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Chapter

Solid-State Fermentation of Cassava Products for Degradation of Anti-Nutritional Value and Enrichment of Nutritional Value

Mohamed Hawashi, Tri Widjaja and Setiyo Gunawan

Abstract

The cassava plant is grown in tropical and subtropical countries, which represents, alongside with its by-products, an important source of food and feed. Hence, this plant has the capacity to promote the economic development of those countries and provide food security. However, cassava has some disadvantages due to the antinutrient compounds produced in its tissues. In addition, the cassava roots have a low protein content. Due to the economic and practical advantages, the solid-state fermentation (SSF) has been used as a cost-effective and efficient processing method to detoxify the cassava products and enrich them in nutrients. This chapter reviews the solid-state fermentation technique of cassava products for the production of valuable components for food and feed applications, microorganisms involved in this process, and key factors used to optimize the SSF process.

Keywords: anti-nutritional value, cassava, nutritional value, processing variables, solid-state fermentation

1. Introduction

Cassava (*Manihot esculenta Crantz*) is grown in tropical and subtropical countries. It is a vital source of food and feed and it can promote economic development and provide food security [1]. Cassava production has been promoted globally by the International Fund for Agricultural Development (IFAD) and the United Nations Food and Agriculture Organization (FAO) to develop cassava strategies [2]. Reports indicate that production rates will reach 300 million tons per year by 2020 [3]. Due to its high drought tolerance, cassava plant cultivation can take place even under critical environmental conditions, with an ideal high yield of approximately 50% for leaves and 6% for roots at plant maturity [4]. Its peel may make up 10–20% of the roots' wet weight [5]. However, cassava has some disadvantages; its tissues contain anti-nutritional compounds and very low protein content [6, 7].

Among all the antinutrients, hydrogen cyanide (HCN) is of great concern, the concentration of which is in cassava and its by-products are much higher than the World Health Organization (WHO) safe limit for human consumption (10 ppm) [8, 9]. Konzo is an irreversible neurological disease associated with intake of HCN [10]. Therefore, a detoxification process is needed to reduce anti-nutritional levels

in order to consume cassava safely. Solid-state fermentation (SSF) has been used as an economical and efficient processing method for enriching and detoxifying cassava and its by-products [11, 12]. Various process parameters such as particle size, moisture content, water activity, pH, the inoculum size, incubation time, concentration of nutrient supplementation, and temperature can affect the microbial growth, enzyme production, and formation of the product during the SSF process [13].

This chapter discusses fermented cassava products through solid-state fermentation for food and feed applications, as well as microorganisms involved in solid-state fermentation and the essential processing variables used to optimize the process.

2. Fermentation processes

Fermentation has been one of the most used technologies to improve the taste and sensory properties of food and continues to be one of the most widely used methods of preserving the food for a length of time [14, 15]. The cassava fermentation process is a strategy to improve nutritional value by enriching protein and detoxifying toxic and anti-nutritional compounds, in particular by reducing toxic cyanogenic glycosides to a safe level of consumption in cassava products as well as reducing post-harvest losses [16–18].

There are two kinds of fermentation, i.e., spontaneous (natural) fermentation and controlled fermentation. For the natural fermentation, the conditions are selected so that to produce the most suitable microorganisms for the production of growth by-products characteristic of a particular type of fermentation [19]. The controlled fermentation is generally used when the natural fermentation is unstable or the bacteria are not able to grow. In this case, specific microbial strains, such as lactic acid bacteria (LAB), yeast, and fungal are isolated, characterized, and preserved for later use as starter cultures [20]. Under optimal growth conditions, these cultures can be used as single or combined starter cultures. As a result, the quality of products and their organoleptic characteristics are well controlled and predictable [20, 21].

However, the fermentation process can be broadly categorized into submerged fermentation (involving soaking in water) and solid-state fermentation (without soaking in water) [22]. The solid-state fermentation (SSF) technique has several advantages over submerged fermentation (SmF). However, the SSF has some constraints. **Table 1** illustrates the advantages and disadvantages of SSF over SmF [23].

