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# The application of hurdle technology in extending the shelf life and improving the quality of fermented freshwater fish (*Pekasam*): A Review

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**Abstract**— Hurdle technology combines several preservation methods to secure the quality of foods by eliminating or controlling the growth of pathogens, making them last longer and, most importantly, safer for consumption. The hurdle approaches used for this *Pekasam* is microbially derived hurdle and physico-chemical hurdles. Inoculation of starter cultures with amine oxidase (AO) activity like lactic acid bacteria (LAB) in *Pekasam* is proven to reduce the accumulation of harmful biogenic amines, especially histamine, for up to 59.9%. This review also involves controlling the water activity and pH of *Pekasam* to a state where it inhibits the growth of microbes. This can be done by adding natural, cheap, and easy to find ingredients like lime juice (*Citrus aurantifolia*) to the basic *Pekasam* recipe. The presence of organic acids in the lime juice act as acidulants; it provides a low pH environment for microbes to retard their growth and therefore reduce the total plate count (TPC) whilst enhancing the flavour of *Pekasam*. However, in a long-ripened *Pekasam*, only the water activity hurdle is strengthened with time. Hence, a proper amount of salt is needed to sustain and maintain the water activity level below 0.94. The use of affordable herbs and spices with antimicrobial properties such as garlic, ginger and onion can prevent the proliferation of some pathogenic microbes, commonly found in *Pekasam*; thus, this helps in increasing the stability of the product. This review aims to outline the application of hurdle technology on fermented freshwater fish quality and shelf life. It focuses on recent accessible applications when combined, providing affordable food which helps those underprivileged people, especially during flash floods and other disruptive calamities such as the COVID-19 pandemic.

**Keywords**— Hurdle technology, fermentation, biogenic amines, starter culture, water activity, pH, antimicrobial spices, shelf life, quality

## I. INTRODUCTION

Floods are considered a regular natural disaster in Malaysia and have been a major recurring issue since it happens nearly every year, especially during the northeast monsoon season

from October to March [1]. Recently, bouts of extreme rainfalls, had affected 11 states (Johor, Kelantan, Melaka, Negeri Sembilan, Pahang, Perak, Selangor, Terengganu, Sabah, Sarawak and the Federal Territory of Kuala Lumpur) in late

December 2021, and continued to the early January 2022, had caused phenomenal crisis with the country being ill-

prepared to curb the flash flood disasters from happening. According to the Department of Statistics Malaysia, overall loss was estimated to be around RM 6.1 billion, with 54 human casualties. The breakdown values of damage costs of public assets and infrastructure amounted at RM2 billion, followed by houses (RM1.6 billion), vehicles (RM1.0 billion), manufacturing industry (RM0.9 billion), business premises (RM500 million) and agricultural industry (RM90.6 million)[2]. To make matters worse, contamination, food supply disruption and water-borne

diseases are often diagnosed in affected flood victims [3], such as typhoid fever, cholera, hepatitis A, and food poisoning [4]. Symptoms of the diseases include diarrhoea, vomiting, fever, abdominal pain, and headache [4]. Food, water safety and hand hygiene are crucial to be taken care of, during and after the disaster to prevent illness from unsafe foods [5]. Therefore, most perishable and unpackaged foods that are exposed to floodwater should not be consumed and must be thrown away immediately. The Ministry of Health Malaysia (MOH), through the Food Safety and Quality Division, also emphasized that any food preparation premises that are affected by floods are not allowed to operate in accordance with the requirements under the Food Act 1983 and Food Hygiene Regulations 2009 [6]. Regarding the above matter, this natural disaster inadvertently has a huge impact on Malaysians, especially for those that are financially categorized under B40; (bottom 40% of the Malaysian household income are those who earn less than RM4,850 per month and are known as the lower-income group) [7]. The effects of the pandemic have placed many among the vulnerable and the poor, under extreme hardship, especially of those who had lost their incomes during lockdowns, with B40 being the hardest hit, from the ongoing aftermath of COVID pandemic and large scales of floods in several states [8].

A review on the application of hurdle technology in extending the shelf-life and improving the quality of fermented food like Pekasam has been conducted as a potential solution, being readily available as safe, affordable and nutritious food for the poor. The difficulties in obtaining food during natural disasters due to the damage caused from floods, that disrupted the conventional cold food supply chain, require food that has a long shelf life with minimal storage equipment and not dependent on chilled and frozen storage facilities. Pekasam is proven as one of the foods that have these characteristics which can be stored at ambient temperature.

