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Effects of Acidic Ingredients on the Microbial Quality, Sensory Acceptance and Shelf Life of Chilli Shrimp Paste

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ABSTRACT

This study was conducted to investigate the effects of acidic ingredients, which was purposely added in traditional preparation of chili shrimp paste (CSP), which helps to enhance its sour flavour and inhibit microbial growth. Despite the recognised role of organic acids in food preservation, specific research on their effects within CSP remains scarce. Four different types of acidic ingredients; lime juice, tamarind juice, sour mango and vinegar were added into CSP according to the amount of acidic ingredients that produced the highest score of sourness taste evaluated by untrained panellists. The microbial quality of CSP with four acidic ingredients and controlled CSP (without acid) were evaluated in order to determine the effectiveness of organic acids in CSP. Then, shelf lives of these CSP were analysed continuously for 7 days at room temperature (28 ± 2°C) and 10 days at chilling temperature (5 ± 1°C). Addition of vinegar in CSP has significantly reduced the microbial content in CSP, inhibited the growth of *Staphylococcus aureus* and increased the shelf-life of the product.

1. Introduction

Traditional foods are defined as products characterized by specific raw materials, long established recipes or distinctive process [1]. These foods are classified based on traditional ingredients, formulations and production or processing methods, as outlined by the AGROCERT standardization, inspection and certification authority in Greek agriculture [2]. Predominantly, traditional foods are offered by vendors, often in streets and public places [3]. Chili shrimp paste (CSP) or 'Sambal belacan', is a widely consumed spicy condiment in the Southeast Asian countries, particularly Malaysia, Singapore and Thailand, due to its versatility in culinary applications [4]. It is traditionally enjoyed as

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a fresh dip, a base for frying rice or cooking vegetables and as an ingredient in kerabu dressing when mixed with desiccated coconut.

The preparation of CSP involves a mixture of raw and bird-eye chilies, roasted shrimp paste, salt and sugar, traditionally homogenized using a pestle in a stone mortar [5]. Modern practices in larger establishments may utilize blenders for efficiency. The inclusion of salt, sugar and various organic acids; namely kalamansi juice, lemon juice, tamarind juice or vinegar, is crucial for flavor enhancement and preservation [6].

Concerns regarding microbial contamination are significant with main ingredients such as chili and shrimp paste. Studies have highlighted the susceptibility of red chilies to fungal contamination [7] and the presence of pathogenic bacteria like *Salmonella* [8] and *Klebsiella pneumonia* [9] in raw chili. Similar microbial risks have been identified in shrimp paste, particularly the presence of *Salmonella*, coliform and yeast and mold which poses a health risk when incorporated into CSP [10-12].

Besides chilies, insufficient heat treatment of shrimp paste indicated the presence of *Salmonella* [13]. The detection of *Salmonella* spp. serotypes in oriental shrimp paste or shrimp paste by [14,15] pose a serious health hazard in CSP because shrimp paste is usually added as a main ingredient in CSP recipes [13]. Based on Regulation 163 (Food Act 1983) and Food Regulations 1985, shrimp paste is shall to contain not less than 15 % sodium chloride and 25 % of protein to maintain the quality and the acidity in preservation [16]. Moreover, on Regulation 39 in Food Act 1983 and Food Regulations 1985 was stated that the microbial standard of total plate count at 37°C for 48 hours should not exceed to 10^6 CFU/g [16].

Hygiene during CSP preparation and handling is paramount, especially since it is often consumed raw or semi-prepared, by-passing heat treatments that could eliminate pathogens. The strategic addition of organic acids is essential for preservation and microbial safety, with the choice of acid dependent on consumer preference and availability. Lime juice (*Citrus aurantifolia*), Tamarind juice (*Tamarindus indica*), Sour mango (*Mangifera petandra*) and Vinegar (acetic acid) are common ingredients added in CSP preparation [17].

Organic acids, including malic, citric and tartaric acids, found naturally in various fruits, along with lactic and acetic acids produced naturally by microorganisms, are leveraged in food preservation. These acids inhibit microbial growth by lowering pH, disrupting proton gradients, acidifying cytoplasm and interfering with nutrient transport across cell membranes [18] and affecting biochemical functioning of the cell [19], thus ensuring the safety and quality of CSP.

Recent interest has been directed toward investigating the physicochemical and sensory attributes of CSP, as well as the enhancement of its formulation and processing techniques. Sobhi *et al.,* [17] conducted a study on the physicochemical and sensory properties of a traditional CSP, finding that a paste with approximately 70 % moisture content, 27°Brix, 4.4 % salt, 10 % sucrose, a thick and chunky texture, a lightness value (L) of 23, redness value (a) over 20 and a yellowness value (b) of 12 was most preferred. The preference was not influenced by the pH range of the samples, which varied from 4.02 - 6.33 [17].