2.1 Solid-state fermentation and its application in cassava products

In recent years, the cassava population has developed numerous processing methods (soaking, boiling, drying, and fermentation) [24–26]. SSF is one of the promising processes of enriching protein and detoxifying of cassava products [27–29].

Fermented cassava products by SSF, such as flour, gari, starch, bread, and biomass contain high protein content that can either be consumed by humans or animals, replacing expensive, conventional protein sources in different parts of Latin America, Africa, and Asia [30]. The major fermented cassava products by SSF can be derived from different parts of the cassava plant, such as roots, peels, and leaves.

2.1.1 Cassava roots

Cassava is grown in many developing countries for its roots as a primary source of carbohydrates and ranks third in the developing countries as the leading source

Parameter	Solid-state fermentation	Submerged fermentation
Substrates	Insoluble substrates (starch, cellulose, pectins, lignin)	Soluble substrates (sugars)
Aseptic techniques	Sterilization of steam and non-sterile conditions	Sterilization of heat and aseptic control
Temperature	Difficult temperature control	Easy temperature control
Water	Low water consumption	High water consumption
pH control	Difficult pH control	Easy control of pH
Industrial level	Relatively small scale, newly designed equipment is needed	The industrial level is available
Inoculation	Spore inoculation, batch process	Easy inoculation, continuous process
Contamination	Contamination risk of low-growth fungi	Contamination risk of single strain bacteria
Energy	Low consumption of energy	High consumption of energy
Equipment volumes	Low volumes and low equipment costs	High volumes and high equipment costs
Pollution (effluents)	No volumes of effluents	High volumes of effluents
Concentration/products	100/300 g/L	30–80 g/L

Table 1.
Comparative characteristics of solid-state and submerged fermentations.

of energy in human diets along with rice and wheat [31]. World production of cassava is estimated at 277 million tons of fresh root in 2017 [32]. Cassava root has several advantages compared to other crop roots, including high productivity, resistance to droughts and pests, flexible harvesting age, and it can be kept in the ground until they are needed [33]. However, cassava root also has certain disadvantages; its tissues contain toxic compounds (a cyanogenic glycoside), low protein content (1% fresh root weight), and short shelf life of 1–3 days [34].

Food processing techniques have been used to convert cassava tubers into flour as an alternative way to preserve the roots after harvesting and then further use it for industrial and traditional purposes [35, 36]. Gari and flour are the most popular fermented food products from cassava roots by SSF. In West Africa, approximately 200 million people consume gari [37, 38]. **Figure 1** shows the production of flour and gari under the solid-state fermentation [11].

The purpose of cassava root fermentation is to increase the low protein content from 2% to about 7% or more than the critical crude protein content [39]. To achieve this goal, several solid-state fermentation techniques have been used. Raimbault et al. [40] reported the principle underlying the SSF procedure for the enrichment of cassava flour. This procedure led to the enrichment of crude protein from 1 to 18–20%, which improved between 1700 and 1900% after 30 h of fermentation. Oboh and Elusiyan [41] studied the effect of solid-state fermentation by *R. oryzae* and *S. cerevisiae* on the improvement of nutritional values of cassava flour produced from two different varieties of cassava root. The nutritional contents of cassava flour were assayed before and after 72 h of fermentation. This study has observed that *S. cerevisiae* was more effective than *R. oryzae* in the nutrient enrichment of cassava flour. The results of this study are presented in **Figures 2–5**. Essers et al. [42] investigated the effect of SSF on the degradation of hydrogen cyanide level in cassava root using six fungal strains, namely *Rhizopus stolonifer*, *Rhizopus*

