.05$) observed between the samples.Based on the microbiological analyses, treatment 1 decreased it microbial count from 1.69×10^8 to 7.70×10^3 over the storage period. In treatment 2, the microbial count also decreased from 1.13×10^7 to 8.07×10^3. For the basis of sensory evaluation, treatment 1 decreased its general acceptability from 6.44 to 5.89 over the storage period. In treatment 2, the general acceptability also decreased from 6.67 to 5.71.According to the overall results, the marinated green mussel was stable for 28 days.

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Introduction

Green mussel (*Pernaviridis*) is a bivalve that belongs to family Mytilidae, and is found in tropical waters ofAsia. It is harvested as a food source due to its dense and fast growth. It is commercially important and has nutritional value. People consume mussels as it is a cheap source of protein and tasty. In every 100 g mussel meat, it contains 11.90 g protein, 3.69 g carbohydrate, 2.24 g fat, 0.507 g fatty acid, minerals and vitamins[1].

Seafoods are perishable of because of their ease of digestibility and the nature of the microbial and systemic enzymes that cause their spoilage[2]. Microbial spoilage in shellfish is mainly caused by bacteria, while chemical spoilage can be caused by oxidation as well as hydrolysis of lipids which results to rancidity[3]. Mussels are highly perishable product that requires intensive care, if the quality has to be maintained for some time after harvesting. Traditional processing technology and preservation methods can inhibit food spoilage[4].

Spoilage in food can be controlled by low temperature storage and some chemical techniques[5,6]. Low temperature storage method inhibits the growth of microorganism and reducing microbial metabolism[7]. In mussel processing, thermal preservation is the primary method in obtaining the desired sensorial characteristics. Moreover,it inhibits the growth of bacteria and enzymatic action, thus, producing a safer and more stable food product. Marinating is also one of the many fish preservation techniques. Marinated fish and other fishery products are preserved through the addition of acetic acid and salt, thus increasing its shelf-life[8]. Vinegar is effective in inhibiting bacterial growth by lowering the pH of the product[9]. Sodium chloride has beneficial effects on sensory, functional and preservation properties and has reported to have a pro-oxidant activity in marinated and salted fishery products[10,11]. Additives such as sugar and spices are also added to obtain various flavors and packed in containers[8].

In the Philippines, mussels are usually sold fresh in the market. There are few new-developed and value added products out of this commodity. Little information is available on post-harvest processing of mussels from Philippines waters[12,13]. There is limited scientific data regarding on green mussel preservation through marinating. Therefore, this study aimed to assess the chemical, microbial and sensory changes in chilled green mussel during chilled storage and the shelf-life of the product.

Materials and Methods

Sample Collection

A total of 10 kg samples were obtained from the local market of Miagao, Iloilo, Philippines. The mussels were placed in a plastic bag and were transported to the laboratory at low temperature (4 °C) for further processing.

Product Formulation

The mussels were washed with clean water with the aid of a brush to remove unwanted materials attaching to its shells. They were packed in net baskets and subjected to hot water bath at 100 °C for 1 minute[13]. The meat was removed from the shells and trimmed away the byssus. Marinades were simmered for 15 minutes together with the other ingredients. The mussels were packed in bottles together with the marinades. Formulation of samples is shown in table 1. There were two variables in this study: Marinated mussel with salt and vinegar; and marinated mussel with salt, vinegar and spices. Triplicate samples were maintained for all treatments.

Product Storage

The product was stored at 2 °C for 28 days. It is indicated that the marinated product has a shelf-life of only a few days when kept at ambient 10 °C, but storing it at1-4 °C can extend the shelf-life to about two months[14]. Product analyses such as chemical, microbial and sensory evaluation was done every 7 days.

Physico-Chemical Analyses

Proximate composition such as moisture, protein, lipids and ash were determinedaccording to the standard method[15].Total Volatile Bases (TVB-N, mg N/100g) was analysed according to the standard Conway micro diffusion method[16]. For pH measurement, 10 g meat samples were homogenized in a 10 ml distilled water solutionand a portable pH meter was used to determine the pH value[17].

Table 1: Formulation of the marinated mussels in two different marinades

Control(%)		Treatment 1Salt and Vinegar (%)		Treatment 2Salt, vinegar and spices (%)	
Mussel	100	Mussel meat	32	Mussel meat	31
		Vinegar	66	Vinegar	66
		Salt	2	Salt	2
				Pepper	0.39
				Fennel	0.10
				Cloves	0.10
				Bay leaves	0.34
				Paprika	0.07

Microbial Analyses

The microbiological quality of the marinated mussels during the storage period was monitored using the total plate count method[18].