Fermented foods like Pekasam are among the safest yet cheapest foods that are accessible, that can help overcome food scarcity and poverty among flood victims; due to its ability to degrade some microbes and eliminate certain harmful chemical substances, despite its minimal processing cost [9]. For example, a 250g of Pekasam, which is equivalent to 2-4 small tilapia fish (depending on the packaged size), can be purchased for as low as RM5.00 at any local Malaysian markets and even through online purchase (e.g., Shopee and Lazada). Utilizing traditional food, familiar to the locals, such as Pekasam, with hurdle technology, for safer food with longer shelf-life can help reduce the risk of food poisoning and food intoxications, especially during occurrence of natural

disasters that disrupt the food supply chain [10]. The application of several "soft hurdles" may reduce the rate of fish deterioration and spoilage, caused by microbial growth and thus mitigate food poisoning and food intoxication.

Hurdle technology can be defined as an amalgamation of more than one preservation technique as a preservation strategy. It is a practical preventive process and approach of food products, which helps in minimizing the risk of food spoilage, food contamination and foodborne illnesses [11]. Hurdle technology works are based on four basic principles, which are homeostasis, metabolic exhaustion, stress reactions and multi-target preservation [12]. Synergistic results can be achieved when hurdles are used in combination of two or more technologies. The same hurdles may have a positive and even negative effect on food depending on its intensity [11].

Homeostasis is an organism's ability to stabilize itself, while metabolic exhaustion is where the microorganisms in foods have completely used up their energy to maintain homeostasis and eventually die [12].

On the other hand, a stress reaction is a condition where some bacteria become more resistant under stress and will release stress-shock proteins to survive [12]. Multi-target preservation can then be defined as the application of multiple hurdles that work synergistically [13]. For food safety, the scope of hurdle technology is more focused on its physico-chemical aspects like temperature, water activity, acidity (pH), redox potential (Eh), chemical preservatives like nitrite, sorbate, sulphite and many more [14].

Hurdle technology is necessary for food safety since consumers are more likely to eat out and favour ready-to-eat foods rather than preparing food from scratch. Moreover, there is an increasing demand for minimally processed food as people are more concerned about their health and diet; fresh and natural foods are preferable due to the escalating concerns of unsafe food among consumers, especially during flash floods and COVID-19 pandemics. Hence, hurdle technology is an emergence of new route of popularising traditional Asian cuisine, at the same time, controlling the growth of desirable microorganisms and retarding spoilage and pathogenic microbes, especially in food products [15].

## II. LITERATURE REVIEW

In this section, an Asian dish, *Pekasam* or fermented freshwater fish, will be discussed, followed by the types of hurdle technology that can be applied to this food product. *Pekasam* is a Malay term for fermented fish (white meat). It usually refers to freshwater fish fermented with salt, table sugar, toasted rice grains and tamarind juice. *Lampam Jawa* (*Puntius gonionotus*) and black tilapia (*Oreochromis mossambicus*) are two of the most common freshwater fishes used for the making of *Pekasam*. [16]. It is named *Pekasam* due to its unique sour flavour adapting from the word '*asam*' in the Malay language, which means sour. This fermented fish is likely to contain lactic acid bacteria, which is good for the body and gives a unique flavour to the *Pekasam* while acting as a natural aid in the preservation of this fermented food [17, 18].

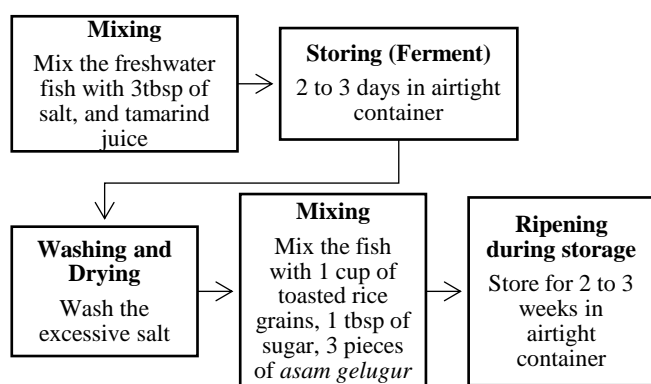
Traditionally fermented fish is very popular at the northern end of the Malaysian peninsula in states such as Perlis, Kedah, Perak, and the Bornean state of Sarawak. It is also widely available in West Kalimantan and Sumatra, Indonesia. There

are many different styles in preparing *Pekasam* due to the influence of different cultures in Malaysia.

A *Pekasam* recipe for 4 - 5 small freshwater fish (**Figure 1**), requires 25% (w/w) or one cup of ground toasted rice, 15% (w/w) or three tablespoons of salt, about one spoonful or 1.5% (w/w) brown sugar, a little bit of tamarind juice (about 1.5% (w/w)) and three pieces or 0.5% (w/w) *asam gelugur* (*Garcinia atroviridis*) [19].

To prepare, the fish is rubbed generously with salt and tamarind juice and stored in a very air-tight container for 2 to 3 days to ferment. After the first fermentation stage, the excessive salt from the fermented fish was washed and dried before mixing it with toasted rice powder, sugar and *asam gelugur* [19]. Next, the fish is kept in a sealed container for another two to three weeks for further fermentation. After this stage, the fermented fish can either be refrigerated or cooked right away and be served as *Pekasam* [19].