Another study by Nadia Sarina *et al.,* [5] focused on enhancing the formulation and production process of CSP based on sensory evaluations concerning acidity, the source of acid and the coarseness of chili paste [6]. They discovered that the optimal pH level was 4.0, kalamansi juice was the most effective acid source, and the preferred coarseness for chili paste was achieved using milling plates set to a 120 µm gap.

Given the limited research on the effects of acidic ingredients in CSP concerning microbial safety and shelf life, this study aims to explore the roles of acidic ingredients in CSP as a preservation method and their sensory acceptance. The objectives of this study were (i) To determine the

accepted amount of acidic ingredients to produce the right sourness taste of CSP; (ii) To determine the effects of four types of acidic ingredients (lime juice, tamarind juice, sour mango and vinegar) in inhibiting the microbial load present in CSP and (iii) To assess the shelf life and pH of CSP with and without acidic ingredients under room temperature and chilled temperatures. This study aimed to elucidate the fundamental principles of using acidity for preservation in one of Malaysia's most beloved traditional foods.

2. Methodology

2.1 Preparation of Chili Shrimp Paste (CSP)

A formulation for producing CSP was reformulated from Dennis's formulation [20] as shown in Table 1 and the experiment was conducted under controlled condition in the laboratory.

Table 1
A standard formulation of CSP [20]

Ingredients	Amount (g)	Percentage
Red chilies	80	59.2
Bird eyes chilies	20	14.8
Roasted 'belacan'	10	7.4
Salt	5	3.7
Sugar	5	3.7
Lime juice / tamarind juice / sour mango and vinegar	10	11.1

All equipment were cleaned and sanitized using boiling water before CSP was prepared. Fresh red chili (*Capsicum frutescence*) and fresh bird's eyes chili (*Capsicum annum*) at commercial maturity stage and fermented shrimp paste (belacan) were purchased from a local hypermarket. All chilies were washed in running tap water, de-stalked manually and blanched for one min in hot water at 100°C. The adopted blanching time was based on preliminary trials that resulted in complete inactivation of peroxidase enzyme in chilies [21]. The wet shrimp paste was chopped into smaller pieces and roasted in the oven at 180°C for 15 mins. The roasted shrimp paste which contained less than 200 CFU/g (est.) was used to reduce initial microbial load in CSP. All the ingredients were then mixed using a household blender (Philips, Malaysia) for 1 min at speed level 1, and continued blending with speed level 2 for 2 mins. The 30 g yield of CSP without any addition of acidic ingredient was obtained consistently from the new formulation in the same batch for comparison purposes.

2.2 Sensory Acceptance of Sourness in CSP Added with Different Acidic Ingredients

CSP was prepared using the new modified formulation, which consists of three different amounts of acidic ingredients in CSP for choosing the most accepted sourness for each type of acidic ingredients as shown in Table 2.

The sensory analysis of CSP was conducted in the teaching restaurant at Faculty of Fisheries and Food Science in Universiti Malaysia Terengganu (UMT), Malaysia. CSP samples were presented with three slices of cucumber as a porter, at room temperature in small container coded with different number of random three digits code. A glass of plain water was provided for rinsing between samples during the sensory assessment to avoid the residual taste between samples. Forty-eight untrained panelists of UMT students were made up of Malaysian adults who are familiar with CSP and consume it regularly. Panelists were asked to rate the acceptability of sour taste of CSP with three acidic

amounts of four types of acidic ingredients using hedonic scale ranging from “dislike extremely” (score 1) to “like extremely” (score 9).

Table 2
Types of optional acidic ingredient and amount added into ‘sambal belacan’ for sensory evaluation

Sample	Acidic amount (mL or g)		
	A	B	C
‘Sambal belacan’ with lime juice	2.5 mL	5.0 mL	6.25 mL
‘Sambal belacan’ with tamarind juice	3.0 g	7.5 g	12.0 g
‘Sambal belacan’ with sour mango	15.0 g	25.0 g	35.0 g
‘Sambal belacan’ with vinegar	5.0 mL	7.5 mL	10.0 mL

2.3 Analysis of pH

The pH of 30 g of CSP added with four acidic ingredients were measured in duplicate using pH meter (WTW Series, INOLAB), with glass electrode at 25°C respectively.

2.4 Microbial Analysis of Selected CSP Added with Acidic Ingredients

Preliminary results showed that the amount acceptance for sourness of CSP added with four acidic ingredients were as follows: 5 ml for CSP with lime juice, 7.5 g for CSP with tamarind juice, 35 g for CSP with sour mango and 10.0 ml for CSP with vinegar. The acceptance sourness of CSP with different acidic ingredients were used during the prepared of new batch CSP for microbial analysis, shelf life study and pH determination.

Initially, 25 g of CSP added with lime juice, tamarind juice, sour mango and vinegar were weighed and placed in a sterile stomacher bag. The samples were then homogenized in 225 ml of 0.1 % peptone water solution using a stomacher making 10^{-1} dilution factor. One milliliter of the homogenized 10^{-1} diluted sample was transferred and mixed with 9 ml of peptone water solution to provide 10^{-2} dilution. The step was repeated until the desired dilution was taken. These serial dilutions containing samples were further analyzed.

