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Development of Oyster Sauce Using Japanese Oysters, Green Mussels, and Clams

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Abstract: *Japanese oysters are rich in nutritional value and contain the fundamental flavor compounds desired in the oyster sauce, while green mussels and clams offer nutritional equivalence and lower cost. In Cambodia, a market analysis presently indicates a lack of commercial oyster sauce incorporating with green mussels and clams. Therefore, this study aimed to develop the oyster sauce produced from Japanese oysters, green mussels, and clams. Four different conditions of oyster sauce were developed such as 100% Japanese oysters (JO), 100% green mussels (GM), 100% clams (Cl), and a mixture of 30% Japanese oysters; 50% of green mussels and 20% clams (JGC). The physicochemical properties of oyster sauce including moisture content, pH, total acidity, total soluble solids, salt content, water activity, consistency were determined. Moreover, the microbiological characteristics (total plate count, yeast and mold count, total coliforms, Staphylococcus aureus, Salmonella), and the sensory attributes were also evaluated. The results showed that the raw materials did not impact significantly the moisture content of oyster sauce ($p > 0.05$), which ranged from 53.52-60.33%. However, the results of pH, total soluble solids, salt content, total acidity, consistency and water activity were significantly were affected ($p < 0.05$) by the raw materials, varying between 5.65-5.79, 45.77-50.10°Brix, 1.05-1.72%, 0.78-1.24%, 11.33-23 cm/min, and 0.88-0.93, respectively. There were non-detected of microbial count in JGC sample; however, the total plate count, yeast and mold, and S. aureus were found in JO, GM, and Cl samples. Among all conditions, JGC sample provided the highest sensory score (6.75) of overall acceptability. For future study, the nutritional profile and shelf-life observation of oyster sauce made from a mixture of Japanese oysters, green mussels, and clams should be further investigated.*

Keywords: Clams; Oyster sauce; Green mussels; Japanese oysters; Sensory evaluation

1. INTRODUCTION

Oyster sauce is a viscous dark brown condiment and renowned for its unique umami taste in many Asian cuisines, including Chinese, Thai, Vietnamese, and Filipino [1]. Oyster sauce traditionally derived from boiled oyster extracts with brine solution, seasoned with soy sauce, sugar, water, caramel coloring and other spices to reduce fishy smell and heated until viscous texture obtain [2]. Oyster sauce concurrently added with chemical preservative, including xanthan gum and typically contains 0.1% sodium benzoate to enhance the freshness and shelf-life of the product, and potentially sustainable to drive innovation and development [3]. Oyster sauce is used for pre-cooked with a variety of foods to enhance the natural flavors; thus, it needs to be resisted the high-temperature and low pH value. After processing, the quality of oyster sauce needs to remain stable during storage, transportation, and re-heated before consumption [4]. The quality of oyster sauces can be varied depends on ingredients, raw materials, processing, formulations, packaging, and storage condition. Different raw materials such as oysters, green mussels, or clams will have slightly different flavor profiles, which will influence the taste of the oyster sauce [5]. Shelled molluscs, especially mussels, oysters and clams are a good source of nutrients and suitable raw materials for producing oyster sauce.

Japanese oyster (*Crassostrea gigas*) is a major maricultural shellfish with tender meat and a strong umami

