

Original Research Article

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## Screening of Starter Culture for the Development of Amaranth (*Amaranthus cruentus*) Incorporated Composite Fermented Milk Drink

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### ABSTRACT

#### Keywords

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In the present study, the acidification activity of different commercial Freeze-Dried, Direct Vat Set starter culture viz., YoFlexExpress1.0, CH-1, YF-L904, STI-13, and CHN-11 in amaranth incorporated milk base was studied. The fermentation potential and acidification activities for each culture were evaluated in terms of acidity development. FD-DVS Culture, CH-1 at 0.009 per cent rate of addition produced the desired acidity 0.9 per cent lactic acid within five hours of incubation, hence selected for development of amaranth incorporated composite fermented milk drink. The acidification activity of all cultures was higher in amaranth incorporated milk than plain milk this may be due to amino acid content in amaranth grain.

### Introduction

In the present investigation, the amaranth grain flour has been incorporated in milk to produce composite fermented milk drink and add value to finished products. Starter culture plays a very important role in the manufacture of fermented milk product. The screening of effective starter cultures is a prime requirement in the development of amaranth incorporated composite fermented milk drink (AICFMD). Presently, there are various

commercial starter cultures available for plain fermented milk drink. However, knowledge and data concerning the fermentation potential of such commercial starter cultures on pseudo-cereal incorporated milk are not traceable.

Therefore, during this part of the study, trials were conducted with different commercial Freeze-Dried, Direct Vat Set (FD-DVS) lactic acid cultures for getting the shortest attainable time of fermentation.

For this, Five kinds of FD-DVS culture were procured from Chr. Hansen and their fermentation characteristics in terms of acidity development in amaranth incorporated milk base and plain toned milk with time were studied (Maurya, 2012). This ensures screening of higher acting cultures and optimisation of its rate of addition for the preparation of AICFMD (Berhe *et al.*, 2018).

From last few years, the uses of FD-DVS lactic acid cultures for the manufacture of various fermented milk products have increased. These cultures are poured directly into culturing vat that contains processed milk and mixed properly by slow agitation for 10-15 min.

The inoculated milk is then incubated at a specified temperature until getting the desired acidity and pH. The DVS cultures offer various advantages for the production of fermented milk products and overcome the varied issues that occurred during the use of liquid bulk starters.

These cultures are convenient for the dairy manufacturer and ensure consistency in the final fermented product, provide a high texture and mouth feel, something that is not normally achievable by using bulk starters (Deepa, 2012). Direct Vat Set cultures are extremely concentrated lactic acid cultures of single, as well as a combination of two or more strains. The cell counts in the DVS culture are  $10^9$ - $10^{11}$ CFU/g (Krishan and Gandhi, 2009).

The grain amaranth is a rich source of vital amino acids such as leucine, isoleucine, valine, alanine, methionine, cysteine, serine phenylalanine and tryptophan (Rastogi and Shukla, 2013). Amaranth grain is also rich in calcium and magnesium and contains a good proportion of vitamins riboflavin, niacin and vitamin E (Liptakova *et al.*, 2017).

Considering the above facts, a study was undertaken to develop AICFMD. The objective of this part of the study was to screen the most suitable FD-DVS culture and its rate of addition that are capable to produce the desired acidity in amaranth incorporated milk within 5 hours of incubation.

## **Materials and Methods**

The study was carried out in the Department of Dairy Technology College of Dairy Science and Food Technology, Raipur (Chhattisgarh).

### **Dairy and non-dairy ingredients**

The raw materials, amaranth grain (*Amaranthus cruentus*), pasteurized toned milk containing 3% fat and 8.5% solids-not-fat (Brand: Amul), and FD-DVS cultures of Chr. Hansen, Denmark as mentioned in Table 1 were used in the study.

