Contents lists available at ScienceDirect

Food Science and Human Wellness

journal homepage: http://www.keaipublishing.com/en/journals/food-science-and-human-wellness

Selected fermented indigenous vegetables and fruits from Malaysia as potential sources of natural probiotics for improving gut health

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A R T I C L E I N F O

Article history:
Received 17 August 2021
Received in revised form 12 October 2021
Accepted 13 January 2022
Available Online 15 March 2023

Keywords:
Ethnic
Leafy vegetables
Fruit
Fermentation
Lactic acid bacteria
Malaysia

A B S T R A C T

In the Peninsular Malaysia and Northern Borneo island of Malaysia, various rich indigenous leafy vegetables and fruits grow and contribute to the nutritional and dietary values of the population. They have high water contents, thus, naturally vulnerable to rapid food spoilage. Food preservation and processing play a vital role in the inhibition of food pathogens in fruits and vegetables that are prevalent in Malaysia. Lactic acid fermentation is generally a local-based bioprocess, among the oldest form and well-known for food-processing techniques among indigenous people there. The long shelf life of fermented vegetables and fruits improves their nutritional values and antioxidant potentials. Fermented leaves and vegetables can be utilized as a potential source of probiotics as they are host for several lactic acid bacteria such as *Lactobacillus confusus*, *Weissella paramesenteroides*, *Enterococcus faecalis*, *Lactobacillus plantarum*, *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*. These strains may be more viable in metabolic systems whereby they can contribute to a substantial increase in essential biologically active element than industrial starter cultures. This review is aimed to address some essential fermented fruits and vegetables in Malaysia and their remarkable reputations as a potential sources of natural probiotics.

1. Introduction

Fermented beverages and foods, whether from plant or animal sources, have been developed, and enjoyed across the globe for many years, are currently of great interests in the scientific community, as well as they are an integral part of the daily diet for many consumers [1-3]. The fermentation method of preservation has allowed forbearers in tropics and arctic regions with limited growing seasons to survive during the harsh drought and winter months. Fermentation is an anaerobic metabolism converting carbohydrates by bacterial enzymes to alcohol or organic acids (Fig. 1) [4-8].

Many practices involving traditional or indigenous fermented foods were done in an artisan method and without any idea of the potential role of the bacteria involved [9]. However, in food bioprocessing, salting and drying are commonly known as the oldest forms of food preservation technologies [10]. Fermentation technologies are thought to have evolved to preserve fruits and vegetables during shortage times; by preserving the food with alcohols and organic acids, as well as by improving the desirable flavour and texture of the food [11,12]. Fermented beverages and foods also aid in decreasing toxicity (remove antinutritional factors) [13] and cooking

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Peer review under responsibility of KeAi Communications Co., Ltd.

Publishing services by Elsevier

http://doi.org/10.1016/j.fshw.2023.02.011
2213-4530/

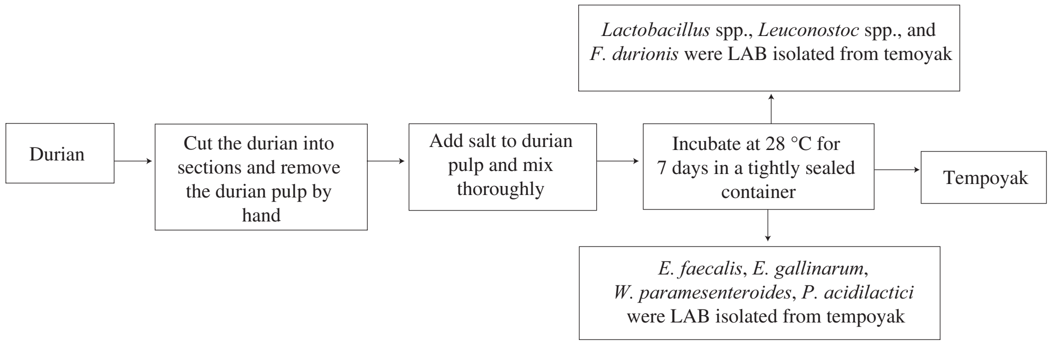

Fig. 1 Natural fermentaion scheme of Temopyak.

time [14]. Consumption of a healthy amount of fruits and vegetables in our daily diet helps prevent illnesses such as hypertension, coronary heart problem and to reduce the risk of strokes as been recommended by the Food and Agriculture Organization and World Health Organization [10,15]. However, consumers usually tend to prefer healthier beverages and foods, that are fresh, extremely nutritious and ready to be consumed [16]. Lactic acid fermentation (LAF) of fruits and vegetables is typically a common practice for enhancing the nutritional and sensory characteristics of food products [17,18]. Previous studies have reported that a majority of potential lactic acid-producing strains were isolated from different traditional naturally processed foods [19-21]. Malaysia's traditional fermented fruits and vegetables can be utilized as potential probiotics as many lactic acid bacteria (LAB) such as *Lactobacillus brevis*, *Lactobacillus confusus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus pantheris*, *Lactobacillus pentosus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*, are found in them [22-24]. Bioavailability of significant amount of essential nutrients, like amino acids, minerals together with acidic nature of vegetables and fruits provides a suitable medium for fermentation by probiotic bacteria [25].

Probiotics, which are mostly LAB and Bifodobacteria including the species belonging to the genera *Lactobacillus* and *Bifidobacterium* [26], can carry out acid fermentation not only to preserve, but also to offer some added nutritional values to the product [25]. Thus, Malaysia's fermented vegetable and fruits possess the quality of not just being a viable food supplement, but can have both direct and indirect impact on human health.

This article review provides an overview of the current work prospects of LAF of both Malaysia's Borneo and Peninsular vegetables and fruits concerning health benefit and human nutrition.

2. Vegetable and fruit based LAF

Fermentation represents one of the ancient and most economical techniques to prolong the shelf life of perishable food before cold storage. Temoyak (fermented durian) is a popular indigenous condiment in Malaysia, made from durian pulp through a natural fermentation bioprocess [27]. It is manufactured by natural LAF after mixing fruit pulp with 1%–2% NaCl, in an airtight vessel at 23–27 °C for about 5–7 days [28]. Tempoyak production typically has long shelf life due to the preservation by lactic acid produced by LAB, as well as addition NaCl during bioprocessing to inhibit growth of undesirable food microbes [29]. *Fructobacillus durionis*, *Leuconostoc* spp., *Lactobacillus* spp., *Lactobacillus durianis* are the microbiota responsible for LAF [28]. The LAF of duration to produce tempoyak has been extensively investigated for decades [30,31]. The basic fermentaion steps involved are summarized in Fig. 2.

With the well-known and successful process of tempoyak (Malaysian indigenous fermented fruit), spontaneous LAF of several other fruits and vegetables has emerged, such as wild mango, mustard leaf, cassava leaves and dabai in both Malaysia Borneo and Peninsular Malaysia (Table 1).

Vegetables and fruits fermenting and characteristics are widely dependent on the types of the raw material involved, fermentation condition and microbe environment.

Tempoyak, processed durian is the most famous investigated lactic acid fermented fruits in Malaysia mainly because of their health benefits and industrial importance.

In recent years, there has been growing interest in the consumption of indigenous fermented Malaysia products both locally and internationally, with the processed vegetable and fruits being manufactured globally, using different famous indigenous type of raw matrices (Table 2). Different kinds of fermented fruit and vegetable are being produced in Malaysia either in at home, villages or small scale for the commercial food sectors. The preservation of the vegetables and fruits is done by naturally occurring lactic acid strains (i.e. spontaneous fermentation), present on the raw materials like *Pediococcus* spp., *Lactobacillus* spp. and *Leuconostoc* spp., etc. However, the use of selected starter cultures like *L. helveticus*, *L. fermentum*, *L. paracasei* and *L. rhamnosus* (all probiotic strains) in fermentation provide some advantages such as process repeatability, microbiological safety and high nutritional values [17,45-47]. In addition, traditional strains, based on individual adaptability to specific plant niche, maybe more viable in the metabolic system, contribute to a substantial increase in essential biologically bioactive element than industrial starter cultures present in the market [17,48,49].

Naturally, fruit and vegetables are rich in phytochemicals, polyphenols, minerals, vitamins, and dietary fibres, which have been designated as healthy foods as numerous studies have stated the beneficial effects of juices on health [50]. However, the combination of these essential nutrient contents, as well as neutral pH, make these matrices suitable for LAF [51,52]. LAF of vegetables and fruits have been proven to be not only an efficient method for

Table 1
Common indigenous fermented vegetable and fruit food product from Malaysia.

