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Production, Organoleptic, and Biological Activities of *Belacan* (Shrimp Paste) and *Pekasam* (Fermented Freshwater Fish), the Ethnic Food from the Malay Archipelago

(Pengeluaran, Organoleptik dan Aktiviti Biologi Belacan (Pes Udang) dan Pekasam (Ikan Air Tawar Yang Difermentasi), Makanan Etnik dari Kepulauan Melayu)

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ABSTRACT

Various popular fermented foods are produced commercially or at the household level. Chemical, physical, and microbiological changes may occur, either desirable or undesirable, in the food matrix during fermentation. Due to the scarcity of information in the literature regarding *belacan* (fermented shrimp) and *pekasam* (fermented freshwater fish) originating from the Malay Archipelago, this review will focus on the physiochemical changes, nutritional, microbiological, sensory, and biological activities of these food ingredients. *Belacan* is primarily composed of fermented blocks of shrimp with salt and usually is added as condiments in a small amount in cooking. In contrast, *Pekasam* consisted of freshwater fish in its original form, often fermented with ground toasted rice and natural acidulants, and can be used as the main dish. Both *belacan* and *pekasam* contain appreciable quantities of umami amino acids and 5′-ribonucleotides such as glutamate, inosinate, and guanylate from the protein degradation by both endogenous proteases and microbial growth. Unfortunately, the breakdown of proteins can contribute to the rise of biogenic amines, which can cause adverse effects on sensitive individuals. The growth of lactic acid bacteria is common, which is often considered safe, but spoilage microorganisms can easily contaminate the product in an unfavourable environment. Therefore, with rich nutrients and biological activities, the appropriate consumption of these food ingredients may contribute to better consumer health while increasing the acceptability of Malay traditional cuisines.

Keywords: *Belacan*; fermented freshwater fish; fermented seafoods; Malaysian ethnic food; *Pekasam*

ABSTRAK

Pelbagai makanan fermentasi yang terkenal dihasilkan secara komersial atau kecil-kecilan. Perubahan secara kimia, fizikal dan mikrobiologi boleh berlaku sama ada diingini atau tidak di dalam matriks makanan ketika proses penapaian. Oleh kerana kekurangan maklumat di dalam kajian berkaitan belacan (udang yang difermentasi) dan pekasam (ikan air tawar yang difermentasi) yang berasal dari Kepulauan Melayu, kertas ini akan memfokuskan kepada nutrisi, mikrobiologi, sensori dan aktiviti biologi ramuan makanan ini. Belacan lazimnya terdiri daripada blok udang yang difermentasi dengan garam dan selalunya ditambah dalam kuantiti yang sedikit sebagai bahan perasa di dalam masakan. Berbeza dengan pekasam yang mengandungi ikan air tawar di dalam bentuk asal, ia lazimnya difermentasi dengan beras bakar yang dikisar dan asidulan semula jadi dan boleh digunakan sebagai hidangan utama. Kedua-dua belacan dan pekasam mengandungi kuantiti amino asid dan 5’-ribonukleotida yang tinggi seperti glutamat, insinasi dan guanilat, berpunca daripada degradasi protein oleh protease endogen dan pertumbuhan mikrob. Malangnya, pecahan protein ini boleh menyumbang kepada peningkatan amina biogen yang boleh menyebabkan kesan buruk kepada individu yang sensitif. Pertumbuhan bakteria asid laktik adalah perkara biasa yang sering dianggap selamat, tetapi mikroorganisma perosak juga boleh membiak dan mencemarkan produk di dalam persekitaran yang tidak sesuai. Oleh itu, berdasarkan kepada kekayaan nutrien dan aktiviti biologi, penggunaan ramuan makanan ini secara bersesuaian boleh menyumbang kepada kesihatan pengguna yang lebih baik di samping meningkatkan penerimaan makanan tradisi Melayu.

Kata kunci: Belacan; ikan air tawar yang difermentasi; makanan etnik Kepulauan Melayu; makanan laut yang difermentasi; pekasam

Introduction

Today, biochemistry defines fermentation as a metabolic process involving bacteria, yeasts, or molds that bring chemical changes and produce energy from organic compounds in anaerobic conditions (Ray & Joshi 2014). Drying or adding salt to food during fermentation preserves the food by lowering its water activity, inducing selective bacterial growth, and contributing to the desired physical changes. Furthermore, beneficial cultures with antimicrobial effects are added to food during fermentation, preventing undesired microbial growth such as spoilage and pathogenic bacteria. Therefore, fermentation also plays a significant role in enhancing food safety and allowing storing of some food in ambient conditions. Apart from that, changes in the food matrix during fermentation produce food with higher organoleptic quality and more desired palatability for consumers (van Boekel et al. 2010).

Each nation's local fermented foods are attributed to its unique culture and ethnicity. Many varieties of fermented food are popular in Malaysia, such as *belacan* (from shrimp) and *pekasam* (from freshwater fish and rice). *Belacan* is fermented shrimp paste which can either be in paste or formed into blocks for selling. Comes in a range of colours from pink to dark brown, its strength is primarily the pungent aroma and taste, derived from several volatile compounds such as glutamate and 5'-nucleotides, such as disodium 5′-innosinate (IMP) and disodium 5′-guanylate (GMP) (Jinap et al. 2010). Other countries in Southeast Asia have their version of *belacan*, known by different names such as *kapi* (Thailand), *ngapi* (Burma), *terasi* (Indonesia), and *andamarang* (Philippines) (Mohd Zaini et al. 2022; Prihanto & Muyasyaroh 2021). There are many origin stories of *belacan*. The most popular theory is that the first ever *belacan* was manufactured over 200 years ago in Malacca, and was known as 'Malacca Cheese' (Representative 1934), while another version has claimed it was invented in Penang Island, Malaysia (Rahman 1973).

