OPTIMIZING VITATO PASTE FERMENTATION: EFFECT OF STRAIN VARIATION, INOCULUM SIZE AND INCUBATION DURATION ON pH DYNAMICS

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ABSTRACT

In Malaysia, VitAto stands out as one of the diverse sweet potato varieties. This experiment aimed to investigate how varying strains, inoculum sizes (0.2%, 0.4%, and 0.6%) and incubation periods (0, 24, 48, and 72 hours) influenced the pH levels during VitAto paste fermentation, specifically utilizing Amylomyces rouxii F0050 and Saccharomycopsis fibuligera Y0021. The cultures underwent incubation at 30°C, with sampling conducted every 24 hours over a span of 72 hours. A universal trend emerged across all cultures observed: a consistent decrease in their individual pH levels. This decline became noticeable following 24 hours of fermentation across both the fungal strain (A. rouxii F0050) and the yeast (S. fibuligera Y0021). Fermentation results showed that the pH at hour 48 [A. rouxii F0050: inoculum size 0.2% - pH 4.57, 0.4% - 4.48 and 0.6% - 4.48; S. fibuligera Y0021: 0.2% - 4.71, 0.4% - 4.64 and 0.6% - 4.68) and hour 72 [A. rouxii F0050: inoculum size 0.2% - pH 4.43, 0.4% - 4.28 and 0.6% - 4.30; S.

fibuligera Y0021: 0.2% - 4.72, 0.4% - 4.61 and 0.6% - 4.64). The investigation highlighted that the pinnacle of solid-state fermentation occurred under specific conditions: utilizing A. rouxii F0050 with an inoculum size of 0.4% w/w, incubated at 30°C for 48 hours. This optimized bioprocessing approach, considering parameters such as strain type, inoculum size, fermentation temperature and incubation period, signifies the potential of VitAto to evolve into functional sweet potato food products.

Keywords: VitAto, solid state fermentation, pH, Amylomyces rouxii F0050, Saccharomycopsis fibuligera Y0021

INTRODUCTION

Sweet potatoes (*Ipomoea batatas* L.), a member of the Convolvulaceae family, stand as one of the world's earliest cultivated vegetables (Tan, 2015). Among the diverse sweet potato varieties in Malaysia, VitAto shines with its vibrant orange hue and remarkable nutritional profile, boasting high levels of vitamins, minerals, carbohydrates, crude fibre, and minimal fat content. 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. Understanding the significance of pH in optimizing microbial growth rates and metabolite production, as highlighted by Shuler and Kargi (2008), is important. Bacteria typically thrive within a pH range of 3 to 8, while yeast and fungi favour slightly more acidic environments, ranging from pH 3 to 6 and pH 3 to 7, respectively.

Solid-state fermentation (SSF) utilizes solid substrates, like food materials, fostering microbial growth and biochemical transformations. This process occurs without free-flowing liquids, sustaining microbial cultures for diverse applications in food, pharmaceuticals and biotechnology, showcasing versatility in industrial bioprocessing. One key benefit of SSF lies in its ability to efficiently utilize diverse solid substrates, reducing wastewater generation and enabling cost-effective production of valuable compounds like enzymes, organic acids and bioactive molecules for various industries (Abdul Manan and Webb, 2017a).

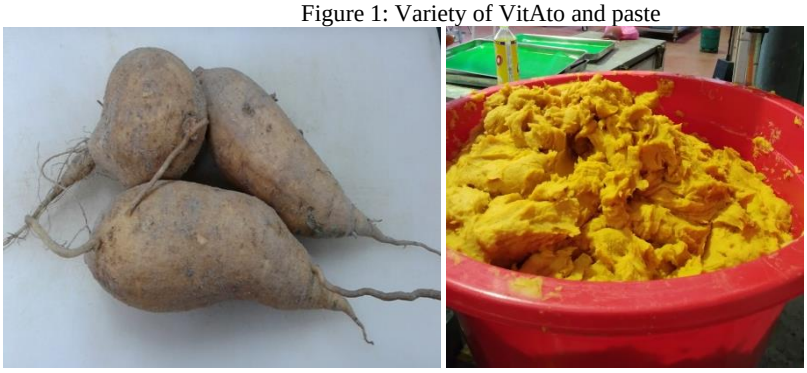
The main objective of this experiment was to scrutinize the impact of various factors - different strains, inoculum size, temperature and incubation period on the pH dynamics during VitAto fermentation, specifically employing *Amylomyces rouxii* F0050 and *Saccharomycopsis fibuligera* Y0021. This investigation sought to shed light on the intricate interplay between these variables in the fermentation process.

