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Local pineapple waste as potential bio-ingredient

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Abstract

The pineapple by-products possess desirable nutritional and physicochemical properties, making it worthwhile to explore their use as a potential functional food element. The study aimed to evaluate the proximate composition and the potential use of MD2 pineapple waste such as peel and pomace, as a growth medium for probiotic bacteria, such as lactic acid bacteria and bifidobacterial. The pineapple pomace was obtained after the pineapple pulp was pressed for the juice. Proximate analysis and the growth of lactic acid bacteria were carried out on the dried and ground pomace. The proximate composition of MD2 pineapple peel and pomace was attained in dry matter (g/100 g) including protein (5.14 g, 6.34 g), total fat (1.14 g,1.09 g), total carbohydrate (85.07 g, 82.75 g) ash (2.42 g, 2.04 g), moisture (6.22 g, 7.79 g), crude fibre (17.4 g,19.39 g) and energy (371 kcal, 366 kcal). The growth performance of *Lactobacillus paracasei* and *Lactobacillus acidophilus* in pineapple peel and pineapple pomace as an alternative bio ingredient in growth media was significantly increased to log 2-log 3 at 72 hrs of incubation and was evidenced by the lowered pH of the growth medium for each bacterium. The results showed that pineapple waste is a good candidate as a bio ingredient to support the growth of *L. paracasei and L. acidophilus* and has the potency to be a potential prebiotic for probiotic strains.

1. Introduction

Pineapple (*Ananas comosus*) belongs to the genus *Ananas* (Bromeliaceae), is a perennial monocotyledonous shrub, and is one of the tropical and subtropical typical fruits (Li *et al*., 2019). The fruit is rich in sugar, proteins, vitamins and minerals and emits a sweet fragrant smell when matured with a sweet and sour taste and crispy juicy flesh (Li *et al*., 2019). Pineapple is high in carbohydrates, and fibres while also rich in vitamins A (carotene) and vitamin C (ascorbic acid) moreover a great source of B6, E and K vitamins, as well as containing, calcium and iron (Devi *et al*., 2015; Sarkar *et al.,* 2020). Furthermore, it is also rich in antioxidants, the phenolic compounds including gallic acid, catechins, epicatechin and ferulic acid (Campos *et al*., 2019; Azizan *et al.*, 2020). These compounds are associated with antimutagenic, anticarcinogenic and antioxidative effects as well as protective effects against cardiovascular diseases and cataracts (Seenak *et al*., 2021). Pineapple leaves have also been reported to have antidiabetic and antioxidant properties on streptozotocin (STZ)-which induces experimental rats (Kalpana *et al*., 2014). The MD2 cultivar is a hybrid pineapple that was developed from two types of pineapples namely the Smooth Cayenne PRI hybrids 58-1184 and 59-443 through a breeding process (Greig, 2004). The MD2 cultivar has a longer shelf life and is the sweetest compared to the pineapple cultivars (Thalip *et al.*, 2015). The flesh contains about (0.05-0.3% acidity) which is less acidic and comprises of 12-19°Brix of total soluble solids and is more fragrant compared to other pineapple cultivars (Achuonjei *et al*., 2003; Wardy *et al*., 2009; Ding and Syazwani, 2016; Siti Rashima *et al.*, 2020). During the pineapple fruit processing approximately 30%-50% of the pineapple biomass is discarded including the crown,

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stem, peel, core pomace and liquid waste (Selani *et al*., 2014). It was estimated that approximately one-third to half of the entire fruit weight is removed throughout the canning process. The disposal of this massive amount of waste is difficult to manage and could cause environmental issues such as in situ pollution that leads to microbial spoilage (Selani *et al*., 2014).