Pekasam is unique to Malaysia, due to the type of freshwater used, additional substrate to induce microbial growth, and accessory ingredients such as tamarind to assist fermentation. They became common dishes or food materials adored by Malaysian due to high sensory acceptance. *Pekasam* is mainly manufactured in the northern region of Peninsular Malaysia, such as Perlis, Kedah, and Perak. *Pekasam* is usually prepared by mixing with ground-roasted uncooked rice as the main source of carbohydrates and aroma (Ezzat et al. 2015). Adding roasted rice can promote the growth of Lactic Acid Bacteria (LAB) while masking the fishy smell (Huda 2012). However, undesirable LAB growth can lead to food deterioration, discolouration, and formation of slime. Therefore, precise fermentation is needed for *pekasam* to ensure the final product is edible. The type of fish that are commonly used to produce *Pekasam* includes Tilapia (*Oreochromis mossambica*), Spotted Gourami (*Trichogaster trichopterus*), Catfish (*Clarias batracus*), Java Barb (*Puntius javanicus*), and Snake Head, which are endemic in Southeast Asia and can live under harsh conditions (Ezzat et al. 2015).

As there are limited reviews concerning Malaysian traditional food in literature, this review aims to summarise the production, physicochemical, biological and sensorial characteristics of *belacan* and *pekasam*. Similar to *kimchi* in the Korean Peninsula, *belacan* and *pekasam* are perhaps the most popular Malay traditional fermented foods with distinct tastes and production methods. Apart from promoting fermented food from the Malay Archipelago, this review also aims to update the current knowledge on the benefits and drawbacks of the consumption of these seafood-based fermented products using traditional methods. As the production of these food ingredients tends to extend the shelf life and utilise less favourable fish products, this will contribute to higher sustainability while reducing food waste as the world is heading into a challenging climate change in the near future.

General Biochemical Reaction during Fermentation of *Belacan* and *Pekasam*

Postharvest biochemical events in seafood concern two major phases - metabolic (enzymatic) and microbial (Hui et al. 2012). To date, there is no specific publication dealing with the complete biochemical changes that happen during the fermentation of *belacan* and *pekasam*. Most of the studies measure the amount of nitrogenous or non-protein nitrogenous compounds (Ezzat et al. 2021, 2015; Hajep & Selamat 2012), which are understandably the most essential components in the sensory experience or the health aspect of the food substance. Therefore, by using these compounds and the process used during the fermentation as a reference, the biochemical events that transpired can be deduced.

Jinap et al. (2010) and Ezzat et al. (2015) have reported that both *belacan* and *pekasam* contain a high amount of umami nucleotides, such as disodium 5′-innosinate (IMP) and disodium 5′-guanylate (GMP). These nucleotides are the product of the breakdown of energy-rich triphosphosphate compounds, such as guanosine triphosphate (GTP) and adenosine triphosphate (ATP). As soon as the fish is deprived of the oxygen required for aerobic respiration (especially if the fish struggled during extraction), the highly branched glucose molecules, glycogen, are converted to ATP, followed by further breakdown into its corresponding products, such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), IMP, and inosine by dephosphorylation and deamination process. Some of these products, such as IMP and GMP, are highly flavourful umami components and suppress certain components, such as sulphurous, fatty, burnt, starchy, bitter, and hydrolyzed vegetable-type flavours (Michael Eskin, Aliani & Shahidi 2013). Eventually, it will also result in the formation of many other by-products, such as formaldehyde, ammonia, inorganic phosphate, and ribose phosphates, and especially lactic acids which are responsible for the tissue contraction and rigor mortis, the post-mortem changes due to stiffening of body muscles. The formation of these components can indicate the low freshness of the seafood product, but in certain areas, are prized for their 'fermented taste'.

The nitrogenous compounds also underwent major changes besides changes in phosphate components. The degradation involved both nitrogenous (mainly muscle protein, such as sarcoplasmic, myofibrillar, and stromal) and non-protein nitrogenous compounds, such as trimethylamine oxide (TMAO) to dimethylamine, formaldehyde, and trimethylamine by endogenous enzyme and bacterial reduction mechanism. Figure 1 illustrated the possible proteolytic changes of *belacan* and *pekasam* production during fermentation. Most fish-based fermentation in Malaysia employs a simple, yet effective salting method. Salting reduces the water content of the fish, introduce chloride ion, and delays putrefaction enzyme activity which leads to increased shelf life. Salt will penetrate tissues and affect the stability of native proteins including enzymes. Additionally, once salt reaches a certain concentration, an irreversible

