

## **CHAPTER 1**

### **INTRODUCTION**

Milk was recognized in Central Asia between 8000 and 6000 BC when the cattle began to be domesticated. British archaeologist Mellart said that during her studies at Çatal Höyük in Turkey, Butter, cheese and ayran could belong to the neolithic period (8000-5000 BC). The first evidence in this regard was found in Ur of Sumerian Civilization, which was established between the Tigris and the Euphrates. We see that the milk and milk cut are processed in the Babylonian thimes reliefs in the era of B.C. 26th century.

Milk is a whitish-colored liquid food stuff which has a distinctive taste, odour and consistency, secreted by mammary glands of female mammals for the nourishment of their young mammalian immediately after birth of mammalian creatures and in sufficient and balanced composition with essential nutrients. Milk must be sterilized or pasteurised to eliminate microorganisms before drinking or using for production. (ASÜD, 2016)

Quality of milk is very important for producing the quality products. High quality product is produced from high quality raw material. Raw milk is the main material in milk and milk products so when we select raw milk, first we must do some analysis. First we must control the critical point, antibiotics, then some analysis like odour, colour, taste, fat, pH, freezing point, protein, dry matter to determine the quality.

There are some products from milk. They are halloumi, yoghurt, white cheese, butter, pasteurized or UHT milk, cheddar cheese. I chose full fat yoghurt, semi skimmed yoghurt, strained yoghurt, high salt halloumi, low salt halloumi, full fat pasteurized fresh milk and semi skimmed pasteurized fresh milk in this study.

Pasteurized fresh milk is the procedure of removing all of pathogenic microorganisms without harming the quality of the nutrients in raw milk and cooled down with

pasteurization process destroying many of the other microorganisms in a limited time and then stored milk.

Halloumi is a traditional Cypriot cheese. If you look at its structure, it is a fresh cheese with a very textured, yellowish white colour (KKTC Turizm Bakanlığı, Northern Cyprus Tourist Guide, 2006). Cow's milk is the main milk used in halloumi cheese production, goat and sheep milk can also be used in small amounts (KKTC Tarım ve Doğal Kaynaklar Bakanlığı, hellim tescili, 2015). The most important feature that distinguishes halloumi cheese from other cheeses is that it is packed after being cooked in a salted manner, which consists of unpasteurized milk.

Yoghurt is a fermented food prepared by pasteurized cow milk, sheep milk, buffalo milk, goat milk or their mixtures, or pasteurized milk, with or without homogenization, if necessary with the addition of milk powder, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. (Turkish standards, 2006)

### **1.1 Fresh milk, yoghurt and halloumi preservation methods**

Heat treatment is one of the most important processes in the preservation and storage of milk and milk products. Pasteurization contains a combination of high temperature applied to raw milk for at least 72°C for 15 seconds or other time-temperature conditions providing a long-term low temperature or equivalent effect applied for at least 63°C. Pathogenic microorganisms are destroyed by pasteurization. In halloumi production, by cooking at 90-95°C for 50-60 minutes, the pathogenic microorganisms which contaminated after the pasteurization are destroyed. Salt is the another way to preserve halloumi cheese throughout shelf life, so after cooking, we add some brine.

### **1.2 The importance of fat in milk and dairy products**

The main ingredient of the milk composition is the milk fat. Milk fat affects the appearance, taste, odor and durability. It is also a source of essential fatty acids, fat soluble vitamins and energy. Depending on many factors, milk fat may change. Milk fat contains much more fatty acids than other animal and vegetable fats. Milk contains triglycerides, phospholipids, free sterols (cholesterol, candles, etc.), free fatty acids, fat soluble vitamins,

more than 400 different fatty acids and fatty acid derivatives. The homogeneous presence in milk facilitates digestion. This is why, it is ideal for children. (“MEGEB, 2012)

If milk fat increased,

- water soluble vitamins (A,D,E,K) will increase
- yield will increase
- taste so quality will increase
- cheese will take better shape, cut easily (Kamber, 2005)

There are three types of milk used at productions namely cow's, sheep's and goat's milk. Fat ratios of these milks are minimum 3.5, 5.5 and 4.15 % respectively. Full fat pasteurised milk's fat is 3 % and the semi-skimmed one is 1.7 %. There are three types of yoghurt we choose in this study, full-fat, semi skimmed and strained, their fats are minimum 3.9, 1.9 and 3.2 % respectively. Salty and semi-salty halloumi's fat 21.8 % and 22.3 % respectively.

### **1.3 Methods of fat analysis**

There are two methods for fat analysis which are quick methods and reference methods. Quick methods are divided into three, butyrometric methods, photometric methods and infrared – spectrofotometric methods.

Butyrometric methods are Gerber Method, Van Gulik Method and Neusal method. Gerber method's principle is after dissolving the milk protein and hard soluble salts of a certain volume by the addition of concentrated sulfuric acid, the fat is separated by centrifugation and the amount of fat is read from the scale of the butyrometer. Van Gullik method provides the most appropriate and quick results in serial analysis in cheese analysis. Sulfuric acid, which is the dangerous chemical is using in the Gerber method, removed out in the neural method.

Fotometric methods are methods that doing by machines. Milkotester method is a method doing by milkotester machine. At less time a lot of sample can be analysed.

Infrared – spectrofotometric methods are IRMA (infra-red-milk-analyser) and milkoscan. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general

term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Röse-Gottlieb method, Mojonnier method and Weibull-Stoldt method are reference methods.

Röse-Gottlieb method is the official method of the Association of Official Analytical Chemists to determine the fat content of milk and its products and was incorporated in the laws of many states. Following extraction of milk fat with the Mojonnier method, defatted solids may be determined by drying the residue which remain in the extraction flask after the solvents are removed. Different techniques may be used to determine solids and results agree with official methods. Weibull-Stoldt method is especially applicable for dairy products containing acidic dairy products and foreign substances other than milk and a high proportion of lactose (Metin, 2006), (Oysun, 1991).

#### **1.4 Method validation**

Method validation ensures that the decision is based on the results of many chemical measurements made in various areas, and that the analytical measurement result is accurate and reliable (repeated) in order to make the right decision. For this reason, the parameters affecting the measurement result of the method should be measured.

The confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Analytical methods need to be validated or revalidated:

before their introduction into routine use;

- whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- whenever the method is changed and the change is outside the original scope of the method (Taylor & Francis, 1998).

##### **1.4.1 Why is method validation necessary ?**

###### **•Ethical**

- Establish fitness-for-purpose on customer's behalf
- Good science

- Commercial
  - “due care” in product liability
- Regulatory/regulatory
  - Legal requirements
  - Consistent application of method
  - Comparability between analysts / laboratories / countries

From ethical point of view, method validation is important because the customer employs the expertise of the laboratory to do an analysis which it could not do by itself. It trusts the laboratory to use a fit for purpose method. The laboratory in turn should apply all aspects of good science to the problem - this includes appropriate validation of the methods used. It is good commercial sense to have some assurance that a measurement will be correct before it is carried out. Validation helps to provide that assurance. The unsatisfactory alternative is to carry the measurement out, detect errors and have to repeat the measurement. It is better to prevent problems from happening rather than have to correct them afterwards. In a production environment, the producer has a duty to have taken all reasonable care to ensure the quality of a product before releasing it to the consumer. Validation provides part of the minimum liability. In some areas, the validation of methods is a regulatory requirement. Compliance with Good Laboratory Practice (GLP), a legal requirement for certain types of study, requires technically valid operating procedures. Likewise methods must be validated according to ISO/IEC 17025 standard. Evaluation of method performance parameters during the validation process yields data that show which parts of the method are stable and which can cause problems in overall performance. Thus validation helps in the design and implementation of suitable quality control procedures. Method validation data provide information which enables the comparability of results from samples analysed in different laboratories and using different.

#### **1.4.2 When do you validate a method ?**

- During method development
- Before using any method for samples
  - verify own ability to match published data
  - verify suitability for analytical requirement
- Change of application / working environment / analyst

- Following period of non-use

Validation usually begins during the method development stage when some performance parameters are evaluated approximately to determine whether the method capabilities are in line with the levels required. Once the method is deemed good enough, the development phase finishes, giving way to more formal validation studies. Published methods may not necessarily be properly validated. The analyst is always advised to check the level of validation again that required and add further validation as needed. The analyst who uses the method routinely will not necessarily be the same with the one who has carried out the validation. Methods are sometimes validated in one part of a laboratory and then transferred to other parts for routine use. Whether the validated method is published or has been developed in-house, the analyst who will actually use it to analyse samples should first confirm that the validation data and subsequent fitness for purpose applies to the method when they are using it. This is sometimes known as verification. A change of use of the method, or use after a period of non-use, requires the validation to be checked. Extending the use of the method to different sample types, or analyte levels, will require the performance to be checked using the new type of samples. The effect of changes to other parameters such as analyst, instrument, or laboratory environment should also be checked.

#### **1.4.3 Who validates a method ?**

- The analyst
  - in house development and validation of new methods
  - verification of the performance of previously validated methods
- The laboratory
  - method development and validation section
- Sectoral / professional / standardiation body
  - validation of methods via interlaboratory study

It is principally the analyst who validates methods although they may work to a standard laboratory protocol to do so. It should be recognised that methods may also be validated by groups of laboratories co-operating in a collaborative trial. Validated methods may be published by sectoral, professional or standardisation bodies.

Large laboratories may have a central development section which develops and validates methods before passing them on to other sections for routine use.

#### **1.4.4 How do you validate a method ?**

- Decide analytical requirements
- Plan and carry out experiments to evaluate performance
- Use data to assess fitness for purpose
- Statement of validation

Method validation is not an accidental activity, it should be both deliberate and planned. The first stage is to examine the problem presented by the customer. Look at the reasons behind carrying out the analysis and find out what it is that the customer hopes to establish from having the work carried out. From this it should be possible to decide which method performance parameters are relevant to the work and what sort of target values are required. From this, a suitable experiment can be designed which can be used to evaluate the performance of candidate methods.

The plan will include details on what is going to be analysed at each stage, what degree of replication is required. It is possible that several parameters may be examined in one set of experiments in which case, the order in which things are done can be important. Once the plan is finalised the method parameters are evaluated and the data used to decide whether the method is fit-for-purpose. The statement of validation is the positive assertion of fitness-for-purpose.

#### **1.4.5 Ensuring Results are Correct**

Method validation is an essential part of the process of ensuring that measurement results reported to customers are correct. However, it is important to have other aspects under control such as satisfactory laboratory design, stable environmental conditions, suitable quality control (QC) procedures (all of these fall under the general heading of quality assurance (QA)). Well trained analysts are also essential to assess the analytical problem (understand the customer needs) and consequently determine the required method performance parameters.

#### **1.4.6 Method Performance Parameters**

Confirmation of identity/ Specificity/ Selectivity

Trueness (bias)

Precision (repeatability, reproducibility)

Limit of detection, limit of quantitation

Linearity, working range

Ruggedness/robustness

Sensitivity

Different method performance parameters will be important in different situations. Trueness has at most importance for calculating absolute values of properties or analytes. It should be noted that the term ‘accuracy’ is often used in documents/standards referring to method validation. Under current ISO definitions, accuracy is defined as the closeness of agreement between a measured value and a true value, and therefore includes the effect of both precision and trueness. Precision is important for all measurements but particularly in comparative studies. Working range will be of interest in most cases. For trace level work, limits of detection and quantitation may be relevant. For planning calibration strategies it may be useful to know over the range where the response is linear. The meaning of “sensitivity” depends on the sector in which it is used. In an instrument sense it refers to the rate of change of response with analyte concentration/property value. Medical and clinical chemists often use it as an alternative to limit of detection. Ruggedness studies, carried out mainly during method development will indicate which parameters need to be controlled in order to preserve performance. This in turn enables suitable quality control strategies to be devised (‘‘Method Validation Course 1125’’, 2015).

The aim of this study is to validate the gerber method to determine total fat amount in milk and dairy products and to estimate measurement uncertainty by using validation data.

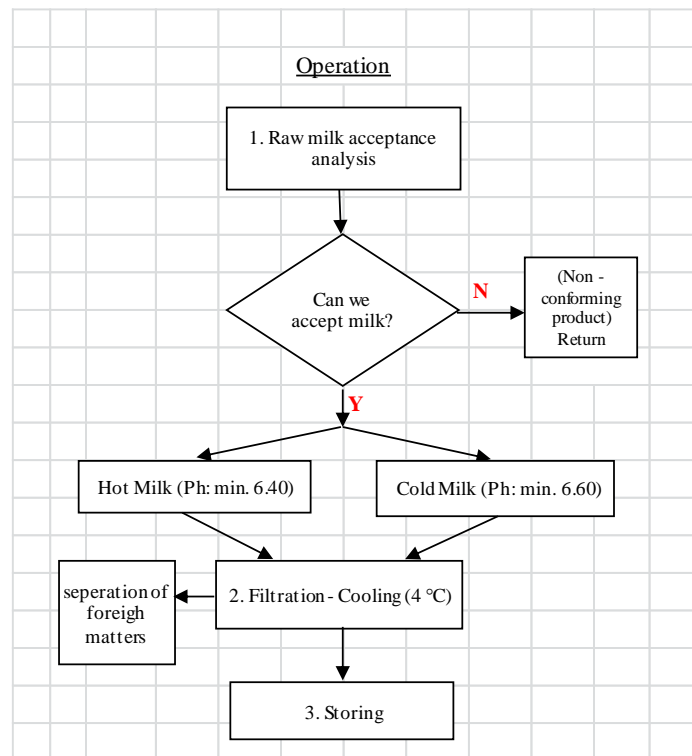


## CHAPTER 2

### THEORETICAL FRAMEWORK

## 2. Milk and Milk Products

### 2.1 Raw milk

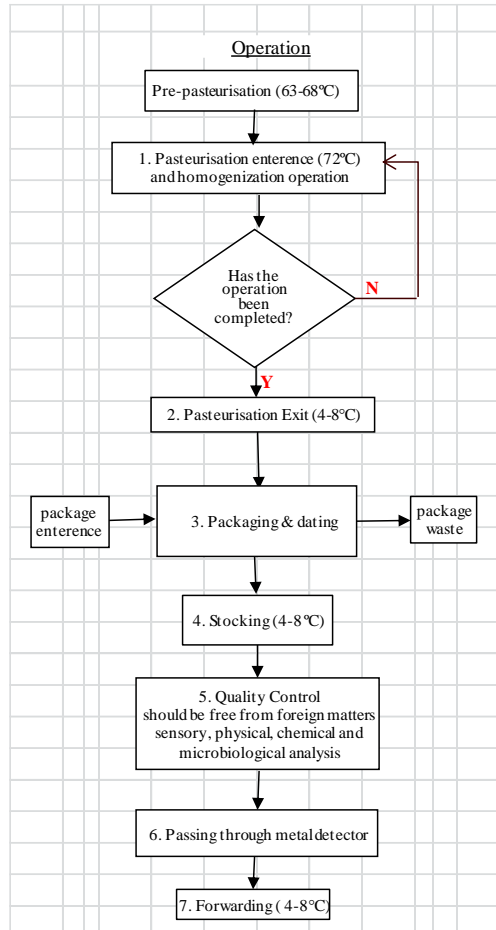


**Figure 2.1:** Raw milk flow diagram

Raw milk selection is the most important step to achieve a quality product. For high-quality milk, antibiotic is critical control point. The milk containing antibiotic should certainly not to be accepted by the factory. Referring to the other quality control fat, dry matter, pH, temperature, the residue and microbiology tests are carried out. However, analyzes made with the existing conditions in the laboratory are the search for presence of coliform in raw milk, temperature, pH, fat, dry matter and antibiotic residue test. Cold temperature should be below 6°C and pH should be minimum 6.60. The temperature of the hot milk should be maximum 24-25°C and the pH should be minimum 6.40. Milk is

filtered through the filters and purified from foreign materials, cooled at 4°C and stored in stirred milk tanks.

