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Research Article

Microbial succession in naturally fermented sliced carrots

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Abstract

Natural fermentation of shredded carrots using two types of carrots (red and crimson coloured) was carried out with 3% salt and 4% mustard powder. The viable number of microorganisms was determined at various time intervals on four different types of media viz. De Man Rogosa Sharpe (MRS), Nutrient agar, Yeast extract peptone dextrose agar (YEPDA) and Acetobacter medium. The viable number of cells initially increased on four media and then came to steady state. Some *Pseudomonas* and *Bacillus* types of microorganism appeared at the beginning of fermentation. However, these microorganisms did not multiply and survive later on. In crimson coloured carrot, some yeast colonies were also observed but these could not be detected later on. As the fermentation of carrots in brine preceded the amount of total sugars (160µg/ml and 200µg/ml) and reducing sugars (90µg/ml and 96µg/ml) first increased in red and crimson coloured carrots at 3rd day and then decreased. The maximum development of 0.03 to 0.9% of total acidity and pH of brine dropped from 6.97 to 3.2 after 15 days of fermentation. From the cultural, morphological and physiological characteristics it was found that three different types of lactic acid bacteria viz. *Lactobacillus* species, *Leuconostoc* species and *Pediococcus* species were present during fermentation. However, fermentation was conducted by heterofermenter *Leuconostoc* species only as is evident from the production of alcohol and volatile acids. Volatile acids were also produced by the action of *Gluconobacter* species after 4th day of fermentation. It was concluded that the fermentation of red and crimson coloured carrots is mainly the function of *Leuconostoc mesenteroides* and *Leuconostoc dextronicum,* since these microorganisms were present and proliferated throughout the fermentation.

Keywords: Microorganisms, viable number, lactic acid bacteria, *Leuconostoc*, India.

Introduction

Fermented food is an important component of diets in many parts of the world because of their high nutritive value and organoleptic characteristics. Lactic acid fermentation of vegetables provides an economic means of producing microbiologically safe products resulting in minimum loss of nutritive value. A large number of vegetable fermentations have been studied extensively to determine the biochemical changes that occur during fermentation and to identify the

microorganisms involved. Most of this fermentation is carried out by hetero fermentative lactic acid bacteria like *Leuconostoc mesenteroides, Lactobacillus plantarum* and *Lactobacillus brevis.* In India many vegetable fermentations are carried out which include preparation of mixed vegetable pickles and fermented spiced beverages. In Northern India, carrots, especially a variety that is deep purple in colour, are fermented to make a traditional ready to serve drink known as 'Kanji' [1]. It is very popular and considered to have cooling, soothing and anti carcinogenic properties that help to prevent against cancer. Carrot (*Daucus carota*) belonging to the family *Umbelliferae* is an important winter vegetable and is considered to be a most nutritive food. It is rich in α- and β-carotene and several other vitamins like thiamine, riboflavin, niacin, vitamin C, folic acid and minerals. They are a rich source of carotene, which is converted to vitamin A in the body. Carrots are widely cultivated all over the world and are eaten both cooked and raw. It is very good for skin, health and building the immunity system.

Materials and Methods

Preparation of carrots for fermentation

Two types of carrots (red and crimson coloured) were procured from local market. The carrots were washed twice with tap water and peeled and again washed with sterilized distilled water. Washed carrots were shredded into pieces (3-4cm in length and 1cm thick).

Fermentation process

Fermentation was carried out in 5L glass bottles in winter season at room temperature. To set up the fermentation on 3 L sterilized distilled water was added to 1.5 kg shredded carrots along with 3% NaCl and 4% mustard powder. The bottles were plugged with cotton. The fermentation was carried out for 3-7 days and samples were withdrawn for microbiological and chemical analysis.

