APPENDIX

APPENDIX-I

Standardization of milk for the preparation of dietetic fermented dairy drink.
**Procedure:**

Standardization is a process of adjusting the composition of milk to the desired level of fat, SNF (Solid Not Fat) or both. If the given sample of milk contains more fat than as required then the excess fat is either removed in the form of cream by separating milk or added to the skimmed milk. In case the fat content is less then the desired output, the calculated quantity of cream is added to have the desired fat level in the milk.

For increasing the SNF calculated quantity of skimmed milk powder is to be added to have the desired level of SNF in milk.

In this experiment, since milk was used and the sample obtained from Student Training Dairy had more than the desired fat and less than the desired SNF, therefore, the calculated quantity of skimmed milk for fat and skimmed milk powder for SNF was added into the milk to have the desired fat and SNF percentage.

**APPENDIX – II**
**Determination of moisture.**

**Principle:**
The sample was heated at specified temperature for specified period of time and the loss in weight was recorded as moisture content of the sample.

**Procedure:**
- 5 g of the sample was weighted and taken in a tared porcelain dish (W₁ g)
- Dish was shaken till the contents were evenly distributed.
- Then dish was placed in hot air oven, maintained at 105° C ± 2° C and dried for at least 2 hours.
- Dish was then cooled in desiccators and weighed.
- The lowest weight was noted (W₂ g).

**Observation:**
- Tared weight of the dish (W g)
- Weight of dish with samples (W₁ g)
- Weight of the dish ± sample after drying in oven (W₂ g)

**Calculation:**

Percent moisture content = \( \frac{\text{Loss in weight}}{\text{Initial weight of the sample}} \times 100 \)

\[ = \frac{W₁ - W₂}{W₁ - W} \times 100 \]

**APPENDIX - III**
Determination of cruds fat.

Principle:
The sample of dietetic fermented dairy drink was placed in a continuous extractor (Soxhlet) and subjected to extraction with ether. The ether soluble substances thus removed are collected in a flask, dried and weighed.
The material extracted include beside the triglycerides, material such as phospholipids, sterols, essential oils, pigments, waxes etc., hence term ‘crude fat’ if the sample contains water-soluble sugars as in molasses, the weighed sample should be washed with water and dried before extraction.

Apparatus:
Soxhlet extractor: the extractor consists of:
   a. The extraction flask.
   b. The extraction carrying the thimble and
   c. The condenser.

Procedure:
   • Extraction flask was weighed accurately ($W_1$ g).
   • Accurately weighed 2 g of the sample transferred into extraction thimble and the thimble was carefully placed in the extractor so that it was within its siphon height.
   • Weighed extraction flask was connected to the extractor carrying the thimble.
   • Sufficient petroleum ether was poured into the extractor to start the siphon and then filled about half the extraction flask.
   • Extraction connected to the condenser and flask was heated on a water bath or electric mental for 3 hours. The heat vaporizes the solvent and condensed in the condenser.
• The condensed solvent falls drop-wise into thimble.
• The solvent extracts the fat present in the food. When the level of the solvent reached the siphon height, the whole of the ether flowed down into the flask below taking along with the extracted fat behind and the process was repeated.
• At the end of 3 hours by which time, at least 10-15 siphoning were taken, flask was removed carefully.
• The extract was transferred in a tarred conical flask and solvent was evaporated.
• Cooled in a dessicator and weighed (W$_1$ g).
• Drying, cooling and weighing or successive weighing not differing by more than 0.0002g were obtained, last weight recorded (W$_2$ g).

**Observation:**

- Weight of sample (W)
- weight of flask (W$_1$)
- weight of flask + fat (W$_2$
- (weight of flask + fat) – (weight of flask) = (W$_2$-W$_1$)

**Calculation:**

Percentage of ether extract was calculated by multiplying the increase in weight of the extraction flask by 100.

$$\text{Percentage fat sample} = \frac{W}{W_2-W_1} \times 100$$

**APPENDIX - IV**

**Determination of protein:**
**Principle:**

Sample was digested with boiling sulphuric acid. The nitrogen of sample was transformed to ammonium sulphate. The acid digest was made strongly alkaline using sodium hydroxide and ammonia released was distilled into boric acid solution. It was then titrated with standard sulphuric acid. The results are transformed by calculation into present of protein in original sample.

