Effect of Dry Salt and Brine on the Fermentation and Colour of Blanched Garlic

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Abstract

Garlic is used for its pungent flavor as a seasoning or condiment all around the world. The bulbs of garlic are mainly composed of water and organic matter. Vegetables and other foods that are processed by lactic acid fermentation bring about a change in nutritive value, vitamins and minerals, etc. Cultures of genus Lactobacillus (from fermented carrot) was added to the peeled blanched garlic treated with different salt concentrations (5% and 8%) and brine (5% and 8%) to achieve desirable properties. The Lactobacillus grew abundantly in blanched garlic with 5% brine, which attained a pH of 3.92 after 30 days. The blanched garlic fermented by Lactobacillus with 5% salt concentration (w/v) was stable at 30°C. The different samples were analyzed for physico-chemical analysis and microbial growth. From these results, it was concluded that the blanched garlic treated with 5% brine showed significantly better quality parameters.

Keywords: Garlic; Blanched; Lactobacillus; Fermentation; Lactic acid

Introduction

Garlic (*Allium sativum*), often called stinking rose is a herb that has been used by humans as a source of antimicrobial agent for the gastrointestinal tract. Garlic was formerly classified in the lily family, it is now included as a member of the Alliaceae [1]. Garlic is known for its odor and flavor. It is actually odorless until its cells are ruptured by being bruised; cut or crushed [2]. It has a complex mixture of many secondary plant products including allicin, diallyl sulfide, dialyl disulfide and allyl mercaptan. As per recent studies from the USDA, an average clove of raw garlic has just under 6 calories, 12 mg potassium, over 5 mg calcium, 4.59 mg of phosphorus, 0.94 mg of vitamin C and small amounts of a variety of other vitamins and minerals [3]. Garlic bulb consists of about water (65%), carbohydrates (28%), protein (2%), fiber (1.5%) and fats (0.15%). It has been used as one of the essential ingredient in the preparation of seasonings, sauces etc. in almost all the cultures. A number of garlic based products are manufactured in the world which includes garlic oil, garlic flakes, fried garlic slices, baked garlic slices, garlic salt and garlic paste [4].

The blanching of fruits and vegetables in water bath before packaging inactivates enzymes present in it [5]. However it also has some negative effect on product quality which includes loss of texture, changes in colour and nutritional losses [6,7]. The antioxidant activity increases or decreases on the application of heat, depending on degradation of polyphenolic compounds or generation of Maillard reaction products [8].

Fermentation has been used as one of the means of improving the keeping quality of food for more than 6000 years [9]. Fermented foods are those foods that are overgrown by edible microorganisms whose enzymes hydrolyse polysaccharides, proteins and lipids to non-toxic products with flavours, aromas and textures pleasant and attractive to the human consumer [10]. According to Hammes [11], fermentation of vegetables can occur in three ways, spontaneous fermentation by natural micro flora, by starter cultures that are added into raw materials and fermentation of heat-treated materials by starter cultures. Fermentation occurs at optimum temperature 20°C - 30°C [12]. Lactic acid fermentation represents the easiest and the most suitable way for increasing the daily consumption of fresh vegetables and fruits.

Due to its beneficial health effects, which include anti-microbial activity, anti-oxidant activity [13], garlic has attracted much more research interest. It is used as condiment in the preparation of dressed olive oils in Spain [14]. It is used an agent for prevention and treatment of cardiovascular, atherosclerosis, hyperlipidemia, thrombosis, and diabetes [15]. Both raw and fermented garlic increases the production of Nitric Oxide Synthase (NOS), a key in the lowering of blood pressure [16]. Garlic enhances the immune system of some people living with HIV by increasing the number of natural killer cells which not only destroy white cells that are infected by viruses but also those which are cancerous. Fermented garlic not only lowers down the cholesterol level but triglycerides as well.

The objective of this study was to observe the effect of addition of different salt and brine concentrations on the fermentation, colour and microbial growth of the blanched garlic.

Materials and methods

Procurement of raw material

Fresh garlic was procured from the local market and was brought to the Department of Food Science and Technology, University of Kashmir. The garlic free from bruises, dirt and other defects were used for the product development.