**Figure 1 Basic *Pekasam* preparation.**



The fermentation processes (Figure 1) are highly crucial in *Pekasam* as they promote the growth of lactic acid bacteria (LAB) and allow the flavour to be fully developed [17, 18]. The presence of LAB, together with a proper storage condition, will help fermented food products last up to several weeks [20-22]. A total of 65 out of 169 LAB were screened and isolated from 23 local fermented fish products, are found in *Pekasam* [23]. This statement proves that *Pekasam* contains acid and proteolytic enzyme producer strains compared to other fermented local fish products, including cencaluk, budu, belacan, salted fish, and anchovies paste (pati ikan bilis) [23].

A significant incidence of strains (*Leuconostoc* sp. and *Lactobacillus* sp.) that exhibited a high osmotolerance and survived up to 15% NaCl are discovered in *Pekasam* [23]. Similarly, high incidences of acid tolerance strains are also observed in samples of *Pekasam*, which mostly belonged to the *Lactobacillus* sp. and *Pediococcus* sp. [23]. The presence of these lactic acid bacteria (LAB) in *Pekasam* give a broad antimicrobial effect towards the pathogenic bacteria, especially *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* spp. [24]. These LAB are also  $\gamma$ -hemolytic and tolerant to various pH (pH 3, 5 and 7.5) and 0.3% (w/v) bile salts which are considered as a bonus [24]. Despite the presence of LAB, a proper processing procedure throughout the preparation of *Pekasam* is still needed in determining the stability and shelf-life of the product. It is because fermented fish products that are produced without proper handling techniques are likely to be

contaminated with *Listeria monocytogenes* and thus become a threat to food safety [25].

**Table 1** shows the types of hurdles technology and examples that can be applied to meat or protein-based products. Most of them cannot be used as the only hurdle but must be combined with other hurdles. Combining food preservation hurdles like physical, physico-chemical and microbially derived hurdles is a synergistic approach towards food preservation and is referred to as hurdle technology [26]. To prolong the shelf life of this traditional food, hurdle technology can be applied along with modifications done during the preparation process. A strategic combination of hurdles leads to metabolic exhaustion and disruption of cellular homeostasis, thereby eliminating or controlling the microbial populations in a product [27].

Table 1. Types of hurdles technology that can be applied to protein-based products and its examples.

| Types of hurdles    | Examples   |
|---------------------|--|
| Physical            | <ol style="list-style-type: none"> <li>1. Heat Processing - Sterilization [27, 28], Pasteurization [28].</li> <li>2. Storage Temperature – Chilling [27], Freezing [29, 30].</li> <li>3. Radiation – Ultraviolet [31, 32], Irradiation [31].</li> <li>4. Microwave [33], Radio frequency [33, 34], High electric field [33].</li> <li>5. Ultra-High Pressure (UHP) [27].</li> <li>6. Packaging – Vacuum [27], active packaging [34], edible coatings [35].</li> <li>7. Modified Atmosphere Packaging (MAP) [27].</li> <li>8. Microstructure [27].</li> </ol>   |
| Physico-chemical    | <ol style="list-style-type: none"> <li>1. Water Activity [27, 36].</li> <li>2. pH [27].</li> <li>3. Redox Potential (Eh) [37].</li> <li>4. Salt (NaCl) [27].</li> <li>5. Nitrite (NaNO<sub>2</sub>) [27, 37, 38].</li> <li>6. Nitrate (NaNO<sub>3</sub> or KNO<sub>3</sub>) [38, 39].</li> <li>7. Carbon dioxide (CO<sub>2</sub>) [27, 34].</li> <li>8. Oxygen (O<sub>2</sub>) [27].</li> <li>9. Ozone [27].</li> <li>10. Organic acids - Lactic acid [27], Acetic acid [27].</li> <li>11. Ascorbic acid [27, 37].</li> <li>12. Smoking [27].</li> <li>13. Phosphates [40].</li> <li>14. Glucono-<math>\delta</math>-lactone (GDL) [27].</li> <li>15. Phenol [41].</li> <li>16. Chelators [42].</li> <li>17. Spices and herbs [27].</li> <li>18. Lysozyme [27].</li> </ol> |
| Microbially derived | <ol style="list-style-type: none"> <li>1. Starter Cultures [27].</li> <li>2. Bacteriocins [27].</li> <li>3. Antibiotics [27].</li> </ol>   |

By implementing multiple hurdles at various processing steps, microbial stability and safety are achieved, and the meat or flesh quality in terms of sensory, nutritive, and economic properties is effectively preserved [26, 27].

Out of all hurdles listed in **Table 1**, the common hurdles used to preserve protein-based products are pasteurization, chilling, freezing, irradiation, vacuum, and modified atmosphere packaging (MAP), microstructure, salt to control water activity, pH, nitrite, lactic acid, smoking and the addition

of Glucono- $\delta$ -lactone [27]. Basically, the types of hurdles used depend on the type of desired final product. Hence, the selection of appropriate hurdles is crucial towards maximizing the stability and shelf-life of specific food products [10].

