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Formulation and Sensory Attributes of the Pickle Made from Buttermilk Treated Green Chilli (*Capsicum annuum*)

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ABSTRACT

Background: The present study aims was to investigate the effect of Direct Vat Set buttermilk (DVSBM) and homemade buttermilk (HMBM) culture on physico-chemical properties of green chilli pickle.

Methods: In the present study the effect Direct Vat Set buttermilk (DVSBM) and homemade buttermilk (HMBM) culture on physicochemical properties of green chillies pickles after 3 months of storage at ambient temperature (25±3°C).

Result: The results revealed that the treatment of chillies with buttermilk *i.e*. HMBM and DVSBM, significantly influenced the properties of green chilli pickle. Therefore, the green chilli pickle treated with HMBM culture maintained higher acidity (0.58%) and lower pH (4.35) with higher retention of firmness and overall acceptability.

Key words: Acidity, Buttermilk, Fermentation, FTIR, Green chilli, Pickle, Probiotics, Sensory characteristics.

INTRODUCTION

Pickling process is generally used for the preservation of food products by anaerobic fermentation in brine (salt water solution) to produce lactic acid, or by dipping in vinegar/ citric acid solution (Behera *et al*., 2020). Picking is a conventional method to developing low cost ready to eat vegetable products with improving their quality attributes (Behera *et al*., 2020; Gadekar *et al*., 2010). In addition, the incorporation of ingredients such as spices and oils may also influenced the nutritive and flavor properties of the products (Shahjahan *et al*., 2005; Chakraborty and Roy, 2018; Shiferew *et al*., 2020). The manufacturing process of pickling is depending on the region (Behera *et al*., 2020). (Generally, pickles contain a range of pH below 4.6, which helps to destroy most of the bacteria. Numerous types of fruits and vegetables pickle manufacturing by addition of microorganisms such as lactic acid bacteria, *Micrococcaceae*, *Bacilli*, Yeasts, and filamentous fungus improving the desired quality and sensory characteristics (Natt and Katyal, 2022). The process of fermentation may involve the oxidation of carbohydrates to generation a range of products; it has preservative effects by retarded the growth of pathogenic micro biota in the foods (Keyeta, 2021; Toldrá, 2023). Pickles contain good amounts of natural and gut friendly Lactic acid bacteria which have beneficial probiotic properties and important in the digestion of food (Sugandhi, 2018). The green chilli pickles are most popular due to its delicious taste, nutritive value and health benefits (Devi, 2018). Chilli is a member of the *Solanaceae* family and belongs to the genus Capsicum. Several varieties such as of green chillies are used to manufacturing the pickle around the world (Saleh *et al*., 2018). In addition, the treatments of buttermilk of green chilli pickle have been used traditionally for the fermentation process. Buttermilk is considered good source of lactic acid bacteria (LAB). LAB are generally recognized as safe for consumption and plays an important role in the detoxification and said to give effect of degeneration of microbes in pickle products (Narayana and Kale, 2019; Sheikha *et al*., 2020). These probiotics also resulted, reducing pH of the pickle, which helps in the prevention against food borne microbial pathogens (Shin *et al*., 2020). Therefore, in India a very limited study has been available on the effects of commercial probiotic culture on the physico-chemical and sensory characteristics of green chilli pickles. Therefore, in present study, the effects of HMBM and DVSBM buttermilk treatments on the physicochemical characteristics of green chilli pickles were investigated.

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MATERIALS AND METHODS

Materials

The green chillies (*Cv. Capsicum annuum*) and stuffing ingredients (mustard seed, fenugreek seed, asafoetida, turmeric and fennel seed were procured from local market of Kundli, Sonipat, Haryana. Pasteurized toned milk (Mother

Dairy brand) was procured from local dairy shop. The freeze dried direct vat set type of commercial starter culture for butter milk (DVSBM) was obtained from a commercial manufacturer, which contains composition of LAB (*Streptococccus, Lactobacillus* and *Bifidobacterium*) microbial strains with a potential to give probiotic curd/buttermilk.

Chemical and reagents

All the analytical grade of chemical and reagents for the analysis were supplied by the Hi-Tech Ind. Pvt. Ltd. Delhi.

Experimental methodology

Cleaning of green chilli

The procured green chillies were directly transferred to the laboratory and washed to remove dust and physical contaminants.

Preparation of buttermilk

The procured toned milk was boiled and cooled up to 40°C. Thereafter, the standard DVS culture (0.12 mg/L) and homemade curd (2% w/v) were added into two different beakers containing milk 400 ml each and allowed to incubate at 37°C for 5 h. Thereafter, the churning process (20 min) was performed to obtained DVSBM and HMBM buttermilk.