**Requirement:**

- Micro kjeldahl digestion flask.
- Micro kjeldahl distillation apparatus
- Erlenmeyer flasks 250 ml/conical flask
- Volumetric flasks (100 ml)
- Pipettes
- Burette – 50 ml
- Potassium sulphate, copper sulphate, 40% NaOH solution, standard sulphuric acid solution 0.1N, standard NaOH solution 0.1 N and mixed indicator.

**Procedure:**

- Weighed accurately 5.0 g of sample in a cupped filter paper.
- Transferred the filter paper and contents to the kjeldahl flask.
- Added 10g digestion mixture (CuSO₄ + K₂SO₄ + selenium oxide) and 25 ml of concentrated H₂SO₄ to it.
- Warmed slowly and carefully to minimize frothing then increased hear and boiled until the solution was clean, continued boiling for further hours.
• Allowed to cool, transferred to a 100 ml volumetric flask and make up the volume using distilled water.
• Added 10 ml of aliquot in the receiver of distillation apparatus and 10 ml of 40% sodium hydroxide.
• Collected ammonia released in 25 ml of 4%. Boric acid, solution containing few drops of mixed indicator. (Mixed indicator gives pink on distillation).
• Distilled for half an hour. Disconnected condenser outlet.
• Titrated the boric acid solution with 0.01N sulphuric acid or 0.01 N Hydrochloric acid.
• Carried out a blank using water or sucrose in place of sample.

Observation:

Volume of 0.1 N H₂SO₄ for sample = A
Volume of 0.1 N H₂SO₄ for blank = B
Weight of sample = W = 2 gm
Volume made (V) = 100 ml
Aliquot distilled (Vₒ) = 10 ml

Calculation:

1ml of 0.01 N H₂SO₄ for = 0.0014 g

Titre volume = A-B ml

Percentage nitrogen = \[ \frac{A-B \times 0.001}{W} \times \frac{V}{Vₒ} \times 100 \]

And percent crude protein = N \times conversion factor (6.25).

APPENDIX - V
Determination of carbohydrate by difference:

Total carbohydrate by difference was calculated by subtracting the sum of percentage of protein, fat, ash and moisture from hundred.

**Calculation:**

Carbohydrate by difference (Nitrogen free extract):

\[
(\text{Percent}) = 100 - [(\text{Moisture (%) + ash (%) + fat (%) + protein (%)})]
\]
Determination of total ash:

Principle:
Ash comprises of the mineral content which was present in the foodstuff which can be determined by igniting unknown amount of dried material in a muffle furnace.

Procedure:
- Took the dried material obtained from determination of moisture in a crucible dish.
- This dried material was ignited on a blue flame of a burner till the smoke was given off.
- The porcelain/crucible dish was then heated in a muffle furnace maintained at 500 ± 5° C for 1-2 hours.
- It was cooled in a dessicator and weight was taken.

Observation:
- Weight of the dish + weight of dried sample = (W₂ g)
- Weight of dish + with of ash = (W₃ g)

Calculation:
Percentage of ash = \[
\frac{\text{Weight of dish after ignition}}{\text{Weight of sample}} \times 100
\]
\[
= \frac{W₃ - W}{W₁ - W} \times 100
\]
**Determination of total solids percentage in product**

**Procedure:**
1. Weighed accurately the clean, dry empty dish with lid.
2. Took 5 ml of the sample and transfer it to weigh quickly with the lid on.
3. Placed the dish uncovered on a boiled water bath at least for 30 minutes.
4. Removed the dish from water bath, wiped the bottom and kept the dish in a hot air oven a silica triangle and heated at 98°-100°C for about 3 hours, placing the lid nearby.
5. After three hours, covered the dish immediately, transfer it to a desiccator, allow it to cool for about 30 minutes.
6. Weighed the dish with the lid on.
7. Returned the dish, uncovered and the lid to the oven and heat for 1 hour.
8. Removed it to the desiccator, cooled and weighed as before, repeat if necessary until the loss of weight between successive weights does not exceeded 0.5 mg.

**Calculation:**
Weight of sample = \((W_1 - W)\) g.
Weight of total solids in sample = \((W_2 - W)\) g.

\[
\text{Percentage of total solid} = \left(\frac{W_2 - W}{W_1 - W}\right) \times 100
\]

Where,
Weight of the dish + lid = \(W\) g.
Weight of the dish + lid + sample = \(W_1\) g.
Weight of the dish + lid + residue after drying = \(W_2\) g.

