Evaluation of Milk Quality

Milk quality control is the use of approved tests to ensure the application of approved practices, standards and regulations concerning the milk and milk products. The tests are designed to ensure that milk products meet accepted standards for Chemical Composition and Purity as well as Levels of different Microorganisms.

Testing milk and milk products for quality and monitoring that Milk Products, Processors and Marketing Agencies adhere to accepted codes of practices costs money. There must be good reasons why we have to have a quality control system for the dairy industry in Kenya. The reasons are:

— *To the Milk Producer*: The milk producer expects a fair price in accordance with the quality of milk she/he produces.

— *The Milk Processor*: The milk processor who pays the producer must assure himself/herself that the milk received for processing is of normal composition and is suitable for processing into various dairy products.

— *The Consumer*: The consumer expects to pay a fair price for milk and milk products of acceptable to excellent quality.

— *The Public and Government Agencies*: These have to ensure that the health and nutritional status of the people is protected from consumption of contaminated and substandard foodstuffs and that prices paid are fair.
to the milk producers, the milk processor and the final consumer.

All the above-is only possible through institution of a workable quality testing and assurance system conforms to national or internationally acceptable standards.

**Milk Testing and Quality Control.**

**Milk Sampling**

Accurate sampling is the first prerequisite for fair and just quality control system. Liquid milk in cans and bulk tanks should be thoroughly mixed to disperse the milk fat before a milk sample is taken for any chemical control tests. Representative samples of packed products must be taken for any investigation on quality. Plungers and dippers me used in sampling milk from milk cans.

![Figure. 1: Equipment used for taking milk samples](image)

Sampling milk for bacteriological tests require a lot of care. Dippers used must have been sterilised in an autoclave or pressure cooker for at least 15mm at 120°C before hand in order not to contaminate the sample. On the spot sterilisation may be employed using 70% Alcohol swab and flaming or scaling in hot steam or boiling water for 1 minute.

**Sample Preservation**

**Chemical Tests**

Milk samples for butterfat testing may be preserved with chemicals like Potassium dichromate (1 Tablet or $\frac{1}{2}$ ml 14%
solution in a ¼ litre sample bottle is adequate.) Milk samples that have been kept cooling a refrigerator or ice-box must first be warmed in water bath at 40 °C, cooled to 20°C, mixed and a sample then taken for butterfat determination. Other preservative chemicals include Sodium acid at the rate of 0.08% and Bronopol (2-bromo-2-nitro-1, 3-propanediol) used at the rate of 0.02%.

If the laboratory cannot start work on a sample immediately after sampling, the sample must be cooled to near freezing point quickly and be kept cool till the work can start. If samples are to be taken in the field e.g. at a milk cooling centre, ice boxes with ice pecks are useful.

Labelling and Records Keeping

Samples must be clearly labelled with name of farmer or code number and records of dates, and places included in standard data sheets. Good records must be kept neat and in a dry place. It is desirable that milk producers should see their milk being tested, and the records should be made available to them if they so require.

Common Testing of Milk

Organoleptic Tests

The organoleptic test permits rapid segregation of poor quality milk at the milk receiving platform. No equipment is required, but the milk grader must have good sense of sight, smell and taste. The result of the test is obtained instantly, and the cost of the test are low. Milk which cannot be adequately judged organoleptically must be subjected to other more sensitive and objective tests.

Procedure:
- Open a can of milk.
- Immediately smell the milk.
- Observe the appearance of the milk.
- If still unable to make a clear judgement, taste the milk, but do not swallow it. Spit the milk sample into a bucket
provided for that purpose or into a drain basin, flush with water.

— Look at the can lid and the milk can to check cleanliness.

Judgement:

Abnormal smell and taste may be caused by:

— Atmospheric taint.

— Physiological taints (hormonal imbalance, cows in late lactation- spontaneous rancidity).

— Bacterial taints.

— Chemical taints or discoloring.

— Advanced acidification (pH < 6.4).

**Clot on Boiling (C.O.B) Test**

The test is quick and simple. It is one of the old tests for too acid milk (pH<5.8) or abnormal milk. If a milk sample fails in the test, the milk must contain many acid or rennet producing microorganisms or the milk has an abnormal high percentage of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected.

**Procedure**

Boil a small amount of milk in a spoon, test tube or other suitable container. If there is clotting, coagulation or precipitation, the milk has failed the test. Heavy contamination in freshly drawn milk cannot be detected, when the acidity is below 0.20-0.26% Lactic acid.