A. *The role of starter cultures (microbially derived hurdles) in reducing biogenic amines in Pekasam to improve its quality.*

Biogenic amines or BAs are nitrogenous, non-volatile, and low molecular weight organic bases produced by bacterial decarboxylation of amino acids that are highly correlated with toxicological symptoms in food products [43, 44]. In short, biogenic amines (BAs) are trace compounds found in foods that are likely to cause harm to consumers' health. The toxicological level of BAs depends on the individual characteristics and the presence of other amines.

Studies by [45] and [46] show that a high number of amines is one of the crucial agents in seafood intoxication, such as histamine poisoning and tyramine toxicity which may lead to diarrhoea, vomiting, red rashes and others. Once these nitrogenous bases are formed, biogenic amines like histamine are difficult to be destroyed even by adjusting their storage temperature, including freezing, cooking, retorting, or smoking [47]. Thus, it is important to ensure the total vasoactive BAs (VBAs) in fermented meat products like *Pekasam* are lower than 200 mg/kg to meet the quality index [48]. On the bright side, these VBAs can act as a chemical indicator or a marker for the hygienic quality and freshness of foods like meats. Therefore, the degree of food fermentation or degradation can be identified easily.

Amines can be separated from food samples by high-performance liquid chromatography (HPLC) using gradient elution (acetonitrile and ammonium acetate). They can be detected with spectrophotometric UV-vis detection at 254 nm [43]. The amines are then extracted in an aqueous acid solution (perchloric acid) and must be derivatized by dansyl chloride [47]. The higher the concentration of biogenic amines found in meat or meat products, the lower the quality of meat will be, making them harmful for consumption. Given that the presence of biogenic amines can cause distinctive pharmacological, physiological, and toxic effects in organisms, especially humans [49], it is crucial to investigate the percentage of BAs in foods, especially in meat products which will be further discussed below.

Research from [50] showed that meats have a huge tendency in carrying biogenic amines along with them, and the most prevalent biogenic amines in meats are tyramine, cadaverine, putrescine, and histamine [51]. This statement is supported by [52], where fish meats like *Pekasam* from black tilapia may contain 18.8-71.3 mg/kg of histamine while Javanese carp *Pekasam* can carry up to 12.7-109.1 mg/kg of histamine. However, the total BAs level may still increase over time.

As a matter of fact, the increasing concentration of some amines in meats may also be due to degrading processes of the meat itself, which are promoted by enzymatic reactions caused by external microbial activity or endogenous tissue activities of the fish [47]. The process of inducing fermentation in foods like *Pekasam* may unintentionally lead to the production of

amines as well [50]. This is also supported by [53], where high concentrations of BAs can be found in fermented foods due to contamination of microflora with amino acid decarboxylase activity.

Inoculation of starter cultures (microbially derived hurdles) with amine oxidase (AO) activity in fermented meat products like *Pekasam* is crucial in preventing the formation of high levels of biogenic amines [54]. For example, studies from [55] and [56] have successfully proven the potential of starter cultures in reducing total BAs accumulation in *som-fug* (a Thai traditional fermented fish sausage) from  $\pm 100$  mg/kg to  $\pm 50$  mg/kg after 120 hours of fermentation. This experiment showed that the accumulation of BAs like cadaverine, putrescine, histamine, tryptamine, phenylethylamine, and tyramine in *som-fug* could be reduced significantly by the incubation with *Lactobacillus sakei* and *Lactobacillus plantarum*. Moreover, inoculation of starter cultures to the raw freshwater fish allows the fermentation process to be better controlled so that the quality characteristics can be standardized [57].

The presence of amine oxidase (AO) in starter cultures is very important as it can catalyze the degradation of BAs through oxidative deamination with the production of aldehyde, ammonia, and hydrogen peroxide. Therefore, the potential role of microorganisms with AO activity has become a particular interest to prevent or reduce BAs accumulation in fermented foods [55]. Examples of starter cultures with AO activity that successfully treat biogenic amines in fermented foods are lactic acid bacteria like *Lactobacillus plantarum*, *Micrococcus varians* and *Staphylococcus carnosus* [58, 59].

Starter cultures that are unable to produce biogenic amines in foods and are important to grow well at the temperature intended for the processing of product are the best to be inoculated into *Pekasam* [60]. In addition, the competitiveness in suppressing the wild amine-producing microflora growth should be considered in choosing the starter cultures [60, 61].

In addition, amine-negative producer bacteria are highly recommended to be used as starter cultures [62]. It is because amine-negative starter cultures can reduce the pH of fermented meat products [62, 63]. At a low pH, the starter cultures will actively compete with the non-starter bacteria during the later phase of ripening and throughout the storage period of meats [63]. This statement is supported by reference [64]. It stated that inoculation of amine-negative strains (*Virgibacillus spp.*) from grasshopper sub shrimp paste could reduce the accumulation of biogenic amines in food samples. Thus, it may avoid excessive biogenic amines production.

