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BATCH CULTURE PRODUCTION OF KENKEY STARTER CULTURE USING MALTED MAIZE AS SUBSTRATE

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INTRODUCTION

Studies carried out under the FRI/Danida project have demonstrated the possibility and advantages of using L fermentum . S. cereviseae and C. tropicalis as starter culture for the controlled fermentation of maize during kenkey production (Halm et al 1996). This procedure leads to a shortening of the fermentation period and a stable microbiological and organoleptic quality of the fermented maize dough and kenkey produced. The use of starter culture together with the application of Hazard Analysis and Critical Control Point quality system develop for commercial production of kenkey (Amoa-Awua and Jakobsen 1997) is expected to offer opportunities for upgrading the traditional food processing activity into the formal sector of the Ghanaian economy.

For supply of starter cultures to commercial kenkey producers it is important that pure cultures are produced cheaply, in an easy-to-use form and by sustainable procedures and are also preserved by simple techniques. To satisfy the above conditions, it is appropriate that the medium used for the production of starter culture is the same as the material used for the production of kenkey i.e. maize rather than imported defined microbiological media. It is also important that the performance of the media used should not pose any disadvantages to biomass yield and the technological properties of the starter culture such as acid production and anti-microbial properties demonstrated for L. fermentum (Olsen et al 1995).

OBJECTIVES

- To develop a cheap fermentable substrate from locally available materials for pilot scale biomass production of L fermentum, C. Krusei and C. tropicalis in continuos and batch culture fermentations.
- To evaluate the growth kinetics and technological properties of the starter cultures produced using the developed substrate.
- To investigate the survival of the starter culture under various storage conditions.

MATERIALS AND METHODS

Cultures

The strain of L. fermentum used, 7-11A had been isolated from fermented maize in a previous study and preserved by freeze drying (Halm et al 1993). Freeze dried cultures of L. fermentum 7-11A were activated by two successive transfers in 10 ml MRS broth and incubated at 30 °C for 24 h. These cultures were inoculated in the fermentation media to a concentration of 10^6 cfu/g.

Growth medium

Maize were obtained from the local markets and from the Kenkey Production Site at Osu Amantra. Different types of flours were prepared from the maize and mixed with different volumes of water into different concentrations and used as substrate for batch culture fermentations. The different types of substrate made were;

1. Whole maize flour
2. Dehulled maize flour
3. Malted maize flour
4. Malted and mashed maize meal

All media were sterilized at 121 °C for 20 min before they were used.

Fermentation

All fermentations were carried out in a 2 litre fermentor (Biostat B, B. Braun, Biotech International, Germany) at a temperature of 32 °C, pH 5.7 and stirred at 50 rpm to simulate anaerobic conditions. Stirring was stepped up to 150 rpm during sampling.

Each fermentation was carried out for 30 hours and samples taken at 2 hour intervals for determination of pH and plate count on MRS.

Down stream processing and preservation

After culturing, the cells were recovered by centrifugation and preservation trials carried out by mixing with an equal weight of maize flour and storing at ambient temperature, and 4 °C.

Analyses

Reducing sugars;

Reducing sugars were determined according to AAOC (1990).

Growth curve;

Concentration of L. fermentum during fermenter runs and storage were determined on MRS incubated anaerobically (AnaeroCult) at 30 °C and 48 h.

RESULTS AND DISCUSSION

A fermentable maize growth medium for cultivation of L. fermentum

L. fermentum failed to grow on all concentrations of sterile media prepared from whole or dehulled maize flour (results not shown). This was attributed to the inability of L. fermentum to break down starch, the principal carbohydrate in maize.

Unpublished results by Halm et al (1993) showed that none of the strains of L. fermentum isolated from fermented maize meal was amylolitic.

To obtain a fermentable substrate from maize a procedure was developed for the endogenous enzymatic break down of the maize starch into sugars utiilisable by L. fermentum based on procedures used in the brewing industry. The fermentable maize media was prepared by the malting of maize to increase the endogenous diastatic activity followed by mashing to break down the starch into fermentable sugars and was prepared as follows:

- Dried maize kernels were soaked in water for 24 h.
- The maize kernels were allowed to germinate by placing them on a wet cloth and covered with a periodically moistened absorbent paper for 72 h.
- The germinated maize kernels were dried in a hot oven at 70 °C for 6 h and milled into flour.
- A 10 % maize medium was prepared by adding 100 g of malted maize flour to 900 ml distilled water. The media was mashed in the fermentor by heating at 60 °C for 30 min.