**Alcohol Test**

The test is quick and simple. It is based on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) results in a positive test.
Evaluation of Milk Quality

Procedure

The test is done by mixing equal amounts of milk and 68% of ethanol solution in a small bottle or test tube. (68% Ethanol solution is prepared from 68 mls 96% (absolute) alcohol and 28 mls distilled water). If the tested milk is of good quality, there will be no coagulation, clotting or precipitation, but it is necessary to look for small lumps. The first clotting due to acid development can first be seen at 0.21-0.23% Lactic acid. For routine testing 2 mls milk is mixed with 2 mls 68% alcohol.

Alcohol-Alizarin Test

The procedure for carrying out the test is the same as for alcohol test but this test is more informative. Alizarin is a colour indicator changing colour according to the acidity. The Alcohol Alizarin solution can be bought ready made or be prepared by adding 0.4 grammes alizarin powder to 1 litre of 61% alcohol solution.

Acidity Test

Bacteria that normally develop in raw milk produce more or less of lactic acid. In the acidity test the acid is neutralised with 0.1 N Sodium hydroxide and the amount of alkaline is measured. From this, the percentage of lactic acid can be calculated. Fresh milk contains in this test also “natural acidity” which is due to the natural ability to resist pH changes. The natural acidity of milk is 0.16 - 0.18%.

Apparatus

- A porcelain dish or small conical flask
- 10 ml pipette, graduated
- 1 ml pipette
- A Burette, 0.1 ml graduations
- A glass rod for stirring the milk in the dish
- A Phenophtalein indicator solution, 0.5% in 50% Alcohol
- N Sodium hydroxide solution.
Procedure

9 ml of the milk measured into the porcelain dish/conical flask, 1 ml Phenolphthalein is added and then slowly from the buret, 0.1 N Sodium hydroxide under continuous mixing, until a faint pink colour appears. The number of mls of Sodium hydroxide solution divided by 10 expresses the percentage of lactic acid.

Resazurin test

Resazurin test is the most widely used test for hygiene and the potential keeping quality of raw milk. Resazurin is a dye indicator. Under specified conditions Resazurin is dissolved in distilled boiled water. The Resazurin solution can later be used to test the microbial activity in a given milk sample.

Resazurin can be carried out as:

- 10 min test.
- 1 hr test.
- 3 hr test.

The 10 min Resazurin test is useful and rapid, screening test used at the milk platform. The 1 hr test and 3 hr tests provide more accurate information about the milk quality, but after a fairly long time. They are usually carried out in the laboratory.

Apparatus and reagents:

- Resazurin tablets
- Test tubes with 10 mls mark
— 1 ml pipette or dispenser for Resazurin solution.
— Water bath thermostatically controlled
— Lovibond comparator with Resazurin disc 4/9

Procedure

The solution of Resazurin as prepared by adding one tablet to 50 mls of distilled sterile water. Rasazurin solution must not be exposed to sunlight, and it should not be used for more than eight hours because it loses strength.

Mix the milk and with a sanitised dipper put 10 mls milk into a sterile test tube. Add one ml of Resazurin solution, stopper with a sterile stopper, mix gently the dye into the milk and mark the tube before the incubation in a water bath, place the test tube in a Lovibond comparator with Resazurin disk and compare it colourimetrically with a test tube containing 10 ml milk of the same sample, but without the dye (Blank).

<table>
<thead>
<tr>
<th>Resazurin disc No.</th>
<th>Colour</th>
<th>Grade of milk</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Blue</td>
<td>Excellent</td>
<td>Accept</td>
</tr>
<tr>
<td>5</td>
<td>Light blue</td>
<td>v. good</td>
<td>Accept</td>
</tr>
<tr>
<td>4</td>
<td>Purple</td>
<td>Good</td>
<td>Accept</td>
</tr>
<tr>
<td>3</td>
<td>Purple pink</td>
<td>Fair</td>
<td>Separate</td>
</tr>
<tr>
<td>2</td>
<td>Light pink</td>
<td>Poor</td>
<td>Separate</td>
</tr>
<tr>
<td>1</td>
<td>Pink</td>
<td>Bad</td>
<td>Reject</td>
</tr>
<tr>
<td>0</td>
<td>White</td>
<td>Very bad</td>
<td>Reject</td>
</tr>
</tbody>
</table>

Gerber Butterfat test

The fat content of milk and cream is the most important single factor in determining the price to be paid for milk supplied by farmers in many countries. Also, in order to calculate the correct amount of feed ration for high yielding dairy cows, it is important to know the butterfat percentage as well as well as the yield of the milk produced. Further more the butterfat percentage in the milk of individual animals must be known
in many breeding programmes. Butterfat tests are also done on milk and milk products in order to make accurate adjustments of the butterfat percentage in standardised milk and milk products.

