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Effect of sodium substitution on lactic acid bacteria and total bacterial population in lime pickle under ambient storage conditions

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Abstract
An attempt has been made to prepare lime pickle using different salt mixtures of NaCl, KCl and $CaCl_2$ and evaluate the pickle samples for total bacterial population and Lactic acid bacteria populations. The purpose of salt mixture was to reduce Na consumption. The results showed that the total bacterial population tends to increase with storage. Minimum bacterial population was observed at 0 day and maximum at 270th day of storage. At the 0 day of storage, T_1 shows highest population of Lactic acid bacteria (171.00) followed by T_2 (150.00), T_6 (147.00) and T_4 (143.00) whereas T_5 showed lowest LAB population (126.67). Similar trend in the LAB population dynamics has been observed at 60th, 90th, 120th, 150th, 180th day, 210th day and 240th day. At 270th day, T_6 showed highest LAB population (34.67) followed by T_1 (31.00). T5 supported lowest LAB population (18) followed by T_3 (21.67), T_2 (23.33) and T_4 (25.67). At 0 day, highest bacterial population was observed in T_1 (8.00) followed by T_2 (7.33), T_4 (6.33) and T_6 (5.67). Lowest bacterial population was observed in T_5 (4.67) followed by T_3 (5.33). At 270th day of treatment, the pickle prepared using salt composition T_1 showed maximum bacterial colonies (180.67) followed by T_5 (171.67), T_2 (168.67) and T_4 (167.33). Lowest bacterial count was observed in T_6 (164.33). Hence, a salt mixture with 50% NaCl, 25% KCl and 25% $CaCl_2$ can be used for pickle preparation.

Keywords: Lactic acid bacteria, salt substitution, bacterial population, LAB, Total Plate Count, Lime Pickle, Microbial dynamics

1. Introduction
Lime pickle is one of the oldest preserved food products which is made from matured lime. Lime pickle is known as 'king of pickle'. The term pickle is derived from the Dutch word 'Pekel", meaning brine [1]. Pickles are made through the natural fermentation of fruits and vegetables, and besides having nutritional values, pickles also act as a food accompaniments and palatability enhancers [2]. The process of pickling involves fermentation which is a primitive preservation method primarily used to enable the long-term storage of foods. Fermentation is a dawdling decomposition process of organic substances persuaded by microorganisms or enzymes that fundamentally convert carbohydrates to alcohols or organic acids [3]. When the fermentation term is used in instance of fruits and vegetables, it is known as pickling. Out of the several approaches to fermentation, lactic acid fermentation, using natural micro flora or lactic acid bacterial (LAB) cultures, is employed throughout the world. Lactic acid (LA) fermentation of vegetables and fruits is a common practice to maintain and rally the nutritional and sensory features of food commodities [4, 5]. Salt is a crucial part of our food habit. Salts not only increase the taste, but it also has an immense role in human nutrition. NaCl is one of the most commonly employed agents for food conservation, allowing considerable increase in storage time by reducing water activity. Salt (sodium chloride) is the oldest food seasoning, which provides one of the important basic human tastes (saltiness) and conserves foods to extend the shelf life. Salt mainly consists of two elements: sodium and chloride. In the pickling industry, salt has historically been used for directing the fermentation of cucumbers, radishes, and carrots [6, 7, 8, 9]. Common salt contains Na^+ (Cation) and Cl^- (Anion). Na^+ (Cation) is mainly responsible for the saltiness in the food. Sodium is a vital element essential in small amounts by the human body, as it helps to control homeostasis and nerve impulses [10]. Sodium chloride is an essential in food as it improves the preservative, technological and sensory quality of food [11]. The extra consumption of sodium present in salt might lead to circumstances such as hypertension and high blood pressure. Approximately one quarter of the world's population suffers from this condition [12].