Numerous research projects were conducted on the capabilities of different starter cultures in reducing biogenic amines. *Bacillus amyloliquefaciens* and *Staphylococcus carnosus* with BAs degradation activity of up to 59.9% and 29.1% respectively can reduce BAs like histamine concentration by 27.7% and 15.4%, respectively [65] and [66]. A culture such as *S. xylosus* was found to be capable of degrading total biogenic amines concentration in fermented anchovies by 16.0% [67]. Furthermore, it is reported that researchers found four isolates of *L. sakei* and one isolate of *L. curvatus*, can degrade BAs like histamine for up to 20–56%; within the period of 30 hours in food products [68].

Unfortunately, studies by [69] and [70] stated that the use of starter cultures is not sufficient in preventing the presence of

biogenic amines in some fermented meat products. This may be due to the action of some starter cultures that may be less effective as they are highly dependent on the hygienic quality of the raw material used. Hence, the use of good quality raw materials, appropriate processing techniques and a great storage condition is a must to prolong the shelf life and improve the quality of meats. Researches from [71-74] also stated that the significant differences in biogenic amines content in fermented meat products are highly dependent on the hygienic quality of its raw materials. Other alternatives that can be used to reduce the biogenic amines in food products are through irradiation, high-pressure processing, and modified atmosphere packaging. However, these techniques are quite pricey and time-consuming [75].

From here, we can conclude that the use of starter cultures (microbially derived hurdles) is a biological and natural way to reduce the accumulation of amines with the ability to extend the shelf-life of fermented meat products, especially *Pekasam*. However, it is fundamental to be supported with good quality raw materials, appropriate processing techniques and a very conducive storage condition.

#### B. Controlling the water activity ( $a_w$ ) and pH of *Pekasam* to prolong its shelf life.

The concept of water activity and pH will be discussed, followed by ways to control them to inhibit microbial growth in *Pekasam*. Water activity ( $a_w$ ) can be defined as the measure of the energy status of water in a system [76]. Classically, it is defined as the amount of water available for microbial growth like bacteria, yeast, and mould [76]. Water activity is the ratio of the vapour pressure of water in a food item ( $p$ ) to the vapour pressure of pure water ( $p_0$ ) at the same temperature; ( $a_w = p/p_0$ ) [76, 77]. It is important to note that water activity and moisture content are two different things. Even though moist foods are likely to have a greater water activity than dry foods, this statement does not apply to all foods. It is because some foods may have the same moisture content and yet have different water activities [78].

Water activity can be measured based on the scale of 0 to 1.0, with pure water having a water activity of 1.00. Thus, the water activity of 0.80 means the vapour pressure is 80 percent of that of pure water [76]. Microbes and moulds vary in their water activity requirements, and this may range from 0.7 to >0.9 [76]. However, unlike moulds, bacteria favour foods with a water activity of 0.95 and above to proliferate as it needs sufficient moisture to support their growth [76]. This statement is proven by [79], where it stated that the rate of growth and the yield of microbes both reduced substantially when the water activity is less than 0.94. From here, it can be concluded that the lower the water activity of food, the less chance for bacteria to grow and the longer the shelf life will be and vice versa.

In contrast, pH is a measure of the degree of acidity or alkalinity of a solution. When the pH shows a value between 0 and 7, this indicates the food is acidic, while values between 7 and 14 indicate the food is in an alkaline state [80]. Just as with water activity, microorganisms have their preferred pH condition at which they can grow and proliferate well. For example, most food spoilage and pathogenic microbes like *E. coli* and *Salmonella spp.* Favour a neutral pH for optimum growth. In contrast, acidophiles and alkaliphiles prefer pH of

less than 5.55 and pH range between 8.0 and 10.5, respectively, to survive [80]. However, these two types of microbes are rarely found in foods [81]. Due to these microbial growth limits, reference [82] concluded that lowering the pH of foods up to 4.2 or 4.6 is found as an effective way to prevent the growth of vegetative pathogens and spore-forming pathogens, respectively and thus help in preserving the foods.

It is important to note that the pH works synergistically with the water activity of a food. This synergistic effect is described in detail by hurdle technology for microbial control. Furthermore, it is an intricate part of the Food and Drug Administration (FDA)'s definition of potentially hazardous foods [83]. Therefore, water activity and pH are important intrinsic factors that work better together, especially in determining the growth of spoilage microorganisms in food. In addition, both factors can be measured easily just by using the commercial water activity meters and pH meters. Thus, controlling the growth of microorganisms in *Pekasam* are predicted to be more effective when both hurdles are combined, and this will be discussed elaborately after this.

