

Effects of Microbial Starter Composition on Nutritional Contents and Pasting Properties of Fermented Cassava Flour

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Fermentation of cassava chips prior to drying and milling processes improves cassava flour quality: i.e. lower cyanogenic content, remove specific odour and colour. Composition of microbial starter applied to the fermentation may affect the microbial population during the fermentation and thus directs the process and the produced flour properties. This study mapped the effects of microbial starter composition on the microbial profiles during the fermentation and the corresponding nutritional contents as well as pasting properties of the produced fermented cassava flour (fercaf). Combinations of *Lactobacillus plantarum*, *Bacillus subtilis* and *Aspergillus oryzae*, that were selected based on their enzymatic activities, were evaluated. The addition of microbial starter was shown to affect the dynamics in microbial population during the fermentation. The addition of lactic acid bacteria accelerated the release of cyanogenic glycoside and starch conversion to simpler sugars, the addition of *B. subtilis* improved the disruption of cassava fibres, whereas the addition of *A.oryzae* was shown to increase the protein content of fercaf. The different microbial starter added to fermentation system also resulted in different pasting properties of fercaf. Microbial starter composition can be designed as such for the production of a particular flour property.

Keywords: fercaf; fermentation; microbial starter; nutrition content; pasting properties.

INTRODUCTION

Cassava is one of the potential carbohydrate sources in the world. It composes of 87% starch (dry basis) while the remaining are fibres, fat, and ash (Emmanuel et al. 2012). However, fresh cassava cannot be stored for long before

used (Tanaka et al. 1983) and it may contain toxic cyanogenic compounds.

An alternative method to prolong its use is by making cassava flour. Traditional cassava flour was prepared by drying the cassava chips/tubers, followed by milling. This type of cassava flour still retains some unwanted characteristics such as strong cassava flavour, moderately high

cyanogenic content and poor in sensory quality thereby it finds limited utilization in food industries.

The fermentation of cassava chips prior to the drying and milling has been shown to improve the properties of flour. The structure of the produced cassava flour are modified: there are disintegration of tissue structure by linamarase (Mkpong et al. 1990) and carbohydrates hydrolysis to simple sugars (Anyogu et al. 2014). Further, the accumulation of microbes may provide additional protein (Obloh and Oladunmoye 2007). Fermented cassava flour was shown to have brighter colour, neutral aroma, softer, and lower cyanogenic content (Meitha et al. 2016; Sobowale, et al. 2007). The product is known as Fercaf, Mocaf, Agbelima, Fufu, or Gari. The term Fercaf will be used in the remaining article.

Some studies on fermented cassava flour production have been performed, such as the effect of fermentation conditions on cyanogenic reduction (Bradbury and Denton 2010; Kresnowati et al. 2014), reactor configuration for fermentation (Lestari et al. 2015; Meitha et al. 2016), or the addition of specific starter culture to improve its nutritional properties (Obloh and Oladunmoye 2007; Sulistyono and Nakahara 2014). In particular, the effects of Lactic acid bacteria, *Bacillus sp.*, *Saccharomyces cerevisiae*, and some fungal as *Rhizopus oryzae* or *Aspergillus niger* have been researched, mostly as single starter (Akindahunsi & Obloh 1999; Amoa-Awua et al. 2014; Anyogu et al. 2014; Gunawan et al. 2015). Microbial interaction during the fermentation and their effects on fermented cassava flour

quality, in particular nutritional and pasting properties, has not been reported yet.

It is the objective of this study is to map the effects of microbial starter compositions on microbial interaction during fermentation and the corresponding nutritional content as well as pasting properties of fercaf. Based on their enzymatic activities, various combinations of *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* as the microbial starter were evaluated. *B. subtilis* causes textural modification by its cellulolytic activity that disrupts cells walls of cassava (Amoa-Awua et al. 2014) and produces glutamate which improves flour's odour. *L. plantarum* has amylolytic activity to hydrolyse starch chains (Giraud et al. 1994) and linamarase activity to cut cyanogenic glycoside structures (Lei et al. 1999). *A. oryzae* may serve as single cell protein in the end product. The addition of microbial starter was confirmed by observing dynamics in microbial population during the fermentation. The quality of produced fercaf was characterized as its cyanogenic glycoside content, nutritional and pasting properties.

