

Master's in Bioengineering

Evaluation of the packaging process and fat content of UHT milk

Master Dissertation

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Abstract

Milk is a product consumed worldwide and its production and commercialization has been increasing annually. To respond to the high demand, the dairy industry needs to not only guarantee the quality of the product, but also the optimization of the production process, being crucial to identify and evaluate the stages that involve significant product losses. Thus, the aim of this project was to evaluate milk losses during the packaging process and fat losses from the arrival of milk to Lactogal until the final product. Regarding milk losses during packaging, the volume of the final product and the microbiological quality of the packages rejected due to splicing were evaluated, to understand if there is excessive filling and rejection.

First, the behavior of the packaging machines was analyzed, and relevant oscillations were registered in the weight of the products. A control was carried out on the effective content of UHT milk and, in spite of the oscillations, all batches were within legal limits. On average, all analyzed machines had a filling above the nominal value, but this was not considered excessive (>1003 mL). Secondly, packages rejected due to splicing were microbiologically analyzed using flow cytometry (D-Count®). Most of the analyzed packages had IBC/mL values within the legal marketing values, and the contamination of the positive samples was due to package damage and not during splicing. As such, it was considered that the two rejected packages from strip and paper splices, and the last three from paper splices in Edge machines, can be placed on the market. Also, an analysis was performed using the traditional method of plate count. The positive samples (>200 IBC/mL) had high counts, in agreement with the results obtained by D-Count®. The suspect samples (100-200 IBC/mL) and some samples in the negative range (0-100 IBC/mL) had zero counts, which may indicate that the cells are in a VBNC state due to the stress suffered after the UHT heat treatment. The third task was related to fat loss throughout the process, and the FTIR principle (MilkoScan®) was used to quantify the percentage of total fat in milk. The results obtained were inconclusive since fat gains were verified in some stages. This can be justified since the equipment used had coefficient of variation $\geq 1\%$ and the recorded values were within this range.

Thus, regarding milk losses during packaging, it was not possible to implement reductions in the filling volume, but a reduction in the number of rejected packages was proposed, which implies a great economic advantage (saving 47304-57816 €/year). As it was not possible to assess fat losses throughout the production process, other methods such as the use of gas chromatography to analyse the fatty acid/triglyceride profile of samples was suggested.

Keywords: UHT milk, losses, microbiology, statistical control, fat.

Resumo

O leite é um produto consumido mundialmente, sendo que a sua produção e comercialização tem vindo a aumentar anualmente. Para dar resposta à elevada procura, é necessário garantir a qualidade do produto e a otimização do processo produtivo, sendo crucial identificar e avaliar as etapas que englobam perdas significativas. Assim, o objetivo deste projeto foi avaliar as perdas de leite durante o processo de embalagem e perdas de gordura desde a receção do leite ao produto final. Relativamente às perdas de leite no embalagem, foi avaliado o volume do produto final e a qualidade microbiológica dos pacotes rejeitados devido aos empalmes, de forma a compreender se existe um enchimento e rejeição excessivo.

Em primeiro, o comportamento das máquinas de embalagem foi analisado, sendo que se registaram oscilações significativas nos pesos dos produtos. Foi realizado um controlo do conteúdo efetivo dos produtos de leite UHT e, apesar das oscilações, todos os lotes encontravam-se conforme os limites legais. Em média todas as máquinas apresentavam um enchimento superior ao valor nominal, porém não foi considerado excessivo (>1003 mL). Em segundo, os pacotes rejeitados devido aos empalmes foram avaliados microbiologicamente através da citometria de fluxo (D-Count®). A maioria das amostras analisadas apresentou valores de CBI/mL dentro dos valores legais de comercialização, sendo que a contaminação das amostras positivas foi proveniente de danos na embalagem e não durante o empalme. Assim, considerou-se que os dois últimos pacotes rejeitados dos empalmes de tira e papel, e os últimos três no caso do empalme de papel nas máquinas Edge, podem ser aproveitados. Também, realizou-se o método de contagem em placa. As amostras positivas (>200 CBI/mL) apresentaram contagens elevadas, o que comprova os resultados obtidos pelo D-Count®. Obteve-se contagens nulas para as amostras suspeitas (100-200 CBI/mL) e negativas (0-100 CBI/mL), o que pode indicar que as células se encontram num estado VBNC devido ao stress sofrido após o tratamento UHT. A terceira tarefa estava relacionada com perdas de gordura ao longo do processo e foi utilizado o princípio de FTIR (MilkoScan®) para quantificar a gordura total (%) no leite. Os resultados obtidos foram inconclusivos uma vez que se verificou ganhos de gordura em algumas etapas. Tal pode ser justificado, pois o equipamento utilizado apresentava um coeficiente de variação $\geq 1\%$ e os valores registados encontram-se dentro desta gama.

Deste modo, não foi possível implementar reduções no volume de enchimento, mas foi proposta uma redução no número de embalagens rejeitadas, o que implica uma poupança de 47304-57816 €/ano. Embora não tenha sido possível avaliar as perdas de gordura ao longo do processo de produção, outros métodos, como o uso de cromatografia gasosa para analisar o perfil de ácidos gordos/triglicerídeos das amostras, foram sugeridos.

Palavras-Chave: leite UHT, perdas, microbiologia, controlo estatístico, gordura.

Declaration

I hereby declare, under word of honor, that this work is original and that all non-original contributions are indicated, and due reference is given to the author and source.

Carolina Columbano Couto

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Notation and Glossary

Units

V	Volume	L
W	Mass	kg
t	Time	s
T	Temperature	°C

Greek letters

ρ	Density	kg/m ³
σ	Standard deviation	-

List of Acronyms

APC	Aerobic Plate Count
CFU	Colony Forming Units
CI	Cryoscopy Index
CIP	Cleaning-in-place
FFA	Free fatty acid
FLW	Food Loss and Waste
FPD	Freezing point depression
FT	Fourier Transform
FTIR	Fourier Transform Infrared
IBC	Individual bacterial count
IFS	International Food Standard
IR	Infrared
SnF	Solids-not-fat
SPC	Statistical Process Control
TAG	Triglyceride
TNE	Tolerable negative error
UHT	Ultra High Temperature
VBNC	Viable But Not Cultivable

1 Introduction

1.1 Background and project presentation

Global milk production reached nearly 906 million tonnes in 2020, with Asia as the main producer (379 million tonnes) followed by Europe (236 million tonnes) (FAO, 2021). It is projected to grow at 1.6% per year over the next decade, reaching 997 million tonnes by 2029, faster than most other main agricultural commodities. These high production numbers are reflected in the high demand for milk, which constitutes an essential component in the diet of approximately 6 billion people (FAO, 2020).

Due to its high demand, the dairy industry is involved in high-volume production, so it is extremely important to control not only the quality of the product alone, but also the production processes (Parfitt et al., 2010). With the increase in market competitiveness, companies increasingly need to offer products and services with higher quality, which is associated with cost reduction. Moreover, in our current reality where about 850 million people live with chronic hunger, reducing the amount of Food Loss and Waste (FLW) is broadly seen as an approach to increase the efficacy of the agro-food framework and improve food security (Cammarelle et al., 2021). Parfitt et al. (2010) refer to ‘food loss’ as a “decrease in edible food mass throughout the part of the supply chain that specifically leads to edible food for human consumption” and that it occurs during production i.e., stages before reaching the consumer, while ‘food waste’ takes place at the retail chain and consumption.

Product loss in the dairy processing industry is about 3% of the processing volume (Halls, 2016). Although this may sound insignificant, in high production dairy industries such as Lactogal (Modivas) which produces an estimated 1.5 million liters of UHT milk and cream per day, it adds up to a daily loss of 45 thousand liters i.e., 16.4 million liters per year. Usually, Lactogal purchases milk from producers at a minimum price of 0.30-0.32 € per liter (depending on the quality of the milk), which translates into a loss of 13.5 thousand € per day or 4.9 million € per year. These values are aggravated when it comes to cream, since this product has an ex-factory cost of approximately 1.9-2.0 € per liter, which is much higher than milk.

Therefore, the identification and evaluation of the critical losses’ points in the UHT milk and cream production process is vital since the implementation of improvements can translate into a significant cost reduction. In this context, an evaluation regarding the fat content and the packaging process, namely the overfilling of packages and the excess rejection of packages during the splicing step, was performed.

1.2 Company presentation

Lactogal Produtos Alimentares S.A. was founded in Portugal in 1996, as a result of the partnership between Agros (*União das Cooperativas de Produtores de leite entre Douro, Minho e Trás-os-Montes, UCRL*), Lacticoop (*União das Cooperativas de Produtores de Leite entre Douro e Mondego, UCRL*) and Proleite/Mimosa. Lactogal is an agri-food company which produces and sells milk and its derivatives, nationally and internationally. Its range of products is constantly growing and is presently comprised by milk, yogurts, cheese, butter, cream, water and juices, aggregating the following brands: Adagio, Agros, Castelinhos, Castelões, Fresky, Gresso, Longos Vales, Milhafre dos Açores, Mimosa, Matinal, Prado Verde, Pleno, Primor, Serra Dourada, Serra da Penha, Vigor. This company leads the Iberian Peninsula market in the dairy sector with 7 factory units. In Portugal, it has three industrial units, four logistics platforms and eight commercial offices. This dissertation was developed in the Quality Control Laboratory of the industrial unit of Modivas - Vila do Conde, which has a total area of 190000 m², of which 40000 m² are covered area, being responsible for the production of 1.5 million liters of UHT milk (Ultra High Temperature) simple, compound, and cream per day.

Food quality and environmental sustainability is a main concern at Lactogal. The company is certified in terms of Quality by ISO 9001 (Quality Management System), as well as by the International Food Standard (IFS) regarding the quality and food security. The industrial unit of Modivas was designed to comply with all quality and hygiene requirements, presenting highly automatized technology, along with an industrial effluent treatment plant in its facilities. This company also has three ponds, which allows the flow regulation of rainwater and the irrigation of green spaces. Effluent treatment with adequate technology is also carried out to ensure that all discharges made to the water network are in accordance with the values required by law. In terms of solid waste, Lactogal promotes the recycling of a large part of the waste generated in the industrial unit.

1.3 Contribution to the work

To assess milk losses during the packaging process, the volume of the final product and the microbiological quality of the packages rejected due to splicing were evaluated. Regarding the analysis of the filling volume, the behavior of the filling machines was evaluated through run charts and a statistical control dictated by the Portuguese regulation was carried out. Also, through the ACCEPT program, control charts for certain filling machines were retrieved and analyzed in order to obtain a viable conclusion about the average filling value. Microbiological analysis of the splices was carried out through flow cytometry and plate count. Also, to assess fat losses throughout the production process, the Fourier-Transform Infrared Spectrometry method was used to evaluate the total fat percentage. Due to the impossibility of performing

this analysis in the reception (milk trucks), the total fat percentage values were retrieved from the SAP program. This program also allowed the traceability of UHT milk from its reception to its packaging.

1.4 Aims of the project

The main goal of this work was to identify and evaluate the critical losses points of the UHT milk production process, in order to implement improvements which aim to reduce losses and costs. In this context, the packaging process was analysed regarding two parameters: the volume of the final product and the microbiological analysis of the rejected packages due to splicing. These evaluations were performed to understand if there was an excess filling and/or rejection of packages, to implement reductions actions. In addition, the fat content of UHT milk was analysed from reception to packaging, in order to identify possible losses in fat during the production process.

2 Context and state of the art

2.1 Milk production process

The microbiological quality is one of the most important aspects for the evaluation of milk. Contamination by microorganisms can occur during the collection, transport and storage of raw milk (Bylund, 2015). Therefore, to guarantee and maintain the quality of milk to the consumer, Lactogal has developed a production process (Figure 1) capable of eliminating the total or large part of the microbial flora present in raw milk.

In addition to the production process, it is important to ensure optimal milking conditions in dairy farms and the selection of healthy cows (without mastitis and/or with other diseases subjected to medication) (Bylund, 2015). Fresh milk from a healthy cow is practically free from bacteria but must be protected against infection as soon as it leaves the udder. Therefore, milking conditions must be as hygienic as possible, the system must be designed to prevent aeration in order to minimize contamination with the surrounding air and the cooling equipment must be correctly dimensioned (Bylund, 2015). The cooling equipment is used to quickly cool the milk to about 3-6 °C, since it leaves the udder at a temperature of 37 °C, to prevent contamination by microorganisms, which thrive and multiply more vigorously at high temperatures (Burke et al., 2018; Bylund, 2015). At low temperatures, the level of activity of microorganisms is lowered, but the bacteria will start to multiply again if the temperature is allowed to rise during storage. Thus, breaking the cooling chain may lead to the development of microorganisms in milk, giving rise to metabolic products and enzymes, which accumulate in milk and alter its organoleptic properties (Bylund, 2015).