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taste. Oysters are normally used in oyster sauce production due to its outstanding umami taste and high nutritional content such as protein (9.70 g/100g), fat (1.80 g/100g), calcium (55 mg/100g), iron (3.60 g/100g) and energy (78 kcal) [6]. Green mussels (*Perna viridis*) are also valuable because of a widely traded and consumed, and also a good source of both essential and nonessential amino acids, vitamins, minerals, and low cholesterol level which contribute to the overall quality and potentially health benefits [7]. Green mussels, like oysters, have a naturally delicious, savory and umami-rich taste, often describes as being somewhere between clams and oysters. This attribute allows green mussels a suitable raw material to make sauce and provide a similar taste to regular oyster sauce [8]. Clams (*Callista erycinai*) are also a great commercial value and regard as a high-quality nutritious shellfish that do not only low in lipid content, but also high content of polyunsaturated fatty acid (PUFA), mineral, and protein [9]. In addition, the nutritional quality, nature, and flavor of clams is not significant difference comparing with oysters and green mussels [10]. In Cambodia, green mussels and clams are easy to find and often cheaper than oysters. In addition, the significant challenge of green mussels in Koh Kong is affected by unstable market demand and price fluctuations. The market price declined from 1,500-2,000 Riel/Kg in 2023 to 600-700 Riel/Kg in 2024, noting to lower profitability. Value-added processing actually represents a possible to enhance product value and income [11]. Hence, by using green mussels and clams alone or combine these shellfish with oysters can give more options and affordable for oyster sauce production, possibly creating a unique and complex flavor profile, especially raw materials to make oyster sauce that are not depending only on oysters. Previous research demonstrated the efficient use of individually frozen (IQF) oysters [12] and another study focused on determination of taurine, free amino acids, 5′-nucleotides and volatile compounds in oyster sauce. However, the development of high-quality oyster sauce using different raw materials have not been conducted yet [13]. Therefore, this study aims to develop and investigate the quality of oyster sauce produced by using Japanese oysters, green mussels and clams.

2. METHODOLOGY

2.1 Sample collection

Japanese oysters were bought from a seafood wholesaler located in Trolok Bek, Tuol Kork district (Phnom Penh, Cambodia). Green mussels were procured from Koh Kong province, and clams were purchased from Dey Huy market where originally from Kampong Cham province, Cambodia.

2.2 Study conditions

The oyster sauce was produced using different raw materials such as Japanese oysters, green mussels, and clams. There were four conditions to make oyster sauce including 100% Japanese oysters (JO), 100% green mussels (GM), 100% clams (Cl), and a mixture of 30% Japanese oysters, 50% of green mussels, and 20% clams (JGC).

2.3 Oyster sauce preparation

The shells of Japanese oysters, green mussels, and clams were firstly removed, cleaned, and boiled at 100 °C for 5 minutes. Next, the boiled samples were blended, followed by adding other ingredients such as garlics, gingers, scallions and red onions until the mixture become homogenous. After that, the mixture was filtered and the caramelized sauce was prepared by heating various ingredients such as dark soy sauce, palm sugar, corn starch, and monosodium glutamate (MSG). Caramel was then poured into the mixture and was heated at 80 °C for 2h until concentrated. Finally, the oyster sauce was filled in a sterilize glass bottle and stored at 4 °C for further analysis.

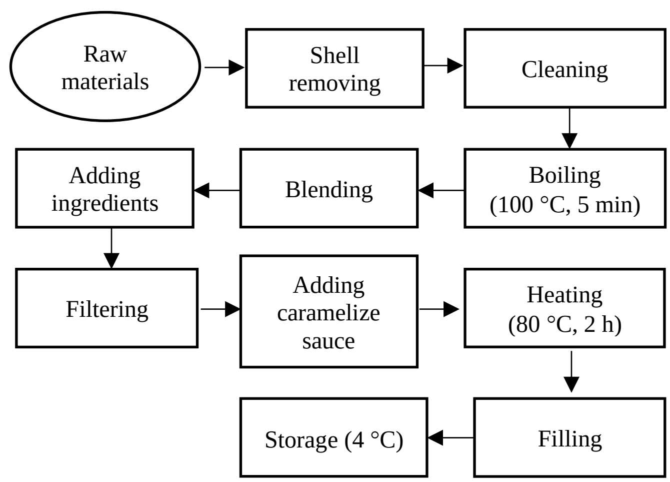

Fig. 1. Oyster sauce processing.