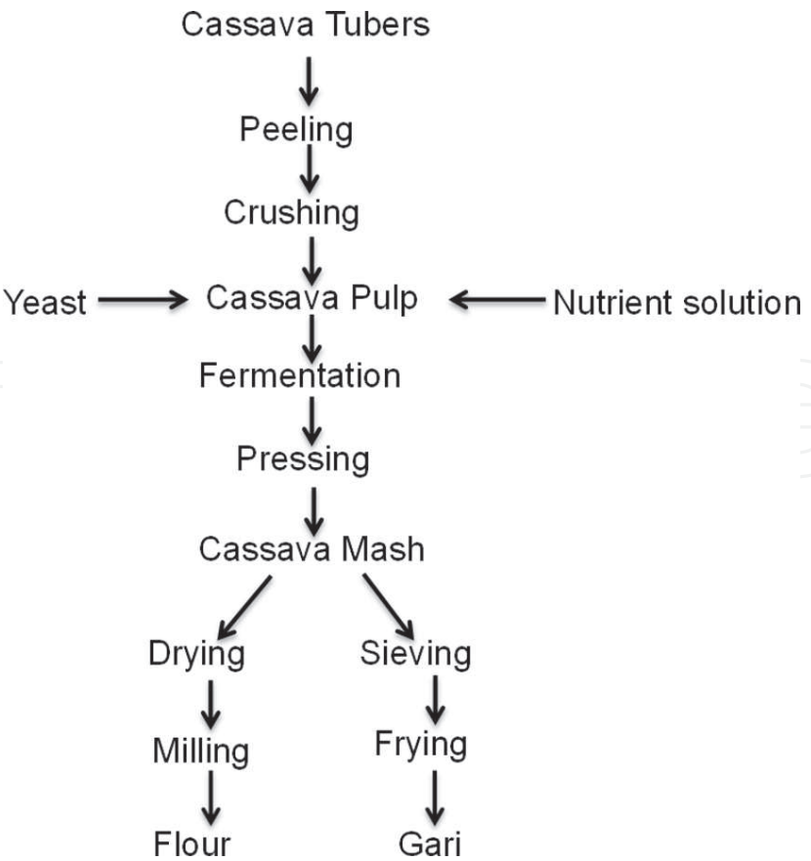

Figure 1.
The production chart of cassava products (flour and gari) under SSF.

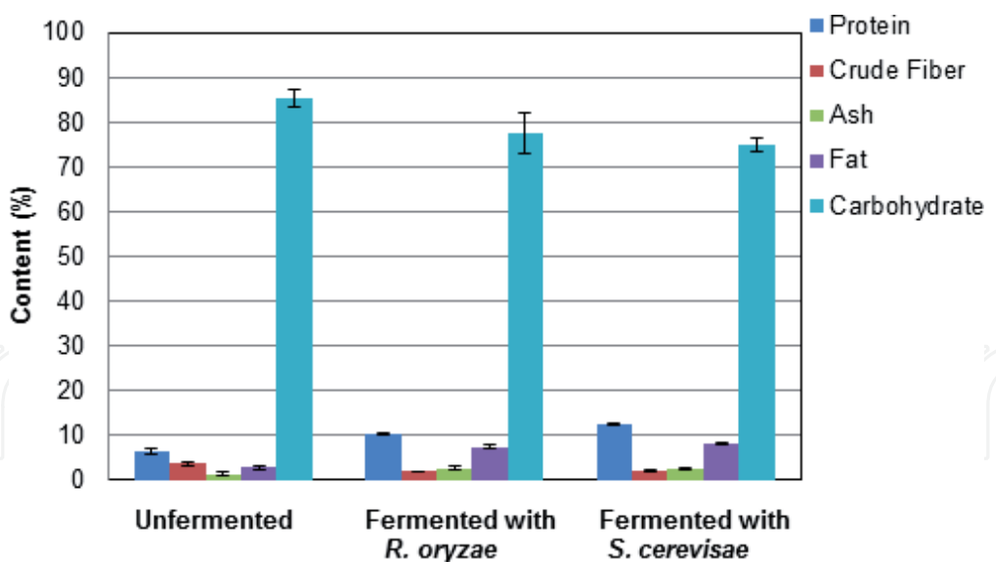

Figure 2.
Proximate composition of the cassava flour obtained from cassava varieties of low HCN subjected to SSF.

oryzae, *Mucor racemosus, Bacillus sp*. *Geotrichum candidum*, and *Neurospora sitophila*. The reduction in cyanide content was more than 60% after 72 h of fermentation.