CSP with different acidic ingredients were analyzed for microbial load in accordance with the USFDA (2006) Bacteriological Analytical Manual for the determination of total plate count (TPC), coliforms count and detection of *Escherichia coli*, *Staphylococcus aureus* count, followed by Australian Standard of Food Microbiology (2008) for *Psychrotrophic* count, *Lactobacillus* count and yeast and mould count and MKMM (2004) for the detection of *Salmonella* spp. Enumerations of agar plates for all microbial analyses were followed counting rules, and these data for the calculations of CFU/ml [22]. Spread plate method was used for all microbial analyses, unless if it was mentioned otherwise.

The media and incubation conditions used were as follows:- Plate Count Agar (Merck) incubated at 37.0 ± 1°C for 48 h for Total Plate Count (TPC); Baird Parker Agar (Merck) incubated at 37.0 ± 1°C for 48 h for *Staphylococcus aureus* count; Potato Dextrose Agar (Merck) plate acidified with tartaric acid (1.2 ml for 100 ml PDA plates) for Yeast and Mould count incubated at 28 ± 1°C for 5 days; Plate Count Agar (Merck) incubated at 7 ± 1°C and 10 days for Psychrotrophic count; MRS agar (Merck, Germany) for 35 ± 1°C and 24 hours.

2.5 Method for Coliform and E. Coli Count

The numbers of coliform and *E. coli* in this experiment were enumerated using Most Probable Number technique (MPN) as the first priority technique. This technique expressed the growth in term of number of positive tubes in LST and BGLB which indicated the presence of coliform. The MPN/ml was calculated by referring the MPN table based on positive tubes of BGLB observed in the study. This MPN technique was divided into three consecutive tests; (a) Presumptive test, (b) Confirmatory test and (c) Completed test. Detail descriptions of these tests were described somewhere else [22].

2.6 Detection of Salmonella

Detection and isolation of *Salmonella* are generally based on pre-enrichment, selective enrichment, plating onto selective differential agar and biochemical and serological confirmation of suspect isolates. The original sample had a dilution factor 10^{-1} after homogenized with 0.1 % buffered peptone water before placed in incubator at 37 ± 1°C for 24 hours for pre-enrichment. After 48 hours, sample was gently mixed and inoculated into two different selective enrichment broth as follow; 1.0 mL into 10 mL selenite cystine broth and incubated at 37°C for 24 hours, while another 0.1 mL was inoculated into 10 mL Rappaport-Vassiliadis soya peptone broth (RVS) and incubated at 37°C within 24 hours.

After 24 hours, samples were sub-cultured from selective enrichment broth using inoculating loop and streaking method. Duplicate Petri dishes, prepared with Xylose Lysine Deoxycholate (XLD) (Oxoid, England) was labelled at each selective broth. These plates were incubated at 37°C for 24 hours. The appearance of typical *Salmonella* spp. colonies on XLD media is pinkish with or without black centers. A few *Salmonella* spp. produce yellow colonies with or without black centers [16]. Characteristics colonies was picked from each plate and inoculated into triple sugar iron (TSI) agar and lysine iron agar (LIA) slants for screening tests. *Salmonella* typically produces alkaline (red) slant and acid (yellow) butt with production of hydrogen sulfur, which gave blackening of agar in TSI agar. In LIA, *Salmonella* typically produces alkaline reaction in butt of tube will generate purple color, and if only distinct yellow in LIA butt, it was considered as negative reaction [22]. Each culture showing presumptive-positive TSI agar and LIA results were maintained on Tryptone Soy Agar (TSA). Cultures were then subjected to biochemical tests [23].

2.7 Shelf-life Analysis

Experiments for shelf life of CSP added with different acidic ingredients were done continuously until day-7 for room temperature sample (28 ± 2°C) and day-10 for chill temperature sample (5 ± 1°C) where total plate count (TPC) and Yeast and mould count (YM) were determined. Besides that, the duplicate measurements of pH were carried out along the storage duration of CSP.

2.8 Determination of Generation Time for Shelf Life Study

The generation time was determined directly from this Eq. (1) and (2),

$$\text{Mean growth rate constant } (k) = [\log N_t - \log N_0] / 0.301t \text{ [18]} \quad (1)$$

$$\text{Mean generation time } (g) = [1/k] \text{ [19]} \quad (2)$$

There also another formulation suggested by [24], where slope was calculated directly from linear equation given from R-squared formula occur in the linear graph.

2.9 Statistical Analysis

Minintab-14 was used to interpret the acceptance level of sour in different CSP prepared for this analysis. The calculated mean values were compared using Fisher's LSD test from one-way ANOVA test results with level of significance defined at $p < 0.05$.