2.4 Physicochemical properties of oyster sauce

2.4.1 Moisture content

The moisture content was determined by the hot air oven method. First, the empty aluminum dish that already dried at 105 °C for 1 h was weighed using an electronic balance. Next, 5 g of sample was weighed in an empty dish and was placed in an oven (Memmert UNE400, Germany) at 105 °C until constant weight. The dried sample was finally re-weighed after cooling in a desiccator until constant weight [14].

$$\text{Moisture content (\%)} = \frac{m_2 - (m_3 - m_1)}{m_2} \times 100 \qquad \text{(Eq. 1)}$$

where:

m_1 = the mass of empty dish (g)
m_2 = the mass of sample before drying (g)
m_3 = the mass of dish with sample after drying (g)

2.4.2 pH value

First, 10 g of sample was diluted with 90 ml of distilled water and the pH was analyzed using a pH meter (LAQUA F-72- HORIBA, China) that had been calibrated with a buffer solution at a pH 7, 4, and 10. The result was recorded when reading constantly on the screen of pH meter [15].

2.4.3 Total titratable acidity

First, 10 g of sample and 10 ml of distilled water was transferred to same conical flask. The mixture was then titrated against 0.1N NaOH solution using phenolphthalein as an indicator until a faint pink appeared. Finally, the volume of NaOH after titration was recorded [16]. Titratable acidity was expressed as the percent of lactic acid and was calculated using the following equation:

$$\text{Total acidity (\%)} = \frac{C_{NaOH} \times V_{NaOH} \times M_{eq} \times 100}{W_{sample} \times 1000} \qquad \text{(Eq. 2)}$$

where:
C_{NaOH} = the normality of NaOH (N)
V_{NaOH} = the volume of NaOH (ml)
W_{sample} = the weight of sample (g)
M_{eq} = the equivalent weight of lactic acid (90 g/eq)
1000 = the conversion factor to convert L to ml

2.4.4 Total soluble solids (TSS)

The total soluble solids of sample were measured using a refractometer (ATAGO, PAL-1, Japan) ranging from 0 – 53 ºBrix. A few drops of sample were placed on the prism, which then closed and the reading was recorded [17].

2.4.5 Salt content

The salt content was investigated using a salt meter (ES-421, Atago, Japan). Then, a few drops of the sample were put on the prism and the result was recorded.

2.4.6 Water activity

Samples were placed in a pre-dried plate that had been dried at 45 °C for 45 min prior to measurement. Plate with sample was then put in a water activity meter (Aqualab 4TE, Benchtop) and measured at the ambient temperature. The result was then automatically shown on the screen of the water activity meter [18].

2.4.7 Consistency

The consistency of sample was measured from a distance (cm) running per minute in a Bostwick consistometer.

2.5 Microbiological analysis of oyster sauce

2.5.1 Sample dilution

The microbial count of the oyster sauce was carried out after one-day storage at 4 °C. First, 10 g of sample was homogenized in 90 ml sterile saline (0.85% NaCl) to obtain 10^{-1} dilution. Then, the serial dilution was done by transferred 1 ml of 10^{-1} dilution to 9 ml of saline diluent to obtain 10^{-2} dilution [19].

2.5.2 Enumeration of bacteria

According to spread plate technique, 0.1ml of diluted sample was added on specific media. Total plate count was performed using plate count agar (PCA), while potato dextrose agar (PDA) was used for determination of yeast and mold count. Total coliforms, *Staphylococcus aureus*, and *Salmonella* was done using Chromogenic Coliform Agar (CCA), Mannitol Salt Agar (MSA), and Xylose Lysine Deoxycholate agar (XLD). The microorganisms were counted after incubation at 37 °C for 24 h. The unit of microbial count was expressed as CFU/g and the number of colonies was calculated by the following formula [20].

$$N = \frac{\text{Average of colonies}}{\text{Dilution factor} \times \text{Inoculum size}} \qquad \text{(Eq. 3)}$$

where:
N = the number of microbial counts (CFU/g)

2.6 Sensory evaluation of oyster sauce

Sensory analyses were conducted using affective method to determine consumer preference for the samples. Oyster sauce was cooked with broccoli for sensory evaluation. The test was evaluated by randomly 79 untrained panelists, recruited from the Institute of Technology of Cambodia. A 9-point hedonic scale (1=extremely dislike; 5=neither like nor dislike; 9=extremely like) was applied for scoring the sensory attributes such as color, consistency, aroma, saltiness, odor, sweetness, flavor and overall acceptability. Samples were served hot and were placed in random order for assessments. Moreover, panelists were given a cup of water to rinse their mouths in between evaluations [21].