Therefore, researchers are interested to transform these by-products into value-added products (Raji and Sarode, 2019). Since the pineapple by-products possess desirable nutritional and physicochemical properties it is worthwhile to explore their use as a potential functional food element to enhance the nutritional quality of foods. Exploiting agricultural by-products would be a better alternative to simple disposal and waste. Furthermore, generating value-added products and functional foods from agricultural wastes can be a cost-effective method in addition to the main product, and could generate side income for agricultural sectors. Utilization of wastes could also solve agriculture waste pollution and at the same time removes the disposal problems towards environmental sustainability. Scientists now are focusing on the development of pineapple by-products, such as producing low-cost fibres, bromelain, enzymes, antioxidants, organic acids, biogas, ethanol and prebiotic oligosaccharides (Awasthi *et al*., 2022). The transformation of pineapple waste into a prebiotic supplementation as an ingredient in food could also be an approach to reducing agricultural waste. Such approaches in recycling agricultural waste can significantly reduce the accumulation of waste and lessen the demand for primary raw materials (Campos *et al*., 2020). A prebiotic is a selectively fermented ingredient that permits a particular effect, both in the composition and/or activity in the gastrointestinal microbiome which presents advantages to the host's well -being (Gibson *et al*., 2004). Fructooligosaccharides, galactooligosaccharides, inulin, resistant starch, and lactulose are several types of prebiotics obtained from different sources such as fruits and vegetables that are non-digestible and possess prebiotic properties (Sah *et al*., 2016). Therefore, this study aimed to evaluate pineapple by-products, namely the peel and pomace nutritional value and their potential as prebiotics for the lactic acid bacteria, and the probiotic strains through in vitro fermentation.

2. Materials and methods

2.1 Preparation of ground pineapple paste

The peel and pomace of MD2 pineapple were obtained from KOSAS (Koperasi Anak Selangor) Farm at Kampung Kundang, Banting, in the state of Selangor, Malaysia. The pomace is the by-product of the pineapple after the process of juice extraction. The fruit pulp was pressed for the juice and the juice was collected for the commercial production of pineapple cordials and the pomace would be the by-product attained from this process. The peel and pomace from the farm were transferred into the laboratory and the pH was checked with a pH meter (S40 Seven Multi TM, Metter-Toledo, Switzerland) and the total soluble solid was recorded using a hand refractometer (Atago, Japan). The samples were then stored in a -80^{o}C freezer prior to use. For testing, the peel and pomace were thawed at room temperature and then washed and rinsed with tap water to remove impurities. After that, they were pressed using a helical press to remove any remaining juices. The pressed peel and pomace were then dried in a hot air drying oven at 50-55°C (Memmert, USA) for about 48-72 hrs. The moisture of the peel/pomace was determined by a moisture analyser. The ideal value of the moisture was between 5.5-6.5%. The dried peel and pomace were then ground into small pieces using a Waring blender and milled with 0.5 µm mesh sieve, Ultra Centrifugal Mill ZM 200 (Retsch, Germany) following the method of Garcia-Amezquita *et al*. (2018) with some modifications. Finally, the powder was packed and sealed in an airtight container and stored at 7-25°C prior to use.

2.2 Proximate analysis

The proximate composition of the pineapple peel and pomace samples including moisture (Method 950.46), ash (Method 923.03), fat (Method 991.36) protein (Method 981.10), and crude fibre (Method 962.09) was determined according to the method of AOAC 1995. The total dietary fibre was determined using the K-TDFR-100A commercial kit (AOAC 991.43) Megazyme International Ireland Ltd. Bray, Ireland (Ramdath *et al*., 2020). The calculation of carbohydrate content in the samples was done according to the method described by Pomeranz and Meloan (2002).

2.3 Moisture

The AOAC method 950.46(B) was used to measure the moisture content of the dried pomace powder.

2.4 Crude protein

The crude protein of peel and pomace were defined by experiments were performed in triplicate (AOAC 981.10).

2.5 Fat

The crude fat was determined by the Soxhlet extraction method of AOAC 991.36.

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2.6 Ash

The ash was determined by AOAC 923.03.

2.7 Crude fibre

The crude fibre content of the sample was determined according to AOAC 962.09.

2.8 Total dietary fibre

Total dietary fibre was examined (AOAC 991.43) using a commercial kit (K-TDFR-100A; Megazyme International Ireland Ltd. Bray, Ireland) on triplicate samples of dried pineapple peel and pomace powder according to the manufacturer's protocol (handbook of Megazyme TDF test kit).

*2.9 Total carbohydrat***e**

The total carbohydrate was estimated by difference to calculate accessible carbohydrates by difference, the other constituents in the food analysed earlier such as protein, moisture, ash and fat are summed and subtracted from the total weight of the food.