Fermented food products	Vegetables and fruits	Other supple-ments	Microbes	Ethnic/State	References
Bambangan	Wild mango (*Mangifera pajang kostermans*) fruits	Salt	*L. plantarum*, *L. delbrueckii*, *L. paracasei*, *P. pentosaceus*	Kadazan and Dusun tribes/Sabah	[32]
Ensabi	Wild brassica (*Brassica juncea* (L.) Czern. var. Ensabi)	Salt and Boiled rice liquid	Remains unknown	Sarawak/Sabah/Malay	[33]
Dabai	Dabai (*Canarium odontophyllum*)	Salt	Remains unknown	Sarawak	[34]
Daun ubi/Dawon tawi	Cassava leaves (*Manihot esculenta* Crantz)	Boiled rice liquid, Coconut milk	Remains unknown	Dayak/Malay	[33]
Jeruk	Ginger, gherkins, leek, chili and leafy tropical fruits and vegetables		*Lactobacillus*, *Leuconstoc*, *Streptococcus* and *Pediococcus*	Malaysia	[35]
Jeruk asam paya	Asam paya fruits (*Eleiodxa conferta*)	Salt and Sugar	*Lactobacillus*, *Leuconstoc*, *Streptococcus* and *Pediococcus*	Malaysia	[36]
Kedondong	Ambrarella (*Spondias dulcis*)	Sugar and Salt	Remains unknown	Native Borneo region	[37]
Tempoyak	Durian	Salt	*W. paramesenteroides*, *E. faecalis*, *E. gallinarum*, *Lactobacillus* spp., *L. mesenteroides*, *P. acidilactici*	Malaysia	[28,29,38]
Tuhau	Tuhau pickle (*Etlingera coccinea*)	Sugar, Salt lime juice, chilli, onions, belacan	Remains unknown	Native Borneo region	[39]
Buah kepayang	Panginum (*Pangium edule*)	Sugar, Salt lime juice, chilli, onions, belacan	Remains unknown	Native Borneo region	[39]
Jeruk lobak	Daikon (*Raphanus sativus* L.)	Sugar	*L. brevis* and *L. plantarum*	Malaysia	[23]
Jeruk bawang puith	Garlic (*Allium sativum*)	Salt, ginger	Remains unknown	Malaysia	[23]
Petai Jeruk	Stinky beans (*Parkia speciosa*)	Salt, chilli	*L. plantarum*, *L. brevis*, *P. pentosaceus*	Malaysia	[23]
Sawi pahit	Napa cabbage (*Brassica rapa* subsp. *pekinensis*)	Salt	*L. plantarum*	Malaysia	[23]
Pickled Kai choy/gai choy	Malaysian Chinese mustard Brassicaceae spp.	Salt, turmeric, sugar, garilic, ginger	*L. plantarum*	Malaysia	[23]
Jeruk maman	Maman (*Cleome gynandra* L.)	—	*L. plantarum*, *L. futsaii*, *L. paralimentarius* and *P. pentosaceus*	Malaysia	[23]
Jeruk rebung	Bamboo shoot (*Bambusa tulda*)	Salt	*L. plantarum*, *L. brevis*	Malaysia	[23]
Mandai	Cempedak/jack fruit (*Atrocarpus champeden*)	—	*L. plantarum*, *P. pentosaceus*, *L. casei*, *L. vaccinostercus*, *L. harbinensis*, *L. perolens*, *L. casei* and *L. paracasei*	Malaysia	[40-42]
Jeruk Mangga	Green mango (*Mangifera indica*)	Salt, sugar, chilli	*P. cerevisiae*, *L. brevis*, *L. plantarum*, *L. mesenteroides*	Malaysia	[43]
Jeruk limau nipis/jeruk limo	Lime (*Citrus* × *aurantiifolia*)	Salt	*L. delbrueckii*	Malaysia	[44]

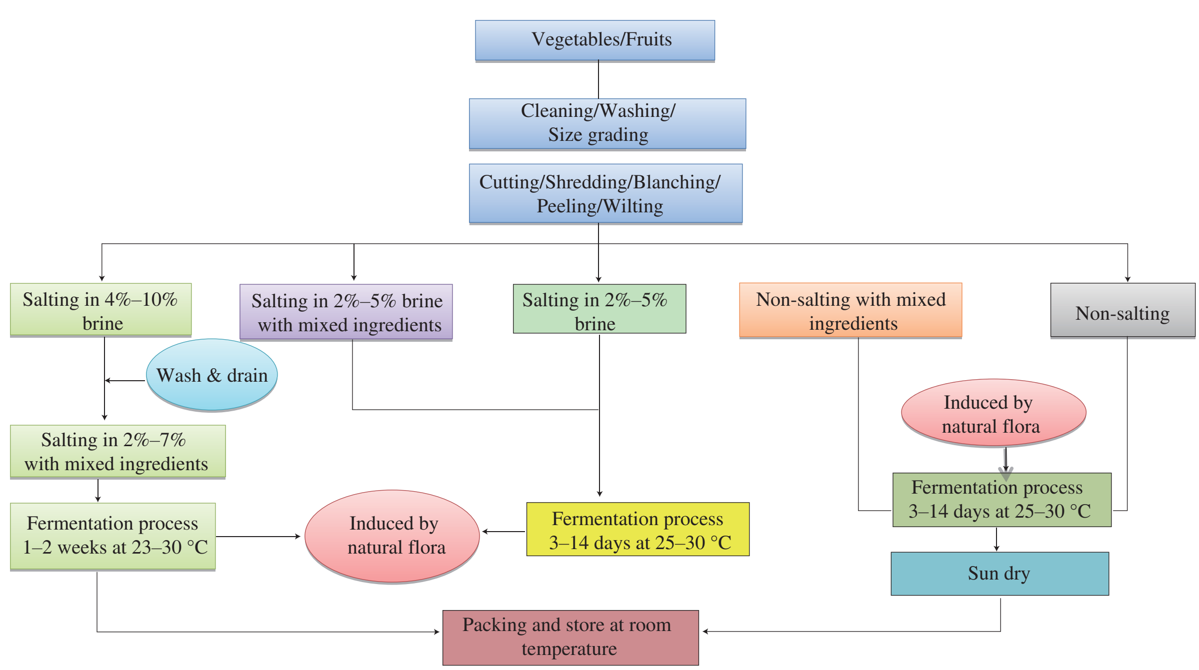

Fig. 2 Common steps in the bioprocess to produce Malaysian fermented fruits and vegetables.

Table 2
Nutritional composition (per 100g) and botanical name of selected vegetables and fruits of Malaysia supply.

