# **ORIGINAL ARTICLE**

# Studies on Effect of Wheat Grass Extract with Buffalo Milk For Dahi Preparation For Microbial Profile of Herbal *Dahi*

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## ABSTRACT

The study was planned to evaluate the impact of wheat grass on microbial profile of dahi prepared by adding wheat grass extract with buffalo milk at different proportion. The average lactobacillus count of fresh sample wheat grass extract added dahi were found to be 2.01, 2.09, 2.07, 2.35, 2.01, 1.98 and 2.00 cfu per g for treatments  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$ , respectively. The average lactococcus count of fresh sample of wheat grass extract added dahi were found to be 2.15, 2.3, 2.2, 2.5, 2.04, 2 and 1.99 cfu per g for treatments  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$  respectively. The coliform and yeast mould count was found within safe zone. It was also observed that the wheat grass extract not effected on the growth of microbs viz. Lactobacillus and lactococcus and coliform and yeast and mould was not increased due to the use of wheat grass extract. Dahi was safe for the consumption purpose. **Keywords:** Dahi, wheat grass extract, microbial counts

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## INTRODUCTION

Fermented foods developed by using the beneficial microorganisms, which are healthy live bacteria and are foods that have gone through a process during which this bacteria converts the starches and sugars in that food into lactic acid and acetic acid. Fermented foods have high nutritional values (vitaminK2, trace minerals, B-vitamins and probiotics), are easy to prepare and are economical. Fermentation is an old food preservation method and was used by the Romans.

In India, *dahi* has been with associated health benefits since time immemorial. Fermented milk contains all the milk components modified through the process of fermentation by lactic acid bacteria (LAB). Fermented foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavor, aroma, and texture which enrich the human diet. The microorganisms involved in *Dahi* fermentation include *Streptococcus thermophilus, S. lactis, S. cremoris, Lactobacillus bulgaricus, L. acidophiuls, L. plantarum* and lactose fermenting yeasts.

When culture is undefined and kept in unhygienic condition it contains mixture of various desirable and undesirable strains of bacteria. Thus the quality of *Dahi* may vary with the type of starter culture used.

Wheat grass is a food prepared from the cotyledons of the common wheat plant (*Triticum aestivum*) belonging to family Gramineae. Wheat grass has high concentrations of chlorophyll, minerals (calcium, potassium, sodium, iron, magnesium and sulphur), and 17 forms of amino acids, vitamins (A, B, C, E and K) and active enzymes [6]. It has some curative properties like anti-cancer activity, anti-ulcer activity, antioxidant activity, anti-arthritic activity, and blood building activity in Thalassemia Major. It has been argued that wheat grass is diseased blood flow, digestion and general detoxification of the body [9]. The use of wheat grass for the development of herbal or health beneficial *dahi* may create the new era of food development. Therefore it was necessary to study the effect of wheat grass extract in the preparation of *dahi* in respect to microbial status of *dahi*.

# **MATERIAL AND METHODS**

The materials used and methods employed during the course of present investigation on preparation of dahi from blends of buffalo milk with wheat grass extract are as under.

# **Collection of buffalo milk**

Fresh and standardized buffalo milk for fat 6 per cent and SNF 9 per cent was procured from Natural Milk Pvt. Ltd. Latur.

# Growing & extracting of wheat grass

The wheat grass are usually grown in small trays. The wheat seeds are washed with the clean water, and soaked in the water for overnight. The soaking of wheat seed results in sprouted wheat seed. The sprouted wheat seed are drained out from water and spread on the layer of soil. Small quantity of water is sprinkle on wheat seed cover with the soil. The wheat seed are allowed to grow for 6-12 days, under indirect sunlight. The grown grass was then cut using scissor, and juice is extracted. [8].

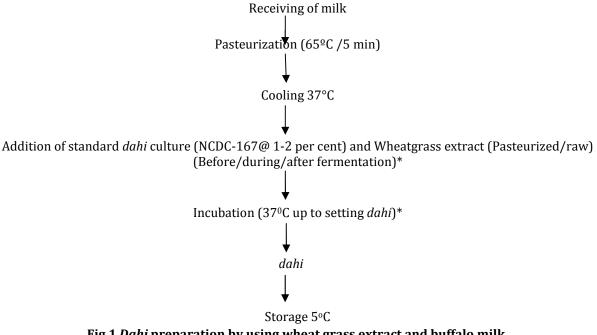
# **Microbial cultures**

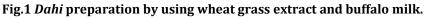
The standard mixed *dahi* culture i.e. Standard *dahi*, contained *streptococcus thermophilus and lactococcus lactis NCDC-167(BD4)* in this study was procured from the National collection of Dairy culture (NCDC), NDRI, Karnal.

# Methods

# Preparation of dahi by using wheat grass extract

The *dahi* was prepared from buffalo milk as per the method described De. [2] in his book Outline of Dairy Technology with slight modification for addition of wheat grass extract. The incubation period and culture rate were optimized on the basis of setting *dahi* as shown in Figure 1.





# Microbiological analysis of dahi

The *dahi* samples were examined for the *lactobacilli* count, *lactococcus* count, coliform count and yeast and mould count.

## **Preparation of sterile dilution blank**

Sterile dilution blank (0.85%) was prepared by dissolving 8.5 g of NaCl in 1 L of distilled water. The pH was adjusted to 7.0 ± 2, followed by autoclaving 9 ml aliquots at 121°C for 15 min.

# **Preparation of sample dilution**

Exactly 1 ml of *dahi* sample was taken and transferred to a sterile 9 ml dilution blank. The content of the tube was mixed well. This representing the first dilution (1:10), subsequently dilutions were prepared by transferring 1 ml in 9 ml sterile dilution blanks.

