MATERIALS AND METHODS

The present investigation entitled "Technological studies and shelf life of Lal Pera prepared from different types of milk", were carried out in the Department of Animal Husbandry and Dairying, Chandra Shekhar University of Agriculture and Technology, Kanpur and B.N.V. College Rath, affiliated to B.U. Jhansi. Cow and buffalo milk were obtained from the dairy of B.N.V. College Rath and University Dairy at Kanpur and Toned milk was purchased from market, processed and sold by milk board Nirala nagar Kanpur in brand name of "Parag"

DETAILED TECHNICAL PROGRAMME OF THE PROJECT:

A. PARAMETERS OF THE STUDY

1. Types of milk -three

   (i) Cow milk (4% fat)

   (ii) Buffalo milk (6% fat)

   (iii) Toned milk (3% fat)

2. Levels of sugar -Three

   (i) 25% of khoa weight

   (ii) 30% of khoa weight

   (iii) 35% of khoa weight
3. **Packaging materials -Two**

   (i) polythene bags

   (ii) parchment paper

4. **Shelf Life of Lal Pera**

   (i) Temp. of storage - Room Temp.

   (ii) periods of storage

   (a) Zero days

   (b) one week

   (c) Two weeks

   (d) Three weeks

B. **FACTORS TO BE STUDIED**

   (i) Yield of Lal pera

   (ii) organoleptic quality

   (a) Flavour

   (b) Body & Texture

   (c) Colour & appearance

   (d) Sweetness

   (III) chemical Quality

   (a) Total solids

   (b) protein

   (c) Fat

   (d) sucrose

   (e) Ash

   (5) **Microbiological Quality**

   (a) Total plate count per gram
(b) Yeast and mould count/g.
(c) Californ count/g.
(xi) Assessment of cost and profit

c. Replication - Three

d. Total No. of samples - 3 x 3 x 2 x 4 x 3 = 216

e. Statistical Design - Factorial completely randomized design.

MATERIAL

MILK:

Buffalo milk having 6 percent fat, cow milk having 4 percent fat and Toned milk having 3 percent fat were standardized using cream and skim milk powder by Pearson square method.

SUGAR:

cane sugar purchased from the market was used as sweetening agent.

EQUIPMENT:

The equipments used for the preparation of lal para were as follows.

1. Gas stove
2. Milk heating vessel (stainless steel karahi)
3. Strainer
4. Muslin cloth
5. Stainless steel laddle
6. A centigrade thermometer.

MANUFACTURING PROCEDURE:
Manufacturing procedure of lal pera is composed of two major parts-

1. Khoa making
2. Lal Pera making

**METHOD OF KHOA PREPARATION:**

Normally 3kg of standradized milk (cow milk having 4% buffalo milk having 6% and Toned milk having 3% fat) was taken per batch and boiled in karahi over a brisk, non smoky fire. The milk was stirred vigorously and constantly with a circular motion by a khunti. During this operation all parts of the pan with which the milk came in contact were lightly scrapped to prevent the milk from scorching. Constant evaporation of moisture occurred and the milk thickened progressively, so for the process was similar to kheer making. However, no sugar was added and milk dehydration continued, Cow milk 2.8 times, buffalo milk 2.5 times and Toned milk 3.0 times), heat coagulation of milk proteins began and the concentrate became progressively “Insoluble”, in water. This stage was marked by an abrupt change in colour. The heating was continued with greater control thereafter and speed of stirring cum scraping was increased. Soon the viscous mass reached a semi solid pasty consistency and began to dry up. Very close attention was paid to the last stage. The final product was ready when it showed sign of leaving the bottom and sides of the karahi and sticking together. The khoa pat was invariably made after removing the pan from the fire and working the contents up and down in to a single compact mass.
METHOD OF LAL PERA PREPARATION:

Freshly made khoa broken into bits and cane sugar was mixed into it at the rate of 25%, 30% and 35% by weight of khoa. Contents were transferred to a karahi and cooked over a very slow non-smoky fire, stirring with a laddle, crushed cardamam was added and the mixture was ready to form balls when tested. Contents were then poured into a tray and left to cool and set. This Lal pera was cut into desired size and shape. This Lal pera was then packed into polythene and parchtment packs.
FLOW DIAGRAM OF LAL PERA PREPARATION

Freshly made khoa was broken into bits

cane sugar was mixed at rate of 25 % , 30 % and 35 % by weight of khoa.

Content were transferred into a karahi and cooked over a very slow non-smoky fire

stirred with a ladle

crushed cardamam was added

Contents were changed into reddish colour.

Contents where then poured into a tray

Cooked to set

cut into size of 50 gm per piece and round flated shape

Lal pera

Packed into Polythene and Parchment paper packs.