**Apparatus for DF test:**
- Gerber butyrameters, 0-6% or 0-8% BF
- Rubber stoppers for butyrometers
- 10.94 or 11 ml pipettes for milk
- 10 mls pipettes or dispensers for Gerber Acid
- 1 mls pipettes or dispensers for Amyl alcohol
- stands for butyrometers

**Gerber water bath Reagents:**
- Gerber sulphuric acid, (1.82 g/cc)
- Amyl alcohol

**Treatment of samples**

Fresh milk at approximately 20°C should be mixed well. Samples kept cool for some days should be warmed to 40°C, mixed gently and cooled to 20°C before the testing.

**Procedure**

Add 10 mls sulphuric acid to the butyrometer followed by 10.94 or 11 mls of well mixed milk. Avoid wetting of the neck of the butyrometer. Next add 1 ml of Amyl alcohol, insert stopper and shake the butyrometer carefully until the curd dissolves and no white particles can be seen. Place the butyrometer in the water bath at 65°C and keep it there until a set is ready for centrifuging. The butyrometer must be placed in the centrifuge with the stem (scale) pointing towards the centre of the centrifuge.

Spin for 5 min. at 1100 rpm. Remove the butyrometers from the centrifuge. Put the butyrometers in a water bath maintained at 65°C for 3 min. before taking the reading. The fat column should be read from the lowest point of the meniscus of the interface of the acid-fat to the 0-mark of the scale and read the butterfat percentage. The butyrometers
should be emptied into a special container for the very corrosive liquid of acid-milk, and the butyrometers should be washed in warm water and dried before the next use.

*Appearance of the Test*

- The colour of the fat column should be straw yellow.
- The ends of the fat column should be clearly and sharply defined.
- The fat column should be free from specks and sediment.
- The water just below the fat column should be perfectly clear.
- The fat should be within the graduation.

*Problems in Test Results*

**Curdy tests:**
- Too lightly coloured or curdy fat column can be due to:
  - Temperature at milk or acid or both too low.
  - Acid too weak.
  - Insufficient acid.
  - Milk and acid not mixed thoroughly.

**Charred tests:**
- Darkened fat column containing black speck at the base is due to:
  - Temperature of milk-acid mixture too high.
  - Acid too strong.
  - Milk and acid mixed too slowly.
  - Too much acid used.
  - Acid dropped through the milk.

*Lactometer Test*

Addition of water to milk can be a big problem where we have unfaithful farm workers, milk transporters and greedy milk hawkers. A few farmers may also fall victim of this illegal practice. Any buyer of milk should therefore assure himself/
herself that the milk he/she purchases is wholesome and has not been adulterated. Milk has a specific gravity.

When its adulterated with water or other materials are added or both misdeeds are committed, the density of milk change from its normal value to abnormal. The lactometer test is designed to detect the change in density of such adulterated milk. Carried out together with the Gerber butterfat test, it enables the milk processor to calculate the milk total solids (\% TS ) and solids not fat (SNF). In normal milk SNF should not be below 8.5\% according to Kenya Standards (KBS No 05-10:-1976).

Procedure

Mix the milk sample gently and pour it gently into a measuring cylinder (300-500). Let the Lactometer sink slowly into the milk. Read and record the last Lactometer degree (°L) just above the surface of the milk. If the temperature of the milk is different from the calibration temperature (Calibration temperature may be=20 °C ) of the lactometer, calculate the temperature correction. For each °C above the calibration temperature add 0.2 °L; for each °C below calibration temperature subtract 0.2 °L from the recorded lactometer reading.

For the calculations, use lactometer degrees, and for the conversion to density write 1.0 in front of the true lactometer reading, i.e. 1.030 g/ml. Clever people may try to adulterate milk in such a way that the lactometer cannot show the adulteration. But look to see if there is an unusual sediment from the milk at the bottom of the milk can and taste to find out if the milk is too sweet or salty to be normal.

Samples of milk from individual cows often have lactometer reading outside the range of average milk, while samples of milk from herds should have readings hear the average milk, but wrong feeding, may result in low readings. Kenyan standards expects milk to have specific gravity of 1.026 -1.032 g/ml which implies a Lactometer reading range of 26.0 -32.0 °L. If the reading is consistently lower than
expected and the milk supplier disputes any wrong doing arrange to take a genuine sample from the supplier.

Freezing Point Determination

The freezing point of milk is regarded to be the most constant of all measurable properties of milk. A small adulteration of milk with water will cause a detectable elevation of the freezing point of milk from its normal values of -0.54°C. Since the test is accurate and sensitive to added water in milk, it is used to detect whether milk is of normal composition and adulterated.