Butter milk pretreatment of green chillies

For the pretreatment of green chillies before pickling were soaked into HMBM and DVSBM buttermilk in different glass containers and were allowed kept in sunlight for 4 to 5 days until green chilli turned green to light pale in colour. Thereafter, the chillies were washed using clean water and allowed to dry for removing of excess water. A lengthwise slit like cut was made on each green chilli manually for stuffing the mix of spices inside the chilli. The without pretreated green chilli was considers as control sample.

Preparation of spice mix

The procured different ingredient and spices such as mustard seed, fenugreek seed, asafoetida, turmeric, fennel seed, were cleaned and roasted at 70°C for 2 min. Thereafter, the spices were coarse grounded using food grade grinder mixture. The optimized spices composition of the ingredients /spices for stuffing in the green chilli pickles is given in Table 1.

Pickling and manufacturing of green chillies pickle

The manufacturing process of the buttermilk treated green chilli pickle is shown in Fig 1. The untreated (without buttermilk) green chili pickle was used as control to comparison with treated pickles. The prepared green chilli pickle was stored for 3 months for physico-chemical analysis.

Determination of pH and titratable acidity (TA)

For the determination of pH and acidity of green chilli samples, 5 g of green chilli pickle was homogenized with 50 ml of distilled water and centrifuged for 10 min at 8000 rpm speed. pH of the sample was determined using digital pH meter (153-S, EUTECH)) at 20°C (Çetin. 2011).

For determination of TA 25 ml sample of supernatant was titrated against 0.1 N NaOH. Phenolphthalein was used as an indicator to mark the endpoint. The results of acidity were expressed as percentage (%) acetic acid and calculated using the equation (Maurya *et al*., 2018).

$$TA\ (\%) = \frac{\text{Vol. of NaOH} \times \text{Molecular wt. of acetic acid } (60.05) \times \text{Normality of NaOH}}{\text{Volume of sample}} \times 100$$

Firmness

The firmness (hardness) of the control and DVSBM and HMBM treated green chilli pickles was determined using a texture analyzer (TA-HD Plus, Stable Microsystems, Goldalming, UK) with 30 kg load cell at 0.2 cm/s of pre and post-test speeds. The average value of the firmness results are expresses in terms of N (Force).

Colour determination

The scale values of colour of the samples were determined using a hand held chroma meter (Konica Minolta Chroma Meter CR400, Japan) in terms of LAB.

Sensory evaluation

The sensory evaluation of green chilli pickle was performed using ranking point descriptive scale (0-15) by 30 semi trained panelists (Food Technologist). The sensory score of pickles was recorded basis on the sensory attributes such as colour, flavor, sourness, pungency, firmness, saltiness and crunchiness.

Fourier-transform infrared spectroscopy (FTIR) analysis

The functional group of the control and buttermilk treated (DVSBM and HMBM) green chilli pickles were identified using FTIR (Opus, 7.2, Ettingen, Germany) at 400 cm^{-1} - 4000 cm^{-1} transmittance with 23 of concentive scans.

Statistical analysis

All the experiments were performed in triplicate and results are expressed in terms of Mean±SD. One way ANOVA with 0.05 of significant level (Duncan Tests) was appointed to significance difference between the data. Origin Pro (20.0) software was used for graphical representation of average data.

Table 1: The optimized spices composition per / kg of green chillies.

Ingredients/Spices	Quantity (Per kg)
Mustard seed	50 g
Mustard oil	50 ml
Turmeric powder	5 g
Fennel seed	15 g
Cumin seed	5 g
Fenugreek seed	15 g
Salt	80 g
Asafoetida	0.1 g

RESULTS AND DISCUSSION

pH and TA

The results of acidity and pH, in control and culture (DVSBM and HMBM) treated green chilli pickles in this study are shown in Fig 2(a) and Fig 2(b). The range of pH and acidity between the samples were recorded from 4.35 to 4.43 and 0.36 to 0.58%, respectively. The significantly ($P<0.05$) higher pH was recorded in control green chilli pickle (4.43) as compared to DVSBM treated (4.42) and HMBM treated sample of pickle (4.35) respectively. Furthermore, significantly ($P<0.05$) higher acidity was recorded in HMBM treated green chilli pickle sample (0.58%) as compared to DVSBM treated pickle (0.42%) and control sample of pickle (0.36%) respectively. Results of present study revealed that the addition of buttermilk could be potential to maintained higher acidity and lower pH in green chilli pickles as compared to the untreated green chilli pickle. Additionally, the variation in curd starter culture may influence the overall physico-chemical, sensory, textural and nutritional properties of pickles (Di Cagno *et al*., 2015). In present study, the higher acidity of pickles was maintained by HMBM treatment with lowest pH due to release of H+ and NH3+ from purification (Raja *et al*., 2020; Sharma *et al*., 2020). The higher acidity and salt concentration of the green chilli pickle can be potential to reducing the risk of microbial and food borne contamination (Aljahani 2020).