Analysed for Organoleptic, Chemical and Microbiological quality tests.
METHOD OF ANALYSIS

A. YIELD OF PERA:

The Yield of pera was calculated by following formula:

\[
\text{Yield (\%)} = \frac{\text{Weight of Lal Pera (gm)}}{\text{Weight of Milk (gm)}} \times 100
\]

B. ORGANOLEPTIC QUALITIES:

Organoleptic qualities of pera judged by a panel of five Judges selected from Department of Animal Husbandry and Dairying C.S. Azad university of agriculture and Technology, kanpur. The quality was judged by using the following 100 points scale as recommended by Sen and Rajorhia (1987) for sandesh as well as pera.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Perfect score</th>
</tr>
</thead>
<tbody>
<tr>
<td>flavour</td>
<td>45</td>
</tr>
<tr>
<td>Body &amp; Texture</td>
<td>30</td>
</tr>
<tr>
<td>colour &amp; Appearance</td>
<td>15</td>
</tr>
<tr>
<td>sweetness</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

The product was considered excellent if it scored 80 and above; very good if scored 70 and above but less than 80; good if scored 60 and above but less than 70; fair if scored 50 to 59 and poor if scored less than 50.

C. CHEMICAL CONSTITUENTS:

1. TOTAL SOLIDS CONTENT (%) OF PERA:

Total solids were determined as per method recommended by ISI (1981) for moisture determination in pera with slight modification.
A clean porcelain dish was heated to a constant weight \((w_1)\) 10gm of finally ground and homogenous sample of Lal pera was accurately weighed in porcelain dish \((w_2)\). Dish was transferred to an automatically controlled electric oven at a temperature of 100\(^0\)C for 3 to 4 hours. During this time the colour of pera changed from white to brown. The dish was then taken out from oven and cooled in a desiccator and weighed to a constant weight \((w_3)\). Total solids were determined by following-

**FORMULA**: \[
\text{Percent T.S.} = \frac{W_3 - W_1}{W_2 - W_1} \times 100
\]

2. **FAT CONTENT (%) OF PERA**:

Fat content of pera was determined by Gerber’s method as described in IS-1224 (part - II) - 1977 for analysis of cheese.

Five gm of pera sample was weighed in beaker and was concentrated into a paste by a glass rod with the help of small amount of hot distilled water. 1ml of ammonium hydroxide was added to it and again it was thoroughly mixed to bring the proteins in solution. The content was transferred into butter butyrometer. The beaker was washed by a little amount of hot distilled water. The washings were also transferred to the butyrometer. Ten ml sulphuric acid (Sp. gravity 1.82-1.825) and one ml of amyl alcohol (Sp. gravity 0.825) were added to it.

Hot distilled water was added, if necessary, to bring the level of limits of graduation on the stem of butyrometer. The butyrometer was stoppered and the contents mixed well by thoroughly shaking. The butyrometer was then transferred in a water bath for five minutes main-
tained at a temperature of $70^\circ$ F. Then the butyrometer was quickly transferred to Gerber's centrifuge and centrifuged for 5-6 minutes at a speed of 1200 r.p.m. The centrifuge was then stopped and the butyrometer taken out from the centrifuge. The butyrometer was again transferred to water bath at a temperature of $70^\circ$ F for 5 minutes. The reading was observed from the graduated stem of butyrometer by manipulating the stopper.

3. PROTEIN CONTENT (%) OF PERA:

Protein content of pera was determined by standard method as recommended by ISI (1961) for milk protein determination.

Five gm of pera sample was taken into a clean Kjeldahl flask. 20 ml of pure nitrogen free sulphuric acid, 10 gm pure potassium sulphate crystals and few crystals of (0.2 gm or 200 mg) pure copper sulphate were added. After this, the flask was kept in the digestion chamber for digestion.

The digested material was taken out from the chamber. In this condition there was no black particles of carbonaceous matter. It was then allowed to cool, diluted with 300 ml distilled water and transferred to distillation flask.

To this a small quantity of pumic powder was also added. Slowly 80 ml of 50 per cent Naoh solution was added to distillation flask so as to form a separate layer at the bottom of liquid separately. 50 ml of N/10 H$_2$SO$_4$ was taken in 500 ml beaker to be used to receive the condensate. Four drops of methyl red indicator was added into it. Distillation was done till the beaker condensate were about 300 ml. Using
usual precautions, the condensate was removed and the excess acid in the distillate was titrated with N/10 Naoh. Thus volume of N/10 H₂SO₄ used by ammonia for neutralization was determined. Percentage of protein in the sample was calculated with the help of following formula:

\[ 1 \text{ml of } N/10 \text{ H}_2\text{SO}_4 = 0.0014 \text{ gm } N_2 \]

I. Percentage of nitrogen in the sample:

Amount of \( N/10 \text{ H}_2\text{SO}_4 \) (ml) required to

\[ \frac{\text{neutralize} \times 0.0014}{\text{Weight of Lal Pera taken}} \times 100 \]

