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COMPARISON AND ESTIMATION OF ADULTERANTS IN VARIOUS PROTEIN FOODS USING ANALYTICAL TECHNIQUES

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ABSTRACT

Aim: This study aims to compare and estimate the presence of adulterants in various protein foods—specifically milk, paneer, and eggs—using a range of analytical techniques to assess food quality, safety, and nutritional value. **Materials and Methods**: Qualitative tests were performed to detect common adulterants such as starch, detergent, synthetic milk, formalin, urea (in milk and paneer), and heavy metals, calcium carbonate, artificial colours (in eggs). Chemicals and reagents used included iodine, sulphuric acid, bromothymol blue, phenolphthalein, sodium hydroxide, ethanol, and more. Analytical instrumentation comprised a double-beam UV-Visible spectrophotometer and precision balances. Quantitative estimation of protein and fat content was achieved via Biuret reagent-based UV spectrophotometry and the Rose-Gottlieb fat extraction method. Method validation adhered to ICH Q2R1 guidelines, including tests for linearity, accuracy, precision, limit of detection, and limit of quantification. **Results**: Milk samples showed no evidence of added starch, detergent, synthetic milk, formalin, or urea. Paneer samples revealed detergent and urea adulteration, while starch and formalin were absent. Egg samples contained calcium carbonate but not heavy metals, artificial dyes, or detergent. Quantitative assays revealed the following protein concentrations: milk (3.30 mg/mL), paneer (3.40 mg/mL), and egg (2.50 mg/mL). Fat content was also measured across samples. Validation parameters confirmed the reliability and precision of the analytical methods employed, with recovery rates generally between 80% and 110% across spiked samples.

KEYWORDS: adulterants, milk, paneer, eggs, protein estimation, fat estimation, UV spectrophotometry, Rose-Gottlieb method, method validation, food safety.

1. INTRODUCTION

Adulterants are substances added to food items to increase quantity and make more profit. These are either intentional or incidental, and they lower the quality, safety, and nutritional value of the food.

Animal protein is the dietary protein obtained from animal sources such as meat, fish, eggs, milk, and dairy products. It is considered high-quality protein because it contains all the essential amino acids in the right proportions needed for human growth and maintenance. It is highly nutritious but should be consumed in balanced amounts to avoid health risks.

Milk is a highly nutritious liquid that contains water, fat, proteins, carbohydrates, vitamins, and minerals, making it an essential part of the human diet. The fat content in milk, usually between 3–6%, provides energy and contributes to its taste and texture. Proteins, mainly casein and whey (3–4%), help in the growth and repair of body tissues. Unfortunately, milk is often adulterated with substances like water, starch, urea, synthetic milk, or detergents to increase volume or thickness. These adulterants reduce nutritional value and can be harmful to health, so chemical tests are needed to ensure milk quality and safety.

Paneer is a popular dairy product made by adding food acids like lemon juice or citric acid to hot milk, which causes it to curdle. The curds are then strained and

pressed to form paneer. It is rich in nutrients, especially protein (about 20–25%) and fat (20–30%), depending on the type of milk used. Casein is the main protein in paneer and is easy to digest. Commercially sold paneer is sometimes adulterated with substances like starch, synthetic milk, dyes, detergent, or non-milk fats to increase weight or improve appearance. These practices affect both the safety and nutrition of paneer, so regular testing is necessary.

Eggs are highly nutritious and consist of two parts: the egg white and the yolk. The egg white is mostly water (about 88%) and contains proteins like ovalbumin, which help with body- building and immune functions. The yolk contains around 32–35% fat, 16% protein, along with vitamins A, D, E, K, and minerals such as iron and phosphorus. Adulteration in eggs includes artificially coloring the yolk, injecting preservatives like formalin, or making fake eggs using chemicals such as gelatin and sodium alginate. These practices reduce the egg's nutritional quality and pose health risks. Therefore, tests for acidity, fat, and protein are important to ensure the freshness and purity of eggs.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents: Iodine, sulphuric acid, bromothymol blue solution, sodium sulphide solution, dilute hydrochloric acid, petroleum ether, sodium hydroxide solution, phenolphthalein indicator, ammonia solution, ethanol, diethyl ether, light petroleum, cresol red solution.