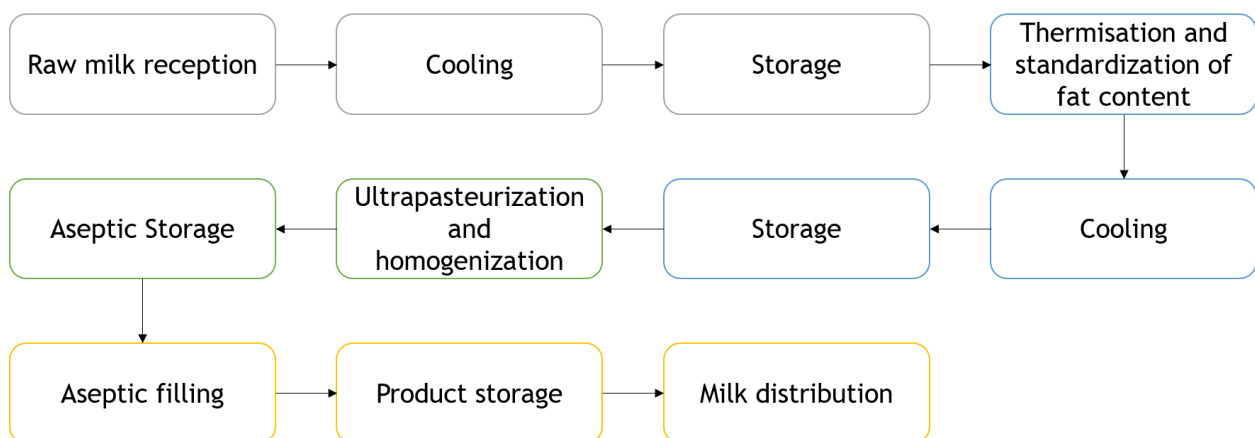


Figure 1 - Layout of the milk production process at Lactogal from reception to distribution.

2.1.1 Reception

The first stage is the reception of raw milk at the factory. Milk reception consists of three basic processes: determination of the raw milk quantity, evaluation of the raw milk quality and cleaning and disinfection of the transport facilities (Spreer & Mixa, 2017). The raw milk is transported by isothermal tank trucks to maintain the low temperature and prevent aeration. Upon arrival, samples of raw milk are manually collected for microbiological and physical-chemical analysis. These analyses include organoleptic inspections (appearance, smell and color), temperature (should be 4-6 °C), presence of inhibitors (e.g. antibiotics), pH (should be between 6.6 and 6.8), alcohol test (determines the heat stability of the proteins, milk must remain stable at an alcohol concentration of 80%) and acidity (must be between 16 and 18 cm³ NaOH/L). If the product is within the parameters analyzed, the milk is received and unloaded from the truck through a filter to retain possible impurities (sand, soil, among others). The quantity to be received is determined by volumetric flowmeters in line and the tank trucks are cleaned at the end of the collection round (Bylund, 2015; Spreer & Mixa, 2017).

In addition, an automatic sample is collected from all tank trucks at the reception to assess parameters such as number of somatic cells and bacteria, protein and fat content and freezing point. These analyzes are carried out in the laboratory of *Associação Interprofissional do Leite e Lactínicos* (Alip). The results of these tests are available on the day after the analysis, in the Lactogal database, and are related with the compensation paid to the farmer.

During transportation, a temperature increase to slightly above 4 °C is unavoidable. Therefore, the milk is usually cooled to below 4 °C in a plate heat exchanger before being stored in isothermal stainless-steel tanks to await processing. The storage tanks are equipped with agitators, a high-level electrode (HL), at the top of the tank, and a low-level electrode (LL), located in the drainage line (Bylund, 2015). The agitation employed should be gentle to prevent aeration of the milk and fat globule disintegration, which exposes the fat to attack from the lipase enzymes in the milk (Spreer & Mixa, 2017). The HL electrode, prevents overfilling by closing the inlet valve when the tank is full, switching the milk supply to the next tank. In contrast, the LL electrode indicates when the tank is completely empty. The signal from this electrode is used to stop or switch drainage to another tank (Bylund, 2015).

2.1.2 Standardization of fat content

In order to obtain a suitable product for consumption, raw milk undergoes a series of treatments before reaching the consumer. In many large dairies it is not possible to pasteurize and process all the milk immediately after reception. Therefore, the milk is usually preheated to a temperature below the pasteurization temperature to temporarily inhibit bacterial growth. This process is called thermisation. In this step, the milk is subjected to a temperature of 63 °C for approximately 15 seconds to decrease the microbial load. To prevent aerobic spore-

forming bacteria from multiplying after thermisation, the milk must be rapidly chilled to 4-6 °C or below and it must not be mixed with untreated milk (Bylund, 2015). After thermisation, milk samples are collected to verify if there were changes in the physicochemical properties, pH, and organoleptic characteristics. In addition, the cryoscopy index (CI) is analyzed to determine whether the milk has been diluted with water, since it indicates the freezing point of milk in relation to the freezing point of water. For an unadulterated milk, CI values greater than or equal to 520 mC in absolute value must be obtained. At the microbiological level, tests are made on aerobic mesophilic flora, aerobic thermophilic flora and thermosetting spores.

Simultaneously with the thermisation process, the clarification, decreaming and standardization of the fat content occurs. Although during reception the milk passes through filters, the purpose of clarification is to remove from the milk all foreign material, such as sand, soil, dust and precipitated protein, which may have entered in the milk during milking, treatment or transport (Burke et al., 2018; Spreer & Mixa, 2017). The absence of this step would cause the particles to form a sediment that would be visible in the final product and could damage the downstream processing equipment (Burke et al., 2018). Decreaming is the mechanical separation of milk into cream and skimmed milk by means of centrifugal forces. The main goal is to produce skimmed milk with the lowest fat content possible, which correlates to good decreaming efficiency (Spreer & Mixa, 2017).

Clarification and decreaming are combined in one operation and are carried out in a disc-bowl centrifugal clarifier (Figure 2) with a three-phase separation (dirt, skimmed milk and cream). The centrifugation speed starts at 2000-3000 rpm, which allows clarification, and with the increase in the centrifugation speed to 5000-7000 rpm, decreaming occurs. Decreaming is based on the fact that fat exists in an emulsified state (polydisperse system) and that the specific density difference between milk fat ($\rho = 0,93 \text{ g/cm}^3$) and skimmed milk ($\rho = 1,035 \text{ g/cm}^3$) is substantial. The milk enters the bottom of the equipment and, under the influence of the centrifugal force, the particles and fat globules begin to separate according to their density. Therefore, the particles that have higher density, will move to the periphery of the equipment and accumulate in an appropriate space. The cream (fat globules) has a lower density than the skimmed milk and therefore moves inwards in the channels, towards the axis of rotation, being forced to move to the upper part of the equipment.

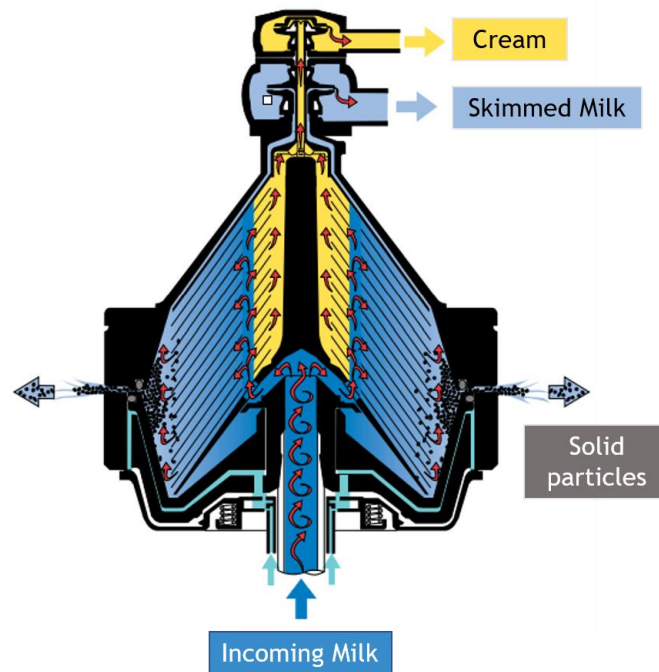


Figure 2- Centrifuge used for clarification of milk (adapted from Bylund, 2015).

The subsequent step to clarification and de creaming is standardization and aims to adjust the fat content of milk, by the addition of cream or skimmed milk, to obtain a certain fat percentage value. Therefore, whole milk must have a fat content of 3.5% (wt/wt), semi-skimmed milk must have a content of 1.5% (wt/wt) and skimmed milk must have a maximum fat content of 0.3% (wt/wt) (Bylund, 2015). In Lactogal, the standardization of milk is automatic (direct in-line standardization), by means of control valves, flow and density meters and a computerized control loop which are used to adjust the fat content of milk and cream to desired values (Figure 3). In order to enable accurate standardization, the pressure in the skimmed milk outlet, the temperature and the fat content in the whole milk before separation must be constant.

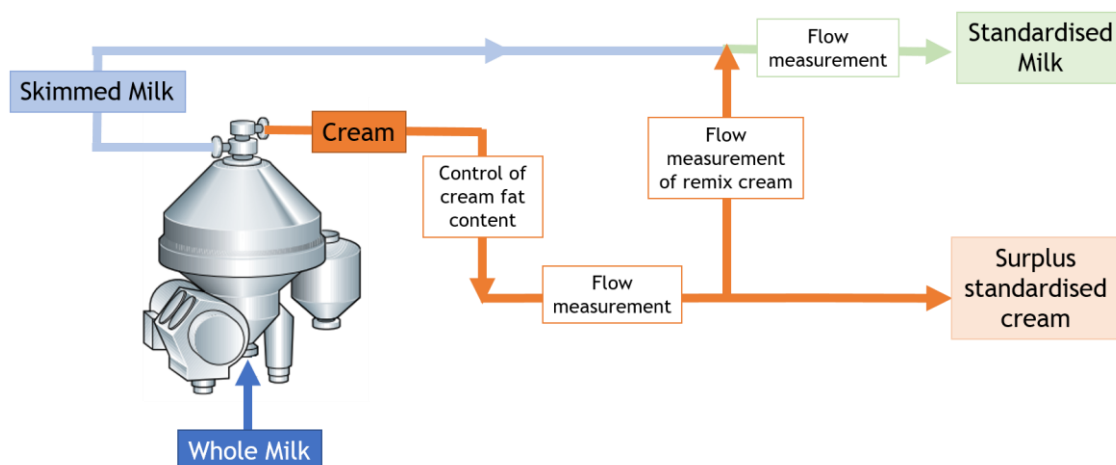


Figure 3 - Direct in-line standardization of cream and milk (adapted from Bylund, 2015).

The clarification, de creaming and standardization of fat usually results in a temperature increase in milk, so the standardized milk is usually cooled to below 4 °C in a plate heat exchanger before being stored in isothermal stainless-steel tanks to await ultra-pasteurization (Bylund, 2015).

2.1.3 UHT treatment

The main aim of heat treatment during the processing of milk is to eliminate pathogenic bacteria, reduce/eliminate spoilage bacteria, inactivate indigenous enzymes (lipases or proteases) and increase shelf-life (Chandan, 2009). With this purpose, heat treatment is repeatedly applied in the dairy industry and is divided into several types such as thermisation (process of exposing products to temperatures between 57 and 68 °C for 5 s to 30 min) and ultra-pasteurization, also called Ultra High Temperature (UHT) (using a temperature range of 135 to 150 °C for 1 to 10 s).

UHT treatment is a continuous process that takes place in a closed system (prevents contamination of the product by airborne microorganisms). The primary goal of the UHT treatment is to produce a milk which is commercially sterile, i.e. free from microorganisms that can grow under the normal conditions of storage (Tamime, 2009). This treatment can be either 'direct', where superheated steam is mixed with milk or 'indirect', where a heat exchanger transfers heat across a partition between the milk and the heating medium (steam or hot water) (Bylund, 2015). In Lactogal both indirect and direct method are employed by plate heat exchangers. In the indirect method a closed hot-water circuit is used as a heating medium. The milk (at 4 °C) is pumped from the storage tank to a plate heat exchanger and heated to about 75 °C. The preheated milk continues to the heating section of the plate exchanger where is heated to 141 °C, and the milk passes through a holding tube for about 6 s. Finally, cooling is done regeneratively in two stages: first against the hot water circuit's cool end, and subsequently against the cold entering product (Bylund, 2015). On the other hand, in the direct method, the product (at 4 °C) is pumped into the preheating section of the plate heat exchanger and heated to 80 °C. After preheating, the product pressure is increased and it continues to a ring nozzle steam injector, where the steam is injected into the product, instantly raising the temperature to 141 °C. The product is held at this temperature for 6 seconds before it is flash cooled in a condenser-equipped expansion chamber (Bylund, 2015). A direct and indirect UHT heating temperature-time profile is shown in Figure 4.

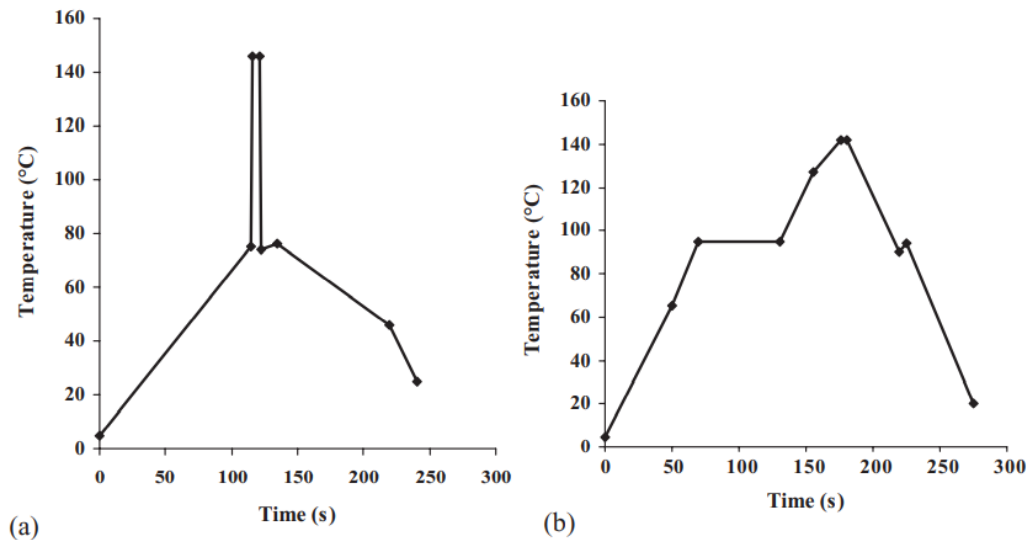


Figure 4 - Temperature-time profile of (a) direct and (b) indirect UHT treatment (retrieved from Tamime, 2009).