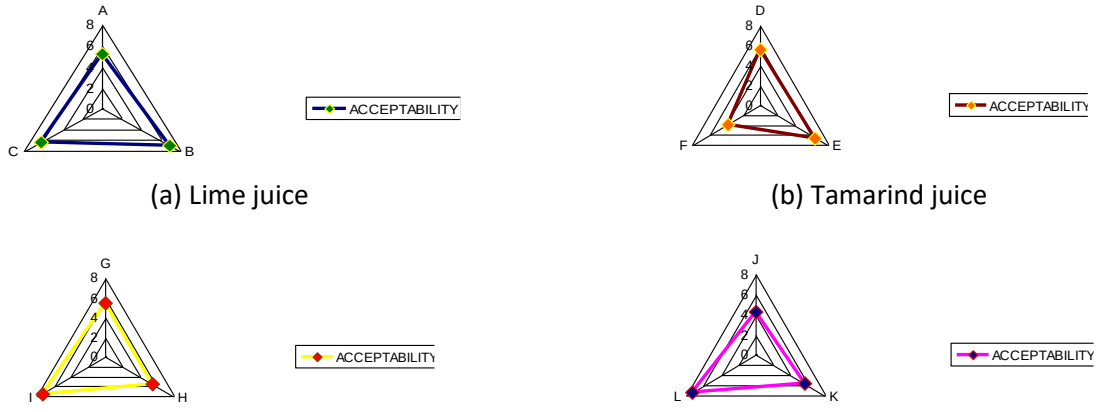
Statistical analysis was carried out by the method described by [25] and [26]. Calculation of statistical analysis was conducted using the software SPSS 16.0. All experiments were carried out in duplicates and analyzed by one-way analysis of variance (ANOVA). Mean and standard deviation values by Duncan's multiple range test with level of significance defined at $p < 0.05$ was to show if there any differences between samples and temperature if storage.

Microsoft Excel 2016 was used to enter the statistical results of microbiological count from SPSS to obtain the bar graph for microbial quality of 'sambal belacan'. The Microsoft Excel helps statistical analysed of generation time of shelf-life analysis by linear regression of R-squared formula that were build up from scatter chart.

3. Results and Discussion

3.1 Sensory Analysis of 'Sambal Belacan'

'*Sambal belacan*' were evaluated for sour taste that depends on acceptance of acidic amount on a 9-point hedonic scale. 12 out of 48 panels were asked to rate different types of optional acidic ingredients added in CSP. Sensory evaluation data of the highest scores acceptance of acidic amount are presented in Figure 1. All data were analyzed using analysis of variance (ANOVA); Fisher's LSD multiple range test was used to separate the mean and statistical significance was determined at $p < 0.05$. The mean scores by acceptability test have been agreed as the suitable acidic amount that was added into CSP as well as a sample for this research.



(a) Lime juice (b) Tamarind juice

(c) Sour mango (d) Vinegar

Fig. 1. The acceptance of acidic amount of CSP incorporated with (a) lime juice, (b) tamarind juice, (c) sour mango and (d) vinegar, respectively

There was significant different within three amount level of lime juice, tamarind juice, sour mango and vinegar for sour acceptance. The outer results as shown by spider web (Figure 1) demonstrated that the highest acidic amount acceptance by panel based on sour taste. For 'sambal belacan' with lime juice, the amount acceptance for lime juice to be added was 5 mL (6.92 $\pm$ 1.24). The amount

acceptance for tamarind juice was 7.5 g (6.50 ± 1.51), sour mango was 35 g (7.42 ± 1.08) while vinegar was 10 mL (7.3 ± 0.89). The most acceptance of acidic amounts were used during the preparation of CSP (Table 3).

Table 3
Acidic amount added in 30 g of 'sambal belacan' formulations

Sample	Acidic amount
Sambal belacan' with lime juice	5.0 mL
'Sambal belacan' with tamarind juice	7.5 g
'Sambal belacan' with sour mango	35 g
'Sambal belacan' with vinegar	10.0 mL

3.2 Microbial Quality of 'Sambal Belacan'

The results of the microbial load analysis of different 'sambal belacan' are shown in Table 3. Earlier study by Fitri Nurdiyana [27] on microbial count in 'sambal belacan' had revealed that *Lactobacillus* spp. and coliform were absent [28,29].