2.7 Statistical analysis

Statistical analyses were determined by using Statistical Package for Social Science (SPSS) for Window version 23.0. All the measurements were conducted in triplicate for each sample and were evaluated as an average ± standard deviations (SD). One-way ANOVA was done to compare between physicochemical properties of each sample using Duncan's multiple range test (DMRT) at a significance level of $p < 0.05$ [22].

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of oyster sauce

3.1.1 Moisture content

Fig. 2 indicates the moisture content of oyster sauce samples. The moisture content of samples ranged from 53.52% to 60.33%, with no significant difference among formulations ($p > 0.05$). This indicates that variation in raw materials, including oysters, green mussels, and clams, did not significantly affect the final moisture content of the oyster sauce samples. Although raw oysters generally possess higher inherent moisture levels than clams and green mussels, the standardized processing conditions and controlled ingredient ratios likely minimized these differences. Consequently, water retention was effectively uniform across all formulations, resulting in comparable moisture contents in the final products [23].

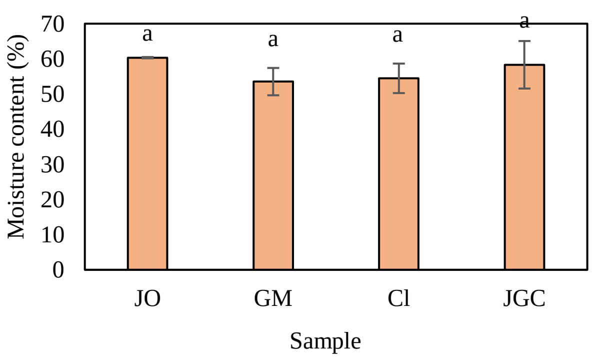

Fig. 2. Moisture content of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.2 pH value

Fig. 3 demonstrates the pH value of oyster sauce samples. The pH of oyster sauce ranged from 5.65-5.79, with a significant difference ($p < 0.05$) among all samples. The JO sample exhibited the lowest pH, while the Cl sample showed the highest pH. The pH values found in this study were higher than Thai community standard (≤4.5) [24]. The observed variations attributed to organic acid composition of raw materials. However, all formulations remained within the mildly acidic range, which is favorable for flavor stability and microbial control in cooked seafood sauces when combined with other preservation factors such as heat treatment and salt content [25]. Accordingly, Mendez-Zamora et al. [26]. reported that interactions between polysaccharides and proteins in raw materials interact occur through attractive and repulsive forces involving polar and non-polar groups, leading to fluctuations in pH.

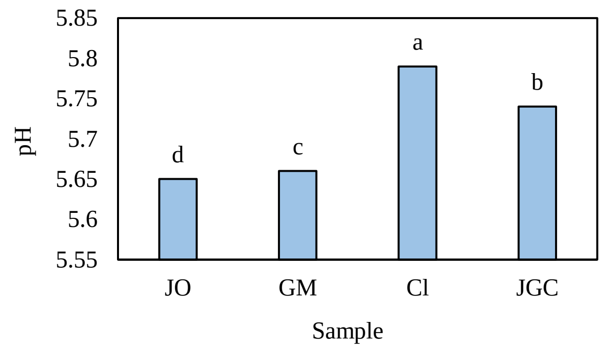

Fig. 3. pH of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.3 Total titratable acidity

Fig. 4 illustrates the total acidity of oyster sauce samples. The total acidity of samples was found between 0.78-1.24%, with significant difference ($p < 0.05$) among all samples. According to the result in this study, JO sample was discovered to be least acidic, while JGC sample found to be the most acidic. GM and Cl samples demonstrated a similar value with no significant differences compared to JO and JGC samples. The differences in biochemical compounds, particularly glycogen, amino acid content, and organic acid in these shellfish and processing can lead to variations in acidity of the oyster sauce [27]. Moreover, the mixed shellfish sample (JGC) may promote greater acid formation due to the combined enzymatic activity and substrate availability during processing, resulting in higher total acidity [28].