Isolation and Enumeration of microorganisms

Samples of brine were withdrawn from each bottle. The samples were diluted by serial dilution techniques and 0.1 ml aliquots of appropriate dilution were spread on four different types of media (MRS, YEPDA, Nutrient agar and Acetobacter medium) in triplicates. The plates containing MRS medium were incubated at 37°C in desiccators, while the plates containing other media were incubated at 30°C for 48 hours.

Morphological characterization

Gram's reaction, cell shape and arrangement and spore formation were examined by using standard techniques.

Biochemical analyses of brine

Biochemical analysis of samples of brine were determined with following parameters such as total sugars [2], reducing sugars [3], alcohol [4], total acidity, volatile acid [5] and pH by using standard methods. Utilization of various sugars (glucose, lactose, xylose, maltose, sucrose, fructose, arabinose, ribose and cellobiose) was tested by using MRS broth with pH indicator bromocresol purple (0.02g/l) without glucose and beef extract. A Durham's tube was also put in each tube in inverted position before autoclave to determine gas production. The tubes were inoculated with different isolates and incubated at 37^0C for 48 h. Observations were recorded for change in colour and gas production.

Results and Discussion

Isolation and Enumeration of microorganisms

The number of different types of microorganisms which were either present at the start of fermentation process or which developed during fermentation was counted by spreading appropriate

dilution of the brine on four different types of media. The number of colonies that appeared on four different types of media after 48 hrs of incubation was counted from a plate containing 100-300 colonies and number of microorganisms per ml of brine was calculated in log number of viable cells (Figs. 1, 2). The total bacterial count varied in both types of carrots were found to be $3.0x10^5$, $2.2x10^5$ and $2.8x10^3$ cells/ml on MRS, YEPDA and Nutrient agar medium at the start of the fermentation and then increased exponentially to $3.4x10^7$, $8.0x10^6$ and $2.1x10^5$ cells/ml after 6 days. On Acetobacter medium, there was no growth observed up to 3rd day; however, on 4th day small, colourless and pinhead colonies appeared which were found to be in the range of $1.0x10^5$ to $1.8x10^5$cells/ml during the rest of the fermentation in both types of carrots. At that time acetic acid bacteria were multiplied because of presence of ethanol and oxidized ethanol to acetic acid between 3rd and 4th day and number increased continuously. On MRS and YEPDA medium two types of colonies were most prevalent in both types of carrots. These were circular, raised, white, small and circular, raised, colourless and small. A few colonies of yeast were also observed at the start of fermentation of crimson coloured carrot.

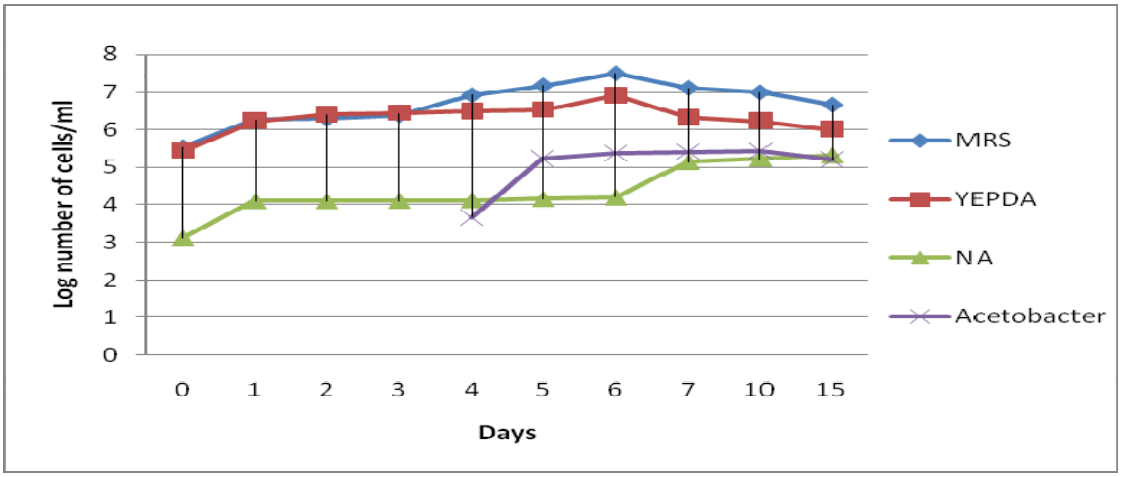

Figure 1. Log Number of viable cells in red coloured carrot.