Total CHO, 100 – (weight in grams [protein+fat+ash+moisture] in 100 g of food (FAO, 2003).

2.10 Energy

The calorie content was determined based on the fat, protein, and carbohydrate contents using the formula:

$$\text{Calories (kcal/100 g)} = (C \times 4) + (P \times 4) + (F \times 9)$$

where, C = carbohydrate content (g/100 g), P = protein content (g/100 g), and F = fat content (g/100 g).

2.11 Microbial fermentation

2.11.1 Probiotic microorganisms

Probiotic strains *Lactobacillus acidophilus* DDS1 NRRL B-3208, *Lactobacillus paracasei* subsp. *paracasei* NTU101 and *Bifidobacterium bifidum* BB12 probiotic strains were obtained from UAS Laboratories, The Probiotic Company (Wasau, Wisconsin, USA). The stock cultures were kept in 20% glycerol and stored at -20°C. The strain was cultured in de Mann Rogosa Sharp (MRS) broth (Oxoid, Basingstoke, UK) and incubated for 24 - 48 hrs at 37°C prior to use. Working cultures were also maintained on MRS agar slants and stored at 4°C and were continuously subcultured monthly. After the incubation periods colony counts were done via the pour plate method by appropriate 10-fold dilutions onto MRS agar and incubated anaerobically for 24–48 hrs using Anaerocult C (Merck) pads. The viable cells of the inoculum were counted in triplicate. The homogenized inoculum (1 mL) was taken to measure the optical density (OD 600nm) of the bacterial suspension. Absorbance (A600 nm) was adjusted to 0.25±0.05 in order to standardize the number of bacteria (10^7–10^8 CFU/mL). These routines were done to ensure the number of cells is within the range number of bacteria (10^7–10^8 CFU/mL) before the fermentation process.

2.11.2 Fermentation

The bacterial cells from 24-48 hrs cultures were harvested by centrifugation (10,000×*g*, 10 mins, 10°C), washed twice with phosphate-buffered saline PBS pH 7.2 (0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4) and suspended in the same buffer. Approximately 1% (w/v) of the homogenised solution was added to 150 mL basal medium that comprised of (1.5-3 g/L) peptone, yeast extract (2 g/L), NaCl (0.1 g/L), K_2HPO_4 (0.04 g/L), KH_2PO_4 (0.04 g/L), $MgSO_4.7H_2O$ (0.01 g/L), $CaCl_2$ (0.01 g/L), $NaHCO_3$ (2 g/L) and Tween 80. The pH was adjusted to pH 7.2 using the phosphate buffer mentioned earlier. This basal medium was slightly modified as proposed by Palframan *et al*. (2003) and Azmi *et al*. (2012). The medium containing peel and pomace were prepared separately in triplicate. The peel and pomace powder were added in 1-2% (w/v) into the basal medium prior to sterilization at 121°C for 15 mins. The cooled pineapple peel and pomace media were then inoculated with the selected stains under aseptic conditions and then statically incubated at 37°C. The pH was measured and the strains were quantified at 0, 24, 48 and 72 hrs (Oberoi *et al*., 2021).

3. Results and discussion

3.1 pH and total soluble solid of fresh sample

Fresh samples of MD2 pineapple waste, peel and pomace were measured for pH and total soluble solids (TSS) (°Brix) and the data are presented in Table 1. The pH value of fresh peel and pomace were 4.72 and 4.86 respectively. The TSS measured from the peel and pomace were 14.8 and 15.4, respectively. The results show that there is a correlation between the (TSS) and pH where increased TSS lowered the pH, and the acidic taste of the peel and pomace. This is in agreement with a previous study by Thalip *et al*. (2015) who reported similar findings on MD2 pineapple flesh, in addition to the high TSS value being associated with the redness and yellowness of the pineapple flesh. Upon ripening the TSS value in pineapples rise due to the breakdown of starch to glucose, sucrose, and fructose. Pineapples consist of a variety of organic acids mainly citric acid, that is responsible for the tart flavour of the fruit. Thus, during the maturity stage of the pineapples, the pH of the fruit would also significantly increases (Thalip *et al*., 2017).

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Table 1. pH and total soluble solids of MD2 pineapples peel and pomace.