### **Preparation of fermented milk base**

The acidification activities of different FD-DVS lactic acid cultures were evaluated in amaranth incorporated milk base and milk without amaranth. For the preparation of this base, amaranth grain flour (*Amaranthus cruentus*) @5% was added in pasteurized toned milk. Plain toned milk and amaranth incorporated milk base samples were heated to 90°C for 5 minutes and cooled to the desired incubation temperature (Chatterji, 2012; Patidar and Prajapati, 1998; Kakade, 2010). The processed amaranth milk base and plain milk were inoculated with different types of FD-DVS starter culture viz., YoFlex Express1.0, CH-1, YF-L904, and combination of STI-13, and CHN-11 at two different rates 0.007 and 0.009 per cent, keeping in view that final desired acidity of 0.9 per cent lactic acid should reach up to five hours of incubation (Mistry *et al.*, 2018).

To facilitate periodical analysis, inoculated samples were filled into 100 ml glass beaker and covered with aluminium foil.

The incubation of product sample inoculated with thermophilic culture YoFlexExpress1.0, CH-1, YF-L904 was carried out at  $42\pm 1^\circ\text{C}$  whereas, the sample inoculated with a combination of thermophilic culture STI-13 and mesophilic culture CHN-11 was incubated at  $37\pm 1^\circ\text{C}$  (Berhe *et al.*, 2018).

### **Acidification**

The fermentation patterns for each culture were evaluated in terms of acidity development up to 5.5 hours of incubation. The final acidity of 0.9 per cent lactic acid in amaranth incorporated milk base up to 5 h of incubation is the criteria used for screening of culture and its rate of addition. The 0.9 per cent acidity of curd was decided based on literature recommended (Hussain *et al.*, 2018) so that the final acidity of the finished product after the addition of sugar and water, etc. should not be below 0.45 per cent of lactic acid (FSSAI, 2017).

### **Titrateable acidity**

The acidity of the product sample was measured by titration method, used for determination of titrateable acidity of condensed milk prescribed in Manual of Methods of Analysis of Milk and Milk Products, Food Safety and Standard Authority of India, FSSAI, 2016 with slight modification.

For analysis, about 10 g of thoroughly mixed product sample was weighed in a porcelain dish and 30 ml warm of distilled water was added to it, after addition of 1.0 ml of phenolphthalein indicator, a sample was then titrated against 0.1 N sodium hydroxide solution until the appearance of a light pink

tinge, which persisted for 30 seconds in the solution. A blank was prepared by diluting 10 g of the sample with 30 ml warm water in another porcelain dish for comparison of colour. The titrateable acidity as per cent lactic acid was calculated as follows.

$$\text{Acidity (as per cent Lactic acid)} = (9 \times A \times N) / W$$

Where,

A = Volume in ml of standard NaOH required for titration

N = Normality of standard NaOH solution

W = Weight in g of a sample taken for the test

### **Results and Discussion**

For validation of acidification activities of different FD-DVS culture in amaranth incorporated milk, the measurement of a titrateable acidity is an important tool. The effect of different starter culture and its rate of addition on acid production over 5.5 hours are presented in Table 2, 3 and Figure 1 and 2.

Table 2 and Figure 1 showed that addition of different culture viz., CH-1, YoFlexExpress1.0, YF-L904, and combination of STI-13 and CHN-11@ 0.007 per cent in plain milk produced only 0.657, 0.645, 0.63, 0.396 per cent lactic acid respectively, Whereas incorporation above, said culture@ 0.007 per cent in amaranth grain flour added milk base produced 0.684, 0.654, 0.642, 0.417 per cent lactic acid respectively, within five hours of incubation at temperature mentioned in section 2.2.

These results showed that inoculation with all above mentioned FD-DVS culture @0.007 per cent rate has not produced the desired acidity in amaranth incorporated milk within 5 hours of incubation; hence further trials were conducted with an increased level of starter culture.