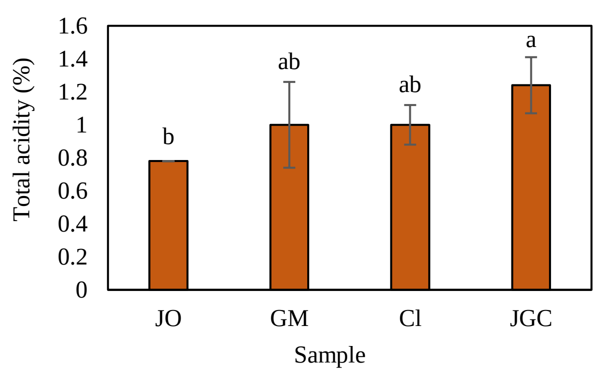

Fig. 4. Total acidity of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.4 Total soluble solids

Fig. 5 shows the total soluble solids of oyster sauce samples. The result was dramatically changed, ranging from 45.77-50.10 ºBrix ($p < 0.05$). The result was in agreement of a patent publication which total soluble solid was 30 to 55 ºBrix [29]. Total soluble solids reflect the overall concentration of dissolved solids such as salt, sugars, amino acids, and soluble proteins, which associated with enhanced umami intensity and overall flavor richness in oyster sauce. Therefore, total soluble solids content varies in oyster sauce samples due to amino acid contained in raw materials [30].

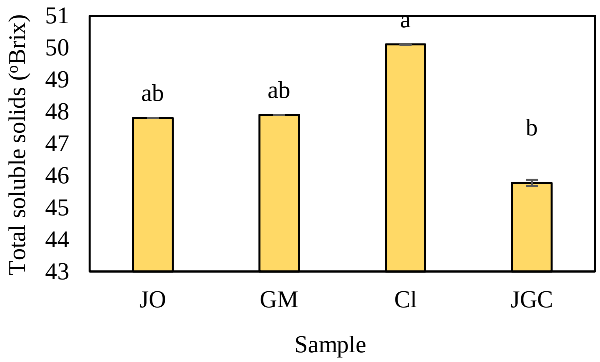

Fig. 5. Total soluble solids of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.5 Water activity

Fig. 6 reveals the result of water activity of oyster sauce samples. There was non-significant difference ($p > 0.05$) among samples. The water activity of samples was between 0.88-0.93, following the Food (Amendment) Regulations 2003, which oyster sauce has a water activity more than 0.85 [31]. The higher water activity observed in JO sample possibly attributed to the higher moisture content and soluble protein of oyster meat, which can retain free water during cooking and concentration [32]. However, processing factors such as salt addition, sugar concentration, and thermal treatment could control water activity, so minimizing the effect of raw material variation [33].

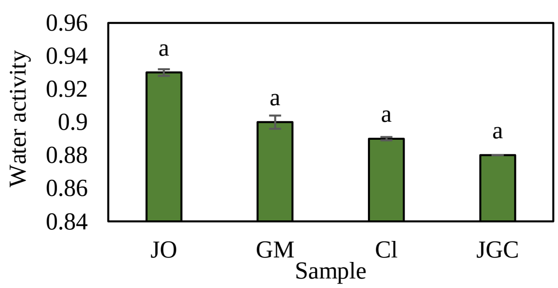

Fig. 6. Water activity of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.6 Salt content

Fig. 7 presents the salt content of oyster sauce samples. The salt content ranged from 1.05-1.72%, with significant differences ($p < 0.05$) among samples. The result indicated that using oysters, green mussels, and clams as raw materials strongly influenced salt levels of final oyster sauce. The green mussel-based oyster sauce (GM) exhibited the highest salt content, followed by JO, JGC and Cl samples. This was in agreement with a report of Nittoseiko Analytech which the salt content of thick sauce is less than 9% [34].