Sensory Evaluation

A total of 10 assessors evaluated the taste, colour, odourand texture of the samples.A taste panel score sheet was used to measure the sensorial quality of the mussel. Samples were retrieved from the refrigerator and held for 30 minutes before they were served to the panelists[19].

Statistical Analysis

All experiments were run in triplicates. Data on physic-chemical, microbiological and sensory evaluation were subjected to one-way analysis of variance (ANOVA). T-test was used to determine significant difference ($p < 0.05$) between samples. All the statistical analyses were performed using the SPSS (version 20) software (SPSS, Inc, Chicago, Illinois).

Results and Discussion

Proximate Analyses

Table 2 shows the proximate composition of the marinated mussel in comparison with the results of other authors. In this study, the protein content of the sample (13.60%)was higher compared to the reported values of the two authors. Lipid content of the sample is 1.66%.However, the moisture content(79.68%) is much lower compared to the values reported by the two authors. It was revealed that the approximate composition of mussel meat can vary slightly with the composition of the food (phytoplankton) in the region where the mussels grow and with seasonal fluctuations during the reproductive cycle[22].

Table 2: Proximate composition of marinated mussel

Parameters	Aveiro *et al.,* [20]	Salan *et al.,*[21]	This study
Protein %	7.55	11.78	13.60 ± 0.02
Lipid %	2.90	1.55	1.66 ± 0.01
Moisture %	85.15	82.82	79.68± 0.04

Physico-Chemical Analyses

Changes in TVB-N value in the marinated mussel samples are shown in Table 3. In treatment 1, TVB-N values had decreased over the 4 weeks of storage from 4.35 – 1.05 mg/100g. In treatment 2,TVB-N values increased from week 1 (4.14 mg/100g) to week 2 (5.38 mg/100g), and decreased further up to week 4 (1.62 mg/100g). There is no significant difference ($p < 0.05$) observed between the samples over the duration of storage. A considerable decrease of TVB-N values from 10.3-6.5 was also determined in marinated sardine fillet with 7% acetic acid and 14% salt[23]. A TVB-N limit of 30-35 mg/100 g fish flesh was established[24] and based on EC guidelines[25] which they considered fishery products unsuitable for human consumption. Also, aquaculture products

are classified based on TVB-N values[4]. Samples with 25 mg/100g TVB-N were identified as very good; 30 mg/100g TVB-N were identified as good; 35 mg/100g TVB-N were identified as marketable; and more than 35 mg/100g TVB-N value were identified as decayed. Based on the results, all samplesincluding control group were still fit for consumption from week 1 to 4.

Table 3: Total Volatile Basic Nitrogen and pH value of marinated mussel

Week	Control		Treatment 1		Treatment 2	
	TVB-N	pH	TVB-N	pH	TVB-N	pH
1	5.15 ± 0.25	7.2± 0.22	4.35 ± 2.35	4.03± 0.06	4.14 ± 0.52	4.2 ± 0.00
2	16.01 ± 0.62	7.3 ± 0.43	2.64 ± 0.62	4.2 ± 0.00	5.38±0.36	4.3 ± 0.06
3	21.13 ± 1.11	7.5 ± 0.01	1.13 ± 0.08	4.4 ± 0.00	2.85 ± 0.06	4.37 ± 0.11
4	31.05 ± 1.33	7.3 ± 0.08	1.05 ± 21.60	4.13 ± 0.06	1.62± 0.894	4.1 ± 0.00

The result of pH analysis showed that both variables have almost similar value of pH, although slight fluctuation has been observed. Treatment 1 was observed to have an increase in pH level from 4.03 to 4.13 over the 4 weeks storage period.Treatment 2 increased from 4.2 (week 1) to 4.37 (week 3), but decreased from 4.37 to 4.1 (week 4). The result of this study shows no significant difference ($p < 0.05$) between the samples. A final pH of 4.43 for a clam (*Anomalocardiabrasiliana*) acidified with acetic acid and thermally treated was reported[26]. Also, an increase of pH from 3.90 - 4.21 in pickled anchovies after 20 days storage was investigated[27]. The pH values measured confirmed the addition of acetic acid in the formulation and limited the growth of microorganisms, ensuring the stability of the mollusk. The acetic acid and salt are diffused into the tissue which helped in the preservation of the mussel. However, pH value is not considered a reliable measure of spoilage and therefore it must be supported by chemical and sensory analyses[28].

