I. INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta)* is one of the carbohydrate sources for food products. It is one of the important sources of starch. Cassava could easily grow under many conditions, even under harsh factors (Hamidi & Banowati, 2018; Lu *et al*, 2020). Many uses that cassava could provide, such as human food products, animal feed, industrial purposes, and alternative fuel (Lu *et al,* 2020). Cassava root is used to be a food source, due to its carbohydrate richness component that yields higher compared to rice and corn (Bala *et al*, 2015). Other than roots, cassava leaves could be used as a food source as well. It has rich nutritional composition, has good phytochemicals with high antioxidant activity (Laya & Koubala, 2020).

Cassava roots could have extended use for food sources. From this cassava roots, it can be produce several type of flour, that are tapioca flour, cassava flour, and modified cassava flour (mocaf). Tapioca flour is made from the starch of the peeled roots that are going to be extracted by the grinded and mixed with water. The starch grains come out and are carried away by the water. The starch grains are precipitated, dried and then grinded to gain the fine white starch granules that are called tapioca. (Roozen & Serventi, 2022). Cassava flour is made from the whole root that is chopped and grinded to make the flour (Zhang *et al*, 2020). Mocaf is made from cassava flour that goes through fermentation before grinded (Seveline *et al*, 2020).

Cassava roots could be fermented with lactic acid bacteria, it could produce a modified fermented cassava root and it is called modified cassava flour or mocaf (Sidqi *et al*, 2022). Mocaf firstly was founded by a professor from Jember University, named Prof.Ir.Achmad Subagio, M.Agr. Mocaf was inspired by potato processing in the Netherlands to make a variety of flour and starch for food sources (Sarprastp, 2021). Mocaf is produced by process fermentation of lactic acid bacteria that modified the cassava's starch. With the fermentation process, LAB breaks down the cellulose cell walls and the starch granules break down completely into monosaccharides and other components such as lactic acid (Seveline *et al*, 2020). Modified cassava flour usually has feature of softer and smoother texture than tapioca flour. Mocaf also has white color, and does not have a smell from the original cassava that is known to have an unpleasant smell because roots contain hydrogen cyanide that is toxic to humans if it's not proceed properly.

Mocaf has advantages to health, such as the higher soluble fiber (3.4%) compared to cassava flour and wheat flour (0.3%). Moreover, the mineral content (e.g calcium) of mocaf is higher than rice flour and wheat flour. Mocaf could decrease the cholesterol absorption, dilute the toxins, increase the short chained fatty acids. Mocaf also has good capacity to expand as wheat type II (medium protein content), and has higher digestibility than tapioca flour (Ratnawati *et al*, 2020; Sahidin, 2018). Moreover, compared to wheat flour, mocaf has lower calories due to lower carbohydrates, also lower in fat and sugar content. Mocaf has higher starch content (87.3%) than tapioca flour (60% - 68%), but has lower protein content (1.2%) than tapioca flour (12%) (Febriani *et al*, 2022; Afifah & Ratnawati, 2017). Mocaf included in gluten-free flour that is safe to be consumed by people who has celiac disease, irritable bowel syndrome, autism (autism spectrum disorder or ASD)(Firdaus *et al*, 2017), diabetes mellitus, also people who sensitive with food gluten-contained. Mocaf can be a substitute for wheat flour, as for people who has disease and could not digest some components in wheat flour (Hamidi & Banowati, 2019; Salim, 2011; Faridah & Yuhelma, 2023; Hasmi *et al*, 2021). If we want to make food products that have high protein content, mocaf could not be used. It has to be mixed with other high protein content, usually mixed with legumes flour (chickpea, lentil or beans), nuts (almonds, etc), and many more. Mocaf has close characteristics to wheat flour, it can be used as a main component to make kinds of cookies (Hasrini *et al,* 2021; Unayah *et al*, 2020; Kristanti *et al*, 2020), pastries (Mumba & Suhartiningsih, 2013), pastas (Febriani *et al*, 2022) and noodles, doughnuts, cakes, and many other products.

From some study, the quality of mocaf is depends on the microbe that is used. Such as different starter culture from Lactic Acid Bacteria (LAB), that usually used is *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus paracasei,* and *Lactobacillus acidophilus* and *also Lactobacillus casei* as well is involved in fermentation of flour (Seveline *et al*, 2020; Handayani *et al,* 2022). Also *Bacillus sp.* is usually used as a single starter as well (Kresnowati *et al*, 2019), usually used *Bacillus subtilis*. *B.subtilis* causes texture modification due to is cellulolytic activity that disrupts cassava's cell wall (Kresnowati *et al*, 2019; Amoa-Awua *et al*, 2014) and produces glutamate that could improve flour's odour. According to Kresnowati *et al* (2019) the combination of *B.subtilis* and *L.plantarum* as co-culture fermentation could give better results for the effect of cyanogenic glucosides and hydrolysis reduction. Longer the time of fermentation, the cassava would be more decayed due to the starch decomposed by microorganisms from the starter (Ningrum & Saidi, 2023).