## 2.2 Pasteurized fresh milk



**Figure 2.2:** Pasteurized fresh milk production flow diagram

After raw milk processing, milk to be processed is first subjected to pasteurization at 63-68°C followed by pasteurization at 72°C. Pathogenic microorganisms are destroyed by pasteurization. If inadequate temperature and time is maintained, pathogenic microorganisms will develop and toxins will not die. Thus, pasteurisation is the critical control point. The milk passing through homogenization is pasteurized on the outlet is 4-8°C. The milk is taken to the tank in the direct filling machine and immediately bottled. Dates bottles filled with milk are stored at 4-8°C. Pasteurized fresh milk are tested in physical (color, taste, smell), chemical (pH, dry matter, fat, moisture), microbiological (*E.*

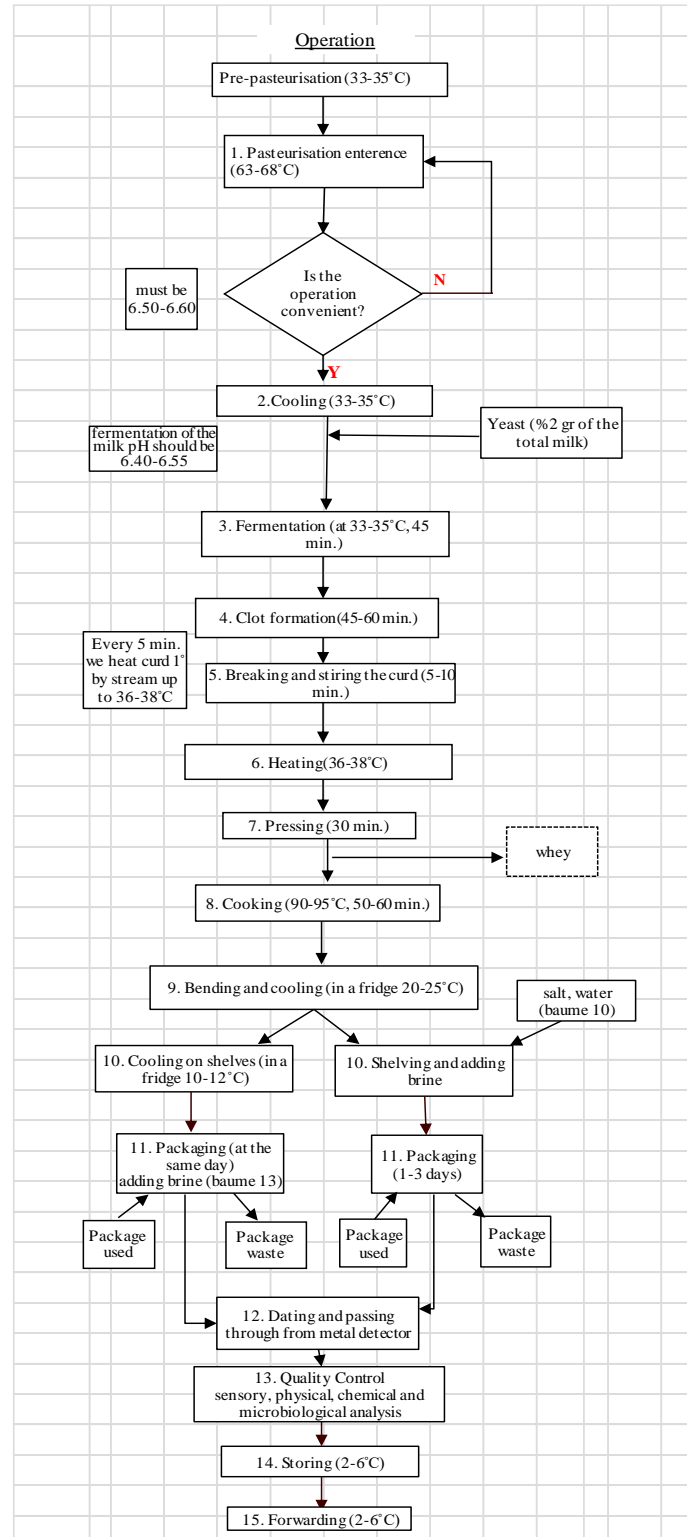
*coli*, coliform, *S. aureus*, mold, yeast). If the results of the analysis are in accordance with the relevant standards, they are shipped at 4-8°C through the metal detector. Metal contamination is a critical control point in pasteurized milk as well as in production.

### **2.3 Halloumi cheese**

The Milk to be used is first Pasteurized at 33-35°C. After pre-pasteurization, pasteurization is performed at 180 bar at 63-68°C to reduce the bacteria in the pod. If the pH is between 6.50-6.60, it is taken to halloumi production boiler and it is cooled to 33-35°C to prepare for fermentation. Ph is expected to fall between 6.40 and 6.55 for fermentation of the milk.

The yeast to be used should be 2% g of milk. For example; 2500 lt super 50 g yeast should be added. The fermentation takes place at 33-35°C for 45 min. After fermentation, clotting formation is expected to take 45-60 min for clot formation. The soft cheese grains that are formed in this stage are called curdling. For shredding and breaking, the mixers in the boiler are used for 5-10 min. At this time, 1°C steam heating increase process is performed in 5 minutes bysteaming. Heating continues until 36-38°C. The resulting screen is lowered to fill the plating tools in which the halloumi cheese patterns are arranged. Then the curd is pressed for 30 minutes so that the curd can bind together to form the halloumi cheese press-clamping is applied. Before pressing pH=6.35-6.42, after pH=6.10-6.24. The mold-pressed strands are cut and placed in trays then put into cooking cages. These cages are placed in a cooking boiler with water inside and preheated to 90-97°C. About 50-60 min. cooking process is applied. The second critical control point in halloumi cheese production is the development of non sterilized pathogenic microorganisms that can result from inadequate temperature and time deviations and toxin formation. For this reason, it is necessary to control the inner temperature of the halloumi cheese from several places towards the end of the cooking process. The internal temperature should be 90°C. The traditional structure of the halloumi cheese must have a whitish/ yellowish color, elastic structure and folded in two. This is followed by cooking, folding and cooling. In order to prevent microbiological contamination and deterioration during folding, the folding and the personnel performing this operation must comply with the necessary hygiene rules. Folding is done when halloumi cheese is hot. Salt is sprinkled on both surfaces of the halloumi cheese. Purposes of adding salt is provide taste formation, adjust pH, to create a

uniform structure and a protective layer on the surface, control microflora, increase product durability.



**Figure 2.3:** Halloumi cheese production flow diagram

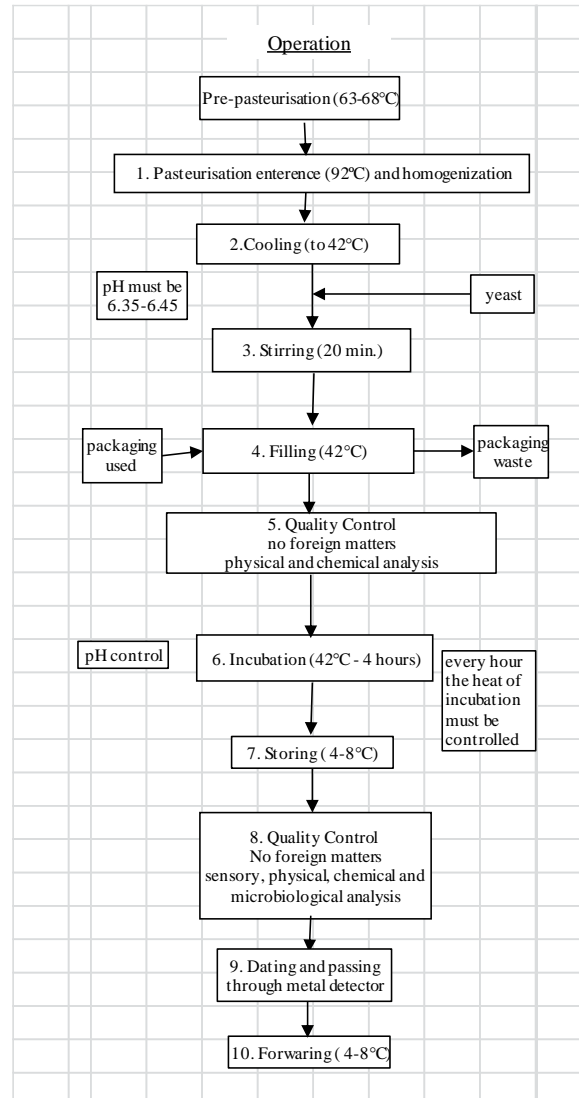
Then the corners are folded together and laid on shelves and cooled down to 20-25°C in the cold room (refrigerator). To prepare the brine salt is added (up to 10% of the water) and water should be 9-13 bome and heated up to 90-92°C thus removing microorganismfrom the brine. After pasteurization process, cooling process should be applied and cooled to 10-12°C. There are two packing ways for halloumi cheese. One of the methods is that it can be packed immediately when the halloumi cheese is cooled down on the shelves at 10-12°C. In this method, the brine is added to the package and gradually sucked into the warm hellim. Another method is to take the container full of hellim brine which is cooled to 20-25°C and packed for 24-72 hours at maximum 6°C. Packing vacuum time is 10 seconds and product weight is 250 g±3%. After packing, the date is stamped. To ensure that no metal contamination is in halloumi cheese production, the products passes through the individual calibrated metal detector for detection. It is important to remember that metal contamination is also a critical control point. Quality control must be made as the packed halloumi cheese is the last product. It should be offered for sale according to the relevant standards and company quality. Products should be kept between 2 and 6°C during storage and shipment. Test are carried out on the taste, structure, odour, colour, appearance in a from of microbiological analyzes, *S. aerues*, mold, yeast, *E. Coli*, coliform, chemical analysis, fat, pH, dry matter, moisture, salt, temperature and sensory analysis. Support can be taken from external laboratories.

## **2.4 Yoghurt**

### **2.4.1 Full fat yoghurt**

After storage of milk, the milk to be used for yoghurt production should be pre-pasteurized at 63-68°C and then for full pasteurization at 92°C for 3 seconds. If sufficient temperature and time are not provided for pasteurization, pathogenic microorganisms do not die, thus toxins develop in yoghurt. So pasteurisation is one of our laws for our production. After pasteurization, homogenization is applied to make the milk homogenous. If the milk fulfills the requirements of the standard, it is cooled to 42°C to add culture. When the pH of the soda is 6.35-6.45 then the culture is suitable to be added. It takes 20 min. to stir the milk. Then the milk is ready for filling the containers after. This is done at 42°C. During the filling, samples are taken to be analysed for quality control. The sample is then sent for

chemical analysis for foreign material control. The incubation and fermentation period is 4 hours at 42°C in a room.

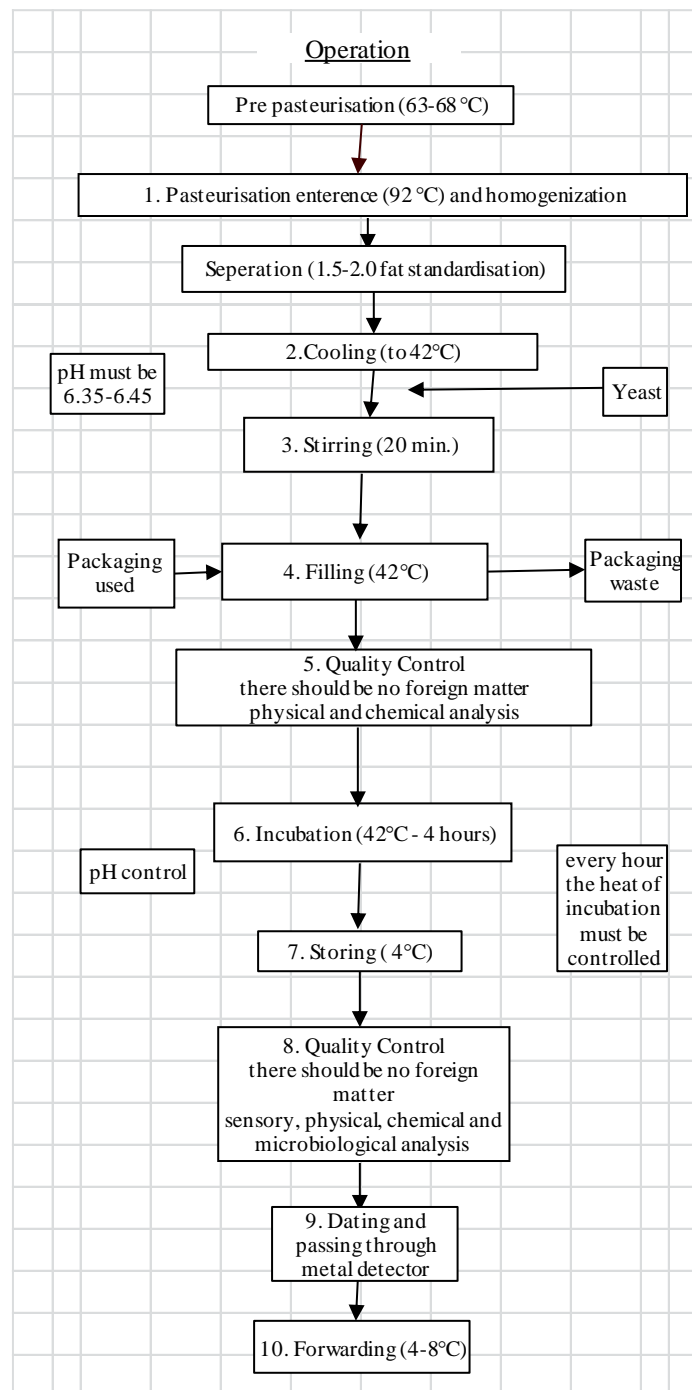


**Figure 2.4:** Full fat yoghurt production flow diagram

During the incubation period temperature is measured every hour. When the pH is reached to required level the incubation period ends. The yoghurt is placed in a fridge and stored at 4-8°C. On the next day, samples are taken and quality is checked. In the quality control, physical (smell, color, taste, structure, appearance) chemical (pH, humidity, dry matter, fat, salt), microbiological (*E. coli*, coliform, *S. aeureus*, mold and yeast) analyzes are made. The anaysis is checked whether the results comply with the relevant standards and quality

limits set by the factory. If the results are appropriate, the date is applied on the container. To check metal contamination during production, then the yoghurt is passed through metal detector. Having passed our critical control point our products are ready for shipment. During the shipment, the temperature must be at 4-8°C.

#### 2.4.2 Semi fat yoghurt

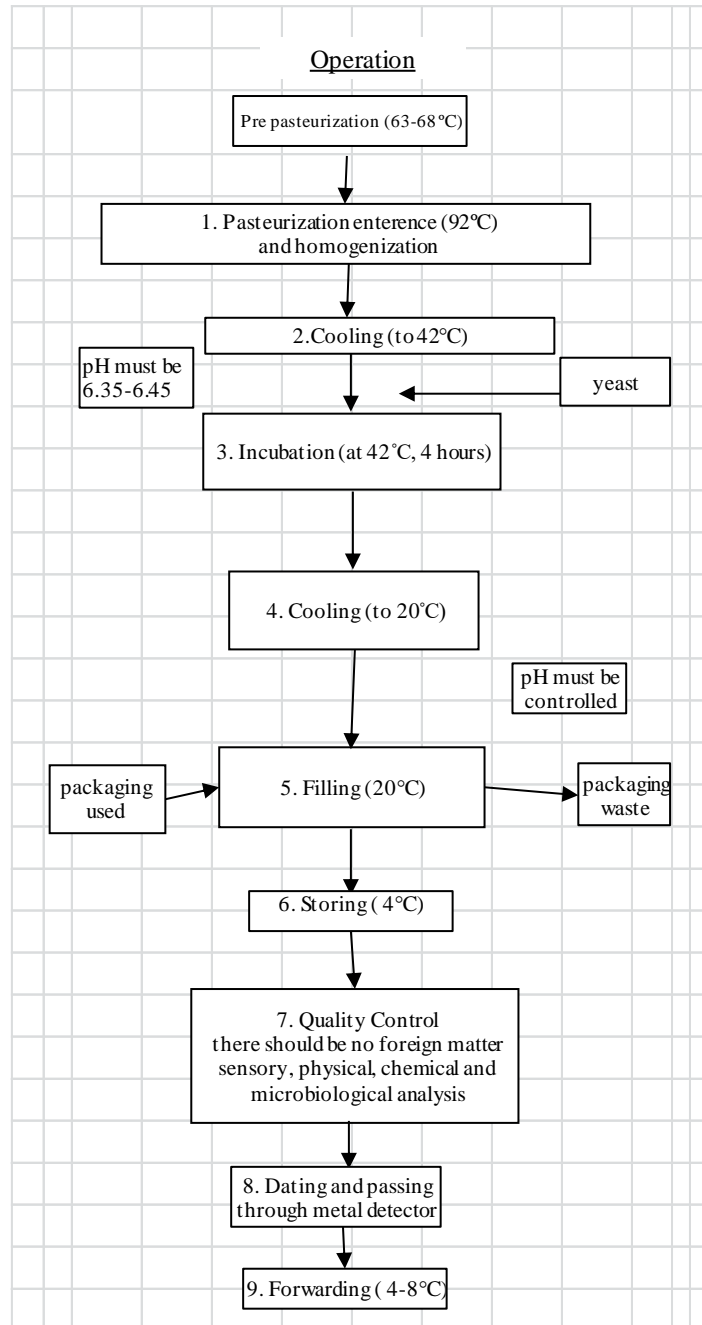


**Figure 2.5:** Semi skimmed yoghurt production flow diagram

Milk to be processed is pre pasturized at 63-68°C. To kill the pathogenic microorganisms 3 sec at 92°C. Pasteurization process is applied. But if heat and time are insufficient, microorganisms do not die and from toxins. Thus, pasteurisation is the critical control point. In order to disintegrate evenly the components of the milk is subjected to homogenization. We then reduce the proportion of milk fat using the separation so that the relevant standards are compatible with the semi-fat yoghurt. In order to add the culture the milk should be cooled down to 42°C. The culture is added when the pH reaches between 6.35 and 6.45. The culture is stirred for 20 min. mixed 42°C is filled in yoghurt bowls. During the filling, samples are taken to pass the quality control. This sample is subjected to foreign matter control and chemical analysis. For the formation of the consistency of the yoghurt, that is to say the completion of the fermentation process, the incubation is carried out at 42°C for about 4 hours. During the incubation, the temperature and pH are controlled every hour. When the pH is 4.30-4.35, the yoghurts are removed from the incubation and stored at 4-8°C. On the next day samples are taken and subjected to quality control tests. Tests are physical (taste, colour, structure, odour), chemical (pH, fat, dry matter, moisture, salt), microbiological (*E. coli*, coliform, *S. aureus*, mold and yeast) tests. If the test results are in accordance with the relevant standards, they are dated and passed through the metal detector. It is important to remember that metal contamination is a critical control point. Then the shipment is prepared. Shipping should be at 4-8°C.