**Table.1** Description of the FD-DVS starter cultures used in the study

Type of Culture	Name of Culture	Stain details
Thermophilic	YoFlexExpress 1.0	<i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> sub sp. <i>bulgaricus</i>
	CH-1	<i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> sub sp. <i>bulgaricus</i>
	YF- L904	<i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> sub sp. <i>bulgaricus</i>
	STI-13	<i>Streptococcus thermophilus</i>
Mesophilic	CHN-11	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Leuconostoc</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Lactococcus lactis</i> subsp. <i>biovardiacetylactis</i>

**Table.2** Titratable acidity of samples inoculated with lactic culture at 0.007% rate

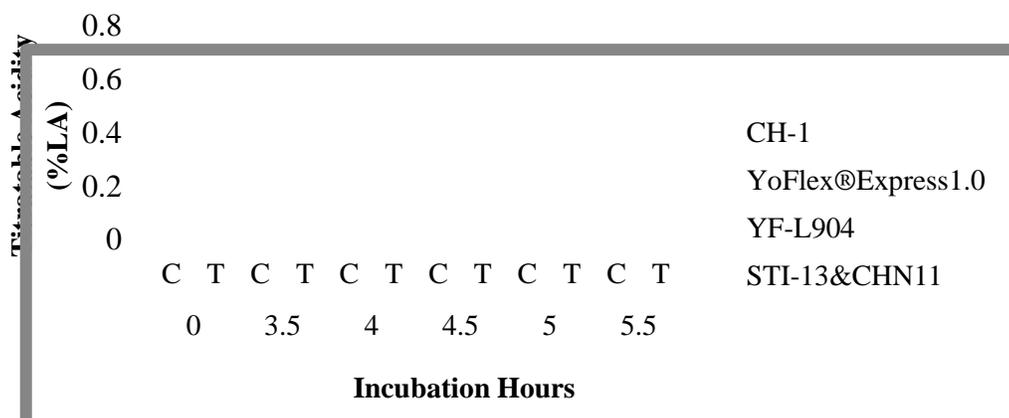
Incubation period (h)	Type of culture →	Titratable Acidity (Per cent lactic acid)			
	Sample↓	CH-1	YoFlex® Express 1.0	YF-L904	STI-13, CHN11
0	C	0.135±0.005	0.135±0.005	0.135±0.005	0.135±0.005
	T	0.135±0.005	0.135±0.005	0.135±0.005	0.135±0.005
3.5	C	0.36±0.005	0.447±0.003	0.414±0.005	0.237±0.008
	T	0.378±0.005	0.468±0.005	0.428±0.002	0.267±0.011
4	C	0.486±0.005	0.558±0.005	0.528±0.003	0.303±0.008
	T	0.501±0.008	0.576±0.009	0.573±0.008	0.33±0.008
4.5	C	0.588±0.003	0.609±0.003	0.591±0.003	0.351±0.005
	T	0.606±0.006	0.615±0.008	0.597±0.003	0.375±0.008
5	C	0.657±0.009	0.645±0.003	0.63±0.005	0.396±0.005
	T	0.684±0.010	0.654±0.008	0.642±0.011	0.417±0.006
5.5	C	0.69±0.006	0.675±0.005	0.657±0.005	0.444±0.008
	T	0.708±0.006	0.693±0.005	0.678±0.006	0.465±0.008

The results are mean value ± SE of three replications.  
Where, C= Plain milk curd, T= Amaranth incorporated milk curd

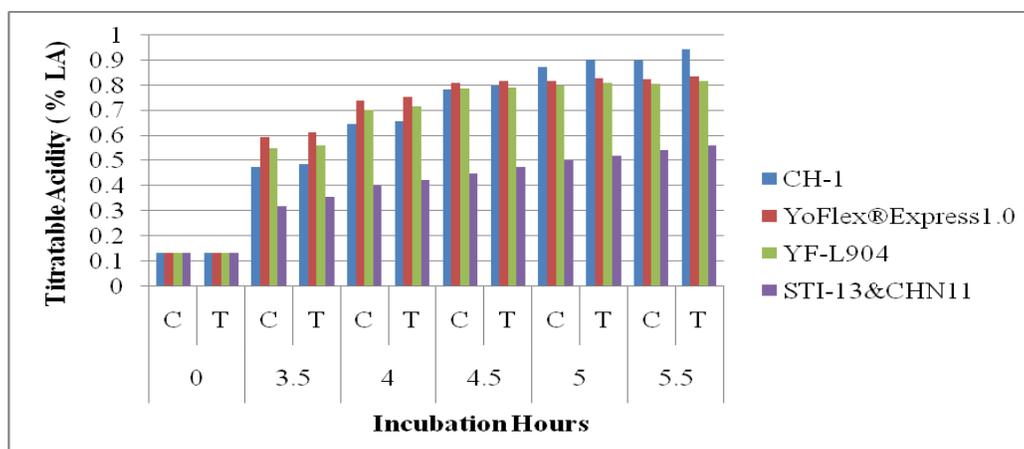
**Table.3** Titratable acidity of samples inoculated with lactic culture at 0.009% rate

