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Determination of nutritional value and the effect of strain, inoculum size, temperature, and incubation period on pH fermentation of *Ipomoea batatas*

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Abstract

Ipomoea batatas, also known as sweet potato, belongs to the Convolculaceae family, and serves as one of the most important food crops worldwide. In the present work, the nutritional values and physicochemical properties of selected sweet potato varieties, namely VitAto (orange), Gendut (yellow), and Anggun (purple) were evaluated. Results indicated that VitAto had the highest content of crude fibre (2.02%), ash (0.65%), and carbohydrate (25%) as compared to Gendut and Anggun. The high carbohydrate content of VitAto makes this variety suitable as a substrate in the development of high nutrition products through food bioprocessing. The combination of parameters studied, such as strain type (*Amylomyces rouxii* F0050), inoculum size (0.4%), incubation temperature (30°C), and fermentation period (36 h) was found to not only affect the growth of microorganisms, but also improved the pH of culture fermentation. This demonstrated that to obtain the required products or metabolites, proper use of microorganisms to hydrolyse carbohydrates or starches into simpler sugars is very important as a source of carbon to grow the microorganisms.

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Introduction

Sweet potato (*Ipomoea batatas*) is one of the oldest vegetables in the world, and has been used for food for centuries (Parle and Monika, 2015). Alam (2021) reviewed that sweet potato possesses various health benefits owing to their remarkable functional and nutritional properties such as proteins, bioactive carbohydrates, flavonoids, anthocyanins, carotenoids, phenolic acids, and minerals (Alam, 2021). Nguyen *et al.* (2021) mentioned that those constituents vary among different sweet potato varieties, and offer several health benefits, such as anti-tumour, anti-diabetic, cardioprotective, anti-obesity, and antimicrobial properties. According to Dora *et al*. (2018), minerals found in sweet potato such as potassium, is beneficial in managing hypertension and heart disease, calcium is important for bone development, and iron is good for pregnant women. Vitamins A, C, and E found in sweet potato are antioxidants that can help prevent premature birth, cancer, and heart attacks, and have anti-aging effects. Souza *et al*. (2020) reported that sweet potato contains a higher nutrient composition of provitamin A, vitamin C, and minerals as compared to rice and wheat. In addition, good nutritional value is one of the important characteristics used to assess the quality of fruits or vegetables (Abdullah Sani *et al*., 2018). In Malaysia, there are various types of sweet potatoes available in the market, with the varieties of VitAto, Gendut, and Anggun being among the most in-demand.

Sweet potato, being rich in nutrients and a good source of energy due to its high carbohydrate content, has the potential to be used as a substrate for solid phase fermentation in the production of nutritious downstream products (Senthilkumar, 2020). Reportedly, sweet potato has a high starch content ranging from 12 - 25%, thus making it an ideal fermentation substrate (Ray and Ward, 2006; Salelign and Duraisamy, 2021). Various technologies and operations are used to convert perishable raw

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materials into value-added food and beverage products that have high stability (Vilela, 2019).

Solid phase fermentation is an alternative fermentation technique where its characteristics allow the use of by-products as substrate support to produce high-value biomolecules (Soccol *et al*., 2017). In recent times, solid phase fermentation technique has been identified as a potential technology in the field of biotechnology. Solid phase fermentation is a process of microbial fermentation that occurs in the absence or near absence of free water, as well as approaching the natural environment where selected microorganisms, especially fungi, are the most suitable microbes (Manan and Webb, 2017). Chilakamarry *et al*. (2022) proposed this type of fermentation as the best tool to transform agro-industrial wastes into value-added products, since it solves the environmental concern of utilising waste products that have low or no economic value. They concluded that solid phase fermentation is economically sustainable, technically feasible, legitimately acceptable, and ecologically helpful. Therefore, the aim of the present work was to study the potential of sweet potato as a carbon source in solid phase fermentation, and the use of selected inocula such as *Amylomyces rouxii* F0050 and *Saccharomycopsis fibuligera* Y0021 in adding value to downstream sweet potato-based products. To our knowledge, this would be the first investigation using these inocula with VitAto, Gendut, and Anggun sweet potatoes varieties.