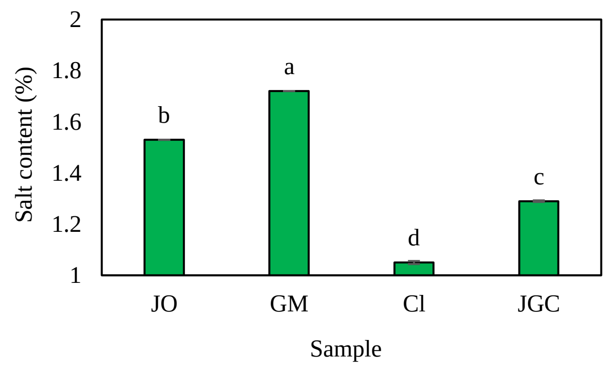

Fig. 7. Salt content of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.1.7 Consistency

The consistency of oyster sauce samples shown in Fig. 8. There was differed significantly among formulations ($p < 0.05$), ranging from 11.33-23.00 cm/min. Consistency of oyster sauce can be related to the degradation of carbohydrate in raw materials, along with the addition of other ingredients such as sugar, salt, soy sauce, and importantly a thickener agent such as starch [35]. A thicker texture is generally preferred in oyster sauce, as it improves consumer perception.

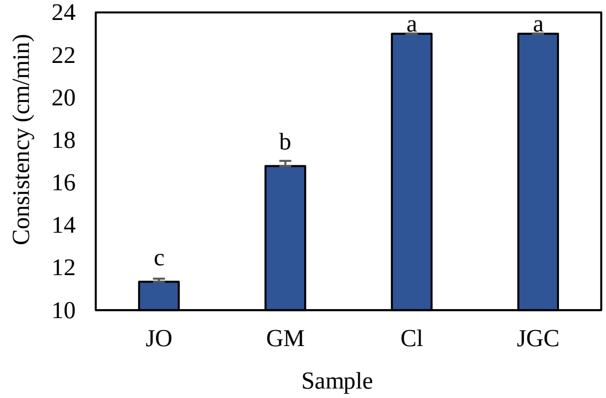

Fig. 8. Consistency of oyster sauce samples. The mean value with different superscripts (a-b) indicates the statistically difference ($p < 0.05$)

3.2 Microbiological quality of oyster sauce

The result of microbiological quality of oyster sauce is presented in Table 1. The oyster sauce produced from clams (Cl sample) exhibited the highest level of total plate count (1.61×10^2 CFU/g) and yeast and mold count (0.64×10^2 CFU/g). Based on the result in this study, the relative higher total plate count and yeast and mold count may associate with its high moisture content and total soluble solids that support microbial survival [36]. In addition, yeast and mold able to contaminate in the samples due to insufficient control of processing conditions because these Fugal are commonly transmitted through air, leading to undesirable changes in sensory evaluation and textural degradation [37]. Meanwhile, low level of *Staphylococcus aureus* was detected in JO, GM, and Cl samples, while none were detected in the JGC sample. The low levels of *Staphylococcus aureus* suggested the enhance microbial stability of oyster sauce sample. According to the Thai Community Standard (Number 466/2547), the microbiological properties such as total plate count, *Staphylococcus aureus*, and yeast and mold count must be less than 1×10^4 CFU/g, 1×10^2 CFU/g, and 10 CFU/g, respectively. The oyster sauce produced in this study met the Thai Community Standard (Number 466/2547) for total plate count and *Staphylococcus aureus*. For the pathogenic bacteria such as total coliforms and *Salmonella* were not detected, confirmed the microbiological safety of samples. Ingredients such as ginger, red onion and garlic exerted antimicrobial activity which could contribute to the antimicrobial capacity [38]. The low and non-detected microorganism likely reflects the impacts of the acidic condition of oyster sauce and heat treatment process [39]. In contrast, the presence of excessive yeast and mold suggests potential post-processing contamination issues, possibly occurring after heating and during the filling stage, which warrants further hygiene controls.