Perishable foods like fish are normally preserved by direct drying under the sun, and it has been practiced since ancient times to reduce its water activity [84]. However, this method is not suitable to be applied on certain fish products (e.g., *Pekasam*) as it may affect the quality [10], texture and even slightly change the concept of *Pekasam* itself. Understanding the concept of moisture sorption isotherm of *Pekasam* is also a must before consideration of heating it [85].

One of the best, easiest, and cheapest ways to control the water activity and the pH of *Pekasam* is by adding an appropriate concentration of lime juice (*Citrus aurantifolia*) to the basic *Pekasam* recipe [86]. The presence of organic acids in the lime juice will help in increasing the acidity level of *Pekasam* and thus reduce its pH [82, 87]. This statement is proven by references [87] and [88], stating that lime juice contains 29% ascorbic acid, 4.12% citric acid, and 4.19% total organic acids. These organic acids, especially citric acids, are widely used as acidulants in food preservation. They can inhibit microorganisms via depression of media pH despite penetrating the bacteria cell wall to disrupt the enzymes, transport permeases, and the bacteria's pumps even at substantially lower concentrations [87]. pH-sensitive bacteria like *Escherichia coli*, *Salmonella spp.*, *C. perfringens*, *Listeria monocytogenes*, and *Campylobacter spp.* are most likely to be affected by pH changes since they are unable to tolerate a wide internal and external pH gradient [87]. On the other hand, the presence of ascorbic acids in the lime juice can act as antioxidant sources for the *Pekasam*, where it preferentially combines with oxygen compounds, thus preventing deterioration of the food by oxygen-free radicals or other oxygenated reactive species [88].

Anaerobes and Gram-positive bacteria like *Staphylococcus spp.*, *Clostridium spp.*, and *Enterococcus faecalis* are susceptible to lime juice that was extracted from 20 lime fruits with minimum inhibitory concentration (MIC) ranging from 64mg/ml–256mg/ml. Besides that, Gram-negative bacteria like *Escherichia coli*, *Salmonella paratyphi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have MIC readings ranging from 128mg/ml–256mg/ml [86]. As for fungi, *Candida albicans* are also susceptible to lime juice with 256mg/ml of MIC [86]. Based on these MIC readings, it is

confirmed that lime juice is capable of controlling the growth of microbes in *Pekasam*.

The addition of 6% (w/w) lime juice and 15% (w/w) palm sugar to a 13.48g eviscerated freshwater fish called *Wadi Betok* are found to be effective in reducing the moisture content and total plate count (TPC) from  $1.58 \times 10^6$  cfu/g to  $0.39 \times 10^6$  cfu/g [89]. Moreover, this combination also increased the good lactic acid bacteria (LAB) count from  $2.90 \times 10^6$  cfu/g to  $2.93 \times 10^6$  cfu/g but inadvertently increased the pH value from pH 5.95 to 5.98 of the fermented *Wadi Betok* as well [89]. This slight increment in pH value is probably due to the inadequate volume of organic acids present in the lime juice, and only part of it was ionized [87]. However, these experimental results are quite invalid to be solely compared to *Pekasam* since the fermentation of *Wadi Betok* does not require the addition of tamarind juice, *asam gelugur* and toasted rice powder as stated in [19]. Furthermore, the fermentation period differs since *Wadi Betok* only requires seven days to prepare, but *Pekasam* needs more than a week to fully ferment [19][89]. Thus, further work must be done to determine which conditions and parameters of lime juice can deliver the optimum reduction in microbial load.

Since pH and water activity work synergistically, the used-up oxygen from microbial respiration will cause the redox potential of the *Pekasam* to decrease [27]. This, in turn, enhances the oxidation-reduction potential (Eh) hurdle, which inhibits aerobic organisms and favours the selection of lactic acid bacteria (LAB) in *Pekasam* [27]. Thus, the presence of these LAB and organic acids from the lime juice will increase the acidity level of *Pekasam* and reduce the pH value. However, in long-ripened *Pekasam*, the nitrite hurdle from the salt will be depleted, and the count of lactic acid bacteria decreases. In contrast, the Eh and pH hurdle will increase again, i.e., all these hurdles will become weak during a longer ripening of *Pekasam* [27]. Thus, only the water activity hurdle is strengthened with time, and it is mainly responsible for the stability of long-ripened freshwater fish.

It is important to ensure that the amount of salt used for the making of *Pekasam* is enough to control the water activity. Reference [27] suggests that a stable fish product must contain at least 4.5g of salt per 100g water. An appropriate concentration of salt will cause the microbial cells to undergo osmotic shock and limit their oxygen solubility, which will then disrupt their cellular enzymes, forcing the cells to expend their energy to exclude sodium ions from the cell, resulting in the loss of water and thereby causing cell death or retarded growth [90][91].

On top of that, the sequence and timing of the lime juice are crucial to be determined before adding it to the *Pekasam* [27, 91]. It is because acid stress may place a large energy demand on bacteria cells, especially *E. coli*. Furthermore, it is theorized that this energy demand can greatly sensitize the cell to successive treatments, especially water activity stress [91]. Thus, research and experiments are needed to determine the best way to add the lime juice for maximum stability of *Pekasam*.