High sodium intake is increasing the risk of heart attack and high blood pressure [13]. Consequences for sodium intake and its effects on human blood pressure were derived from scientific research, animal studies and other human surveys [14, 15]. The mechanism of the effect of salt on blood pressure could be due to the rise in plasma sodium or to the upsurge in extracellular fluid volume. Higher dietary sodium intake is also related to bone disease (Doyle, 2008). Pickling is done in presence of high concentration of salt solution in which the fruit pieces are dipped to ensure fermentation. Pickles contain salt at about 15-20% levels making it one of the high salt containing foods. The biggest drawback with pickles is the presence of high concentration of sodium ion (Na^+) which may lead to adverse effects on human health and on food business. Many food products have been commercialized in its low salt version. The only form of salt that does not contain sodium is the low-sodium alternatives are the replacement with Potassium, Magnesium and Calcium ion instead of sodium. Eating excess salt raises the amount of sodium in our bloodstream and disturbs the delicate balance, reducing the ability of our kidneys to remove the water. The fractional replacement of NaCl by KCl or $CaCl_2$ seems to provide an alternative for reducing sodium content. Increased potassium intake is reported to guard stroke, high blood pressure, heart rhythm complications, kidney failure, and even osteoporosis [16]. The additional use of KCl and $CaCl_2$ to partially replace NaCl could be helpful in reducing sodium content [17]. However, the use of KCl is mainly limited by its bitter and astringent taste [18]. Some people have reported a metallic after taste and therefore choose not to use KCl in food. But a mixed concentration of Na, K and Ca can help to reduce the total salt intake in our body. Basically in this work, we have made an attempt to observe the changes in population dynamics of Lactic Acid bacteria (LAB) which is beneficial in nature and is known to have a desirable effect on human health. Beside that it is known to have some antagonistic property towards detrimental pathogens including bacteria. The total bacterial population depicts the presence of both harmful and beneficial bacteria. Therefore, the present work has been undertaken to investigate the possibility of replacing sodium chloride by potassium and Calcium salts and develop low sodium lime pickle and to study the effect of NaCl substitution microbiological attributes of the pickle.

2. Materials and methods

2.1. Site of experiment: The present study was accompanied in Post Graduate Laboratory, Department of Pomology and Post-Harvest Technology, Faculty of Horticulture and Central Instrumentation Centre Lab of Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal.

2.2. Source of pickling materials: The lime fruits were fresh, ripe yellow and were free from pests, diseases and blemishes. Lime fruits were purchased from local market for pickle preparation. The chemical used were of Laboratory grade.

2.3. Design for deciding the salt mixture for pickle preparation in the experiment

The following design was used for deciding the salt mixture for pickle preparation in the experiment.

Design: Randomized Block Design (RBD)
Software used: SPSS

Table 1: Treatment details showing different salt proportion used for curing purpose.

Treatment	NaCl (%)	KCl (%)	$CaCl_2$ (%)
T_1	100	0	0
T_2	50	50	0
T_3	50	0	50
T_4	0	100	0
T_5	0	50	50
T_6	50	25	25

2.4. Procedure for lime pickle preparation: The lime pieces were chopped into 4-6 pieces. The lime pieces were dipped in salt solution for fermentation. Pickles were prepared by using the standardized procedure. The prepared pickles were stored in glass jars which were cleaned appropriately and were sterilized in boiling water at room temperature. During the complete storage period it was ensured that the pickle was stored in aerated, dry and hygienic conditions.

2.5 Lactic Acid bacteria and Total Plate count upto 210th day of pickle storage

The entire microbiological aspects of the experiment were performed in Post Graduate Lab, Department of Plant Pathology, UBKV. Microbiological analysis for the pickle was carried out by the method of Ranganna (1977) [19]. All the enumerations of Bacteria and *Lactobacillus* were carried out following serial dilution technique using specific media. Plates were incubated at 34±1 °C for 48 hours and colony forming units (CFU/g) were recorded. Observations for microbial count were made at prescribed intervals. The principle behind this is that the population of total bacterial population tends to decline with the decimal reduction in the concentration of the sample analyzed. Generally in a culture the microbial population was expected to be higher in 10^{-1} which tends to decline with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} sample concentration. Higher the sample concentration higher would be the expected microbial concentration. As the colonies have a habit to coalesce or merge at higher concentration hence the readings for Lactic Acid Bacterial Count and Total Bacterial Population Count has been studied at 10^{-5} concentration.

3. Results and discussion

Table 2 shows the effect of different salt proportion on Lactic Acid Bacteria (LAB) population in the lime pickle sample which has been studied upto 270th day of preparation. Varying proportion of NaCl, KCl and $CaCl_2$ has been used for the curing purpose. At the 0 day of storage, T_1 shows highest population of Lactic acid bacteria (171.00) followed by T_2 (150.00), T_6 (147.00) and T_4 (143.00) whereas T_5 showed lowest LAB population (126.67). This can be attributed due to the fact that KCl and $CaCl_2$ have a negative response on any sort of microbial growth. At 30th day of storage, T_1 have highest LAB population (164) followed by T_2 (147.67), T_6 (145.33) and T_4 (144.33). T_5 have lowest LAB population (121) followed by T_3 (124.67). Similar trend in the LAB population dynamics has been observed at 60th, 90th, 120th, 150th, 180th day, 210th day and 240th day. At 270th day, T6 showed highest LAB population (34.67) followed by T_1 (31.00). T_5 supported lowest LAB population (18) followed by T_3 (21.67), T_2 (23.33) and T_4 (25.67). At 270th day of storage, LAB population using T_1 and T_6 salt proportion is at par. This clearly shows that NaCl and KCl have positive influence on LAB population whereas addition of $CaCl_2$ in

salt mixture resulted in reduction in LAB population. This can be due to the unique ability of $CaCl_2$ to reduce the water activity of salt cured lime pieces.