### 2.4.3 Strained yoghurt



**Figure 2.6:** Strained yoghurt production flow diagram

After the raw milk treatment, the milk is pre-pasteurized at 63-68°C. Then pasteurization is done at 92°C to kill the bacteria. If time and temperature are insufficient, pathogenic microorganisms will not die and will develop toxins. The milk is cooled to 42°C Then the culture can be added when the pH is 6.35-6.45. Incubation is completed in the boiler for 4 hours at 42°C giving steam. This is what differentiates the most important stage of the

production of other strained yoghurt from other types of yoghurt. Then 20°C cooling is applied and 20°C should be maintained. The yoghurts are stored at 4°C. After 1 day samples are taken for quality control. The tests are physical (structure, odour, taste, colour), chemical (pH, dry matter, moisture, fat, salt), microbiological (*E. Coli*, Coliform, Mold, Yeast, *S. Aeureus*). If the obtained results are in accordance with the standard regulations. They are dated and pass through metal detectors for shipping. Metal presence is our critical control point. Shipping should be between 4-8°C.

## **2.5 Factors influencing the composition of milk**

Genetic characteristics: Each dairy breed produces nutrients in specific proportions. The influence of animal breed on the fatty acid profile in particular has been comprehensively investigated.

Volume of milk and stage of lactation: An increase in volume produced per animal results in composition changes, such as altered fat and protein profiles and decreased nutrient density which has a significant impact on the processing and retail industry.

Feeding regime: Increased knowledge about the impact of feeding on the quality and quantity of milk production has led to more sophisticated diet formulations for cattle. Since the 1980s feeding regimes of dairy cattle have been modified to induce changes in fat percentage, saturation, protein and lactose content, and to increase the amount of unsaturated fatty acids (for both health and manufacturing reasons). Alterations in grain feeding schemes, as well as the administration of biohydrogenated fat supplements, among others, have been used in the dairy industry to bring about these changes. Manipulation of the nitrogen content of raw milk has also been well reviewed. Research has shown that forage-to-concentrate ratio plays a significant role in the proportion of nitrogen in milk. Reducing the amount of forage, while increasing the amount of concentrate administered, increases both protein content and yield, while the amount and source of protein and fat in the diet has also been shown to alter the final protein composition. The amount of vitamins and other constituents in bovine milk is also directly influenced by factors such as feeding, genetics (cow breed) and stage of lactation (Schönfeldt, 2011).

## **2.6 Methods to determine the fat content**

Methods used to determine the fat in milk and dairy products are in the following:

### 1) Quick methods

- a) Butyrometric methods: Gerber method, Van Gulik method, Neusal method
- b) Photometric methods: Milkotester
- c) Infrared-spectrophotometric methods: Milkoscan

### 2) Reference methods

- a) Röse-Gottlieb method
- b) Mojonnier method
- c) Weibull-Stoldt method

### 1) Quick methods

#### a) Butyrometric methods:

- **Gerber method:**

Fat is the most important constituent of milk as it is used as a basis for fixing the purchase and sale price of milk. It helps to detect adulteration like watering and skimming of milk. The Gerber Method is a primary and historic chemical test to determine the fat content of raw and processed milks. The Gerber Method is the primary testing method in Europe and much of the world.

#### **Application area of Gerber Method**

- All types of milk
- Untreated and pasteurized milk with a fat content of 0-16% for milk which contains a suitable preservative as well as for homogenized milk.

#### **The principle of Gerber Method**

The determination of fat content according to Gerber involves running off the fat into a special measuring vessel, the butyrometer, and determining its volume as a percentage by mass. The fat is present in the milk in the form of small globules of various diameters, from 0.1 to 10 micrometers. The globules of fat form a consistent emulsion with the milk liquid. All globules of fat are surrounded by a protective coating, a fat globule membrane which is made up of phospholipids, a fat globule coat protein and hydrate water. This protein coating the fat globules prevents them from coalescing and stabilizes the emulsified state. In order to completely isolate the fat, the protection coating around the fat globules

must be destroyed. This is done with concentrated sulphuric acid of 90-91% by mass. The sulphuric acid oxidizes and hydrolyzes the organic components in the protective coating around the fat globules, the lactoprotein fractions and the lactose. This produces a high heat of reaction in addition to the heat of dissolution. The butyrometer gets quite hot. The oxidation products turn the resulting solution brown. The released fat is then isolated by centrifuging, whereby the addition of amyl alcohol facilitates phase separation and a sharp delineation is produced between the fat and the acid solution. The fat content of the milk can be read off as a mass percent content on the butyrometer scale.

### **History of Gerber Method**

The butyrometric determination of fat content in milk was developed by Dr. Niklaus Gerber in 1892 and incorporated into official regulations as a sulphuric acid process in 1935. The rapid testing method appears both in German standards (e.g. DIN 10479) and international standards (e.g. ISO 2446).

### **Advantages of Gerber Method**

- Omission of the need for time-consuming calibration of the measuring gauge
- Relatively low investment costs and hence low costs in performing quick tests on individual samples
- It can be used on all types of milk

### **The disadvantages of using Gerber Method**

The disadvantage is the use of very corrosive, concentrated sulphuric acid, which necessitates the observation of special precautions and the disposal of the sulphuric acid mixture in an environmentally suitable way.

### **Tools and materials used in Gerber Method**

Gerber centrifuge, pipets (1 ml, 10.75 ml), gerber butyrometers and plugs, sulphuric acids (%90-91 H<sub>2</sub>SO<sub>4</sub> with density  $d_{20}= 1.818\pm0.003$  g/mol and %90-91 H<sub>2</sub>SO<sub>4</sub> with density  $d_{20}=1.5$  g/mol), amyl alcohol ( $d_{20}= 0.811\pm0.003$  g/mol). ("Laboratory catalogue for milk analysis")

- **Van Gulik method:**

#### **Aim and application area**

It is the fast and easy determination of the amount of fat by butyrometric method in cheese. However, it is not recommended to use this method in some cheeses such as blue moldy cheeses, long-matured cheeses, cheese made from homogenized milk, cheeses with low and high fat content, cheeses with additives, cheeses made of different milk from cow's milk.

#### **Principle of method**

The inclusion of non-fat components in the cheese with concentrated  $H_2SO_4$  and amyl alcohol, the ratio of fat (fat in 100 g of cheese) as determined by a special Van Gulik butyrometer.

#### **Tools and materials used in Van Gulik Method**

Analytical scales (at least 0.1 mg in sensitivity), gerber centrifuge, water bath, Van Gulik cheese butyrometer and beaker, pipette and burette (1ml, 10ml), sulfuric acid (at  $20^\circ C$   $d_{20} = 1.522 \pm 0.005$  g/ml), amyl alcohol (at  $20^\circ C$   $d_{20} = 0.808-0.818$  g/ml)



**Figure 2.7:** Van Gulik Cheese Butyrometer

#### **Processes of Van Gulik Method**

Butyrometer beaker is weighed 3 g at a sensitivity of 0.005 g from the cheese sample and placed firmly on the bottom of the butyrometer. 10 ml of  $H_2SO_4$  is placed from the top of

the butyrometer and the top mouth is sealed with a special plug. Butyrometer 65-70°C water bath in the occasional bottom of the cheese is expected to melt completely. After complete dissolution of the cheese, remove the top plug and shake gently, adding 1 ml amyl alcohol. H<sub>2</sub>SO<sub>4</sub> is then added to the line 35 at the neck of the butyrometer. The top of the butyrometer is dried with a small blotting paper and closed again with the plug. Shake well from bottom to up. Butyrometer is waiting in the water bath for five minutes. The centrifugal butyrometers are placed facing and centrifuged at 1000-1200 rpm for 10 minutes. The butyrometers are kept in the water bath at 65-70°C for 4-5 minutes. At the end of the procedure, the % fat ratio is read directly from the butyrometer scale. The result obtained gives % fat content of the cheese at 100g.

- **Neusal method:**

The sulfuric acid used in the Gerber method is a dangerous substance. So there are some studies that do not require sulfuric acid, one of them is the Neusal method.

#### **Tools and materials used in Neusal Method**

Gerber centrifuge, gerber butyrometer, pipette (9.7 ml for milk), water bath (adjustable to ±65°C), butyrometer statif with bridge, neusal power (1 kg), isobutyl alcohol (860 ml)

#### **Preparation of Neusal Solution**

1000 g of Neusal powder is dissolved in 2.4 l of water. The solution is heated slightly, cooled to room temperature. 860 ml of isobutyl alcohol is added at room temperature. 3360 ml of solution are obtained. This solution is diluted with the same amount of water (3360 ml). Mix well, filter if necessary, filter paper or cotton, stored in a cool place. Deionized or distilled water should be used when preparing the solution.

#### **Processes of Neusal Method**

12 ml neural solution is placed in the butyrometer. 9.7 ml of milk is added and the butyrometer plug is closed. Shaken vigorously, the bottom is several times. It is kept in a water bath at 65±2°C for 5 min. The mixture is centrifuged for 5 minutes at 1000-1200 rpm. It is placed in a water bath set to 45°C for a short time.

**b) Photometric methods :**

In order to determine the amount of fat in milk by photometric method, tools similar to those described in the milkotester automatic fat analyzer were developed.

The milkotester automatic device has been widely implemented for routine analysis in recent years due to the large number of samples analyzed in a short time.



**Figure 2.8:** Milkotester

**Application area**

Whole milk, homogenized milk, skimmed milk, whey, cream

**Principle of machine**

Place the milk sample beakers on the bant. These beakers move forward automatically every 30 seconds. A mixer mixes the samples thoroughly, then 2 ml of milk is sucked through the milk pipette and the sample is transferred to a milk reservoir. Wherein a sprayer is mixed with a metered amount of dilution solution, which is solvent-soluble in the milk proteins. Then the mixture is homogenized in the high-pressure pump so that the desired size of the fat beads is reached. The homogenized mixture is sent to a micro cuvette. The optical density is measured by means of a photocell. As the light beams transmitted pass through the sample, this transition is more or less spread depending on the number of fat beads. The resulting turbidity is measured photometrically. The percentage of fat in the display is expressed directly up to 1/100 in numerical order. Results are passed on the strips or cards in a writing machine connected to the instrument.

The error limits on milkotester automatically increase as the fat level of the sample increases. At %2.2 fat grade, it varies from  $\pm 0.005\%$  to  $\pm 0.011\%$ , at %8 fat grade

$\pm 0.2016\%$  to  $\pm 0.041\%$ . According to these data; the milkotester automatic device is found to be more sensitive than the Gerber method, which has a margin of error of 0.05%.

### c) Infrared – spectrophotometric methods:

**Milkoscan:** This electronic instrument provides us the ability to quickly analyze milk samples for components (i.e. fat, protein, lactose, total solids and solids non-fat.). In basic terms, the instrument provides milk component results through infrared light measurement.



**Figure 2.9:** Milkoscan

## 2) Reference methods

- **Röse-Gottlieb method:**

This method used as reference method in international standards. It is a method of determination based on weight.

The fat which is free from the result of treatment of the protein around the fat spheres with ammonia is extracted with the aid of an organic solvent. after the extraction of an organic solvent, it is dried and amount find as gravimetrically.

### Application area

Whole milk, skimmed milk, UHT milk, churn, whey, sweetened and unsweetened darkened milk, milk powder, cream

### Tools and materials used in Röse-Gottlieb Method

Ammonia solution ( $d_{20} = 0.91$  g/ml, %25 (by weight)), ethyl alcohol (%94-97 (by volume)), diethyl ether ( $K_n = 34-35^\circ\text{C}$ , no peroxide and antioxidant inside), petroleum ether ( $K_n = 30-60^\circ\text{C}$ ), solvent mixture (ether and petroleum ether mixture by amount 1:1), NaCl (%0.5), scale (0.1 mg sensitivity), Röse-Gottlieb extraction container or Mojonnier



tube and plugs, statif (for extraction balloon), centrifuge(500-600 rpm), distillation balloon(flat bottom, round, 200-250 ml volume, weight <150 g), distillation scheme, boiling stone, water bath (adjustable to 60-70°C), drying cabinet(adjustable to 102±2°C)



**Figure 2.10:** Mojonnier fat extraction tube and plug

### **Processes of Röse-Gottlieb Method**

The sample is mixed well before analysis, and the oil is distributed homogeneously. The sample which connect clotted cream, is heated to 40°C, Mix well, cool to room temperature again. weighing is done at room temperature. The following quantities are taken for analysis.

Milk, whey, churn: 10-11 g

Skimmed milk: 10-11 g

Cream:

10% fat: 5 g

30% fat: 2 g

40-50% fat: 1 g

Sweetened condensed milk: 3.0-3.5 g

Unsweetened condensed milk: 4-5 g

Milk powder (fatty): 1.0-1.1 g

Milk powder (unfatty): 1.5-1.6 g

The method is usually worked in parallel. The purity of the chemical substances effect the result; blind trial should be performed using 10 ml of water instead of fat. If the blind test gives a value, this value must be taken into account in the calculation of the analysis results. The sample weighed in the above amounts in the extraction vessel is completed to 10 ml with distilled water. The cream sample is finished with 0.5% salt solution 10 ml instead of water. In the milk powder, the milk powder-water mixture is mixed regular intervals for 15 minutes at 60-70°C water bath. Add 1.5-2 ml ammonia solution with a safe

pipette and mix. It is recommended to use either concentrated ammonia (35%) or 3 ml in acidic products. 10 ml to prevent formation of emulsion after cooling alcohol is added, mixed. Add 10 ml of alcohol to better monitor the separation of the layers. In order to better observe the separation of the layers, two drops of congo red solution or phenolphthalein may be added. With the addition of 25 ml of diethyl ether, the lid of the extraction container is sealed and the contents mixed well for one minute, the lid should not come into contact with the mixture. 25 ml of petroleum ether are added, followed by stirring in the same manner. In order to remove the diethyl ether-petroleum ether layers from the water phase, the extraction vessel is allowed to stand for 2 hours or centrifuged at 500-600 rpm for 5 minutes. The cover is removed, the upper part is dried in the drying cabinet with boiling stones and weighed 0.1 mg. The second extraction is carried out by addition of 5 ml of ethyl alcohol, 15 ml of diethyl ether and 15 ml of petroleum ether while the processes described previously are also applied. In the third extraction 30 ml of solvent mixture is added instead of the individual substances. At the opening of the extraction vessel, the sealer is washed with a small amount of solvent mixture. The second and third extracts are also collected in the distillation flask. The distillation balloon is connected to the distillation apparatus and the solvent is distilled off from the oil at 60-70°C on a water bath. The distillation balloon is dried for 1 hour in the drying cabinet at 102±2°C. In a clean environment, cool to room temperature and weigh to 1 mg. The constant weight is checked again by 60 minutes each time. this process continues until the weight reduction is less than 1 mg.