MATERIALS AND METHODS

Microbial Starter Culture Preparation

A.oryzae ITB L24, *B. subtilis* ITB B128, and *L. plantarum* ITB B188 were obtained from the culture collection of Microbiology and Bioprocess Technology Lab., Chemical Engineering Dept., Institut Teknologi Bandung, Indonesia. Commercial starter was kindly provided by Mr Cahyo Hendradi, Koperasi Gemah

Ripah Loh Jinawi, Trenggalek, East Java. This starter composes of 5 different species of *Bacillus* as was reported in Meitha et al (2016).

Inoculum was prepared by growing each of the microbial species in the appropriate medium towards its logarithmic phase. *A. oryzae* was grown on Potato Dextrose Broth (PDB) at 30°C, *B. subtilis* was grown on Sodium Broth (NB) at 30°C, whereas *L. plantarum* was grown on deMan, Rogosa, and Sharpe (MRS) media at 37°C. 200 mL of these solutions, containing 10⁹ cfu/mL microorganisms, was mixed with 200 g of cassava chips and demin water to make a total volume of 2 L and was incubated for 24 hours at the appropriate temperature before being inoculated at the main fermentation. The commercial starter culture was prepared by mixing 3 g of dried starter microorganism with media composed of 2 L of demin water, 200 g of cassava, and 6 g of sugar; and incubating it at 30°C for 24 hours before being inoculated at the fermentation. The starter culture compositions used in the experiments were varied as following: control experiment without starter addition (Run 1); commercial starter (Run 2); 50% *L. plantarum* and 50% *B. subtilis* (Run 3); 50% *L. plantarum* and 50% *A.oryzae* (Run 4); 50% *B.subtilis* and 50% *A.oryzae* (Run 5); 33.3% *L. plantarum*, 33.3% *B. subtilis* and 33.3% *A.oryzae* (Run 6).

Cassava Chips Preparation

Fresh cassava tubers were obtained from local market in Bandung (West Java, Indonesia) and were processed within 24 hours. 5 kg of fresh cassava were used in

each fermentation experiment. The tubers were peeled, washed, and chipped of about 0.1 cm thick before further used.

Fermentation

The fermentation was conducted in a large vessel in which the temperature was controlled at 34°C for 96 hours. Freshly prepared cassava chips were put into the system along with 2 L of inoculum solution. Afterwards, demin water was added into the system to give a final volume of 20 L. Samples were taken every 4 hours during the first 24 hours and further every 24 hours until the end of fermentation. The fermented cassava chips were dried at room temperature using a fan before being milled into flour.

Analysis

Microbial population was determined from the fermentation broth by Total Plating Count (TPC) method. *A.oryzae* concentration was determined using *Aspergillus Flavus Paraciticus* Differentiation Agar (AFPA) at 30°C for 48 hours (Elbashiti et al. 2010). *B. subtilis* concentration was determined using Manitol Egg Yolk (MYP) agar, into which Polymixin was added to suppress the growth of gram negative bacteria, at 37°C for 48 hours (Peng et al. 2001). *L. plantarum* concentration was determined using deMan, Rogosa, and Sharpe (MRS) agar at 37°C for 48 hours (Coghetto et al. 2016).

The cyanogenic content was analysed from the cassava chips samples following HCN titration method (Gunawan et al. 2015). Nutritional and pasting properties were determined from fermented cassava

flour: the protein content was analysed following Biuret method (Okafor 2004), starch content was determined following its complete hydrolysis (Gunawan et al. 2015) and Nelson-Somogyi method to measure its glucose concentration, Dextrose Equivalent (DE) was determined following Lane and Eynon's method (Dokic et al. 2004), the swelling power was measured based on the method of Leach (Kusumayanti et al. 2015). Pasting properties such as measure pasting temperature, peak viscosity, and final viscosity, were characterized using Rapid Visco Analyzer (Niba et al. 2002).

RESULTS

Microbial Dynamics and Interaction during Fermentation

Dynamics in the microbial population during the fermentation of cassava chips are shown in Fig. 1. Growth of microorganisms was observed and in all variations steady conditions or the stationary phases were achieved within 12 hours of fermentation. These stationary phases were achieved faster than the previous results of (Gunawan et al. 2015) which indicated the stationary phase of *L.plantarum* growth on cassava chips at 24 hours.