Table 2: Lactic Acid Bacteria population (log CFU) under ambient storage conditions.

Treatments	0 day	30th day	60th day	90th day	120th day	150th day	180th day	210th day	240th day	270th day
T_1	171.00	164.00	160.00	141.00	124.00	101.00	85.33	58.00	48.00	31.00
T_2	150.00	147.67	145.33	130.33	112.33	95.33	75.33	43.00	39.00	23.33
T_3	129.67	124.67	121.33	108.00	94.00	77.67	69.33	41.00	36.67	21.67
T_4	143.00	143.33	140.33	115.00	100.00	89.67	74.67	45.00	39.00	25.67
T_5	126.67	121.00	118.00	102.33	81.00	82.67	67.00	36.00	30.33	18.00
T_6	147.00	145.33	142.00	120.33	105.67	85.00	82.33	60.00	52.00	34.67
C.D.	15.24	15.79	13.33	11.60	12.04	N/S	5.31	2.95	2.43	1.41
SE(m)	4.77	4.95	4.18	3.63	3.77	4.78	1.66	0.92	0.76	0.44
C.V.	5.72	6.08	5.25	5.27	6.35	9.35	3.81	3.40	3.22	2.98

Table 3 shows the effect of different salt proportions on the total bacterial population in lime pickle sample at ambient storage upto 270th days. The total plate count showed significant variation of total bacterial population among different treatments. At 0 day, highest bacterial population was observed in T_1 (8.00) followed by T_2 (7.33), T_4 (6.33) and T_6 (5.67). Lowest bacterial population was observed in T_5 (4.67) followed by T_3 (5.33). T_1 was at par with T_2 which clearly indicates that the presence of higher amount of NaCl and no $CaCl_2$ provides suitable environment for bacteria to survive. Similar trend was observed at 30th, 60th, 90th, 120th, 150th, 180th, 210th day and 240th day of treatment. At 270th day of treatment, the pickle prepared using salt composition T_1 showed maximum bacterial colonies (180.67) followed by T_5 (171.67), T_2 (168.67) and T_4 (167.33). Lowest bacterial count was observed in T_6 (164.33). The reason behind lowest bacterial population in T_5 and lower bacterial population at T_6 can be attributed to the salt composition that was used during the curing procedure. T_5 salt mixture contain 50% $CaCl_2$ because of which the pickle prepared was having lowest bacterial count. $CaCl_2$ is known to be a good curing agent that can substantially reduce the available water in tissue of pickle pieces thus reducing the water activity (a_w) and resulting in lower total bacterial population. This result is similar to Mani *et al*. (2017) [20] who worked on population dynamics of total bacteria and Lactic acid bacteria in mango pickle under ambient storage conditions. Singh *et al*. (2017) [21] found out the same pattern of population change while working with mix-veg pickle.

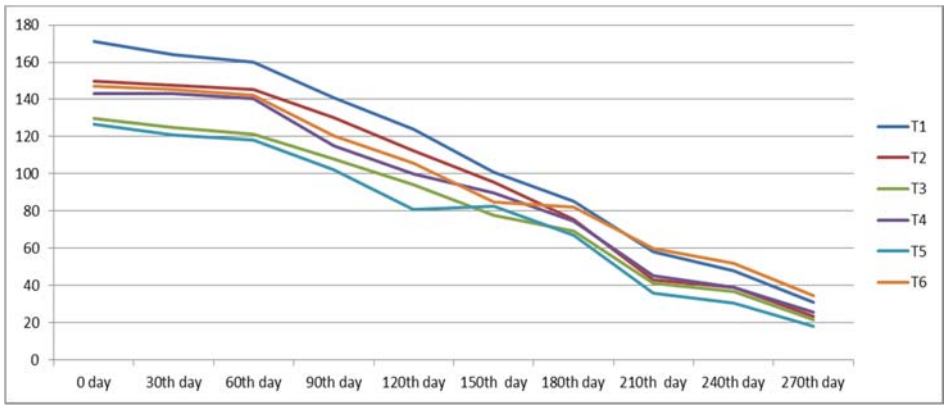

Fig 1: Lactic Acid Bacteria population (log CFU) under ambient storage conditions.