Materials and methods

Preparation of sweet potato samples

VitAto, Gendut, and Anggun sweet potatoes were obtained from the Gene Bank Research Farm at MARDI Station, Kundang, Selangor. They were then cleaned, peeled, soaked, and ground into a paste form using a laboratory grinder (Waring Commercial model 32BL79, USA). Ground samples were packed in sealed plastic bag prior to storage in a freezer (Faber model FZ 2180U, Italy) at -20°C (Teh *et al*., 2018). These samples were then used in proximate studies to determine the contents of protein, fat, crude fibre, water, ash, and carbohydrate.

Proximate analysis

Proximate analysis was carried out according to Maisarah *et al*. (2014) to determine the contents of protein, fat, crude fibre, ash, and carbohydrate, while the moisture analysis was conducted according to Suhaime *et al*. (2019).

Microorganism

Two microbial strains namely *A. rouxii* F0050 and *S. fibuligera* Y0021 were used and obtained from the Collection of Functional Food Culture, Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor.

Inoculum production

Three types of inocula namely (1) mould inoculum (*A. rouxii* F0050), (2) yeast inoculum (*S. fibuligera* Y0021), and (3) a mixture of mould and yeast inocula (*A. rouxii* F0050 and *S. fibuligera* Y0021) were used for the solid phase fermentation of VitAto sweet potato. The production of these inocula followed the method described by Merican and Quee-Lan (2004) (Figure 1).

Preparation of sweet potato for solid phase fermentation

Sweet potatoes were cleaned, peeled, soaked, and cut into cubes measuring 1 × 1 × 1 cm using a cutting machine (Hallde model RG 61, Sweden) (Ortiz-Viedma *et al*., 2018). The cubes were weighed to 50 g, placed into 250 mL flask, and pasteurised at 118°C for 20 min (USPTO, 2017). Pasteurised flasks were then left to cool at room temperature. The mixed inoculum of fungus and yeast resulted from the addition of 0.075% inoculum of *S. fibuligera* Y0021 to the inoculum of *A. rouxii* F0050. The production process was similar to the previous step, with yeast added at the end of the process.

The solid phase fermentation parameters used were temperature (27, 30, and 33°C), incubation time (0, 12, 24, 36, 48, 60, and 72 h), size (0.2, 0.4, and 0.6%), and inoculum strain (*A. rouxii* F0050 mould inoculum; *S. fibuligera* Y0021 yeast; and a mixture of mould and yeast strains - *A. rouxii* FTCC F0050 and *S. fibuligera* Y0021). The sweet potato culture was divided into several fermentation cultures, where M1: mould monoculture at 27°C; M2: mould monoculture at 30°C; M3: mould monoculture at 33°C; Y1: yeast monoculture at 27°C; Y2: yeast monoculture at 30°C; Y3: yeast monoculture at 33°C; MY1: mixed culture at 27°C; MY2: mixed culture at 30°C; and MY3: mixed culture at 33°C. pH analysis of fermentation products was performed following the AOAC (2000) method.