**APPENDIX - VIII**

**Determination of titrable acidity.**
**Principle:**

Natural acidity in dairy drink is due to its constituents such as casein, albumin, citrates, phosphates and CO$_2$. This acidity can be measured by titrating dairy drink against a standard alkali using an indicator like phenolphthalein and is expressed in term of lactic acid.

**Procedure:**

1. Thoroughly mix the sample.
2. Pipette out 20 ml of the sample into a beaker.
3. Add few drops of phenolphthalein indicator and titrate against the standard alkali.
4. Repeat the experiment to get concordant values.

**Calculation**

The reactions between lactic acid and sodium hydroxide are simple acid base neutralization reaction.

\[ \text{CH}_3\text{CH} (\text{OH}) \text{ COOH } + \text{NaOH} \rightarrow \text{CH}_3\text{CH} (\text{OH}) \text{ COONa } + \text{H}_2\text{O} \]

Lactic acid \hspace{1cm} sodium lactate

From this it is evident that 1 gm equipment of sodium hydroxide reacts with 1 gm equivalent of lactic acid (90 g)

\[ 1000 \text{ ml of N NaOH} = 90 \text{ g lactic Acid} \]
\[ 1 \text{ ml of N NaOH} = 0.09 \text{ g Lactic Acid} \]
Thus 0.09 is the factor for lactic acid corresponding to 1 ml of N NaOH percent lactic acid in the sample can be calculated as

\[
\text{Percent lactic acid} = \frac{0.09 \times V \times N \times 100}{W}
\]

Where,
0.09 = factor for lactic acid corresponding to 1 ml of normal alkali.
V = Volume of standard alkali used in titration.
N = Normality of standard alkali used in titration.
W = Weight or volume of the milk sample taken for titration.
Dietetic Fermented Dairy Drink Score Card

Name of the judge:

Replication No:

You are requested to please award your score rating as observed by you in the product on its critical examination by you, against the following parameters.

Guidelines:

- Examine the flavour and taste of the given samples.
- Examine the consistency of samples.
- Examine the colour and appearance of the samples.
- Examine the overall acceptability of the samples.

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Average................

Remarks............

Date................... Signature.............
APPENDIX - X

MICROBIAL ANALYSIS OF DAIRY DRINK SAMPLES

The microbial analysis is yeast and count, and presumptive coliform test of prepared dairy drink sample were determined by “I.S. method (9001 / 2007)”. Microbial analysis was one before making the products just to check that strict hygienic conditions were free from microbial contamination.

Cleaning and sterilization of glassware’s:

Washing of glass wares thoroughly with detergent or sulphuric acid, glassware after drying was wrapped with brown paper or in newspaper and kept an electric hot air oven for sterilization at 160° C-180° for 3-4 hours and medias sterilized in autoclave at 15lb pound pressure at 121°C and open only in laminar flow. Laminar flow was precaution was taken to avoid contamination from outside.

Yeast and mould count

Potato Dextrose Agar

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<tr>
<td>Dextrose</td>
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<td>Agar</td>
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</tr>
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<td>Distilled water</td>
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<td>pH</td>
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**Procedure:**

Prepare the samples and dilutions accordingly. Transfer 1 ml portion into duplicate petridishes. Adjust pH to 3.5 during plating by adding sterile Tartaric acid 10% (few drops) to each plate. Mix the contents by rotation. After the plates cool down and the medium is set, invert them and incubate at 25°C for 5 days. Select suitable plates and count the number of yeast and mould colonies. Using the dilution factor compute the yeast and mould count of the sample. Express the results as yeast and mould count per gram.
Coliform count

Violet red bile agar

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<td>Peptone</td>
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<tr>
<td>Lactose</td>
<td>9.0g</td>
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<tr>
<td>Bile saltsNo.3</td>
<td>1.5g</td>
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<td>Neutral red</td>
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<td>Crystal violet</td>
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<td>Sodium chloride</td>
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<td>Disodium phosphate</td>
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<tr>
<td>Agar</td>
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<td>Distilled water</td>
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</tr>
<tr>
<td>pH</td>
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Procedure:

Thoroughly mix the product and place 11g of the product in a sterile wide – mouthed container. Then add 99 ml of heated (40°C) sterile 2% (m/v) sodium citrate solution and shake the mixture until homogeneous dispersion has been obtained. This constitutes the 1:10 dilution of the product. Prepare further tenfold dilution in the sterile dilution. Use a pipette to transfer 1 ml of the liquid product or the appropriate dilutions to the center of the two petridishes. Use a fresh pipette to inoculate each dilution.