In addition, Oboh and Akindahunsi [11] investigated the effect of solid-state fermentation with *S. cerevisiae* on the nutritional and antinutrient contents of cassava products (flour and gari). After 72 h of fermentation, the results revealed that the content of protein and fats in cassava flour increased by 10.9 and 4.5%, respectively. The protein and fat content of fermented gari also improved by 6.3% and 3.0%. In contrast, the content of cyanide in flour and gari decreased to 9.5 and 9.1 (mg/kg),

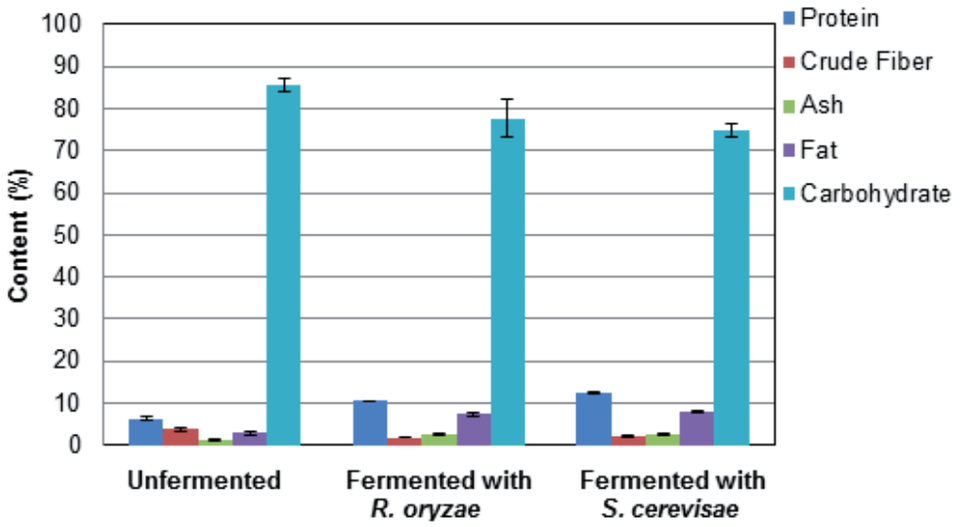

Figure 3.
Proximate composition of the cassava flour obtained from cassava varieties of medium HCN subjected to SSF.

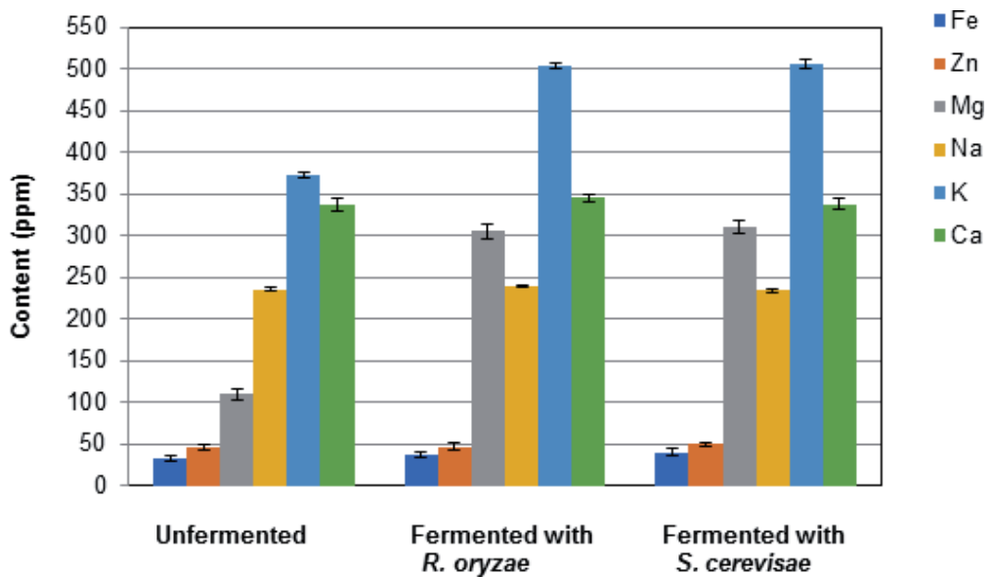

Figure 4.
Mineral contents of the cassava flour obtained from cassava varieties of low HCN subjected to SSF.

respectively. However, the tannin content, crude fiber, and ash content of the cassava products did not change significantly under SSF.