Plants	Scientific Name	Region	Utilized for	CHO (%)	Protein (%)	Fat (%)	Dietary fibre (%)	P (mg)	K (mg)	Mg (mg)	Ca (mg)	Fe (mg)	Vitamin C (mg)
			Vegetables										
Bamboo shoot	*Bambusa tulda*	Malaysia	Jeruk rebung	6.92	3.69	0.48	3.97	700	20	40	1300	1.57	1.42
Cucumber (Timun Dayak)	*Cucumis sativus*	Sarawak/Sabah	Jeruk	0.6	0.6	2.1	0.8	109	206	12	14	1	0.0
Green onion (Bawang)	*Allium wakegi*	Sarawak/Sabah	Jeruk	9.34	1.10	0.10	1.7	29	146	0.13	23	0.21	7.4
Napa cabbage (sawi putih)	*Brassica rapa* L.	Malaysia	Sawi putih	4.7	2.1	0.7	—	70	—	—	147	6.8	89
			Flower buds										
Garlic	*Allium sativum*	Malaysia	Jeruk bawang puith	33.1	6.36	0.5	2.1	153	401	25	181	1.7	31.2
Etlingera	*Etlingera coccinea*	Sabah	Tuhau (Dusun)/tepus (Iban)	2.46	0.44	0.37	7	286	194	327.1	100	0.32	1.05
			Leafy vegetables										
Cassava leaves	*Manihot esculenta Crantz*	Sarawak	Daun ubi/ Dawon tawi (Bidayu and Malays	33.7	29.28	3.19	16.25	22	56	0.42	39	2.02	—
Cabbage	*Brassica oleracea*	Malaysia	Jeruk	3.4	1.6	0.2	0.9	9	103	12	40	0.6	53.0
Wild Malaysian brassica	*Brassica Juncea* (L.) *Czern* var. *Ensabi*	Sarawak	Ensabi iban/Ensabi Dayak	4.51	2.56	0.47	2	48	136	13	118	0.87	25.3
Maman leaves	*Cleome gynandra* L.	Malaysia	Jeruk maman	4.5	4.8	0.8	4.3	49	478	48	189	2.6	64
			Fruits										
Wild mango	*Mangifera pajang kostermans*	Sarawak/Sabah	Bambangan (Sabah) Malay Sarawak (asam embang/buah embawang) Iban (buah mawang)	21.0	1.13	1.98	5.26	17	120	—	7	0.4	46.31
Asam Paya	*Eleiodxa conferta*	Sarawak	Jeruk asma paya	11.8	0.8	3.1	1.7	10	227	22	26	5.5	0.6
Ambarella (unripe)	*Spondias dulcis*	Sarawak/Sabah	Kendong	10.4	1.76	0.34	20.25	4520	—	11.76	32.76	0.8	5.50
Kepayang (Pangium)	*Pangium Edule*	Sarawak	Kepayang/peyang Dusun (pangi)	10.0	1.4	6.8	—	26	355	17	7	0.5	3.4
Dabai	*Canarium odontophyllum*	Sarawak	Jeruk dabai	22.1	3.8	26.2	—	65	810	106	200	1.3	—
Petai (bitter bean)	*Parkia speciosa*	Malaysia	Jeruk petai	13.2	6.0	1.6	—	115	341	29	108	2.2	—
Nenas (Pine apple)	*Ananas comosus*	Malaysia	Jeruk	—	1	1	4	8	109	12	13	0.3	47.8
Durian	*Durio zibethinus*	Malaysia	Tempoyak	30.9	2.5	0	13	39	478	48	189	2.6	6.4
Cempedak (Jack fruit)	*Atrocarpus champeden*	Malaysia	Mandai	25.4	1.9	0.4	1.5	41	407	27	37	1.9	14
Mangga (Mango)	*Mangifera indica*	Malaysia	Jeruk Mangga	15	0.82	0.38	1.6	14	168	10	11	0.16	36.4
Limau nipis (Lime)	*Citrus × aurantiifolia*	Malaysia	Jeruk limau nipis/jeruk limo	10.9	1.76	0.83	0.32	20	1.30	1.88	26	0.3	30
			Root and tuber										
Radish	*Raphanus sativus* L.	Sarawak/Sabah	Jeruk lobak	2.4	0.7	0.1	1.6	14	71	---	38	0.9	23.4

manufacturing both edible and inedible produce, but also to offer some added nutritional values to the food product such as attractive aroma, better taste, high biological value, and health benefits [53-56]. Some of these processed fruits and vegetables contain lycopene, β-carotene, anthocyanins, phenolic which act as a powerful antioxidant, to reduce the risk of cancer [57], macuar degeneration with age [58], cardiovascular disease, as well as coronary afflictions [59]. In another instance, flavonoids and vitamin C from fermented vegetable were linked to the prevention of free radical formation and lowering blood pressure in the body [60,61]. Processed vegetables have gained a reputation for their beneficial properties on intestinal health, immunity and social well being [62]. However, to improve the stability of the food products obtained and their nutritional value, prebiotics are added to their composition [57]. The significance of LAF vegetables and fruits as a sustainable food technology for the world has become well acknowledged by the Food and Agriculture Organization (FAO) [63,64]. The most studied fermented vegetable and fruit products are classified as follows.

Vegetable juice: carrot, turnips, tomato pulp, grapes and lemon.

Flower bud: bell peppers, garlic, cucumbers, and onions.

Fruits: durian, lemons, unripe mangoes, Dabai, apples, and Ambrarella.

Tuber and root vegetables: sweet potatoes, ensabi leaves, turnips, radishes, and onions [65].

3. Traditional Malayisa fermented foods as novel sources of natural probiotics cultures

In the Peninsular Malaysia and Northern Borneo island of Malaysia, ethnic fermented fruit and vegetable products like Bambangan, Jeruk, Tuhau, and Tempoyak are prepared from highly perishable fruits and vegetables for consumption and further storage. *L. plantarum*, *L. delbrueckii*, *L. paracasei*, *Weissella*, *Leuconostoc*, *Streptococcus*, *Oenococcus*, *Pediococcus*, and *Enterococcus* are found to be predominant LAB involved in traditionally fermented fruits and vegetables (Table 1). Some of the LAB spp. might also possess functional and protective properties that render them interesting candidates to be used as a starter culture to optimize and control the production of processed vegetables and fruits products [19,66,67].

3.1 Bambangan (Asam embang/Buah mawang)

Bambangan is a local indigenous Sabah Borneo fermented condiment made from wild mango (*Mangifera pajang kostermans*) fruits, with brownish skin flesh, grow in the forest of Borneo island [32]. To the Sarawak Malays, the brown wild mango is called asam embang or buah embawang, while the Sarawak Iban call it buah mawang. It is traditionally processed by mixing the bambangan cubes and its grated seed with salt (2%–5%) and allowed to ferment at room temperature (25–30 °C) for 1–2 weeks in a tightly closed container. The process of bambangan remains a cottage industry among Dusun and Kadazan race (ethnic people in Sabah). Bambangan is consumed with deep-fried fish with plain white rice. *L. plantarum*, *L. delbrueckii*, *L. brevis*, *L. paracasei* and *P. pentosaceus* were identified to be isolated from bambangan (fermented wild mango) [68].

3.1.1 Jeruk

Jeruk is a type of fermented pickle locally made from Malaysia. It is made from vegetables and fruits, which are too pungent or sour to be consumed in excess or fresh during the surplus seasons. The most common vegetables are ginger, gherkins, onion, cucumbers, leek, bamboo shoots, chilli, and leafy tropical vegetables depending on the availability as well as season. Young immature fruits commonly pickled include pineapple, papaya, kedong, and mango. Jeruk is one of the favourites and economic food for low-income rural groups. To make Jeruk, the pickled fruits and vegetables are separated, cut into small pieces and soaked in a tightly closed jar for 2 days in 4%–10% brine. Then, they are washed and the liquid is squeezed off. The medium is then soaked again for 3 days in 6% brine and 2% sugar in a tightly closed container, followed by fermenting at room temperature for weeks or months. *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus* spp. were LAB isolated from jeruk [35].

3.1.2 Jeruk asam paya (pickled swamp fruit)

It is a type of fermented pickle locally made from native Sarawak. To make jeruk asam paya, the pickle asam paya fruits (*Eleiodoxa conferta*) are separated, washed and soaked in a tightly closed jar at 25–30 °C for 2–3 days with salt, sugar and then squeezed to drain off the liquid. The medium is then soaked again for 3 days in 2% sugar in a closed airtight earthen pot and stored for weeks or months for fermentation. *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus* spp. were LAB isolated from jeruk asam paya [36].

3.1.3 Jeruk lobak (pickled daikon)

Jeruk lobak (daikon/radish pickle) is a prominent pickle root vegetable in the native Borneo region and Malaysia. Fresh lobak (*Raphanus sativus* L.) is washed and cut into smaller pieces and mixed with 5% salt (NaCl) and 1%–2% sugar. All of the ingredients are mixed well and then kept fermented at 23–25 °C for 2 weeks, then dried under hot sun for one week. In addition, sugar is added optionally depending on the recipe. Jerk lobak can be added to the chicken porridge and Johor laksa (Spaghetti dish). Rahman et al. [23] isolated a new *L. brevis* and *L. plantarum* from this source.

3.1.4 Jeruk petai (stinky bean pickle)

Jeruk petai is a famous pickled fruit in Malaysia. Fresh petai (*Parkia speciosa*) is washed and then soaked and boiled for several hours until the skin can be separated from the seed. Cleaned petai seed is mixed with 5%–7% salt, layered in a jar. The petai seeds are then washed, soaked with 5%–7% salt in a sealed jar container at 25–30 °C for 3–7 days. In addition, chilli is added for a spicy taste (optionally). If it is still not salty, add a little more salt. *L. plantarum*, *L. brevis*, *P. pentosaceus* are the predominant strains in jeruk petai [23].