II. Percent of protein:

Percent of nitrogen in the sample \( \times 6.38 \)

4. SUCROSE CONTENT (%) LAL PERA:

Sucrose content of Lal pera was determined by volumetric method recommended by Lane-Eynon. The following method was followed:

A. PREPARATION OF SOLUTION:

Weighed 40 gms of well mixed sample of the product and transferred to a 100 ml beaker. Added in beaker about 50 ml of hot water (80-90°C). The content was mixed well and transferred to a 250 ml measuring flask, washed it with successive quantity of distilled water at 60°C until the volume was 120-150 ml. Mixed well and cooled down to room temperature, the re-after added 5 ml of dilute ammonium solution mixed well and allowed to stand for 15 minutes. Thereafter exactly equivalent volume of dilute acetic acid was added to neutralize the previously added ammonia. Mixed again and added 12.5 ml of zinc acetate solution followed by 12.5 ml of potassium ferrocyanide solution, mixed again
and made up the volume exactly 250 ml allowed to settle and filtered. this solution was marked B₁.

In a 100 ml volumetric flask took exactly 50 ml of solution B₁ and added 5 ml concentrated hydrochloric acid and heated at 68°C for 5 minutes, cooled down the solution and neutralized with sodium hydroxide solution and made up the volume to 100 ml. This solution was marked as A₁. Diluted the solution B₁ and A₁ so that the volume of 10 ml of Fehling's solution was 15 and 50 ml marked the solution as B₂ and A₂ respectively.

B. STANDARD METHOD OF TITRATION:

Pipetted out 10 ml of Fehling's solution into a 300 ml conical flask and allowed to run from the burette almost whole of the prepared solution B₂ required to effect reduction of all the copper. Gently boiled the content of the flask for 2 minutes, at the end of this period, added, without interrupting boiling, 1 ml of methylene blue indicator solution. When the content of the flask was boiling added, the prepared solution drop by drop from the burette till blue colour of the indicator just disappeared. Repeated the titration using the solution A₂.

C. Calculation:

\[
\text{sucrose (\%) by wt} = \frac{20 W₁}{W₂} \left( \frac{2 f₂}{V₂} - \frac{f₁}{V₁} \right)
\]

Where,

\[W₁ = \text{Wt in mg of sucrose corresponding to 10 ml of Fehlin's solution,}\]

\[W₂ = \text{Wt in gm of the material taken for the determination.}\]

\[f₂ = \text{Dilution factor for solution A₂ from A₁}\]
\( \frac{f_1}{V_1} = \) Dilution factor for solution \( B_2 \) from \( B_1 \)

\( V_1 = \) volume in ml of solution \( A_1 \) Corresponding to 10 ml of Fehling’s solution.

\( V_2 = \) volume of ml of solution \( B_2 \) corresponding to 10 ml of Fehling’s solution.

6. **ASH CONTENT (%) OF PERA**:

   Took 5 gm of sample in a weighed silica dish (w) and found its weight (\( w_1 \)). Added 6 ml of concentrated HNO₃, heated to dryness, charged over a burner, finally ignited at a temperature below dull redness (so that chloride may not be lost) to make the ash free from carbon, cooled in a desiccator and took its weight (\( w_2 \)).

**Formula**:

\[
\text{% Ash} = \frac{W_2 - W}{W_1 - W} \times 100
\]

Where:

\( W = \) weight of Lai Pera in gms.

\( W_1 = \) Weight of silica disc with samples in gms.

\( W_2 = \) Weight after ashing silica disc with ash in gms.

**BACTERIOLOGICAL QUALITIES**

**Preparation and dilution of the samples for microbiological analysis**:

Using all aseptic precautions 1 : 10 dilution of pera samples was made in standared saline solution in a presterilized pestle and mortar. From the initial dilution subsequent decimal dilutions were made for plating.
1. TOTAL PLATE COUNT/GM OF PERA:

Total bacterial count was done on plate count agar medium using 1:100 and 1:100 dilutions. Proper dilution was transferred induplicate sterilized petri plates. 10 ml of melted and cooked to $45^0\text{C}$ plate count agar medium was poured in each petri plate. After thoroughly mixing. The plates were left for sometimes on the bench for solidification of the medium. All essential precautions were taken to avoid external contamination during plating. The inverted plates were placed in incubator maintained at $37\pm1^0\text{C}$ for 48 hours. After the incubation period. The colonies made by bacteria on the plates were counted with the help of colony counter. The number of bacteria was calculated by multiplying the number of dilution with number of colonies counted (Standard methods for the Examination of Dairy products, 1978).