Pour about 15 ml of the VRB agar at the 45±1°C into each petridish. Mix immediately after pouring by rotating the petridish sufficiently to obtain evenly dispersed colonies after incubation. Allow to solidify on a cool horizontal surface.
After complete solidification, pour about 4 ml of the VRB agar at 45°C ± 1°C on to the surface of the inoculated medium and allow to solidifying. Prepare a control dish with 15 ml of the medium to check its sterility. Incubate the plates in an inverted position for 24 hrs at 37°C.

Select the plates with more than 10 and fewer than 150 colonies. Count the dark red colored colonies with a diameter of at least 0.5 mm. Calculate the number of coliform organisms per gram or per ml.
APPENDIX - XII

Energy Value

Energy value of the food sample can be calculated by multiplying the figure for percentage of protein, fat and carbohydrate by 4, 9 and 4 respectively and adding the figures obtained.

Calculation:

Calorific value (Kcal/g) = (% protein × 4) + (% fat × 9) + (% carbohydrate × 4)
APPENDIX - XIII

FORMULA USED FOR STATISTICAL ANALYSIS

Analysis of Variance:

\[ G = T_1 + T_2 + T_3 + \ldots + T_n \]

\[ = R_1 + R_2 + R_3 + \ldots + R_n \]

1. Correction factor (C.F.) = \( G^2/rt \)
2. Treatment S.S = \( T_1^2 + T_2^2 + T_3^2 + \ldots + T_n^2 - C.F \frac{r}{t} \)
3. Replication S.S = \( R_1^2 + R_2^2 + R_3^2 + \ldots + R_n^2 - C.F \frac{t}{r} \)
4. Total S.S = Sum of square of each observation – C.F
5. Error S.S = Total S.S – S.S. due to treatments– S.S. due to replications

Where,

\[ G = \text{Grand total} \]
\[ T = \text{Treatment} = 12 \]
\[ R = \text{Replication} = 5 \]
\[ S.S = \text{Sum of Squares} \]

3 x 4 Factorial Design:

Factors: Two (F and C)

F- 3 levels i.e. \( F_1, F_2 \), and \( F_3 \).
C- 4 levels i.e. \( C_1, C_2, C_3 \), and \( C_4 \)

Treatment combinations - \( F_1C_1, F_1C_2, F_1C_3, F_1C_4, F_2C_1, F_2C_2, F_2C_3, F_2C_4, F_3C_1, F_3C_2, F_3C_3, \) and \( F_3C_4 \).

Interaction table (FxC):
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<th>$C_2$</th>
<th>$C_3$</th>
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<th>Mean</th>
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<td>Sum of $F_2C_2$&lt;br&gt; $m_{22}$</td>
<td>Sum of $F_2C_3$&lt;br&gt; $m_{23}$</td>
<td>Sum of $F_2C_4$&lt;br&gt; $m_{24}$</td>
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<tr>
<td>$F_3$</td>
<td>Sum of $F_3C_1$&lt;br&gt; $m_{31}$</td>
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</table>

### Formulas

**S.S. due to (fat) =** \( F_1^2 + F_2^2 + F_3^2 - CF \)

\[ \frac{C \times r}{F \times r} \]

**S.S. due to (ratio Corn flakes) =** \( C_1^2 + C_2^2 + C_3^2 + C_4^2 \)

\[ \frac{F \times r}{F \times r} \]

**Interaction S.S. (F×C) =** \( m_{11}^2 + m_{12}^2 + \ldots + m_{34}^2 - CF - SS(F) - SS(C) \)

\[ \frac{r}{r} \]

**Error S.S. = Total S.S. - S.S. due to replications - S.S. (F) - S.S. (C) - Int. S.S. (F×C)**

Where,

- $F =$ Types of fats = 3
- $C =$ Ratio of Cornflakes = 4
## Skeleton of ANOVA Table

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<th>Source of variation</th>
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<th>S.S.</th>
<th>M.S.S.</th>
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<th>F“tab” 5%</th>
<th>F“tab” 1%</th>
<th>Result</th>
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Critical difference (C.D) for treatment

\[
S.E.(T) = \sqrt{2 \times E.M.S.S./r}
\]