The level of fermentable sugars determined as percentage reducing sugars increased from 0.51-1.1.3 % in the maize kernels to 11 - 16.1 % in the 10 % malted mashed maize substrate in three samples of media analysed.

Biomass yield and preservation of L fermentum as starter culture

The concentration of biomass in 10 % malted mash maize media during 24 h of fermentation is presented in Table I. As seen from the table, the cells inoculated at a level of 10^6 increased to a level of 10^{10} within 13 h of fermentation. Similar rates of growth were observed in two replicate fermentations (Results not shown). Lei and Lammert (1997) observed a maximum cell concentration of 10^9 cfu/g in 17 h of

fermentation of the same strain of L. fermentum when grown in DeMan and Ragosa Medium in the same fermenter in arun in which the pH was not controlled. Thus the malted mashed maize media may be considered as an appropriate and rich growth medium for the cultivation of L. fermentum.

Table I
Biomass yield of L. fermentum in mashed malted maize media.

Fermentation time in h.	Cell concentration in cfu/g
0	5.7x 106
1	5.5 x 106
2	9.0 x 106
3	3.7 x 107
5	8.7 x 107
8	1.1 x 108
10.5	4.6 x 109
13	1.3 x 1010
15	9.5 x 109
23	1.3 x 1010
24	1.5 x 1010

The harvested cells of L. fermentum were preserved either directly by refrigeration at 4 °C or mixed with an equal volume of whole maize flour and stored at 4 °C or by dehydration of the mixed harvested cells and maize flour at 42 °C for 72 h followed by storage at room temperature or refrigeration at 4 °C. It was observed that there was a tremendous loss of cell viability during the dehydration of mixed biomass and maize flour. At a drying temperature of 42 °C, a six fold reduction in viable cell concentration from 10^9 to 10^3 cfu/g was observed. At drying temperatures above 50 °C near total loss of cell viability was observed. The possible reduction in loss of cell viability during dehydration through the use of protectants such as skim milk and glycerol used as

Table II

Viability of first sample of L. fermentum during storage

L. fermentum as starter culture	Storage period						
	week 0	week 1	week 2	week 3	week 4	week 7	week 8
Harvested cells stored at 4 °C	3.9 x 109	3.8 x 109	2.1 x 108	7.4 x 108	4.1 x 108	2.5 x 108	3.0 x 108
Harvested cells mixed with maize flour and stored at 4 °C	1.4 x 109	2.9 x 109	1.7 x 109	4.6 x 108	nd	1.2 x 109	4.0 x 108
Dry biomass and maize flour stored at 4°C	nd	3.6 x 103	3.8 x 103	3.2 x 104	1.6 x 104	5.4 x 104	1.3 x 104
Dry biomass and maize flour stored on the shelf (25 °C)	1.5 x 103	1.9 x 103	2.2 x 103	3.1 x 103	1.7 x 104	2.5 x 103	nd

Table II

Viability of second sample of L. fermentum during storage

L. fermentum as starter culture	Storage period				
	week 0	week 1	week 4	week 9	week 11
Harvested cells stored at 4 °C	3.6 x 108	5.1 x 108	3.9 x 109	1.1 x 108	3.1 x 108
Harvested cells mixed with maize flour and stored at 4 °C	4.1 x 108	3.6 x 108	1.1 x 109	6.3 x 108	2.6 x 108
Dry biomass and maize flour stored at 4°C	2.2 x 104	7.0 x 103	2.5 x 104	2.8 x 104	3.5 x 104
Dry biomass and maize flour stored on the shelf (25 °C)	nd	2.0 x 103	1.0 x 104	nd	2.7 x 103

cryo-protectant for the freezing of lactic acid bacteria are the subject of current investigations.

The storage stability of two sets of samples of L. fermentum under the various storage conditions mentioned above are shown in Tables II and III. Virtually no loss of cell viability was observed after 2 months storage of the two sets of starter cultures preserved in various forms under the various cell conditions i.e. shelf and refrigerated storage of wet or dry mixture of biomass and maize flour or refrigerated storage of harvested cells containing residual substrate. These results, especially the shelf storage of L fermentum for at least two and a half months, are rather promising because lactic acid bacteria are unable to survive for more than 3 weeks plates kept under refrigeration

CONCLUSION

L. fermentum has successfully been produced in batch culture fermentations using a cheap growth medium prepared by malting and mashing maize. Even though considerable loss of cell viability occurred during the drying of the biomass mixed with maize flour, storage of harvested cells either in the wet or dry form mixed or not mixed with maize flour was successful over a two months period at refrigeration temperatures. Shelf storage of the dried cells in maize flour did not result in loss of cell viability over the two storage period investigated.

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