MATERIAL AND METHOD

Substrate preparation

VitAto (Figure 1) sourced from Impiana Juara Resources Sdn. Bhd. at Projek Pembangunan (Persekutuan) in Kota Tinggi, Johor, underwent meticulous preparation. The sweet potatoes underwent thorough cleaning, peeling and soaking before being processed into a paste using a bowl cutter, as detailed by Sabeetha et al. 2016.

Figure 1: Variety of VitAto and paste



Microorganisms

The research study incorporated two distinct microorganism strains: *A. rouxii* F0050 and *S. fibuligera* Y0021. These strains were procured from the Collection of Functional Food Culture (CFFC), Enzyme and Fermentation Technology Program at the Food Science and Technology Research Centre, Institute of Malaysian Agricultural Research and Development (MARDI) in Serdang, Selangor.

Fermentation condition

The SSF process for VitAto employed two distinct inoculum strains: fungal (*A. rouxii* F0050) and yeast (*S. fibuligera* Y0021). The size of the inoculum emerged as a crucial factor influencing this SSF. The study investigated three varying inoculum sizes: 0.2%, 0.4% and 0.6%. Furthermore, the impact of the incubation period during SSF was assessed, with cultures undergoing incubation

for 72 hours. Sampling intervals were set at 0, 24, 48 and 72 hours, allowing insight into the fermentation's progression over time. Consistently, the SSF maintained an incubation temperature of 30°C throughout the experimentation process.

pH analysis

The pH measurement of the samples followed a specific protocol: 10 mL of fermented sample supernatant was placed into a test tube for analysis. Utilizing a pH measuring instrument, specifically the Mettler Toledo model MP220 from Switzerland, the pH values were determined following the AOAC (2000) guidelines. Each sample underwent pH analysis three times to ensure accuracy and consistency in the results.

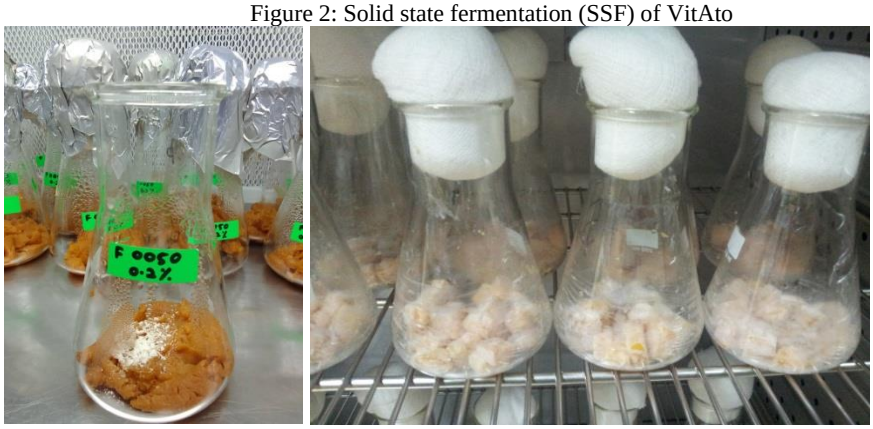
Statistical analysis

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 (SAS Institute, 1985).

RESULT AND DISCUSSION

The observed pH dynamics during the fermentation process unveiled intriguing patterns across different cultures and inoculum sizes. The pH decreases across all cultures commenced after 24 hours of fermentation for both *A. rouxii* F0050 and *S. fibuligera* Y0021 (Figure 2). Notably, a consistent decline in pH was observed until the 48 hours, particularly evident in the fungal culture at 0.4% inoculum (4.28 ± 0.02) [showed significant difference ($p>0.05$) compared to yeast culture at 0.2%, 0.4% and 0.6% inoculums]. This pattern tapered off thereafter until the 72 hours, with pH ranges varying among different inoculum sizes for both fungal and yeast cultures (Figure 3). This trend of increasing acidity over time signals the proliferation of microorganisms within the cultures, aligning with findings by Ajayi et al. (2016) and emphasizing pH as a vital indicator for microbial growth, as highlighted by Ainaa et al. (2016).