Table 4
Microbial quality in 'sambal belacan' with different acidic ingredients

Sample	Microbiological Tests (CFU/g or MPN/mL)			
	Total Plate Count	Psychrotrophic bacteria count	Yeast and Mold count	*S. aureus* count
'Sambal belacan' with lime juice (SBLJ)	2.776 ± 0.25^{b}	2.301 ± 0.00^{d}	N.D^{c}	1.000. ± 1.41^{cd}
'Sambal belacan' with tamarind juice (SBTJ)	2.540 ± 0.34^{b}	3.848 ± 0.02^{b}	2.500 ± 0.28^{b}	2.000 ± 0.00^{bc}
'Sambal belacan' with sour mango (SBSM)	4.292 ± 0.06^{a}	5.700 ± 0.07^{a}	3.160. ± 0.17^{a}	4.213 ± 0.07^{a}
'Sambal belacan' with vinegar (SBV)	2.239 ± 0.34^{b}	2.151 ± 0.21^{d}	2.540 ± 0.09^{b}	N.D^{d}
'Sambal belacan' without acidic ingredients (SB control)	3.785 ± 0.04^{a}	3.263 ± 0.08^{c}	2.573 ± 0.38^{ab}	2.690 ± 0.12^{ab}

Note: N.D = Not detected. Values are mean ± SD of two replicates, values followed by different letters in the same column differs significantly ($p < 0.05$)

However, there is no detection of *Salmonella* spp*.,* Lactobacillus spp*.,* coliform and *Escherichia coli* in prepared 'sambal belacan' for this study. Besides, compared to the results shown in Fitri Nurdiyana [26] study, all microbial count in present study are lower than previous one [28].

Throughout the table, 'sambal belacan' added with vinegar (SBV) show the lowest count of microbial load, followed by 'sambal belacan' with lime juice (SBLJ), 'sambal belacan' with tamarind juice (SBTJ), 'sambal belacan' without any optional acidic ingredient (SB control), whereas 'sambal belacan' with sour mango (SBSM) demonstrated the highest count respectively. The highest count of microbial load in 'sambal belacan added with sour mango may reason by initial microbial load and microflora contained in sour mango. According to FDA/IFT (2003), initial microbial load of raw materials were contaminated with microorganisms before preparation of natural antimicrobials in the acidic ingredients have caused these inconsistent results [30].

The ability of acetic, citric and lactic acid to prevent growth of *Listeria monocytogenes* during extended incubation at 7 to 35^{o}C while tartaric acid in tamarind can reduce the population of

Salmonella spp. and *Listeria monocytogenes* in raw shrimp [30]. In this finding, lime juice and tamarind juice showed the moderate inactivation effects of acidic ingredients than vinegar. It shows that citric acid was less inhibitory than acetic acids.

Acetic acid contained in vinegar has the highest concentration of acidic value than other optional acidic ingredients that incorporated with 'sambal belacan'. However, different organisms have demonstrated different rankings for the inhibitory effects of organic acids [30]. So, it is possible to successfully predict the effect of changing from one organic acid to another in this study.

3.3 Shelf-life of 'Sambal Belacan'

'Sambal belacan' is a type of foods that are perishable by nature. It is well known that conditions used to process and store foods may adversely influences the quality attributes in foods [30]. Physical, chemical and microbiological changes the leading causes of food deterioration [30]. As the consequence of these mechanisms, *'sambal belacan'* may be altered to such an extent that they are either rejected by the consumer or it may become harmful to the person consuming them. There are many factors have been evaluated to influences the microbial growth. There can be divided into intrinsic and extrinsic factors. Intrinsic factors are those that are characteristic as the food itself, such as moisture content, pH and acidity, nutrient content, biological structure, redox potential or naturally occurring and with the addition of antimicrobials and competitive microflora. Extrinsic factors are those that refer to the environmental surrounding the food. The example of extrinsic factors are types of packaging or atmosphere, effects of time and temperature condition on microbial growth, storage and holding conditions, processing steps and product history [30].

This study was used the temperature and period (time) of storage that analyzed the chemical and microbiological changes in different *'sambal belacan'*. Perhaps, with the standing of different reaction will be an initial developing specific procedure will prolong the shelf-life of *'sambal belacan'*. Samples stored at room temperature (28 ± 2^{o}C) were analyzed until day 0 to day 7; while samples stored at chill temperature (5 ± 1^{o}C) were analyzed from day 0 to day 10. pH was analyzed as a part of chemical analysis, meanwhile Total Plate Count (TPC) and Yeasts and moulds count (YMC) are part of microbiological analysis for the shelf-life of *'sambal belacan'*. According to FDA (2003), the need for time and temperature control is primarily determined by the potential food for contamination with pathogenic microorganisms of concern including processing influences and the potential for subsequent growth or toxin production [31].

Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety (IFT/FDA, 2003). Figure 2 shows the results of the total plate counts (TPCs) of 'sambal belacan' samples at different temperature during storage. Initially, the TPCs of the 'sambal belacan' without acidic was 6.2 x 10^3 CFU/g respectively. The additions of acidic ingredients have reduced the TPC of the 'sambal belacan' either at room temperature or at chill temperature. However, the addition of sour mango into 'sambal belacan' will pose a serious health hazard to consume because there are highest TPC during 2-day storage at room temperature.