Pineapple Waste	pH	Total Soluble Solid (°Brix)
Peel	4.72	14.8
Pomace	4.86	15.4

3.2 Proximate composition

The proximate composition which includes protein, fat, total dietary fibre crude fibre, moisture, carbohydrate and calorie content of the pineapple peel and pomace are shown in Table 2. All the data obtained were expressed in g/100 g.

3.2.1 Crude protein composition

The crude protein content was 5.14±0.06 and 6.34±0.3 g/100 g for pineapple peel and pomace respectively. As observed the crude protein content of the pineapple peel was lower compared to the pomace. This contradicted the findings of Sah *et al*. (2016) whose values were 6.89±013 and 3.91±0.09 g/100 g for peel and pomace respectively. This discrepancy is probably due to a few factors particularly the different maturation index of the fruit and the different methods and techniques of sample preparation. The amount of crude protein from freeze-dried pineapple pomace from a study done by Selani *et al*. (2014) was 4.71±0.28 g/100 g while the crude protein from the solid pineapple waste reported by Abdullah and Mat (2008) accounted for 5.18±00 g/100 g. The protein content in the peel is usually higher compared to the pomace because the peel still contains some of the flesh while the pomace has been thoroughly pressed for the pineapple juice. Overall, the protein contents in both peel and pomace were too low to be regarded as a good source of protein

The World Health Organization (WHO, 2007) recommended a daily protein intake, which is 0.8 g/kg of body weight per day, for all age groups and disregarding gender, health status and physical activity. This recommendation is based on short-term nitrogen balance studies. For people with limited, average, and higher physical activity, respectively, dietary intake of protein is 1.0, 1.3 and 1.6 g/kg body weight per day is advised to meet functional needs such as boosting skeletal-muscle protein accretion and physical strength. Healthy people can consume protein for an extended period at 2 g/kg BW per day, and well-adapted subjects can consume up to 3.5 g/kg BW per day in the absence of any adverse influence (Wu, 2016).

3.2.2 Total dietary fibre

The total dietary fibre content was high in the peel and pomace 57.82 g/100 g and 55.76 g/100 g respectively. The values are to that of pineapple peel and pomace powders in Sah *et al*. (2016), they were 57.76 g/100 g and 46.19 g/100 g, respectively. Current recommendations for dietary fibre intake for adults in most European countries and in the US are between 30–35 g per day for men and between 25–32 g per day for women (Stephen *et al*., 2017). The finding showed that pineapple peel and pomace are good sources and options for dietary fibre. Dietary fibres are composed of soluble and insoluble dietary fibre and soluble dietary fibre is closely related to prebiotics. Prebiotics is valuable and is a food for probiotic which inhabit the human and animal colon. Probiotics play an important role to maintain the good health and well-being of the host (Davani-Davari *et al*., 2019). Altan *et al*. (2011) reported that as low as 3%-5% of dietary fibre was able to bring a positive effect on fish growth, though a too high fibre level for example more than 12% may lessen the digestibility of dry matter and the efficiency ratio of other nutrients.

3.2.3 Carbohydrate

The total carbohydrate content in the pineapple peel was found to be 85.07 g/100 g while for pomace it was

Table 2. Proximate analysis of pineapple peel and pomace powders by oven-dried technique.

Proximate analysis	Pineapple Waste		Test Method/Technique
	Peel	Pomace	
Moisture, g/100 g	6.22±0.16	7.79±0.09	AOAC 16th Ed.950.46
Ash, g/100 g	2.42±0.06	2.04±0.01	AOAC 16th Ed.923.03
Total Fat, g/100 g	1.14±0.03	1.09±0.04	AOAC 16th Ed.991.36
Protein, g/100 g	5.14±0.06	6.34±0.3	AOAC 16th Ed.981.10
Crude Fibre, g/100 g	17.4±0.08	19.3±0.24	AOAC 16th Ed.962.09
Total Dietary Fibre, g/100 g	57.82±0.33	55.76±1.2	K-TDFR-100A; Megazyme International Ireland Ltd. Bray, Ireland.
Total Carbohydrate (%CHO = [100 -(%moisture + (%Ash +fat + %protein	85.07±0.09	82.75±0.22	Method No. STP/Chem/A06 based on Promerance Food Analysis: Theory and Practice, 2nd Ed. (page 637)
Energy, kcal/100 g	371±0.00	366.33±0.58	Method No. STP/Chem/A03 based on Pearson's The Chemical Analyses of Foods 6th Ed. (page 578)

Values are presented as mean±standard deviation (n = 3).