2.1.2 Cassava peels

Cassava wastes, such as peels and leaves and starch residues make up 25% of the total cassava plant [43]. Cassava peel is the leading waste from the cassava plant, but its use is limited due to the high content of cyanide and fiber as well as low protein and therefore disposed of it after cassava processing into food or other industrial products [44, 45]. Many efforts have been made using SSF techniques to enrich the protein content and degrade the cyanide level of cassava peels for animal feed.

Bayitse et al. [12] studied protein enrichment of cassava residue using *Trichoderma pseudokoningii* under solid-state fermentation for 12 days, urea, and ammonium sulfate was used as a nitrogen source, and the moisture content ranged from 60 to 70%. The result showed an improvement in crude protein content of 12.5% using urea as a nitrogen source, and a moisture content of 70%, as compared

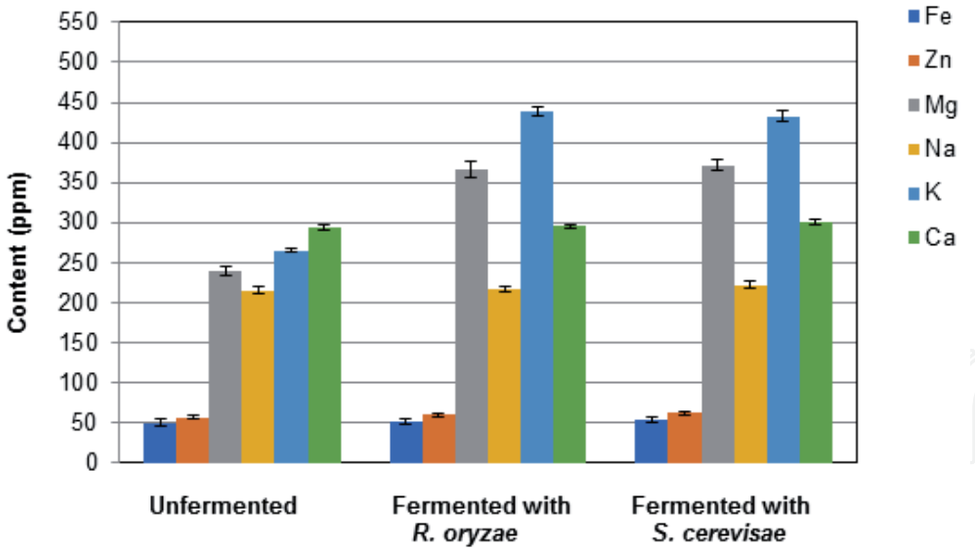

Figure 5.
Mineral contents of the cassava flour obtained from cassava varieties of medium HCN subjected to SSF.

to 8.89 and 6.37% improvement observed with ammonium sulfate as a nitrogen source, and without using nitrogen source. The study observed a decrease in cyanide content, but it did not attribute it to the fermentation effect of *Trichoderma pseudokoningii*, rather it stated that the reduction could have been as a result of the pre-processing of cassava peels.

Iyayi and Losel [43] also evaluated protein improvement of cassava peels using different types of microorganisms and fermentation time (*Saccharomyces cerevisiae, Aspergillus niger, Rhizomucor miehei,* and *Mucor strictus*). The solid-state fermentation of cassava peels by *S. cerevisiae* produced the highest protein content from 5.6 to 16.74% for 21 days. Also, they reported the maximum fermentation period for the protein enrichment of cassava peel to be from 12 to 15 days, after which no significant change was observed, which is in line with the work reported by Bayitse et al. [12].