2.2 Instrumentation: Double-beam UV-Visible spectrophotometer of Lab India with quartz cuvettes was used for absorbance measurements in UV spectroscopic analysis. UV WIN software was used to analyse the samples.

Analytical balance with 0.1 mg sensitivity was used for accurately weighing standards and chemicals for spectroscopic procedures.

Standard volumetric flasks, pipettes, funnels, and burettes were utilized for all solution preparations and titrations.

2.3 Qualitative estimation of adulterants present in Milk

Milk is often adulterated with substances like starch, detergent, synthetic milk, formalin, and urea.

Starch

Starch is added to increase thickness but causes indigestion and is detected by a blue color with iodine.

Detergent

Detergent is mixed for frothiness and whiteness, leading to stomach irritation, and can be identified by persistent foam on shaking with water.

Synthetic milk

Synthetic milk, prepared using water, detergent, urea, and oil, is highly toxic and shows a soapy smell and lather on heating or shaking.

Formalin

Formalin is added as a preservative but is carcinogenic and detected by a violet-blue ring when concentrated sulfuric acid is added.

Urea

Urea is used to enhance protein content, harms the kidneys, and gives a deep blue color with bromothymol blue.

2.4 Qualitative estimation of adulterants present in Paneer

Paneer is often adulterated with starch, detergent, synthetic milk, and formalin to increase bulk, whiteness, or shelf life.

Starch

Starch is added to increase solid content, which causes indigestion and is detected by blue color with iodine.

Detergent

Detergent may be used to give a frothy appearance and smooth texture, but it leads to gastrointestinal irritation and forms persistent foam on shaking with water.

Synthetic milk

Synthetic milk prepared from water, oil, detergent, and urea is sometimes used in paneer preparation; it is highly toxic and shows a soapy smell or lather on heating.

Formalin

Formalin is added as a preservative to extend shelf life, but it is carcinogenic and can be detected by the violet-blue ring test with concentrated sulfuric acid.

2.5 Qualitative estimation of adulterants present in Eggs

Eggs may be adulterated with heavy metals, detergent, calcium carbonate, and artificial colors.

Heavy metals

Heavy metals like lead or cadmium cause toxicity and organ damage, and can be detected by atomic absorption or chemical tests for metal ions.

Detergent

Detergent is used to clean or shine shells, leading to gastric irritation, and is detected by persistent foam when the sample is shaken with water.

Calcium carbonate

Calcium carbonate is added to increase shell weight, which may cause gastric problems, and can be detected by treating with dilute HCl where effervescence confirms its presence.

Artificial colors
Artificial colors are mixed to darken yolk, leading to allergies, and can be detected when yolk placed in water diffuses color abnormally.

2.6 Titration Method (Titratable Acidity)

2.6.1 Preparation of Standard Casein Solution

10 ml of standard casein solution was pipetted into a conical flask. Then, 1 ml of phenolphthalein indicator was added to the flask. The mixture was titrated against 0.1 N NaOH solution until a faint pink colour appeared. The same procedure was then repeated using a milk sample.

2.6.2 Preparation of Standard Leucine Solution

10 ml of standard leucine solution was pipetted into a conical flask. Then, 1 ml of phenolphthalein indicator was added to the flask. The mixture was titrated against 0.1 N NaOH solution until a faint pink colour appeared. The same procedure was then repeated using a paneer sample.

2.6.3 Preparation of Standard Bovine Serum Albumin Solution

10 ml of standard Bovine Serum Albumin solution was pipetted into a conical flask. Then, 1 ml of phenolphthalein indicator was added to the flask. The mixture was titrated against 0.1 N NaOH solution until a faint pink colour appeared. The same procedure was then repeated using an egg sample.

The amount of Standard (Casein, Leucine, BSA) was calculated using the formula.

$$N_2 = \frac{N1 \times V1}{V2},$$

Where: N_1 = Normality of **s**tandard solution, N_2 = Normality of sample, V_1 = Final burette reading – Initial burette reading, V_2 = Volume of sample.

$$\textbf{Lactic acid } \% = \frac{N1 \times V1 \times Eq.wt\ lactic}{V2 \times 1000} \times 100$$

2.7 Estimation of Fat Content in Protein Samples Using the Rose–Gottlieb Method

The Rose–Gottlieb method was used to estimate fat content by solvent extraction following protein digestion. For milk, 10–11 g of the sample was weighed into a Mojonnier flask; smaller amounts were used for high-fat products like paneer or egg. A blank was run using the same reagents but with water; the blank residue was ensured to be ≤ 2.5 mg.