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82.75 g/100 g. The value of total carbohydrates in pomace was quite similar to the value reported by Sah *et al*. (2016), which was 84.53 g/100 g though the carbohydrate content in peel was 55.87 g/100 g which is lower compared to our study. For example, if a person consumes 2,000 calories per day, between 900 and 1,300 calories should be from carbohydrates, which translates to between 225 g and 325 g of carbohydrates per day. Wilson (1994) recommended 40% of dietary digestible carbohydrates for tilapia while the energy utilisation efficiency of digestible carbohydrates in common carp and Nile tilapia was found to have a (linear relationship) (Schrama *et al*., 2018; Phan *et al*., 2019).

In humans, a healthy, balanced diet consists largely of foods that contain carbohydrates and dietary fibre. Fish nutrition has generally overlooked the importance of a suitable fibre ratio in fish feed and its beneficial outcome on digestion and metabolism (Sulaiman *et al*., 2018). However, when taken in the proper ratio, its benefits can be just as significant as those of the commercial prebiotics now being used to encourage the growth of advantageous bacteria in the digestive system, which has a positive impact on how well feed is utilized by animals such as fish (Markowiak and Śliżewska, 2018). The majority of researchers believe that fibre and carbohydrates have negative effects when incorporated into the diet (Wilson, 1995; Montagne *et al*., 2003; Cummings *et al*., 2004; Tripathi and Mishra, 2007; Eshaghzadeh *et al*., 2015). However, the benefits of incorporating fibre sources in fish meals have been successfully established by Yarahmadi *et al*. (2014) who also reported that doing so could improve fish performance.

Beamish *et al*. (1986) reported that carnivorous species such as salmonids when fed with an extra carbohydrate diet will suffer mortality due to high levels of liver glycogen. Meanwhile herbivorous and omnivorous species such as carp and tilapia are more susceptible to utilising dietary carbohydrates as an energy source (Kamalam *et al*., 2017).

3.2.4 Crude fibre

As for humans consuming a high-fibre diet is related to better gastrointestinal function, lower risk of heart disease, easier weight loss, reduced hypertension, lower risk of some types of cancer and lower in serum cholesterol concentrations (Soliman, 2019). Dietary fibres can be categorized into two types namely soluble fibre and insoluble fibre (Anderson, 1984). Most researchers consider that fibres in the fish diet have a negative effect on fish. Therefore, the significance of a healthy fibre ratio in feed and how it affects nutrition, metabolism, and digestion have not been well investigated (Wilson,1995; Tripathi and Mishra, 2007). However, fibres may in some ways be as significant as commercial prebiotics being presently used. It could be used to encourage the growth of good microbes in the digestive system, which in turn encouraged fish to consume more feed. Various researchers (Yarahmadi *et al*., 2014; Eshaghzadeh *et al.,* 2015; Lumsangkul *et al*., 2021) had successfully proven the advantage of adding fibre into fish pellets and recommended further investigation into its benefits in stimulating fish growth and immunity. Sulaiman *et al.* (2018) demonstrated that without compromising its growth, feed efficiency, or body composition, the hybrid tropical carp could withstand 15% dietary crude fibre. However, they also reported that the development of the fish and feed regulation was severely reduced when fish were fed with 20% fibre. In addition, Anderson (1984) stated that a dietary fibre level of above 10% seemed to affect the dietary protein and energy retention for omnivorous tilapia, thus dietary fibre levels over 10% are not advised.

The amount of crude fibre of pineapple peel and pomace was found to be measured at 17.4 g/100 g and 19.3 g/100 g, respectively. Anderson (1984) claimed that a decrease in gastrointestinal passage time (GPT) and food digestibility is related to the growth reduction in carnivorous rainbow trout fed on 10% fibre. Insoluble fibre can be included in feed formulations at a maximum of 15.5% without negatively influencing animal growth performance or health Bonvini *et al*. (2018).

