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A novel method to produce low salinity and healthier Indian lemon (*Citrus limonum*) pickle

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ABSTRACT

Pickle is used as a household meals supplement in almost all Indian families irrespective of their socioeconomic status. Indian lemon (Citrus limonum) has been one of the main ingredients in pickle and the practice has been carried over for generations till today. The objective of the study was to device a novel method to produce healthier, low salinity Indian lemon pickle without compromising on organoleptic qualities by using Bacillus coagulans. Three salinity concentrations of lemon pickles were prepared, 5%, 10% and 15%. Addition of Bacillus coagulans to the 5% pickle sample resulted in the end product which was qualitatively on par with the other two high salinity pickle samples. The organoleptic qualities of the low salinity pickle were found to be same as the high salinity pickle samples, sustaining same levels of palatability and shelf life. Quantification of LAB (Lactic Acid Bacteria) was done in the low salinity pickle sample and found to be 56 x 10^4 CFU. Titratable acidity of the three salinity samples was found to be 18.6 °T, 23.1 °T and 35.8 °T for 5%, 10% and 15% salinity pickle samples respectively. Production of low salinity, highly healthy pickle will be a boon for average Indian households by which side effects of continuous pickle consumption such as high blood pressure can be reduced significantly.

INTRODUCTION

Indian pickles are made from certain individual varietals of vegetables and fruits that are chopped into small pieces and cooked in edible oils like sesame oil or brine with many different Indian spices like asafetida, red chili powder, turmeric, fenugreek and plenty of salt. Some regions also specialize in pickling meats and fish. Vegetables can also be combined in pickles to make mixed vegetable pickle (1). Some varietals of fruits and vegetables are small enough to be

used whole. The most common Indian-style pickles are made from mango and lime. Others include cauliflower, carrot, radish, tomato, onion, pumpkin, palm heart, lotus stem, rose petals, ginger, Indian gooseberry, garlic, green or red chili peppers, kohlrabi, gunda, kerda, zimikand (purple yam), karonda, karela (bitter melon), jackfruit, mushroom, eggplant, cucumber and turnip as suggested by Gorbach *et al* (4). Homemade pickles are prepared in the summer and kept in the sun during daytime while stored in porcelain or glass jars with airtight lids. The high concentrations of salt, oil and spices act as preservatives. Many commercially produced pickles use preservatives like citric acid and sodium benzoate. Even using the same main ingredients, Indian pickles come in a wide variety of flavors due to differences in spices and process (3). A mango pickle from South India may taste very different from one made in North India. In the southern states, sesame oil is preferred, while mustard oil is preferred in northern states for making pickles (7).

Lime is one of the most common ingredient for preparing pickle. In cooking, lime is valued both for the acidity of its juice and the floral aroma of its zest. It is a very common ingredient in authentic Mexican, Southwestern United States, Vietnamese and Thai dishes. It is also used for its pickling properties in ceviche. The use of dried limes (called black lime or loomi) as a flavouring is typical of Persian cuisine and Iraqi cuisine, as well as in Gulf-style baharat (a spice mixture that is also called kabsa or kebsa). Lime is an essential ingredient of any cuisine from India and many varieties of pickles are made e.g. Sweetened lime pickle, salted pickle, Lemon Chutney. Lime leaves are also a herb in South, East, and Southeast Asia.

Bacillus coagulans is a lactic acid forming bacterial species within the genus *Bacillus*. The organism was first isolated and described in 1932 and was elaborated in the fifth edition of Bergey's Manual of Determinative Bacteriology. It was initially considered to be a spore-forming *Lactobacillus*. Since Bacillus coagulans exhibits characteristics typical of both genera *Lactobacillus* and *Bacillus*, its taxonomic position between the families *Lactobacillaceae* and *Bacillaceae* was often debated. However, in the seventh edition of Bergey's, it was finally transferred to the genus *Bacillus*. DNA-based technology was used in distinguishing between the two genera of bacteria which are morphologically similar and possess similar physiological and biochemical characteristics.

B. coagulans is a Gram-positive rod (0.9µm by 3.0µm to 5.0µm in size); catalase positive, spore-forming, motile, a facultative anaerobe. B. coagulans may appear Gram-negative when entering the stationary phase of growth according to Fujiwara *et al* (2). The optimum temperature for growth is 50 °C; range of temperatures tolerated is 30°C - 55°C. IMViC Tests VP and MR (methyl-red) tests are positive.

Bacillus coagulans has been added by the EFSA to their Qualified Presumption of Safety (QPS) list and has been approved for veterinary purposes as GRAS by the U.S. Food and Drug Administration's Center for Veterinary Medicine, as well as by the European Union and is listed by AAFCO for use as a direct feed microbial in livestock production (10). Its main use is thus its veterinary applications, especially as a probiotic in pigs and shrimp. There are also references to use of this bacterium in humans, especially in improving the vaginal flora, improving abdominal pain and bloating in Irritable Bowel Syndrome patients and increasing immune response to viral challenges. The bacterium has also been assessed for safety as a food ingredient. Spores are

activated in the acidic environment of the stomach and begin germinating and proliferating in the intestine.

Pickles are restricted in various households because of their nature to increase the blood pressure. The high salt content in all varieties of pickle tends to push up the blood pressure when the consumer has a prolonged consumption of it. Pickles of different varieties have different levels of salt concentration in them and they are not uniform in nature. Although the concentration of NaCl (Sodium Chloride) can differ in the pickle varieties, the continuous consumption is not recommended in any case (9). The presence of Sodium Chloride in the pickle makes the elderly people more vulnerable to hyper blood pressure. The relationship between age and blood pressure is directly proportional, i.e. blood pressure increases with the increasing age (5). Advancement of age produces less rigid blood vessels which results in increase of blood pressure. In these cases, continuous intake of pickle varieties which are rich in Sodium Chloride will surely be counterproductive.