Maximum allowed value in blind trial = 1mg

Calculation of the result:

Amount of fat in% g:

$$Y = (A - B) / E \times 100$$

A: Weighing

B: Blind trial

E: Sample quantity

The result is indicated up to 1/100 (two digits after the comma). The difference between the results of the parallel test can be calculated in the cases where 0.03% of whole fat milk,

skimmed milk, low-whey, whey, sweetened and unsweetened darkened milk do not exceed 0.2% in full-fat milk powder, non-fat milk powder, cream at 0.2%.

- **Mojonnier method:**

This is the chemical method of choice for total milk fat because of its inherent analytical capacity. It can provide a very precise measurement of milk fat. Because of its precision, the ether extraction procedure is the preferred chemical reference method for milk fat. Ether and alcohol are used to extract the fat from milk. The ether extract is decanted into a dry weighing dish and the ether is evaporated. The extracted fat is then dried to a constant weight and is expressed as fat percent by weight (“Laboratory test procedures”,2013).

#### **Procedure of mojonnier method**

- Weight 10 g milk to Mojonnier flask



**Figure 2.11:** Mojonnier fat extraction flask

- Add 1,5 ml of  $\text{NH}_4\text{OH}$  and shake vigorously
- Add 10 ml of 95% ethanol and shake for 90 sec
- Add 25 ml of ethyl ether and shake for 90 sec
- Cool if necessary, and add 25 ml of petroleum ether and shake for 90 sec
- Centrifuge for 30 sec at 600 rpm



**Figure 2.12:** Centrifuge for mojonnier extraction

- Decant ether solution from flask into previously weighed Mojonnier fat dish



**Figure 2.13:**Mojonnier fat dish

- Perform 2nd & 3rd extractions in the same manner (ethanol, ethyl ether, petroleum ether, centrifuge, decant)
- Evaporate solvent in the dish on a hotplate <100°C in
- Cool dish to room temperature and weigh

#### Calculations

$$\% \text{Fat} = 100 \times \{[(\text{wt dish} + \text{fat}) - (\text{wt dish})] - (\text{avg wt blank residue})\} / \text{wt sample}$$

Blanks must be prepared every day

Use 10 ml of water instead of milk

The blank should be <0.002 g

Duplicate analyses should be <0.03% fat

- **Weibull-Stoldt method:**

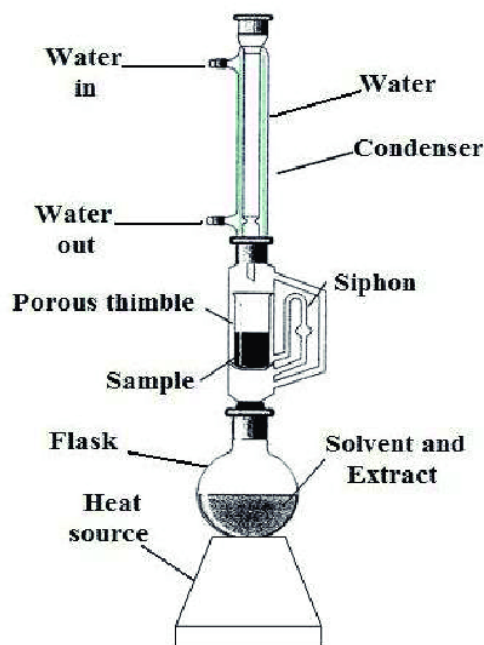
The fat is very stable to hot HCl. however, proteins and carbohydrates are easily hydrolyzed. Therefore, by the end of the heating with HCl, the water insoluble oil is separated and separated from the solutes, is washed with hot water from the filter, filtered and dried and extracted with ether. The ether is evaporated or distilled off, the remaining fat is dried and gravimetrically determined.

#### Application area

Acidified milk and cream, yoghurt, ice cream, coagulated whey products, dried whey products

### Tools and materials used in Weibull – Stoldt Method

Sensitive scales (at least 0.1 mg in sensitivity), drying cabinet, breaker, glass baguette, glass bead, clock glass, filter paper, funnel, distillation balloon, Soxhlet or twisselmann extractor, water bath, fume hood



**Figure 2.14:** Soxhlet extractor

### Processes of Weibull – Stoldt Method

Weigh 5g of the homogenized cheese sample into the beaker and make up to 80 g with distilled water. Before the water is given, very few parts are mixed; then all are added. 80 ml of 25% HCl is added. It is closed with a clock glass, and there is a the glass bead, is started to boil slowly. First it is foaming strongly. It is boiled for 30 minutes, on the otherhand you check that if there is any unresolved sediment. When completely dissolved, it is diluted twice with warm water at the initial volume. Previously filtered through the folded filter paper moistened with hot water. The filter containing the fat portion is washed at least three times with warm water in the beaker. After fully filtering, the filter paper is dried on the watch glass at 102°C for 2-3 hours. The dried filter paper which is dried for 2-3 hours, is extracted into the pre-dried and weighed extraction flask with diethyl ether in the Soxhlet or Twisselmann extractor. The fat balloon is allowed to dry for one hour at 102°C, preferably in the recumbent position. Cool at room temperature for at least 30 minutes and weigh. The drying process continues until the constant weighing.

Try to be carried out together with the blind trial in order to check the purity of the chemicals.

The result is calculated with following formula on weight basis:

$$\text{Fat (\%)} = (A - B) * 100 / E$$

A: Remaining fat at balloon (g)

B: Remaining residur at balloon at blind trial (g)

E: Sample quantity (g)

(Metin, 2006), (Oysun, 1991).

## **2.7 Method validation**

Method validation is necessary for all kind of laboratory analysis. Validation has three important parts, these are:

1. the specific intended use or application, is the analytical requirement which derives from the problem that the analysis is intended to solve; this is clarified during the discussions between the laboratory and the customer as part of contract review.
2. the objective evidence is usually generated data from planned experiments, from which the appropriate method performance parameters are calculated;
3. the confirmation is taken as a satisfactory comparison of the performance data with what is required, i.e. demonstrating that the method is fit for purpose. Advice on how to do method validation is laid out in a number of guides - the actual procedures may vary from sector to sector. It is always worth following any guidance available for the particular sector, so that validation procedure is compatible with that in peer laboratories. Where particular conventions have been followed these should be stated. There are a number of different definitions of validation but they are broadly in line with the definition of ISO Guide 99:2007 (International vocabulary of metrology – Basic and general concepts and associated terms (VIM)) which defines validation as, verification, where the specified requirements are adequate for an intended use and verification as, provision of objective evidence that a given item fulfils specified requirements (“Method Validation Course 1125”, 2015).

### **2.7.1 Method Performance Acceptability Criteria**

In method validation studies, several performance characteristics may be investigated, depending on the type of method and its intended use. These are summarized below:

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components. In drug assays, specificity takes into account the degree of interference from other active ingredients, excipients, impurities, degradation products, or matrices, and ensures that a chromatographic peak corresponds to a single component. Specificity can be demonstrated by the resolution between peaks of interest. Modern chromatographic methods typically include a peak-purity test based upon photodiode-array detection or mass spectrometry.

Accuracy is the closeness of test results to the true value. For drug substances, accuracy measurements are obtained by comparing test results to the analysis of a standard reference material or to a second, well-characterized method. For drug products, accuracy is evaluated by analyzing synthetic mixtures (containing all excipient materials in the correct proportions) spiked with known quantities of analyte. Guidelines recommend that data be collected from a minimum of six determinations over at least three concentration levels covering the specified range. The data should be reported as the percent recovery of the known, added amount, or as the difference between the mean and true value with confidence intervals (such as  $\pm 1$  SD). Accuracy determination ranging 70-130% of expected content will satisfy requirements. Statistical analysis can be applied using a one sample t-test.

Precision measures the degree of agreement among test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision is commonly described in terms of repeatability, intermediate precision, and reproducibility. Repeatability is investigated by analyzing a minimum of six determinations using the same equipment and sample, covering the specified range of the procedure, or a minimum of six determinations at 100% of the test concentration and reported as percent relative standard deviation (RSD). Intermediate precision refers to the agreement among the results from a single laboratory, despite potential variations in sample preparation, analysts, or

equipment. Reproducibility refers to the agreement among the results from different laboratories. Results are reported as % RSD, and the percent difference in the mean values between the analysts must be within specifications. Less than 2% RSD is often recommended, but less than 5% RSD can be acceptable for minor components.

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be quantified with acceptable precision and accuracy under the stated operational conditions of the method. In a chromatography laboratory, the most common way to determine both the LOD and the LOQ is using signal-to-noise ratios (S/N), commonly 3:1 for LOD and 10:1 for LOQ. An appropriate number of samples must be analyzed to fully validate the method performance at the limit.

Linearity is the ability of a method to provide results that are directly proportional to analyte concentration within a given range. Range is the interval between the upper and lower concentrations of an analyte that have been demonstrated to be determined with acceptable precision, accuracy, and linearity using the method. The range is normally expressed in the same units as the test results obtained by the method (for example, nanograms per milliliter). Guidelines specify that a minimum of five concentration levels be used to determine the range and linearity, along with certain minimum specified ranges depending on the type of method. Data to be reported generally include the equation for the calibration curve line, the coefficient of correlation ( $R^2$ ), standard deviation of relative residuals and the curve itself.

Ruggedness is a measure of a method's capacity to obtain comparable and acceptable results when perturbed by small but deliberate variations in procedural parameters; it provides an indication of the method's suitability and reliability during normal use. During a ruggedness study, method parameters (such as eluent composition, gradient, and detector



settings) are intentionally varied to study the effects on analytical results. Common chromatography parameters used to measure and document robustness include critical peak pair resolution ( $R_s$ ), plate number ( $N$ ) or peak width in gradient elution, retention time ( $t_R$ ), tailing factor ( $T_F$ ), peak area (and height) and concentration.

Dynamic range is the range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity ("Analytical Procedures and Method Validation", 2000).

### **2.7.2 How should methods be validated?**

The laboratory using a method is responsible for ensuring that it is adequately validated, and if necessary for carrying out further work to supplement existing data. Usually national or international organizations, such as AOAC International, ISO, have undertaken the interlaboratory validation of the method in a method performance (collaborative) trial. The extent of laboratory internal validation and verification depends on the context in which the method is to be used. If a method is being developed which will have wide-ranging use, then collaborative studies involving a group of laboratories is probably the preferred way of carrying out the validation. However, it is not always a suitable option for industrial laboratories, since those that might be interested could be competitors. Whether or not methods validated in a single laboratory will be acceptable for regulatory purposes depends on any guidelines covering the area of measurement concerned. The type of method and its intended use indicates which validation parameters need to be investigated. The laboratory has to decide which performance parameters need to be characterised in order to validate the method. Characterisation of method performance is an expensive process and inevitably it may be constrained by time and cost considerations. Some of the parameters may have been determined approximately during the method development stage. Often a particular set of experiments will yield information on several parameters, so with careful planning the effort required to get the necessary information can be minimised. Validation requirements may be specified in guidelines within a particular sector of measurement relevant to the method and it is recommended that where these are available they are followed.

### **2.7.3 Method Validation Strategy**

The necessity for laboratories to use a ‘fully validated’ method of analysis is now universally accepted or required within many sectors of analysis. Most method validation guides start with discussions on how criteria such as specificity, accuracy and precision of the method shall be established. The analytical problem, requirements of the customers and choices of analytical principles are seldom mentioned in this context. The first step in a ‘full validation procedure’ therefore should be to identify and document ‘customer requirements’ and the analytical problem, what is analytically and economically possible and other specific requirements on sampling, laboratory environment, external environment, etc.

This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; turnaround time; and environmental, health and safety requirements. Faced with a particular analytical problem, ideally, the laboratory should firstly agree with the customer an analytical requirement, which defines the performance requirements that a method must achieve to solve the analytical problem. In response to this requirement, the laboratory can evaluate existing methods for suitability and if necessary develop a new method. This iterative process of development and evaluation continues until the method is deemed capable of meeting the requirement; further development is unnecessary and the analytical work can proceed. This process of evaluation of performance criteria and confirming that the method is suitable.

Here are some recommendations for the use of a singlelaboratory method validation:

- Wherever possible and practical, a laboratory should use an analysis method whose performance characteristics have been evaluated through a collaborative trial that conforms to an international protocol.
- When such methods are not available, an in-house method must be validated before being used to generate analytical data.
- Single-laboratory validation requires the laboratory to select appropriate characteristics for evaluation (e.g., selectivity, calibration, accuracy, etc.).

- Evidence that these characteristics have been assessed must be made available.

During method validation, the parameters, acceptance limits and frequency of ongoing system suitability tests or quality control checks should be defined. Criteria should be defined to indicate when the method and system are beyond statistical control. The aim is to optimize these experiments so that, with a minimum number of control analyses, the method and the complete analytical system will provide long-term results to meet the objectives defined in the scope of the method.

#### **2.7.4 Transferring Validated Routine Methods**

When validated methods are transferred between laboratories the receiving laboratory should demonstrate that it can successfully perform the method and their validated state should be maintained to ensure the same reliable results in the receiving laboratory. This means the competence of the receiving laboratory to use the method should be demonstrated through tests, for example, repeat critical method validation experiments and run samples in parallel in the transferring and receiving laboratories. Typical instances when method transfer occurs are from the Research and Development (R&D) laboratory to the Quality Control (QC) laboratory. Currently, there is no official document available that can be used as a guide for performance demonstration of the receiving laboratory. However, the USP has published an article where the most common practices of method transfer are described : comparative testing, co-validation between two laboratories or sites, complete or partial method validation or revalidation, and the omission of formal transfer, sometimes called the transfer waiver. The transfer should be controlled by a procedure. The recommended steps are: (1) designate a project owner; (2) develop a transfer plan; (3) define transfer tests and acceptance criteria (validation experiments, sample analysis: sample type, replicates); (4) describe rationale for tests; (5) train receiving laboratory operators in transferring laboratory on equipment, method, critical parameters and troubleshooting; (6) repeat 2 critical method validation tests in routine laboratory; (7) analyze at least three samples in transferring and receiving laboratory; and (8) document transfer results.

### **CHAPTER 3**

#### **RELATED RESEARCH**

Method validation has received considerable attention in the literature and there are several guidelines available for analytical and bio-analytical aspect and they are as follows:

- a. The United States FDA established two industrial guidelines. First one for the validation of analytical methods (this guidance provides recommendations to applicants on submitting analytical procedures, validation, data and samples to support the documentation of the identity, strength, quality, purity and potency of drug substances and drug products) and second one for the validation of bioanalytical methods (this guidance applies to bioanalytical methods used for human or non-human clinical, pharmacological, toxicological studies and preclinical studies-based on bioanalytical procedures such as chromatography, immunology and microbiology).
- b. ICH developed two guidelines for method validation that were later merged in one: Q2-R1. It discusses the considered characteristics (terminology and definitions) and methodology to be used during the validation of the analytical procedures.
- c. International Union of Pure and Applied Chemistry published “Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis”. This guideline provides minimum recommendations on procedures that should be employed to ensure adequate validation of analytical methods.

The above-described guidelines are mainly focused on seven common parameters that should be considered during bio-analytical method validation in order to establish the method “fit-for-purpose” (Naz et al., 2014).

There are various researches about method validation in literature. Some of researches are summarised below:

De Langen has studied determination of fat in meat and separable fat by the Gerber test. A modified Gerber fat test for the determination of fat in meat and separable fat is described. A factor of 0.935 is applied to correct for the difference between the S.G. of butterfat and body fat. Average recovery of purified animal fat was 99.9 per cent. Samples containing more than 3 per cent fat showed lower variability with the Gerber test than with the solvent extraction method. For routine work on animal tissue and fat the Gerber test was found to be more rapid and reliable than the solvent extraction method.