The sample was gently mixed and warmed if needed (35–40 °C), then cooled to approximately 20 °C. 2 mL of ammonia (~25%) was added and mixed thoroughly. Then, 10 mL of ethanol (≥94%) was added and mixed again. 25 mL of diethyl ether was added and shaken well, followed by 25 mL of petroleum ether, and shaken again.

The extraction process was repeated three times, or until the fat was fully removed.

All organic layers were combined into a pre-weighed dish. The solvents were evaporated using a rotary evaporator or a water bath. The remaining fat residue was then dried in an oven at 102 ± 2 °C to constant weight.

Optionally, the residue was dissolved in warm petroleum ether to confirm it was fat. Finally, the residue was cooled, weighed, and the fat percentage was calculated based on the weight difference and sample mass.

Formula: Fat % = Final weight of flask – initial weight of flask / sample x 100

2.8 Estimation of Protein by UV Spectrophotometric Method

2.8.1 Preparation of Standard Casein Solution

10.0 mg of casein was dissolved in 10.0 mL of distilled water. Standard solutions were prepared at concentrations of 2, 4, 6, 8, and 10 mg/mL. From each standard solution, 1.0 mL was transferred into labelled test tubes. To each tube, 4.0 mL of Biuret reagent was added. The absorbance was measured.

Preparation of Sample (Milk)
Fresh milk was taken and transferred into a beaker. 100 mL of distilled water was added, and the mixture was stirred well. The resulting filtrate was used as the sample solution. 1.0 mL of the sample solution was taken into a test tube, and 4.0 mL of Biuret reagent was added.

The absorbance of the standards and the milk sample was recorded. Calibration curve was plotted, and the concentration of protein in the sample was determined using the regression equation.

The Assay % was calculated using the formula
Assay % = (P.Y / T.Y) × 100
Where: P.Y = Practical yield (from sample absorbance), T.Y = Theoretical yield (from standard curve)

2.8.2 Preparation of Standard Leucine Solution

10.0 mg of leucine was dissolved in 10.0 mL of distilled water. Standard solutions of 2, 4, 6, 8, and 10 mg/mL were prepared. 1.0 mL from each standard was transferred into labelled test tubes, and 4.0 mL of Biuret reagent was added to each. Absorbance was measured.

Preparation of Sample (Paneer)
About 2–5 g of paneer was weighed and homogenized in 20–30 mL of distilled water or 0.1N NaOH using a blender or mortar and pestle. The homogenate was then filtered or centrifuged to remove fat and solid particles. The clear supernatant or filtrate was collected and used as the sample. 1.0 mL of the filtrate was added to a test tube along with 4.0 mL of Biuret reagent. The absorbance of

the standards and paneer sample was recorded. Calibration curve was plotted, and the protein concentration was determined using the regression equation.

The Assay % was calculated using the formula.
Assay% = (P.Y / T.Y) × 100

2.8.3 Preparation of Standard BSA (Bovine Serum Albumin) Solution

10.0 mg of BSA was dissolved in 10.0 mL of distilled water. Standard solutions at concentrations of 2, 4, 6, 8, and 10 mg/mL were prepared. 1.0 mL from each solution was pipetted into labelled test tubes, and 4.0 mL of Biuret reagent was added. Absorbance was recorded.

Preparation of Sample (Egg)

A fresh hen's egg was broken and the egg white and yolk were separated. They were homogenized separately or together (as whole egg). About 1.0 g of the homogenized egg sample was weighed and transferred into a beaker. 100 mL of distilled water was added, and the mixture was stirred well. The filtrate was used as the sample solution.

1.0 mL of this filtrate was mixed with 4.0 mL of Biuret reagent in a test tube. The absorbance of standards and the egg sample was measured. Calibration curve was plotted, and the concentration of albumin was determined using the regression equation.

The Assay % was calculated using the formula.
Assay% = (P.Y / T.Y) × 100

3. Method Validation (ICH Q2(R1))

The method was validated according to international guidelines.

