# Development of Instant Microbial Starter for Production of Fermented Cassava Flour: Effect of Vacuum Drying Temperature, Carrier Media, and Storage Temperature

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**Abstract.** Cassava is an important crop for tropical countries such as Nigeria, Brazil, Thailand, and Indonesia. The potential utilization of cassava in the food industry can be enhanced by processing cassava into fermented cassava flour (*fercaf*), which has been shown to have a neutral color and aroma as well as low cyanogenic content. The use of specific microbial starter in the cassava chip fermentation for fercaf production will direct the fermentation process, maintaining a high quality of the produced flour. Thereby, the availability of an easy-to-use microbial starter is important for the production of fermented cassava flour. The aim of this study was to evaluate vacuum drying methods in the preparation of microbial starter for fermented cassava flour production. In particular, the effects of carrier media, drying and storage temperature on cell viability in dry starter were tested. The results showed that different methods should be applied to different microbial species. *Bacillus subtilis* and *Aspergillus Oryza* should be prepared using fercaf as the carrier media at a drying temperature of 55 °C, whereas *Lactobacillus plantarum* starter should be prepared using skim milk as the carrier media at a drying temperatur of 40 °C. Apart from *B. subtilis*, the starters should be stored in a refrigerator.

**Keywords:** *Carrier; cassava; fercaf; fermentation; microorganism; starter; storage; vacuum drying.*

## 1 Introduction

Indonesia is one of the largest cassava producing countries in the world. In 2015, cassava production in Indonesia reached 25 million ton [1]. The main problem with cassava is its HCN content and short shelf life. One way of eliminating the HCN content and extending cassava shelf life is by processing cassava into flour. One of the processed cassava products is fermented cassava flour (fercaf). Fercaf is reported to have decreased HCN levels, extended shelf life, thereby expanding cassava's potential use [2,3]. Fercaf can also be

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regarded as a second-generation modified cassava flour (Mocaf), with standardized starters, production process, and more hygienic quality.

One factor that affects the quality of fercaf is the microbial composition that works on the fermentation process [4,5]. However, there are problems related to starters, such as the limited accessibility and the high price of commercial starters, makes it impractical to be applied in community.

Scientifically, the method for preparing instant starter may share many similarities with methods for preparing microorganisms for probiotics. However, the microorganisms used as starter for fermented cassava flour production are not necessarily probiotics, i.e. microorganisms that when consumed maintain or restore beneficial bacteria to the digestive tract and thus need to be able to survive and multiply in the host [6]. In this respect, less sophisticated and thus cheaper methods may be required for instant starter preparation.

Previous studies have been carried out on instant starter production by using conventional dryer, spray dryer and freeze dryer [7]. Some microorganisms can be dried using the conventional drying method but *Lactobacillus plantarum*, a lactic acid bacteria species that cannot form endospores, cannot be dried by conventional drying [7]. Further, conventional drying is susceptible to contamination. The other two drying methods, i.e. spray drying and freeze drying, are relatively costly. On the other hand, vacuum drying could be a good alternative, since it operates under vacuum conditions, resulting in a mild operation temperature and therefore takes less time than conventional drying. Since vacuum drying ovens are hermetically sealed to prevent leakage and maintain vacuum pressure, external contamination is also prevented from penetrating into the oven. Based on these properties, vacuum drying is a potential candidate for preparing dried microbial starter.

This paper describes the determination of the vacuum drying parameter, i.e. drying temperature, and carrier matrices/media for the microorganisms, in order to get the best instant starter for the production of fercaf. Furthermore, the effect of storage temperature of the produced instant starter was also investigated to ensure the viability of the instant starter during storage (post-drying process).

## 2 Materials and Methods

### 2.1 Microorganisms

*B. subtilis* ITB B128, *L. plantarum* ITB B188, *A. oryzae* ITB L24 were obtained from the culture collection of the Laboratory of Microbiology and Bioprocess Technology, Chemical Engineering, Institut Technologi Bandung. One slant agar was used as inoculum in 100 ml of liquid culture. Natrium broth (NB) was

used to grow *B. subtilis* at 30 °C, deMan, Rogosa, and Sharpe (MRS) broth was used to grow *L. plantarum* at 37 °C, PDB (potato dextrose broth) was used to grow *A. oryzae* at 30 °C. These media were obtained from OxoidTM (Thermo Fischer Scientific, UK).