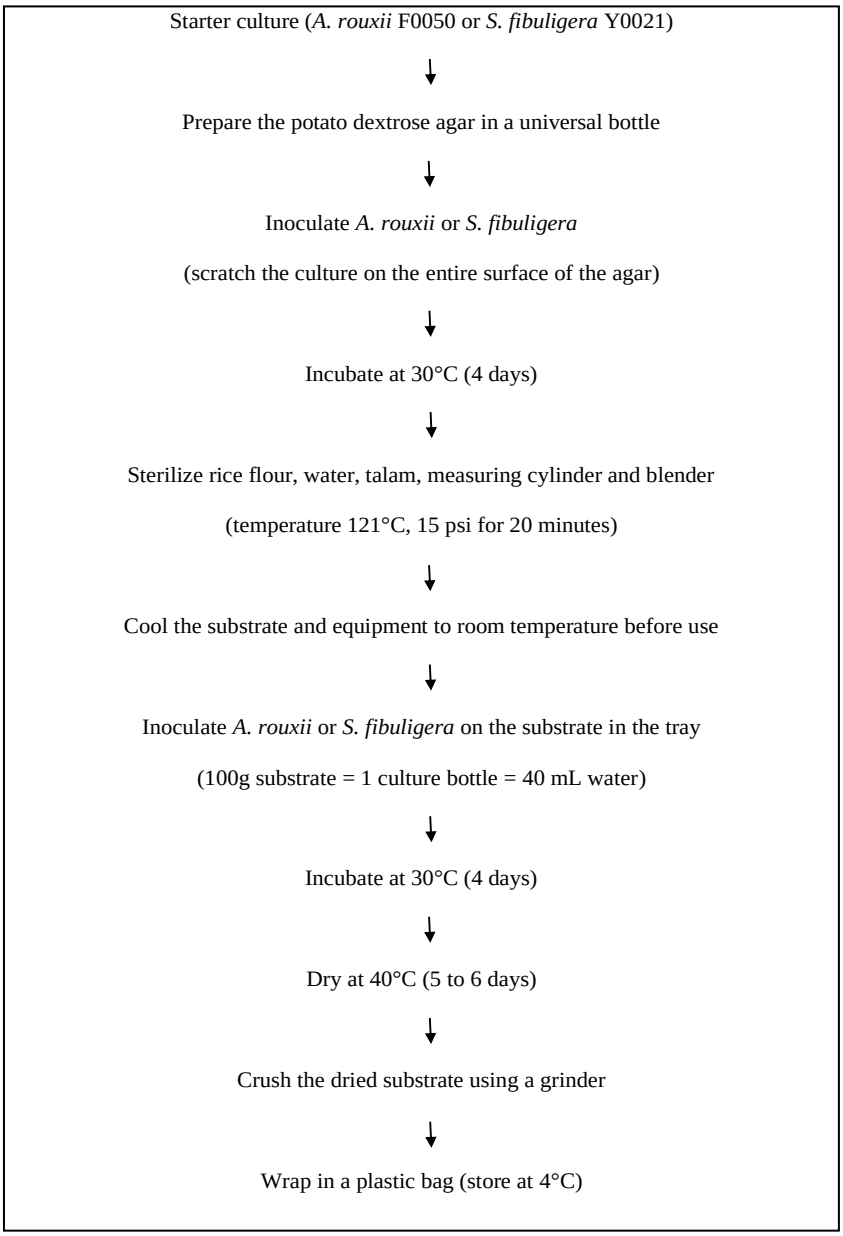

Figure 1. Sweet potato inoculum production process.

Statistical analysis

The experiment was conducted using a completely randomised design (CRD). The entire data obtained were analysed using analysis of variance (ANOVA) method. Significant differences for each data that gave significant results at the $p < 0.05$ level were determined by performing Duncan's test, using a statistical analysis system.

Results and discussion

Proximate analysis was conducted to determine the contents of protein, fat, crude fibre, water, ash, and carbohydrate in all the three varieties of sweet potato, namely VitAto, Gendut, and Anggun.

Proximate analysis

Referring to Table 1, Gendut had a protein content of 1.26 ± 0.05% which was significantly different ($p < 0.05$) from that of Anggun (1.05 ± 0.04%) and VitAto (1.03 ± 0.06%). However, there was no significant difference ($p > 0.05$) between the protein contents of Anggun and VitAto. The protein contents of these three sweet potato varieties ranged from 1.0 - 2.5%, which was consistent with the range reported by Dansby and Bovell-Benjamin (2003). The protein contents of these varieties were higher than that of sweet potato in Rwanda, which ranged from 0.71 - 0.91% (Rose and Vasanthakaalam, 2011). The fat contents of the three sweet potatoes ranged from 0.04 ± 0.01 to 0.07 ± 0.01%. Gendut had higher

fat content of 0.07 ± 0.01% as compared to VitAto (0.05 ± 0.02%) and Anggun (0.04 ± 0.01%). This range of fat content was found to be lower than the range reported by Tumuhimbise *et al*. (2013) which was around 0.2 - 0.17%, 0.54 - 2.22% (Sanoussi *et al*., 2016), and 0.17 - 0.63% (Alam *et al*., 2016).