Table 1. Microbiological properties of oyster sauce

Formulations	Unit	Samples				Standard [Thai Community Standard (Number 466/2547)]
		JO	GM	Cl	JGC	
Total plate count	CFU/g	0.23×10^2	0.36×10^2	1.61×10^2	ND	1×10^4
Yeast and mold count	CFU/g	0.05×10^2	0.03×10^2	0.64×10^2	ND	10
Total coliforms	CFU/g	ND	ND	ND	ND	-
Staphylococcus aureus	CFU/g	0.06×10^2	0.08×10^2	0.01×10^2	ND	1×10^2
Salmonella	CFU/g	ND	ND	ND	ND	-

3.3 Sensory evaluation of oyster sauce

Fig. 9 demonstrates the result of sensory evaluation of oyster sauce. Sensory evaluation is an important parameter to investigate the preferences of consumer on products characteristics. The result in this study reveals that JGC sample received higher scores of overall acceptability which was 6.75, followed by GM, JO, and Cl samples. The overall score of GM, JO, and Cl was 6.7, 6.5, and 6.37, respectively. The mixture of Japanese oysters, green mussels, and clams can create a better sticky texture that much acceptable as a condiment and unique flavor combination that is distinct from traditional oyster sauce. The flavor profile of oyster sauce likely influenced by the combined flavors of the sweetness of the mussels and clams, the brininess of the salt water, and the umami taste of the Japanese oysters. In addition, clams and mussels have a milder flavor profile that can be overpowered by the stronger flavors of other ingredients in a dish. Umami contains in oysters, green mussels, and clams can be perceived through the interaction with umami receptors in the oral cavity, resulting a potential to increase the food acceptance, especially flavor attribute [40]. Based on sensory result, JGC sample is the most promising product compared to other formulations in all sensory attributes. Therefore, this study suggests that the combination of Japanese oysters, green mussels, and clams offers a more appealing sensory score compared to sauces made from a single shellfish type.

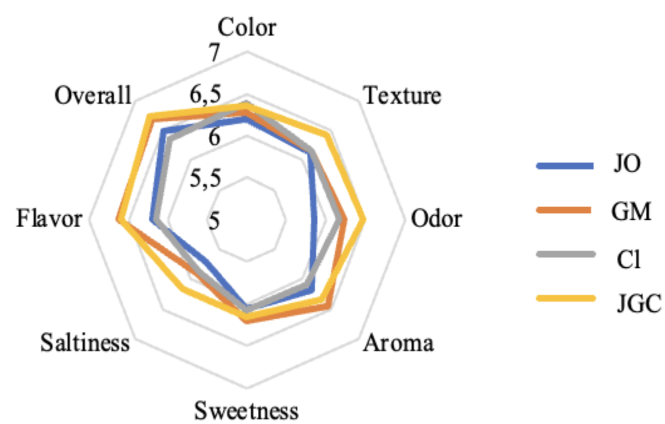

Fig. 9. Sensory evaluation of JO, GM, Cl, and JGC samples.

4. CONCLUSIONS

In conclusion, this study reveals that the moisture content of the oyster sauce remained consistent despite varying raw materials such as Japanese oysters, green mussels, and clams. However, pH value was higher than the Thai standard, while total soluble solids, water activity, salt content, and consistency were within established range. The result of microbial analysis of oyster sauce also aligned with the Thai standard, indicating good quality and safety.

The sensory evaluation clearly indicated that the JGC sample achieved the highest overall acceptability of 6.75. The shelf-life observation of oyster sauce made from Japanese oysters, green mussels, and clams should be considered for future research.

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