## 2.2 Carrier Preparation

Cassava (*Manihot esculenta*) based materials were evaluated as carrier media, such as cassava cubes, cassava chips, and fercaf. The cassava used was obtained from a local market, whereas the fercaf was prepared in the laboratory following [5]. For comparison the most commonly used carrier medium, skim milk (Merck, Germany) [8], was also tested. Fercaf and skim milk powder were sterilized by roasting for ± 5 min, the cassava chips and cubes were sterilized by using an autoclave at 121°C for 15 min.

## 2.3 Production Process of Instant Starter

The liquid microbial culture fermentation was concentrated using a centrifuge. The concentrate was mixed with the carrier media, i.e. fercaf, cassava cubes, cassava chips, or skim milk, at 10% of 100 ml of liquid culture.

Subsequently, vacuum drying was conducted using a Lab Companion OV-01 (Jeio Tech, Korea) vacuum drying oven in which the pressure of the chamber was set in the range of 0-0.1 MPa, with temperature variation of 40 °C and 55 °C. The drying process was conducted until the sample was considered to be dry based on visual inspection.

Samples were taken to evaluate the drying performance before and after drying. The dry cultures were stored in a refrigerator (± 4 °C) and room temperature. The viability of the dried cells was analyzed shortly before drying and after drying, at the second, the fourth, the sixth, the eighth week of storage.

## 2.4 Sample Analysis

The microbial viability of the sample was analyzed using total plate count (TPC). 0.1 g of sample was rehydrated with 0.9 mL of sterile water and subsequently serial dilution of the solution was conducted. The diluted samples were then spread into agar of specific media and subsequently the plates were incubated at specific conditions. Natrium agar (NA) was used to determine the *B. subtilis* population with incubation conducted at 30 °C for 48 hours. DeMan, Rogosa, and Sharpe agar (MRSA) was used to determine the *L. plantarum* population with incubation conducted at 37 °C for 48 hours. Potato dextrose agar (PDA) was used to determine the *A. oryzae* population with incubation

conducted at 37 °C for 72 hours. The obtained colonies were counted and cell viability was calculated following Eq. (1).

$$\%viability = \frac{\sum cell\ life - t}{\sum cell\ life\ before\ drying} x100\% \quad (1)$$

## 3 Results and Discussions

In general, the cell viability shortly after drying decreased from the viability before drying. The cell viability shortly after drying ranged from 0.05% to 14.02% of its initial value (Tables 1-2). Carrier medium, drying temperature, and storage temperature are some factors that affect the value of cell viability.

### 3.1 The Effect of Carrier Media

The obtained results showed that the cell viability after drying was influenced by the carrier media used in this study. In general, fercaf and skim milk provided good viability compared to the other carrier media, i.e. cassava cubes and cassava chips (Figure 1). The highest viability value shortly after drying for *A. oryzae* was 1.07%, obtained when fercaf was used as the carrier media. The highest viability value shortly after drying for *L. plantarum* was 9.14% obtained when skim milk was used as the carrier media. Meanwhile, for *B. subtilis* the highest viability value shortly after drying was 14.02% with cassava cubes as the carrier medium, but the difference with the viability of *B. subtilis* cell dried by cassava cubes and fercaf was not significant, as can be seen in Table 1.

Based on the results shown in Table 1 and realizing the morphology of each carrier medium, it is suggested that the powder morphology of fercaf and skim milk provides a large surface area that may coat and thus protect the cell walls of the microorganisms. Therefore, the intracellular parts of the cells can be maintained. On the other hand, both cubes and chips have a macroscale dimension that results in a low surface area, resulting in poor protection of the cell walls and correspondingly the intracellular parts of the microorganism cells, making them susceptible to the heat from the oven during the drying process. Based on this research it can be concluded that different types of carrier media have a significant effect on the cells for production of instant microbial starter.