VitAto had the highest content of crude fibre with 2.02 ± 0.08%, followed by Anggun (1.89 ± 0.07%) and Gendut (1.46 ± 0.12%). The crude fibre content of VitAto showed a significant difference ($p < 0.05$) as compared to Anggun and Gendut. The range of crude fibre content for these three sweet potatoes was around 1.46 ± 0.12 to 2.02 ± 0.08%, which was lower than the range reported by Senanayake *et al*. (2013) for Sri Lankan sweet potato varieties (2.1 - 13.6%), but higher as compared to four Rwandan sweet potato varieties (0.11 - 0.14%) (Rose and Vasanthakaalam, 2011). The percentage range of moisture content for these three varieties was around 73.27 ± 0.16 to 76.04 ± 0.15%. This range was higher as compared to the moisture percentage range reported by Rose and Vasanthakaalam (2011) for four Rwandan sweet potato varieties, which ranged from 62.58 - 64.34%, and Alam *et al*. (2016) which ranged from 70.95 - 72.96%. VitAto had a higher percentage of carbohydrates with 25.0 ± 0.2% as compared to Gendut (22.37 ± 0.18%) and Anggun (22.28 ± 0.17%). The carbohydrate content in the VitAto showed a significant difference ($p < 0.05$) as compared to the Gendut and Anggun. The content of carbohydrate was higher as compared to the average of other sweet potato varieties around the world, which is around 21.56% (Ellong *et al*., 2014) and 21.1 - 24.5% (Alam *et al*., 2016). Although the carbohydrate content is high, sweet potatoes have low glycaemic index, and are very suitable for diabetics and overweight patients (Ali *et al*., 2020). However, Sato *et al*. (2018) reported that the sugar content of sweet potato is higher than cassava.

Table 1. Proximate composition of VitAto, Gendut, and Anggun sweet potatoes.

Composition (%)	VitAto	Gendut	Anggun
Moisture	73.27 ± 0.16^{c}	75.68 ± 0.21^{b}	76.04 ± 0.15^{a}
Ash	0.65 ± 0.02^{a}	0.61 ± 0.08^{a}	0.60 ± 0.02^{a}
Protein	1.03 ± 0.06^{b}	1.26 ± 0.05^{a}	1.05 ± 0.04^{b}
Fat	0.05 ± 0.02^{ab}	0.07 ± 0.01^{a}	0.04 ± 0.01^{b}
Crude fibre	2.02 ± 0.08^{a}	1.46 ± 0.12^{b}	1.89 ± 0.07^{a}
Carbohydrate	25.0 ± 0.20^{a}	22.37 ± 0.18^{b}	22.28 ± 0.17^{b}

Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

Effect of strain selection

Fermentation is a metabolic process of organic compounds through enzyme reactions or complex organic catalysis produced by microorganisms. This reaction involves the conversion of carbohydrates such as starch or sugar into organic acids, alcohols, and other metabolites. The pH change of the culture is influenced by the selection of microbial strain, inoculum size, temperature, and incubation period of the fermented culture.

All cultures fermented using all three types of inoculum strains showed a decrease in pH up to 72 h (Figure 2). The pattern of pH decrease was found to be inversely proportional to the moisture produced during the fermentation process. It was observed that the pH started to decrease after 12 h of fermentation for cultures M1, M2, M3, and MY3, while for cultures MY1 and MY2, the pH decrease started to occur after 24 h. The use of yeast inoculum for monocultures Y1, Y2, and Y3 showed an insignificant decrease in pH until 72 h. Results indicated that the culture fermentation process using mould inoculum produced more acidic environment as compared to yeast inoculum. An increase in the concentration of organic acids causes the acidity of the pH of the culture. Microorganisms such as *A. rouxii* and yeast can degrade starch to sugar while maintaining their growth, and with the occurrence of ethanol and acid syntheses, the pH of the culture also decreases (Abe *et al*., 2004). According to Ezeama and Amajor (2015), the decrease in pH during the fermentation process indicates that mould and yeast possess tolerant properties to the resulting acid.