3.2.5 Fat

The fat contents were generally low the amount of fat in the pineapple peel and pomace was estimated to be 1.14 g/100 g and 1.09 g/100 g, respectively. The total calories of human consumption suggested by the World Health Organization and the Dietary Reference are about 20-35% of total fat intake (FAO, 2010). A total of 20% is the minimum amount of total fat that is adequate for essential fatty acids, fat-soluble vitamins and total energy, moreover preventing atherogenic dyslipidemia that happens due to low-fat, high carbohydrate diets and high risk of coronary heart disease (FAO, 2010).

3.2.6 Ash

It is very important to measure the ash content in food as it can affect the food's characteristics in terms of physiochemical and nutritional properties. It is also crucial to determine the safety of the food because the ash content determines the amount of toxic minerals present in the food. The ash content in food is varied and can be an indication of how much processing has taken place as natural foods have a lower ash content

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compared to more processed food. The ash content in the pineapple peel and pomace was recorded at 2.42 g/100 g and 2.04 g/100 g respectively of dry matter, which means they are at a safe level for consumption (Ismail, 2017).

3.2.7 Energy

The estimation of energy requirement is based on a factorial approach, which expresses energy requirement/ expenditure, as well as its various components, as multiples of basal metabolic rate (BMR) (RNI, Ministry of Health Malaysia, 2017). The energy requirement also known as the energy demand is the amount of food energy required to maintain a body's size, composition, and a degree of essential and desirable physical activity consistent with long-term good health (FAO, 2001). This comprises the energy required for children's healthy growth and development, the implantation of tissues during pregnancy, and the production of milk during nursing in a manner that promotes the well-being of both mother and child (WHO, 2016).

The energy average allowance for men of reference size (77 kg) is 2,300 kcal/day; for women, it is 1,900 kcal/day (National Research Council (US) Subcommittee, 1989). The energy or calorie contained in 100 g of pineapple peel and pomace were estimated accordingly based on the fat, protein, and carbohydrate contents, using calculation as mentioned in the Methods section and the results are 371 kcal and 366.33 kcal respectively.

3.3 Propagation of selected probiotics strain in pineapple peel and pomace

The selected probiotic strains *L. acidophilus* DDS1 NRRL B-3208, and *L. paracasei* subsp. *paracasei* NTU101 were examined for the ability to ferment pineapple peel and pomace. The basal media as mentioned previously was separately supplemented with 1-2% of pineapple pomace and peel separately and was inoculated with the strains obtained from the pellet of 24 -48 hrs full-grown strains under aseptic conditions. The growth kinetics of the strains are depicted in Figure 1 while the pH is shown in Figure 2. Generally, all of the strains grew effectively in the medium containing peel or

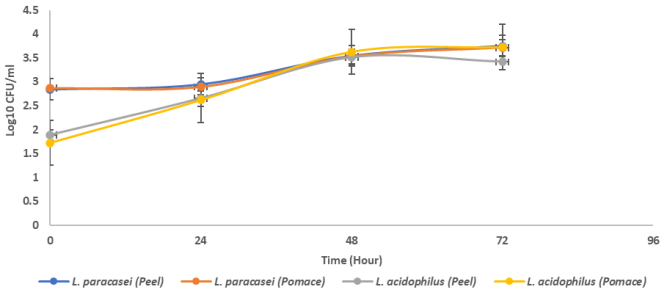

Figure 1. Growth curve and proliferation of probiotic strains in $\log_{10}$ CFU/mL. Values are presented as mean±standard deviation (n = 3).

pomace as the sole carbon source. The number of bacterial cells was increasing by at least one log prior to the incubation period. In both the pineapple peel and pomace media, the bacterial growth was at least more than one log compared to the negative control. The growth curves and proliferation values in $\log_{10}$ CFU/mL of probiotics growing in peel and pomace medium during incubation are presented in Table 3. It is clear that the tested probiotic strains could utilize pineapple waste as a growth medium for propagation. At 72 hrs of incubation in peel and pomace medium, *L. acidophilus* DDS1 NRRL B-3208 produced 3.417 and 3.725 $\log_{10}$CFU/mL) respectively while *L. paracasei* subsp. *paracasei* NTU101 produced 3.748 and 3.714 $\log_{10}$CFU/ mL, respectively peel and pomace respectively.