The obtained results are in agreement with Antara, *et al.* [9], who showed that there was no significant difference between the viability of lactic acid bacteria preserved by vacuum drying using different media, i.e. maize starch floor, rice floor, and cassava starch floor (tapioca), all of which have a powder morphology.

**Table 1** Effect of carrier media on viability of instant starter cells of various microorganisms shortly after drying at 40 °C.

| Carrier Media | %Viability After Drying | | |
|---|---|---|---|
| | ***B. subtilis*** | ***L. plantarum*** | ***A. oryzae*** |
| fercaf | 8.45 ± 4.80 | 4.33 ± 2.94 | 1.07 ± 0.22 |
| Cassava Cubes | 14.02 ± 10.75 | 0.84 ± 0.25 | 0.11 ± 0.03 |
| Cassava Chips | 2.62 ± 2.62 | 1.47 (not in duplo) | 0.93 ± 0.53 |
| Skim milk | 5.04 ± 2.28 | 9.14 ± 5.42 | 0.06 ± 0.02 |

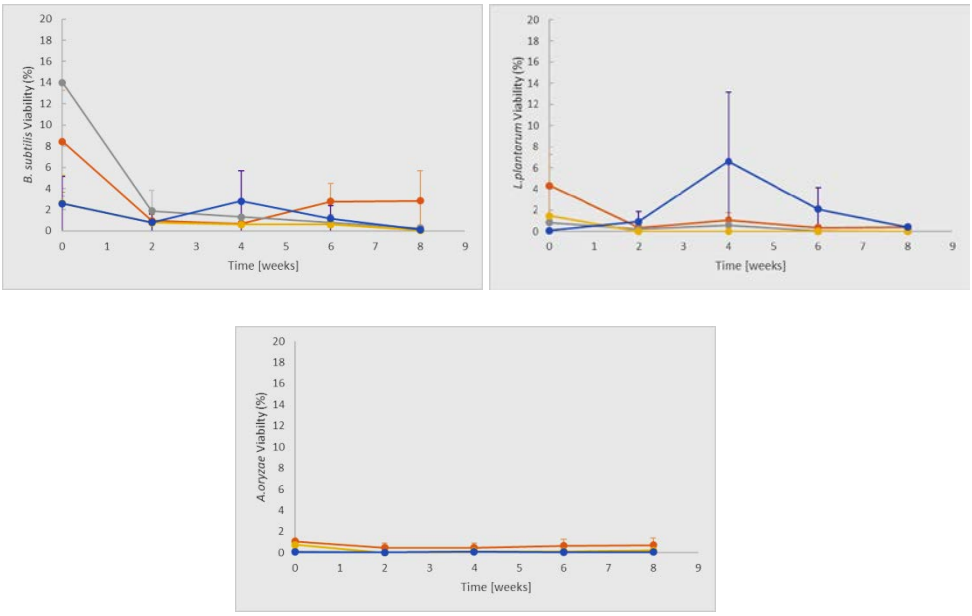


**Figure 1** The effect of carrier media on instant starter viability (data obtained by vacuum drying at 40 °C with the addition of 10% carrier media; different colors indicate different carrier media: orange for fercaf, grey for cassava cubes, yellow for cassava chips, and blue for skim milk).

## 3.2 The Effect of Drying Temperature

Vacuum drying experiments were conducted by varying the process temperature at 40 °C and 55 °C. The effect of drying temperature on instant starter viability can be seen in Table 2.

Table 1 shows that cell viability shortly after drying at 40 °C is generally lower than shortly after drying at 55 °C. This can be caused by the time difference required for the drying process. Drying at 55 °C was faster than drying at 40 °C,

thus the time required for drying at 55°C was shorter. A longer drying process will cause further damage to the cell wall. DNA is very sensitive to drying due to cell component damage that affects cell reproduction in cell inactivation processes [10]. A different trend was observed for *L. plantarum.* This may be related to the inability of this species to form endospore: brief exposure to a higher temperature may kill most of cells. Indeed Jordan & Cogan [11] reported the D-value of this species at 55°C to be 8.6, which means that exposing a culture of this bacteria for 8.6 minute to 55 °C will reduce its population by 90%.