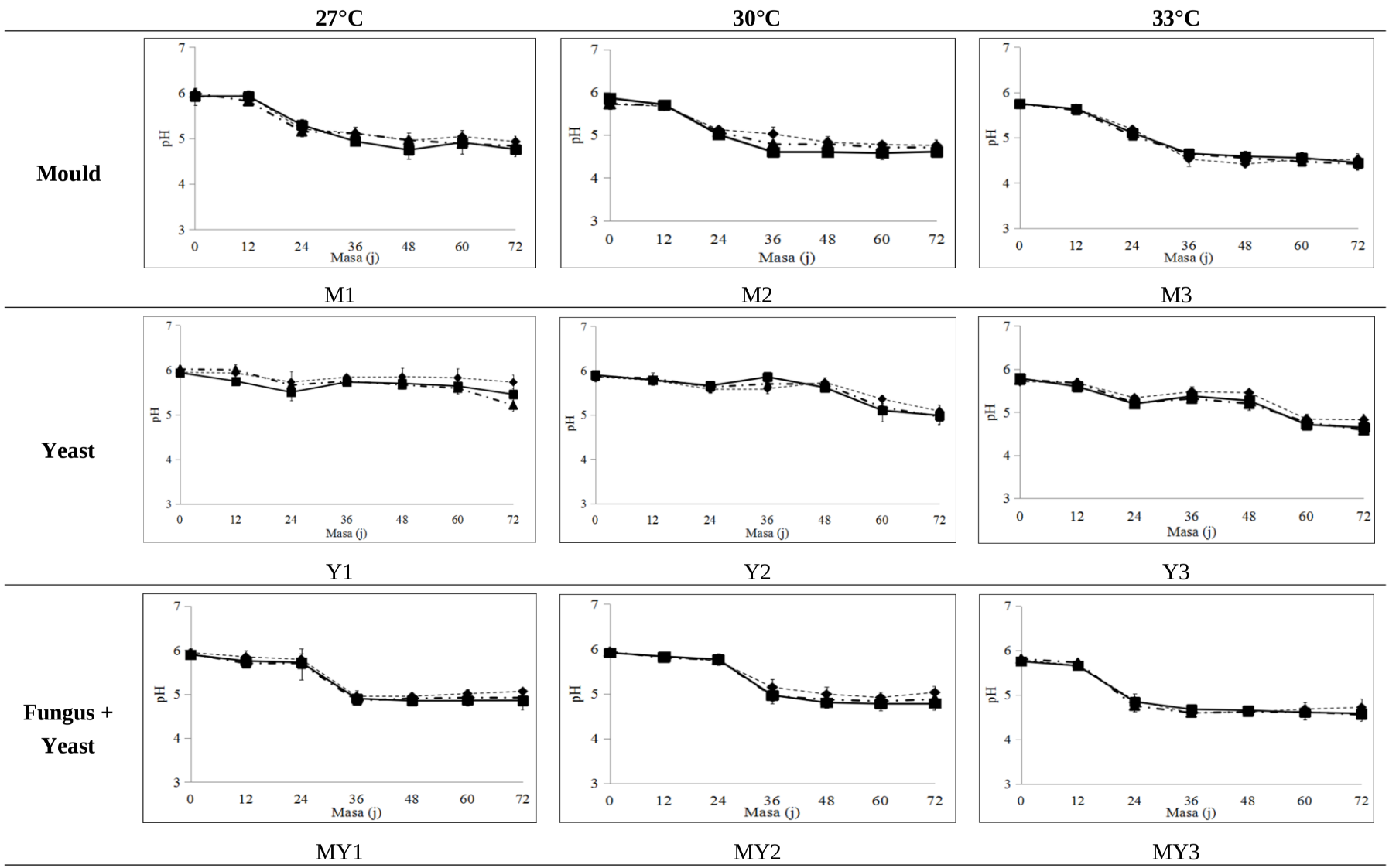

Figure 2. Effect of strain, temperature, inoculum size, and incubation period on pH of VitaTo sweet potato fermentation cultures. M1: mould monoculture at 27°C; M2: mould monoculture temperature at 30°C; M3: mould monoculture at 33°C; Y1: yeast monoculture at 27°C ; Y2: yeast monoculture at 30°C; Y3: yeast monoculture at 33°C; MY1: mixed culture at 27°C; MY2: mixed culture at 30°C, and MY3: mixed culture at 33°C. Inoculum size: (♦): 0.2%, (■): 0.4%, and (▲): 0.6%. Error bars indicate ± mean standard deviation (SD) of triplicates (n = 3).