Linearity: linearity was done using Standard solutions (casein, leucine, bovine serum albumin) dilutions (2–10 µg/mL).

Accuracy: Accuracy was done using spiked standard addition method.

50, 100, 150 % spiked samples were prepared (standard + sample), absorbance was measured and accuracy was calculated.
% Recovery = amount of found / amount expected x 100

Precision: 6 samples of 100% Standard solutions (Casein, Leucine, BSA) were prepared and absorbance was recorded 217nm, 220nm, 278nm.
% RSD = (SD / µ) × 100

LOD & LOQ: 6 blank samples were prepared & absorbance was measured
LOD = 3.3 x σ / S, LOQ = 10 x σ / S

According to ICH guidelines, LOD <0.15 µg/mL, LOQ < 0.45 µg/mL.

4. RESULTS

4.1 Qualitative estimation of adulterants.

4.1.1 Table 1: Milk sample.

S.No.	Adulterant	Observation
1	**Starch**	No blue color → starch absent.
2	**Detergent**	No persistent foam → detergent absent.
3	**Synthetic Milk**	Red litmus didn't turn blue → synthetic milk absent.
4	**Formalin**	No violet/blue ring → formalin absent.
5	**Urea**	No blue color → urea absent.

4.1.2 Table 2: Paneer sample.

S.No.	Adulterant	Observation
1	**Starch**	No blue color observed — starch not present.
2	**Formalin**	No violet or blue ring seen — formalin not detected.
3	**Detergent**	Persistent foam observed — detergent detected.
4	**Urea / Synthetic Solids**	Blue color observed — urea present.

4.1.3 Table 3 Egg sample

S.No.	Adulterant	Observation
1	**Heavy Metals 10**(Lead, Mercury, Cadmium)	No dark brown/black → heavy metals absent
2	**Detergents**	No stable foam >10 mins → detergent absent
3	**Calcium Carbonate**	Effervescence → $CaCO_3$ present
4	**Artificial Colour in Yolk**	No color change in solvent → dye absent

4.2 Titration Method

Table 4: Estimation of Titrable acidity in Samples.

Sample	V1	V2	N1	N2	%Lactic acid
Milk	8.50ml	10ml	0.100N	0.0850	0.766%
Paneer	7.20ml	10ml	0.100N	0.0720	0.649%
Egg	3.40ml	10ml	0.100N	0.0340	0.306%

4.3 Fat Content by Rose–Gottlieb Method

Table 5: Estimation of Fat content in Samples.

Sample	Amount of sample	Initial weight of flask	Final weight of flask	% Fat
Milk	10	67	68	10%
Paneer	4.3	67.04	67.40	8.37%
Egg	4.9	70.4	70.8	8.16%

4.4 UV Spectrophotometric Method

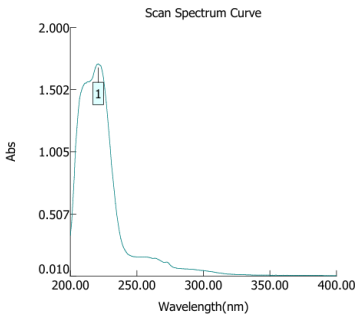

Fig.1: Casein spectra.

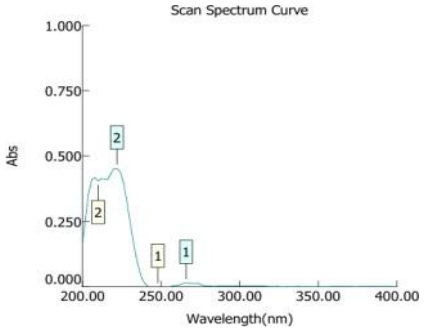

Fig.2: Leucine spectra.

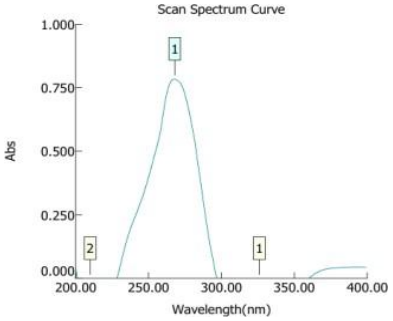

Fig.3: BSA spectra.