Firmness

The results of texture analysis in terms of firmness of the control, and buttermilk treated green chilli pickle are shown in the Table 2. The results showed that the firmness was

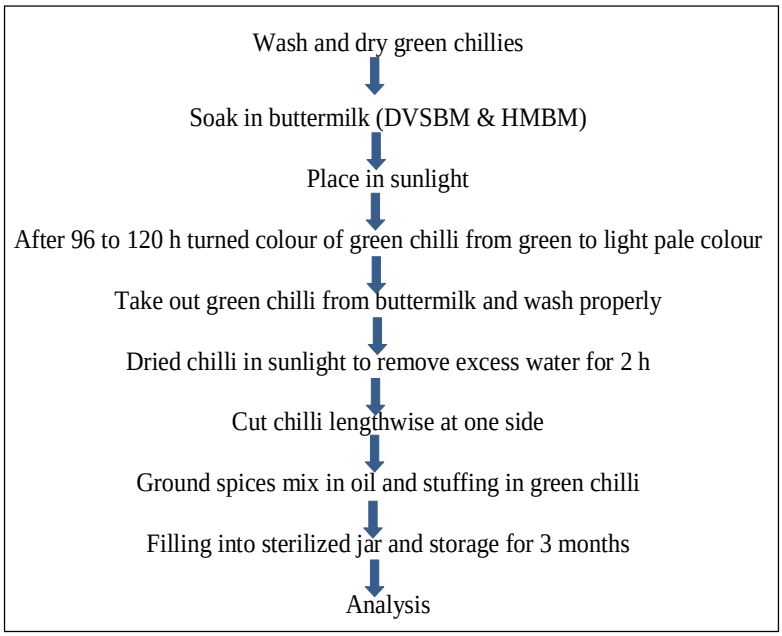
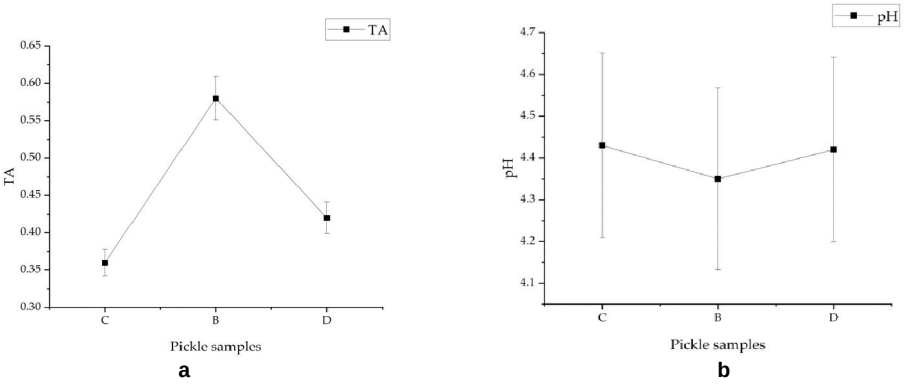


Fig 1: Manufacturing process of green chilli pickle.



Where; C= control (fresh green chilli pickle); B= Homemade buttermilk treated green chilli pickle; D= Commercial DVS buttermilk treated green chilli pickle

Fig 2: Titrable acidity and pH of different green-chillies pickle.

significantly (P<0.05) reduced in control pickle from 7442.75 N-3322.45 N. Whereas, the HMBM treated green chilli pickles was significantly maintained the firmness of green chilli pickles (Kumar *et al*., 2017). The results of the firmness were also supported by the overall acceptability of the consumers. The results are line with the previous findings of Johanningsmeier *et al*. (2005) and Ragul *et al*. (2017) in cabbage and brine pickle using LAB starter culture (Al-Shawi and Alneamah, 2021; Behera *et al*., 2020).