Inhibitor Test

Milk collected from producers may contain drugs and/or pesticides residues. These when present in significant amounts in milk may inhibit the growth of lactic acid bacteria used in the manufacture of fermented milk such as Mala, cheese and Yoghurt, besides being a health hazard.

Principle of the method: The suspected milk sample is subjected to a fermentation test with starter culture and the acidity checked after three hours. The values of the titratable acidity obtained is compared with titratable acidity of a similarly treated sample which is free from any inhibitory substances.

Materials:
- test tubes
- Starter culture
- 1 ml pipette
- water bath
- material for determination of titratable acidity

Procedure:
- Three test tubes are filled with 10 ml of sample to be tested and three test tubes filled with normal milk.
- All tubes are heated to 90 °C by putting them in boiling water for 3 - 5 minutes.
— After cooling to optimum temperature of the starter culture (30, 37, or 42°C), 1 ml of starter culture is added to each test tube, mixed and incubated for 3 hours.

— After each hour, one test tube is from the test sample and the control sample is determined.

Assessment of results:

— If acid production in suspected sample is the same as the normal sample, then the suspect sample does not contain any inhibitory substances;

— If acid production as suspect sample is less than in the normal milk sample, then, the suspect sample contains antibiotics or other inhibitory substances.

**Quality Control of Pasteurised Milk**

When milk is pasteurised at 63°C for 30 min in batch pasteuriser or 72°C for 15 seconds in heat exchanger, continuous flow pasteurisers, all Pathogenic Bacteria are Destroyed, thereby rendering milk safe for human consumption. Simultaneously various enzymes present in milk, and which might affect its flavour, are destroyed.

In order to determine whether or not milk has been adequately pasteurised, one of the enzymes normally present in milk phosphatase, is measured. A negative phosphatase result indicates that the enzyme and any pathogenic bacteria have been destroyed during pasteurisation. If it is positive, it means the pasteurisation process was inadequate and the milk may not be safe for human consumption and will have a short shelf life.

— Test tubes
— 5 mls pipettes
— 1 ml pipettes
— 100 ml volumetric flask
— 500 ml volumetric flask
— water bath at 37°C
Reagents

— **Buffer Solution:** Is mixed by 0.75g anhydrous sodium carbonate and 1.75g Sodium bicarbonate in 500 ml distilled water.

— **Buffer-substrate Solution:** Place 0.15 g of di-sodium paranitrophenylphosphate (the substrate) into a clean 100ml measuring cylinder. Add the buffer solution to make to 100 ml mark. Store this buffer-substrate solution in a refrigerator and protected against light. It should not be used after one week. Prepare a fresh stock.

Procedure

Pipette 5 mls buffer-substrate solution into a test tube, stopper and warm the solution in the water bath at 37°C. Add to the test tube 1ml of the milk to be tested, stopper and mix well and place in water bath at 37°C. Prepare a blank sample from boiled milk of the same type as that undergoing the test. Incubate both the test samples and the blank sample at 37°C for 2hrs. After incubation, remove the tubes and mix them thoroughly. Place one sample against the blank in a Lovibond comparator “All Purposes” using A.P.T.W. disc and rotate the disc until the colour of the test sample is matched and read the disc number.

**Interpretation:**

<table>
<thead>
<tr>
<th>Disc Reading after 2 hrs. incubation at 37°C</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>Properly pasteurised</td>
</tr>
<tr>
<td>10-18</td>
<td>Slightly under pasteurised</td>
</tr>
<tr>
<td>18-42</td>
<td>Under Pasteurised</td>
</tr>
<tr>
<td>&gt; 42</td>
<td>Not Pasteurised</td>
</tr>
</tbody>
</table>

**Production of Quality Milk**

History records cows being milked as far back as 9000 B.C. In Florida, cows have been milked since the settlement of St.
Augustine in 1565. However, it was not until colonial times in 1611 that dairy cows arrived in Jamestown. From that time until 1850, dairying changed very little. Most cattle were dual purpose (used for dairy and beef purposes) and were kept to satisfy family food needs. Milk and dairy products were in short supply and for the most part unavailable to those not living on or near the farm.

Milk production was seasonal, creating periods of excess as well as deficiency in the family milk supply. Stabilisation of these production fluctuations by storage and/or further processing into butter, cheese, or other milk products was precluded by the lack of refrigeration. Consequently, marketing of milk, butter, and cheese was limited to towns which could be reached by horse-drawn wagons. Over the years modern technology has rectified these problems and today a wide array of safe, wholesome dairy products are available to people throughout the developed world.