Without any starter culture addition, lactic acid bacteria were observed to grow well and dominated the microbial population during the fermentation (Fig. 1a). In agreement with previous observation (Achi and Akubor 2000; Brauman et al. 1996; Fayemi and Ojokoh 2014), it showed that lactic acid bacteria were an endogenous microbial species in

cassava fermentation and also the dominant microorganism in natural fermentation of cassava. The micro-environment of cassava fermentation suited well for the growth of this facultative anaerobic microorganism. *L.plantarum* was also observed to grow well in all fermentation variations.

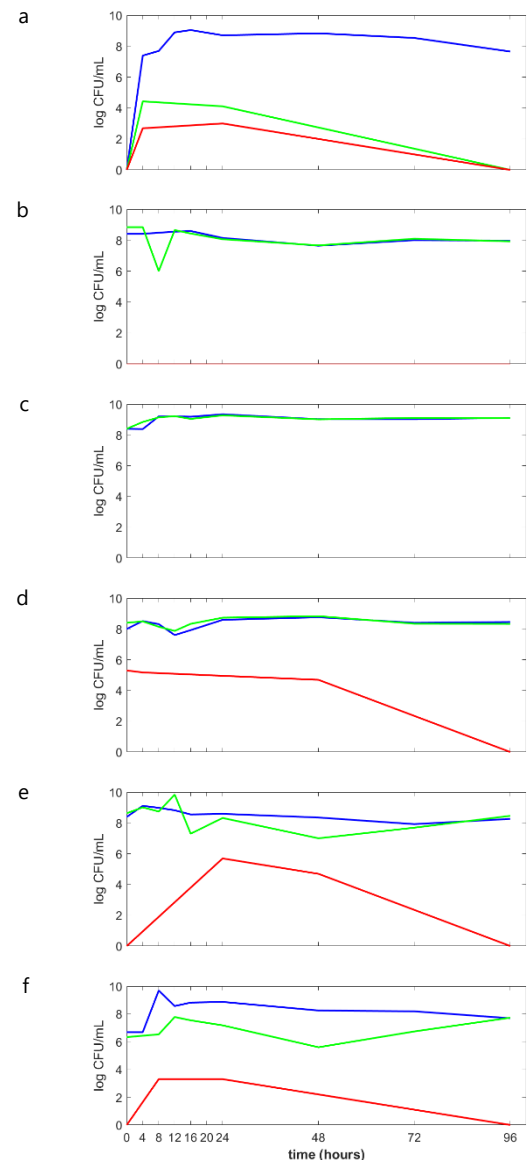


Fig. 1: Time Profile of Microbial Concentration during the Fermentation (*L.plantarum* (blue), *B. subtilis* (green), *A.oryzae* (red)) for Run 1 (a), Run 2 (b), Run 3 (c), Run 4 (d), Run 5 (e), and Run 6 (f)

High concentration of *L.plantarum* was measured from all samples, despite the starter culture composition added in the beginning of fermentation (Fig. 1).

B. subtilis was observed to grow well in all fermentations with added starter cultures (Fig. 1). Indeed this species was reported to have the ability to degrade cellulose and starch (Amoa-Awua et al. 2014) that supports its growth competitiveness during the fermentation, upon its addition in the onset of fermentation. *A. oryzae* was only detected in fermentations in which it was added as the starter culture (Fig. 1). Slow fungal growth resulted in overall low fungal concentration. The low measured concentration may also be the effect of its tendency to live in solid substrate, whereas in this study the microbial population was measured only from the liquid phase.

No clear trend of interaction between species of added microorganism was observed. The addition of *B.subtilis* was not clearly seen to inhibit or accelerate the growth of *L.plantarum* or *A.oryzae* and vice versa.

Effects Starter Composition on Cyanogenic Content of Fermented Cassava Flour

The cyanogenic content of cassava chips decreased during fermentation process, even at natural fermentation in which no starter culture was added into the fermentation. As was reported previously (Brauman et al. 1996; Gunawan et al. 2015; Kresnowati et al. 2014), time affects the reduction of cyanogenic content in cassava chips. In general, the

cyanogenic content significantly decreased in the first 24 hours of fermentation (Fig. 2), whereas the rate of cyanogenic content removal was much slower afterwards. The larger scale of fermentation implied in the experiment caused slower cyanogenic content removal compared to our previous experiments (Kresnowati et al. 2014), in which significant reduction in the cyanogenic content occurred within the first 3 hours of fermentation. It indicated that scale affected cyanogenic reduction process performance. By the end of 96 hours fermentation, up to 87% of cyanogenic content could be removed giving cyanogenic content of cassava chips in the range of 2-6 ppm (Fig. 2). These cyanogenic contents are lower than the safe cyanogenic content of WHO standard, 10 ppm.