Microbial Analyses

Throughout the storage period of 28 days, bacterial counts were <100 CFU/g, indicating the good microbiological quality of the mussels and the adequate hygiene used during processing of the product. As shown in table 4, treatment 1 has a result of 1.69×10^8 in week 1 and decreased to 7.70×10^3. While the microbial count of treatment 2 also decreased from1.13×10^7 to 8.07×10^3 during the 4 weeks of storage. There is no significant difference ($p < 0.05$) observed between the samples. Based on the results, it can be explained that the acetic acid and salt are responsible for inhibiting the growth of bacteria since these ingredients are known to preserve fishery products for several days or months, thus extending the shelf-life of the mussels.

Table 4: Microbial Count (log CFU/g) of marinated mussel

Week	Control	Treatment 1	Treatment 2
1	5.33×10^8	1.69×10^8	1.13×10^7
2	4.89×10^7	3.62×10^7	2.11×10^6
3	3.22×10^8	1.31×10^6	6.22×10^6
4	8.21×10^8	7.70×10^3	8.07×10^3

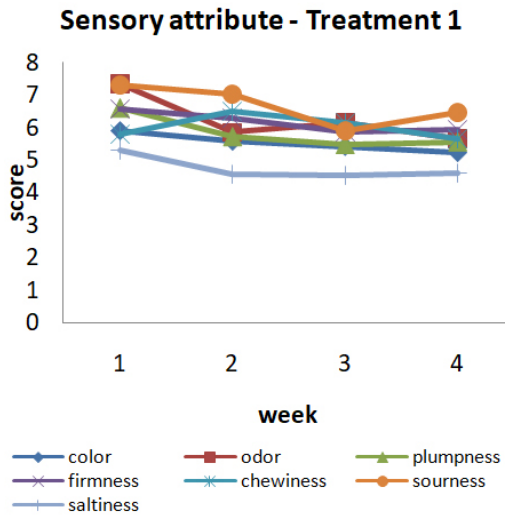
Sensory Analysis

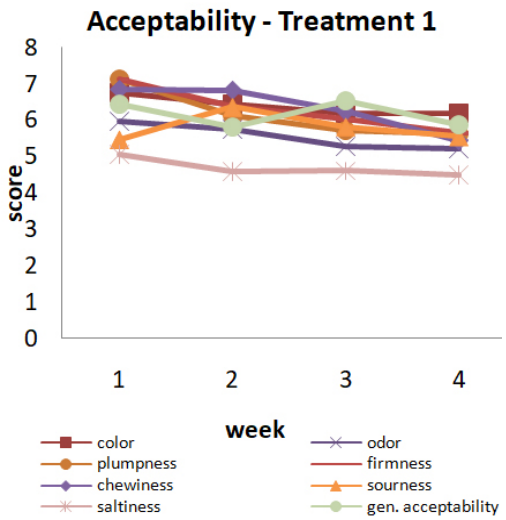
Table 5 shows the mean scores for the sensory evaluation of the two treatments. For treatment 1, the general acceptability decreased from 6.44 to 5.89. Panelists commented that the sample was very sour. But they observed that the texture was still firm throughout the 28 days of storage. For treatment 2, general acceptability also decreased from 6.67 to

5.71. Panelists observed that the color of the sample was very dark. This might be due to the spices added to the sample. The average overall sensory scores of marinated mussels decreased continuously with time, with some fluctuation. Since panel members were not exactly the same for some test where 1-3 substitutes were taken. It is suspected that this also contributed to the variation in average overall scores between tests. Based on the results of the statistics, there is no significant ($p < 0.05$) observed between the two samples in terms of the sensorial attribute and acceptability of the mussel. Control samples were not included in the sensory evaluation since the samples were already deteriorating even at the first week of storage. Unpleasant odor was already observed and some panelists rejected the samples during the sensory testing.