Production of quality milk is the concern of:

- consumers of dairy products
- retail distributors (super markets)
- milk and milk product processors
- dairy cooperatives
- state regulatory departments
- veterinarians, and
- dairymen.

From the list it's obvious that very few of us are left out. Whether we derive a living from the dairy industry through employment or otherwise, most of us are at the very least consumers of dairy products.

**Milk Composition and Nutritional Value**

The Code of Federal Regulations, Title 21, Section 131.110 provides the following definition of milk:

"Milk is the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows."
Nearly 12% of the American household’s total food expenditure is for dairy products. Milk and milk products alone provide 10% of the total available calories in the United States food supply, and in addition, represent one of the best natural sources of essential amino acids for human nutrition. These nutritional attributes of milk have long made it a mainstay particularly in the diet of growing children. There are estimated to be some 8 to 10,000 different types of milk products available thus making it an exceptionally versatile raw product.

Milk is composed of water, fat, protein, lactose and minerals (ash). The concentration of these components will vary between cows and breeds. Total milk solids refers specifically to fat, protein, lactose and minerals. This is to be differentiated from solids-not-fat milk (SNF), a frequently used term which describes the total solids content minus fat. SNF milk is known to most people as “skim milk”. The nutritional as well as economic value of milk is directly associated with its solids content. The higher the solids content the better its nutritional value and the greater the milk product yields.

**Flavour and Odour Characteristics**

Consumer acceptance is greatly affected by flavour. There are several factors which may produce off-flavours and/or odours in milk. Some of the more common causes of flavour and odour problems are:

- Feed and weed flavours
  - wild onion or garlic
  - strong flavoured feedstuffs such as alfalfa silage
- Cow-barny flavours - which result when milk is obtained from unclean or poorly ventilated environments, improperly cleaned or sanitised milking equipment
- Rancid flavours - presence of free fatty acids (FFA)
  - due to excessive agitation of milk during collection or transport
breakdown of the milk fat component by proteolytic and lipolytic enzymes present in raw milk

- Malty flavours, high acid flavours
  - bacterial contamination
- Oxidised flavours
  - exposure of milk to sunlight
  - contact of milk with oxidising agents such as rust, copper, and chlorine
- Foreign flavours
  - fly sprays, medications, etc.

Process of Delivering Milk

A multitude of events take place in the process of delivering milk from the farm to the dinner table and all are designed to provide the consumer with a wholesome, nutritious and safe product. The production of quality milk and milk products begins on the farm and continues through further handling, processing and distribution.

Milk processing has three primary objectives:

- destruction of human pathogens through pasteurisation
- keeping the quality of the product without significant loss of flavour, appearance, physical and nutritive properties, and
- selective control of organisms which may produce unsatisfactory products

Milk processing plant procedures seek to:

- prevent further bacterial contamination of raw materials
- reduce bacterial numbers in milk
- protect the finished product from recontamination through careful handling, proper packaging and storage
Pasteurisation is the means whereby raw milk is rendered safe for human consumption. It is the process of heating milk to a sufficient temperature for a sufficient length of time to make it free of pathogens, however, not totally free of bacteria.

Bacteria in Milk

As stated earlier, certain organisms are capable of surviving pasteurisation and/or refrigeration processes. These bacteria are an important concern because they reduce product shelf-life. Those of major significance are:

- **Thermoduric bacteria**
  - common in raw milk
  - they survive pasteurisation and include
    - Enterococci
    - Micrococci
    - Brevibacterium
    - Lactobacilli

- **Psychrotropic bacteria**
  - common dairy product contaminants
  - these grow at refrigeration temperatures
  - they do not survive pasteurisation
  - can produce off-flavours

- **Spore-formers**
  - common contaminants
  - survive pasteurisation
    - Clostridial spp.
    - Bacillus spp.

The primary source of these bacteria is the environment: air, dust, dirty equipment, operators, etc. Therefore, proper cleaning and sanitising procedures are necessary for quality control. Grade A milk quality standards allow a maximum of 100,000 bacteria/ml. in raw bulk milk. Chronic offenders of these limits risk losing their license to sell milk to the Grade A market. Most dairies are able to maintain bacteria counts...
between 5 to 10,000 per ml. When high counts become a problem it is generally due to one or more of the following:

- improper cleaning of milking equipment (the most common cause of high bacteria counts in milk)
- improper cooling of milk
- occasionally, a herd experiencing a high prevalence of infection due to Strep ag. or Staph sp.