Ezekiel and Aworh [13] evaluated the effectiveness of SSF with *Trichoderma viride* on the reduction of cyanide content and enrichment of the crude protein content of cassava peel by optimizing the fermentation conditions such as moisture content, pH, particle size, nitrogen source, and incubation temperature. The optimum SSF conditions were found at the initial moisture content of 60% (v/w), the particle size of 4.00 mm, a pH of 6.0, 30°C of temperature, and ammonium sulfate (10 g N/kg substrate) as nitrogen sources. After 8 days of fermentation, the cyanide content was reduced by 71% and improved the crude protein content from 4.2 to 10.43% at optimized conditions.

In another study by Ruqayyah et al. [45], the application of response surface methodology was used to optimize SSF conditions (moisture content, inoculum size, and pH) with *P. tigrinus* to enrich the crude protein content of cassava peel. A maximum protein content of 89.58 (mg/g) was obtained at 75% (v/w) moisture content, 7% (v/w) inoculum size, and pH of 5.3 with a fermentation time of 15 days. The optimum level resulted in a significant enrichment of the protein content by 55.16%.

Oboh [46] investigated the effect of solid-state fermentation of cassava peel with a mixture of *Saccharomyces cerevisiae* and two strains of lactic acid bacteria, *Lactobacillus delbrueckii* and *Lactobacillus coryniformis* to improve the nutritional value and detoxification of cassava peel. The chemical composition of cassava peel has been analyzed before and after fermentation. The results showed the effective

performance of the SSF technique in removing cyanide by 86% after 7 days of fermentation. On the other hand, the mineral composition of the cassava peel did not change during the fermentation. The results of this study are presented in **Table 2**.

2.1.3 Cassava leaves

Cassava leaves are an extremely rich source of proteins, vitamins, and minerals that exceed some of the other green vegetables [47, 48]. The production of cassava leaves is estimated at 10 tons of dry leaves per hectare, which has a similar yield with the roots [49]. Cassava leaves are consumed in most Southeast Asian and African societies, such as Indonesia, Malaysia, Congo, Madagascar, and Nigeria [50, 51]. However, cassava leaves contain both nutritive (33.8–37.4% protein content) and anti-nutritional compounds [301.04–192.47 (mg/100 g) HCN content] [52]. Boiling, soaking, steaming, drying the sun, drying the oven, and cooking are the most common methods for processing cassava leaves in African and Asian countries [53].

The origin of HCN in the cassava leaves is a two-step process [54, 55]. First, the linamarin, a cyanogenic glycoside, which represent 93% of cyanogenic glycosides found in cassava (7% is lotaustralin), is hydrolyzed by linamarase (a beta-glycosidase) into glucose and cyanohydrin. Then, in the second step, the cyanohydrin is decomposed, either enzymatically or not, to HCN and acetone. The nonenzymatic pathway depends on pH. At pH > 6, the HCN is liberated, but at an acidic pH (~5), the process is much lower, and the resulting HCN is therefore relatively lower in concentration. However, this approach did not assure full hydrolysis of cyanogens. The partial breakdown of the leaf cells only partially releases linamarase resulting in only a certain proportion of the cyanogenic compounds being converted to HCN. This implies that a proportion of the cyanogens remain present in the leaves after processing and resulting in the release of HCN directly into the human body upon consumption.

The conventional methods have been proven to be ineffective for lowering the cyanide content in cassava leaves to the safe limit, at the same time causing a significant loss of protein and essential nutrients, which is highly desired from the cassava leaves [56–60]. Hence, the establishment of a universally acceptable method that produces edible leaves with low cyanide level while maintaining

Composition	Fresh	Naturally fermented	Fermented with a mixed culture
Crude protein (%)	8.2 ± 0.1	11.1 ± 0.3	21.5 ± 1.2
Crude fiber (%)	11.7 ± 0.5	6.5 ± 0.5	11.7 ± 0.5
Fat (%)	3.1 ± 0.4	3.5 ± 0.2	2.1 ± 0.1
Ash (%)	6.4 ± 0.4	6.0 ± 0.2	7.2 ± 0.2
Carbohydrate (%)	64.6 ± 0.2	67.3 ± 0.4	51.1 ± 0.4
Moisture (%)	5.1 ± 0.3	5.7 ± 0.2	6.4 ± 0.4
Ca (ppm)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Na (ppm)	00.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Zn (ppm)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
K (ppm)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
HCN (mg/kg)	45 ± 0.3	24 ± 0.2	6.1 ± 0.4