Effect of inoculum size

Analysis of the three different percentages of inoculum used in this fermentation found that the inoculum size of 0.4 and 0.6% did not significantly affect the pattern of pH decrease for both cultures, whether it was a mould monoculture (pH around 4.42 ± 0.14 to 4.93 ± 0.128) or a mixed culture of mould and yeast (4.55 ± 0.135 to 5.063 ± 0.065) as compared to yeast monoculture (4.586 ± 0.078 to 5.723 ± 0.165) after fermentation was carried out for 72 h. This indicated that any increase in the percentage of inoculum used in the fermentation culture will also increase the production of acid, and this condition further affects the decrease in pH of the culture. This has been proven by Wardani *et al*. (2017) where the role of inoculum concentration of *Streptococcus thermophilus* and *Lactobacillus acidophilus* caused changes to viscosity and acidity, and a significant decrease in pH in yogurt fermentation.

Effect of infusion temperature

Studies on incubation temperature showed that the decrease in pH occurred relatively slowly at 27°C as compared to cultures incubated at 30 and 33°C. In addition, the fermentation temperature was found to show no significant difference ($p > 0.05$) for cultures Y1, Y2, and Y3 (pH range around 4.587 ± 0.078 to 5.723 ± 0.165 of 72 h fermentation) in terms of pH decrease as compared to other cultures. Results also indicated that cultures (mould inoculum: pH range around 4.42 ± 0.14 to 4.757 ± 0.131; and mixed inoculum: pH range around 4.55 ± 0.135 to 5.027 ± 0.145) incubated at 30 and 33°C did not show significant differences ($p > 0.05$) between each other up to 72 h of fermentation. It is worth noting that a temperature of 30°C is the optimum temperature for most mould and yeast growth (Aliyah *et al*., 2017).

Effect of incubation period

The decrease in pH started to occur after 12 h of fermentation across all mould, yeast, and mixed cultures. In addition, the pH decrease pattern continued until 36 h, and then started to level off until 72 h of fermentation. Notably, this trend was most evident in the M2 culture (incubation temperature, 30°C; inoculum percentage, 0.4%; 4.61 ± 0.123); followed by cultures M3 and M1 (pH range around 4.627 ± 0.16 to 5.12 ± 0.125); MY1, MY2, and MY3 (pH range around 4.677 ± 0.1 to 4.95 ± 0.131); and Y1, Y2, and Y3 (pH range around 5.317 ± 0.049 to 5.937 ± 0. 072). Abbas (2019) reported that a decrease in pH occurred during the first three days of fermentation of VitAto with various microorganisms (bacteria, moulds, and yeasts) due to the presence of organic acids such as lactic and acetic acids. Mould monoculture was found to produce higher acid as compared to mixed culture and yeast monoculture. As the fermentation process progressed, it led to the production of more acid. This indicated that there was an increase in the population of microorganisms in the culture when the fermentation period increased (Ajayi *et al*., 2016). According to Ainaa *et al*. (2016), pH also plays a role as one of the indicators for the growth of microorganisms.

Conclusion

Among the three varieties tested, VitAto exhibited the highest crude fibre, ash, and carbohydrate contents as compared to Gendut and Anggun varieties. As VitAto had the highest carbohydrate content, this variety was selected as the carbon source for the subsequent fermentation process. The present work demonstrated that the pattern of pH decrease occurred up to 36 h, and then started to level off until 72 h of fermentation, mainly for the M2 culture. Mould monocultures were found to produce higher acid as evidenced by the decrease in pH value. This indicated an increase in the population of microorganisms in the culture as the fermentation period increased.

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