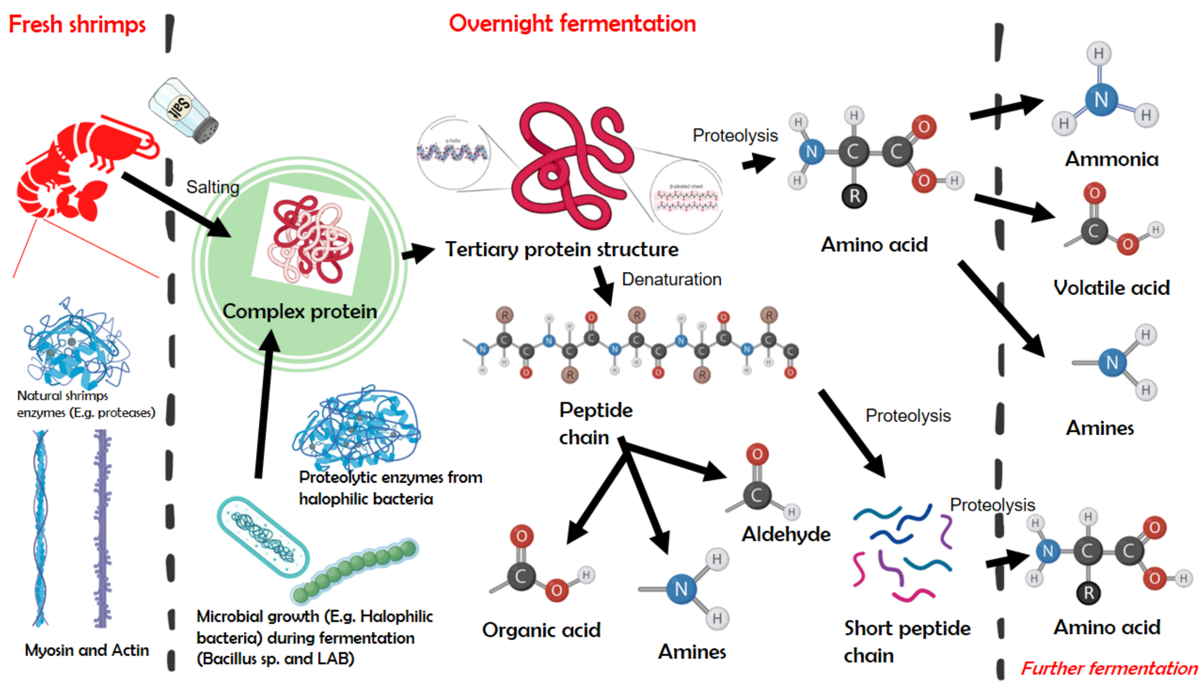

FIGURE 1. The proteolytic events that occurred in during seafood fermentation such as *belacan* and *pekasam*

change to the fish muscle protein occurred, leading to desirable organoleptic properties and the growth of halophilic bacteria.

As the substrate consists of natural endogenous and microbial enzymes, such as protease and lipases, it aids in the production of essential amino acids or volatile compounds that are responsible for the development of flavour, taste, and aroma (Abu Bakar 2002; Hajep & Selamat 2012; Jaksuma 2018). Proteases, such as cathepsin A, C, and D, trypsin-like enzymes, and aminopeptidase assist in releasing many amino acids that contribute to the flavour (Hui et al. 2012). *Pekasam* is unique in the sense that it involved the addition of ground toasted rice which is essential to provide aroma and carbon source to the resulting LAB growth. Therefore, *pekasam* often possesses tangy or acidic characteristics due to the organic acids produced from the heterofermentative bacteria, and enhanced by the addition of dried tamarind, the acidic-fragrant fruit that is endemic in tropical countries (therefore known as acid-assisted fermentation) (Ezzat et al. 2021).

THE PRODUCTION OF *BELACAN* AND *PEKASAM*

BELACAN

The general method to produce *belacan* is demonstrated in Figure 2. During the processing of *belacan*, whole krill shrimps of *Acetes* species including the head and shells are washed and cleaned before being mixed with 4-10% (w/w) salt (Hajep & Selamat 2012). This is followed by microbial lactic acid fermentation overnight at a temperature ranging from 30 °C to 32 °C (Abu Bakar 2002) and then sun-dried for at least several hours to reduce the bacterial count and evaporate the water. Finally, the mixture is pounded to obtain a cohesive pastry texture and the proceeding process greatly depends on the manufacturer.

Usually, the paste will be filled into a wooden or earthen container for further fermentation for up to months to inhibit microbial growth, lower the water activity, and forms a thick paste with 13% to 20% of salt content. The containers used are believed to enhance organoleptic qualities by providing optimal fermentation conditions (Representative 1934). For example, the Japanese have long been using wood barrels for the development of *koji* for soy sauce fermentation as they can absorb a small amount of water, are resistant to mold growth, impart flavours, and are microbe-friendly. To develop the best quality *belacan*, care must be taken to not include other non-related seafood, such as small fish, as it might cause unwanted rancidity and sensorial qualities of the final product (Ali 2022). Additionally, Abu Bakar (2002) proposed covering the mixture with paper to absorb the excess liquid. This paper needs to be replaced frequently, as the proliferative spoilage microorganism might grow in *belacan* and reduce the quality of the mixture. Then, the closed

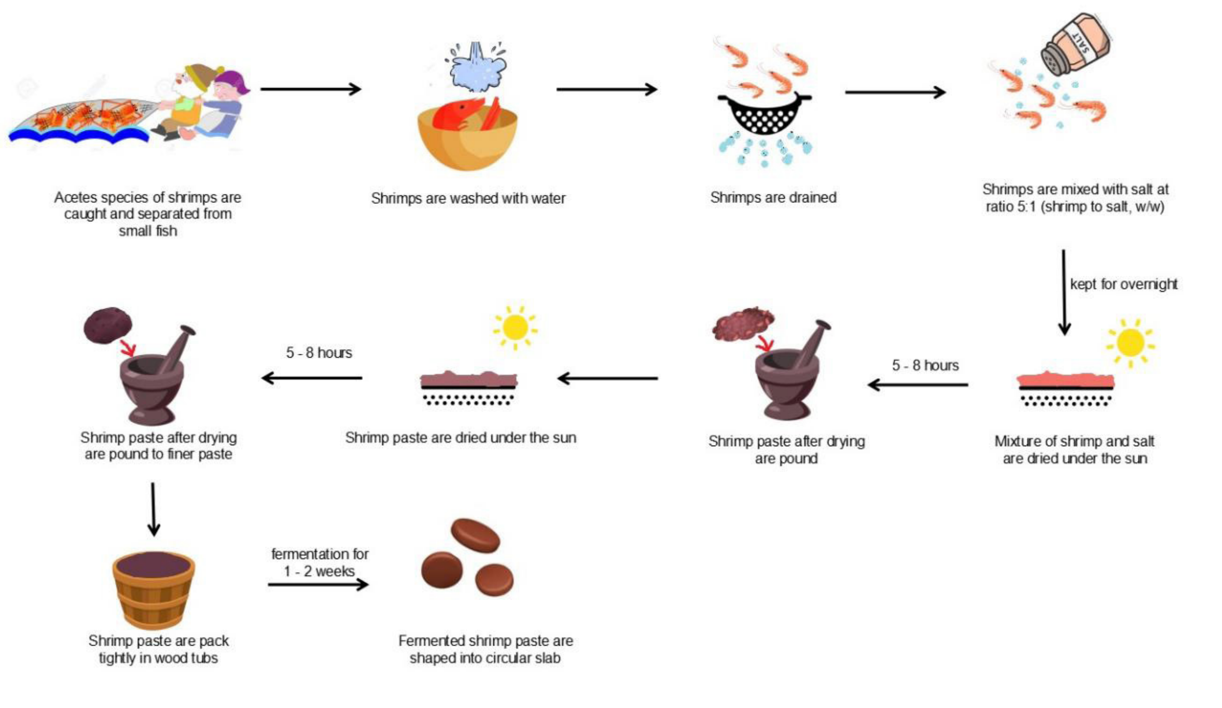

FIGURE 2. Process of making *belacan* including fermentation of shrimp in wood tubs

container is inverted to establish an anaerobic system for fermentation.