We can conclude that both water activity and pH are considered physico-chemical hurdles that work better together. Therefore, lowering the water activity (below 0.94) and the pH (below optimum level) are some of the great ways to prolong the shelf-life of *Pekasam*. This can be done by adding a proper

concentration of affordable freshly squeezed lime juice into the *Pekasam* recipe.

### C. Antimicrobial function of spices in improving the quality of *Pekasam*.

This section will discuss the role of herbs and spices and their antimicrobial properties in traditional food preservation. Reference [92] shows that fermented meat products have a huge tendency to be contaminated with pathogenic bacteria like *Clostridium botulinum*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Escherichia coli*. In addition, reference [93] stated that *Listeria* spp. was found in 14 (56%) of 25 fermented fish samples purchased from the Malaysian wet market, and 12% were contaminated with *Listeria monocytogenes*. This type of foodborne pathogens can cause fatal illness with clinical manifestations like sepsis or meningitis in immunocompromised patients or neonatal babies and flu-like illness or abortion during pregnancy in women. To overcome this problem, antimicrobial herbs and spices are essential to serve as additional preservatives to this fermented food product.

Herbs and spices from vegetables, roots and stems can be classified as physico-chemical hurdles used in food preservation. The term spices refer to the parts of a plant other than the leaves and flowers, such as the roots, twigs, seeds, fruits, and barks and most of them are usually dried [94, 95]. On top of that, reference [96] defined spices as aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition. They are true to name, and from them, no portion of any volatile oil or other flavouring principle has been removed [96]. Despite providing culinary characteristics, desirable flavour, texture and aroma, these condiments act as palatability amplifiers for food. Spices can also inhibit microbes due to their antibacterial and antioxidant properties [97-103]. Some of the antimicrobial compounds that are present in spices and herbs are eugenol, thymol, thymol and carvacrol, vanillin, allicin, cinnamic aldehyde, and allyl isothiocyanate that are, respectively, present in cloves, thyme, oregano, vanilla, garlic, cinnamon, and mustard [104, 105].

Spices are chosen over other chemical preservatives like benzoic and sorbic acids because they are natural, residue-free and generally recognized as safe (GRAS) and innately better tolerated in the human body and have inherent superiorities for food industries [106, 107]. It is because spices like turmeric, ginger, black paper, curcumin, and many more are proclaimed to have antioxidant, anti-inflammatory, and wound-healing properties [108]. Therefore, spices could be one of the best and cheap candidates to discover and develop new antimicrobial agents against foodborne and human pathogens.

In terms of *Pekasam*, instead of serving them plain, as fermented freshwater fish, it is better to put a little twist to the basic recipe by incorporating garlic, ginger and onion for better antimicrobial effects and providing better taste and flavour. Garlic (*Allium sativum*) belongs to the Liliaceae family [109], and the active component was identified as allicin, a diallyl thiosulfinate (2-propenyl-2-propenethiol sulfonate) [110]. Garlic is one of the most powerful vegetable herbs as it inhibits almost all types of bacteria like *Bacillus*, *Campylobacter*,

Clostridium, Enterobacter, Enterococcus, Escherichia, Klebsiella, Lactobacillus, Proteus, Pseudomonas, Salmonella, Serratia, Staphylococcus, Streptococcus, Vibrio, Yersinia and many more [111]. Reference [112] stated that the viable cell concentration of *L. monocytogenes* declined by a factor of 10 (from  $10^6$  to 1cfu/ml) in 7 hours at 37°C when 1% (w/v) clove was added to a broth model system. However, 1% (w/v) clove is not enough to inhibit *L. monocytogenes* in food products that are high in protein, and fat but low in water content like soft cheese, meat slurry and cooked beef [97, 98, 112, 113, 114]. A fat coat could form on the surface of bacteria cells, creating a barrier to prevent the inhibitory substances of spices from penetrating and disrupting the cells [112]. Hence, future researches should test out the appropriate concentrations of garlic and study the inhibitory effects over a longer storage time in Pekasam to access the potential spices as a preservative.

In contrast, ginger (*Zingiber officinale*), which belongs to the Zingiberaceae family [115], is widely used as an ingredient in food, pharmaceutical, cosmetic, and other industries due to its antimicrobial properties. Some volatile compounds which are responsible for antimicrobial activities in ginger were  $\alpha$ -pinene, borneol, camphene, and linalool [116]. The presence of ginger in Pekasam may help inhibit the growth of bacteria like *Bacillus*, *Flavobacterium*, *Leuconostoc*, *Proteus*, *Salmonella*, *Staphylococcus* and *Streptococcus* [117]. Reference [118] proved that 2% (w/w) ginger could reduce the total viable count (TVC) of tomato paste from 13.45 log cfu/g to 11.54 log cfu/g at the 8th week of storage. Reference [119] has also confirmed that ginger's 0.0625-3.0% (w/w) delivers a great antibacterial action against common food contaminating bacteria. A combination of 4% (w/w) ginger and 2% (w/w) garlic powder are proven to give a greater microbial inhibitory effect since the total viable count (TVC) of the studied tomato paste dropped from 13.45 log cfu/g to 4.69 log cfu/g over the period of storage [118].