Colour properties

The results of the changes in the colour scales in the green chilli pickle after addition of DVSBM and HMBM culture are shown in Fig 3. The results revealed that the pre-treatment of DVSBM and HMBM were significantly reduced the lightness (L*) of green chilli pickle due to increased rate of fermentation with retention of higher green (a*) natural colour of green chilli. The control green chilli pickle prepared using oil was showed significantly (P<0.05) higher yellowness (28.84) and lightness (39.28) with lowest in green (1.18) colour followed by pickle treated with HMBM and DVSBM. The yellowness was higher in control sample due to addition of oil. The significantly highest yellow colour in control samples might be possible due to presence of mustard oil, which contains fatty acid compositions such as oleic acid, erucic acid, linolenic acid and linoleic acid (Lee *et al*., 2015; Sharif *et al*., 2017). The findings are supported with the previous researcher; those maintained the color properties of pickles using LAB (Raghul *et al*., 2017; Behera *et al*., 2020).

Sensory analysis

The results of the consumer acceptability of the different green chilli pickles are showed in Table 3. The results indicated non-significant effects on the overall acceptability of the green chilli pickles. Therefore, green chilli pickle prepared using HMBM had the highest overall acceptability (10.4) as compared to DVSBM treated green chilli pickle and control. The significantly lowest overall acceptability based on the consumer scores was recorded in green chilli pickle treated with DVSBM culture due to reducing the score of saltiness, bitterness, texture, flavor, colour etc. Therefore, several researchers were stated that the addition of buttermilk as starter culture (LAB) is desirable to improving sensory attributes of the vegetable pickles (Sultana *et al*., 2014; Behera *et al*., 2020). The results are in line with previous findings of Alan *et al*. (2019) in LAB rich gherkin pickle.

FTIR analysis

The control and HMBM treated pickle samples structured similar types of functional groups (Fig 4). The OH- bond was recorded between 3339.0.84 cm^{-1}- 3348.285 cm^{-1} in treated and untreated green chilli pickle samples, which corresponding to hydroxyl group presence in the sample. Therefore, the aldehyde group (C-H) stretching bend was shifted at lower in DVSBM treated green chilli pickle (2683.035 cm^{-1}) as compared to pickle (2721.936 cm^{-1}). The mono substitute of alkyne (Ca≡C) stretching was shifted at 2118.642 cm^{-1} in HMBM treated green chilli pickle, which showed more stronger bond as compared to DVSBM treated (2093.668 cm^{-1}) and control (2091.210 cm^{-1}) due to its higher acidic nature.The strong N-H and O=C=O stretching band peak at 2300.668 cm^{-1} was only appeared in DVSBM treated green chilli pickle, which denoted the stretching of the amine salt and trapped carbon dioxide in the sample (Jeong *et al*., 2018). Amide-I mode and C=C stretching of phenolic functional groups was observed at same spectra range between 1634.437 cm^{-1} to 1635.690 cm^{-1} in all the pickle samples may due to presence phenolic groups in chilli and spices mix (Troconis-Torres *et al*., 2012). A peak at 1158.763 cm^{-1} contributed by primary, secondary and tertiary alcohol, aliphatic ether groups due to C-O stretching (Hilares *et al*., 2018). In conclusion the results indicated that the treatment of DVSBM treated green chilli is more affected the functional groups as compared to HMBM treatment.