**Role of Somatic Cells in Milk**

Somatic cell counts represent another important milk quality parameter. Milk with high somatic cell concentrations reduces cheese yields due to the lowered casein content. In addition, high cell count milk generally contains increased amounts of proteolytic and lipolytic enzymes (lipase). These presence of these enzymes in milk increases the potential for off-flavours and odours.

Somatic means body and thus a somatic cell is a body cell. There are three types of somatic cells typically found in milk: epithelial cells, macrophages, and polymorphonuclear leukocytes (PMN). Cell types found in milk obtained from non-infected glands are predominantly epithelial cells and macrophages. Milk from infected glands, however, generally contains high concentrations of PMN's with little or no increase in other cell types.

Consequently, somatic cell counts are an important indicator of udder health. Somatic cell counts are made available to dairymen from a variety of sources including milk quality laboratories operated by state and local regulatory departments, dairy cooperatives, DHIA-SCC programme, and veterinary diagnostic laboratories.

Mastitis causes a shift in the composition of milk. In addition to lowered amounts of casein, lactose and fat levels are decreased particularly in milk with somatic cell counts in excess of 2 million. Because the bacterial quality and somatic cell content of raw milk are important to product shelf-life, flavour and yields (particularly cheese), milk processors strive
to obtain the highest quality raw product possible from their producers.

**Antibiotic Residues in Milk**

Antibiotic residues pose a significant public health threat. Consequently, milk in Florida is routinely monitored by dairy cooperatives and the Florida Department of Agriculture and Consumer Services, Division of Dairy Industry. The official test in current use is the Bacillus stearothermophilus disc assay. It is particularly sensitive for penicillin but can detect other inhibitors as well. The vast majority of antibiotic residues in milk occur by accident. Dairymen can avoid residue problems by:

- properly identifying treated cows
- informing milkers of the need to withhold and the method for withholding milk
- keeping an accurate record of dates and times of treatment
- following label directions and veterinarians' advice for withholding times
- having milk tested from suspect cows if uncertain about treatment or withholding time
- having tank milk tested when it is suspected of having milk containing antibiotic residue
- isolate purchased cows and test their milk for residue prior to their entry into the milking herd.

**Dairy Cooperatives**

Dairy cooperatives are organised by dairymen for the purpose of marketing milk. Thus, instead of buying milk direct from farms' milk processors buy their raw product from the dairy cooperative. Dairymen are in turn paid by the cooperative for the milk they produce. Florida's dairies supply approximately 80% of the state's marketing demands. Therefore, at certain times of the year some milk must be imported to satisfy processing needs.
During other times of the year, milk production is in excess of market demand and milk must be exported out-of-state. These daily marketing difficulties could be both expensive and time-consuming problems for dairymen. Consequently, the majority of producers belong to milk marketing cooperatives. Cooperatives serve the dairymen by promoting dairy products, providing an effective lobby for political concerns, and informing members on a variety of dairy industry issues such as water quality, waste management, and milk pricing.

**Different Animal Milk Detection in Dairy Products**

The adulteration of food products is a significant problem in the food production. This is how fraudulent producers try to cheat consumers and authorities. The adulteration affects all commodities in the food processing. Most frequently, such products are adulterated that are produced in big quantities and further, the expensive products whose adulteration brings a profit.

**Detection of Adulteration**

First of all, it is necessary to familiarise with the composition of individual milks from different species and to find the suitable marker components for the detection of adulteration with other species. The determination of fat, crude protein, lactose, ashes, and total dry matter in cow, ewe and goat buffalo milk and colostrum was dealt with by Hadjipanayiotou. The highest contents of fat, crude protein, ashes, and total dry matter were found in ewe milk followed by goat milk, the lowest contents of these components having been discovered in cow milk.

We also obtained the basic composition of buffalo milk. Table 1 presents the milk yields of individual animal species. It is apparent that the yield of cow milk is significantly higher than those of other species. It also follows from Table 1 that the highest content of non-protein nitrogen (NPN) can be found in goat milk and the lowest one in cow milk.
Evaluation of Milk Quality

Table 1. Output of cow, ewe, goat and bu.alo milks and their composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine</th>
<th>Ewe</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>44</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Milk output (kg/day)</td>
<td>21.09 ± 7.86</td>
<td>2.45 ± 0.549</td>
<td>3.49 ± 0.504</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>38.0</td>
<td>55.2</td>
<td>45.2</td>
</tr>
<tr>
<td>Crude protein (N × 6.38) (g/kg)</td>
<td>33.0</td>
<td>58.0</td>
<td>41.1</td>
</tr>
<tr>
<td>NPN (g/kg)</td>
<td>2.18</td>
<td>2.70</td>
<td>2.91</td>
</tr>
<tr>
<td>NPN (expressed in % of crude protein)</td>
<td>7.43</td>
<td>4.66</td>
<td>7.13</td>
</tr>
</tbody>
</table>

It follows from the literature that the detection of adulteration by the substitution of one milk type for another one is made by protein analysis. This problem is a very complicated one as it is necessary to take into account that the composition of milk and milk proteins is very variable, both between individual types of milk and within one type. It depends on the breed or on the lactation level.