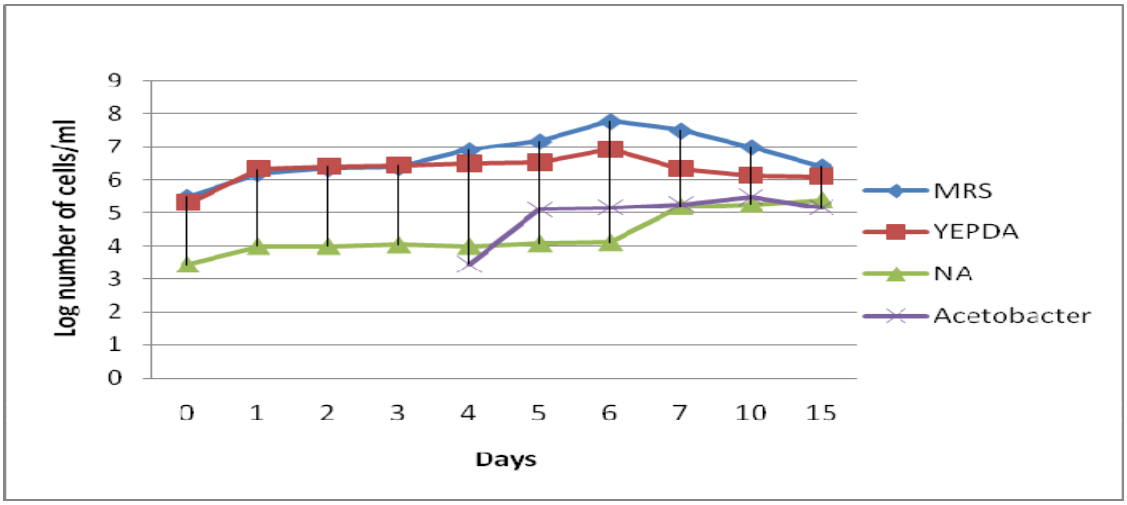

Figure 2. Log Number of viable cells in crimson coloured carrot.

Microbial successions and characterization

The different types of microorganisms were isolated from fermented carrots (red and crimson coloured) brine and it was observed that at the start of fermentation, different types of microorganisms were present belonging to the genus *Pseudomonas* species, *Bacillus* species, *Leuconostoc* species and a few yeast. However, these microorganisms survived for the initial few

days. During the fermentation of carrots, some lactic acid bacteria and acetic acid bacteria were also observed which belonging to the genus *Lactobacillus, Leuconostoc* species, *Pediococcus* species and *Gluconobacter* species**.** Only five selected bacterial isolates (Isolate Nos. 1, 2, 3, 4, and 5) were characterized on the basis of their morphological and biochemical characteristics. The four isolates were g+ve, coccobacillus, non-spore forming bacteria. These bacteria were present throughout the fermentation. In case of red carrot *Leuconostoc mesenteroides was* found to be most prevalent species and in crimson carrot *Leuconostoc dextronicum* was the predominant species. The fermentation of cauliflower and cucumber was reported to be due to *Leuconostoc mesenteroides* followed by other lactic acid bacteria at low temperature and low salt concentration. Processing and storage of lye-treated carrots fermented by using a mixed starter culture of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* was compared with a single culture of *L. plantarum* [6]. The bacterial isolates were tested for various biochemical characteristics. Four isolates (Table 1) were catalase negative and could not solubilised $CaCO_3$ and one isolate no-4 was catalase positive and solubilised $CaCO_3$. It was belonging to the genus *Gluconobacter* species. The effects of different temperature and with varying salt content were employed in the fermentation of both carrots. It was found that all isolates showed good growth at temperature 15°c and 37°c and 4 and 6.5% salt concentration. Lactic acid fermentation of carrot as a method of preservation using different lactic acid bacteria, viz. *Lactobacillus plantarum, Pediococcus cerevisiae* and *Streptococcus lactis* subsp. *diacetylactis* as such and in sequence at different temperatures and with varying salt content (2, 2.5 and 3%) were employed in the fermentation of carrot [7].