# Lactobacillus count

Lactobacilli count of dahi was determined by using pour plate method employing MRS agar [10]. The prepared plates were incubated at 37°C for 48-72 hrs and counts were expressed as log cfu/ml of sample. Agar was prepared by dissolving 42.5 g MRS broth in a 1 litre distilled water and agar powder was added to broth at the rate of 1.2% and the media was sterilized by autoclaving at 121°C for 15 min. Ten milliliters of membrane filtered sterile 20% solutions of sorbitol was added to 90 ml of MRS agar (2% final 33 concentration) just before pouring the agar medium. Plates were incubated at 37°C for 72 h. After the incubation colony forming units were calculated and expressed as log cfu/ml.

#### Lactococcus count

*Lactococcus* of *dahi* was determined by using pour plate method employing M 17 agar as a medium agar [1]. Agar was prepared by dissolving 52.25 g M 17 agar with glycerophosphate powder in 1 litre of distilled water and the media was autoclaved at 121°C for 15 min. Plates were incubated aerobically at 30°C for 24 h. After the incubation colony forming units calculated and expressed as log cfu/ml.

# **Coliform count**

Enumeration of coliform count of *dahi* was determined by using pour plate method described by Hought *et al.* [3] employing Violet Red Bile Agar (pH 7.4  $\pm$  0.1). The prepared plates were incubated at 37°C for 48 h. Colonies with dark red coloration were counted and they were expressed as log cfu per ml of sample.

#### Yeast and mold count

*Dahi* was ascertained for yeast and mold counts as per the method suggested by Marshall [5] using potato dextrose agar and pH of media adjusted to  $3.5 \pm 0.1$  using tartaric acid solution. The prepared plates were incubated at 22°C for 3-5 days and counts were expressed as log cfu per ml of sample.

## **Statistical analysis**

The data obtained in the present investigation was tabulated. The data were analyzed statistically by using Completely Randomized Design (CRD) as per Panse and Sukhatme [7].

## **RESULT AND DISCUSSION**

# Microbial analysis

## Lactobacillus count

From the table no.1 it was observed that the average *lactobacillus* count of fresh sample wheat grass extract added *dahi* were found to be 2.01, 2.09, 2.07, 2.35, 2.01, 1.98 and 2.00 cfu per g for treatments  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$ , respectively. The raw wheat grass extract mixed treatments were increased for *lactobacillus* count as compared to control and pasteurized treatments but count  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$  and  $T_7$  was not significantly differed whereas control *dahi*  $T_1$  was shown similar count of *lactobacillus* like all of the *wheat grass extract* added *dahi* samples indicate that *lactobacillus* can grow in the mixture media of wheatgrass extract and milk.

## Lactococcus count

From the table no.1 it was observed that the average *lactococcus* count of fresh sample of wheat grass extract added *dahi* were found to be 2.15, 2.3, 2.2, 2.5, 2.04, 2 and 1.99 cfu per g for treatments  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$  respectively. When the *lactococcus* count was compared with *lactobacillus* count, it was observed that both *bacilli* and *cocci* were found near about in equal proportion in *dahi*.

#### **Coliform count**

The Table no.1 shows the coliform count of *dahi*. The range for coliform found in between 0 to 1.25 cfu per g. The higher count for coliform was observed in treatment  $T_4$  (1.25 cfu per gm) and lower in treatment  $T_2$ ,  $T_3$ ,  $T_5$  and  $T_6$  (0 cfu per gm). It was seen that the coliform count remained within the limit (maximum 100 c.f.u./gm) prescribed by IS: 9617 [4] indicated that all treatments were prepared at hygienic condition and suitable for consumption.

## Yeast and Mould count

The Table no.1 shows the yeast and mould count of fresh *dahi*. The yeast and mould was found in control treatment only which had count 0 cfu/gm in each. The mixed *dahi* was blank for yeast and mould except treatment  $T_4$  (0.50). It was seen that the yeast and mould count remained within the limit (maximum 100 c.f.u./gm) prescribed by IS: 9617 [4]. All treatments were prepared at hygienic condition and at par to each other.

## Bacterial count of selected treatments of fresh dahi

The fresh product prepared was subjected to microbiological analysis with respect to *lactobacillus* count, *lactococcus* count, yeast and mould count and coliform count. The microbial load of fresh product were estimated and expressed in  $\log_{10}$  (cfu/gm) in Table No.1.

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Treatments	Lactobacillus	Lactococcus	Coliform count	Yeast & Mould
	count	count		count
T <sub>1</sub>	2.01	2.15	0.25	0.00
T <sub>2</sub>	2.09	2.30	0.00	0.00
T <sub>3</sub>	2.07	2.20	0.00	0.00
$T_4$	2.35	2.50	1.25	0.50
T <sub>5</sub>	2.01	2.04	0.00	0.00
T <sub>6</sub>	1.98	2.00	0.00	0.00
T <sub>7</sub>	2.00	1.99	0.25	0.00

#### Table No.1 Microbial evaluation of wheat grass extract added dahi

# CONCLUSION

It was observed that the *wheat grass* extract not effected on microbial count viz. *lactobacillus, lactococcus,* coliform and yeast and mould and also the quality of herbal *dahi*. All microbial count range was safe for the consumption purpose. It could be concluded that the delicious *dahi* with pleasant aroma can be prepared by using the standard mixed *dahi* culture i.e. Standard *dahi,* contained *streptococcus thermophilus and lactococcus lactis NCDC-167(BD4)* alone or in combination containing viable lactobacilli counts and lactococcus count more than 10<sup>6</sup>c.f.u./gm.

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