PEKASAM

Fermentation of fish is an important way of preserving fish, especially in situations where drying of fish is not possible. For *pekasam*, the fermentation process is carried out by LAB which is responsible for the taste and for improving the microbiological stability and safety of food. The process of making *pekasam* can be divided into two stages of fermentation (Figure 3). For the first stage of fermentation, the fish will be washed using water, thoroughly cleaned, and drained properly. This step is essential as to the higher levels of slime present in freshwater fish, due to their habitats, such as paddy fields or low-current waters. Apart from washing away the microorganisms, the washing procedure can reduce the presence of TMAO that is associated with the fishy odor of the fish. The fermentation of *pekasam* can easily go bad, characterized by a foul odor due to the higher fat and microbial content of freshwater fish. After the washing step, the fish will be kept in a well-sealed container with plenty of salt under chilling temperature. This is to ensure that the water activity of raw fish can be reduced to the minimum level.

After the first stage of fermentation is complete, the fish will undergo second fermentation for a few weeks with ground toasted rice grains, brown sugar, water, and natural acidulants (typically below pH 2.5) such as dried tamarind slices (*Tamarindus indica)* or dried slices of the *Garcinia atroviridis* fruit (Ezzat et al. 2015). This assists the acidification process to reduce spoilage in a process known as acid-assisted fermentation. Typically, the fish is placed first at the bottom of the container, followed by one layer of toasted rice grain

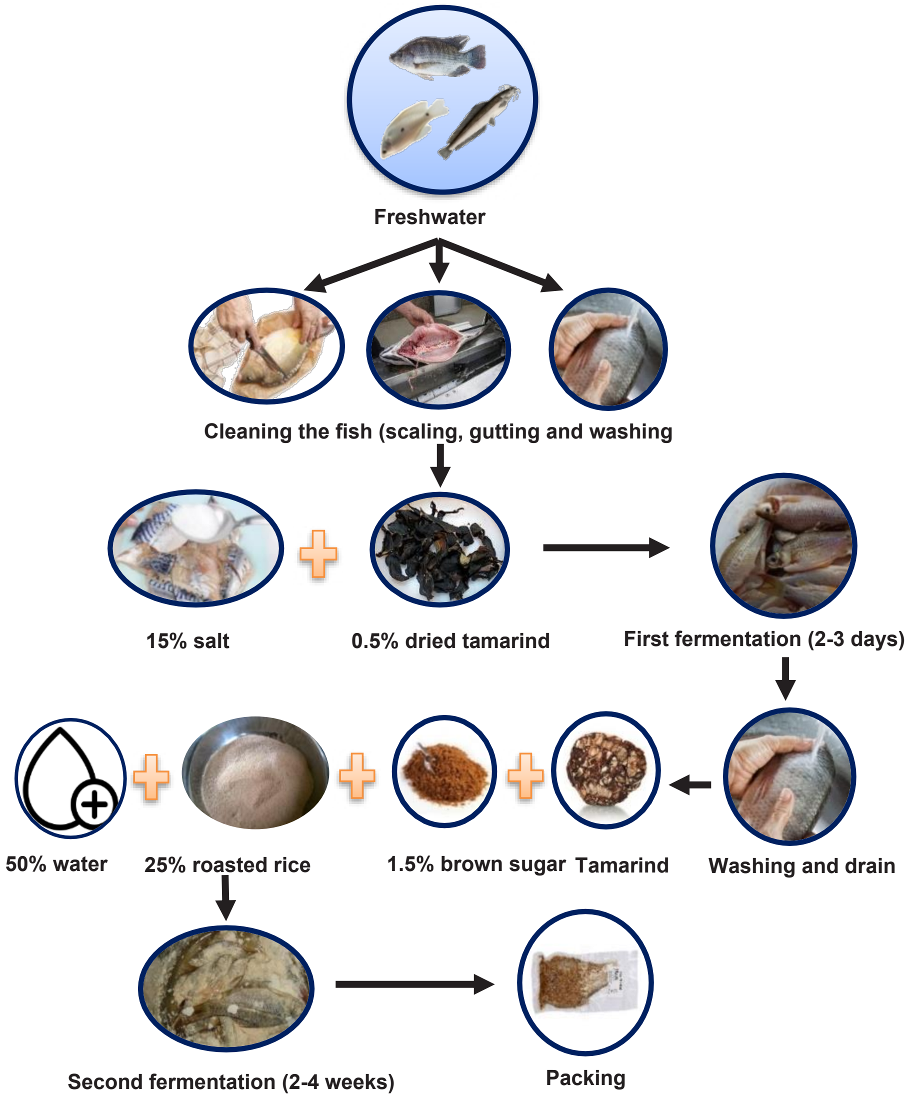

FIGURE 3. The production of *pekasam*, the fermented fish that is popular in the Northern States of Malaysia

powder and a few pieces of dried tamarind slices. The steps are repeated until the container is filled with the fish. The container must be closed properly without no gaps between the cover and the body of the container to ensure that no contamination can occur. Normally, the fermentation process takes longer for larger fish than for smaller fish with more extended fermentation periods that may result in higher-quality products (Ezzat et al. 2015).