**Table 2** Effect of drying temperature on viability of instant starter cells of various microorganisms shortly after drying at 40 °C and 55 °C.

| Microorganisms | %Viability after drying | |
|---|---|---|
| | 40 °C | 55 °C |
| *B.subtilis* | 5.04 ± 2.28 | 9.48 ± 3.13 |
| *L. plantarum* | 9.14 ± 5.42 | 0.48 ± 0.06 |
| *A.oryzae* | 0.06 ± 0.03 | 12.95 ± 3.57 |

## 3.3 The Effect of Storage Temperature

The average range of instant starter cell viability values of various microorganisms stored at room temperature for eight weeks after drying at 40 °C was 0.02-6.62% while for drying at 55°C it was 0.05-8.79% (Figure 2). The average range of instant starter cell viability values of various microorganisms stored at refrigerator temperature for eight weeks of storage after drying at 40 °C was 0.04-12.68% while for drying at 55 °C it was 0.10-17.10% (Figure 2). Overall storage in a refrigerator (± 4 °C) provided better cell viability than storage at room temperature.

This is consistent with the results from a previous research, in which dry culture of *Streptococcus thermophillus* and *Lactobacillus acidophillus* yielded higher culture viability at 4 °C storage temperature [12]. The main cause of decreased cell viability during storage is the occurrence of lipid oxidation [13]. Essential macromolecules such as lipids and proteins are degraded naturally during the storage process. Lipids and proteins in cells sustain oxidation and denaturation respectively during storage. At higher temperatures, an increase in lipid oxidation causes the saturated fatty acid composition to increase in the cell membranes, resulting in decreased cell membrane fluidity. This leads to leakage of cell membranes, which in turn results in a decline in cell viability during storage. Long-term storage of dry cultures is strongly influenced by the storage

conditions, i.e. temperature and moisture content [13]. Storage at 4 °C may inhibit lipid oxidation and cell damage.

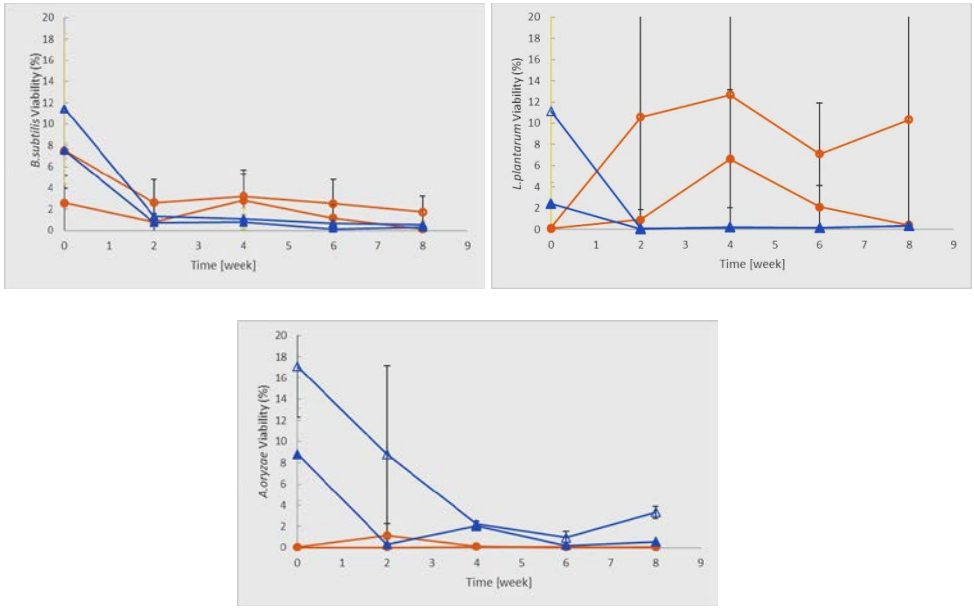


**Figure 2** The effect of drying and storage temperature on instant starter viability (drying temperature is indicated by different symbols: 40 °C (circles) and 55 °C (triangles); storage conditions are indicated by open symbols (refrigerator) and closed symbols (room temperature)).