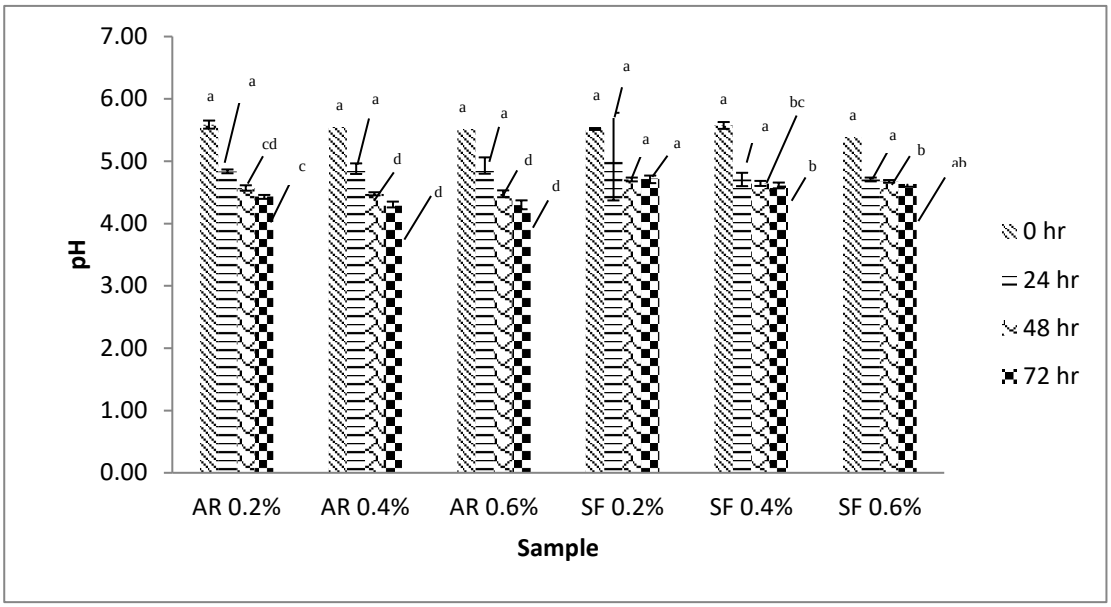
Figure 2: Solid state fermentation (SSF) of VitAto



These findings reflect the dynamic nature of SSF, highlighting the temporal evolution of microbial populations and their metabolic activities. The decreasing pH aligns with microbial proliferation and the accumulation of organic acids as by-products of their metabolic processes. Notably, the differences observed in pH levels among inoculum sizes and cultures underscore the nuanced influence of these variables on fermentation dynamics.

SSF's significance in this context lies in its ability to sustain microbial growth and biochemical activities on solid substrates, like sweet potato-derived VitAto, without the need for free-flowing liquids. This approach is particularly advantageous in utilizing food materials as substrates, reducing waste and cost while facilitating the production of valuable compounds like enzymes or bioactive molecules (Abdul Manan and Webb, 2017b). Moreover, the pH fluctuations observed in this study serve as crucial indicators of microbial growth and metabolic activity, providing essential insights into optimizing SSF processes for efficient product development in various industries, including food and pharmaceuticals.

Figure 3: Effect of inoculum size and incubation period on pH of VitAto sweet potato fermentation cultures (*Amylomyces rouxii* F0050: AR; *Saccharomycopsis fibuligera* Y0021: SF)



CONCLUSION

The utilization of *A. rouxii* F0050 strains in the fermentation process exerted a more pronounced effect on lowering the pH compared to the yeast culture counterpart. This notable decline in pH levels indicates a substantial increase in acid production as the fermentation progresses. The findings suggest a direct correlation between the microbial population's growth and the escalating acidity over time. Such changes in pH serve as a valuable indicator of the thriving microbial community within the culture medium. The observed trend underscores the dynamic nature of the fermentation process, wherein the proliferation of microorganisms, particularly under the influence of *A. rouxii* F0050, drives the heightened production of acidic compounds. This phenomenon elucidates the important role of microbial activity in altering the environment during VitAto paste fermentation, elucidating the potential significance of strain selection for optimizing product characteristics in sweet potato functional food development.

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