MICROBIOLOGICAL AND TOXICOLOGICAL CHARACTERISTICS OF *BELACAN* AND *PEKASAM*

As *belacan* tend to have higher water activity, this likely promotes the growth of halophilic bacteria, LAB, and certain fungi that contributed to the strong pungency of *belacan* (Table 1). The 13 species of LAB inside the *belacan* exhibits probiotic properties that resist acidic and alkaline condition with a high cell hydrophobicity (Haitham et al. 2017). However, as *belacan* is typically roasted or cooked before consumption, further research is needed to verify the viability of the LAB under certain food preparation. In a study by Bolanle (2014), up to 154 LAB isolates on selective MRS agar were obtained from one sample of *belacan* with 18 of them identified as *Lactobacillus*. Out of the 18, only 6 genera consisting of *L. plantarum* and *L. rhamnosus* managed to survive the digestive model system and demonstrated antimicrobial activities against certain food pathogens (Nunal, Florece & Treyes 2016). A similar study was conducted on *pekasam* using 40 isolates, whereby 3 isolates from *Bacillus*, *Pediococcus*, and *Lactobacillus*

TABLE 1. The function of microorganisms exists in *belacan* and *pekasam*, and their role of type of other seafood-based fermentation

Items			Remarks	Outcomes	References
Microorganisms	*Belacan*	Mold	Break down protein into amines and ammonia	HR/AFP	(Hui et al. 2012)
		Yeast	Too few findings on this matter except for the presence of mesophilic yeasts	HR/AFP	(Cheok et al. 2017; Sobhi et al. 2012)
		Non-LAB	Produce histamine from histidine, hazardous when exceeds 500 mg/kg	HR	(Afifi, Mohtar & Alias 2014)
		LAB	Resilience under acidic hydrochloric acid in the stomach to reach the gastrointestinal tract	BH	(Haitham et al. 2017)
			High cell hydrophobicity to colonize the non-secretary epithelium in the gastrointestinal tract	BB	(Haitham et al. 2017)
			Strong fishy smell, production of peptides, and free amino acid for flavour and umami taste	AFP	(Abu Bakar 2002; Hajep & Selamat 2012; Kim et al. 2014)
			Produce volatiles and strong aroma	AFP	(Abu Bakar 2002)
			Convert histidine into histamine	HR	(Afifi, Mohtar & Alias 2014)
			Promote the fermentative microorganism	MC	(Rhee, Lee & Lee 2011)
			Create low pH environment to control pathogen	MC	(Ilyanie, Huda-Faujan & Ida Muryani 2020)
			Tolerant to alkaline bile salt to reach the gastrointestinal tract	BH	(Haitham et al. 2017)
	Pekasam	Mold	Too few findings on this matter except for the presence of mesophilic molds	HR/AFP	(Cheok et al. 2017; Sobhi et al. 2012)
		Yeast	Too few findings on this matter except for the presence of mesophilic yeasts	HR/AFP	(Cheok et al. 2017; Sobhi et al. 2012)
		Non-LAB	Halophilic bacterial action may lead to the formation of biogenic amines	HR	(Ezzat et al. 2015)
		LAB	May lead to the formation of biogenic amines, involved in aroma development, prevents the growth of pathogenic bacteria, and act as probiotics	BB/HR/AFP/MC	(Ezzat et al. 2015; Ida Muryany et al. 2017; Tan, Lim & Wan Mustapha 2017)

BB: beneficial to human beings; HR: health risk exists; AFP: aroma of flavour promoter; MC: microbial control showed antimicrobial properties against bacterial pathogens while being γ-hemolytic (no ability to cause hemolysis, an important trait of probiotics) and resistance to a wide range of pH (Ida Muryany et al. 2017). The LAB isolated from *pekasam* tends to be salt-, acid-, and thermo-tolerance, which are desirable traits for food production (Mansor et al. 2014). Due to the presence of a wide range of LAB, the application of *belacan* can go beyond the food, such as a biocontrol for plant growth (Mohd Zaini et al. 2022). Interestingly, the growth of LAB in *pekasam* is highly dependent on the amount of protein present in the substrate used. In a study by Mahyudin, Ibadullah and Saadin (2015), they found that the highest protein-fish (in this case, *patin*) showed a significantly higher *Lactobacillus* growth compared to the other freshwater fish species. This finding was supported by Tan et al. (2017), who reported that different *pekasam* fish species lead to significantly different microflora profiles. Although the mechanism is unclear, higher growth of *lactobacillus* is desirable in terms of successful fermentation. Likely, different types of amino acids, proteins, methods of production, and endogenous microflora affected the growth of bacteria, as they rely heavily on nitrogen sources (or protein from fish) and other growth parameters such as salinity, temperature, and pH. *Lactobacillus* may outgrowth putrefactive microorganism, and produced lactic acid that acts as a flavourant and natural preservative. However, more research is needed to find the statistical correlation between the ratio of the substrate, the number of LAB, and the effect on the organoleptic properties of *belacan* or *pekasam*.

Much research focused on the presence of LAB, rather than other microbial types such as yeast and mold. Apart from easier identification, LAB is important in processing (reduction in pH) and health benefits (as probiotics and being safe). However, yeast and mold possess their role as well, and certain pathogenic strains can affect the well-being of humans. For example, *Saccharomyces cerevisae*, the most popular yeast, has been implicated in various biological processes in food products, such as in the development of organoleptic properties, and the production of bioactive metabolites and enzymes (Liszkowska & Berlowska 2021). Likewise, mold such as *Aspergillus* and *Penicillium* species also play major roles in ripening and enzymatic activities in fermented food products. To date, there is limited information on the roles of yeast and mold in the production of *belacan* and *pekasam*.

Certain bacterial actions may lead to the decarboxylation of amino acids or transamination reaction and lead to the production of histamine, tyramine, cadaverine, putrescine, and related metabolites, especially if the bacteria survive the high salt condition (Moon et al. 2013). To sensitive individuals, these metabolites may cause adverse reactions (scombroid fish poisoning) similar to allergic reactions, such as headaches, itchiness, tingling, and others. For example, the presence of *Bacillus* sp. will lead to the decarboxylation of histidine amino acid to histamine, which often is used as the indicator of freshness. Being the combination of protein-rich fish substrate and fermentation, *belacan* and *pekasam* are prone to the production of biogenic amines. In a study comparing the number of biogenic amines in 62 food samples of Malaysia, *pekasam* and *belacan* were found to contain the highest amount of these substances (Saaid et al. 2009). Padilah et. al (2018) found that 47% of *belacan* in Northern Malaysia contained more than 50 mg/kg histamine level, with the highest amount recorded at 143 mg/kg. Furthermore, fish and shrimp paste has been implicated to contain high levels of histamine, with over 26% of the samples exceeding 500 ppm, which is 5 times the allowable limit under Good Manufacturing Practice (Tsai et al. 2005). In general, *belacan* tends to contain higher histamine than *pekasam* weight-by-weight (Ezzat et al. 2015). However, *belacan* is not being consumed in a large amounts like *pekasam* which would lessen its impact.