Right after the UHT process, the milk is homogenized by an aseptic homogenizer incorporated at the end of the UHT equipment, to break down the fat globules and distribute them evenly, avoiding the formation of cream. The ultra-pasteurized milk is provisionally stored in an aseptic tank with a capacity of 20000 L. This intermediate storage is very useful in cases of unexpected stops at the filling machines (stores the surplus milk during stoppage) or when packaging of two products is occurring simultaneously (Bylund, 2015).

2.1.4 Aseptic Packaging

According to Bylund (2015), aseptic packaging can be defined as “a procedure consisting of sterilization of the packaging material or container, filling with a commercially sterile product in a sterile environment, and producing containers which are tight enough to prevent recontamination”. Thus, in this step, the milk is packed in sterilized and hermetically sealed carton packages by the filling machines of Tetra Pak, which are fully automated.

The machines have a compartment for the packaging material that is rolled up in the form of a coil to facilitate transport and operations within the industry and optimize the storage location. The packaging produced by this company consist of six layers (Figure 5): the first two inner layers are made of polyethylene, followed by aluminum, polyethylene, cardboard and again polyethylene (Tetra Pak, 2020). Each of these layers has different functions in the packaging: the card, made of renewable material, guarantees stability and resistance; polyethylene ensures that there is no direct contact between the milk and the other layers and helps protect against external humidity; and aluminum protects the milk from oxygen and prevents the passage of light, thus maintaining the nutritional value and the organoleptic characteristic of the product. The result is a package that guarantees the protection of milk

against light, air, water and microorganisms, maintains the integrity of the milk and increases shelf life. Each individual paper package is united by a polyethylene strip (Tetra Pak, 2020).

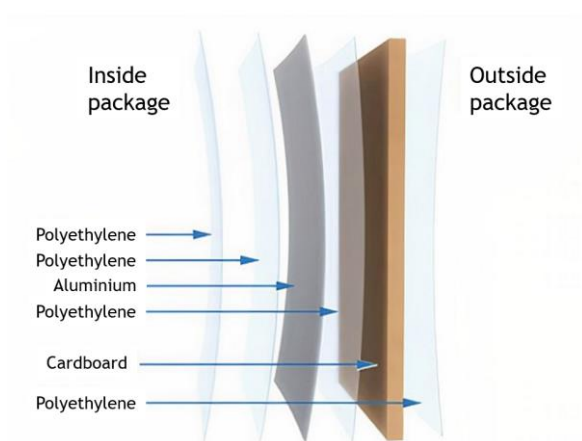


Figure 5 - Tetra Pak packaging (adapted from Tetra Pak, 2020).

During the packaging process, when the paper roll or strip (components of the UHT packaging) are about to run out, they are joined with a new roll of the respective material; this amendment is called a splice. Splices are performed manually by the machine operator that ensures the correct union and quality of the roll to be used. The operator must disinfect his hands before the splice and the roll is further sterilized with 30% hydrogen peroxide (H_2O_2) followed by a heating step at 70 °C for six seconds, where the packaging material is compressed by rollers, and subjected to hot air treatment (125 °C) to evaporate the residual H_2O_2 (Tamime, 2009). Despite these disinfection steps, when the splicing is carried out, the packaging machine automatically rejects a certain number of packages, depending on the machine and the type of splice (paper or strip), designated in this dissertation as rejected packages. This procedure is implemented for the sake of food quality, to ensure that the sterility conditions of the final product are not altered, either by the manual contact of the operator or by the incorrect packaging.

The last step, before going to the market, is the palletizing and storage of the product in a quarantined state, so that all necessary tests can be carried out. When all the microbiological, physicochemical, and organoleptic results are known and if the product is fit for consumption, it is released and distributed to the market.

2.2 Statistical Process Control in the Food Industry

Statistical Process Control (SPC) is an industry-standard methodology for measuring and controlling quality during the manufacturing process and, ideally, improving a process through statistical analysis (Lim et al., 2014). Therefore, it is characterized as a methodology that acts preventively on the production process, making use of techniques and statistical analysis to

evaluate the behaviour of the manufacturing process, allowing the implementation of corrective and improvement actions. The implementation of the SPC methodology has several benefits, which include accurate assessment of process capability, predictive, preventive and diagnostic of process control, timely detection of process drift or “special causes” of variation, quantifiable customer quality assurance and effective and systematic documentation of quality related data (Grigg, 1998; Grigg & Walls, 1999).

SPC was initially popularised in 1950 in the Japanese manufacturing industry by W. E. Deming, who elaborated on the principles developed by W. Shewart in 1920 (Lim et al., 2014). The basic philosophy of Deming was that quality and productivity increase as variability decreases and, because all things vary, statistical methods of quality control must be used to measure and gain understanding of the causes of the variation (Lim et al., 2014). The food industry was one of the pioneer industries to implement SPC methods. Most of the applications of SPC were applied in the control of the packaging process, where food producers continuously faced problems reducing process variations and detailing accurate net weight. Since then, many efforts to improve filling process control through statistical methods have been made and have led to important savings (Lim & Antony, 2019a).

2.2.1 SPC usage in food packaging

Weight and measures control is very important since the underfilling of packages is a significant issue in consumer protection, as the costumer takes in on trust that purchased food products are of the stated weight. Also, there are potentially significant financial considerations for the food producer, since the overfilling of packages entails costs that can be avoided (Grigg, 1998). The control of package weight and quantity is regulated, in Portugal, by the Regulation of Metrological Control of Quantities of Pre-package Products (Decreto-Lei n.º 310/91, 1991). This control is mandatory and exercised by the Portuguese Quality Institute. As a rule, the control needs to be exercised, at least, once a year for each packer, importer, product (with identical characteristics) and nominal quantity (Decreto-Lei n.º 310/91, 1991). This control includes a series of rules, which indicate the sample collection, the control site, the verification of the effective content and acceptance of the batch. According to *Decreto-Lei n.º 310/91 (1991)*, the control must be carried out by statistical methods and is exercised on the average and effective content of the pre-package samples.

The declared weight/volume of the package is known as the “nominal quantity”, and if the actual content is less than the nominal quantity stated, the difference is referred to negative error. For any given weight/volume there is an associated tolerable negative error (TNE), which represents a permissible amount by which some packages may be underfilled (Table 1). According to the Regulation, there is a set of three rules that packers must comply with (Decreto-Lei n.º 310/91, 1991):

1. The actual contents of the packages shall be not less, on average, than the nominal quantity.
2. Not more than 2.5% of the packages may be non-standard, for example, have negative errors larger than the TNE specified for the nominal quantity.
3. No single package may be inadequate, for example, have a negative error larger than twice the specified TNE.

Table 1 - Tolerable negative errors (Decreto-Lei n.º 310/91, 1991)

Nominal quantity (Qn)	Tolerable negative error (TNE)	
	As percentage of Qn	g or mL
5-50	9.0	-
50-100	-	4.5
100-200	4.5	-
200-300	-	9.0
300-500	3.0	-
500-1000	-	15.0
1000-10000	1.5	-

In order to ensure that all three requirements are met for any sample, there are two basic methods which organizations can employ. The first method is to pack to a mean target weight which is higher than the nominal quantity. This method will result in the overfilling of packages at the expense of the packer but is not uncommon. In many cases, when the product is relatively cheap, such as milk, this is not seen as a problem (Grigg & Walls, 1999). However, this strategy can result in two main issues. First, any substantial overfilling is a violation of the regulation, and second, although a sufficiently high mean weight may be achieved in this manner, if the process variance is not controlled, more than the allowed proportion of packages may fall below the minimum TNE values (Grigg & Walls, 1999).

The second method involves the use of SPC to routinely monitor and control the mean and variability of package quantities. To this end, continuous sampling and control charts must be used to accurately determine the average fill level and the inherent variability of the process. Usually, in high production industries, the control charts are automatically plotted and analyzed by statistical software programs (Grigg et al., 1998). For this purpose, Lactogal uses the program ACCEPT, which is a software for metrological control of pre-packaged products, which guarantees the fulfillment of the legal criteria of its products, eliminating losses resulting from overfilling. Also, it allows to verify, in real time, if the legal criteria for each batch are being met since the program statistically analyzes the filling process and issues batch reports that can be used as documents in audits (ACCEPT, 2021).

2.2.2 Control Charts

A control chart is one of the most technically sophisticated tools of SPC, and it is considered the core tool of the technique. They are also known as Shewhart charts since they were initially proposed by W. Shewhart (Lim & Antony, 2019b; Montgomery, 2013).

A control chart is a graphical display of the quality parameters that have been measured typically through a range of time in a continuous manner. It usually contains three lines, that are known as control limits. The centreline represents the average value of the measured parameters, in which the process is under control. The chart also presents two lines corresponding to the upper and lower limit, which will delimit an area that comprises values of a process still under control. Any point that extrapolates such a region indicates that the process is out of control, or in an out-of-control state, indicating that investigations, feedback plans and corrective action are required (Lim & Antony, 2019b; Peña-Rodríguez, 2003). In addition, even though all points are in the control region, if a systematic situation is observed, where the points have some special configuration that excludes the randomness of the data, the process may be out of control, since processes under control are characterized by randomness (Montgomery, 2013).

The control charts test the proposition that process/product data are consistent and uniform, and that variation is caused by inherent or normal causes. If the hypothesis is rejected, that means that unusual causes of error are present, requiring further investigation of the root causes and, to avoid any nonconforming products manufacture, corrective actions must be taken (Montgomery, 2013). Although control charts usually employ historical data, the main purpose is to prevent the production of defective items and facilitate the process prediction as it has the capability of reflecting the trend of the process (Lim & Antony, 2019b). There are different ways to construct a control charts, but the common steps usually are: the identification of process characteristics with observations or calculations; calculate the process mean; determine the standard deviation; calculate the upper and lower control limits and finally, plot the control limits and connect the consecutive points (Lim & Antony, 2019b).

2.2.2.1 Types of Control Charts

Control charts can be classified according to the quality characteristics analyzed in the process, that is, variable control charts and attributes control charts. Variable control charts are used when it comes to quality characteristics that can be expressed in numerical terms, on a continuous scale of measurement (Lim & Antony, 2019b; Montgomery, 2013). Most food manufacturing process parameters measured use these types of data, so variable control charts will be the focus of explanation. Of this category of control charts, the most used are:

1. **X-bar (average chart):** in this chart the averages of the samples are plotted, to control the average values of the studied characteristics. Therefore, it is possible to monitor the average level of the process from the variability of the samples (Montgomery, 2013).
2. **R-bar (amplitude chart):** the amplitude chart reveals the variability within the same sample. The X and R charts should be used together, if the sample size is relatively small, to ensure an efficient monitoring of the process (Montgomery, 2013).
3. **S-bar (standard deviation chart):** in this type of chart, the standard deviation values are plotted, which indicate the variability of the sample measurements. They are preferred when the number of samples are higher than 10 or 12, since for values greater than these the amplitude (R) loses the efficiency. This chart should also be used in conjunction with the average chart (Lim & Antony, 2019b; Montgomery, 2013).
4. **Individual chart:** in cases where the sample consists of an individual unit, this chart is used. This can occur when the production rate is very slow and it is impracticable to wait for the accumulation of samples to carry out the analysis, or when the standard deviation obtained is extremely small. In addition, the chart of individual measures can be used when several measures are taken in the same product unit (Montgomery, 2013).

Attributes control charts are used for quality characteristics that cannot be measured on a quantitative scale, for example, the number of defective units, the number of defects in a unit or the number of complaints received from dissatisfied customers (Lim & Antony, 2019b). Similar to the variable control chart, the attribute control chart consists of several types of charts, depending on the purpose of the process control and the type of data. In this category, the most used are the proportion defective chart (p-Chart) and the number defective chart (np-Chart). Nevertheless, the patterns and rules to indicate an out-of-control condition are similar for both variables and attribute control charts (Lim & Antony, 2019b; Montgomery, 2013).

In addition, a powerful tool in process improvement is the Run Chart. This chart is often used at the beginning of a project, since it can detect trends in processes, whether there is a degradation or improvement over time, before gathering sufficient data to calculate reliable control limits. The run chart is a line chart of at least 10 point data plotted over time with the mean value as a horizontal line (Lim & Antony, 2019b).

2.3 Milk microbiology

Due to its chemical composition, milk is an ideal medium for the growth of microorganisms (Bylund, 2015). One of the main concerns in the quality of milk is the permanent risk of contamination by microorganisms, since the contaminated milk constitutes losses for the producer, industry and consumer. Microbiological contamination is associated

with changes in the product, such as color, viscosity, odor, taste and nutritional changes (Tamime, 2009). Therefore, the microbiological quality of milk is an important criterion in the quality of the final product.