Table 2.
The effect of fermentation on the chemical composition of cassava peels.

maximum nutritional content is challenging and still far away from being established. Among the efforts made so far, Morales et al. [61] proposed a solid-state fermentation of cassava leaves, reducing the cyanide content while improving the nutritional value of the processed leaves. SSF was performed using *Rhizopus oligosporus*, and babassu mesocarp flour was the substrate used, supplemented by cassava leaf flour. The solid-state fermentation decreased the total cyanide content of the cassava leaves by 94.18%, also SSF increased the quantity and quality of crude protein content by 15%, resulting in the relative nutritional value of 98.18% for food, which is equivalent to casein (100%). Furthermore, Kobawila et al. [62] investigated the effect of alkaline fermentation on the reduction of cyanide level in cassava leaves to produce ntoba mbod. The dominant microflora in the fermentation of the cassava leaves was *Bacillus subtilis, Bacillus macerans,* and *Bacillus pumilus*. These bacteria can utilize cyanide acid for their nutrition [63]. Thus, they are responsible for the reduction of the cyanide content in the medium of fermentation (~70% removal). However, the report did not provide the effect of the fermentation process on the protein content of cassava leaves.

One of the essential criteria for the solid-state fermentation is the selection of an appropriate microorganism [64]. Several research works have explored different types of microorganisms mainly fungi, yeasts, and bacteria, as well as different substrates to favor the metabolism of the microorganisms in SSF of cassava products. Examples of microorganisms associated with solid-state fermentation of cassava products for food and feed applications are summarized in **Table 3**.

Microorganism	Substrate	Product	References
Rhizopus oryzae	Cassava root	Gari	[29]
Rhizopus oryzae (TISTR 3052), *Rhizopus oryzae* (TISTR 3058), *Rhizopus delemar* (TISTR 3534), and *Rhizopus delemar* (TISTR 3190)	Cassava flour	Bread	[65]
Panus tigrinus (M609RQY)	Cassava peels	Animal feed	[66]
Aspergillus niger and *Panus tigrinus*	Cassava peels	Poultry feed	[67]
Rhizopus oryzae and *Saccharomyces cerevisiae*	Cassava pulp	Animal feed	[68]
Saccharomyces cerevisiae, Aspergillus niger, Rhizomucor miehei, and *Mucor strictus*	Cassava leaves	Animal feed	[43]
Rhizopus oryzae	Cassava pulp	Lactic acid production	[69]
Lactobacillus plantarum and *Rhizopus oryzae*	Cassava root	Cellulase production	[70]
Bacillus sp., *Mucor racemosus, R. oryzae, Neurospora sitophila R. stolonifer* and *Geotrichum candidum*	Cassava root	Cassava flour	[71]
Rhizopus stolonifer LAU 07	Cassava peel	Feed supplements	[72]
Rhizopus sp.	Cassava starch and leaves	Lactic acid and ethanol productions	[73]

Table 3.
Examples of microorganisms associated with the SSF of cassava products.

3. Environmental factors

The process control of the solid-state fermentation parameters is closely related to the metabolic regulation of microorganisms [74]. Based on the metabolic needs of the fermentation microorganisms, the control of water activity, oxygen content, temperature, and pH are the main solid-state fermentation parameters [23]. In the solid-state fermentation process, the water, gas, and heat caused by the growth microbes are the dominant factors that determine the environmental changes. The environmental factors can affect the microbial growth and formation of the product during the SSF process [75, 13]. Therefore, the physical-chemical parameters must be controlled.