The pH of the media was observed to decrease through the fermentation period (Figure 2) because lactic acid bacteria break down carbohydrate substrates for propagation and vitality and the major fermentation

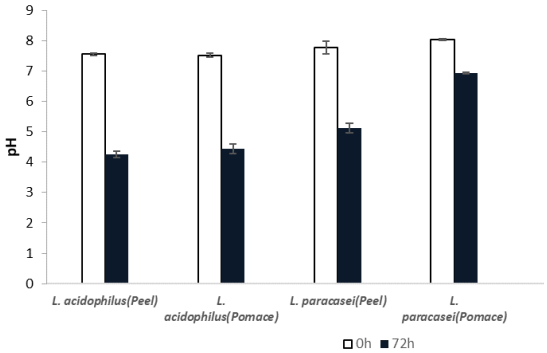

Figure 2. pH of the probiotic strains tested on 0 hr and 72 hrs in pineapple peel and pomace. Values are presented as mean±standard deviation (n = 3).

Table 3. Propagation of probiotics strains *L. paracasei* subsp. *paracasei* NTU101, *L. acidophilus* DDS1 NRRL B-3208 in pineapple peel and pomace at 0 hr and 72 hrs.

Probiotic Strains	Log_{10} CFU/mL			
	Peel		Pomace	
	0 hr	72 hrs	0 hr	72 hrs
Lactobacillus paracasei subsp. *paracasei* NTU101	2.843±0.03	3.748±0.0	2.869±0.05	3.714±0.16
Lactobacillus acidophilus DDS1 NRRL B-3208	1.886±0.10	3.417±0.02	1.723±0.32	3.725±0.03

Values are presented as mean±standard deviation (n = 3).

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products are mainly lactic acid, acetic acids and short-chain fatty acids (Cummings, 1987). The peel and pomace powders in this study supported the growth of the probiotic organisms due to the high dietary fibre content, which other studies have reported to be mostly cellulose, hemicellulose, lignin, fructans, pectin, and pectic substances (Huang *et al*., 2011; Chitturi *et al*., 2013) in addition some studies have reported pectin-derived oligosaccharides (Gullón *et al*., 2013). The prebiotic strains lowered the pH in the peel and pomace medium to a final value that ranged from pH 6.93 to 4.26.

4. Conclusion

The pineapple peel and pomace dried powder contain high amounts of dietary fibres and carbohydrates, that can be contemplated as an outstanding source of bio-ingredients for the growth of probiotic strains, in particular, the *L. acidophilus* DDS1 which showed the best growth in this study. Moreover, the ash content was at a safe level for consumption while the protein and fat contents were negligible. Although the crude fibre content was quite high it did not exceed 20 g/100 g. Therefore, it is advisable to lessen the amount of the peel and pomace if they are to be used in the fish feed. This study has proven that the probiotic bacterial strains, were able to grow on the pineapple peel and pomace, as evidenced by the exponential increase in CFU/mL and the lowered pH of the growth medium after 72 hrs of incubation, the latter indicating that the probiotics strain fermented the pineapple waste and consequently producing various organic acids. In conclusion, this study has shown that the waste from the pineapple processing industry, namely the peel and pomace have the potential to be used as a prebiotic for probiotic bacterial strains that can be beneficial for human and animal health.

Probiotics usually produce short-chain fatty acids namely acetic acid, propionic acid and butyric acid as a byproduct of the fermentation. In vitro fermentation of the pineapple by-product such as peel and pomace in this study exhibited an increasing number of bacterial growth. A significant characteristic of the substrate as an ingredient for the growth of microbes is the ability to improve the proliferation of the microbes successfully. In this study *L. acidophilus* DDS1 is a tested probiotic strain candidate that exhibited promising outcome, due to the increasing number of bacterial colonies and decreasing pH of the pineapple waste substrate, peel and pomace during the fermentation period, 0 to 72 hrs. In conclusion, the peel and pomace of the pineapple are potential bioingredients to support the growth of *L. acidophilus* DDS1. In the future, further studies on pineapple waste as a prebiotic source should be explored.

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