Incubation period (h)	Type of culture →	Titratable Acidity (Per cent lactic acid)			
		CH-1	YoFlex® Express 1.0	YF-L904	STI-13, CHN11
	Sample √				
0	C	0.135±0.005	0.135±0.005	0.135±0.005	0.135±0.005
	T	0.135±0.005	0.135±0.005	0.135±0.005	0.135±0.005
3.5	C	0.477±0.005	0.594±0.005	0.549±0.005	0.318±0.008
	T	0.486±0.005	0.612±0.010	0.561±0.011	0.357±0.008
4	C	0.645±0.008	0.738±0.005	0.702±0.005	0.402±0.008
	T	0.657±0.005	0.753±0.008	0.717±0.008	0.423±0.005
4.5	C	0.783±0.005	0.81±0.005	0.789±0.003	0.45±0.005
	T	0.798±0.011	0.819±0.005	0.792±0.009	0.477±0.010
5	C	0.873±0.005	0.816±0.008	0.801±0.005	0.501±0.008
	T	0.903±0.008	0.828±0.009	0.81±0.010	0.522±0.005
5.5	C	0.9±0.005	0.825±0.008	0.807±0.006	0.543±0.008
	T	0.945±0.009	0.837±0.005	0.819±0.009	0.561±0.008

The results are the mean value ± SE of three replications.  
Where, C= Plain milk curd, T= Amaranth incorporated milk curd



**Figure.1** Titratable acidity of samples inoculated with lactic culture at 0.007% rate



**Figure.2** Titratable acidity of samples inoculated with lactic culture at 0.009% rate

From the Table 3 and Figure 2, it was observed that addition of different culture viz CH-1, YoFlexExpress1.0, YF-L904, and combination of STI-13, and CHN-11@ 0.009 per cent rate in plain milk produced 0.873, 0.816, 0.801, 0.501 per cent lactic acid respectively, Whereas incorporation above, said culture@ 0.009 per cent rate in amaranth incorporated milk yield 0.903, 0.828, 0.81, 0.522 per cent lactic acid respectively within five hours of incubation at temperature mentioned in section 2.2. These results showed that except CH-1, no other culture have produced the desired acidity in amaranth incorporated milk base within 5 hours of incubation, therefore the FD-DVS culture CH-1 containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* sub sp. *bulgaricus* and 0.009 per cent rate of culture addition was selected for development of AICFMD.

The ideal FD-DVS culture and optimum inoculum level were selected based on the rapid fermentation rate to eliminate the risk of microbial contamination and shorten the production cycle time (Ganguly, 2013).

According to Holset al (2018) milk is deficient in the free amino acid and short peptides that are necessary for the growth of lactic acid bacteria thus required exogenous source of amino acid further, amino acid such as methionine, leucine, cysteine and valine stimulate the growth of lactic acid bacteria.

On other hands, Rastogi and Shukla (2013), reported that grain amaranth is a rich source of vital amino acids such as leucine, isoleucine, valine, alanine, methionine, cysteine, serine phenylalanine and tryptophan. From the Figure 1 and 2 it was confirmed that all FD-DVS lactic culture used in the present investigation resulted in faster acid production in amino acid-rich amaranth incorporated milk than plain milk without amaranth.

Incorporation of FD-DVS lactic culture CH-1@ 0.009 per cent rate in plain milk yields 0.873 per cent lactic acid and that in amaranth incorporated milk 0.903 per cent lactic acid whereas, no other culture produced the desired acidity within 5 hours of incubation. Therefore the FD-DVS culture CH-1 containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sub sp. *bulgaricus* and 0.009 per cent rate of culture addition was selected for the development of AICFMD.

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