In agreement with previous observation (Ampe et al. 1994), the result showed that the addition of starter culture improved the reduction of cyanogenic content. It accelerated the reduction of cyanogenic content and moreover increased the percentage of cyanogenic content removal (Fig. 2). The addition of starter culture containing *L.plantarum* and *B.subtilis* (run 3) and *L.plantarum* and *A.oryzae* (run 4), for example, increased the cyanogenic content removal by 10% and 9%, correspondingly. Indeed some strains of *B.subtilis* were reported to have the ability to degrade cyanogenic compounds in cassava (Abbas et al. 2010). Thereby the presence of this species gave positive effect on cyanide reduction of cassava chips.

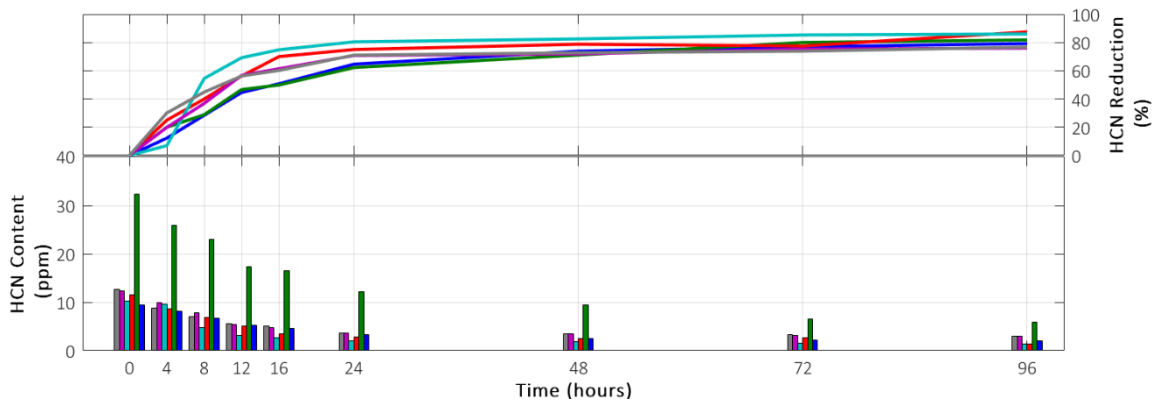


Fig. 2: Time Profile of Cyanogenic Content during the Fermentation (Run 1 (dark blue), Run 2 (green), Run 3 (red), Run 4 (cyan), Run 5 (purple), Run 6 (grey))

The decrease was even more apparent when *L.plantarum* was added as starter culture (Fig. 2). *L.plantarum* metabolism produced lactic acid which decreased the pH of fermentation. This brought the fermentation to the optimal condition for endogenous linamarase activity, around pH 5.5 (Brauman et al. 1996), and thus increasing the cyanogenic glycoside degradation in cassava chips. Lactic acid bacteria might also produce linamarase and pectinase which aided the degradation process (Brauman et al. 1996).

Effects of Starter Composition on Nutritional Contents of Fermented Cassava Flour

Along with the increase in microbial concentration throughout the fermentation, the starch content of the produced fercaf was observed to decrease (result not shown) and respectively the Dextrose Equivalent (DE) was observed to increase (Fig. 3a). The microbial amyolytic activity catalysed the hydrolysis of starch and produced simpler sugar as was observed in the increase DE along the fermentation. The decrease in starch

content of the produced cassava flour due to fermentation was also reported previously (Gunawan et al. 2015; Sulistyo and Nakahara 2014) and was attributed either to its consumption for the carbon source of microbial metabolisms or its conversion for metabolic products.