At room temperature, shelf-life for 'sambal belacan' added with vinegar are safely to consume within day 7 followed by SBLJ for 5 day. However, 'sambal belacan' added with sour mango already shows the deterioration in day 1, followed by 'sambal belacan' without acidic in day 2, and SBTJ in day 3. While in chiller storage, all sample safe to consume until day 10. The addition of vinegar not only increases the keeping quality of 'sambal belacan' but also give the lower rate of generation time.

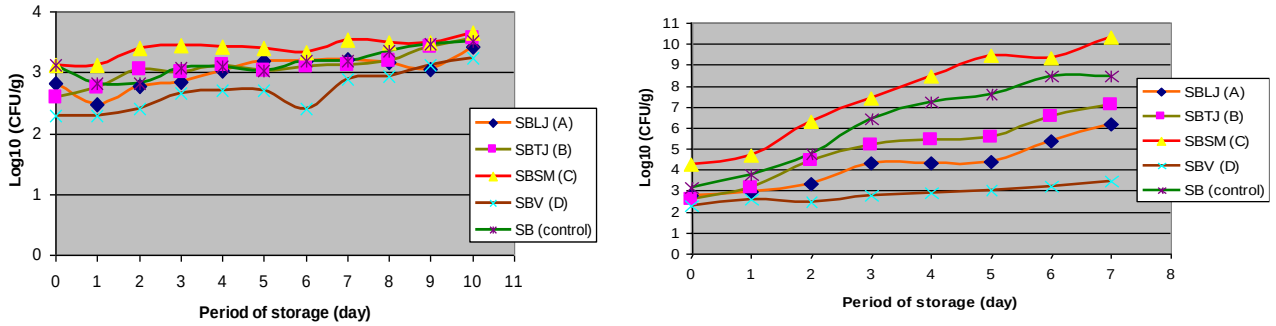


Fig. 2. Shelf life of 'Sambal belacan' at chill and room temperature based on TPC, respectively

During the storage, the TPC for SBSM increased to 7.53 $\log_{10}$ CFU/g at room temperature while the TPC was decreased if stored at chill temperature to 3.39 $\log_{10}$ CFU/g, which is higher than 'sambal belacan' control. There were no significant differences between SBLJ, SBTJ and 'sambal belacan' without acidic (control). Furthermore, SBV showed the lower growth rate of TPC that was 2.84 $\log_{10}$ CFU/g at room temperature; while 2.695 $\log_{10}$ CFU/g of TPC at chill temperature. Therefore, storage at chill temperature will help to improve the quality of food from spoilage and prolonged the shelf-life of 'sambal belacan'. However, the results showed for 'sambal belacan' stored at room temperature was exceeding the safe limit for total plate count (TPC), which was less than 10^5 CFU/g.

Furthermore, Figure 2 shows the shelf-life of 'sambal belacan' for yeasts and moulds counts at chill temperature and at room temperature. Initially, the yeasts and moulds count were 1.85 x 10^3 CFU/g for 'sambal belacan' without acidic. For the samples at chill temperature, there were no significant differences between SBLJ (3.08 $\log_{10}$ CFU/g), SBTJ (3.30 $\log_{10}$ CFU/g) and SBV (3.17 $\log_{10}$ CFU/g), but these three samples obviously were significantly different with SBSM (4.06 $\log_{10}$ CFU/g) and 'sambal belacan control' (4.16 $\log_{10}$ CFU/g) in the observation of yeast and mould count during 10-days storage.

Meanwhile, at room temperature, the results showed that the growth rate for yeasts and moulds count was higher than the samples storage at chill temperature. Similar trend to chilled storage, SBSM almost had the highest count of yeasts and moulds with 7.01 $\log_{10}$ CFU/g, while differ significantly to SBV, which had the lowest count (3.06 $\log_{10}$ CFU/g) of yeasts and moulds.

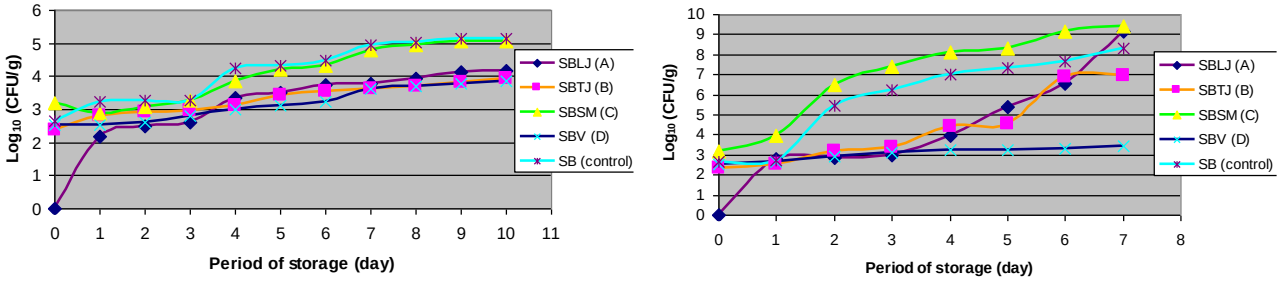


Fig. 3. Shelf life of 'Sambal belacan' at chill and room temperature based on Yeast and Mould Count, respectively