2.3.1 Microbial composition and main contamination factors

Microorganisms present in milk can be classified into two main groups: desirable and undesirable. The desirable microorganisms are used for the transformation of milk into fermented products such as yoghurts or cheese. On the other hand, undesirable microorganisms cause food deterioration and public health problems, thus limiting the product durability (Chandan, 2009; Tamime, 2009). These can be classified as spoilage or pathogenic microorganisms, although some can play a dual role (Tamime, 2009). Spoilage microorganisms are capable of hydrolyzing milk components such as fat, lactose or protein by action of enzymes such as lipases, esterases or peptidases, to yield compounds suitable for their growth. While they are not a great public health threat, such reactions can lead to organoleptic and nutritional changes in milk, making the milk unfit for consumption (Tamime, 2009). Pathogenic microorganisms are those that can be transmitted to humans through milk, inducing food poisoning, thus posing a threat to human health. In addition, they can be responsible for some changes in milk, such as color, odor or aroma (Chandan, 2009).

Most microorganisms isolated from raw milk are bacteria, however viruses, protozoan parasites, algae, yeasts, and molds have also been linked to raw milk. Bacteria associated with milk and dairy products are generally classified by their resistance to heat (thermo or non-thermophilic) and colling (psychrophilic) or by their ability to form spores (spore or nonspore-forming bacteria) (Chandan, 2009).

As previously mentioned, contamination by microorganisms can occur during the collection, transport and storage of milk (Bylund, 2015). Although milk secreted by the udder is virtually sterile, it is infected by a small number of bacteria present in the teat channel. However, in case of bacterial udder inflammation (mastitis) or other diseases, poor hygiene conditions or improper storage, milk is heavily contaminated with bacteria and may even be unfit for consumption (Bylund, 2015; Chandan, 2009). The degree of infection and the composition of the bacterial population depends on several factors such as: animal diseases and diet, milking conditions and environment, cleanliness of surfaces and equipment in contact with milk, sanitation and refrigeration practices, storage and processing conditions, as well as climatic and geographic factors (Chandan, 2009; Tamime, 2009).

Bacteria that belong to the normal udder flora of animals are called lactic acid bacteria, namely *Lactobacillus sp.*, *Lactococcus sp.* and *Leuconostoc sp.* These bacteria are characterized by not producing spores and predominantly produce lactic acid from carbohydrates such as lactose, being able to grow rapidly in milk especially at temperatures above 20 °C (Bylund,

2015; Robinson, 2002). Pasteurization at a low temperature (e.g, for 15 seconds at 72 °C) can kill mesophilic lactic acid bacteria; however, it does not kill thermophilic lactic acid bacteria like *Streptococcus thermophilus* (Robinson, 2002). Even though this group of bacteria can spoilage milk, it is also useful in the production of fermented dairy products. On the other hand, coliform bacteria (*Enterobacteriaceae* family) can be found digestive track of animals and are classified as spoilage microorganism (Bylund, 2015). Both *Escherichia coli* and *Klebsiella aerogenes* belong to this family and usually grow rapidly in milk, particularly above 20 °C. They metabolize lactose and break down milk protein, resulting in the formation of gas (CO₂ and H₂) leading to an off-flavor and smell of milk (Bylund, 2015; Chandan, 2009).

The microorganisms that normally contaminate milk grow over a wide temperature range and can be divided into mesophilic, thermophilic and psychrophilic. The group of mesophilic bacteria is defined by an optimal growth temperature between 30 to 37 °C (supporting a maximum of 50 °C), while thermophilic bacteria have an optimal growth between 50 and 55 °C, being able to multiply up to 60 °C (Gleeson et al., 2013). The predominant mesophiles in milk are spore or non-spore-forming bacteria, such the genera *Bacillus*, *Microbacterium*, *Micrococcus*, *Enterococcus*, *Streptococcus* and *Arthrobacter*. This group comprises the majority of contaminants, both spoilage and pathogenic (Gleeson et al., 2013). The thermophilic bacteria are normally found in small numbers in milk but can reach large populations when milk is kept at high temperatures. The genera *Bacillus* and *Clostridium* are the most important thermophilic microorganisms in milk (Gleeson et al., 2013; Tamime, 2009). As for psychrophiles, they have an optimal growth temperature lower than 20 °C and tolerate a maximum of 25 °C; developing, typically, when milk is subjected to long periods of storage at low temperatures (2-6 °C). Psychrophilic bacteria found in milk are Gram-negative (*Pseudomonas*, *Aeromonas*, *Chromobacterium* and *Flavobacterium spp.*) or Gram-positive (*Bacillus*, *Clostridium*, *Corynebacterium* and *Streptococcus*), capable of producing thermostable hydrolytic enzymes that can maintain their activity even after heat treatment (Chen et al., 2003; Cousin, 1982). It is also relevant to mention that this group of bacteria can spoil milk in a very short time without causing its acidification (Cousin, 1982).

Due to the ability of these bacteria to produce enzymes, such as proteases and lipases, milk undergoes a series of changes due to the degradation of protein and milk fat. This can cause changes in taste and odor or shorten the shelf life of the product, which leads to economic losses and possible problems for public health (Robinson, 2002; Tamime, 2009). Since most of the microorganisms involved are destroyed by ultra-pasteurization, milk deterioration is usually caused by post-pasteurization contamination. However, some of the spoilage microorganisms are resistant to heat (spore-forming) and produce thermostable enzymes that carry over to the finished product (Chandan, 2009).

Pathogenic bacteria and/or those that produce toxic substances are present in raw milk by external contamination or by bovine mastitis. The most prevalent pathogenic bacteria found external to the udder are *Salmonellae* and thermotolerant *Campylobacter* strains. Because they have been linked to multiple outbreaks of illness and to antibiotic-resistance, *Salmonellae* are a major source of concern for the dairy sector (Tamime, 2009). Regarding the pathogenic bacteria involved in mastitis, the most common are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Listeria* spp. and *E. coli*. *S. aureus* can produce heat-stable enterotoxins that cause food poisoning, while *S. agalactiae* causes bacteremia and meningitis, which are potentially fatal to infected infants (Chandan, 2009; Tamime, 2009).

2.3.2 Microflora of UHT milk

The contaminants present in UHT milk can result from three sources: post-process contamination, the survival of heat-resistant spore-formers and viable but not cultivable bacteria (Tamime, 2009).

Post-process contamination can occur through poor surface and equipment hygiene, low quality water and poor hygiene of the workers or external agents (Tamime, 2009). The major sources of spoilage bacteria in UHT milk are related to packaging problems, involving the aseptic filling process or faulty seams or pin-holes in the packaging, and processing equipment (Varnam & Sutherland, 2001). Filling equipment is a common source of psychrophiles in packaged milk, even when it is effectively sanitized. These microorganisms may enter the filler through vacuum system or from containers (Varnam & Sutherland, 2001). In addition, storage tanks can also be a source of psychrophiles, pseudomonads and heat-resistant spore-formers, since they are able to strongly adhere to the surface of tanks and the existence of microscopic fissures in the walls can protect organisms from the sanitizing procedures (Tamime, 2009).

The spores capable of surviving UHT process are mainly *Geobacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus sporothermodurans* and *Paenibacillus lactis* (Robinson, 2002; Tamime, 2009). *G. stearothermophilus* has the greatest survival potential, but as this organism is a thermophile (unable to grow below 30 °C), spoilage problems may only occur in packages held at elevated temperatures (Varnam & Sutherland, 2001). *B. sporothermodurans* is a main contamination problem in UHT milk since it has a high heat resistance, mesophilic growth and it is extremely difficult to remove from contaminated equipment. For the inactivation of this organism, it is required heating conditions of 150 °C for 6 s (Tamime, 2009).

In addition, due to UHT treatment, bacteria can enter a physiological state called viable but not cultivable (VBNC). Unlike normal cells that are culturable on media and develop into colonies, VBNC cells are living cells that have lost the ability to grow on suitable media but

are not regarded as dead cells. While dead cells have a damaged membrane and are metabolically inactive, VBNC cells have an intact membrane containing undamaged genetic information and are capable of respiratory and enzymatic activity (Zhao et al., 2017). Cells enter the VBNC state as a response to stress, such as high or low temperatures, suggesting that this is an adaptive strategy for long term survival of bacteria, being able to exit from dormancy when the environmental conditions become hospitable (Ayrapetyan & Oliver, 2016).

According to Gunasekera *et al.* (2002), in a commercially available pasteurized milk, the viable cell counts determined from colony forming unit (CFU) was significantly lower than direct viable counts, which suggests that a significant subpopulation of cells might be in a VBNC state. Thus, the total number of viable bacteria in a sample will be underestimated by the CFU count method. Underestimation or non-detection of viable cells in quality control in the food sector may pose major threats to the public, especially for bacterial species causing human infections. In addition, it may also limit shelf life and cause early spoiling of products, being important to use detection methods (Ayrapetyan & Oliver, 2016; Gunasekera et al., 2002). There are several approaches for determining the total and viable count of bacteria in alternative to culture-based methods, of which the most used is flow cytometry due to its simplicity and fast processing (Jepras et al., 1995; Silva et al., 2004).

2.3.3 Microorganism counting techniques

Bacterial counting is one of the most important tools for monitoring microbiological quality. The reference method for enumeration of microorganisms is the standard plate count, however, despite being a very simple method, it is very time consuming. In this way, new technologies have been developed and, over the years, the standard plate count method has been replaced by faster methods with less influence of human errors due to their automation, such as flow cytometry.

2.3.3.1 Traditional methods

The standard plate count, also known as aerobic plate count (APC) is one of the reference methods and is used for estimating the bacterial population that can grow aerobically at mesophilic temperatures (Mendonca et al., 2020; Tamime, 2009). Plate counts are based on the use of selective or general media, formulated for specific microbial groups or organism to grow and form colonies that can be counted visually. The results reflect the number of colonies that can emerge under the given physical and chemical conditions, such as available nutrients, atmosphere or temperature, and are expressed in colony forming unit (CFU) per milliliter (CFU/mL) (Marshall, 1992).

The advantages of this method are its simplicity, ease of execution and it does not require sophisticated equipment or specialized technicians. However, there are several

disadvantages, such as the exclusive count of colonies. Colonies are aggregates of living microbial cells, thus it's not possible to compare the results with direct counts. Plate counts underestimate the presence of microorganism since is only able to enumerate the viable and cultivable cells, as VBNC do not grow in a culture medium (Marshall, 1992; Tamime, 2009). On the other hand, only microorganisms capable of developing under the specific conditions of the medium will be detected. In addition, this method requires a minimum of 72 hours of incubation to obtain the results, thus becoming a problem for the industry as it is very time consuming (Marshall, 1992).

2.3.3.2 Flow cytometry

The limitations of the traditional methods, such as long incubation times, caused an intense investigation to develop other faster and more automated methods, such as flow cytometry. Flow cytometry is a quantitative technique for cell analysis, developed in the 1970s, which allows the simultaneous measurement of multiple physical, chemical and biological characteristics of cells in suspension. This technique is used in many applications such as pharmacology, microbiology, molecular biology and genetics (Adan et al., 2017). Flow cytometry has the ability to perform rapid measurements of the optical and fluorescence characteristics of individual cells in homogeneous or heterogeneous populations or any other particle such as microorganisms, nuclei and chromosome preparations in a fluid stream when they pass through a light source (Adan et al., 2017; Jepras et al., 1995).

D-Count[®] is a flow cytometry system, provided by bioMérieux, used by Lactogal for microbiological analysis to quantify the total flora present in the final product, including bacteria and molds (BioMérieux, 2021). The viability criteria of this equipment are the enzymatic activity of the microorganism and its membrane integrity. Only viable microorganisms, with active metabolism, can cleave the fluorescent substrate, resulting in the release of a fluorochrome. Since the interest of using this device falls on counting viable cells, the equipment automatically labels the microorganisms with active metabolism, including those growing in conditions of stress or in the presence of growth inhibitors (BioMérieux, 2021). Therefore, the samples are incubated with a non-fluorescent viability substrate, ChemChrome V26, which crosses the membrane of viable cells by passive transport. In the microorganism's cytoplasm, the ChemChrome V26, that is a fluorogenic ester, is converted to carboxyfluorescein by esterase activity (Parthuisot et al., 2000). Thus, only microorganisms with active metabolism are able to cleave the substrate, leading to the release of a fluorescent fluorochrome. In addition, only microorganisms with an intact cytoplasmic membrane can retain the substrate (Veal et al., 2000). Hence, it is possible to guarantee that only viable microorganisms, including those that are non-culturable, are marked and later counted (Adan et al., 2017; BioMérieux, 2021; Jepras et al., 1995).

The underlying principle of the D-Count[®], and flow cytometry in general, is related to light scattering and fluorescent emission, which occurs as light from the excitation source strikes the moving particles (Figure 6). This equipment consists of the following core systems: fluidics, optics (excitation and collection) and electronics (detectors) (Adan et al., 2017). In the fluidics system, a flow cell is responsible to guide cells and particles through the centre of the illumination spots with micrometre precision. The system employs a laminar sheath flow in the flow cell, into which the labelled sample is injected. This leads to a narrow sample stream by hydrodynamic focusing, in which each single cell in the sample is drawn to the centre of the flow cell, traveling at the same speed (Adan et al., 2017; BioMérieux, 2008).