MATERIALS AND METHODS

Pickle preparation

Lemon fruits that had to be used for pickle preparation were procured from Guduvancherry, a locality near SRM University, Chennai. The lemons were ripe, dark yellow in colour and without any microbial or insect infections. Procured lemon fruits were washed with running water and then with distilled water. The lemon fruits were then cut in to smaller pieces using a pre-sterilized knife and placed in a container. About 300 grams of the cut lemon pieces were divided in to 3 equal portions with each having 100 grams weight. Each portion was added with different salt concentrations, 5%, 10%, and 15% respectively. The additional ingredients such as red chili powder and sesame oil were added in same proportion to all the three salt concentration pickle samples (acidify.htm).

Addition of bacteria

The bacteria had been procured from IMTECH (Institute of Microbial Technology), Chandigarh, India, (MTCC number – 492). The bacterial species had been obtained in an active slant and was sub-cultured with Nutrient agar media before being used. After the pickle ingredients were added in to the container, Loopful of *Bacillus coagulans* (8×10^4 CFU) was added to the sample containing least salinity concentration, i.e. 5%. The uniformity of bacterial addition was maintained so that the results correspond in an acceptable way.

Pickle end product

The pickle mixture along with the above mentioned ingredients was allowed to remain in shade for 10 days. The pickle samples were maintained at room temperature away from direct sun light. Occasional stirring was done to ensure that the blending had been perfect. A lot of emphasis was laid on blending as it is concerned with mixing of the pickle ingredients and the interaction between *Bacillus coagulans*, pickle.

Assessment of fermentation effect by bacteria

After 10 days are over, the three pickle samples of varying salinity concentrations were assessed for the comparative effect of bacterial fermentation. In the time period of ten days, the pickle

formation was complete and it was fit for consumption. The least salinity concentration, 5% was assessed for the fermentative effect by *Bacillus coagulans.*

Quantification of LAB (Lactic Acid producing Bacteria) and titratable acidity

The LAB (Lactic Acid producing Bacteria) was quantified in the the least salinity concentration, 5%. The titratable acidity of all the three pickle samples with varying salinity was determined in triplicate titrimetrically by using 0.1 M NaOH with phenolphthalein as an indicator. The volume of the phenolphthalein used up in milliliters to neutralize the 0.1M NaOH of the acid in 10 ml of the product expressed in Toerner's degree (^oT)

Assessment of organoleptic characteristics

After the pickle formation and quantification of bacteria, titratable acidity the various organoleptic characteristics were assesses for all three salinity samples. Organoleptic characters that were assessed were colour, flavour, taste, consistency and salinity.

RESULTS

The pickle formation was complete and flawless in all the three different salinity concentrations. The Quantification of LAB (Lactic Acid Bacteria) was done in the low salinity pickle sample, 5% and found to be 56 x 10^4 CFU.

Figure 1. Pickle samples during 1st day of preparation (in ascending order of salinity)

Titratable acidity of the three salinity samples was found to be 18.6 °T, 23.1 °T and 35.8 °T for 5%, 10% and 15% salinity pickle samples respectively.

Figure 2. Pickle samples after 10 days (in ascending order of salinity)

The organoleptic characteristics of least salinity sample, 5% was found to be on par with the higher salinity pickle samples. The colour of the pickle was pale yellow, flavour was characteristic lime, taste was limited sourness, consistency was slightly viscous and salinity was just enough for palatability.

Figure 3. 5% Salinity concentration sample at the end of 10 days

The organoleptic characteristics of least salinity sample, 10% was found to be quite close with the 5% and 15% salinity pickle samples. The colour of the pickle was yellowish, flavour was characteristic lime, taste was limited sourness, consistency was viscous and salinity was just enough for palatability.

Figure 4. 10% Salinity concentration sample at the end of 10 days

Figure 5. 15% Salinity concentration sample at the end of 10 days

The organoleptic characteristics of least salinity sample, 15% was found to be in line with the 5% and 10% salinity pickle samples. The colour of the pickle was yellowish, flavour was characteristic lime, taste was sourness, consistency was more viscous and salinity was enough for palatability.

DISCUSSION AND CONCLUSION

Lemon pickles are usually preferred by many Indian families as they have one of the highest shelf life periods among all varieties of pickle (6). The reason behind the longevity of Indian lemon's high shelf life is the chemical constituent nature of it, rich in citrus juice. Lemon has a distinct sour taste due to the presence of citric acid and Indian public has a lineage towards that particular aspect of lemon fruit. Lemon is also relatively cheap in comparison with other fruits and vegetables used for preparing pickles making it an ideal candidate for lower income groups. The availability of lemon throughout the year makes sure that there is no seasonal problem associated with it as in the case of other fruits and vegetables with which the pickle is produced or prepared.

The effect of *Bacillus coagulans* in the case of least salinity sample, 5% was very evident. Although the salinity was least, the organoleptic characteristics (colour, flavour, taste, consistency and salinity) were almost same as observed in the case of higher salinity concentrations (10% and 15%). The results conclusively prove the point that *Bacillus coagulans* had replaced salinity to produce the desired effects in the pickle. The bacterium was successfully able to bring about the results in our case of Indian pickle (*Citrus limonum*).

These results can play a crucial role in reducing the health risk of a various Indian household population as it demonstrates the substitution of salt by *Bacillus coagulans* as far as Indian lemon pickle is concerned. The idea could be expanded and tried with all other fruits and vegetables used to produce pickles. When commercially implemented it could go down well with niche, middle class and even lower middle consumers for its potential health benefits to reduce the risk of high blood pressure and related disorders.

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