3.1.5 Jeruk maman (pickled maman)

Jeruk maman, is a popular traditional fermented leafy vegetable food product in Perlis, Kelantan,Terengganu and several states in Malaysia. The leafs of maman (*Cleome gynandra* L.), belonging to the family Capparaceae, are cleaned, washed, wilted. Water from cooked rice is mixed to provide fermentable sugar to ensure that adequate acidity is created during the production. The mixture is maintained at 23–25 °C for 3–4 days fermentation. Jeruk maman is often used as flavoring vegetable in chicken or fish stews and a variety of traditional cooking recipes that are popular. *L. plantarum*, *L. futsaii*, *L. paralimentarius* and *P. pentosaceus* microflora were reported from Jeruk maman [23].

3.1.6 Jeruk limau nipis/jeruk limo (lime pickle)

Limau nipis or limo (pickled lime) is a salt pickled fruit in Malaysia. Ripened limes are collected, washed and immersed in warm water (60–65 °C) for 5 min. They are then cut into suitable pieces, submerged in 5%–10% salt in a closed container at 25–30 °C for 1 day, before allowing for natural fermentation. After fermentation, limes are removed and sun dried until the skin becomes brown. Borah et al. [44] isolated a new *L. delbrueckii* strain from this product.

3.1.7 Jeruk mangga (green mango pickle)

Mangga (pickled mangoes) is a popular pickled fruit in Malaysia. Fresh unripe mangoes are washed in clean water and drained. Outter skin of the mangoes are removed and sliced into long pieces. The sliced mangoes are submerged in brine solution in an earthen pot at 23–25 °C for 2 days. After the exuded solution is drained off, fermented naturally mango slices are mixed with chilli and sugar. The ingredients were mixed well and then fermented at 23–25 °C for one day and then consumed as a condiment. Microflora that take part during jeruk mangga fermentation are *P. cerevisiae*, *L. brevis*, *L. plantarum*, *L. mesenteroides* [43].

3.1.8 Jeruk rebung (pickle bamboo shoot)

Jeruk rebung a common processed food of Malaysia is prepared from the tip of young bamboo shoots. The main source of this food ingredient is the middle parts of the young bamboo shoots (*Bambusa tulda*). The outer skin, uppermost and lower part of the shoots are removed. The middle part of the shoots is sliced into sections and submerged in water and left to ferment naturally at 23–25 °C for 4–7 days. The sour taste of jeruk rebung is as a result of acid produced by LAB during process. In addition, NaCl is added optionally to have a sour taste. The dish can be fried after adding a little salt so that later there will be a unique taste to the fried jeruk rebung, this fried dish can be consumed with rice. Rahman et al. [23] isolated a new *L. plantarum* and *L. brevis* strains from this product.

3.1.9 Mandai (cempedak/jack fruit)

Mandai is the fermented inner-skin of jack fruit or cempedak (*Atrocarpus champeden*) product, commonly consumed by the Banjarese people in Malaysia. The main source of the microbes for spontaneous fermentatiton process is the epidermis of cempedak (jack fruit). The outer skin of the cempedak fruits were removed and epidermis are cleaned, immersed in 5%–16% salt solution in an earthen pot. The fermentation is carried out at 23–25 °C for 7–14 days. The process is achieved by certain plant derived micoflora of LAB such as *L. plantarum*, *P. pentosaceus*, *L. casei*, *L. vaccinostercus*, *L. harbinensis*, *L. perolens*, *L. casei* and *L. paracasei* [40-42]. It is usually used as a seasoning and cosumed as a side dish with rice.

3.1.10 Sawi pahit

Sawi pahit is a well known traditional fermented vegetable foodstuff to the Bidayuh in Sarawak (Malaysia Borneo). Leaves of white mustard (*Brassica rapa* subsp. *pekinensis*), belonging to the family Brassicaceae, are obtained, washed, wilted, cut into smaller pieces, mixed with 5% salt (NaCl). The mixture is kept at 23–25 °C for 4–7 days in an airtight container. In addition, porridge is added optionally to have a sour taste. Sawi pahit can be added to steam fish. Rahman et al. [23] isolated a new *L. plantarum* strains.

3.1.11 Tempoyak

Tempoyak, an ethnic durian pulp (*Durio zibethinus*) fermented condiment obtained through spontaneous LAF in Malaysia. Tempoyak is prepared from the mixture of durian pulp with a small quantity of salt and maintained at ambient temperature in a sealed vessel for approximately one week. The total acidity content of tempoyak is found to be around 2.8%–3.6%. However, the salt concentration remarkably affects the aroma, sourness, colour, and sweetness of the product, with the most preferred tempoyak being with 2% NaCl [69]. The sour taste of tempoyak is due to the total acidity produced by LAB strains during production. *W. paramesenteroides*, *E. faecalis*, *E. gallinarum*, *L. plantarum*, *L. brevis*, *L. fermentum*, *L. mali*, *L. mesenteroides*, *L. casei*, *P. acidilactici* are the predominant strains in tempoyak [28,29,36].

3.1.12 Pickled Kai choy/Gai choy

It is an ethnic low salted, fermented and acidic leafy vegetable food product of Malaysia, especially the Malaysian Chinese. During fermentation of Kai choy, harvetsed leafy vegetables (Brassicaceae spp.) are washed, cleaned, wilted and mixed with 4%–8% NaCl. The mixture is kept at 23–25 °C for 7 days in an airtight container. Sugar, turmeric, garlic, ginger are optionally added to give the dish a distinct flavour. Kai choy is often used in several types of cusine in Malaysia such as spicy sour meat and fish as well as other dishes. *L. plantarum* microflora was reported from Kai choy [23].

3.2 Unexplored Malaysia traditional food sources as potential novel sources of natural probiotics culture

Till date, many rare indigenous Malaysian fermented fruit and vegetables foods have been selected by numerous authors across the globe to isolate novel lactic acid strains as natural sources of probiotics. However, many potential lactic acid strains are still unexplored. There is plentiful prospect available for microbiologists to explore these fermented diets, such as buah kepayang, dabai, daun ubi and ensabi etc., for the isolation of new lactic acid strains for their potential role to ehance diet quality and sensory properties.

3.2.1 Buah kepayang

Buah kepayang (Pangium pickle) is a common pickled fruit in Sarawak (Malaysia), especially in the Dayak area. The fruit pickles (*Pangium edule*) are usually left to ripen until the flesh falls away, after which the seeds are removed. In the most common method, the seed is washed, cleaned and boiled (at least more than three hours) to make it easier to break open their skin. After the cooling phase, the seeds are broken into the skin and only the brown meat inside was taken, placed inside a basket, tied to the riverbank, submerged in the river for 5–7 days (to completely remove the intoxicating substance). Watery rice, salt and pork are sometimes added to proceed fermentation at 25–27 °C. However, brown meat is mixed with NaCl, allowed to ferment at 25–27 °C in an earthware container for 30 days, but it may be elongated even more than 6 months. Buah kepayang is mostly used as a seasoning for chicken, meat, and various other diets. The microbiological aspects of buah kepayang are still unknown [39].

3.2.2 Dabai (Canarium odontophyllum)

Dabai (dabai pickle) is a popular pickled fruit in Borneo Malaysia especially Sarawak. Fresh dabai is washed, soaked with hot water in an earthenware container for 10 min. After the removal of the exuded water, the dabai is mixed with the salt and maintained at 25 °C for one month in an earthenware container for fermentation. Salted dabai can be added to rice and it can be cooked together to create a unique local delicacy called dabai fried rice. The microbiological aspects of the dabai pickle are still unknown [34].

3.2.3 Daun ubi/Dawon tawi

Daun ubi (Cassava leaves) is another common customarily fermented leafy dish product of the natives in Sarawak (Malaysia), especially by the Dayak. Mustard leaves are clean, washed, squeezed with 2.5%–4.5% salt. Water from cooked rice is mixed to provide fermentable sugar to ensure that adequate acid is created during the production. The mixture is maintained for 3–4 days fermentation, along with coconut milk sometimes at room temperature. This dish is consumed with plain white rice. The microbiological aspects of daun ubi are still unknown [33].

3.2.4 Ensabi (wild Malaysian brassica leaves)

Ensabi is a dish prepared by Malays and some natives in Sarawak and Sabah (Malaysia), especially the Iban, Dayak, and Kenyah. In Sarawak the Dayak and Iban called it Ensabi, the Kenyah called it Ambek. Wild Malaysian brassica leaves, locally called ensabi, are obtained, washed, rinsed with water and roots of ensabi vegetables are removed. The vegetable is dried, wilted overnight, squeezed with 2%–4% salt. Water from boiled rice is added to serve as an appetizer during the 7 days fermentation at 25 °C. The dish can be fried after adding a little sugar, so that later there will be a sweet taste to the fried ensabi, this fried dish can be consumed with bleary fish [33]. The microbiological aspects of the ensabi pickle are still unknown.