In the optics system, a laser beam is focused to a small spot inside the flow cell. Light scattered from cells can be detected in a forward direction range (forward scatter), while side scatter and fluorescence light is collected by the objective at a right angle. Carboxyfluorescein has an excitation maximum that closely matches the 488 nm blue line of the argon-ion laser, and an emission peak at 517 nm (Hibbs, 2004). Thus, while passing through the flow cell one-by-one, the cells are individually illuminated by an argon laser at a constant wavelength of 488 nm (BioMérieux, 2008). Despite the maximum emission peak of carboxyfluorescein is 517 nm, the D-Count features an emission setup at 515 nm. This slightly difference may exist because the fluorescence intensity of fluorescein is influenced by environmental factors such as pH, and the pH for maximum emission is 8.5 (Hibbs, 2004). As milk normally has a pH range between 6.5-6.7 at 25 °C, the emission wavelength may decrease. The emission spectrum is detected by photomultipliers and the fluorescent light is separated to two colour channels (green and red) by means of dichroic mirror and optical filters. The intensity in each channel is analysed and recorded for each single particle separately. The classification is performed in real time, while cells are still passing the flow cell (BioMérieux, 2008; Jepras et al., 1995).

Finally, there is also the electronic component that acquires, processes, analyses and stores data on the computer, converting light signals to electrical signals. On the computer, it is possible to analyse the data, and the electrical signals are transformed into numbers of bacteria (BioMérieux, 2008; Jepras et al., 1995). The results are expressed in individual bacterial count (IBC) and are presented in number of bacteria per mL of milk (IBC/mL).

Regarding other methods of counting microorganisms, flow cytometry has several advantages. It avoids the need for culturing or enrichment procedures, and can be both qualitative and quantitative (Gunasekera et al., 2000). It is a much faster and direct method, being able to scan up to 40 samples per hour. This leads to a reduction of production cycle times and inventories, as well as implement just-in-time manufacturing processes. Also, it minimizes the risks and costs associated with potential in-process contamination while continuing to guarantee and enhance the quality of end products (BioMérieux, 2021).

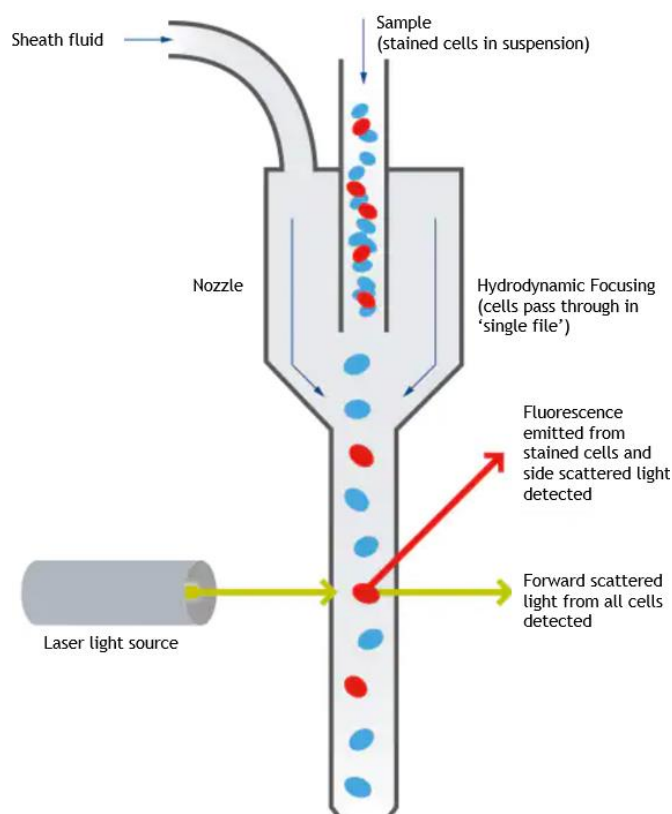


Figure 6 - The underlying working principle of D-Count® (adapted from Abcam, 2021).

2.4 Chemical composition of cow milk

Milk is a colloidal suspension and its often called the “perfect food”, since it is rich in key nutrients, containing a heterogenous family of proteins, the carbohydrate lactose, minerals, vitamins, enzymes, and emulsified globules of fat. It plays a key role in nourishment, priming the immune system and establishing an essential gut microflora in new-born mammals (Foroutan et al., 2019). The composition of cow milk may vary according to genetic factors (breed), age, nutrition or even by the environment (season and thermal effects) (Harding, 1995). Table 2 discriminates some of the physical and chemical characteristics of raw cow’s milk.

Table 2 - Physicochemical properties of cow milk (Chandan, 2009)

Characteristic	Cow milk
pH	6,60
Buffer value (pH 5,1)	$3,59 \times 10^{-2}$
Density at 20 °C (g/cm ³)	1,03
Viscosity (cP)	1,87
Acidity (%)	0,14

2.4.1 Milk major constituents

Generically, cows' milk contains about 87,4% of water and 12,6% of total solids, the latter composed by lactose (4,6%), fat (3,9%), protein (3,2%), and other solids such as minerals, enzymes and vitamins (0,9%) (Harding, 1995). The solid constituents are present in different physical forms: lipids and fats are emulsified in water, protein is colloiddally dispersed and lactose is dissolved. These characteristics facilitate the separation of the major constituents of milk (Harding, 1995; Tamime, 2009).

Lactose is a disaccharide composed of d-glucose and d-galactose and is the second most abundant constituent in milk. Carbohydrates, such as lactose, are an important source of energy and are responsible for the sweet taste of milk (Chandan, 2009; Tamime, 2009). During the heat treatment of milk, lactose undergoes a variety of changes, which include isomerisation and degradation via the Maillard reaction. Maillard's reaction is a chemical reaction between a reducing sugar and an amino acid under heat, resulting in a brownish or burnt colour of milk and a caramelized flavour, decreasing the nutritional value of milk (Bylund, 2015; Chandan, 2009). In addition, lactose serves as substrate for the lactic acid bacteria which perform lactic fermentation leading to the acidification of milk (Bylund, 2015; Guetouache et al., 2014).

Milk fat is the primary source of energy and is mainly a mixture of triglycerides, but it also presents 1-2% (wt/wt) of phospholipids, steroids, carotenoids and fat-soluble vitamins (A, D, E and K) (Chandan, 2009). It is present in milk in small spherical globules, 1-20 μm in size, in the form of an emulsion. The fat emulsion is stabilized by an adsorbed layer of proteins and phospholipids, that prevents fat from separating (Chandan, 2009). Due to its lower density than the remainder of the milk, the fat globules tend to form a layer of cream on top of the milk and to coalesce when subjected to heating and/or lowering of the pH (Bylund, 2015; Tamime, 2009). To prevent fat from rising (in the long-life milk), it's necessary to submit the milk to a homogenization step, where the fat globules increase in number and considerable decrease in diameter (less than 1 μm) (Guetouache et al., 2014). Thermal degradation of lipids is not observed, since the required temperature for decomposition of fatty acids (>200 °C) is significantly superior to the range of temperature used during heat treatment (Tamime, 2009).

Proteins present in milk are usually divided in two classes: caseins, which represent 80% of total milk protein and soluble whey (or serum) proteins, which represent 20% of total milk protein (Chandan, 2009; Tamime, 2009). Caseins are a colloidal substance with low solubility and are presented in the form of micelles of calcium phosphoserine. These micelles are thermostable up to a temperature of 140 °C but are easily degraded by proteolytic enzymes and in the presence of acid their precipitation tends to occur. There are several types of casein such as α -s1, α -s2, β , κ and γ -casein (Chandan, 2009; Guetouache et al., 2014). Whey proteins also have different types such as blood serum albumin, α -lactalbumin, β -lactoglobulin,

immunoglobulins, and proteose peptones (Tamime, 2009). These proteins are thermolabile and denature completely at a temperature of 80 °C (Bylund, 2015; Chandan, 2009).

Despite being in small quantities in milk, minerals greatly influence its characteristics. Calcium, potassium, sodium chloride, magnesium and phosphorus are the most important minerals present in milk (Guétouache et al., 2014). They are present in an equilibrium of colloidal and soluble state and some are fundamental in the structure of the casein micelles, providing the stability of milk (Chandan, 2009; Guétouache et al., 2014). In milk, minerals can also be found in fat-soluble vitamins A, D, E and K and water-soluble vitamins such as vitamins of group B and C. The fat-soluble vitamins are usually lost during decreaming as they get concentrated in the cream fractions during separation, being necessary to restore the vitamin content of skimmed milk (Guétouache et al., 2014; Tamime, 2009). While B vitamins are stable during the heating process, most of the C vitamin content is destroyed during pasteurization (Chandan, 2009). In addition to all the constituents described above, milk contains a large number of enzymes (approximately 60), such as proteases, lipases, peroxidases and phosphatases (Chandan, 2009; Tamime, 2009). Their role is the degradation of the original constituents of milk and their activity is influenced by pH, temperature and access to the substrate (Tamime, 2009).

In the dairy industry it is extremely important to control the physicochemical composition of milk during all stages of the production process, to ensure that the product is suitable for the consumer. Traditional standard methods can be used for the analysis of the different components of milk. For example, for fat determination, the standard method is based on either weight or volumetric analysis, involving the destruction of the fat globule, separation, drying and weighting (FOSS, 2020). In addition, for the total solids determination usually is employed the drying oven method, which simply removes all water from the sample, and the weighted results from before and after the heating step are subtracted (Bylund, 2015; FOSS, 2020). These methods are very time consuming, little automated and inaccurate. Thus, food industries tend to use more cost-effective, faster and non-destructive techniques, such as the Fourier Transform Infrared Spectroscopy (Capuano & van Ruth, 2015).

2.4.2 Fourier Transform Infrared Spectroscopy

Infrared (IR) spectroscopy is the study of the interaction of infrared light with matter. This analysis allows the identification of the molecules present in a sample and their concentration (Smith, 2011). The peaks in the IR spectrum of a sample represent the excitation of vibrational modes of the molecules in the sample. Thus, the peaks are associated with the various chemical bonds and functional groups present in the molecules and the amount of infrared energy absorbed by a compound is proportional to its concentration (Ismail et al., 1997). IR spectroscopy is one of the most important analytic techniques available in the food

industry, since it provides qualitative and quantitative information in a fast, cost-effective and non-destructive way, does not require the use of polluting chemicals, and can be carried out even by minimally trained personnel (Capuano & van Ruth, 2015).

There are several types of infrared spectrometers in the world, but the most widely used are FTIRs (Fourier Transform Infrared). The FTIR technique has been gaining interest for raw milk quality control, especially because of its high level of analytical capacity, low sample manipulation and use of fewer reagents, resulting in less time, lower costs, and a higher number of samples that can be analysed at the same time (Coitinho et al., 2017). This technique employs the Fourier Transform (FT) that is a mathematical procedure which relies on the fact that every periodic function may be split into a sum of sine functions and every sine function can be defined by two values: its frequency (wavelength) and its amplitude (intensity). The FT calculation allows the conversion of raw data into the full spectrum of the sample (Smith, 2011).

MilkoScan FT1[®] is an equipment, used by Lactogal, that controls and standardises liquid dairy products while simultaneously screening for abnormalities (FOSS, 2021). The equipment allows the composition analysis of dairy products like milk, cream, whey, yoghurt and more. The samples are analysed regarding their fat, protein, lactose, total solids, solids-not-fat (SnF), freezing point depression (FPD), total acidity, density, free fatty acid (FFA), citric acids, casein, urea, sucrose, glucose, fructose and galactose composition. This analysis is performed simultaneously, including screening for adulteries and added water, in 30 seconds (FOSS, 2021).

The MilkoScan FT1[®] consists of two main parts: the measuring unit and a computer for the control of the overall operation. This equipment employs a purpose built FTIR interferometer (FOSS, 2020). The interferometer records the light intensity caught by the detector as a function of optical path difference generated by sliding a moving mirror (Figure 7). The plot of light intensity versus optical path difference is called an interferogram (FOSS, 2020; Smith, 2011). Measuring the minute displacement of this mirror is achieved by means of a laser beam which follows the same path as an IR beam. However, at this level, the interferogram refers to the position of the moving mirror and not the wavelength that is of interest. The infrared beam from the IR source hits the beam-splitter, which sends half the beam to a fixed mirror and the other half to a movable mirror. From the mirrors the IR beams reflect and recombine before they reach the detector. All IR frequencies travel through the interferometer at the same time, and rapid small distances movements of the mirror enables simultaneous generation of the entire IR spectrum (FOSS, 2020). In a few seconds, the interferogram is collected by the spectrometer, processed through the FT calculation, and converted into a full spectrum of the sample. Thus, the IR spectrum of a compound is one of its most characteristic physical properties and can be regarded as its "fingerprint". The peaks,

or absorbance bands, of the full spectrum are compared to the data base of the equipment, and the sample compounds and their concentration are identified. For example, the absorption at $5.7\text{ }\mu\text{m}$ is due to stretching vibration in the C=O bonds of the carbonyl group. This measurement is a measure of the number of fat molecules, regardless of the length and weight of the individual fatty acids (FOSS, 2020).

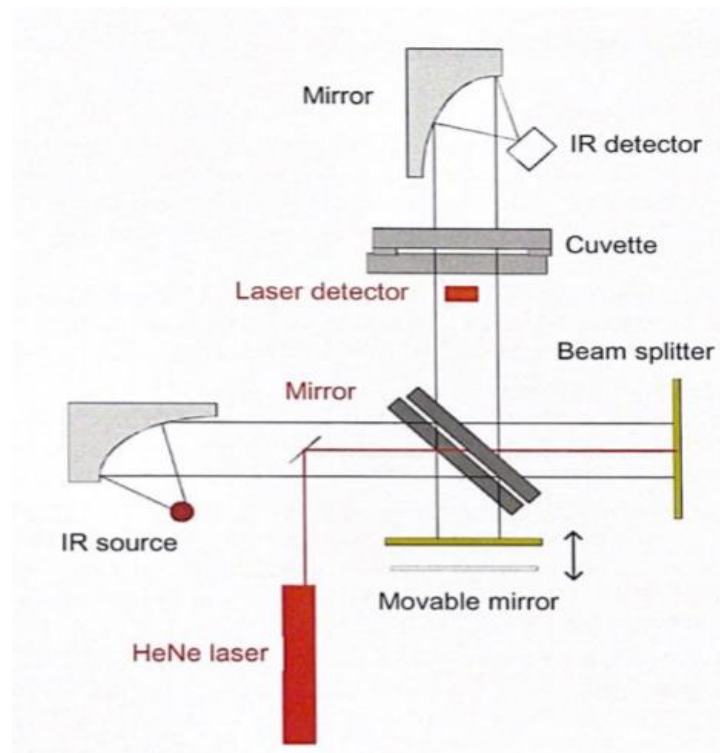


Figure 7 - The underlying working principle of MilkoScan FT1® (FOSS, 2020).