C.D. = S.E. (T.) \times t (5%) on error degrees of freedom

Where,

- \( S.E. \) = Standard Error
- \( E.M.S.S. \) = Error Mean Sum of Square

Critical difference (C.D) for Fat (F)

\[
S.E.(F) = \sqrt{2 \times E.M.S.S./rxc}
\]

C.D. = S.E. (f.) \times t (5%) on error degrees of freedom
Where,
S.E. = Standard Error
E.M.S.S. = Error Mean Sum of Square

Critical difference (C.D) for cornflakes (C)
\[ S.E.(T) = \sqrt{2 \times E.M.S.S. / r \times f} \]
C.D. = S.E. (c.) \times t (5\%) on error degrees of freedom

Where,
S.E. = Standard Error
E.M.S.S. = Error Mean Sum of Square

Critical difference (C.D) for Interaction (F\times C)
\[ S.E.(T) = \sqrt{2 \times E.M.S.S. / r} \]
C.D. = S.E. (m.) \times t (5\%) on error degrees of freedom

Where,
S.E. = Standard Error
E.M.S.S. = Error Mean Sum of Square
**INPUT SHEET FOR THESIS**
**M-LEVEL DATA**

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<th><strong>Author’s Name</strong></th>
<th>Kirti Srivastava</th>
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<td><strong>Academic Degree</strong></td>
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**Summary Statement**
The study revealed that the dairy drink added with corn flakes powder were found to have a high feasibility and a pleasant taste with increase in fibre and energy content.

**Note**

**Availability**
Central library, Sam Higginbottom Institute of Agriculture Technology & Sciences, Allahabad.

**Language Code of Descriptors**
English

**Keywords**
Milk fat, cornflakes powder, spices, dairy drink, skimmed milk and organoleptic quality.

**AGROVAC Descriptors**
(To be filled by DIPA)

**Language Code of Abstract**
English

**Abstract**
A recent trend established in the dairy industry involves combining dairy ingredients with other food components, which has provided an excellent way to utilize the nutritionally valuable components and to exploit from the synergy between the characteristics of the cereal and dairy sources. The nutritional and therapeutic attributes of fermented milk have the potential to reduce the role of medicine for maintaining normalcy in human health, thus leading to a tremendous growth of global market to yoghurt and similar fermented milk products. The present study was undertaken with the objectives to develop suitable technology for preparation of low fat dietetic fermented dairy drink, to ascertain the level of fat in milk suitable for the production of low fat dietetic fermented dairy drink. To study the effect of different levels of cereals on the quality of dietetic fermented dairy drink, to evaluate the organoleptic quality, chemical quality, microbiological quality, energy value of dietetic fermented dairy drink and cost of the product. Three different percentage of milk fat 0.5%, 1.5%, and 3% indicated as F₁, F₂, and F₃ respectively and four different level of cornflakes powder 4%, 6%, 8% and 10% indicated as C₁, C₂, C₃, and C₄ respectively were compared to each other. Sensory evaluation of the prepared dairy
drink was carried out by using the nine point hedonic scale. Product was tested for moisture, fat, protein, carbohydrate, ash, total solids and acidity, energy value and cost of the product was also worked out for different treatment combinations. The data obtained during investigation were statistically analyzed by using factorial design and critical difference between combinations. Amongst the different treatment combinations of dietetic fermented dairy drink, F3C3 having 3 percent milk fat and using 8 percent level of cornflakes powder was found to be superior in term of flavour and taste, as well as overall acceptability over the other treatment combinations. Among the different treatment F1C1 having 0.5 percent milk fat and 4 percent level of cornflakes dairy drink contained the highest percentage of moisture (91.80). The treatment combination F3C4 having 3 percent milk fat and 10 percent level of cornflakes powder contained the highest percentage of fat (15.1). The treatment combination F2C4 using 1.5 percent milk fat and 10 percent level of cornflakes powder contained the highest percentage of protein (25.1) and carbohydrate (10). Amongst the treatment combinations F3C4 having 3 percent milk fat and 10 percent level of cornflakes contained the highest percentage of total solids (14.29). The cost wise, dietetic fermented dairy drink prepared was also more economical as compared to the dairy drink available in present day market. The microbial quality for different treatment combinations was found to be satisfactory.