Addition of optional acidic ingredients, except for 'sour mango' were reduced the population of yeasts and moulds, either at room temperature or in chiller and improve keeping quality of 'sambal belacan'. Moreover, vinegar is a good antimicrobial agent which helps to improve the keeping quality. It also will increase the shelf-life of sample by decreasing the population and the growth rate of microorganisms. The presence of bacteria of SBV according to public health significance was below the prescribed limit. Therefore, it can be concluded that prepared 'sambal belacan' with vinegar past stored up to 7-days at room temperature and 10-days at chill temperature was microbiologically safe

for yeasts and moulds count. High count of yeasts and moulds in 'sambal belacan' as escalation day of storage may indicate by temperature abuse, inadequate processing, or by post-processing contamination. Food ingredients that were exposed to mould growth may contain hazardous mycotoxins.

3.4 pH Analysis

Until now, there are no established data related to the effectiveness of acidification in retarding the growth of pathogenic bacteria and other toxin-producing organisms towards 'sambal belacan'. The ability of microorganisms to grow or survive in acidic environments depends on the proton concentration which is determined by pH. All pH 'sambal belacan' incorporation with acidic ingredients in this research was is under pH lower than pH 5.0, except for the control sample.

Organic acids are most effective as preservation in the undissociated state. However, pathogenic bacteria can survive and even grow in such medium acids food, such happening on SBSM [32]. Acid treatment proved to be more effective in diminishing the population of *Vibrio vulnificus* in half-shelled oysters compared to ultrasound and ozone treatments [32]. Figure 4 presents the pH analysis of various 'sambal belacan' at different temperatures.

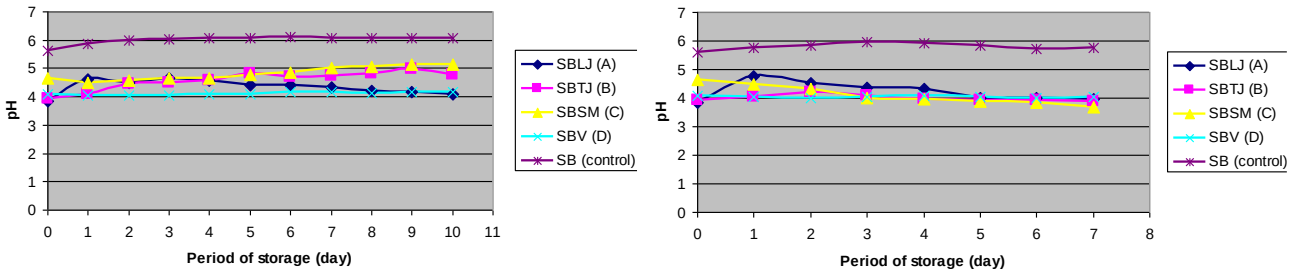


Fig. 4. pH analysis of CSP at chill and room temperature, respectively

From Figure 4, the pH at chill temperature was increased significantly but at the room temperature, the pH was increased at the beginning day but become decreased at day 7. Statistical analysis data have been approved that pH at room temperature are lower than pH at chill temperature. There are no significant differences between all 'sambal belacan' samples added with acidic at room temperature. However, there is differs significantly between all type of 'sambal belacan' at chill temperature.

Range for the pH at room temperature among 'sambal belacan' with acidic is pH 4.00 for SBV to pH 4.22 for SBLJ. At chill temperature, the pH of 'sambal belacan' show the lower result in SBV (pH 4.11) and SBSM is the have the higher increasing of pH, that is 4.82. It shows that different organisms have demonstrated different ranking for the inhibitory effects. The changes of pH level are because of the breakdown reaction of glucose and protein.

The pH for 'sambal belacan' without any optional acidic ingredient is higher than the pH for 'sambal belacan' added with lime juice, tamarind juice, sour mango and vinegar. So, it is not surprising that organic acids were used to lower the pH of primarily 'sambal belacan' (without acidic). The percentage dissociation increase the pH will become less acidic which relatively ineffective as preservatives anymore [19].

There are no differences between room temperature and chill temperature for 'sambal belacan' without acidic; either at room temperature or at chill temperature. Since the results shows the pH lower than pH 7.0, it was concluded that all 'sambal belacan' are acidic based. Furthermore, acidic added into 'sambal belacan' have a strength acidic pH proportionate to 'sambal belacan' without

acidic. SBV has the lowest pH range than the others, which mean that the sample is high of pK_a because the preservative is 50 % dissociated. Vinegar is most effective in acid foods because the percentage of undissociated acid is high [33]. This is because the organics acids was accentuated the pH inhibition the growth rates and limit the magnitude of inhibition [33].