The quantitative determination of milk proteins is complicated by the existence of genetic and nongenetic polymorphism, and by the technological treatment and processing of milk. Thermal denaturation or proteolysis, that is common with the manufacturing of many milk products, incurs a risk of complex formation, the formation of insoluble new compounds, smaller peptides and amino acids whose analysis is fairly complicated. The information on the occurrence and quantity of individual proteins or derived compounds is, for the reasons mentioned above, very important for the estimation of processing, quality, and adulteration. Protein content in individual milks and their abundance in casein and whey fractions is shown in Table 2.

Table 2. Protein composition of bovine, ewe, goat and bu.alo milk

<table>
<thead>
<tr>
<th>Component (g/100 g)</th>
<th>Bovine</th>
<th>Ewe</th>
<th>Goat</th>
<th>Bu.alo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>3.2</td>
<td>4.6</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Caseins</td>
<td>2.6</td>
<td>3.9</td>
<td>2.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Whey proteins</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Many studies were published on bovine casein. Its composition in both raw and processed milk is well known. However, few studies deal with the composition of milk casein in other types of milk (e.g. goat, ewe or buffalo). Jensen stated that caseins make 82, 87, 80, and 77% of proteins in ewe, buffalo, bovine and goat milk, respectively. Casein micelles consist of four caseins: aS1-, aS2-, β- and k-caseins that occur in cow milk in the ratio of 39:10:36:13.

In milk are also present the products of proteolysis of all four primary caseins. γ-Casein and some proteosopeptone compounds are fragments of β-casein, originating from the action of plasmin, the endogenous alkaline milk protease. λ-Caseins are presumably fragments of αS1-caseins, having also originated through plasmin cleavage. Glycomacropeptides and para-k-caseins are fragments of k-caseins emerging as a result of chymosin action.

Whey proteins contain proteins soluble at pH 4.6 and 20°C. To these proteins belong β-lactoglobulin (β-Lg), α-lactoalbumin (α-La), immunoglobulins (IgG, IgA, IgM) and serum albumin (BSA). The following minor proteins are also present: lactoferrin, lactoperoxidase, enzymes, protein compounds of milk fat globule membrane (MFGM), proteosopeptone compounds, and glycomacropeptides. Well known are the primary sequences α-La, β-Lg and BSA. In bovine milk, β-Lg and α-La occur approximately at a ratio of 3:1. β-Lg is the main whey protein in all types of milk studied. Its highest abundance was found in ewe milk, the lowest one in goat milk. In ewe milk, immunoglobulins are contained in significant amounts; after β-Lg they represent the second largest fraction. In bovine and goat milks, the second most represented whey fraction is α-La.

The discovery of two variants of β-lactoglobulin in cow milk by Aschaffenburg and Drewry in 1955 generated considerable interest in the research of milk proteins. The polymorphism of milk proteins is caused either by the substitution of amino acids or by their deletion. All caseins and main whey proteins show genetic polymorphism that can
affect milk composition and some parameters of milk processing. For this reason, genetic variants of milk proteins are considered to be potential selection criteria in cattle husbandry. The identification of the genetic variant of milk protein also enables the determination of adulteration with various animal milk types.

**Analysis of Milk Proteins and their Genetic Variants**

For the detection of adulteration of one type of milk with another one, namely the following methods are used: electrophoresis, isoelectric focusing (IEF), capillary electrophoresis (CE), reverse phase high-performance liquid chromatography (RP HPLC) and ion exchange high-performance liquid chromatography (IE HPLC), hydrophobic interactive chromatography (HIC), immunochemical methods (ELISA), PCR techniques, and mass spectrometry.

**Electro-migration Methods**

**Electrophoresis**

Electrophoresis plays a significant role in the research of milk proteins and genetic variants of main milk protein components. The classification of caseins was carried out by electrophoretic analysis; minor casein components \( Y_1, Y_2, Y_3 \) and \( \text{para-}\kappa \) caseins were detected.

Polyacrylamide gel electrophoresis was used for the analysis of milk proteins. This employs the separation of individual molecules both according to their electric charge and size. In PAGE, the proteins have a negative or a positive charge, depending on the buffer conditions. They migrate with a speed based on their charge and size. The advantage of this technique lies in the fact that the individual groups of milk proteins are well separated and that the genetic protein variants and the levels of their phosphorylation can be detected.