The validity of the application of a correction factor of 0.935 (calculated directly from the S.G. differences) to the Gerber test readings for the determination of fat in meat and separable fat was tested by comparing the recoveries, uncorrected and corrected, from 4 purified fat samples. Mean results of 6 estimations and the standard deviations are 0.66, 0.61, 0.96, 0.50. A comparison was made of results from meat and separable fat samples by the solvent extraction method and the Gerber test using the correction factor with the latter. The range, mean, and standard deviation calculated from 12 estimations on each sample. Assuming that the solvent extraction gives the true fat content, the good agreement between the 2 methods indicates the validity of the correction factor, and the precision and accuracy of the Gerber procedures. Standard deviations of estimations by the Gerber test were appreciably lower than those obtained by the solvent extraction method except with the 2 samples containing less than 3 per cent fat. This was probably due to error in reading low values on the butyrometer scale calibrated in 0.1 per cent divisions (De Langen, 2012).

Kleyn et al. (2001) has studied determination of fat in raw and processed milks by the Gerber method in a collaborative study. The Gerber method is used worldwide as a simple and rapid method for determining fat in raw and processed milks. However, the volume of the test portion used in the method has not been internationally agreed upon. A collaborative study was conducted to evaluate performance of the Gerber method using either a weighed test portion (11.13 g) or a 10.77 mL test portion delivered by pipet. For each method, laboratories received 10 test samples: 5 raw and 5 pasteurized homogenized

milks, 2 of which were blind duplicate pairs. Eleven and 10 laboratories participated in the evaluation of aliquot addition by weight and pipet, respectively. Mojonnier ether extraction (Method 989.05) was used as the reference method. Interlaboratory study statistics were similar between methods of test portion addition and between raw and processed materials; therefore, summary interlaboratory study statistics were pooled. The fat content of milk samples ranged from 0.96 to 5.48%. Absolute reproducibility and repeatability were not affected by fat level, and pooled statistical performance (invalid and outlier data removed) was (g fat/100 g milk)  $s(r) = 0.026$ ,  $s(R) = 0.047$ ,  $r = 0.074$ , and  $R = 0.132$ . Relative standard deviations increased with decreasing fat content, and were summarized by fat level: 1-2% fat milk, mean = 1.437,  $RSD(r) = 1.809\%$ ,  $RSD(R) = 3.271\%$ ; 2-6% fat milk, mean = 4.156,  $RSD(r) = 0.626\%$ ,  $RSD(R) = 1.131\%$ . Compared with ether extraction, test results by the Gerber method were slightly lower (0.02% fat) using a weighed test portion and significantly lower (0.06% fat) using a 10.77 mL volume addition by pipet. A trend toward underestimating fat content at lower fat concentrations (1-2% fat) was observed with the weighed test portion but not when a pipet was used. The Associate Referee recommends that the Gerber method using a weighed test portion be adopted as First Action with applicability limited to whole milk (Kleyn et al., 2001).

Peeler et al. (1989) has studied precision parameters of standard methods of analysis for dairy products. The available collaborative studies for standard methods of analysis for various constituents of milk and milk products were examined in an attempt to assign specific repeatability and reproducibility precision parameters to these methods. The different collaborative assays for the primary constituents (moisture/solids, fat, protein), the nutritionally important elements (calcium, sodium, potassium, phosphorus), and miscellaneous analytes/physical constants (ash, lactose, salt, freezing point) produced different estimates of the precision parameters for the same method. A suitable summary of the precision estimates from collaborative studies is given by the reproducibility relative standard deviation,  $RSDg$ , which is relatively constant within a product and permits comparisons across products. An estimate of the variation of  $RSDR$  for an analyte from a number of collaborative studies is presented in terms of the median and 90% interval (the range of the centermost 90% of values). These estimates are only informative when a substantial number of independent studies are available for pooling the independent

estimates to form a distribution of RSDR values. The RSDR for the determination of the primary constituents of milk and milk products is characterized by a median RSDR of 1% and a 90% interval of 0.3-3%, with RSDR estimates occasionally occurring below 0.3% and above 4%. These overall estimates appear to be independent of analyte, matrix, and method and apply to concentrations of primary constituents that range from about 2 to 80%. The repeatability relative standard deviation, RSD<sub>r</sub>, is unstable, although it tends to converge to about 0.5-0.7 X RSDR. Too few collaborative assays are available to characterize RSDR for the determination of certain other constituents (acidity, ash, lactose, salt, and the nutritionally important elements) unless RSDR values for different analytes, methods, and matrixes are pooled on the basis of similar analyte concentrations. When pooled, the RSDR values are generally better than predicted from the Horwitz equation,  $RSDR (\%) = 2 \exp (1-0.5 \log_{10} C)$ , where C is the concentration expressed as a decimal fraction; all but one of 661 RSDR values are within the upper empirical limit of twice this curve (Peeler et al., 1989).

Horwitz et al. (1990) has studied precision parameters of the methods required for nutrition labeling. Part I. Major nutrients. Major components of foods and feeds are fat, protein, and carbohydrates. Fat and protein are determined by direct measurements that are interpreted as the quantity of the constituent. Carbohydrates are usually calculated by difference. For this calculation, values for moisture/solids, ash, and fiber are also needed. The readily available collaborative studies for the determination of these major components are reviewed in an attempt to assign precision parameters to validated methods of analysis. When a number of studies for the same analyte, in the same food, by the same method are available, it is seen that the precision parameters among laboratories (standard deviations, SR; relative standard deviations, RSDR) and the ISO maximum tolerable difference functions (repeatability value, r; reproducibility value, R) are not characterized by any conventional distribution. The precision data are best summarized as a median or average parameter and the interval containing the centermost 90% of reported values. Typically, the precision of methods of analysis can be expressed as a function of concentration only, independent of analyte, matrix, and method. The average RSDR value from each collaborative data set can then be used as the numerator in a ratio containing, as the denominator, the value calculated from the Horwitz equation:  $RSDR = 2 \exp (1 - 0.5 \log$

C) where C is the concentration as a decimal fraction. A series of ratios consistently above 1, and especially above 2, probably indicates that a method is unacceptable with respect to precision. By this criterion, only the protein (Kjeldahl) determination is unqualifiedly acceptable with a 90% interval for RSDR of 1 to 3% at C values above about 0.01 (1 g/100 g). Fat, moisture/solids, and ash are acceptable down to limiting concentrations in the region of 1 to 5 g/100 g, if a test portion large enough to provide at least 50 mg of weighable residue or volatiles is specified. Measurements of individual carbohydrates and fiber-related analytes have unexpectedly poor precisions among laboratories. The variability, although high, may still be suitable for nutrition labeling. Reliability of analyses for the control of labeling of the primary nutrients must be achieved through quality assurance programs that require strict adherence to the directions of empirical methods and the use of suitable reference materials for absolute methods (Horwitz et al., 1990).

Gurd et al. (2018) has studied determination of fats, oils and greases in food service establishment wastewater using a modification of the Gerber method. Discharges from food service establishments (FSEs) are a major source of fat, oil and grease (FOG) which cause blockages in sewer networks. Previous research has identified that current methods are unsuitable for quantifying FOG in FSE wastewater owing to interference from surfactants in detergents, and protein from food residuals which emulsify FOG. A novel quantification method, based on the dairy industry Gerber method, has been developed which negates the impact of surfactants. Moreover, the method allows free and emulsified oil to be quantified separately providing greater insight into FOG management strategies. Trials in synthetic and real FSE wastewaters indicate the novel method is more reliable than standard liquid–liquid and solid phase extraction in FOG-rich systems.

The development of a modified Gerber method for FOG measurements in FSE wastewater, coupled with a free oil pre-measurement, has been demonstrated to enable more consistent FOG recovery levels than typically observed in the current standard methods. Furthermore, the addition of a casein precipitation stage has enabled application to non-dairy systems and negated the impact of surfactant on the reliability of FOG measurement in FSE wastewaters experienced in the other methods. Whilst the LOQ of the new method is higher than



standard liquid–liquid extraction techniques it has both excellent recovery and precision down to below the 100 mg/LFOG level making it suitable for discharge monitoring. The technique is simple, inexpensive and rapid in comparison to standard methods enabling more consistent sampling to be undertaken. Furthermore, the simple separation of free and emulsified oil contents proposed in this method has the opportunity to greatly enhance insights into management options and support innovation in the sector (Gurd et al., 2018).

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 Materials

Milk, yoghurt and halloumi samples containing different levels of fat were used as material for validation experiments. All samples were also analyzed by an accredited laboratory for the fat content. These values were used as reference values for the calculations.

##### a) Preparing of the milk samples

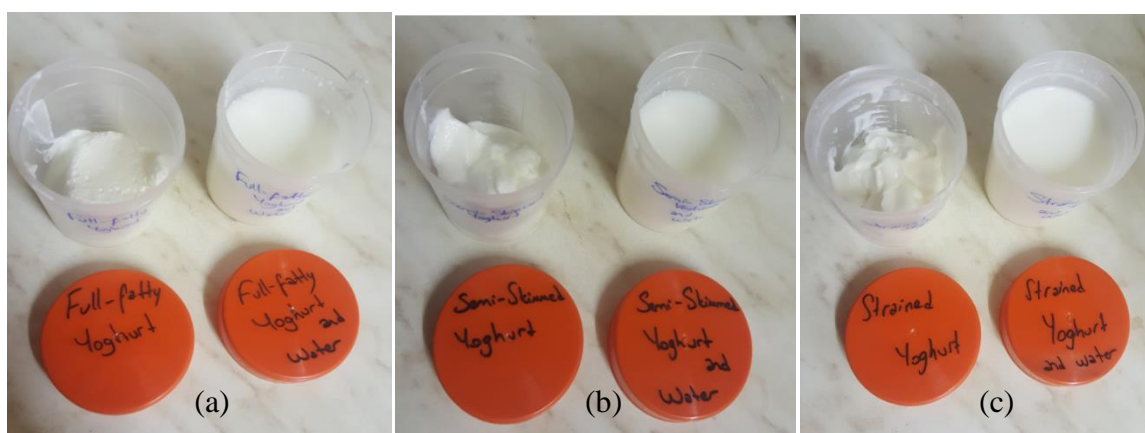
Raw milk was initially heated to 63-68°C for 30 minutes and then to 72°C for 15 seconds in a continuous system. After homogenization, pasteurized milk was immediately cooled down to 4-8°C and stored in cold storage maintained at 4±1°C. For method validation studies, two types of milk were used, i.e. full fat milk containing approximately 3% fat and semi-fat milk with 1.7% (Figure 4.1 a and b).



**Figure 4.1:** (a) Pasteurized full fat fresh milk (b) Pasteurized semi fat fresh milk

### b) Preparing of the yoghurt samples

Milk to be used for yoghurt production should be pasteurized at 63-68°C for 30 minutes and then at 92°C for 3 seconds. After homogenization, milk was cooled to 42°C; yoghurt culture was added and mixed by stirring for 20 minutes. Milk was transferred to yoghurt cups and hold at 42°C for 4 hours in a fermentation room. The yoghurt samples were placed in a fridge and stored at 4-8°C. On the next day, we took the yoghurt samples for fat analysis. The yoghurt samples were diluted 1:1 with distilled water before analysis. In this study, full fat yoghurt, semi skimmed yoghurt and strained yoghurt (Figure 4.2 a, b and c) were used for fat analysis.

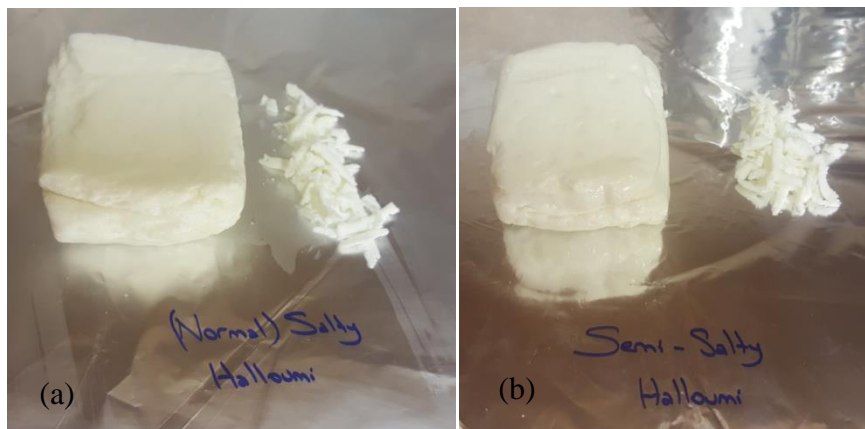


**Figure 4.2:** (a) Full Fat Yoghurt (b) Semi Skimmed Yoghurt (c) Strained Yoghurt

### c) Preparing of the halloumi samples

Milk was pre-pasteurized at 33-35°C for 15 minutes. After pre-pasteurization, full pasteurization was performed at 180 bar at 63-68°C for 30 minutes. Wait the milk pH to be between 6.50-6.60, then milk taken to halloumi production boiler and cooled to 33-35°C. 2% of the rennet solution was added. The fermentation takes place at 33-35°C for 45 min. After fermentation, curd is allowed to rest 45-60 min for solid mass formation. To cut curds, the mixers in the boiler was used for 5-10 min. During cutting process, hot stream was given to curd to increase temperature to 36-38°C. Then curd was pressed for 30 minutes on a special trays to remove whey and get compact structure. Pressed curd was cut and cooked at 90-97°C in whey. Cooking takes 50-60 min. till inner temperature of halloumi cheese reaches to 90°C. After that folding was done when cheese was still hot. For salty halloumi, cheese was waited in the brine of 10 baume, for 1 day, for less salty

halloumi (Figure 4.3 a and b), cheese was waited half a day in the brine of 13 baume. The next day, halloumi was packaged and analyzed. Halloumi was chopped before the fat analysis.



**Figure 4.3:** (a) High-Salt Halloumi (b) Low- Salt Halloumi

#### **d) Preparation of calibration standards**

Raw milk was directly analyzed to determine fat content. And then diluted with water to prepare calibration standards at 5 different levels i.e, 3.0, 2.6, 2.3, 2.0, 1.7% of fat.

#### **4.2 Reagents and other materials/equipments**

**Gerber centrifuge:** A centrifuge with heating appliance was used for analysis. It has 8 compartments and a time setting (Figure 4.4). The cycle was 1000-1200 rpm.



**Figure 4.4:** Gerber centrifuge with heat

**Pipets:** Manual and automatic pipets were used to transfer amylalcohol, sulphuric acid and samples.

**Sampling container:** Generally 40 ml sampling container was sufficient.

**Gerber butyrometer:** They are glass butyrometers, known as cheese butyrometer, or milk butyrometer, with a distinct opening according to the product characteristics, a clear opening, a rubber stopper, and a with no breaker (without glass) or two open breakers (Figure 4.6). Butyrometers, which are equipped with rubber plugs and are also used in cheese analysis as well as in cream and fat analysis. Milk butyrometers can also be used such as liquid milk, buttermilk, homogenized diluted yoghurt, etc. (Figure 4.5).



**Figure 4.5:** Milk butyrometer and the plug



**Figure 4.6:** Cheese butyrometer and the plug

**Sulphuric acid:** The acid to be used in the gerber method should be in 90-91% of purity (Figure 4.7). The density of the acid is also very important parameter for the quality of the product. If the density of the acid is high, the fat will burn, while if it is low, the fat will not be digested.

For example, 90-91%  $\text{H}_2\text{SO}_4$  with a density of  $1.818 \pm 0.003$  g/mol was used for milk and yoghurt fat analysis, however 90-91%  $\text{H}_2\text{SO}_4$  with a density of 1.5 g/mol were used for cheese fat analysis.



**Figure 4.7:** Sulfuric acid

### Preparation of diluted sulphuric acid

The preparation of the sulfuric acid to be used in the experiment should be as follows; 40 ml of purified water is poured into the balloon and 54 ml of sulfuric acid is added after that 6 ml of water to dilute the sulfuric acid required for fat analysis. 6 ml of water is added and mixed.

**Amyl alcohol:** The amyl alcohol to be used for the analysis should be pure and its density should be  $0.811 \pm 0.003$  g/mol at  $132^{\circ}\text{C}$  (Figure 4.8). Quality of the amyl alcohol should also be checked before starting the analysis. Therefore 10 ml of  $\text{H}_2\text{SO}_4$  which has a density of 1.817-1.821 g/mol is added into the milk butyrometer. 11 ml of distilled water and 1 ml of amyl alcohol are added in order to form a layer there on. The butyrometer plug is closed and mixed well; centrifuged at 1000 rpm for 5 minutes. If the fat layer is not formed, the amyl alcohol is considered suitable for analysis.