3.2.5 Tuhau

The (*Etlingera coccinea*) is a traditional side dish made by the natives in Sabah (Malaysia Borneo), especially by the Dusun who lives in the rural areas. Tahau pickle is prepared from fresh tuhau, which is collected, cleaned with water, pounded with mortar and pestle to maintain the aroma. The pickle is properly mixed with lime juice, chilli, onions, belacan, salt, and sugar. The mixture is allowed to stand for 1 or 2 days before consumption. This dish can be consumed or accompanied by marinade fish, plain white rice and nasi-goreng (fried rice). The microbiological aspects of tuhau pickle are still unknown [39].

3.2.6 Kedondong

Kedondong is a type of fermented pickle locally made from native Sarawak (Malaysia Borneo). Kedondong pickle (ambarella pickle) is made from unripe kendong (*Spondias dulcis*) fruit, which is collected, peeled, cut into quarters, washed and then drained off. The mixture is transferred into an airtight vessel, with an added sprinkle of coarse sugar to give a nice taste, followed by a small amount of salt or chilli. The mixture is maintained at 25 °C for 7–14 days. The dish is used as an appetizer or sometimes as a dish in some vegetarian cafes. The microbiological aspects of kedondong pickle are still unknown [37].

3.2.7 Jeruk bawang putih (garlic pickle)

Jeruk bawang putih is widely used local fermented side dish with *Allium sativum* in both Peninsular Malaysia and native Borneo region. It is prepared by boiling sugar, salt as well as ginger together. The mixture is placed in the airtight jar, left to ferment at 25 °C for 1–2 weeks after cooling. Pickled garlic not only raises the appetite but also makes kerabu rice (herb rice) dishes to be more delicious. The microbiological aspects of Jeruk bawang putih are still unknown [23].

4. Probiotic microbial cells

4.1 LAB as source of probiotic

Lactobacillus is a very heterogeneous genus in the LAB group which is generally associated with food and feed production [70,71]. It is used as starters and probiotics inoculants in processed food [72-74]. The genus *Lactobacillus* are classified as Gram-positive bacteria that is characterized by the formation of lactic acid, constituting the majority of the group of LAB species [75,76]. Major genera such as *Enterococcus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, *Oenococcus*, *Streptococcus*, with *Lactobacillus* are also considered in the LAB-probiotics group because of their LAF ability [77,78]. *Lactobacilli* is widely employed as a starter, silage inoculants, as well as probiotics in processed food [72,79]. The *Lactobacillus* genus belongs to the major part of the LAB group, which are Gram-positive species producing lactic acid through fermentation [80,81]. LAB-probiotics include *Oenococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Weissella* and *Leuconostoc* species [25,82]. *Lactobacillus* are rodshaped, often organized into chains, representing the major group within the family Lactobacillaceae. They are aerotolerant, strictly fermentative, but grow well in anaerobic environments. *Lactobacilli* can be divided into heterofermentative and homofermentative species based on the fermentation potential of the carbon source [25]. The homofermentative species convert glucose almost entirely to lactic acid, while heterofermentative, ferment glucose into lactic acid, ethanol, and CO_2 [72,83]. LAB can affect the flavour of processed foods in various ways. During the metabolic process, lactic acid is generated because of the fermentation of fermentable carbohydrate. As a result, the sweetness taste may decrease as sourness and acidity increase [84-86,52].

Lactobacilli prefer acidic environments because their primary catabolite is lactic acid (pH 5.5–6.5) [87]. The species originated from a wide range of environmental niches such as animals, plants, milk, insects and birds [88]. Because of the wide variety of environment, *Lactobacillus* possesses a broad range of versatile metabolites they can use as substrates within the lactic acid group [80]. It is widely applied in food preservation and starter culture for the production of dairy, fruit, meat, cereals, fish, vegetable, as well as animal feedstocks in the form of silage [75,76,89]. Many species of *Lactobacillus* are frequently selected as a potential probiotic strain due to their preventive and therapeutic properties in humans [25,90,91]. The health properties associated with these strains include lowering of gastrointestinal symptoms in lactose-intolerant consumers, relieving constipation, prevention and treatment of travellers' and infantile diarrhoea, as well as provide bioactivity against *Helicobacter pylori*. Although *Lactobailli* is often described as traditional inhabitants of the human tract, they are more likely to be autochthonous of the oral cavity or fermented diet [92-95]. *L. fermentum* is considered a human probiotic because of its efficacy and proven history of safe use in processed food. Besides, it is also one of the best known species of the genus *Lactobacillus* in a LAB group, and have been isolated from fermented vegetable, fruits, and dairy [96,97].

The *Pediococcus* spp. are non-spore forming microbes with coccoid shaped, facultatively anaerobic, non-motile, homofermentative, Gram-positive, catalase-negative with sugar degradation characteristics [98,99]. They belong to the family Lactobacillaceae, order Lactobaillales, class Bacilli and phylum Firmicutes. *Pediococci* are found along with *Lactobacilli* and *Leuconostoc* spp. in plant habitats. They have more in common physiologically with these organisms than with the *Streptococci*, which are more associated with animal habitats. The genus constitute of 12 species at the period of this study: *P. stilesii*, *P. siamensis*, *P. pentosaceus* (with 2 subspecies intermedius & pentosaceus), *P. parvulus*, *P. inopinatus*, *P. ethanolidurans*, *P. damnosus*, *P. claussenii*, *P. cellicola*, *P. argentinicus*, *P. acidilactici* and *P. lolii.* Species of *P. urinaeequi*, *P. dextrinicus* have been reported to be reclassified in the genera *Aerococcus* and *Lactobacillus* respectively [100,101]. Within this genus, members of Pediococcus (*P. damnosus* and *P. claussenii*) are well known for their negative role in ethanol fermentation such as beer [102-104]. The majority species of the *Pediococcus* (mostly *P. acidilactici* and *P. pentosaceus*) are used in the food sector as starter cultures for processed products, as well as probiotic products because of their preservative activities of pediocin [105]. In addition, it has been claimed that *P. pentosaceus* is one of the frequent species isolated from food source, beverages, pickles, meat, dairy as well dairy products [106-108]. *P. pentosaceus* has been considered as one of the food-grade strains because of its potential role as starter cultures in processed food [109-111]. Besides, several investigations have also stated that strains of *P. stilesii* to be the bacteriocinogenic LAB in food preservation [112].

4.2 New conceptualization on definition of probiotics

The word 'probiotics' actually means 'for life', but in terms of healthy or beneficial microbes, probiotics can be stated as 'viable or unviable microbial cells (ruptured or intact; spore or vegetative) that is potentially healthful to the host' [113]. According to the definition, the probiotic concept are classified into three classes; referred to as 'pseudo probiotic' (viable and inactive cell), 'ghost probiotic' (dead/non-viable/inactive cell in forms ruptured), as well as "true probiotic" (active and viable microbial cell). Each are classified into two primary classes, based mainly on their site of action (*in vitro* & *in vivo*). For example, if a probiotic microbial cells produce healthy bioactive molecule and metabolite in a dietary matrix, it should be considered as "true probiotic external"; whereas, when this active and viable microbial cells are transformed into the body to perform its specific biofunction in a target organ, it is referred to as a "true probiotic internal". If the ealier probiotic cells become inactive (but still viable) in diet, the class is known as "pseudo-probiotic vegetative external". In contrast, ruptured probiotic microbial cells in diet are referred to as "ghost probiotic ruptured – external"; however, when microbial cell fragments of these probiotics are consumed in a diet or supplement to impart their health benefits, the probiotic is regarded as "ghost ruptured internal".

4.3 Selection criteria and application for probiotics

Before a probiotic can benefit human health, it must fulfil several criteria as presented in (Fig. 3) [114]. It must have good technological properties so that it can be produced and incorporated into food products properties without losing viability and functionality; it must survive through the upper gastrointestinal (GI) tract and arrive as alive at its site of action, and it must be able to function in the gut environment.