Table 3: Total Bacterial population (log CFU) under ambient storage conditions.

Treatments	0 day	30th day	60th day	90th day	120th day	150th day	180th 1day	210th day	240th day	270th day
T_1	8.00	18.33	42.33	71.00	100.67	119.67	142.67	164.33	171.00	180.67
T_2	7.33	22.33	41.33	69.00	95.33	114.00	138.00	160.33	164.00	168.67
T_3	5.33	23.33	38.67	64.67	84.33	107.00	128.67	153.33	157.67	164.67
T_4	6.33	20.33	43.00	68.67	93.67	112.67	137.33	155.67	161.00	167.33
T_5	4.67	22.33	35.00	63.00	81.00	104.00	124.67	148.00	158.00	171.67
T_6	5.67	16.33	40.00	64.33	86.67	111.00	130.33	150.67	159.33	164.33
C.D.	1.64	3.76	1.33	1.05	2.62	2.81	1.89	1.98	4.82	1.49
SE(m)	0.51	1.18	0.42	0.33	0.82	0.88	0.59	0.62	1.51	0.47
C.V.	14.27	9.96	1.80	0.85	1.57	1.37	0.77	0.69	1.61	0.48

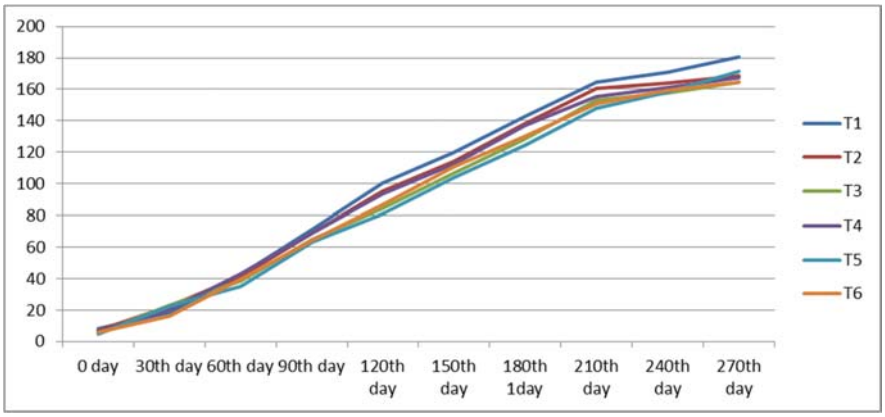

Fig 2: Total Bacterial population (log CFU) under ambient storage conditions.

Summary

The lime pickle which was prepared after curing with different salt mixture was evaluated for total bacterial population and lactic acid bacteria (LAB) population. The results showed that the total bacterial population tends to increase with storage. Hence minimum bacterial population was observed at 0 day and maximum at 270th day of storage. At the 0 day of storage, T_1 shows highest population of Lactic acid bacteria (171.00) followed by T_2 (150.00), T_6 (147.00) and T_4 (143.00) whereas T_5 showed lowest LAB population (126.67). Similar trend in the LAB population dynamics has been observed at 60th, 90th, 120th, 150th, 180th day, 210th day and 240th day. At 270th day, T_6 showed highest LAB population (34.67) followed by T_1 (31.00). T5 supported lowest LAB population (18) followed by T_3 (21.67), T_2 (23.33) and T_4 (25.67). At 0 day, highest bacterial population was observed in T_1 (8.00) followed by T_2 (7.33), T_4 (6.33) and T_6 (5.67). Lowest bacterial population was observed in T_5 (4.67) followed by T_3 (5.33). At 270th day of treatment, the pickle prepared using salt composition T_1 showed maximum bacterial colonies (180.67) followed by T_5 (171.67), T_2 (168.67) and T_4 (167.33). Lowest bacterial count was observed in T_6 (164.33).

Conclusion

A conclusion can be drawn from this that the incorporation of $CaCl_2$ in the salt mixture which is to be used for curing purpose directly helps to suppress the bacterial population even at 270th day (9 month) of storage. But it also affects the growth of desirable Lactic Acid Bacteria (LAB) in the pickle. Hence, a salt mixture with 50% NaCl, 25% KCl and 25% $CaCl_2$ can be used for pickle preparation. This salt mixture would not only help to minimize Sodium (Na) consumption but can also ensure a balanced microbial population throughout its ambient storage period to satisfactory level.

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