**Figure 4.8:** Amyl alcohol

## 4.3 Method

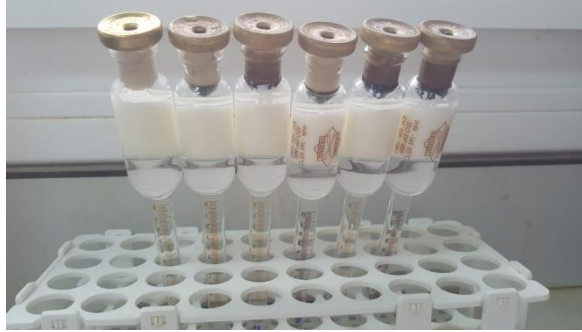
### 4.3.1 Determination of fat content in milk

- Dairy butyrometers are placed on special supports with their mouths pointing upwards. Then sequentially and carefully; 10 ml of sulfuric acid first and then 11 ml of sample adjusted to  $20^{\circ}\text{C}$  is added with pipet on the inner wall of bottom tube of the butyrometers. Lastly we add 1 ml of amyl alcohol. Care should be taken to add milk and amyl alcohol; milk and amyl alcohol should be extremely slowly added and leach from the butyrometre side, in order to avoid wetting the butyrometer mouth and to form a layer according to their density.

- The butyrometer's lid is thoroughly and tightly sealed with a dry rubber plug. If the adjusting bracelet is used, it must be ensured that the metal bracelet is firmly placed in the throat of the butyrometer and firmly tied (Figure 4.9).
- The butyrometer is slowly shaken and overturned until the existing clot is completely dissolved and no whiteness remains in the container (Figure 4.10). The butyrometer heats up during the dissolution of the clot. For this reason, a clean rag or shake bridged stand, or a rinsing machine can be used to be protected against temperature and possibly the risk of fracture of the butyrometer. During the analysis, goggles or even proper clothing should be used.
- If the centrifuge to be used does not contain the heating appliance, we wait until the clot is completely dissolved; then the batches are placed in to a 65°C water bath for 5 minutes. If the centrifuge contains a heating appliance, then there is no need to wait for the process in a water bath. The butyrometers are then placed on the centrifuge table in such a way that the plugs face outwards. To ensure equilibrium with centrifugal ratios, equilibrium must be established by placing a pre-calibrated butyrometer, which can be used in the same way as the counter-sample butyrometer or sample weight.
- The batch of the butyrometers is centrifuged for 5 minutes at a centrifugation rate of 1000-1200 rpm with the graduated portion coming down. Then, in a water bath of 65-70°C, the graduated part should be kept waiting for 5 minutes. In the availability of heated centrifuges, there is no need to use for water bath. The centrifugation process must be completed and an indefinite readout must be obtained.
- In order to perform read at the bottom the stopper must be pushed by turning the stopper in the mouth to bring the lower limit of the fat column to one of the degree lines so that reading can be done. Each section of the butyrometer corresponds to 1 g of fat in 100 g or 100 ml of milk, depending on the amount of sample taken. When 11 ml of the sample was taken, the result will be g fat/100 g milk (Figure 4.11).
- When the analysis is complete, the butyrometer should be emptied into a separate container and left in hot water for a while to be rinsed with cold water in order to protect the content from the effect of acid. If this is not done, then cleaning of fat



becomes difficult, because the fat will be remained in the neck area of the butyrometer area, since the fat will condense. In case like these, it is necessary to keep the butyrometers in mild hot water or hot water with sodium and then wash them several times.



**Figure 4.9:** Prepared butyrometers for analysis of milk fat.



**Figure 4.10:** Mixture of samples prepared on the butyrometer for analysis of milk fat



**Figure 4.11:** Sample prepared in the butyrometer for the analysis of milk fat ready to read after centrifugation

#### 4.3.2 Determination of fat content in yoghurt

- Milk butyrometers are placed on special supports with their tops pointing upward.
- 10 ml of sulfuric acid is added first, then 11 ml of homogenized diluted yoghurt adjusted to 20°C and finally 1 ml of amyl alcohol are then added in sequence and carefully into the butyrometers. More distilled water can be added if necessary. When all these are added, care should be taken not to wet the butyrometre's mouth and thus not to form a layer of additives. In order to be able to do this, yoghurt and amyl alcohol should be poured very slowly to leach from the side wall of butyrometer.
- Once the butyrometer has been filled, its mouth is covered with a dry, solid rubber plug. The two ends are compressed to the thickest part of the conical plugs.
- The butyrometer is slowly stirred and overturned until the resulting clot is completely dissolved and there is no in the butyrometer. During this process, a rug rinse bridged, or stirrer must be used, which will heat the butyrometer.
- When the non-greasy components are completely dissolved, the butyrometers are placed immediately in a hot water bath at 65°C, with the partition neck facing

upwards and wait for five minutes. There is no need to wait in the water tank if the used centrifugal heating system is included.

- The butyrometers are placed on the centrifuge table in such a way that the plugs come to the outside. Even if the sample of yoghurt is single, it should be balanced by placing a pre-calibrated butyrometer on the other side, which can be used with a yoghurt or a specimen weight.
- The butyrometers placed are centrifuged for five minutes.
- The butyrometers are then removed from the centrifuge, with the laminar section facing upwards, and left in a 65°C water bath for about five minutes. If heated centrifuges are used, there is no need for water bath. When centrifugation is complete, reading should be performed.
- During reading; the plug is slowly pushed and pulled to bring the concave lower limit of the oil column to "0" or tangent to any partition line. The column between the lowest tangential point and the lower boundary of the upper concave edge of the mass of fat is read.
- The result read from the butyrometer scale is multiplied by 2, and the amount of fat is determined as a percentage. The reason for this multiplication is 1:1 dilution during sample preparation.

#### **4.3.3 Determination of fat content in halloumi**

- 3 grams of grated cheese is weighted out.
- It is placed in a glass-bottom unit of butyrometer (Figure 4.12).
- 10 ml of diluted sulfuric acid is poured onto the sample.
- The butyrometer is placed in the 70°C bath water and the cheese is thoroughly melted by shaking.
- If cheese pieces are left in the butyrometer, 1 ml of amyl alcohol is added and shaken.
- The butyrometer is filled with the same sulfuric acid up to 35 parts and the mouth is covered with a rubber plug and centrifuged for 10 min.
- It is held in a 65°C water bath for 5 minutes. This process is unnecessary if the centrifuge is heated.
- The amount of fat is read as % from the butyrometer scale.



**Figure 4.12:** Butyrometer -prepared samples for cheese fat analysis

#### 4.3.4 Linearity and Limit of Detection (LOD) Determination

Matrix-matched calibration standards were prepared by using raw milk sample at seven different concentrations 1.7, 2.0, 2.3, 2.6 and 3.0 %.

The linearity of calibration curve was determined by computing correlation coefficient (R) and standard deviation of relative residuals (S<sub>rr</sub>) by using below formula 4.1:

$$S_{(y/x)w} = \left\{ \frac{\sum_i w_i (y_i - \hat{y}_i)^2}{n - 2} \right\}^{1/2} \quad (4.1)$$

Where:

$y_i$  is the response obtained from injecting  $x_i$  analytical standard.

$\hat{y}_i$  is the point corresponding with  $x_i$  on the regression line

**n** is the total number of standard injections (e.g. when the calibration is made at three level with duplicate injections, then **n** is replaced with  $n \cdot k = 3 \cdot 2$  equal to 6).

**m** is the number of replicate injections made to determine the analyse concentration

**b** is the slope obtained from the weighted linear regression.

$y_o$  is the mean response ( $m$  replicate injections) used to calculate the concentration value  $X_o$

$w_o$  is the weighing appropriate to value of  $y_o$

$w_i$  is the weighing appropriate to value of  $y_i : x_i$  pairs

$\bar{y}_w$  is the arithmetic mean of the weighted  $y_{iw}$  responses from all standard injections  $(y_i w_i / n)$ .

$\bar{x}_w$  is the arithmetic mean of the weighted  $x_{iw}$  concentrations of standards  $(w_i x_i / n)$ .

Limit of detection is the minimum concentration or mass of the analyte that can be detected with acceptable certainty, though not quantifiable with acceptable precision. LOD is also estimated through calibration curve (Tiryaki, 2016).

## CHAPTER 5

### RESULTS AND DISCUSSION

#### 5.1 Results

For the recovery studies, milk, yoghurt and halloumi samples were taken during the production and sent to an accredited laboratory to be analyzed for fat content. These values belonging to the samples were used as reference values in calculations according to following formulas (Table 5.1):

Recovery (R)= Analysis result / Reference value \*100

Accuracy (%) =  $\Sigma \text{Recovery}_{1-n}/n$

Precision (rsd/ RSD)=Standard Deviation of Recoveries\*100/Average Recovery

**Table 5.1:** Reference values for the samples

Matrices		Fat Content, %		
		Time 1	Time 2	Time 3
Milk	Full-fat	3.30	2.80	2.90
	Semi-fat	1.70	1.50	1.90
Yoghurt	Full-fat	3.96	3.63	3.60
	Semi-skimmed	1.32	1.50	2.20
	Strained	3.08	3.19	3.30
Halloumi	High- salt	24.00	22.00	21.00
	Low- salt	22.50	20.00	23.00

##### 5.1.1 Validation Results of Fresh Milk

The results obtained from fresh milk for 2 different levels in 6 replications at 3 different times were summarized in Table 5.2.

When we look at recoveries and the repeatability values of the daily fat analysis of fresh milk, recoveries were found as 97, 100 and 98 % for day 1, day 2 and day 3 of fat levels with relative standard deviations (RSD) of 3, 4 and 2 % respectively.

Fat recoveries from fresh milk were changing from 93 % to 107 %. Average recoveries of three different days were found as 97 and 100 % for full and semi fat milk samples, with 2 and 4% RSD respectively.

### **5.1.2 Validation Results of Yoghurt**

Yoghurt at three different levels were analysed in 6 replications and at three different time. Table 5.3 represents the recoveries from yoghurt at three different levels and three different time.

When we look at recoveries and the repeatability values of the daily fat analysis, recoveries were found as 105, 98 and 96 % for day 1, day 2 and day 3 and relative standard deviations 8, 5 and 8 % respectively.

Fat recoveries from yoghurt are changing from 82 % to 121 %. Average recoveries of three different days were found as 99, 98 and 101% for full, semi-skimmed and strained yoghurt samples, with 3, 13 and 4% RSD respectively.

### **5.1.3 Validation Results of Halloumi**

Halloumi at two different levels were analysed in 6 replications and at three different time. Table 5.4 represents the recoveries from halloumi at two different levels and three different time.

When we look at recoveries and the repeatability values of the daily fat analysis, recoveries were found as 96, 101 and 100 % for day 1, day 2 and day 3 and relative standard deviations 2, 1 and 3 % respectively.

Fat recoveries from halloumi were changing from 93 % to 105 %. Recoveries of three different days were found as 99 and 99 % for different types of halloumi cheese samples with 3 and 3% RSD respectively.

**Table 5.2:** Recoveries from fresh milk at two different levels and three different time.

	1.7%						3%						Ravg	SD	RSD
time-1	100	100	94	100	94	100	97	94	94	97	94	97	97	3	3
time-2	107	107	100	100	100	107	100	96	100	96	93	100	100	4	4
time-3	100	100	100	100	95	100	100	96	96	100	96	96	98	2	2
Ravg	100						97								
SD	4						2								
RSD	4						2								

**Table 5.3:** Recoveries from yoghurt at three different levels and three different time.

	1.9%						3.20%						3.90%						Ravg	SD	RSD
time-1	121	106	106	106	121	121	97	104	104	104	104	110	101	101	96	96	96	101	105	8	8
time-2	88	88	98	98	88	98	100	100	100	100	94	100	105	99	105	105	99	99	98	5	5
time-3	81	100	82	82	90	90	103	97	103	97	103	103	100	100	100	100	94	94	96	7	8
Ravg	98						101						99								
SD	13						4						3								
RSD	13						4						3								

**Table 5.4:** Recoveries from halloumi at two different levels and three different time.

	21.80%						22.30%						Ravg	SD	RSD
time-1	98	93	98	98	100	100	94	94	96	98	96	94	96	2	2
time-2	102	100	102	102	102	100	100	100	102	102	100	100	101	1	1
time-3	102	96	98	98	100	96	100	98	105	102	102	100	100	3	3
Ravg	99						99								
SD	3						3								
RSD	3						3								

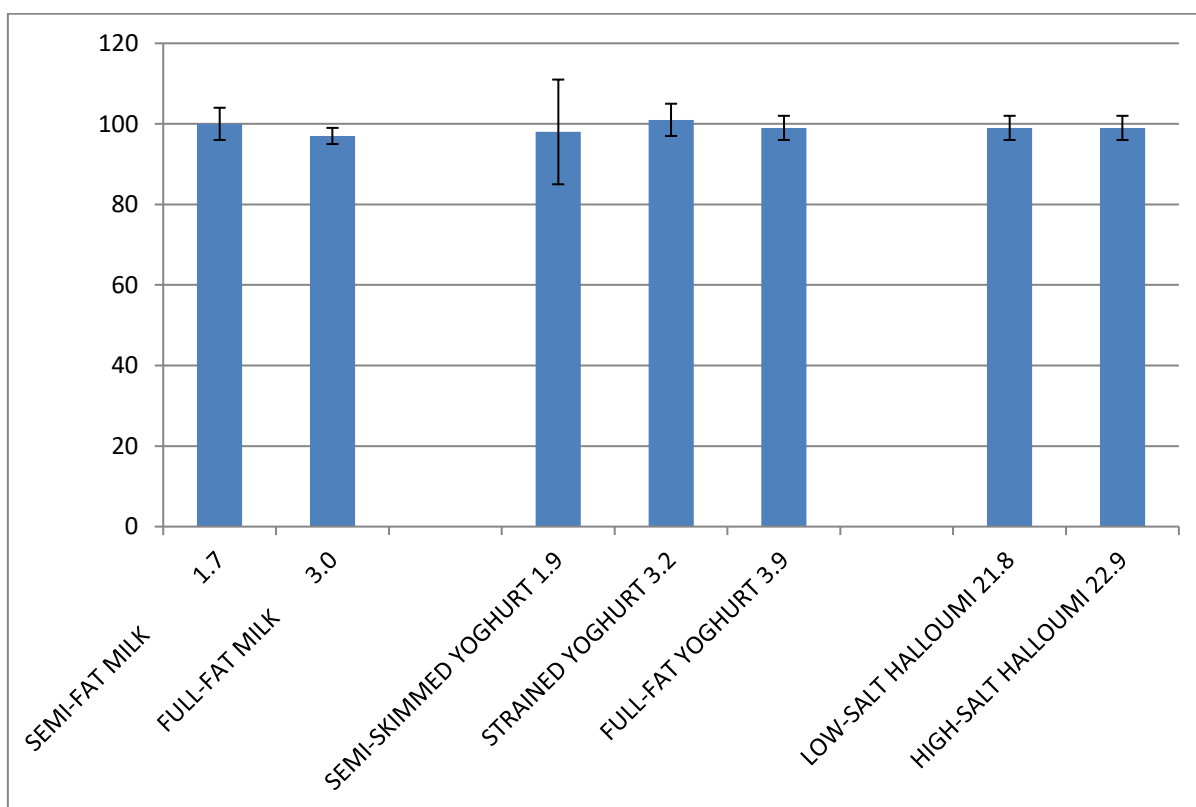


#### 5.1.4 Overall Recovery and Reproducibility of the Method

Recovery studies were carried out to determine the accuracy of the method.

Mean recovery for milk, yoghurt and halloumi were found as 98, 100 and 99% respectively (Figure 5.1). Reproducibility values were calculated as relative standard deviation of mean recoveries obtained from three different time analysis of the milk, yoghurt and halloumi samples. Reproducibility values were 3, 8 and 3% for milk, yoghurt and halloumi samples respectively. Finally overall recovery, in other words, accuracy of the Gerber method is 99% with 4% of RSD (n=126) for milk, yoghurt and halloumi.