Procurement of chemicals

The chemicals used for physico-chemical and microbial analyses were obtained from the Himedia and Sigma Aldrich.

Preparation of product

Garlic bulbs were separated into the individual cloves, wherein the small and defective cloves were discarded and the fresh cloves were peeled and washed under tap water. The cloves were blanched in water at 90°C for 15 minutes. The surface moisture was removed by spreading the cloves on stainless steel trays for few hours. The cloves were then divided into four equal portions. Two portions were filled in two identical glass jars (B_1 and B_2) of 500 cc capacity. The materials in these jars were covered with 8% and 5% brine respectively. The other two portions were mixed with 8% and 5% table salt respectively and then filled in glass jars (B_3 and B_4) of same dimensions and capacity as above. Before filling them into glass jars, naturally fermented carrot pickle were added as source of innoculum to all the glass jars. Fermentation was carried out at 22°C for 10 days. All glass jars were monitored continuously for 30 days.

Sampling of garlic cloves

The sampling of the fermenting garlic product was done before fermentation and 15 and 30 days of fermentation by random sampling technique. The samples were taken from bottom, middle and upper portion of each glass jars.

Chemical analysis

The chemical analysis of fermenting garlic, treated with different salt and brine concentrations was carried out at regular intervals of time. Fermented garlic cloves along with brine were homogenized (Wise TIS homogenizer HG-15A). The sample was incubated at room temperature for 30 minutes and then centrifuged at 16000 rpm for 20 minutes. The supernatant obtained was used for the analysis of pH. The pH of the samples was determined by digital pH meter (HI 2215 pH / ORP meter).

Acidity of samples was determined by titrimetric method. An aliquot of sample was diluted and was titrated against 0.1 N Sodium hydroxide to a faint pink colour using few drops of 1% phenolphthalein solution as an indicator. The percent acidity (expressed as percent lactic) was analyzed according the method of Ranganna [17] and was calculated as:

$$\text{Acidity (\%)} = \frac{N \times V \times Eq.\ Wt \times Vol.\ made}{W \times Vol.\ of\ aliquot \times 1000} \times 100$$

Where,

N = Normality of titrant

V = Volume of titrant used

Eq. wt = Equivalent weight of acid

W = weight of sample taken for estimation

Microbiological analyses

Microbial analysis was carried out using pour plate technique. The sample was prepared in the saline solution (SS, 0.85%) and was homogenized (Wise TIS homogenizer HG-15A).The serially diluted samples (10^1 - 10^7) was poured on MRS agar. The plates were incubated at 21°C for 48 h before counting LAB colony forming units.

Statistical analysis

The statistical analyses were performed using the IBM SPSS statistics 21. The differences among treatments were evaluated statistically by one-way analysis of variance (ANOVA) and Duncan's multiple tests. All data were two sided at the 5% significance level and are reported as means ± standard deviations (SDs).

Sensory evaluation

Sensory evaluation of the blanched garlic prepared from different treatments was done by using nine-point hedonic scale.

Results and Discussion

Chemical changes in fermented garlic

The data obtained for chemical changes of fermented garlic is given in Table 1. In garlic, as a result of the growth of the inocula, the consumption of substrate was considerable. The major acid produced during fermentation was lactic acid and was found highest in B2 with a mean value of 2.28 and lowest in B_4 with the mean value of 1.65 after 30 days of fermentation. The acetic acid may also be produced during fermentation as reported by Castro et al. [18]. The degradation of citrate by *L. plantarum* may produce acetic acid during fermentation of garlic. The initial degradation of citrate produces acetate and oxaloacetate. The oxaloacetate gets decaboxylated to pyruvate which is further precipitated to acetate by the enzyme acetate kinase [19].