The number of active and viable microbial cells in probiotic dietary products at the period of intake is the most critical value for these products, as it determines their efficacy [115,116]. It has been recommended that probiotic products should be taken regularly to deliver a sufficient quantity of viable cells to the intestine [117,118]. Moreover, the dose of 10^6–10^8 CFU/mL is accepted as the minimum and satisfactory levels. In addition, probiotic microorganisms are quite sensitive to some environmental stresses such as microbial antagonism, temperature, food matrix, oxygen, pH as well strains of probiotics [25].

4.4 Potential mechanisms of action of probiotics

Probiotics have numerous, diverse effect on the host. The basic mechanism of action by which the probiotics confer nutritional value on consumers includes modulating the mucosal function barrier, decreasing epithelial cells apoptosis, improving the mucin building [119,120]. On the other hand, probiotics also help in

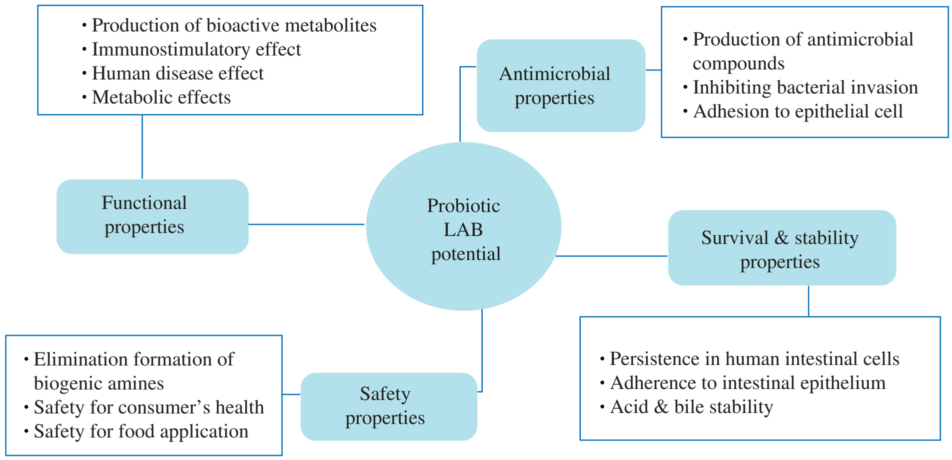

Fig. 3 Basic properties for the selection of probiotic microbial cells.

Table 3
A comprehensive summary of the output of the processing conditions applied to vegetable and fruit prior to LAF or storage of treated and fermented beverages and vegetables.

Reference	Product	Method	Processing condition	Remarks/Outputs
[128]	Fresh mango	(Non thermal sterilization)	Mixing mango with distilled H_2O (1:2, *m/V*); squeezing; ultraviolet assisted ultrasonic (US-UV)); fermentation (37 °C, 12 h) by a mixed probiotic strains (10^8 CFU/mL) of 1:2:1 (*V/V*, *L. bulgaricus*, *S. thermophlus*, *L. plantarum*); cold storage (4 °C, 30 days) in the dark	US-UV pretreatment improved the essential qualities of fermented mango + storage stability+ prolonged its shelf life during storage compared to fresh juice & traditional thermal processing.
[129]	Apple	Non thermal/Thermal	Impregnation of apple with the probiotic; pretreatment: air drying (AD), freeze drying (FD), freeze drying followed by microwave vacuum drying (FD+MVD) and air drying followed by explosion puffing drying (AD+EPD); powered samples; Homogenization; incubation (37 °C, 48 h); Storage (25 °C, 120 days)	FD+MVD exhibited a better drying method for the production probiotic enriched dried apple snacks in color, texture, sensory quality, cell viability and storage stability; Probiotic cell in FD+MVD-dried samples remained above 1×10^6 CFU/g for 120 days at 25 °C. Bacterial viability in FD+MVD-dried samples turned out to be significantly higher than FD-dried samples during storage for 120 days.
[130]	Litchi	Thermal	Treated litchi; heat treatment (pH 4.56, 95 °C; 1 min); high hydrostatic pressure (HHP) sterilization (pH 4.56, 500 MPa, 2 min, 25 °C); *L. casei* fermentation (5.6 pH, 30 °C, 18 h)	Fermented HHP-treated litchi juice displayed a better color, flavor, overall acceptance than heat treat; HHP fermented juice exhibited improvement of total phenolic and antioxidant capacity; Both HHP and Heated fermented litchi juice enriched cell viability.
[131]	Broccoli	Thermal treatment	Pretreatment (pack processing & direct hot water blanching); homogenized (4 h, 25 °C); stored (-20 °C) until use; fermentation (30 °C for 15 h for pre-treatment samples; 24 h, 30 °C for control samples); stored (4 °C, 25 °C) for 14 days.	Both pre-heating fermented broccoli-based products enhance the sulforaphane content. Pack pre-heating treatment (65 °C, 3 min) exhibited better sulforaphane yield, remained stable (93% retention) during storage at 4 °C for 2 weeks.
[132]	Mixed vegetable juices	Non thermal	Pretreatment (ultraviolet (UV) and ultrasonic (US)); fermentation (*L. plantarum*)	Both UV & US of mixed vegetable juices ensured safety microbial safety with increase *in vitro* bio-accessibility of bioactive compounds; fermented juice exhibited to improvement of the bioactive profile
[133]	Huyou	Non thermal/Thermal treatment	Sterilized dimethyl dicarbonate (DMDC); pasteurized; fermentation (*L. plantaum* L1: *L. fermentum* L2)	Fermented DMDC-sterilised huyou juice exhibited better color, odor and overall acceptability in the sensory evaluation; both DMDC sterilized and pasteurized fermented huyou juices maintain cell viability
[134]	Yacon, litchi, longan (YLL)	Thermal/Non thermal	Bleaching (100 °C, 2 min); soaking (0.4% vitamin C); squeezing; filtering; thermal treatment (TT) (100 °C, 30 s); HHP sterilization (300, 400, 500 MPa, 15 min, 25 °C); stored (20 °C) till use; fermentation (*L. rhamnosus*, 30 °C, 18h), *Gluconacetobacter xylinus* (30 °C, 24 h, 160 r/min)	Both processing of juice complied with aseptic standard as well microbial safety; HPP preserved the nutritional and aroma properties of juice; TT lowered the flavor molecule and taste of the juice. Fermentation of HPP juice improved the aroma, flavor and taste. HPP co-fermentation enriched the quality of YLL juice
[52]	Coconut juice	Thermal	Coconut water filtration; pasteurized (90 °C /5 mins); fermentation (*L. lactis*, 30 °C, 48 h, Stored (4 °C, 4 weeks)	TT Fermented coconut juice exhibited no change in sensory evaluation; TT fermented juice maintain cell viability during (4 weeks, 4 °C).
[135]	Pomegranate juice (PJ)	Thermal	Pasteurization (65 °C, 20 min); fermentation (*L. plantarum*, *L. acidophilus*, and *S. cerevisiae* , 25 °C, 48 h); HPP (400, 500 and 600 MPa) for (3, 5, 7 and 10 min), 20 °C; TP (65 °C, 20 min), stored (4 °C) until use.	HHP fermented PJ and pasteurized fermented PJ maintain stable pH, titratable acidity and total soluble solids. HPP fermented PJ shows higher levels of bioactive compounds and volatile compound than thermal one.

inhibiting uncontrolled cancer cell growth, inhibiting bacteria spore outgrowth by the host [121,122]. Probiotic strain cultures support the function as an anti-inflammatory agent in the intestine, inhibiting cell damage by scavenging the free radicals [123-125]. However, the mechanism of action may vary from one probiotic strain to another and are, in most cases, probably a combination metabolic processes, thus making the investigation of the responsible mechanisms a very difficult and complex task.

5. Processing technologies before probiotic microbial fermentation

Vegetables and fruits are commonly subjected to biological, chemical and physical (thermal and non-thermal) processes before utilization, consumption and preservation of the product quality [126]. Thus, the processing is done to ensure the safe lactic flora but also maintains the sensory quality of the product which might be affected by bio-enzyme activity and chemical reaction. The efficacy of processing is strongly dependent on the complex composition of the matrix, as well as their lactic flora diversity, which may trigger the choice of process and process factors [77,127]. Physical processes with different intensities are the most widely practised pre-treatments of fruit and vegetable before LAF, due to their simple equipment and easy operation. A comprehensive summary of the output of the processing conditions applied to vegetable and fruit before LAF or storage of treated and fermented beverages and vegetables is shown in Table 3.

Besides, thermal and non-thermal process (Fig. 4), other physical treatments are applied to vegetables and fruits before drying, such as infrared heating, skin puncturing and peel abrasion.