The increases in DE at experiments with added starter cultures were higher than that of control experiment (Fig. 3a). This may be related to the increased in total microbial population in the system (Figure 1) that increased the rate of starch hydrolysis into simpler sugars, part of which was consumed by the microorganisms. As *L. plantarum* was the dominant microorganism during the control fermentation, the result might also indicate that *B. subtilis* and *A. oryzae* provided higher amyolytic activity compared to *L. plantarum*. Beside the enzymatic amyolytic activity, the produced lactic acid might also play a role in the hydrolysis of starch into simpler sugars (Brauman et al. 1996). However, high decrease in starch associated with lower yield of produced flour. Thus, the particular process that resulted in high

starch degradation is not wanted.

Fermentation was also aimed to increase the protein content of cassava flour. Without prior fermentation, the protein content of cassava flour was measured in the range of 1.0 – 2.3% (wet basis). The control fermentation resulted in a decrease in the protein content of the produced flour (Fig. 3a). The protein content rapidly decreased from 1.0% to 0.4% within the first 24 hours of cassava fermentation and remained relatively constant afterwards. Only when *A. oryzae* was added as starter culture (Run 4, 5, 6), the protein contents were observed to increase. Comparable increases in protein content were observed at these three experiments.

Other literatures reported that cassava fermentation, in particular using *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Leuconostoc mesenteroides* or *L. plantarum* as starter culture, would result in an increase in the protein content (Gunawan et al. 2015; Tefera et al. 2014) that was attributed to microbial protein or the extracellular enzymes that were excreted during the fermentation. The decrease in protein content after cassava fermentation was only reported by (Sobowale et al. 2007), which showed that traditional cassava fermentation decreased protein content from 1.65% to 1.61%, whereas the addition of *L. plantarum* starter would further decrease the protein content to 1.26% or 1.14%.

The obtained results showed that despite high protein content of the microbial cells, the growth of microorganisms did not lead to an increase in the protein content of the

produced flour. Indeed, the nature of this cassava fermentation was suspended fermentation; thereby microorganisms were suspended in the fermentation broth except for the fungi *A. oryzae* which tended to grow in the surface of the cassava chips. This also explained why only fermentations in which *A. oryzae* was added as starter culture caused an increase in the protein content. Other possible explanation for the decreasing protein content was the ability of *B.subtilis* to produce protease that degraded protein into ammonia and carbon dioxide (Amoa-Awua et al. 2014). Further, considering that no additional media was added into the fermentation, the decrease in protein content in the other fermentations might be caused by its consumption by the microorganisms for the Nitrogen source for their growth.

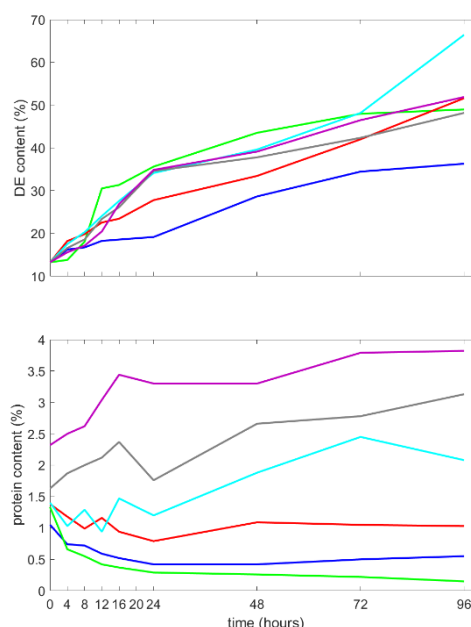


Fig. 3: Profile DE Value (a) and Protein Content (b) during the Fermentation (Run 1 (dark blue), Run 2 (green), Run 3 (red), Run 4 (cyan), Run 5 (purple), Run 6 (grey))

Effects of Starter Composition on Physical Properties of Fermented Cassava Flour

Swelling power is positively related with the ability of amylose to bind with water molecules. It indicates the starch granules capability to swell during cooking process. It also relates to food quality, particularly texture and product elasticity (Kim and Seib 1993). In general, increase in swelling power index was observed at run 2, 3, 4, 5, and 6 (Fig. 4), in which high concentration of *B. subtilis* was observed (Fig. 1). On the other hand, decrease in the swelling power index was observed at the natural fermentation (Figure 4) when insignificant concentration of *B. subtilis* was observed (Fig. 1). *B. subtilis* was reported to have cellulolytic activity (Amoa-Awua et al. 2014) which is able to disrupt cassava tissue hence supports the penetration of water molecule into the starch granule and facilitates amylose-water bounding. The increase of swelling power along those fermentation also strengthen prior hypothesis as the concentration of *B. subtilis* increased along the fermentation period (Fig. 1).