3 Materials and Methods

3.1 Packaging process analysis

The packaging unit features 17 packaging machines in which four different types of packages are used: Base, Edge, Slim-Cap and Slim-Leaf. It is important to mention that 1 L machines predominantly pack skimmed, semi-skimmed and whole milk, while 0.2 L machines generally pack chocolate milk and cream. Table 3 summarizes the different existing machines, the type of packaging used and the number of rejected packages due to paper and strip splices.

Table 3 - Description of the packaging machines used at Lactogal.

Volume	Machine number	Packaging Type	Rejected packages		Visual Representation
			Paper	Strip	
1 L	601, 602, 603, 606, 612, 613	Base	4	3	
	604, 607, 619, 620	Edge	10	6	
	609, 610, 611	Slim-Cap	5	3	
0.2 L	615, 616, 617, 618	Slim-Leaf	3	3	

The packaging process analysis can be subdivided in two works, the first related to the effective package content and the second to microbiological contamination analysis due to splices.

3.1.1 Effective package content

In order to perform the destructive control of UHT milk and cream, in batches where the number of units exceeds 100, 20 consecutive samples should be collected at random (Decreto-Lei n.º 310/91, 1991); thus, 20 samples of each packaging machine were collected and analysed. After collection, the samples were weighed (Digital Scale, METTLER), and the batch information was recorded. In addition, to determine the effective package content its necessary to know the weight of the empty packaged, designated in this dissertation as tare. To calculate the average tare, its required to analysed 10 samples when the tare is lower than 10% of the gross

weight, which is the case; hence of the 20 samples collected, the first 10 were used to further analyse the tare, volume and density, while the last 10 were returned to production.

The volume was measured using volumetric flasks of 1.0 L and 0.2 L (LINEX) removing or adding the necessary volume to reach the mark represented in each flask, and the density was measured using a densimeter (GERBER Instruments). For both measurements, the samples were at a temperature of 20 °C (Decreto-Lei n.º 310/91, 1991). After determining these parameters, the tare was measured after drying. To control the volume of milk and cream without destroying the package, Lactogal determines the effective content through calculations. Thus, through the values obtained from the gross weight (W_{gross} (g)), density (ρ (g/cm³)) and average tare weight (W_{tare} (g)), it was possible to calculate the volume of each collected sample ($V_{effective}$ (mL)) by Equation 1. This volume was subsequently compared to the volume determined by the volumetric flasks, to prove the reliability of the method used by Lactogal.

$$V_{effective} = \frac{W_{gross} - W_{tare}}{\rho} \quad \text{Equation 1}$$

For the control of the effective content of pre-packages, two sampling plans are foreseen: one for non-destructive control (does not imply the destruction of the pre-package) and other for destructive control (assuming the opening or destruction of the pre-package). Lactogal performs daily the non-destructive control of the pre-packaged samples in accordance with all restrictions and rules mentioned in the *Decreto-Lei n.º 310/91 (1991)*. Thus, the destructive control was performed, and the batch will be considered accepted if the arithmetic mean of the effective contents of the pre-packaged sample ($\bar{x}$) is greater than (Decreto-Lei n.º 310/91, 1991):

$$Q_n - \frac{s}{\sqrt{n}} \cdot t_{(1-\alpha)} \quad \text{Equation 2}$$

Q_n represents the nominal quantity, s represents the standard deviation of the samples of each batch, n represents the number of samples for the present verification and $t_{(1-\alpha)}$ represents the random variable of the student distribution, depending on the number of degrees of freedom, $\delta = n - 1$ and on the confidence level, $(1-\alpha) = 0.995$. For a sample size of 20 per batch, the batch will be accepted if $\bar{x} \geq Q_n - 0.640 s$.

In addition, run charts were plotted for each packaging machine analyzed using the program Minitab and the control charts, for the machines 602, 607, 609 e 613, created by the ACCEPT software were studied for the sample collection period.

3.1.2 Microbiological analysis of the splices

In order to reduce the number of rejected packages, contributing to the reduction of the company's waste and losses, the last 2 rejected packages of each splice from the 1 L machines were collected. Since the Edge machines have a higher number of rejected packages, the last 3 rejected packages of each paper splice were collected. The samples were incubated (Memmert) at 30 ± 1 °C for 3 days and analysed using the automatic D-Count® equipment (Biomérieux). If the result of a sample was positive or suspect, the sample was further analysed by plate count and for package damage. Also, to establish a relationship between the results obtained by D-Count® and those obtained by traditional methods, some negative samples were analysed by the two methods.

For using D-Count®, some reagents/solutions required previous preparation in the laboratory. Also, it was necessary to perform a cleaning and calibration step, which is performed automatically by the equipment. The preparation of reagents, cleaning and calibration method are described in Annex I, section D-Count®: Reagents, cleaning and calibration. The application used to analyse the samples was “A0720-03”, which corresponds to the analysis of white milk. In the analysis, 500 µL of each sample were placed in test tubes of the equipment. To ensure the correct measurement and validation of the samples under study, a positive control (tube with 200 µL of raw milk) and a negative control (empty tube) were used. As the samples were analysed, the results in IBC/mL appeared on the monitor coupled to the D-Count®.

For the detection and enumeration of cultivable cells, the streaking and agar incorporation method was performed. The streaking method is schemed in Figure I. 2, Annex I. To detect the presence of aerobic microorganisms, 1 mL and 0.1 mL of sample were added, with the aid of graduated pipette (VWR Internacional, LLC), in Petri dishes (VWR Internacional, LLC) using the MPCA medium. After homogenization and solidification of the medium with the sample, the plates were incubated at 30 ± 1 °C for 3 days. At the end of the incubation period, it was verified whether the streaks were positive or negative and the colonies that grew in the culture medium were counted. For detecting the presence of anaerobic microorganisms, 1 mL and 0.1 mL of each sample were placed in screw cap test tubes, with the aid of a graduated pipette. The RCA medium was added to incorporate the samples, and after solidification of the medium, the test tubes were incubated at 30 ± 1 °C for 3 days. To control the sterility of the medium, a plate with MPCA medium and a test tube with screw cap with RCA medium (negative controls) were placed. In Table 4, the culture media used as well as its composition, microbial flora and associated brand are present.

Table 4 - Culture media composition and brand used for plate count method.

Culture Medium	Composition (g/L)	Microbial group	Brand
Milk Plate Count Agar (MPCA)	Tryptone (5.0); yeast extract (2.5); glucose (1.0); skim milk powder (1.0); agar (10.0)	Aerobic mesophilic and termophile	Liofilchem S.r.l.
Reinforced Clostridial Agar (RCA)	Casein peptone (10.0); yeast extract (3.0); meat extract (20.0); Dextrose (5.0); sodium chloride (5.0); sodium acetate (3.0); soluble starch (1.0); cysteine (0.5); agar (15.0)	Anaerobic	Scharlau

To understand the cause of contamination of the positive samples, the packages were subjected to a damage analysis. Therefore, the positive and suspect samples were cut in the cross-section and a dye, erythrosine, was applied inside. After a quick drying step, the outer layer of the package (polyethylene layer) was removed, and it was verified whether the dye penetrated the card layer. The presence of pigmentation in the card layer, proves that the package has suffered damage and that there are pin-holes in the package, which can be the source of contamination.

In addition to the microbiological analysis, the pH of all samples was measured, at a temperature of 20 °C, with the aid of a calibrated potentiometer (HANNA Instruments). In Figure 8, a summary of the procedures applied to the rejected packages is described.

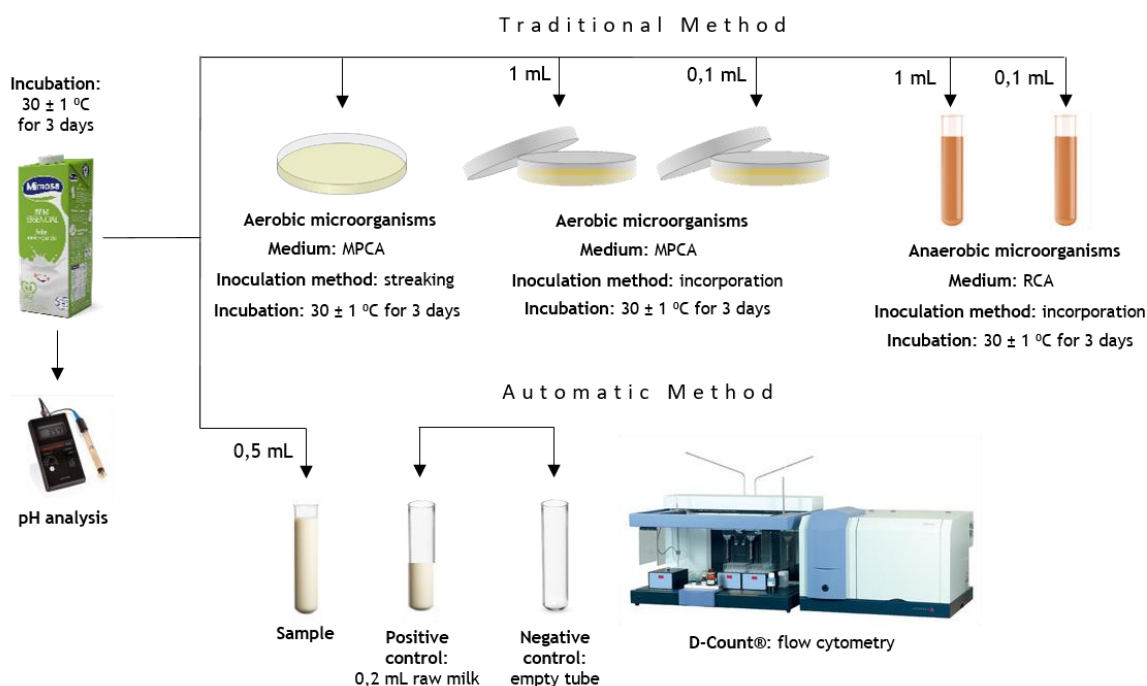


Figure 8 - Outline of the analyses carried out on the rejected packages.

3.2 Quantification of fat content

To verify whether there were fat losses throughout the UHT milk production process, from the tank truck bringing the raw milk to the final product, samples from all batches of raw and thermised milk were collected in sterile flasks of 100 mL (FL Medical) for 3 days, as well as 5 final products (of different batches) on the 3rd day. It is important to mention that no samples were retrieved after the UHT treatment because this process is performed in a closed system to ensure aseptic conditions. In Figure 9 the sequential process of sampling is represented.

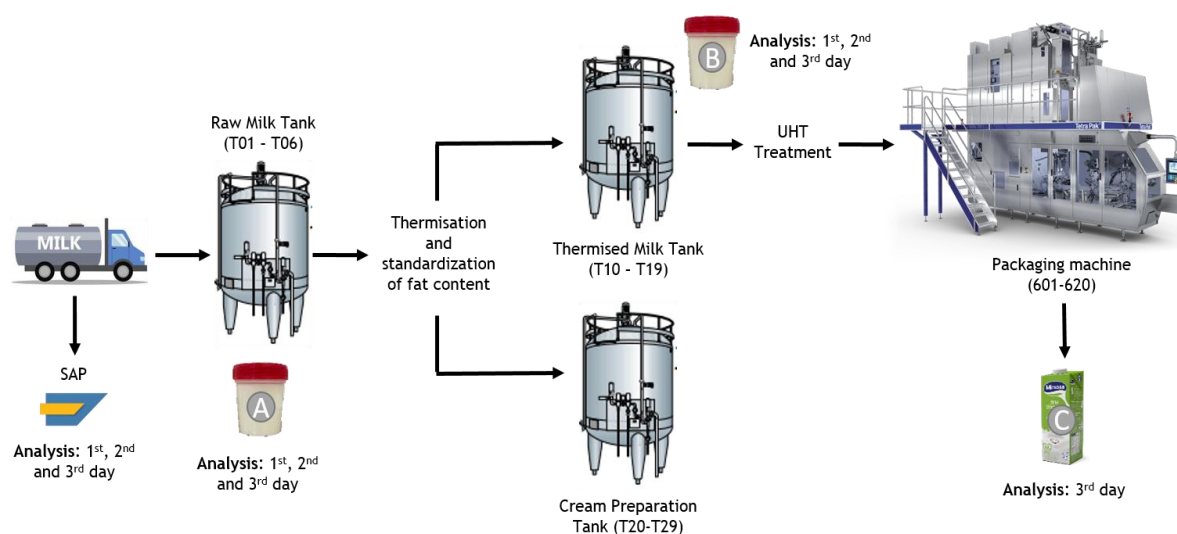


Figure 9 - Layout of the production process and the respective UHT milk sampling site: A - Raw milk; B - thermised milk; C- Final product (package taken from the packaging line, depending on production).