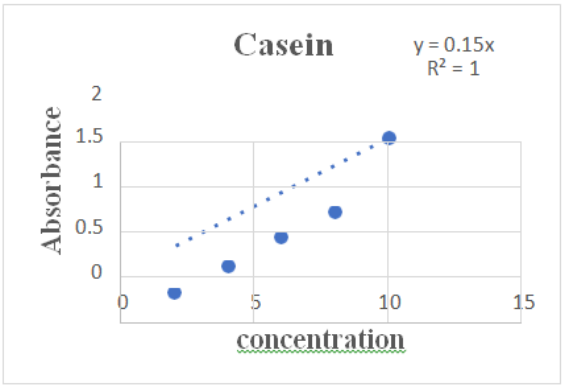

Fig.4: Standard graph of Casein.

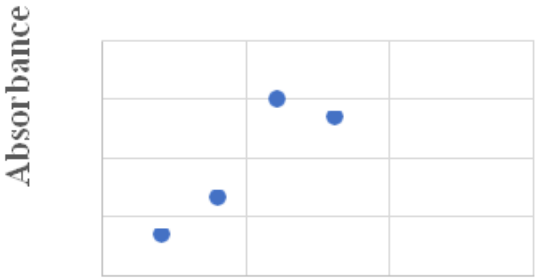

Fig.5: Standard graph of leucine.

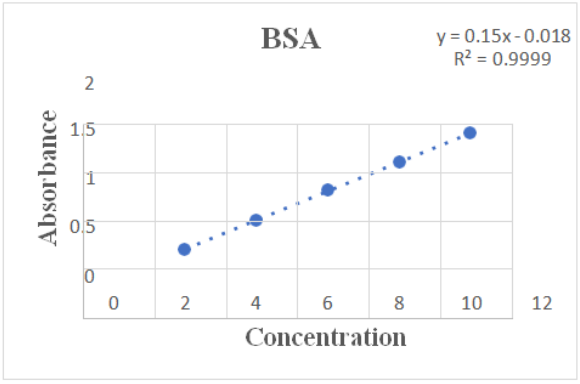

Fig.6: Standard graph of BSA.

4.4.2 Accuracy (Recovery study)

Table 6: Accuracy of Ascorbic acid using Spiked recovery method (Milk).

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50	35	33.9	96.85%
100	46	46.6	101.3%
150	58	58.2	100.3%

Table 7: Accuracy of Ascorbic acid using Spiked recovery method (Paneer)

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50	45	44.5	98.8%
100	50	50.4	100.8%
150	62	60.8	98.06%

Table 8: Accuracy of Ascorbic acid using Spiked recovery method (Egg).

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50	37	36.4	98.37%
100	48	48.2	100.41%
150	54	55.2	102.22%

Accuracy was done using spiked standard addition method. Spiked samples are prepared as 50% = 0.5 x Cs, 100% = 1 x Cs, 150% = 1.5 x Cs

Where Cs is concentration of sample: Milk (23 µg/mL), Paneer (25 µg/mL), Egg (24 µg/mL), Accuracy: Recovery studies showed results between 80–110%.

% Recovery = amount of found / amount expected x 100

4.4.3 Precision

Table 9: Precision of Standards.

S. No	Casein	Leucine	Bovine serum albumin
1	0.305	0.489	0.183
2	0.306	0.488	0.182
3	0.307	0.487	0.181
4	0.304	0.485	0.180
5	0.303	0.486	0.184
6	0.305	0.487	0.185
Mean	0.305mg/ml	0.487mg/ml	0.183mg/ml
S. D	0.00221mg/ml	0.00226mg/ml	0.0028mg/ml
(RSD%)	0.72%	0.46%	1.56%

6 solutions of standard with same concentration were prepared & absorbance was recorded at 217nm, 220nm, 278nm respectively. Precision was calculated using the formula.

% RSD = (SD / µ) × 100

4.4.4 LOD & LOQ

Table 10: LOD & LOQ of Standards.

SAMPLE	LOD	LOQ
Casein	0.0174 µg/mL	0.053 µg/mL
Leucine	0.0548 µg/mL	0.1663 µg/mL
BSA	0.0740 µg/mL	0.2248 µg/mL

6 blank samples were prepared & absorbance was measured.