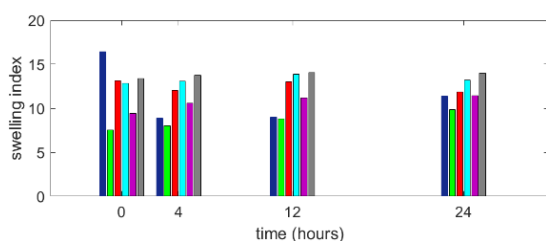


Fig. 4: Time Profile of Swelling Index during the Fermentation (Run 1 (dark blue), Run 2 (green), Run 3 (red), Run 4 (cyan), Run 5 (purple), Run 6 (grey))

Effects of Starter Composition on Pasting Properties of Fermented Cassava Flour

Pasting temperature indicates the minimum temperature required to cook starch products. Higher pasting temperature indicates more energy needed to process the flour, whereas lower pasting temperature represents lower stability of starch granules towards processing temperature, making it easier for pasting the flour during the cooking (Abbas and Khalil 2010). The difference in pasting temperature can be affected by the interaction between starch granules and non-starch components (Liu 2005). In agreement with this, we observed that the pasting temperature of fercaf was lower than tapioca (Figure 5a), the flour produced from the extraction of cassava starch. Natural fermentation (Run 1) did not significantly change the pasting temperature (Fig. 5). The possible explanation for this is that the starch granules were not significantly damaged or disrupted during the natural fermentation, whereas the number of intact granules influences the pasting temperature (Liu 2005). Indeed the concentration of *B. subtilis*, which was suspected to cause the damage in starch granule, was observed to be low in natural fermentation (Fig. 1). In other runs where high concentration of *B. subtilis* was observed, i.e. Run 2, 4, and 5, the pasting temperature was observed to decrease considerably (Figure 5a).

Other important pasting properties of flour are peak, final, and setback viscosities. Peak viscosity of the flour is related to its water holding capacity and represents the equilibrium between the

number of swollen granules and leaked polymer from the starch granules. High peak viscosity indicates the stronger starch granules. Final viscosity represents the viscosity of a product after being cooled. Setback viscosity is related with amylose composition of the flour and represents the tendency of flour retro degradation (Niba et al. 2002). Due to its fibre content, the peak viscosity of unprocessed cassava flour is lower than that of tapioca starch (Figure 5b). One previous study reported that fibre content has a capability to prevent water access towards starch granules, thus it will produce lower peak viscosity (Niba et al. 2002). Microbial activities during the fermentation of cassava chips led to degradation of cassava fibre and hydrolysis of cassava starch. Accordingly, the peak viscosities of the produced flour were observed to increase and that the addition of starter culture accelerated the increase of peak viscosity (Fig. 5b).

Fermentation increased the final viscosities of the produced flour (Fig. 5c). The final viscosities of the unfermented cassava flour (time 0) was lower than tapioca, but 4 – 12 hours of fermentation, the final viscosities of the produced flour were similar or exceeding the final viscosity of tapioca. On the other hand, no clear trend of setback viscosities was observed (Fig. 5d). The changes in final viscosities and setback viscosities of the produced flour due to fermentation were somehow smaller than the changes of the corresponding peak viscosities.

The obtained results clearly indicated that fermentation of cassava chips modified the pasting properties of the

produced flour. Considering that these viscosities are important properties in food processing industries. It is therefore important to set the targeted viscosities and design the fermentation time accordingly.