Table 5: Mean scores for the sensory evaluation of the two variables

	Treatment 1				Treatment 2			
	Week				Week			
	1	2	3	4	1	2	3	4
Color	5.89±1.21	5.57 ± 1.31	5.41±1.08	5.22±1.1	6.13±1.85	5.54±1.33	5.08±1.24	4.31±1.65
Acceptability	6.76± 1.27	6.44±1.43	6.17±1.5	6.18±0.82	6.07±1.05	5.59±1.10	5.26±1.5	4.43±2.06
Odor	7.35 ± 0.99	5.58±1.43	6.16±1.0	5.65±1.06	5.95±1.64	5.77±1.76	6.47±1.20	6.31±1.8
acceptability	5.97± 1.79	5.74±1.41	5.27±1.86	5.22±1.65	6.82±0.89	5.46±1.14	5.41±1.26	5.65±0.80
Plumpness	6.59 ± 1.27	5.73±1.4	5.49±1.17	5.57±1.14	5.9±1.31	5.93±1.10	5.59±1.78	4.98±1.05
Acceptability	7.14±1.52	6.12±1.43	5.72±1.29	5.62±0.98	6.5±1.24	6.4±1.26	5.59±1.66	4.83±0.78
Firmness	6.57 ± 0.77	6.29±1.86	5.85±1.05	5.93±1.05	6.8±0.77	6.35±1.67	5.5±1.35	5.75±1.02
acceptability	7.12± 0.94	6.36±1.47	6.03±1.04	5.65±1.59	6.79±0.8	6.37±1.11	5.72±1.54	6±1.40
Chewiness	5.79±1.38	6.5±1.53	6.15±0.84	5.66±1	6.03±1.5	6.51±1.34	5.4±1.27	5.78±0.97
Acceptability	6.84±1.03	6.82±0.84	6.23±1.04	5.43±0.716	6.7±1.10	6.64±1.03	6.17±1.19	5.65±1.27
Sourness	7.32±1.82	7.04±0.45	5.91±1.16	6.48±0.9	6.73±1.82	6.65±1.31	6.28±1.27	6.87±0.82
Acceptability	5.45±1.54	6.36±1.30	5.81±1.7	5.53±1.44	5.86±1.38	5.51±1.47	6.06±1.24	5.61±1.44
Saltiness	5.31±1.23	4.58±1.47	4.54±1.58	4.61±1.48	5.2±1.89	4.35±1.64	4.48±1.6	4.7±1.8
Acceptability	5.07±1.23	4.58±1.47	4.61±1.73	4.49±1.44	5.11±1.9	4.35±1.65	4.84±1.68	4.8±1.85
Gen. Accept.	6.44 ± 1.04	5.82±1.61	6.53±1.10	5.89±1.27	6.76±0.86	5.84±1.46	5.91±1.30	5.71±1.09





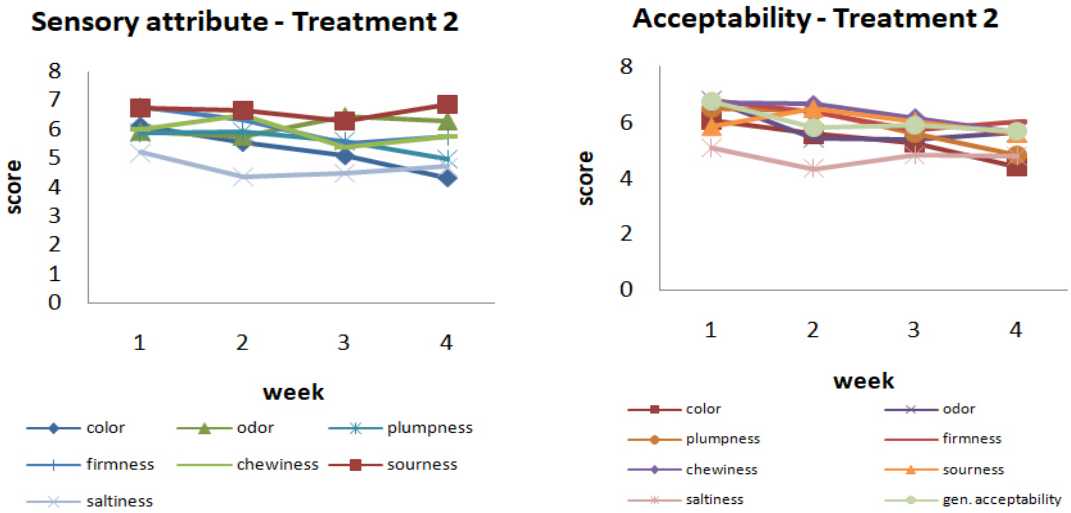


Fig. 1: Mean scores for the sensory evaluation of the two treatments

Based on the data of microbiological and physico-chemical analyses, it showed that the product was stable for 30 days in chilled storage at 2 °C. Marinating is an effective method of preservation for mussels since it can inhibit the growth of microorganisms. Chilling also helped in the preservation of the product. Further studies may be conducted to determine the optimum conditions of marinated mussel beyond 28 days storage and its maximum shelf-life.

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Conflict of Interest

The authors declare no conflict of interest.

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