In *belacan*, putrescine and tyramine were reported to be at 658 and 242 mg/kg, respectively. They also detected a high amount of putrescine averaging 137 mg/kg (n=15) with the highest at 314 mg/kg. In contrast, cadaverine and tyramine levels were quite low at an average of 50.1 and 8.64 mg/kg, respectively (Padilah et al. 2018) (Table 2). In contrast, the total biogenic amines in *pekasam* is below 500 mg/kg, which is significantly lower that the permitted limit of 1000 mg/kg (Ezzat et al. 2015). Interestingly, the highest biogenic amines in *pekasam* are cadaverine and putrescine, which accounted for more than 60% of total biogenic amines. These two biogenic amines are less studied and therefore no limit is imposed by many countries, as indicated in Table 2. Javanese carp demonstrated higher putrescine than tilapia although both fish contain a similar amount of arginine (precursor of putrescine). This may be due to the presence of different microbial flora with more pronounced arginine carboxylase (Ezzat et al. 2015) which also quantified trans- and cis-urocanic acids in *pekasam*, which theoretically can be converted into biogenic amines inside human body. The findings also indicated that *pekasam* contains much lower trans-urocanic acids than Indian mackerel, therefore could be much safer than the latter (Zare et al. 2013). However, these values are depended on the types of fermentation, substrate, and type of fish used, as different biogenic amine values were obtained from Saaid et al. (2009). Nevertheless, from a safety perspective, *pekasam* is quite safe to be consumed due to its low biogenic amines and urocanic acids.

In preserved food, low levels of mutagens such as nitrosamines and N-nitroso compounds may form during salting and drying procedures. Additionally, intake of excessive salt has been implicated in many diseases, including induction of mucosal damage and destruction of the mucosal membrane lining. In *belacan*, the genotoxic effect using 4 different extraction solvents were conducted, using three different samples of *belacan* from various region. A few samples showed mutagenicity induction at lower levels, but typically their action is nullified by a metabolic induction system when tested using the *Umu* test (mutant of *Salmonella typhi* in rats) (Ghazali et al. 2012). Similar metabolic nullification was also observed by Abdullah (2012) using the *Ames* test. However, it still contains high levels of arsenic and lead, exceeding the recommendation by the Malaysian Food Act 1983 and Food Regulations 1985. As seafood can easily accumulate a toxic amount of heavy metals (such as the infamous case of Minamata Disease), care must be taken during consumption (Ngah & Yahya 2012). Especially in *pekasam*, the common freshwater fish usually inhabit the agricultural irrigation systems that are widely exposed to heavy metals and pesticides. Nevertheless, as for *belacan*, the amount of harmful heavy metals (As, Pb, Cd, and Hg) taken daily in the population is considerably lower than provisional tolerable weekly intakes outlined by FAO/WHO due to the small amount used during the cooking, and the small size of the shrimps that is less likely to accumulate a large number of xenobiotics. Therefore, although *belacan* is listed as one of the ‘high-risk’ Malaysian fermented food due to its high salt level (Ghazali et al. 2012) and was shown to cause DNA damage *in vitro* in a previous study (Ghazali et al. 2005), the consumption of *belacan* at a moderate level should be safe. This is in line with the adage of toxicology by *Paracelsus*, the father of toxicology - “the amount is what makes it toxic”.

Apart from heavy metal contamination, the bacterial population in fermented food is also exposed to the risk of antibiotic resistance. Although this occurrence is likely to happen in all fermented foods, the traditional methods of producing *belacan* and *pekasam* mean that there is less stringent monitoring during unwanted bacterial growth. This phenomenon has been shown in certain traditional fermented foods, such as dairy products in India (Thumu & Halami 2012), *dadih* and meat in Indonesia (Sukmarini et al. 2014), and vegetables in China (Nawaz et al. 2011). In case there are incidences whereby LAB obtained the antibiotic resistance genes, these genes are transferable via horizontal gene transfer (transformation, conjugation, or transduction). As a result, other bacteria genera may acquire those genes and spread the resistance to other unwanted species.

TABLE 2. The presence of biogenic amines and the reference limit for the safe intake imposed by European, Canada, Switzerland, Brazil, Australia, and New Zealand

	Histamine	Tyramine	Cadaverine	Putrescine	References
Belacan	5.77 - 143 mg/kg	4.16 - 20.0 mg/kg	9.56 - 134 mg/kg	61.8 - 314 mg/kg	(Padilah et al. 2018)
Pekasam	42 - 66.8 mg/kg	39.3 - 65.5 mg/kg	101.2 - 136.7 mg/kg	69.2 - 133.5 mg/kg	(Ezzat et al. 2015)
Reference/limit	50 - 200 mg	600 mg	N/A	N/A	(Biji et al. 2016)

ORGANOLEPTIC AND NUTRITION OF *BELACAN* AND *PEKASAM*

The Malaysian Food Act 1983 and Food Regulations 1985 (2014) recommended that *belacan* shall contain not less than 25% protein and 40% and 35% moisture content and ash, respectively. The moisture content tends to be quite high to ensure the *belacan* did not turn too dry, brittle, and hard. In a study by Kim et al. (2014), *belacan* has high moisture at around 27%, although this figure can vary widely as high as 47%. Ilyanie, Huda-Faujan and Ida Muryani (2020) also obtained similar moisture content at around 32.16%, which is significantly lower than another native Malaysia fermented food of *cencalok* at 67.44%. However, the moisture content is considerably lower than the average moisture content in other shrimp fermentation, ranging from 56.1 - 70.9% (Kim et al. 2014). This might be contributed to the repeated drying procedures during its production. As a result, the physicochemical properties can greatly vary as well, as moisture has been demonstrated to play essential biochemical and microbial roles in food.