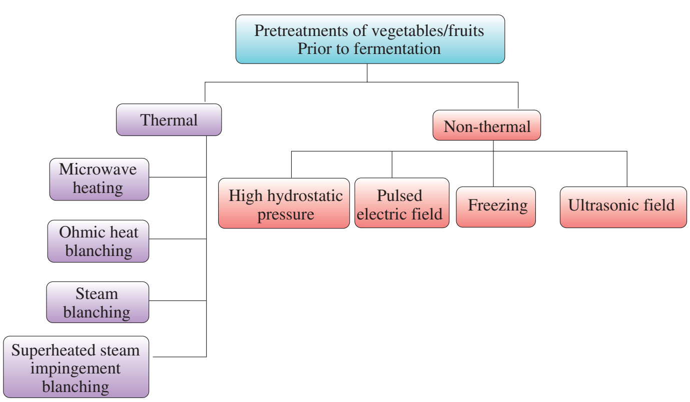

Fig. 4 Pretreatment techniques of vegetables and fruits before the fermentation process.

Yong et al. [136] reported the effects of different mechanical and non-chemical pretreatment on the drying performance of dragon fruit. The study demonstrated that mechanical pretreatment (drilled holes and pin holes) can be a viable treatment that offers notable improvement in the drying rate of dragon fruit. The drying rate increased with an increase in the diameter and density of the holes. It was also reported that drill hole pretreatment lowers the drying time to complete the drying process, and decrease browning of pepper and red pigment loss [137]. Adiletta et al. [138] investigated that, physical wax abrasion of the goji berries peel reduced the dry time (to 15 h) and the pretreated sample had better colour, higher antioxidant activity, maintaining the same sugar content compared. Adiletta et al. [139] also stated that the peel abrasion with sand paper lined in a rotating drum and found treated sample reduced drying time (up to 1/3) as compared to untreated control. Moreover, either skin puncturing and peel abrasion are not suitable for industrial application as it is a tedious, complex and costly process.

Infrared radiation energy, with a suitable wave length range, could penetrate directly into the material or desired compounds thereby leading to blanching and the energy transfer is highly efficient [140]. The previous study has shown that high moisture removal was achieved through infrared radiation heating of grape seed by 7.20%, improved the microbial reduction, free fatty acid content (10.39%) and peroxide value respectively when compared with the control sample [141]. Its advantage application over conventional drying methods, such as higher energy efficiency, less process period, as well as better quality in the finished product [142-144]. However, the infrared energy does not penetrate deeply and heats up only a few centimeters beneath the surface of the sample, causing severe water loss of the samples and has been the bottleneck for drying application.

6. Role of common ingredients used for fermenting fruits and vegetables

6.1 Ingredients favouring microbial growth activities

The functions of some added ingredients in lactic acid fermented fruits and vegetables are to improve the development of lactic microbial flora. They have 4 major contributions, such as (i) source of essential bionutrients (energy/nitrogen source), which influences the speed of the metabolic process (ii) they supplying pleasant aromatic active compound to processed product (iii) inhibiting the food borne pathogen by increasing the acidity (iv) aides in improving sensory profiles of lactic acid fermented product [54].

Aside from adding flavour, cooked rice liquid provides fermentable sugars to some vegetables such as mustard leaves (ensabi & daun ubi), to ensure that sufficient acid is produced during the metabolic process [33,145]. In Malay style *Pangium edulele* fermentation, *P. edulele* has undergone thermal treatment to remove their toxic substance before being submerging in water. They are then added with watery rice to enhance the LAF process. Honey is often recommended for utilising in traditional lactic acid fermented fruit metabolic processes, as it has high sugar content, which is a good source to produce LAB [146-148]. It also supplies nitrogenous and mineral source for lactic microbes' activity and biochemical reactions [149].

6.2 Antiseptic property of ingredient

Aromatic plants (herbs & spices) have been utilized as a food ingredient to some lactic acid fermented fruits and vegetable, not only to improve the sensory properties of the end product but also to increase the shelf life [134]. Certain spices such as garlic, not only show antibacterial and nitrate reduction properties [150] but also give special favour to lactic acid fermented pickle fruit [151]. Other antiseptic active ingredients derived from diverse sources such as plants (e.g., essential oil (EO) from clove, rosemary, oregano, basil, cinnamon, thyme), natural polymers (e.g., chitosan), algae, microbes (e.g bacteriocins, nisin, natamycin), organic acids (e.g., sorbic, citric acid) as well as, animal tissues (e.g enzymes) provide natural bioactive compounds with antibacterial and antioxidant properties, which limit the growth of undesirable microbes while promoting the growth of LAB [152]. For instance, the combination of LAB with EO thyme showed a good positive effect on the shelf life and safety of the sliced apples and lamb's lettuce product, when applied as biocontrol agents without affecting their quality parameters [153].

6.3 *Addition of salt*

In addition to the development of the brine and enhancing flavour, salt (NaCl) has an important microbiological role. The optimum salt concentration for fruits and vegetables varies, depending on the type of fruit or vegetable [154]. The replacement of 1.7% NaCl and 1.7% $CaCl_2$ with 0.85% KCl + 0.85% NaCl on the quality of salgam production from black carrot did not change the microbiological, sensory and chemical properties of the final food product [155]. Moreover, the utilization of KCl + NaCl improved LAF in salgam. Another function of salt is to draw water out of microbes and slow the growth of pathogens [156] as well as to reduce unwanted chemical reactions like lipid oxidation and enzymatic browning [157].

6.4 *Addition of vinegar*

Another important ingredient widely used in lactic acid fermented fruits and vegetables is vinegar. Specifically, vinegar is used for the preservation and picking of fermented beverages and vegetables [158], but is mostly associated with traditional fermented pickle made of vegetables such as tursu [159]. For instance, the addition of vinegar in traditional tursu production provides a low pH level for initial fermentation condition. This condition encourages the growth of LAB and inhibits the growth of yeast [160].

7. Beneficial health effects of fermented vegetables and fruits

Fermented fruits and vegetables, enjoyed across the globe, conveys nutritional health benefits through LAF. Traditional fermented food plays a vital role in affecting the final flavour and extended shelf life [161,162]. Therefore, over the past few decades, food technologists have increasingly reported the nutrition benefit of lactic acid fermented vegetables and fruits. Owing to the anti-nutritional reduction, and nutritional values and medical benefits, especially for human safety.

7.1 *Reduction of anti-nutritional factors*

Most fermented fruits and vegetables contain naturally accruing anti-nutritional and toxin biocompound such as chymotrypsin, trypsin, lectins, spaonins, oxalates, glycosides, phytates and tannins [13]. These biocompounds reduce the nutritional value of fruits and vegetables by interfering with the digestibility of carbohydrate, proteins and mineral bioavailability [163]. However, these can be detoxified or removed by the action of natural or pure strain during the fermentation system [163]. For example, the fermentation bioprocess that produced the Malaysia Borneo product, daubi ubi, removes the toxins from the leaves of *Manihot esculenta*, since fermentation is an important step in ensuring that cassava leave is safe for consumption.

7.2 *Improving nutritional values and safety*

Nutritional values of foods can be increased by the fermentation process, which might provide an attractive and functional addition to food products [52,163]. The foodstuffs constitute the multiplication