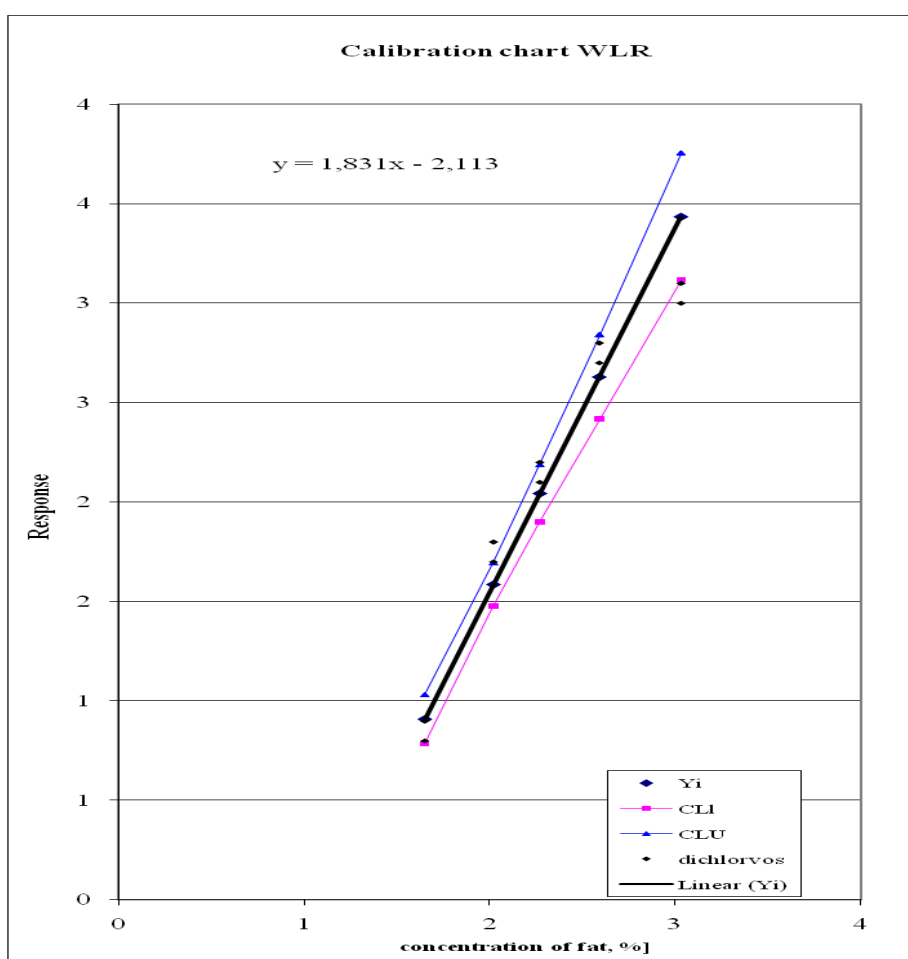
Gerber method for the determinatin of fat in milk, yoghurt and halloumi was succesfully validated, since the accuracy and precision of the method were with in the acceptable ranges.



**Figure 5.1:** Mean recoveries and RSDs for milk, yoghurt and halloumi samples at different fat levels

### 5.1.5 Linearity and Limit of Detection (LOD)

Linearity and LOD are important parameters to be determined in method validation experiments. One simple way to estimate linearity and LOD is use of the calibration curves in matrix. The standard deviations of relative residuals (Srr), which is a decisive parameter in internal quality control of linearity, should be  $\leq 0.1$  ( Gozek et al., 1995; Miller & Amburs, 2000). This was the case in the study that correlation coefficient  $R^2$  was 0.929 and Srr was 0.09. Typical LOD value for fat calculated from the calibration curve was found 0.453%.



**Figure 5.2:** Calibration curve of fat analysis by using butyrometer in matrix

## 5.2 Discussion

For three of the matrices, milk, yoghurt and halloumi, recoveries averaged as 99 % over the validation range with a relative standard deviation of 4 % (n=126). All these findings comply with the values recommended for mean recovery range (82–121%) and reproducibility (RSD 4%). Our findings are also in accordance with the Gerber method's validation criteria.

De Langen (2012) has studied determination of fat in meat and separable fat by the Gerber test. A modified Gerber fat test for the determination of fat in meat and separable fat is described. A factor of 0.935 is applied to correct for the difference between the S.G. of butterfat and body fat. Average recovery of purified animal fat was 99.9 per cent. Samples containing more than 3 per cent fat showed lower variability with the Gerber test than with the solvent extraction method. For routine work on animal tissue and fat the Gerber test was found to be more rapid and reliable than the solvent extraction method. We also have got higher RSDs with semi-skimmed yoghurt fat content which was 13%.

The validity of the application of a correction factor of 0.935 (calculated directly from the S.G. differences) to the Gerber test readings for the determination of fat in meat and separable fat was tested by comparing the recoveries, uncorrected and corrected, from 4 purified fat samples. Mean results of 6 estimations and the standard deviations are 0.66, 0.61, 0.96, 0.50. A comparison was made of results from meat and separable fat samples by the solvent extraction method and the Gerber test using the correction factor with the latter. The range, mean, and standard deviation calculated from 12 estimations on each sample. Assuming that the solvent extraction gives the true fat content, the good agreement between the 2 methods indicates the validity of the correction factor, and the precision and accuracy of the Gerber procedures. Standard deviations of estimations by the Gerber test were appreciably lower than those obtained by the solvent extraction method except with the 2 samples containing less than 3 per cent fat. This was probably due to error in reading low values on the butyrometer scale calibrated in 0.1 per cent divisions (De Langen, 2012).

Kleyn et al. (2001) has studied determination of fat in raw and processed milks by the Gerber method, collaborative study. The Gerber method is used worldwide as a simple and

rapid method for determining fat in raw and processed milks. However, the volume of the test portion used in the method has not been internationally agreed upon. A collaborative study was conducted to evaluate performance of the Gerber method using either a weighed test portion (11.13 g) or a 10.77 mL test portion delivered by pipet. For each method, laboratories received 10 test samples: 5 raw and 5 pasteurized homogenized milks, 2 of which were blind duplicate pairs. Eleven and 10 laboratories participated in the evaluation of aliquot addition by weight and pipet, respectively. Mojonnier ether extraction (Method 989.05) was used as the reference method. Interlaboratory study statistics were similar between methods of test portion addition and between raw and processed materials; therefore, summary interlaboratory study statistics were pooled. The fat content of milk samples ranged from 0.96 to 5.48%. Absolute reproducibility and repeatability were not affected by fat level, and pooled statistical performance (invalid and outlier data removed) was (g fat/100 g milk)  $s(r) = 0.026$ ,  $s(R) = 0.047$ ,  $r = 0.074$ , and  $R = 0.132$ . Relative standard deviations increased with decreasing fat content, and were summarized by fat level: 1-2% fat milk, mean = 1.437,  $RSD(r) = 1.809\%$ ,  $RSD(R) = 3.271\%$ ; 2-6% fat milk, mean = 4.156,  $RSD(r) = 0.626\%$ ,  $RSD(R) = 1.131\%$ . Compared with ether extraction, test results by the Gerber method were slightly lower (0.02% fat) using a weighed test portion and significantly lower (0.06% fat) using a 10.77 mL volume addition by pipet. A trend toward underestimating fat content at lower fat concentrations (1-2% fat) was observed with the weighed test portion but not when a pipet was used. The Associate Referee recommends that the Gerber method using a weighed test portion be adopted as First Action with applicability limited to whole milk (Kleyn et al., 2001).

## **CHAPTER 6**

### **CONCLUSION AND RECOMMENDATIONS**

All the testing laboratories wish to demonstrate that they operate a management system, are technically competent and are able to generate technically valid results. They have to also comply with international standards such as ISO 17025 and ISO 9001 and operate in accordance with them. The use of international standards will facilitate cooperation between laboratories and other bodies and also in the harmonization of standards and procedures.

Many factors contribute accuracy and precision of the test results performed by a laboratory, such as accommodation and environmental conditions, test and calibration methods and method validation, equipment, sampling and handling of test and calibration items. Laboratory should validate the methods to confirm that the methods are fit for the intended use.

In this study, Gerber method was successfully validated for fat analysis in all three matrices, milk, yoghurt, halloumi and all fat levels, method can be used for routine analysis by implementing internal quality control measures during its use.

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## **APPENDICES**



AB-0566-T
17/2160
09.10.2017

#### NANOLAB LABORATUVAR HİZMETLERİ

#### MUAYENE VE ANALİZ RAPORU

Tarih : 09.10.2017

Rapor No : 17/2160  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)

Numune Alma Tutanağının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Taze Süt  
Numune Kod Numarası : 17/2160

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1- Yağ Tayini(%)	3,3 ± 0,05					TS 1330	

#### Kısaltmalar:

U: Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerleri birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Beyan GÜLER YEŞİLİK



Tasdik Olunur  
Tarih, İmza, Mühür  
Laboratuvar Müdürü  
Turgay ZABUN

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Bilal FATLİSİZ

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AB-0566-T
17/2159
09.10.2017

## NANOLAB LABORATUVAR HİZMETLERİ

### MUAYENE VE ANALİZ RAPORU

Tarih : 09.10.2017

Rapor No : 17/2159  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)  
Numune Alma Tutanağının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Taze Diyet Süt  
Numune Kod Numarası : 17/2159

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1- Yağ Tayini(%)	1,7 ± 0,05					TS 1330	

#### Keskinlikler:

U: Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Begüm EROLER YERLİLİK



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**NANOLAB LABORATUVAR HİZMETLERİ**

**MUAYENE VE ANALİZ RAPORU**

Tarih : 09.10.2017

Rapor No : 17/2163  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)  
Numune Alma Tutarının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Yarım Yağlı Doğal Yoğurt  
Numune Kod Numarası : 17/2163

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1.*Yağ Tayini(%)	1,32 ± 0,06					TS 1330	

**Kısaltmalar :**

U:Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.  
Not: 1: "" işaretli analizler akreditasyon kapsamındadır.  
Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.  
Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.  
Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.  
Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.  
Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.  
Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Begüm GÜLER YEŞELİK



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Bülent KATILISÖZ

**NANOLAB LABORATUVAR HİZMETLERİ**

**MUAYENE VE ANALİZ RAPORU**

**Tarih : 09.10.2017**

**Rapor No** : 17/2161  
**Analizin Amacı** : Özel İstek  
**Numuneyi Gönderen** : ŞEÇİL ARÇAY (KIBRIS)

**Numune Alma Tutanağının Tarihi ve Sayısı** : 04.10.2017  
**Numunenin Cinsi, Seri-Parti/No** : Gıda Süzme Yoğurt  
**Numune Kod Numarası** : 17/2161

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1-*Yağ Tayini(%)	3,08 ± 0,06					TS 1330	

**Kısaltmalar :**

U:Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "\*" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

**Kimyasal Analiz Bir.Sor.**

**Begüm GÜLER YEŞİLLİK**



**Numune Kabul ve Rapor.Bir.Sor.**

**Bülent TATLISÖZ**



AB-0566-T
17/2162
09.10.2017

#### NANOLAB LABORATUVAR HİZMETLERİ

#### MUAYENE VE ANALİZ RAPORU

Tarih : 09.10.2017

Rapor No : 17/2162  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)  
Numune Alma Tutarının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Tam Yağlı Doğal  
Numune Kod Numarası : 17/2162

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1-*Yağ Tayini(%)	3,96 ± 0,06					TS 1330	

#### Kısaltmalar :

U:Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "+" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Begüm GÜLER YERLİLİK



Numune Kabul ve Rapor.Bir.Sor.  
Bülent TATLISÖZ

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**NANOLAB LABORATUVAR HİZMETLERİ**

**MUAYENE VE ANALİZ RAPORU**

Rapor No : 17/2158  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)  
Numune Alma Tutanağının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Hellim  
Numune Kod Numarası : 17/2158

Tarih : 09.10.2017

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1-*Yağ Tayini(%)	24,0 ± 0,44					TS 1330	

**Kısaltmalar :**

U:Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "\*" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Begüm GÜLER YEŞİLİRK



Numune Kabul ve Rapor.Bir.Sor.  
Bilgin TATLISÖZ



**NANOLAB LABORATUVAR HİZMETLERİ**

**MUAYENE VE ANALİZ RAPORU**

Tarih : 09.10.2017

Rapor No : 17/2157  
Analizin Amacı : Özel İstek  
Numuneyi Gönderen : ŞEÇİL ARÇAY (KIBRIS)  
Numune Alma Tutanağının Tarihi ve Sayısı : 04.10.2017  
Numunenin Cinsi, Seri-Parti/No : Gıda Hellim Az Tuzlu  
Numune Kod Numarası : 17/2157

Analiz	Sonuç	Tespit Limiti	Ölçüm Limiti	G.K. (%)	Ölçüm Belirsizliği	Analiz Metodu	Değerlendirme
1- Yağ Tayini(%)	22,5 ± 0,44					TS 1330	

**Kısaltmalar :**

U: Uygun, UD: Uygun Değil, DY: Değerlendirme Yapılmadı

Beyan edilen genişletilmiş ölçüm belirsizliği, standart belirsizliğin k=2 olan genişletme katsayısı ile çarpımı sonucunda %95 oranında güvenilirlik seviyesi sağlamaktadır.

Yapılan muayene ve analiz sonucunda yukarıda belirtilen değerler tespit edilmiştir.

Not: 1: "\*" işaretli analizler akreditasyon kapsamındadır.

Not: 2: Bu analiz raporunun hiç bir bölümü tek başına veya ayrı ayrı kullanılamaz.

Not: 3: Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

Not: 4: Bu rapor laboratuvarın onayı olmadan çoğaltılamaz.

Not: 5: Limit değerlerin birimleri bulgu birimleriyle aynıdır.

Not: 6: Gerekliğinde "Ölçüm Belirsizliği" ve "Geri Kazanım" oranları analiz sonucu ile birlikte verilir.

Not: 7: Bu analiz raporu adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

Kimyasal Analiz Bir.Sor.  
Begüm GÜLER YEŞİLLİK



Numune Kabul ve Rapor.Bir.Sor.  
Bilal TATLISOZ



## NANOLAB LABORATUVAR HİZMETLERİ

AB-0566-T

17/6373

08.11.2017

## MUAYENE VE ANALİZ RAPORU

Rapor / Revizyon No : 17/6373/00

Analiz Amacı : Ürün

Numuneyi Gönderen : GÜLGÜN SÖT ÜRÜNLERİ (KIBRIS)

Numunenin Cinsi : TAZE SÖT

Üretim - Son Kul. Tarihi : 26.10.2017-31.10.2017

Sıcaklık : 5 °C

Tutanağın Tarihi - Sayısı : 02.11.2017 -

Seri - Parti No : -

Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 07.11.2017

Num. Geliş Tar./Alım Yön. : 02.11.2017 /-

Lot No : 0129A8

Miktar : 1000 mL

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	U/UD
1- Yağ Tayini (%)	2,80 ±0,06	AOAC 2000.18 / TS 1864		0,06			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.

2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.

3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.

4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.

5. İmzasız ve mühürlü raporlar geçersizdir.

6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır

7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L.: Ölçüm Limiti.