For protein, the value is often below 40% due to the degradation of shrimp protein during fermentation. Meanwhile, ash content always exceeds 30% due to the unregulated addition of salt, which increases the mineral content of *belacan*. The amount of different mineral in belacan in descending order is sodium, calcium, phosphorus, magnesium, potassium, iron, and zinc. As the source is shrimp, *belacan* is rich in protein (>35%), peptides, and free fatty acids due to autolysis reaction by natural shrimp enzymes or enzymes from microbial fermentation (Kim et al. 2014). The content of amino acids can reach up to 27174 mg/100 g, with 11145 mg/100 g of essential amino acids (EAA). Glutamic acid dominated the amino acid profile at over 5000 mg/100 g, followed by aspartic acid (2800 mg/100 g) and leucine (2450 mg/100 g). These amino acids, particularly glutamic acid, are the major fatty acids for umami taste, while others can contribute to bitter, sour, and sweet taste (Jinap et al. 2010). This value is also in range with previous studies (Jinap et al. 2010; Mizutani et al. 1992). Moreover, the glutamate value showed the potency of *belacan* as a flavour enhancer as compared to others such as beef concentrate (7300-9600 mg/100 g), autolysed yeast powder (4900 mg/100 g), kombu (2250 mg/100 g) and soup stock and oyster sauce (3000-8000 mg/100 g) (Lau & Mok 1995).

5'-nucleotides were also found in significant amounts, which vary quite significantly between 11 different *belacan* samples (0.85 - 42.25 µg/g). The lowest value (0.85 µg/g) was shown by powdered *belacan*, which was produced via spray drying that likely destroyed the sensitive 5'-nucleotides (Jinap et al. 2010). In terms of the percentage of fat compared to other components, fat only accounts for around 1% of the total macronutrient (Ilyanie, Huda-Faujan & Ida Muryani 2020; Kim et al. 2014). *Belacan* tends to contain the highest amount of polyunsaturated fatty acids (PUFA) (47%), followed by SFA (31%) and MUFA (21%). Unfortunately, cholesterol content can be quite high, up to 51 mg/100 g (Kim et al. 2014), although dietary cholesterol was shown to not be the main determinant for cardiovascular-related diseases due to its poor absorption during digestion. There are no particular values are recommended for *pekasam* in the Malaysian Food Act 1983 and Food Regulations 1985 (2014), except for the required amount of salt shall contain not less than 10%. This might be caused by the highly varied method of production and the substrates used, unlike *belacan* which usually rely on one type of substrate (shrimp), as demonstrated in Table 3.

Biochemically, the sensory of *belacan* is largely influenced by the composition of amino acids and volatile compounds. As mentioned before, *belacan* contains a rich amount of amino acids, which possess their own characteristics such as sweet, bitter, salty, sour, and umami (Jinap et al. 2010). The synergistic relationship between these components produced an enhanced organoleptic experience, up to 32 folds higher as judged by the sensory panellist. Free glutamate, the main amino acid responsible for umami taste, was found to be as high as 530 mg/100 g in *belacan* and increase up to 4207 mg/100 g when integrated into cooking. It is suggested that *belacan* should be roasted before application, to kill the bacteria and enhance its flavour (Hutton 2005).

A study by Jinap et al. (2010) using a quantitative descriptive analysis using known reference materials to find a correlation between 17 sensory attributes, showed that the positive experience by the trained food panellist is directly proportional to the concentration of *belacan*. However, certain tastes, such as bitter, sweet, and astringency did not score highly. The study also indicated that the type of food used with *belacan* significantly influence the acceptance by the panellist. For example, a mere 0.45% *belacan* is optimal for *asam pedas* (tamarind flavoured dish with *belacan*), but up to 18% is required for *sambal belacan* (chilli *belacan*). Moreover, the panellist described that *belacan* enhanced the aroma and shrimp-like characteristics in the aroma department and salty and meaty in the flavour department. *Belacan* also tend to score highly in food with lower water content, such as fried rice and sambal *belacan*. Although sambal *belacan* requires a high concentration of *belacan*, care should be taken to not overuse the ingredient as the subsequent study showed some displeasure among the participants. In another study by Cheok et al. (2017), they found that *sambal belacan* contains up to 24 different volatile compounds, which are mainly terpene, limonene, and butanoic acid that can contribute to the fresh, sweet citrusy, and cheesy sensory perception. Further irradiation and thermal treatment retain up to 23 and 19 of these volatile compounds, respectively.

The main factor in the acceptability of *pekasam* is different from *belacan*. This may be due to the different consumption styles; *belacan* is added as a flavour enhancer in a small amount while *pekasam* is eaten whole as part of a meal. In a study conducted by Ezzat et al. (2021), they found that the type of fish played a major role in determining their acceptability, regardless of the type of fermentation used (i.e., natural or acid-assisted fermentation). Although the toasted ground rice or the acidity of tamarind slices may contribute to flavour, it was found that the flesh/muscle texture of fish played a greater role - as panellist favors the tender fish texture of Javanese carp compared to black tilapia. In terms of the organic acid presence, it was found that lactic acid was dominant, followed by succinic and acetic acids (Ezzat et al. 2021). LABs likely contributed to a significant portion of lactic acid, while acidulants contributed to others, although LAB may also produce other acids too via heterofermentative pathways. The major volatile compounds extracted in *pekasam* using methanol and hexane are carboxylic acids, which contributed to the rancid and 'goaty' off-flavour. The other three major volatile compounds are organic peroxide, ester, and alkaloids, which can give a floral, fruity, and fishy smell, respectively (Tan, Lim & Wan Mustapha 2017).