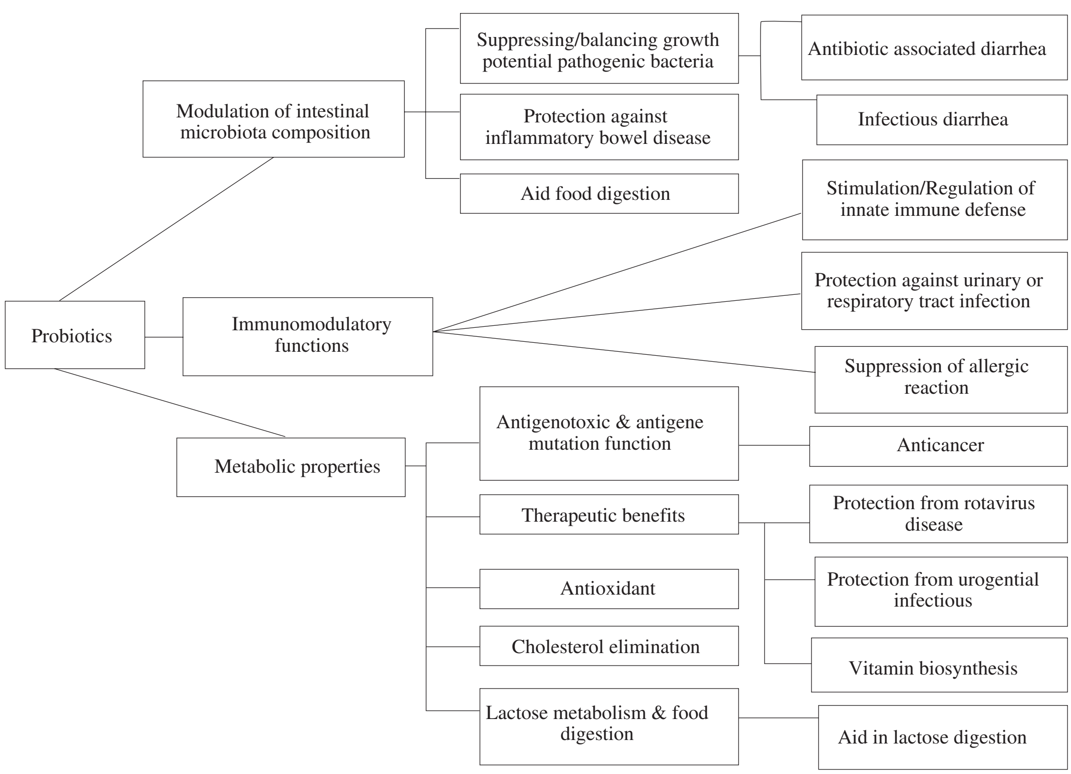

Fig. 5 Health benefits of probiotic LAB.

of the minerals, amino acids, vitamins concentration as well as antioxidant activities of the starting product. For instance, tempoyak (lactic fermented product consumed in Malaysia) is high in mineral and vitamin. A wide variety of antimicrobial substances (lactic acid, butyric, propionic, acetic, formic, bacteriocins, hydrogen peroxide) are produced during the metabolic process, decrease the pH [164,165]. It has been considered that these acids are responsible for the inhibition of the growth of non-desirable microbes that may decompose or spoil the food [166,167].

Apart from its nutritional profile, fermented food also possesses a variety of enzymes many of which have the potential to influence the quality of foods. Amylase in fermented food product degrades the starch to simple sugar (glucose). Glucose plays a remarkable role in maintaining the health of the body. Similarly, phytases are produced by LAB can degrade the phytic into a digestible compound. This decrease in phytate can increase the bioavailability of zinc, iron, calcium by large fold folds in fermented food [168].

7.3 Therapeutic benefits

There are a lot of myths about the therapeutic properties of processed food products. In Borneo Malaysia, the Dayak and Malay ethnic groups firmly believed that eating fermented foods can protect them from illness. Tempoyak (a fermented durian product in Malaysia) has been reported to possess immunostimulatory properties [169].

However, there are some scientific proofs to these assertions; including the following:

(i) The lowering of the pH inhibits the development of spoiling microbes and reduced deterioration in the food.

(ii) Most non-specific antimicrobial and bacteriocins substances are produced by some strains of LAB (*L. acidophilus*) [170].

(iii) The exopolysaccharides produced from LAB have been reported to have beneficial physiological effects on consumer's health [171-173].

(iv) Compounds such as bacteriocins, H_2O_2 as well as weak organic acid (lactic & acetic acid) in fermented foods have been reported to have an immunostimulatory effect to suppress the growth of pathogens, bacterial vaginosis and other sexually transmitted diseases [174-176]. Overview health benefit of probiotic LAB to the host are depicted in Fig. 5.

The fermentation process is the ancient practice of removing and detoxifying anti-nutrient from Buah kepayang (Pangium pickle).

8. Molecular typing tools used for analyzing LAB in fermented vegetables and fruits

In addition to the conventional or classic approaches (plate count, microscopy), several well-characterized molecular methods like species-specific PCR, real-time quantitative PCR (qPCR), Random amplification of polymorphic DNA (RAPD), Repetitive extragenic palindromic sequence-based PCR (rep-PCR), Amplified fragment length polymorphism (AFLP) Denaturing gradient gel electrophoresis (DGG), Temperature gradient gel electrophoresis (TGGE), Amplified ribosomal DNA restriction analysis (ARDRA), and 16S rDNA sequencing are applied to classify and identified various types of LAB flora, isolated from fermented vegetables and fruits [177-179]. For instance, Chen et al. [180] identified 178 LAB isolated from fermented watermelon (xi-gua-mian) from a different region in Taiwan using RFLPs and 16S rDNA sequencing gene. The results confirmed *L. plantarum* has the most populated LAB in xi-gua-mian obtain from southern Taiwan, whereas *P. pentosaceus* is the dominant species in Northern Taiwan. A PCR-DGGE method followed by 16S rRNA sequencing gene fragments eluted from the interested bands on denaturing gradient gels was applied to study changes in the microbial flora of two industrial kimchi (salted cabbage) and mixed ingredient samples during 1-month processing at 4 °C, as well as 10 °C. *Leuconostoc* genus is reported as predominant LAB flora at 4 °C over *Lactobacillus*. *W. confusa* was also found throughout the processing in both samples at 4 and 10 °C. *Leuconostoc gelidum* was the predominant LAB identified at 4 °C in both samples [181]. A combination of RFLP and 16S rDNA sequencing was applied to characterize 2 non-identical LAB species (*E. durans & E. Faccalis*) from red dragon fruit juice. Despite the fact it is used in food fermentation, the threat it poses to human health must not be overlooked [182]. Tamang et al. [183] isolated 60 LAB flora from soijim, soibum, seidon and mesu (processed bamboo shoot) samples, and investigated their genotypic applying DNA-DNA hybridization, sequencing analysis of 16S rDNA, species-specific PCR, rep PCR and RAPD-PCR. *E. durans*, *Leuconostoc citreum*, *L. lactis*, *L. fallax*, *P. pentosaceus*, *L. mesenteroides*, *L. curvatus*, *L. plantarum*, *L. brevis* are predominant LAB flora associated with bamboo shoots fermentation. Hong et al. [184] also used two SDS-PAGE and PCR DGGE based method to investigate LAB diversity in the salted Chinese cabbage (Kimchi). In addition, the PCR-DGGE approach detected a wider diversity of LAB species, including non LAB species. Likewise, using a combination of MALDI-TOF MS finger printing and phenylalanyl tRNA synthase sequencing gene was applied to identify LAB flora from Vietnamese fermented vegetables [185]. The use of MALDI-TOF MS finger printing to identify LAB isolates resulted in a comprehensive and detailed description of the diversity of LAB found in associated Vietnamese fermented foods. A previous study has investigated the microbial strain population dynamics in a fermented capper berry using a combination of RAPD, TGGE and 16S rDNA [186].

9. Future trends: towards probiotics applications

Fermented dairy-based products are regarded to be a good matrice for the delivery of probiotics and recently, fermented vegetables and fruits have shown their suitability as novel non-dairy probiotic foods. In general, a genus of *Lactobacillus* is applied in most probiotic functional and industrial activities (Table 1). Sequencing gene technology and genomic tools will play a role in the rapid screening of LAB flora and allow for high throughput study of the mechanism and functionality of LAB as probiotics [187,188].

10. Conclusion

In the Peninsular Malaysia and Borneo region, fermented vegetables and fruits are linked to various cultural, social and economic trends of different ethnicities. Studies suggest that vegetables and fruits could serve as an appropriate delivery for natural probiotic species. Fermented vegetables and fruits comprising of a diverse group of prebiotic components, which can stimulate and enhance probiotic strains production. The volume of additives and dietary ingredients in the processed foodstuffs such as monosodium glutamate, honey, sugar and salt should comply with acceptable standards set by the target market regulations. Mixed culture production with greater variability can be substituted by pure culture to attain large-scale fermentation. Though some issues remain unaddressed, it is feasible that processed foods, handed down for several generations, may play a pivotal impact on world food security. In-depth investigations on the characteristics and microbial composition of fermented fruits and vegetables from traditional and indigenous sources in both Peninsular Malaysia and Borneo could lead to further applications of these natural probiotic cultures in the mainstream global food industry.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgements

The authors want to thanks Sofia Abdullah, Fanta Abby Jawen, Timila Jahie, Ngieng Ngui sing and Universiti Malaysia Sarawak for the support of this research.

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