LOD = 3.3 x σ / S & **LOQ = 10 x σ / S**

4.4.5 Assay of Protein content in Samples Assay was calculated using calibration curve.

Table 11: Protein % in samples.

Sample	Concentration(mg/ml)	Amount found(mg/g)	% Assay
Milk	3.30 mg/ml	140.0 mg/g	3.30%
Paneer	3.40 mg/ml	170.0 mg/g	17.0%
Egg	2.50 mg/ml	125.0 mg/g	12.5%

5. DISCUSSION

The findings of this study highlight both the prevalence and risks of adulteration in commercially available protein foods such as milk, paneer, and eggs. These results underscore the effectiveness of the selected analytical and spectrophotometric methods in identifying both qualitative and quantitative adulteration. The application of validated methodology, stringent guidelines (such as ICH Q2R1), and robust recovery/precision testing support the reliability of these findings. Furthermore, the quantitative analysis confirmed variable protein and fat content among the tested foods, revealing that while all three food samples provide notable protein, the detected levels reflect the potential influence of adulteration and processing practices.

6. CONCLUSION

Among the tested protein food samples, paneer was more vulnerable to adulteration, particularly with detergent and urea, while milk and egg samples largely met quality standards except for the detection of calcium carbonate in eggs. Advanced analytical techniques, combined with method validation, provide a rigorous approach for the standardized detection and quantification of food adulterants. These results emphasize the need for routine, widespread monitoring to protect public health and maintain nutritional integrity.

In terms of protein content, the study found that paneer had the highest concentration measured at 3.40 mg/mL, closely followed by milk at 3.30 mg/mL, while eggs showed a somewhat lower concentration of 2.50 mg/ml. This comparison highlights that although all three foods are valuable sources of protein, paneer and milk offer marginally greater protein density per millilitre than egg within the tested samples. Routine quantification of protein levels, alongside adulterant detection, remains essential for accurate food quality assurance and dependable nutritional labelling.

REFERENCES

1. Neelima N, Chitta S, Anusha K, Prasanthi G. Detection of adulterants in milk and milk products. *International Journal of Current Microbiology and Applied Sciences*, 2018; 7(8): 3502-3513.
2. Food Safety & Standards Authority of India (FSSAI). Manual of methods of analysis of foods - Milk and milk products. Food Safety & Standards Authority of India, 2016.
3. Yadav S, Agarwal N, Dhaneshwar S, Shukla K. Detection of milk adulteration by physico-chemical methods. *Indian Journal of Dairy Science*, 2017; 70(5): 586-595.
4. Sharma P, Rai DK, Saini RK. Analytical approaches for detection of milk adulteration: A review. *Journal of Food Science and Technology*, 2021; 58(2): 373-38.
5. Kumar V, Aggarwal R, Bhardwaj A. Detection of adulterants in paneer samples in local market. *International Journal of Chemical Studies*, 2019; 7(3): 1619-1622.
6. Arya S, Mahajan N, Sharma A, Mandal PK. Instrumental methods for analysis of food quality and safety. *Indian Food Industry Magazine*, 2020; 39(2): 46-53.
7. International Conference on Harmonisation (ICH). Guideline Q2(R1): Validation of analytical procedures: Text and methodology, 2005.
8. Fakude T, Pike G, Jansen van Rensburg J. Adulteration detection in eggs: Methods and health impacts. *Food Control*, 2022; 133: Article 108502.
9. Zafar T, Islam J, Niazi S. Adulteration in eggs: Techniques for detection. *Journal of Pharmacy and Pharmaceutical Sciences*, 2020; 9(1): 58-66.
10. Sreedhar A, Raghavendra V, Varma RP. Validation of quantitative methods in food analysis. *International Journal of Pharmaceutical Sciences Review and Research*, 2018; 51(2): 83-89.
11. Singleton VL, Rossi JA. Colorimetric analysis methods for adulterants in milk and foods. *Journal of Agricultural and Food Chemistry*, 2017; 65(3): 357-363.
12. Pandey P, Mishra P, Shukla S. Food adulteration: Analytical techniques for detection and safety. *Asian Journal of Dairy and Food Research*, 2021; 40(1): 96-103.