Tamine et al. proved the addition of bovine milk (25% and more) into the goat Kish product by Page technique. The analysis was based on the mobility of bovine \( \alpha_{s1} \)-casein. This
technique was also used for the identification of bovine milk in ewe yoghurt. The marker component was bovine para-k-casein and 1% addition of bovine milk was successfully detected.

For the detection of adulteration also SDS and urea electrophoresis can also be used. Urea and anion solvent sodium dodecyl sulphate (SDS) possess the ability of dissolving various types of proteins and of decomposing polymer proteins to polypeptide components. SDS binds to individual protein molecules and gives them a strong negative charge thus removing the differences in the total charge. The electrophoretic separation takes place only on the basis of their molecular weights. On the contrary, with the application of urea, proteins are separated according to their charge.

Tamine et al. used for the cow milk detection in goat Kish product besides the above mentioned PAGE also SDS electrophoresis. A good separation was achieved of k-, β-, α-caseins of cow and goat milk. Nevertheless, the detection of adulterants was not successful due to the extensive proteolysis of the sample. By urea-PAGE electrophoresis, the presence of 5% and more of bovine milk in ewe and goat milk was detected. The detection was based on the analysis of bovine αs-casein.

**Isoelectric Focusing (IEF)**

The protein separation according to their isoelectric points is especially suitable for the analysis of caseins that form many genetic variants. For example, by using IEF instead of PAGE electrophoresis for the analysis of the genetic variants of bovine β-casein, the procedure is significantly simplified. If for the same determination PAGE electrophoresis is to be used, it has to apply both alkali and acid PAGE for the differentiation of A variants from B, C and D, and A1, A2, and A3 variants.

IEF is an EU reference method for the determination of the presence of cow milk and caseinate in cheeses made from ewe, goat or buffalo milk or their blends. It is based on the identification of γ-caseins after plasminolysis. The method is
suitable for the sensitive and specific detection of raw and thermally processed cow milk and caseinate in fresh and ripened cheeses made from ewe, goat or buffalo milk or their blends.

This detection is based on \( \gamma_2 \)- and \( \gamma_3 \)-caseins determination. Their isoelectric points lie between pH 6.5 and 7.5. By using two milk reference standards (with 0 and 1% of cow milk), the samples positive for the presence of cow milk can be detected. In the case, that the amounts of bovine \( \gamma_2 \)-casein and \( \gamma_3 \)-casein are equal or greater that their amounts in 1% standard, the presence of cow milk is confirmed. The method allows the detection of 0.5% addition. The disadvantage is that it is impossible to determine the adulteration with goat milk in ewe milk and vice versa. This may be changed by selecting an appropriate marker analyte, e.g. para-k-casein.

**Capillary Electrophoresis (CE)**

Capillary electrophoresis is a modification of electrophoresis that is carried out as a carrier-free electrophoresis via free capillary. CE is a quickly developing technique that enables a rapid casein and whey protein separation with a high resolution and good quantification. The use of CE resulted in the development of expedient and automated analyses with a very high resolution and with the demand for only very small amounts of samples and buffers. De Jong et al. carried out a complete analysis of caseins and whey proteins in bovine, ewe and goat milk by capillary electrophoresis. Based on this analysis, he succeeded in identifying adulterants (starting from 1%) of bovine, ewe and goat milk in all milk blends.

The employment of capillary zone electrophoresis in the detection of adulterants of cow milk in ewe and goat milk is described in the paper of Cattaneo et al. \( \alpha_{3i} \)-Casein fraction was the marker component for the detection of cow milk and a successful detection was carried out of the addition of cow milk to ewe or goat milk starting from 8%. Lee et al. used the detection of \( \alpha_{3i} \)-casein fraction with this technique. The authors succeeded in improving the detection and they were
able to determine 1% of adulterants in raw and reconstituted milk.

Whey fraction was also studied for the detection of cow milk presence in goat milk and cheeses. As suitable marker analytes, caprine α-lactalbumine and bovine β-lactoglobuline A were determined. Minimal detectable amount of cow milk was 2% in milk mixtures and 4% in cheeses.

An unusual method using CE analysis of ethanol-water protein fractions with isoelectric iminodiacetic acid buffer was also described as a possibility to fast identify and quantify cow milk adulterants in goat and ewe cheeses. The authors declare that the amount of cow milk in goat and ewe cheese can be estimated with the relative standard deviation of 6-7%, based on electroforegrams and statistical PLS (partial last squares) multivariable regression.

REFERENCES