All collected samples were analysed, in triplicate, in MilkoScan FT1[®] (FOSS). This equipment allows the measurement of various parameters such as: percentage of fat, protein, lactose, defatted dry extract and cryoscopic point. For this analysis, the sample was homogenised and directly inserted in the equipment (with at least 8 mL) and a 30 s reading is performed, resulting in the fat percentage values through the FTIR principle. Although it was not possible to manually collect milk samples from the milk trucks and analyse the percentage of fat in MilkoScan FT1[®], this percentage is available in SAP (System Analysis Program).

SAP is a management system where it is possible to analyse all traceability information. Therefore, through the batch number of the final product it is possible to know the batches and volumes of the thermised and raw milk and the milk trucks that were used to originate the final product. Thus, through SAP and the analysis performed by MilkoScan FT1[®], it was possible to gather all the traceability information for the final products analysed, building a process scheme for the 5 final products (Annex IV).

4 Results and Discussion

4.1 Control of the milk packages effective content

The aim of the work was to perform the destructive control and check if it is within the desired standards. Also, the behaviour of the filling process was studied to identify possible trends. In addition, the values of tare and density obtained were compared to the values used by Lactogal (in the ACCEPT program) to confirm their veracity and the product volume was evaluated to check if the filling process is occurring by excess.

4.1.1 Destructive Control

A data sheet was created in Excel, where all the registered weights of the packages per batch were placed, as well as the density and tare measured (Annex II - Figure II. 1). The sheet allows the automatic analysis of the statistical control (by the destructive control sampling plan), indicating whether the batch in question is within legal parameters. This sheet can be used by Lactogal in future controls. In total, 38 batches of 1.0 L machines (17 of the Base machines, 9 of Edge and 12 of Slim-Cap) and 6 batches of 0.2 L machines (Slim-Leaf) were analyzed. Of all analyzed batches (20 samples each), no value was below the lower specification limit (985.0 or 191.0 mL). In addition, the average batch volume was always higher than $\bar{x} \geq Q_n - 0.640 s$. Hence, all batches were within the legal requirements and can be accepted.

It is possible to conclude that for the year 2021, the control of the effective content of pre-packages (by the destructive control plan) was exercised, according to *Decreto-Lei n.º 310/91 (1991)*, and all analyzed batches were compliant and can therefore be accepted.

4.1.2 Machine behavior analysis

To analyze the behavior of each machine, run charts were constructed (Annex II - Run Charts) with the weight data from the first batch (20 samples) collected for each machine. These are sequential graphs of the weight data over time and were used to check if there are trends in the data throughout the production process. The charts were analyzed in terms of clusters, mixtures, trends and oscillations.

Clusters are groups of points in one area of the chart. Clusters may indicate special-cause variation, such as measurement problems, lot-to-lot or set-up variability, or sampling from a group of defective parts. On the other hand, mixtures are characterized by frequent crossing of the mean line. Mixtures often indicate combined data from two populations, or two processes operating at different levels. A trend is a sustained drift in the data, either up or down. A trend can be caused by factors such as worn equipment, a machine that does not hold

a setting, or periodic rotation of operators. Oscillation occurs when the data fluctuates up and down, which indicates that the process is not steady. If the p-value for mixtures, clusters, trends or oscillations is less than 0.05, that indicates their presence in the data (Minitab, 2019). Table 5 represents the approximate p-values obtained for each machine through run charts.

Table 5 - Approximate p-values for clustering, mixtures, trends and oscillation. The highlighted data represents the values with $p < 0.05$.

Type	Machine	Approximate P-value			
		Clustering	Mixtures	Trends	Oscillations
Base	601	1.000	0.000	1.000	0.000
	602	0.833	0.167	0.500	0.500
	603	0.972	0.028	0.867	0.133
	606	0.989	0.011	1.000	0.000
	612	0.916	0.084	0.867	0.133
	613	1.000	0.000	1.000	0.000
Edge	604	1.000	0.000	1.000	0.000
	607	0.519	0.481	0.711	0.289
	619	0.179	0.821	0.500	0.500
	620	1.000	0.000	1.000	0.000
Slim-Cap	609	0.967	0.033	0.952	0.048
	610	0.695	0.305	0.133	0.867
	611	0.999	0.001	0.987	0.013
Slim Leaf	615	0.835	0.165	0.711	0.289
	616	0.387	0.613	0.133	0.867
	617	1.000	0.000	1.000	0.000
	618	0.997	0.003	1.000	0.000

Analyzing Table 5, it is possible to verify that 58.8% of the machines presented mixtures and 53.9% presented oscillations ($p\text{-value} < 0.05$). These values are quite high, since more than half of the existing machines at Lactogal present an out-of-control behavior. This may be due to the machine itself, as during filling the package is sealed through two transverse sealing jaws. These jaws move down at the same speed as the cylinder roll and seal the bottom of the cylinder, so that it can be filled with product. Thus, these jaws may be poorly calibrated, and have slightly different speeds which can cause oscillation in the filling and consequently of the weight (frequent crossing of the mean line). Thus, it is necessary to study in greater detail the effect of the transversal jaws on the oscillation of the weights, especially on the machines: 601, 606, 613, 614, 620, 609, 611, 617 and 618.

4.1.3 Tare and density control

Lactogal uses the program ACCEPT to control the effective content of pre-packages. In this software, the tare for each type of packaging, as well as the density values for whole, semi-skimmed, skimmed and chocolate milk, are already established. To confirm these values, the tare was measured from the collected samples using a digital scale (the same scale was always used to minimize errors) and density was measured using a densimeter. The average of the values obtained, as well as the difference between the average and the value defined in ACCEPT, are shown in Table 6 and 7 for tare and density, respectively.

Table 6 - Average tare and comparison with the values defined in ACCEPT.

	Packaging	Average Tare (g)	N _{samples}	ACCEPT Tare (g)	Difference (g)
1 L	Base	30.5 ± 0.4	80	30.4	0,1
	Edge	26.2 ± 0.1	80	26.1	0.1
	Slim-Cap	29.3 ± 0.2	100	28.9	0.4
0.2 L	Slim-Leaf	8.8 ± 0.1	70	8.6	0.2

The differences between the average tare obtained and the values defined in ACCEPT, are not significant. The largest difference belongs to the Slim-Cap packages (0,4 g), but it does not justify its change in the program. Changes could be made if differences greater than 1 g were obtained.

Table 7 - Average density and comparison with the values defined in ACCEPT.

	Milk	Average Density (g/cm ³)	N _{samples}	ACCEPT Density (g/cm ³)	Difference (g/cm ³)
1 L	Skimmed	1.0334 ± 2.42x10 ⁻⁴	10	1.0335	2.0x10 ⁻³
	Semi-Skimmed	1.0316 ± 1.96x10 ⁻⁴	17	1.0320	4.0x10 ⁻⁴
	Whole	1.0290 ± 0.0	3	1.0310	2.0x10 ⁻³
0.2 L	Chocolate	1.0510 ± 0.0	6	1.0510	0.0

Regarding density, similar results were verified since the differences were insignificant and even non-existent for chocolate milk. Thus, we can conclude that both the tare and density values are correctly defined in the ACCEPT program and can be used to calculate the volume of samples.

4.1.4 Volume Control

To understand if the filling process was occurring by excess, the volumes of the collected samples from all batches were studied. To this end, for each type of machine, the average values of volumetric weight (determined by Equation 1 in the Materials and Methods section) and net volume (determined by volumetric flasks) are shown in Figure 10 for Base, Edge, Slim Cap and Slim-Leaf machines.

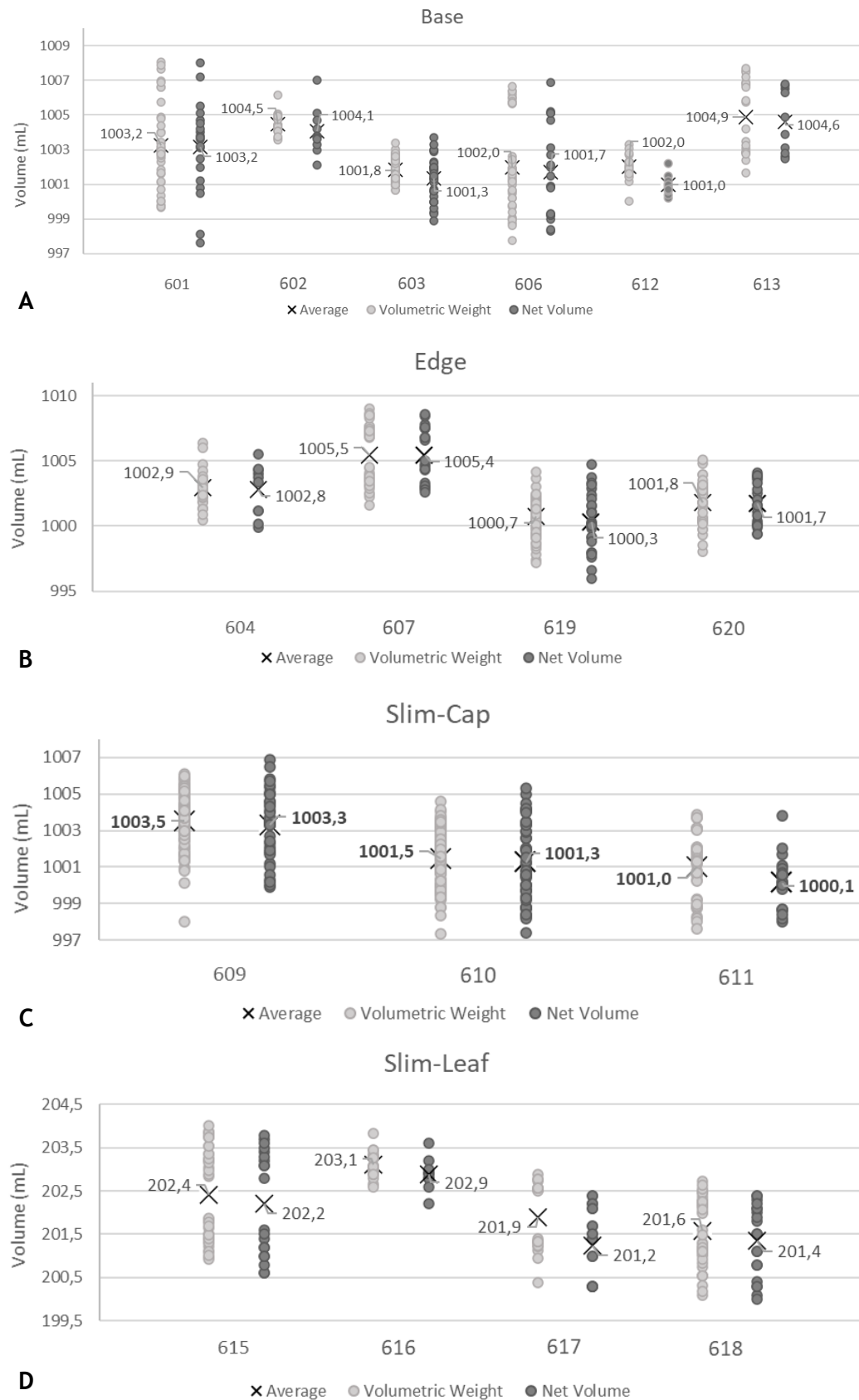


Figure 10 - Average, volumetric Weight and net volume of: A - Base machines; B - Edge machines; C - Slim-Cap machines and D - Slim-Leaf machines.

Analyzing the differences between volumetric weight and net volume, it is possible to verify that, for all machines, the average net volume was always lower than the average volumetric weight. However, the differences are not very significant since the largest difference was registered in machine 612, corresponding to 1 mL. This variation can be explained by human error since the net volume was measured using volumetric flasks. Although during the measurement the milk was carefully drained into the flask, a few millimeters may remain in the package, giving rise to these variations. However, from the results it is possible to conclude that the calculation of the volumetric weight is an accurate method to determine the effective contents of the packages.

Regarding the overfilling analysis, an overfill is only considered if the machines register an average volume greater than 1003 mL (for 1 L machines) and 205 mL (for 0.2 L machines). Although all machines had an average volume higher than the nominal (1000 or 200 mL), only the machines 601, 602, 607, 609 and 613 registered an overfilling (>1003 mL). Thus, these machines were studied in more detail and more samples were taken, to check whether a filling reduction could be carried out.

Table 8 - Volume analysis of the machines with opportunity for improvement.

Type	Machine	N _{samples}	Maximum (mL)	Minimum (mL)	Average Volume (mL)
Base	601	100	1008.1	999.5	1002.9 ± 2.1
	602	80	1007.5	1001.6	1004.3 ± 1.5
	613	60	1007.7	1000.0	1004.0 ± 2.3
Edge	607	60	1009.0	1000.7	1004.2 ± 2.6
Slim-Cap	609	100	1006.1	998.0	1003.7 ± 1.5

It was verified that the average volume of machine 601 was less than 1003 mL, excluding this machine from the possibility of overfilling. The remaining machines maintained an average volume higher than 1003 mL. However, it is possible to observe a large discrepancy between the minimum and maximum volume registered. This difference is in line with the results obtained through the Run Charts, in which a typical oscillation of values and a frequent crossing of the average line was verified (values oscillate between being above and below the average).