Biochemical analysis

The following parameters were examined at different time intervals 0, 3, 7 and 15 days. The initial total sugars concentration in red carrot fermentation was found to be 14µg/ml and 20µg/ml in crimson carrot and this dramatically increased to 160µg/ml and 200µg/ml after 3rd day of fermentation and decreased later on. The maximum amount of reducing sugars was found to be 90µg/ml ^{in} red carrot and 96µg/ml in crimson carrot on 3rd day. The reducing sugars were utilized by microorganisms and organic acids were produced and pH of brine dropped from 6.97 to 3.2 after 15th day of fermentation. On other hand, the total acidity was increased regularly from 0.03% to 0.70% in red carrot and 0.05% to 0.91% in crimson carrot after 15 days. In fermentation of whole carrots, acidity was reported to be 1.1 to 1.3% acids as lactic acid with a pH of about 3.3 was developed in 15 days[8]. However, the maximum amount of alcohol was 2% and 2.5% in both types of carrots on 3rd day and later on decreased to 1.6 % and 1.8 % after 15 days of fermentation. At that time maximum increase in volatile acids was on 3rd day, and acetic acid bacteria were developed and oxidized ethanol to acetic acid. Thus, it indicates the involvement of acetic acid bacteria for the oxidation of alcohol to acetic acid.

Table 1. Morphological, cultural and physiological characteristics of different bacteria isolated from red and crimson coloured carrots.

Characteristics	Isolate No.				
	1	2	3	4	5
Colony characteristics	Circular, raised,white and small	Circular, raised, colourless and small	Irregular, flat, colourless and large	Circular, raised, colourless and small	Circular, raised, colourless and pinhead
Cell morphology	Coccobacilli (pairs or small chains	Cocci (pairs or tetrads)	Large rods(long chains)	Coccobacilli (pairs or small chains	Single/Small roads
Catalase test	-	-	-	-	+
Sugars fermentation					
Arabinose	-	±	-	-	-
Ribose	±	-	+	+	-
Xylose	±	-	±	+	-
Glucose	+	+	+	+	+
Fructose	+	+	+	+	-
Sucrose	+	±	+	+	-
Maltose	+	±	+	+	-
Cellobiose	-	-	-	-	-
Lactose	±	+	+	±	-
Effect of salt concentration 4%	+	+	+	+	+
6.5%	+	+	+	+	-
8%	-	±	-	-	-
Growth Temperature 15°C	+	+	+	+	+
37°C	+	+	+	+	+
45°C	-	±	+	-	-
Production of dextran in the presence of 5% sucrose	-	-	-	-	-
Solubilisation of $CaCo_3$	-	-	-	-	+
Genus	*Leuconostoc mesentroids*	*Pediococcus sp.*	*Lactobacillus sp.*	*Leuconostoc dextranicum*	*Gluconobacter sp.*

Conclusion

In the present investigation, carrot fermentation was carried out under natural conditions. As the fermentation of carrots in brine preceded the amount of total sugars, reducing sugars and alcohol first increased and then decreased due to the production of volatile acids and pH was also dropped from 6.97 to 3.2 after 15 days. *Leuconostoc mesenteroides* and *Leuconostoc dextronicum* were found to be most prevalent species in red and crimson carrot. The acetic acid bacteria genus *Gluconobacter* species were also isolated in both types of carrots.

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