Samples	0 days	15 days	30 days
	Lactic acid	**Lactic acid**	**Lactic acid**
B_1	1.43 ± 0.01[a]	1.62 ± 0.015[a]	1.82 ± 0.015[a]
B_2	1.73 ± 0.01[b]	1.98 ± 0.020[b]	2.28 ± 0.025[b]
B_3	1.47 ± 0.01[a]	1.66 ± 0.005[b]	1.87 ± 0.015[b]
B_4	0.98 ± 0.011[c]	1.31 ± 0.01[c]	1.65 ± 0.011[c]

Table 1: Percentage of Lactic acid and Acetic acid after 0, 15 and 30 days of fermentation, Values [abc] are means ± SD and different letters within a column indicates significant differences (p < 0.05) among samples by Duncan's test.

The pH value of fermented blanched garlic ranges from 3.86 - 4.00 after 30 days of fermentation and is shown in Figure 1. In blanched garlic B_3, the pH of the brine decreased gradually from 5.37 to 4.0 as a result of the growth of the inoculum. However, there was an initial decrease in pH of the medium in case of blanched garlic B_1 which then possibly rose due to the diffusion into the garlic of acid in the brine. There was a green, blue and orange-red discoloration of blanched garlic at the end of the fermentation. The green coloration was also earlier reported by Sano and Joslyn et al. [20] and Lukes [21] in garlic products like in garlic puree but it remains unidentified.
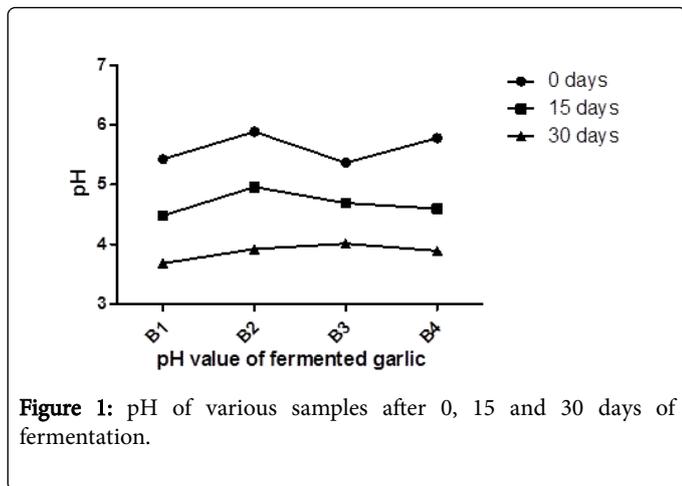
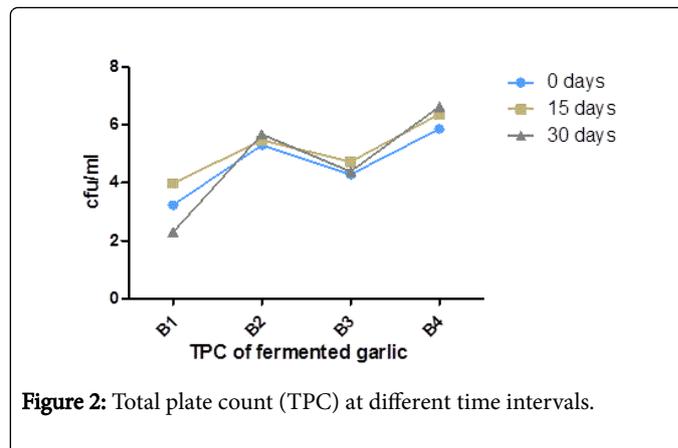**Figure 1:** pH of various samples after 0, 15 and 30 days of fermentation.

Figure 2: Total plate count (TPC) at different time intervals.

Microbial changes in fermented garlic

Microbiological stability of packed fermented garlic requires pasteurization or other treatments as methods of preservation. There was the secondary fermentation by yeasts during the storage of fermented blanched garlic. It was also observed in beets and carrots by Mc Feeters et al. [22]. In raw garlic the average counts of aerobic mesophilic bacteria expressed in cfu/g ranged from 1.4×10^2 to 1.7×10^3. It was in accordance with the study carried out by Fuselli et al. [23]. Limited microbial growth was detected during the storage of blanched garlic fermentation by *L. plantarum* in B_1. This is because of the preserving effect of low pH and high salt concentration which not only inhibits *L. plantarum* growth [24], but also the growth of yeasts in the fermented product. The total numbers of viable bacteria in different samples at different periods were shown in Figure 2. Treatment B_4 showed maximum count and sample B1 showed minimum count. The garlic pickle with 5% brine solution showed very little difference in microbiological load after 15 and 30 days as compared to that of 0 days of storage for all samples. It was seen that after 30 days of storage, the blanched garlic pickle B_4 grew maximum 6.63×10^3 cfu/g of bacteria and B_1 with minimum 2.30×10^3 cfu/g of bacteria. These counted viable loads in a food product might be considered hygienic according to the FAO / WHO expert consultation or microbiological specifications who mentioned that maximum 5×10^4 mesophilic aerobic microorganisms are safe for human consumption and the values at or above 10^6 are unacceptable [25].