8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Bilgi Sorumlusu(V)

Tasdik Olunur

08.11.2017  
Turgay ZABUN  
Müdür

Bülent TATLISOZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

EK-T KYS.PR.17 İlk Yayın Tarihi: 28.11.2011 Revizyon Tarihi/No: 13.11.2015/02

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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A. Kahveci Mh. Çankaya Cd. Vadi İş Mrkz. N-2 E K: 2 No: 25 Beylikdüzü - İSTANBUL

Tel.: 0212 855 48 10 - 855 02 53 Fax: 0212 855 48 33

Sayfa No 1/1



## NANOLAB LABORATUVAR HİZMETLERİ

AB-0566-T
17/6372
08.11.2017

## MUAYENE VE ANALİZ RAPORU

Rapor / Revizyon No : 17/6372/00

Analiz Amacı : Ürün

Numuneyi Gönderen : GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)

Numunenin Cinsi : DİYET SÜT

Üretim - Son Kul. Tarihi : 27.10.2017-01.11.2017

Sıcaklık : 5 °C

Tutanağın Tarihi - Sayısı : 02.11.2017 -

Seri - Parti No : -

Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 07.11.2017

Num. Geliş Tar./Alım Yön. : 02.11.2017 /-

Lot No : 0129A9

Miktar : 1000 mL

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	U/UD
1- Yağ Tayini (%)	1,50 ±0,03	AOAC 2000.18 / TS 1864		0,03			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılmadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Bilmi Sorumlusu(V)

Tasdik Olunur

08.11.2017

Turgay ZABUN

Müdür

Bülent FATİSÖZ  
Nurhan Kabul ve  
Düzenleme Birimi  
Sorumlusu

EK-1 KYS.PR.17 İlk Yayın Tarihi:28.11.2011, Revizyon Tarihi/No:13.11.2015/02

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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Sayfa No 1/1

**NANOLAB LABORATUVAR HİZMETLERİ**

AB-0566-T
17/6370
08.11.2017

**MUAYENE VE ANALİZ RAPORU**

Rapor / Revizyon No : 17/6370/00  
 Analiz Amacı : Ürün  
 Numuneyi Gönderen : GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
 Numunenin Cinsi : YARIM YAĞLI YOĞURT  
 Üretim - Son Kul. Tarihi : 25.10.2017-30.10.2017  
 Sıcaklık : 5 °C  
 Tutanağın Tarihi - Sayısı : 02.11.2017 -  
 Seri - Parti No : -  
 Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 06.11.2017  
 Num. Geliş Tar./Alım Yön. : 02.11.2017 /-  
 Lot No : -  
 Miktar : 610 g

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	U/UD
1- Yağ Tayini (%)	2,04 ±0,04	AOAC 2000.18 / TS 1864		0,04			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçerli değildir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar: D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla GAYLI  
Kimyasal An.  
Birim Sorumlusu(V)

Bülent TATLISOZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur

08.11.2017  
Turgay ZABUN  
Müdür

EK-1 KYS.PR.17 İlk Yayın Tarihi:28.11.2011 Revizyon Tarihi:13.11.2015/02  
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Sayfa No 1/1



## NANOLAB LABORATUVAR HİZMETLERİ

AB-0566-T
17/6371
08.11.2017

### MUAYENE VE ANALİZ RAPORU

Rapor / Revizyon No :17/6371/00

Analiz Amacı :Ürün

Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)

Numunenin Cinsi :SÜZME YOĞURT

Üretim - Son Kul. Tarihi :26.10.2017-31.10.2017

Sıcaklık :5 °C

Tutanağın Tarihi - Sayısı :02.11.2017 -

Seri - Parti No : -

Analiz Baş.-Bitiş Tarihi :02.11.2017 – 06.11.2017

Num. Geliş Tar./Alım Yön. :02.11.2017 /-

Lot No :-

Miktar :610 g

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	U/UD
1-*Yağ Tayini (%)	3,19 ±0,07	AOAC 2000.18 / TS 1864		0,07			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birim Sorumlusu(V)

Tasdik Olunur

08.11.2017  
Purgay ZABUN  
Müdür

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

EK-1 KYS.PR.17 İlk Yayın Tarihi:28.11.2011 Revizyon Tarihi:13.11.2015/02  
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www.nano-lab.com.tr

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Sayfa No 1/1





## NANOLAB LABORATUVAR HİZMETLERİ

### MUAYENE VE ANALİZ RAPORU



Test  
TS EN ISO/IEC 17025  
AB-0566-T

AB-0566-T

17/6369

08.11.2017

Ürün

Rapor / Revizyon No : 17/6369/00  
Analiz Amacı : Ürün  
Numuneyi Gönderen : GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi : TAM YAĞLI YOĞURT  
Üretim - Son Kul. Tarihi : 26.10.2017 - 30.10.2017  
Miktar : 610 GR

Tutanağın Tarihi - Sayısı : 02.11.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 07.11.2017  
Num. Geliş Tar./Alım Yön. : 02.11.2017 /-  
Sıcaklık : 5°C  
Numune Ambalajı : Orjinal

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.-Yağ Tayini (%)	3,63 ± 0,08	AOAC 2000.18 / TS 1864		0,08		Min:3,8	UD

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme. U: Uygun. U.D.: Uygun Değil. D.Y.: Değerlendirme Yapılamadı. G.K.: Geri Kazanım. Ö.B: Ölçüm Belirsizliği. Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Tasdik Olunur  
08.11.2017  
Turgay ZABUN  
Müdür

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

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Fax: 0212 855 48 33

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1



## NANOLAB LABORATUVAR HİZMETLERİ

### MUAYENE VE ANALİZ RAPORU



Test  
TS EN ISO/IEC 17025  
AB-0566-T

AB-0566-T

17/6374

08.11.2017

Ürün

Rapor / Revizyon No : 17/6374/00  
Analiz Amacı : Ürün  
Numuneyi Gönderen : GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi : HELLİM PEYNİRİ  
Üretim - Son Kul. Tarihi : 26.10.2017 - 25.10.2017  
Sıcaklık : 5°C  
Numune Ambalajı : Orjinal

Tutanağın Tarihi - Sayısı : 02.11.2017 -  
Seri - Parti No : 29A9 - 6/1  
Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 07.11.2017  
Num. Geliş Tar./Alım Yön. : 02.11.2017 /-  
Lot No : 0129A8  
Miktar : 250 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.*Yağ Tayini (%)	22,00 ± 0,46	AOAC 2000.18 / TS 1664		0,46			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılmadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi(Sorumlusu(V))

Tasdik Olunur  
08.11.2017  
Turgay ZABUN  
Müdür

Bülent TANIRSOZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

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Fax: 0212 855 48 33

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1



## NANOLAB LABORATUVAR HİZMETLERİ

### MUAYENE VE ANALİZ RAPORU



Test  
TS EN ISO/IEC 17025  
AB-0566-T

AB-0566-T

17/6375

08.11.2017

Ürün

Rapor / Revizyon No : 17/6375/00

Analiz Amacı : Ürün

Numuneyi Gönderen : GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)

Numunenin Cinsi : HELLİM PEYNİRİ AZ TUZLU

Üretim - Son Kul. Tarihi : 25.10.2017 - 24.10.2018

Sıcaklık : 5°C

Numune Ambalajı : Orjinal

Tutanağın Tarihi - Sayısı : 02.11.2017 -

Seri - Parti No : 29A8 - 6/1

Analiz Baş.-Bitiş Tarihi : 02.11.2017 - 07.11.2017

Num. Geliş. Tar./Alım Yön. : 02.11.2017 /-

Lot No : 0129A8

Miktar : 250 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1- Yağ Tayini (%)	20,00 ± 0,42	AOAC 2000.18 / TS 1864		0,42			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Tesdik Olunur  
08.11.2017  
Turgay ZABUN  
Müdür

Bülent TATLISIZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

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Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1



**NANOLAB LABORATUVAR HİZMETLERİ****MUAYENE VE ANALİZ RAPORU**

AB-0566-T

17/7623

14.12.2017

Rapor / Revizyon No :17/7623/00  
Analiz Amacı :Ürün  
Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi :TAZE YAĞLI SÜT  
Üretim - Son Kul. Tarihi :07.12.2017 - 12.12.2017  
Sıcaklık :5°C  
Numune Ambalajı :Orjinal

Tutanağın Tarihi - Sayısı :13.12.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi :14.12.2017 - 14.12.2017  
Num. Geliş Tar./Alım Yön. :13.12.2017/-  
Lot No :0133A6  
Miktar :1000 ML

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.-Yağ Tayini (%)	2,90 ± 0,06	AOAC 2000.18 / TS 1864		0,06			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur

15.12.2017

Turgay ZABUN

Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

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**NANOLAB LABORATUVAR HİZMETLERİ****MUAYENE VE ANALİZ RAPORU**

AB-0566-T
17/7622
14.12.2017

Rapor / Revizyon No :17/7622/00  
Analiz Amacı :Ürün  
Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi :TAZE DIYET SÜT  
Üretim - Son Kul. Tarihi :06.12.2017 - 11.12.2017  
Sıcaklık :5°C  
Numune Ambalajı :Orjinal

Tutanağın Tarihi - Sayısı :13.12.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi :14.12.2017 - 14.12.2017  
Num. Geliş Tar./Alım Yön. :13.12.2017/-  
Lot No :0233A5  
Miktar :1000 ML

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.-Yağ Tayini (%)	1,90 ± 0,04	AOAC 2000.18 / TS 1864		0,04			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adı-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L.: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur  
15.12.2017  
Turgay ZABUN  
Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

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Tel.: 0212 855 48 10 - 855 02 53 Fax: 0212 855 48 33

**NANOLAB LABORATUVAR HİZMETLERİ****MUAYENE VE ANALİZ RAPORU**

AB-0566-T
17/7626
14.12.2017

**Rapor / Revizyon No** :17/7626/00**Analiz Amacı** :Ürün**Numuneyi Gönderen** :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)**Numunenin Cinsi** :YARIM YAĞLI DOĞAL YOĞURT**Üretim - Son Kul. Tarihi** :07.12.2017 - 12.12.2017**Sıcaklık** :5°C**Numune Ambalajı** :Orjinal**Tutanağın Tarihi - Sayısı** :13.12.2017 -**Seri - Parti No** : -**Analiz Baş.-Bitiş Tarihi** :14.12.2017 - 14.12.2017**Num. Geliş Tar./Alım Yön.** :13.12.2017/-**Lot No** :1133A2**Miktar** :610 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1-Yağ Tayini (%)	2,20 ± 0,05	AOAC 2000.18 / TS 1864		0,05		Min:1,5 Max:2	UD

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur

15.12.2017

Turgay ZABUN  
Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

**NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.** [www.nano-lab.com.tr](http://www.nano-lab.com.tr)  
A. Kahveci Mh. Çankaya Cd. Vadi İş Mrkz. N-2 E K: 2 No: 25 Beylikdüzü - İSTANBUL  
Tel.: 0212 855 48 10 - 855 02 53 Fax: 0212 855 48 33

**NANOLAB LABORATUVAR HİZMETLERİ**

**MUAYENE VE ANALİZ RAPORU**

AB-0566-T
17/7625
14.12.2017

Rapor / Revizyon No :17/7625/00  
 Analiz Amacı :Ürün  
 Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
 Numunenin Cinsi :SÜZME YOĞURT  
 Üretim - Son Kul. Tarihi :06.12.2017 - 11.12.2017  
 Sıcaklık :5°C  
 Numune Ambalajı :Orjinal

Tutanağın Tarihi - Sayısı :13.12.2017 -  
 Seri - Parti No : -  
 Analiz Baş.-Bitiş Tarihi :14.12.2017 - 14.12.2017  
 Num. Geliş Tar./Alım Yön. :13.12.2017/-  
 Lot No :0233A4  
 Miktar :610 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1- Yağ Tayini (%)	3,30 ± 0,07	AOAC 2000.18 / TS 1864		0,07			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
 Kimyasal An.  
 Birimi Sorumlusu(V)

Bülent TATLISÖZ  
 Numune Kabul ve  
 Düzenleme Birimi  
 Sorumlusu

Tasdik Olunur

15.12.2017

Turgay ZABUN

Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

**NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.** [www.nano-lab.com.tr](http://www.nano-lab.com.tr)  
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**NANOLAB LABORATUVAR HİZMETLERİ****MUAYENE VE ANALİZ RAPORU**

AB-0566-T
17/7624
14.12.2017

Rapor / Revizyon No :17/7624/00  
Analiz Amacı :Ürün  
Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi :TAM YAĞLI YOĞURT  
Üretim - Son Kul. Tarihi :07.12.2017 - 12.12.2017  
Sıcaklık :5°C  
Numune Ambalajı :Orjinal

Tutanağın Tarihi - Sayısı :13.12.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi :14.12.2017 - 14.12.2017  
Num. Geliş Tar./Alım Yön. :13.12.2017/-  
Lot No :0330A5  
Miktar :610 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.-Yağ Tayini (%)	3,60 ± 0,07	AOAC 2000.18 / TS 1864		0,07		Min:3,8	UD

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D.: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur

15.12.2017

Turgay ZABUN  
Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

**NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.** [www.nano-lab.com.tr](http://www.nano-lab.com.tr)  
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**NANOLAB LABORATUVAR HİZMETLERİ**

AB-0566T
17/7628
14.12.2017

**MUAYENE VE ANALİZ RAPORU**

Rapor / Revizyon No :17/7628/00  
Analiz Amacı :Ürün  
Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi :Helim Peyniri  
Üretim - Son Kul. Tarihi :27.11.2017-26.11.2018  
Sıcaklık :5 °C

Tutanağın Tarihi - Sayısı :13.12.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi :14.12.2017 – 14.12.2017  
Num. Geliş Tar./Alım Yön. :13.12.2017 /-  
Lot No :33A1-PN: 5/1  
Miktar :250 g

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	U/UD
1- Yağ Tayini (%)	21,0 ±0,4	AOAC 2000.18 / TS 1864		0,4			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır.
7. Kısaltmalar; D: Değerlendirme, U: Uygun, U.D: Uygun Değil, D.Y.: Değerlendirme Yapılamadı, G.K.: Geri Kazanım, Ö.B: Ölçüm Belirsizliği, Ö.L: Ölçüm Limiti.
8. \* İşaretili analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birinci Sorumlusu(V)

Bülent TATLISOZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur  
19.04.2018  
Fergay ZARFİN  
Müdür

EK-1 KYS.PR.17 İlk Yayın Tarihi:28.11.2011 Revizyon Tarihi/No:13.11.2015/02

NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.

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Sayfa No 1/1



**NANOLAB LABORATUVAR HİZMETLERİ****MUAYENE VE ANALİZ RAPORU**

AB-0566-T
17/7627
14.12.2017

Rapor / Revizyon No :17/7627/00  
Analiz Amacı :Ürün  
Numuneyi Gönderen :GÜLGÜN SÜT ÜRÜNLERİ (KIBRIS)  
Numunenin Cinsi :AZ TUZLU HELLİM PEYNİRİ  
Üretim - Son Kul. Tarihi :16.11.2017 - 15.11.2017  
Sıcaklık :5°C  
Numune Ambalajı :Orjinal

Tutanağın Tarihi - Sayısı :13.12.2017 -  
Seri - Parti No : -  
Analiz Baş.-Bitiş Tarihi :14.12.2017 - 14.12.2017  
Num. Geliş Tar./Alım Yön. :13.12.2017/-  
Lot No :33A2-PN: 6/1  
Miktar :250 GR

Analiz	Sonuç	Metot / Cihaz	G.K. (%)	Ö.B.	Ö.L.	Ref. Değer	D
1.-Yağ Tayini (%)	23,0 ± 0,5	AOAC 2000.18 / TS 1864		0,5			DY

1. Bu analiz raporunun hiçbir bölümü tek başına veya ayrı ayrı kullanılamaz.
2. Bu rapor adli-idari işlemlerde ve reklam amacıyla kullanılamaz.
3. Analiz sonuçları yukarıda belirtilen numune için geçerlidir.
4. Bu rapor, laboratuvarın onayı olmadan çoğaltılamaz.
5. İmzasız ve mühürlü raporlar geçersizdir.
6. Limit değerlerinin birimleri bulgu birimleriyle aynıdır
7. Kısaltmalar; D: Değerlendirme. U: Uygun. U.D.: Uygun Değil. D.Y.: Değerlendirme Yapılamadı. G.K.: Geri Kazanım. Ö.B: Ölçüm Belirsizliği. Ö.L: Ölçüm Limiti.
8. \* işaretli analizler akreditasyon kapsamındadır.

Damla ÇAYLI  
Kimyasal An.  
Birimi Sorumlusu(V)

Bülent TATLISÖZ  
Numune Kabul ve  
Düzenleme Birimi  
Sorumlusu

Tasdik Olunur

15.12.2017

Turgay ZABUN

Müdür

Ek-1.KYS.PR.17

İlk Yayın Tarihi: 28.11.2011

Revizyon Tarihi / No: 05.09.2017 / 03

Sayfa No 1/1

**NANOLAB LABORATUVAR HİZM. KİMYA GIDA DANIŞMANLIK ÇEVRE EĞİTİM SAN. VE TİC. LTD. ŞTİ.** [www.nano-lab.com.tr](http://www.nano-lab.com.tr)  
A. Kahveci Mh. Çankaya Cd. Vadi İş Mrkz. N-2 E K: 2 No: 25 Beylikdüzü - İSTANBUL  
Tel.: 0212 855 48 10 - 855 02 53 Fax: 0212 855 48 33