Several studies have proven that onion (*Allium cepa*) possess great antibacterial and antifungal properties, which can be used to treat human pathogenic organisms. In addition, onion contains many secondary metabolites like flavonoids, polyphenols, organic sulphur, and saponins that are effective in controlling the growth of bacteria [120]. Furthermore, the presence of high sulphuric compounds (about 60%) in onions also helps inhibit the growth of bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Staphylococcus aureus* [121]. This statement is supported by reference [120], where it stated that onion juice at 100%, 50%, 20% and 10% of concentrations have very strong effects on the growth of *Staphylococcus aureus*, which results in total plate count (TPC) value of only  $2.9 \times 10^6$  cfu/g to no growth at all on the Methicillin-resistance *Staphylococcus aureus* (MRSA) test over the period of storage.

Other spices like clove, cinnamon, cumin and herbs such as oregano, thyme can exhibit significant antimicrobial activities against food spoilage bacteria like *B. subtilis* and *P. fluorescens*; pathogens like *S. aureus*, *V. parahaemolyticus*, and *S. typhimurium*; harmful fungi like *A. flavus* and *A. niger*, and even antibiotic-resistant microorganisms [121]. Therefore, these spices and herbs could be used to decrease the possibility of food spoilage and even food poisoning. However, sensory evaluations are needed to be done to investigate the acceptance among consumers.

Before incorporating plant-based condiments into any food, especially fermented foods like Pekasam, it is important to note that most spices and herbs are thermostable. Still, some of them are not [122]. Thus, to preserve the quality of spices and herbs and prevent them from being destroyed, it is important to know the suitable timing for the ingredients to be added while preparing the Pekasam. Furthermore, good quality spices and herbs will give a significant antimicrobial effect, at the same time, providing nice flavours to the food. Therefore, the loss of flavour would be a useful physico-chemical indicator for losing antimicrobial potency [122-123]. To extend the shelf life and improve the quality of fermented freshwater fish like Pekasam, a combination of microbially derived hurdle and physico-chemical hurdles is necessary to prevent it from spoilage.

This can be done by reducing the accumulation of biogenic amines like histamine in Pekasam by inoculating starter cultures high in amine oxidase activity (AO). The physiological responses of microorganisms during food preservation are the basis for the application of advanced hurdle technology. The disturbance of the homeostasis of microorganisms is the key phenomenon of food preservation. Synergistic effects of different hurdles can lead to the enhancement of food safety and even prolong the shelf-life of food products. The presence of desirable Lactic acid bacteria can function as probiotics, and be added during ripening of the products [124].

In addition, controlling both water activity, salt and the pH of Pekasam is a must to inhibit the growth of both Gram-positive and Gram-negative bacteria and even some food spoilage fungi. This is possible by adding cheap and easy to find local ingredients with high percentage of organic acids like lime (*Citrus aurantifolia*) juice during the fermentation of Pekasam. Furthermore, incorporating affordable and accessible spices with antimicrobial properties like garlic, ginger, and onion powder into the recipe of Pekasam will also help enhance the food quality with respect to the longer shelf life of this fermented freshwater fish. Besides that, spices are natural, accessible, affordable, and most importantly, highly useful for underprivileged people like the B40 groups to reduce the case of food poisoning among themselves, especially during flash floods.

Physical hurdles may be applicable in high-income countries, using state of the art technologies. They may charge higher costs to the end consumers, which not many poor consumers cannot afford. However, traditional fermentation recipes like Pekasam provide fast, cheap, convenient, and low-cost technology, easily adopted among poor people. The government of Malaysia can help eradicate poor nutrition through improved dietary practices. This can be done by campaigning on easy step by step cooking class videos with instructions, available in the major languages of poor people of Malaysia for impactful change, at the same time, reducing dependence on empty calories or low-quality high fat processed foods which are cheaper, but not nutritiously beneficial, or shelf-stable/prolonged shelf life..

#### IV. CONCLUSIONS

*Pekasam* with a longer shelf-life can be one of the safest yet cheapest foods to consume, especially during this monsoon or flash floods season regardless of COVID-19 pandemic. Future researchers are suggested, to test out how

long *Pekasam* would last after applying all the hurdles discussed. In addition, future works on sensory testing are also recommended on innovating *Pekasam* to investigate its acceptance among consumers. We can conclude that by incorporating spices with antimicrobial properties like garlic, ginger, and onion into the *Pekasam* recipe, the stability of *Pekasam* can be improved, thus prolonging its shelf-life.

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