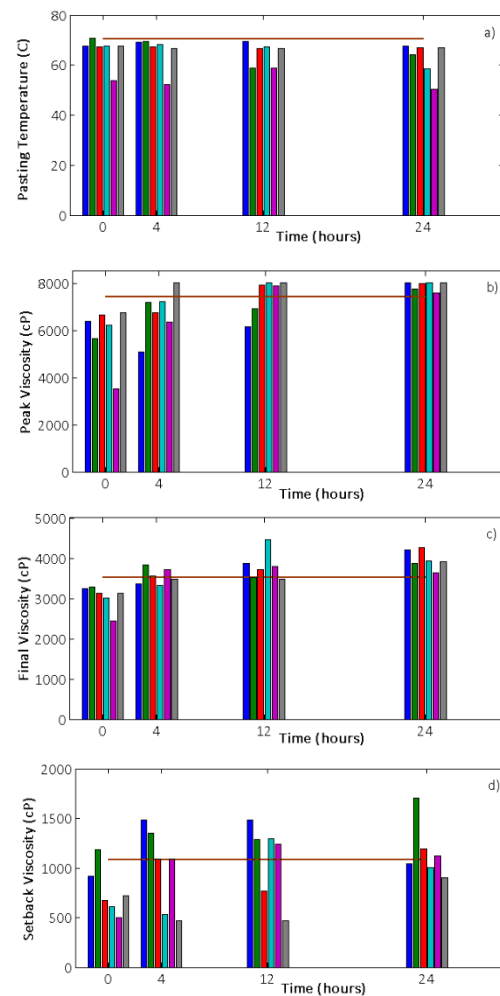


Fig. 5: Time Profile of Fercaf Pasting Properties: Pasting Temperature (a), Peak Viscosicites (b), Final Viscosities (c), and Setback Viscosities (d) during the Fermentation (Run 1 (dark blue), Run 2 (green), Run 3 (red), Run 4 (cyan), Run 5 (purple), Run 6 (grey), Tapioca (straight line))

DISCUSSIONS

Overall results showed that fermentation of cassava chips, prior to drying and milling changed the properties of the produced flour. When no microbial starter was added to the fermentation, *Lactobacillus* as the endogenous microbial species in cassava fermentation dominated the population. The presence of *Bacillus* in the fermentation was shown to accelerate the reduction of complex carbohydrates such as starch, pectin, cellulose, or the cyanogenic glycoside, which further induced specific physical and pasting properties of the flour. The presence of *Aspergillus* in the fermentation was shown to increase the protein content of the produced flour, an important nutritional quality of cassava flour that needed to be improved.

The answer to the question, which starter composition should be applied for production of fermented cassava flour, is not distinct one. Although there was no clear trend of interaction between species of added microorganism such as the addition of *B.subtilis* was neither inhibit nor accelerate the growth of *L.plantarum* or *A.oryzae*, increased in *Bacillus* population during fermentation caused both positive and negative effects to the fermented cassava flour production. The ability of *Bacillus* species in degrading complex carbohydrate accelerates the reduction of cyanogenic content of the produced flour but also accelerates degradation of starch into simple sugars. Simple sugars are easier to dissolve in the fermentation broth, causing lower yield of the produced fermented cassava flour. In

the end, besides choosing the appropriate the microbial starter composition we need to carefully decide how far the fermentation should progress or in other words how long the fermentation should be.

For health reason, cyanogenic content of the produced fermented cassava flour should be the main consideration. Without considering further reduction of cyanogenic content during the following drying process, short fermentation time of about 4 hours was sufficient to achieve safe cyanogenic level of 10 ppm. However, the implemented fermentation time should be re-inspected when high cyanogenic cassava or the so-called bitter cassava was used as the raw materials.

Mixed starter culture containing *Lactobacillus plantarum*, *Bacillus subtilis* and *Aspergillus oryzae* was considered to give the optimum results: low cyanogenic content, low yield loss, high protein content, acceptable swelling power and other pasting properties. On the other hand, fermentation time should be limited to less than 12 hours to minimize yield loss. Nonetheless, it should be possible to produce specific properties of fermented cassava flour by designing specific microbial starter composition and fermentation time.

CONCLUSIONS

Fermentation of cassava was shown to modify the characteristics of the produced flour (fercaf), both the nutritional contents and pasting properties. The composition of microbial starter used in the fermentation was shown to affect the

dynamics in microbial population during the fermentation and their corresponding activities. Although endogenously presents in cassava, the addition of lactic acid bacteria accelerated the release of cyanogenic glycoside and starch conversion to simpler sugars. The addition of *B. subtilis* improved the disruption of cassava fibres. On the other hand, the addition of *A. oryzae* was shown to increase the protein content of fercaf. The degree of starch conversion and cell wall structure disruption further determined the pasting properties of fercaf. Overall, it is possible to produce different types of fermented cassava flour, each with defined nutritional and pasting properties, by implementing a specific microbial starter culture composition and setting a specific fermentation time.

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