TABLE 3. The summary of the nutritional content of *Belacan* and *Pekasam*

Nutrients	*Belacan*	*Pekasam*	Reference(s)
Protein	Not less than 25%	15%-25%	(Hajep & Selamat 2012; Huda, 2012; Kim et al. 2014; Food Act 1983 and Food Regulations 1985, 2014)
Fat	~1%	3-8%	(Huda 2012; Ilyanie, Huda-Faujan & Ida Muryani 2020; Kim et al. 2014)
Carbohydrate	11%	N/A	(Ilyanie, Huda-Faujan & Ida Muryani 2020; Kim et al. 2014)
Ash	Less than 35%	6-14%	(Huda 2012; Food Act 1983 and Food Regulations 1985, 2014)
Moisture	Less than 40%	57-73%	(Huda 2012; Food Act 1983 and Food Regulations 1985, 2014)
Salt	Not less than 15%	Not less than 10%	(Food Act 1983 and Food Regulations 1985, 2014)
Free glutamic acid	~5000 mg/100 g	Vary 200-300 mg/100 g	(Ezzat et al. 2015; Jinap et al. 2010; Kim et al. 2014)
5′-Nucleotides	0.85 - 42.25 µg/g	N/A	(Jinap et al. 2010)

*Vary due to the wide range of substrates being used

BIOLOGICAL ACTIVITIES

As the research on *belacan* and *pekasam* is in infancy, many of their biological activities are still uncharacterised. In Table 4, several authors showed that *belacan* and *pekasam* (or their equivalents) possessed antioxidant abilities. Antioxidants are compounds that donate an electron to free radicals, neutralising them and thus preventing damaging chain reactions. Due to metabolic (generation of ATP in mitochondria) and environmental (UV light) stress, the production of free radicals can reach a dangerous level and disrupt biological structures such as cell membranes and DNA. Typically, the exogenous antioxidants come from the plant-based product due to its rich polyphenols content. In the case of *belacan*, the calorimetric measurements showed an increase in antioxidative capacity, which is mainly attributed to the development of Maillard reaction products (MRPs), or browning, due to the presence of free fatty acids. Shorter fermentation time did not produce significant changes in antioxidant capacities, which suggested that the proteolytic processes in seafood-based fermentation occurred slowly over time. The increase in antioxidant capacities was shown to be proportional to the increase in free fatty acids and MRPs, especially after most of the fatty acids were liberated after 12 months of the fermentation period (Peralta et al. 2008). Similar observation on the impact of browning on the increase of antioxidant abilities was also observed in other studies (Benjakul et al. 2005; Jing & Kitts 2004). In contrast, the antioxidant activities of *pekasam* are mainly attributed to the presence of certain amino acids, such as hydrophobic amino acids (Najafian & Babji 2018)2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid or certain peptides (Ma et al. 2022).

Some LAB can produce fibrinolytic enzymes that aid in the treatment of cardiovascular diseases. As the blood clotting ability is enhanced via the fibrin fibre synthesis, an effective fibrinolytic enzyme from a few fermented foods was shown to improve the anticoagulant abilities of the blood. Out of 13 Asian fermented foods, shrimp paste was shown to possess the strongest fibrinolytic activity (Wong & Mine 2004), synthesised by the novel *Bacillus* sp. present in the product (Anh et al. 2015; Hua et al. 2008). Unfortunately, the information on these two items is still relatively scarce, and more research is needed to elucidate their health impact on humans.

Although fermented food is trendy, most research is concentrating on western fermented foods such as dairy products (cheese and yogurt), wheat (sourdough), and alcoholic beverages (Rahim et al. 2023). In the instances where other types of fermented foods are discussed, it usually concentrates on the ethnic foods of developed countries, such as *kimchi* (Korea), *soy sauce* (China), and *natto* (Japan).

In South Korea, a recent national survey indicated that the frequent consumption of ethnic fish-related food managed to improve allergic responses such as atopic dermatitis in the general population (Park & Bae 2016). Although no mechanism was elucidated, frequent exposure to allergens may 'train' the immune responses to be tolerant and passed the beneficial trait to the next generation. This shows the tremendous potential of seafood-related fermented foods while improving sustainability as leftover or under-utilised seafoods are being preserved for longer (Mohd Zaini et al. 2022).

TABLE 4. The biological activities of *belacan* and *pekasam*, or their equivalence in other Southeast Asian countries. Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric reducing ability of plasma

	Biological activities	Test(s)	Remarks	References(s)
Belacan or equivalent*	Antioxidants	DPPH, hydrogen peroxide, and lipid peroxidation	Increased after prolonged fermentation, due to the formation of Maillard compounds	(Peralta et al. 2008)
		DPPH	14 samples of shrimp paste showed 4.12 – 14.5 TE/g protein antioxidative activity	(Prapasuwannakul & Suwannahong 2015)
		DPPH, ABTS, FRAP	Ka-pi (Thailand-fermented shrimp) possessed antioxidative properties that increase over time	(Pongsetkul et al. 2015)
	Fibrinolytic/ Thrombolytic	*In vitro* enzymatic digestion	*Bacillus* sp. isolated from shrimp paste produced a unique fibrinolytic enzyme	(Anh et al. 2015; Hua et al. 2008)
		In vitro enzymatic digestion	Resistant to pepsin and trypsin digestion and possess anticoagulant activity	(Wong & Mine 2004)
	ACE inhibitory activity		Possessed antihypertensive activity due to the presence of di-peptides Ser-Val and Ile-Phe	(Kleekayai et al. 2015)
Pekasam or equivalent*	Antioxidant	DPPH and ABTS	The hydrophobic amino acids in fish peptide extract contributed to antioxidant activity	(Najafian & Babji 2018)
		Molecular docking, DPPH, ABTS	Tilapia skin hydrolysate contains high antioxidant activity and is water-soluble, stable, and non-allergenic for food application	(Ma et al. 2022)

Conclusion

Belacan and *pekasam* are the two precious ethnic foods in the Malay Archipelago. They are being used in a variety of traditional and modern dishes and are known to enhance sensorial properties due to their rich umami-related amino acids and volatile compounds. However, due to the presence of certain amino acids and microbial action, many potentially allergenic compounds in form of biogenic amines may form that can affect the immunocompromised population. Nevertheless, to those who can tolerate them, these fermented foods are a privilege on top of having certain nutritional properties for health.

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