2. YEASTS AND MOULDS COUNT/GM OF PERA:

For yeasts and moulds count 1:10 dilution of pera suspension was transferred in duplicate sterilized petriplates. 10 ml. melted potato dextrose agar medium was poured in each petri plate after thoroughly mixing. The plates were left for some time on the bench for solidification of the medium. All essential precautions were taken to avoid external contamination during plating. The inverted plates were placed in incubator maintained at $22\pm1^0\text{C}$ for 3 to 5 days. After incubation period the colonies made by yeasts and moulds on the plates were counted with the help of colony counter (standard Methods for the examination of Dairy products, 1978)
3. **COLIFORM COUNT/GM OF PERA** :

For coliform count 1:10 dilution was transferred in duplicate sterilized petri-plates. Ten ml melted and cooled to $45^\circ C$ violet red bile salt agar medium was poured in each petri-plate. After thoroughly mixing the plates were left for some time on the bench for solidification of medium. All essential precautions were taken during plating. The inverted plates were placed in incubator maintained at $37 \pm 1^\circ C$ for 24 hours. After incubation period the colonies made by bacteria on the plates were counted with the help of colony counter (Standared Methods for the Examination of Dairy products, 1978).

4. **Cost and profit per kg of lal pera** :

For calculating the cost and profit per kg of Lal Pera the rates of cow, buffalo and Toned milk, cane sugar, heating medium, labour charge, packaging material and miscellaneous charges etc. were taken into account. The rate of milk was considered on prevailing rates of milk in the market (Buffalo milk Rs. 14 00 Kg. per Kg, Cow milk Rs. 11.50 per kg. and Toned milk Rs. 9.50 per kg. The rate of cane sugar (Rs. 16.00 per kg.), heating medium charges (Rs. 2.50 per hour) labour charges (Rs. 50/8 hours per day), packaging material Rs. 0.40 and 1.06 per bag and miscellaneous charges Rs. 2.00 were taken as prevailing in the market.

**Note:** Rates of fat and S.N.F. per kg during study period were Rs. 48.00 and Rs. 32.00 respectively. These rates were of milk Board of Kanpur. The rates of milk were fixed as per rate of fat and S.N.F.
Rates of heating medium per burner was Rs. 2.50 / hours as per the rates of NOIDA, Kanpur.

F. STATISTICAL ANALYSIS:

1- INTRODUCTION:

In order to study the effects of three types of milk (M) three sugar levels (S) two packaging material (P) four storage period (D) and their interaction effects on the different characteristics of pera, an experiment was conducted and the data were collected. The analysis of variance of these datre were worked out on the basis of factorial completely randomized design.

2. ANALYSIS OF VARIANCE :

In few cases, the factor of storage period was not there. The structure of analysis of variance (in such cases) was as given below.

<table>
<thead>
<tr>
<th>Sources</th>
<th>D.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
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<tr>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>S</td>
<td>2</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
</tr>
<tr>
<td>M X S</td>
<td>4</td>
</tr>
<tr>
<td>M X P</td>
<td>2</td>
</tr>
<tr>
<td>S X P</td>
<td>2</td>
</tr>
<tr>
<td>M X S X P</td>
<td>4</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>53</strong></td>
</tr>
</tbody>
</table>
In the remaining cases the date of the treatment combinations of the levels of all the four factors were available and the structure of analysis of variance for such cases was as under:

**ANALYSIS OF VARIANCE**

<table>
<thead>
<tr>
<th>Sources</th>
<th>Df.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
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</tr>
<tr>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td>S</td>
<td>2</td>
</tr>
<tr>
<td>P</td>
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<tr>
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<td>MXSXPXD</td>
<td>8</td>
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<tr>
<td>Error</td>
<td>108</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>161</strong></td>
</tr>
</tbody>
</table>

3. CRITICAL DIFFERENCES:

In order to compare different treatment combinations, the relative critical difference was used. Any differences of the two means
equal to or greater than the critical difference was declared as significant. This critical difference was calculated with the help of the following expression:

\[
\text{C.D. at 5\% level} = \sqrt{\frac{2 \text{V}_E}{n}} \times t_{5\%} \text{ for Error D.F.}
\]

Where,

\[
\text{V}_E = \text{Error mean square}
\]

\[
n = \text{Number of observations to which the means were based.}
\]

4. TRANSFORMATIONS:

In order to fulfill the condition for the analysis of variance, the following transformations of the data were used, wherever they were suitable.

\[
\begin{align*}
\text{if } x &= \text{Original value} \\
y &= \text{Transformed value} \\
(a) & \quad Y = \log_{10} x \\
& \text{for back transformation} \\
x &= 10^Y \\
(b) & \quad Y = 10 + \log_{10} \\
& \text{for back transformation} \\
x &= 100 \times \left( \frac{X}{100 - X} \right)
\end{align*}
\]

Where,

\[
K = (10)^{Y-10} \left( \frac{K}{K + 1} \right)
\]