3.1 Water activity and moisture content

The unique feature of solid-state fermentation is that there is almost no free water in the substrate [76]. However, microorganisms can grow depending upon the water activity of the substrate [64, 75]. The growth of fungi and some yeast usually requires a water activity value between 0.6 and 0.7 [77]. In addition to meet the microbial physiological requirements, the water content level plays a decisive role in the variation of the three-phase structure relating to water retention, permeability, and thermal conductivity. The degree of swelling in the SSF system was low at a lower moisture level and hence increased water stress reduces nutrient solubility. On the contrary, the higher level of humidity results in changes in substrates that reduce porosity, thus contributing to stickiness and reduced gas exchange [78, 79]. According to Grover et al. [80], the required moisture content should range between 60 and 80% for an efficient SSF system.

3.2 Temperature

The fermentation temperature affects microbial growth, spore germination, and the formation of product [81]. Heat generation in solid-state fermentation system is more problematic than in liquid fermentation. Due to poor heat conductivity and accumulation of metabolic heat in the material combined with substrate shrinkage and decreased porosity, gas convection is severely impeded. Previous studies showed that the significant resistance to heat transfer in solid-state fermentation was low conduction efficiency [82, 83].

Therefore, moisturizing is a common measure of temperature control. In addition, routine operations (e.g., forced ventilation and jacket cooling) all can solve these problems [84]. The evaporative cooling is one of the main solid-state fermentation temperature control measures [85, 86]. In general, the aeration could reduce the temperature gradient of the medium [23]. The forced ventilation can take away more than 80% of the heat generated from the substrate [84]. From the current investigation, it is difficult to maintain the temperature at an ideal range in SSF system. To reach this aim, the main strategy used in large-scale solid-state fermentation is to combine ventilation and humidity [77].

3.3 Oxygen concentration

The gas environment is a critical factor that significantly affects the relative levels of biomass and the production of an enzyme [23]. Oxygen uptake rate (OUR) and carbon dioxide production (CDPR) can be used to assess the state of the solid-state fermentation process. However, different microorganisms cause these

assessments to vary. Ghildyal et al. [87] studied the impact of the gas concentration gradient on product yield in a tray solid-state fermentation bioreactor. The results showed that the variations of O_2 and CO_2 concentration gradients were visible, which severely affected product yield. The yield decreased when gradient increases. Gowthaman et al. [88] also studied the impact of gas concentration gradient on the product in a packing bed bioreactor. The results showed that the gas concentration gradient could be eliminated and the ability of mass transfer can be enhanced by forced ventilation, which increased enzyme activity.

3.4 pH value

In general, if the initial pH value of the medium is adjusted, the variations of pH value during the solid-state fermentation process need to be considered [89]. During the fermentation process, the pH values change drastically. The reason is that organic acids including citric and lactic are secreted during the fermentation process, which decreases the pH [23]. While the increase in pH was rationalized in terms of organic acid decomposition and protein degradation in the raw materials into amino acids and peptide fractions [90]. The pH values are difficult to determine by conventional detection in SSF due to the low water content of the substrate. Nitrogen-containing inorganic salts (such as urea) are often used as sources of nitrogen to offset the pH variation in the fermentation process [91, 92].

In the study conducted by Ezekiel and Aworh [13] to evaluate the effect of pH on protein enrichment and soluble sugars of cassava peel by *Trichoderma viride*, the fungus was grown in a controlled pH medium of 4.– 6.0 with an incubation time of 8 days. The optimal growth condition was observed at pH 6.0. The protein increased in cassava peels from 230 at pH 4.0 to 270 (mg/gm) at pH 6.0. Also, the sugars yield at pH 5.0 and 6.0 was five times higher compared to pH 4.0. According to the study, the growth rate of the fungi at pH below five was affected by high acidity, leading to reduced bio-conversion of sugars into protein.

4. Conclusions

The results discussed in this chapter highlighted the importance of the SSF technique applied to cassava to improve its nutritional value. The solid-state fermentation using microbial protein is beneficial for the reduction of cyanide contents while the content of protein and other nutrients is increased compared to those obtained by the conventional approaches, i.e., soaking, boiling, and drying. Thus, the SSF technique for processing cassava products is better suited for developing societies and rural communities in the African and Asian countries that do not have easy access to available protein sources.

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Conflict of interest

The authors declare no conflict of interest.

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