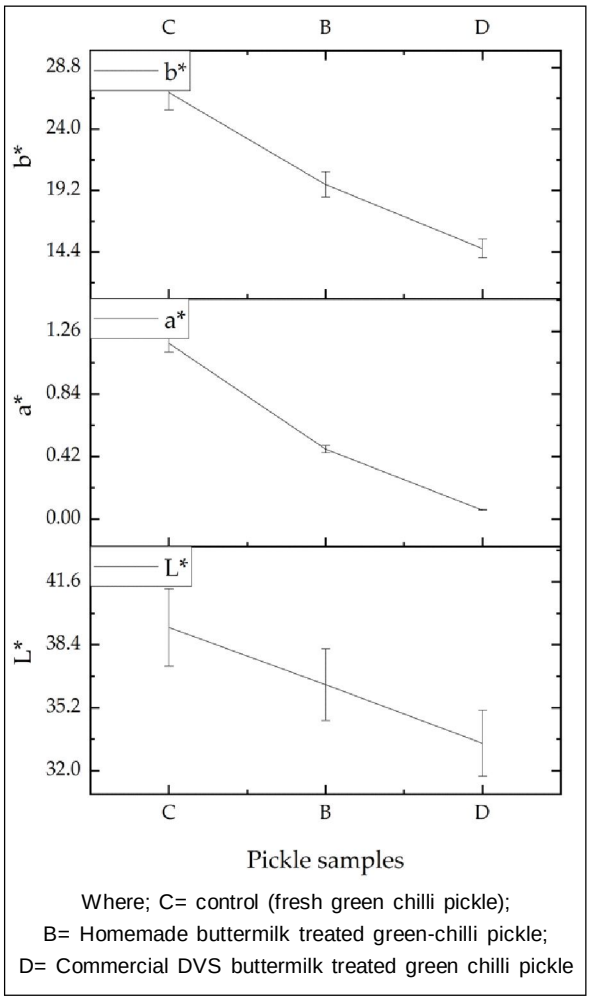
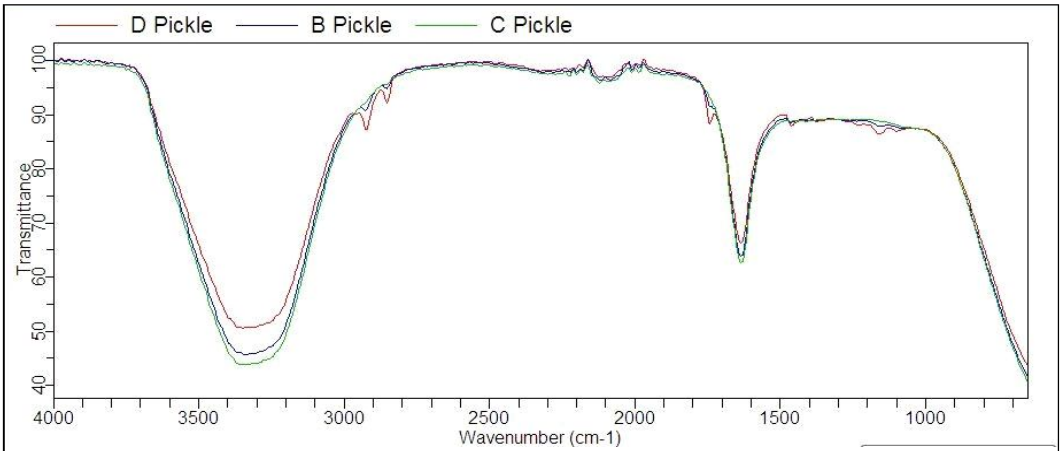


Fig 3: Colour measurement of different green-chilli pickle.



Where; C= control (fresh green chilli pickle); B=Homemade buttermilk treated green-chilli pickle; D= Commercial DVS buttermilk treated green chilli pickle.

Fig 4: FTIR spectra of green chillies pickles.

Table 2: Firmness of green chilli pickles.

Pickle samples	Firmness (N)
Fresh green chilli	7442.75±561.31
C	3322.45±452.33
B	11241.46±468.32
D	8212.24±452.33

Mean±SD, Where; C= control (fresh green chilli pickle); B= Homemade buttermilk treated green chilli pickle; D= Commercial DVS buttermilk treated green chilli pickle.

Table 3: Sensory analysis and consumer acceptability of green chilli pickle samples.

Sensory parameters	C	B	D
Sourness	5±2.7	4.9±2.7	4.9±2.3
Saltiness	8±1.8	8±1.97	5.8±1.5
Spiciness	6.5±1.8	7.1±2.4	6.3±2.1
Bitterness	0.5±1.05	1.1±3.8	1.3±2.6
Crunchiness	7±2.9	7.4±3.7	6.3±2,7
Firmness	6.4±3.2	7.2±3.5	6.6±2.7
Off flavor	0	0	2.4±3.2
Pungency	6.4±3.5	5.6±3.1	5.5±3.5
Overall acceptability	10.1±2.7	10.4±3.6	10.3±2.9

Mean±SD, Where; C= Control (fresh green chilli pickle); B= Homemade buttermilk treated green-chilli pickle; D= Commercial DVS buttermilk treated green chilli pickle

CONCLUSION

The findings of present study revealed that the textural, physicochemical and sensory properties of green chillies pickle was improved by the pre-treatment of buttermilk (DVSBM and HMBM) as compared to control chilli (without pre-treatment) after 3 months of storage. Moreover, the green chilli pickle treated with HMBM culture was significantly effective to improving the quality such as higher acidity, firmness, lower pH with retention of good colour and sensory characteristics (consumer overall acceptability) of the pickle followed by DVSBM, due to the presence of the probiotic bacteria. The results of FTIR spectra showed stronger functional groups in DVSBM treated pickle peak at 2300.66 cm^{-1} and HMBM (2118.642 cm^{-1}) as compared to control pickle of green chillies may indicated the presence of fermented (LAB) bacteria. The effects of buttermilk treatments on the microbial load of green chilli pickle should be also investigating in future.

Conflict of interest: None.

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