Until now there is no research about *Pediococcus acidilactici* that is used for mocaf fermentation. From the *pediococcus* group usually used is *P.pentosaceus t*hat has already been observed in several studies, one of them is Isa *et al* (2020) study. *P.pentosaceus* is used beside *L.plantarum*, the most popular used LAB for mocaf fermentation. It is good to observe how *P.acidilactici* works on mocaf fermentation. The strains of *P.acidilactici* has been used for animal-based and plant-based food and beverage product (Abbasiliasi et al, 2017; Bhagat et al, 2020; Fugaban et al, 2022; Surachat et al, 2021; Todorov et al, 2023). Their sugars fermentations have abilities associated with the specific substrates metabolization and potential applications in different areas of the food industries (Todorov et al, 2023). Their strains are able to metabolize a variety of carbohydrates, including glucose, ribose, fructose, galactose, and many more (Anastasiadou et al, 2008; Todorov et al, 2023). Also it could ferment the lactose, sucrose, and maltose (Holzapfel, 2015). Some previous studies have investigated that the fermentation process could be depending on the time of fermentation and also the starter. The importance of time fermentation is vital since they have a huge impact on the quality and composition of the final product (Maleke *et al*, 2020). During fermentation, because of microbial activity that produces enzymes that break down organic compounds into simpler compounds, H_2O and heat energy, the water evaporates during the fermentation process. Also the starch component is decreased due to the amount of hydrolyzed starch into sugars (Diniyah et al, 2018). The ash content and HCN is decreased as well (Apriliani & Mulyadi, 2022). For whiteness is increased along with the different amount of starter concentration (Apriliani & Mulyadi, 2022). As for the starter concentration, more concentration is applied in fermentation, it could increase the quality of the mocaf itself, as stated in Apriliani & Mulyadi (2022) and Hargiyanti and Mulyadi (2022) experiment. In this research, we observe the time fermentation on mocaf production using *P.acidilactici* if the time fermentation could affect the quality of flour such as whiteness, protein content, ash content, water content, and other standard quality parameters.

Pediococcus is a genus that belongs to the *Lactobacilliaceae* family. It is gram-positive lactic acid bacteria, non-motile, non-spore forming, catalase-negative, facultative anaerobe, with coccus-shaped or tetrads arranged bacteria. It is usually used as probiotics (*Porto et al,* 2017). *Pediococcus acidilactici* is a lactic acid bacteria that produces lactic acid, and belongs to the *Pediococci* genus. It is usually used most in industry, from the food and beverage industry to the pharmaceutical industry (Holland, Crow, & Curry, 2011). Beside being used for food industry lactic acid production, it is used in industry because of its ability to produce antimicrobial activity. *P.acidilactici* could produce pediocin that shows bactericidal activity against several bacteria, because of that *P.acidilactici* acquires the latter property. Hence, it can be used as a biopreservative agent to prevent food spoilage and food-borne disease (Todorov *et al,* 2022). *P.acidilactici* has strong tolerance towards acidic and bile salt conditions, in order to survive in the acidic stomach conditions (i.e. in stomach)(Song et al, 2017; Erkkilä & Petäjä, 2000).

1.2 Research Scope

The scope of this study are:

1. Produce mocaf flour using several starters (*P. acidilactici* and *L. plantarum*) with different time fermentation (12, 24, and 48 hours).
2. Analyses the mocaf quality parameters by using physicochemical analysis (water content, protein content, ash content).

1.3 Research Question:

From the background and objective mentioned above, several research questions are made:

1. Can mocaf flour be produced using *P.acidilactici?*
2. Do different starters of LAB affect the quality of mocaf flour?
3. Do different time fermentation affect the mocaf flour quality?

1.4 Hypothesis

From the research questions, some hypotheses are formulated:

1. H_0: *Pediococcus acidilactici* can be used as starter to produce mocaf
   H_1: *Pediococcus acidilactici* cannot be used as starter to produce mocaf
2. H_0: Different time fermentation will result in different quality of mocaf.
   H_1: Different time fermentation will not result in different quality of mocaf.