Sensory evaluation of product

The mean scores for colour, flavour, texture and overall acceptability of garlic pickles are presented in Table 2. The consumer's acceptability of developed pickles was evaluated by a taste-testing panel. The nine point hedonic rating test was used to determine the acceptability. The panelists were selected from the Technical staff and students of the Department of Food Science and Technology.

The coded samples were served to the panelists and were asked to assign appropriate score for characteristics colour, flavour, texture and overall acceptability of processed fermented blanched pickles. The results were later evaluated by Analysis of variance and Duncan's New Multiple Range Test Procedures of the Statistical Analysis System (SAS).

Sample	Sensory attributes			
	Colour	**Flavour**	**Texture**	**Overall acceptability**
B_1	6.5[a]	6.3[a]	7.65[b]	6.81[a]
B_2	8.0[c]	8.1[c]	7.80[b]	7.96[b]
B_3	7.5[b]	6.2[a]	7.50[b]	7.06[b]
B_4	7.3[b]	7.4[b]	6.90[a]	7.20[b]

Table 2: Mean sensory score of fermented blanched garlic pickle. Sample [abc] means having the same letter suffix do not differ at 5% ($P < 0.05$) level of statistical significance.

In addition to physico-chemical analysis, the physical properties such as colour, flavour, texture and visual fungal growth of the garlic pickles were also studied and is shown in Table 3. There were discoloration and undesirable changes in flavour in some of the treatments and may be is due to the enzyme catalyzed oxidation reactions. This was in accordance with the studies done by Joslyn and Sano [26].

Storage period (days)	Treatment	Colour	Flavour	Texture	Visual fungal growth
0	B_1	No change	No off flavour	Firm	No growth
	B_2	No change	No off flavour	Firm	Do
	B_3	No change	No off flavour	Firm	Do
	B_4	No change	No off flavour	Firm	Do
15	B_1	No change	No off flavour	Firm	No growth

	B_2	No change	No off flavour	Firm	Do
	B_3	No change	Off flavour	Slightly soft	Do
	B_4	No change	No off flavour	Slightly soft	Do
30	B_1	No change	No off flavour	Slightly soft	No growth
	B_2	No change	No off flavour	Firm	Slight
	B_3	Change[#]	Off flavour	Soft	moderate
	B_4	Change[*$]	No off flavour	Soft	Do

Table 3: Physical properties of blanched garlic pickle during fermentation. The symbol [#]indicates the change of colour to green [*]indicates the change to blue and [$]indicates change to orange red.

However it was reported that the pickles would be assessed after six months of storage in jars for taste, keeping quality etc. The pickles became soft after three months, but otherwise remained satisfactory up to six months of storage. There was slight to moderate fungal growth in case of blanched garlic with 5% brine and 8% salt respectively as seen visually [27].

Conclusion

Lactic acid fermentation of vegetables is an important technology, which has ensured the suppression of the growth of hazardous microorganisms in foods particularly in regions where adequate means of preservation are lacking. Fermentation has great potential to improve the hygienic condition of foods. In controlled fermentation of garlic using a starter culture of *L. plantarum*, a blanching step is important besides preventing the appearance of unwanted effects. There was no fungal growth at any of the temperatures studied. This study also found that a formulation containing blanched garlic in 5% salt (w/v) retained physical stability. Moreover, it is possible to suggest that this formulation may be effective in maintaining healthy cholesterol levels and blood pressure in normal range.

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