## 4 Conclusions

The type of carrier media, in particular the morphology of the carrier media, and drying temperature applied during vacuum drying affect the cell viability of microbial starter. Fercaf was shown to be a good alternative carrier medium. In general, oven vacuum drying at slightly higher temperature, 55°C, was shown to provide better cell viability than drying at 40 °C. In relation to storage, using a refrigerator was shown to maintain higher cell viability compared to storage at room temperature.

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## References


[1] Statistics Indonesia (BPS), *Productivity of Cassava*, www.bps.go.id, 2015. (Text in Indonesian) (16 September 2017)

[2] Kresnowati, M.T.A.P., Bindar, Y., Aliwarga, L., Lestari, D., Prasetya, N. & Tanujaya, A., *Effects of Retting Media Circulation and Temperature on the Fermentation Process in Soft Texture and Low Cyanogenic Content Cassava Flour Production*, ASEAN Journal of Chemical Engineering, **14**(2), pp. 67-76, 2014.

[3] Meitha, A., Bindar, Y. & Kresnowati, M.T.A.P., *Effects of Cassava Chips Fermentation Conditions on the Produced Flour Properties*, ASEAN Journal of Chemical Engineering, **16**(1), pp. 50-58, 2016.

[4] Brauman, A., Keleke, S., Malonga, M., Miambi, E. & Ampe, F., *Microbiological and Biochemical Characterization of Cassava Retting, a Traditional Lactic Acid Fermentation for Foo-foo (Cassava Flour) Production*, Applied Environmental Microbiology, **62**(8), pp. 2854-2848, Aug. 1996.

[5] Kresnowati, M.T.A.P., Listianingrum, Zaenuddin, A. & Trihatmoko, K., *The Effects of Microbial Starter Composition on Nutritional Contents of Fermented Cassava Flour*, AIP Proceeding, **1669**, International Conference of Chemical and Material Engineering, 2015 .

[6] Martin, M.J., Lara-Villoslada, F., Ruiz, M.A. & Morales, M.E., *Microencapsulation of Bacteria: A Review of Different Technologies and Their Impact on the Probiotic Effects*, Innovative Food Science and Emerging Technologies, **27**, pp. 15-25, 2015.

[7] Burhan, K.H., Ricky, Rudly, C. & Kresnowati, M.T.A.P., *Evaluation of Solid State Fermentation and Conventional Oven Drying for Development of Microbial Starter for Fermented Cassava Flour Production*, Proceeding of International Seminar on Chemical Engineering in conjunction with Seminar Teknik Kimia Soehadi Reksowardojo (STKSR) 2017, pp. 185-192, 2017.

[8] Berny, J.F. & Hennebert, G.L. *Viability and Stability of Yeast Cells and Filamentous Fungus Spores during Freeze-Drying: Effects of Protectants and Cooling Rates*, Mycologia, **83**(6), pp. 805-815, 1991.

[9] Antara, N.S., Trisna, I P.G.U.S. & Suter, I K., *Production of Urutan Inoculum Powder from Pediococcus Acidilactici U318 Pure Culture with Various Fillers*, Agrotekno, **15**(1), pp. 1-4, 2009. (Text in Indonesian)

[10] Potts, M., *Desiccation Tolerance of Prokaryotes*, Microbial Rev, **58**(4), pp. 755-805, Dec. 1994.

[11] Jordan, K.N. & Cogan, T.M., *Heat Resistance of Lactobacillus spp. Isolated from Cheddar Cheese*, Letters in Applied Microbiology, **29**, pp. 136-140, 1999.

[12] Wang, Y.C., Yu, R.C. & Chou, C.C., *Viability of Lactic Acid Bacteria and Bifidobacteria in Fermented Soymilk after Drying, Subsequent Rehydration and Storage*, Int Journal Food Microbiol, **93**(2), pp. 209-217, Jun. 2004.

[13] Santivarangkna, C., Kulozik, U. & Foerst, P., *Inactivation Mechanisms of Lactic Acid Starter Cultures preserved by Drying Process*, J. Appl. Microbiol., **105**(1), pp. 1-13, Jul. 2008.