3.5 Generation Time

Most bacteria have a generation time of 1 – 3 hours, others require more than 24 hours per generation [19]. The results of the generation time for total plate count and yeasts and moulds are shown in Table 5 at two different storage temperatures. Generation time for samples at room temperature was faster than the chill temperature. Compared to microbiological analysis, yeasts and moulds grow much fastest than the population on total plate count. Again, incorporation of vinegar has been used to prolong the shelf-life and quality of 'sambal belacan'. SBV was taken 7.29 days to double if stored at chill temperature, 1.98 days at room temperature for total plate count; while for yeasts and moulds, there took 1.96 days to double at room temperature and 2.37 days at chill temperature. Bacteriostatic effect from refrigeration temperature can prolonging the shelf-life of 'sambal belacan' if stored at chill temperature [34].

Table 5
Generation time for microbial growth, yeast and mould of 'sambal belacan'

Temperature / Sample	Generation time (day)	
	Total Plate Count	Yeast and Mould
28^0C		
SBLJ	0.650 ± 0.14^{b}	0.280 ± 0.04^{b}
SBTJ	0.470 ± 0.07^{bc}	0.435 ± 0.04^{b}
SBSM	0.325 ± 0.04^{c}	0.325 ± 0.06^{b}
SBV	1.975 ± 0.04^{a}	1.955 ± 0.18^{a}
SB (control)	0.360 ± 0.04^{c}	0.740 ± 0.57^{b}
5^0C		
SBLJ	4.425 ± 0.09^{c}	0.940 ± 0.09^{d}
SBTJ	4.050 ± 0.07^{d}	2.065 ± 0.05^{b}
SBSM	3.385 ± 0.04^{e}	1.210 ± 0.14^{c}
SBV	7.290 ± 0.04^{a}	2.370 ± 0.03^{a}
SB (control)	5.105 ± 0.02^{b}	1.140 ± 0.06^{c}

Note: Values are mean ± SD of two replicates, values followed by different letters in the same column differs significantly ($p < 0.05$)

'Sambal belacan' added with lime juice and tamarind juice has a slower rate for bacteria to double at total plate count analysis. However, tamarind juice is not the best antimicrobial; which inhibit or slower growth of yeasts and moulds at both temperatures. Sour mango as well did not show any benefit of the addition towards 'sambal belacan'. Samples stored at room temperature were doubled in count less than 1 day except for SBV. Total plate counts have slower ability to double if stored at chill temperature. However, yeasts and moulds count much faster and no significant differences between different storage temperatures respectively.

As stated earlier, time alone at room temperatures can be used to control food safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen of concern in the product. Normally, TPC contained a vary types of microbial load. So, they have more resource to compete within the other microflora in the same plate, while yeasts and moulds is the only one of microorganisms grow in the plate during experimentation. The generation time yeasts

and moulds are faster than the microbial to double in total plate count. The generation time was slower in SBV while there are faster to double in SBSM.

4. Conclusions

The microbiological stability and public health safety of medium acid foods are predominantly determined by the ability of pathogenic microorganisms and fungi to survive in the presence of organic acids within the food's aqueous phase. The study demonstrated that all acidic ingredients significantly inhibited the growth of *Lactobacillus* spp., reduced coliform counts, and Escherichia coli in 'sambal belacan'. Notably, vinegar was the most effective in reducing microbial load and extending the shelf life of the product compared to other acidic additives. The microbial load in 'sambal belacan' with vinegar (SBV) was reduced to 2.239 ± 0.34 $\log_{10}$ CFU/g from an initial count of 3.785 ± 0.04 $\log_{10}$ CFU/g in the untreated paste. Furthermore, the addition of vinegar effectively suppressed the proliferation of *Staphylococcus aureus*.

The study found that 'sambal belacan' containing vinegar exhibited a slower bacterial growth rate, taking 7.29 ± 0.04 days to double in bacterial count under chilled conditions and 1.98 ± 0.04 days at room temperature. Consequently, 'sambal belacan' with vinegar remained stable up to day 7 at room temperature and day 10 in chilled storage. Additionally, storing 'sambal belacan' at chilled temperatures significantly prolonged its shelf life compared to room temperature storage, attributed to a slower generation time of microorganisms under cooler conditions.

These findings highlight that pathogenic bacteria exhibit reduced growth in vinegar, making it a superior preservation agent among the acidic options explored in this study. The utilization of vinegar in 'sambal belacan' (pH 4.05) not only enhances shelf life but also minimizes the risk of foodborne illnesses, positioning 'sambal belacan' treated with vinegar as less likely to become vectors for health hazards. Vinegar showcased the most potent microbial inactivation effect, thereby improving the microbial quality and shelf life of 'sambal belacan', with lime juice and tamarind juice providing moderate efficacy in comparison. Future study will explore the combined effects of acidic ingredients with other preservation methods, such as vacuum packaging, high-pressure processing (HPP) or modified atmosphere packaging (MAP), on the shelf life and safety of 'sambal belacan'.

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