To verify if there was an actual overfilling on these machines (602, 607, 609 and 613), the control charts (Annex II - Control Charts) retrieved from ACCEPT for the month of March (month where the samples were collected) were analyzed. In this analysis, it was verified that all machines have an average volume lower than 1003 mL (602 - 1002.27±1.29 mL; 607 - 1002.08±0.60 mL; 609 - 1001.93±0.44 mL; 613 - 1001.95±1.13 mL). The decrease in the average filling volume is due to the increased sampling (1032-1576 samples). These results support the conclusion that the filling values are quite oscillatory. Also, the minimum volume recorded by the machines ranged between 994.8 and 997.7 mL, so if a reduction in the filling volume was

performed, due to the unpredictability of the process, some packages could result in an underfilling lower than the legal values (985 mL). In conclusion, it is not possible to carry out reductions in the filling volume of the machines under analysis and the oscillation of the packages weights should be investigated in more detail.

4.2 Loss analysis regarding splicing

A splice, as mentioned earlier, happens when the packaging material is about to run out and it is joined to a new roll of the same material to continue the packaging process. This process can occur for either the paper or the strip roll. The splice is performed manually which could lead to contamination of the packaging material when the operator is incorrectly sanitized. Therefore, to ensure the microbiological quality of the product and its correct packaging, there are a few packages that are rejected before and after splicing. The number of rejected packages varies depending on the type of splicing (paper or strip) and the type of machine (Base, Edge or Slim-Cap), as defined in the Table 3 in the Materials and Methods section. It is important to mention that, although the number of rejected packages is defined by the machine system, the machine can change this number in case of wrong formations. That is, if the package is not correctly formed, the machine continues to reject packages until it is correct.

To reduce the numbers of rejected packages in the 1.0 L machines, the last packages (furthest from the splicing) were studied, as they are less likely to have been contaminated during splicing and therefore, can potentially be introduced in the market. Hence, the last two rejected packages of each machine and type of splicing were microbiologically analysed, except for the rejected packages from paper splice of the Edge machines in which the last 3 were studied, due to its greater amount.

4.2.1 Distribution of samples

In total, 300 splices were studied, 196 of which were of paper and 107 of strip. The reduced number of strip splices compared to paper is because paper splicing is performed approximately every hour, while strip splicing is performed every 2 hours. Of the studied splices, 139 were from Base machines (88 of paper and 51 of strip), 110 from Edge machines (71 of paper and 39 of strip) and 54 from Slim-Cap machines (37 of paper and 17 of strip). This variation is due to the number of machines within each type and the frequency of use. There are a total of 6 Base machines, while there are only 4 and 3 Edge and Slim-Cap machines, respectively. Also, Base and Edge machines are used more frequently than Slim-Cap machines.

As mentioned earlier, the number of rejected packages may vary with each splice due to wrong packaging formation. Therefore, during the sample collection, the number of rejected

packages was registered to verify if they were in accordance with the defined in the machine system. The number of strip and paper splices analyzed for each machine, as well as the number of non-conforming splices (when the machine rejected more packages than defined in the software), is shown in Table 9.

Table 9 - Number of total and non-conforming splices.

Type	Machine	Paper splice			Strip splice		
		n total	n non-conforming	%	n total	n non-conforming	%
Base	601	18	4	22.2	11	0	0.0
	602	15	5	33.3	9	0	0.0
	603	17	3	17.6	10	0	0.0
	606	16	7	43.8	10	0	0.0
	612	11	2	18.2	5	0	0.0
	613	11	3	27.3	6	0	0.0
Edge	604	17	17	100.0	8	0	0.0
	607	26	26	100.0	15	0	0.0
	619	15	15	100.0	9	0	0.0
	620	13	13	100.0	7	0	0.0
Slim-Cap	609	15	0	0.0	7	0	0.0
	610	13	1	7.7	6	0	0.0
	611	9	0	0.0	4	0	0.0

In the case of the Base machines, the strip splices were all conforming, that is, 3 packages were always rejected. On the other hand, in the paper splices, of the 88 splices studied, 24 were non-conforming (27.3%), in which 5 packages were rejected instead of the defined 4. The machine with the highest percentage of non-conforming splices was machine 606, followed by machine 602, 613, 601, 612 and 603. These differences may be associated with the machine calibration. If the machine is poorly calibrated, the splicing will be performed incorrectly (union is not established in the proper place). Thus, the packages formed after the splicing are malformed, and the machine needs to readjust the packages at the cost of further rejection. Hence, it is necessary to study in greater detail the calibration of machines 606 and 602.

For Edge machines, all 39 strip splices were compliant (6 packages were always rejected). Regarding the paper splices, all were non-conforming (100.0%) which resulted in the rejection of 12 packages instead of 10. This is because the edge machines change the filling speed during the rejection of the packages due to splicing (transient filling). Consequently, the number of rejected packages can vary according to the filling speed and this variation results in the rejection of two extra packages than established (10).

Regarding Slim-Cap machines, similarly to the others, all strip splices were conforming (3 packages were always rejected). Of the 37 strip splices, only 1 was non-conforming (2.7%),

in which 6 packages were rejected instead of 5. This event becomes almost insignificant, so it can be concluded that the Slim-Cap machines are well calibrated.

4.2.2 Bacterial contamination analysis: D-Count®

To verify if the last rejected packages of strip and paper splices present sufficient microbiological quality to be introduced in the market, microbiological analysis through flow cytometry (D-Count®) was employed. The classification of IBC by D-Count® (total viable cells) is the following: (i) **negative** - values less than 100 IBC/mL, indicating that the milk from the batch has good quality and can be commercialized, (ii) **suspect** - values between 100 and 200 IBC/mL, these samples need to be studied by plate count methods to verify their commercialization and (iii) **positive** - values greater than 200 IBC/mL, indicating that the milk is contaminated and can't be commercialized. In addition, in the suspect and positive samples, a physical analysis of the package was performed, to verify if the source of contamination was due to package damage (existence of pin-holes) or splicing. In total, 676 packages were analyzed, of which 462 belong to the paper splicing and 214 of strip. The data was analyzed based on each type of machine (Base, Edge or Slim-Cap).

4.2.2.1 Base

For microbiological analysis, the 3rd and 4th packages (and the 5th of non-confirming splices) from the paper splice and the 2nd and 3rd packages from the strip splice were studied. In total, 218 packages (176 from paper and 102 from strip slices) were analyzed. The distribution of the milk samples, according to the level of bacterial contamination, for paper and strip splices in each Base machines is shown in Table 10 and 11, respectively.

Table 10 - Distribution of milk samples (n), according to the level of bacterial contamination, from rejected packages due to paper splice in Base machines.

Individual Bacterial count (IBC/mL)	601		602		603		606		612		613	
	n	%	n	%	n	%	n	%	n	%	n	%
0-25	24	66.7	20	66.7	29	85.3	32	100.0	22	100.0	21	95.5
25-50	12	33.3	8	26.7	5	14.7	0	0.0	0	0.0	1	4.5
50-100	0	0.0	1	3.3	0	0.0	0	0.0	0	0.0	0	0.0
100-200	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
> 200	0	0.0	1	3.3	0	0.0	0	0.0	0	0.0	0	0.0
Total	36	100.0	30	100.0	34	100.0	32	100.0	22	100.0	22	100.0

Regarding the paper splices, it is possible to verify that all collected samples were negative, except one sample in the machine 602. The positive sample corresponds to the 3rd package, which, as it is closer to the splice, could justify its contamination. However, the 3rd package of the remaining machines, and even on the machine 602, always presented negative

results, meaning that it is unlikely that the contamination is related to splicing. Thus, the package corresponding to this sample was subjected to a damage analysis, as mentioned in the Material and Methods section. The dye used (erythrosine) penetrated the card layer, which means that there were pin-holes in the package. This damage can be explained since, when the machine rejects the packages, they fall from a height of around 60 cm, which often leads to package damage causing immediately leaking or acidification after the incubation period. Thus, it is possible to conclude that sample contamination was due to packaging damage, not contamination during splicing.

Table 11 - Distribution of milk samples, according to the level of bacterial contamination, from rejected packages due to strip splice in Base machines.

Individual Bacterial count (IBC/mL)	601		602		603		606		612		613	
	n	%	n	%	n	%	n	%	n	%	n	%
0-25	10	45.5	14	77.8	15	75.0	17	85.0	10	100.0	10	83.3
25-50	10	45.5	2	11.1	3	15.0	3	15.0	0	0.0	2	16.7
50-100	1	4.5	0	0.0	2	10.0	0	0.0	0	0.0	0	0.0
100-200	0	0.0	1	5.6	0	0.0	0	0.0	0	0.0	0	0.0
> 200	1	4.5	1	5.6	0	0.0	0	0.0	0	0.0	0	0.0
Total	22	100.0	18	100.0	20	100.0	20	100.0	10	100.0	12	100.0

In the analysis of strip splices, it is possible to conclude that machine 601 presented a positive sample (2nd package), machine 602 a positive (2nd package) and a suspect sample (3rd package), and the other machines always obtained negative results. The positive and suspect samples were analyzed for packaging damage. The positive sample demonstrated the existence of pin-holes, which justify their contamination, while the suspect sample didn't demonstrate damage. Suspect samples need to be studied using traditional plate count methods, as milk fulfils the microbial criteria and can be commercialized when the number of colonies is lower than 100 CFU/mL after 2 days at 30°C incubation (Kracmarová et al., 2018). This value was verified, which means that the sample could be commercialized.

Thus, for all machines of the Base format, we can conclude that the last two rejected packages of paper and strip splicing have sufficient microbiological quality to be introduced in the market. This results in a significant cost reduction in terms of the reuse of milk and packaging, since splicing takes place every hour (paper) or every two hours (strip), and there are several machines of this format.

4.2.2.2 Edge

The 10th, 11th and 12th packages from the paper splice and the 5th and 6th packages from the strip splice were studied for bacterial contamination. In total, 288 packages (210 from

paper and 78 from strip slices) were analyzed. The distribution of the milk samples, according to the level of bacterial contamination, for paper and strip splices in each Edge machine is shown in Table 12 and 13, respectively.

Table 12 - Distribution of milk samples according to the level of bacterial contamination in rejected packages due to paper splice in Edge machines.

Individual bacterial count (IBC/mL)	604		607		619		620	
	n	%	n	%	n	%	n	%
0-25	49	96.1	68	89.5	34	77.3	38	97.4
25-50	2	3.9	7	9.2	7	15.9	0	0.0
50-100	0	0.0	0	0.0	0	0.0	0	0.0
100-200	0	0.0	0	0.0	0	0.0	0	0.0
> 200	0	0.0	1	1.3	3	6.8	1	2.6
Total	51	100.0	76	100.0	44	100.0	39	100.0

Of the samples collected from paper splices, there were 5 positive samples, 1 on the machine 607 (10th package), 3 on the 619 (one on the 10th, and two on the 11th package) and 1 on the 620 (10th package). All showed pin-holes on the packaging, which suggests that the source of contamination is not from the splicing, but from the damage suffered.

Table 13 - Distribution of milk samples according to the level of bacterial contamination in rejected packages due to strip splice in Edge machines.

Individual bacterial count (IBC/mL)	604		607		619		620	
	n	%	n	%	n	%	n	%
0-25	13	81.3	27	90.0	16	88.9	14	100.0
25-50	3	18.8	1	3.3	2	11.1	0	0.0
50-100	0	0.0	0	0.0	0	0.0	0	0.0
100-200	0	0.0	1	3.3	0	0.0	0	0.0
> 200	0	0.0	1	3.3	0	0.0	0	0.0
Total	16	100.0	30	100.0	18	100.0	14	100.0

Regarding the samples collected from strip splices, there were 1 positive and 1 suspect samples on the machine 607 (both from 10th package). Like the previous ones, the positive sample showed pin-holes on the packaging, while the suspect sample didn't. Therefore, the suspect sample was further studied by plate count technique and its commercialization was verified. Thus, for all machines of the Edge format, we can conclude that the last three rejected packages of paper and the last two of strip splices can be introduced in the market.

4.2.2.3 Slim-Cap

The 4th and 5th packages from the paper splice and the 2nd and 3rd packages from the strip splice were subjected to microbiological analysis. In total, 110 packages (76 from paper

and 34 from strip slices) were analyzed. The distribution of the milk samples, according to the level of bacterial contamination, for paper and strip splices in each Slim-Cap machines is shown in Table 14 and 15, respectively.

Table 14 - Distribution of milk samples according to the level of bacterial contamination in rejected packages due to paper splice in Slim-Cap machines.

Individual bacterial count (IBC/mL)	609		610		611	
	n	%	n	%	n	%
0-25	25	83.3	21	80.8	19	95.0
25-50	2	6.7	5	19.2	1	5.0
50-100	2	6.7	0	0.0	0	0.0
100-200	0	0.0	0	0.0	0	0.0
> 200	1	3.3	0	0.0	0	0.0
Total	30	100.0	26	100.0	20	100.0

In the analysis of paper splices, only one sample on the machine 609 was positive (4th package). The package was analyzed for damage, and pin-holes were found.

Table 15 - Distribution of milk samples according to the level of bacterial contamination in rejected packages due to strip splice in Slim-Cap machines.

Individual bacterial count (IBC/mL)	609		610		611	
	n	%	n	%	n	%
0-25	13	92.9	11	21.6	7	87.5
25-50	1	7.1	1	2.0	1	12.5
50-100	0	0.0	0	0.0	0	0.0
100-200	0	0.0	0	0.0	0	0.0
> 200	0	0.0	0	0.0	0	0.0
Total	14	100.0	12	100.0	8	100.0

Of the strip slices analysed, all samples were negative. Therefore, for all machines of the Slim-Cap format, we can conclude that the last two rejected packages of paper and strip splicing can be introduced in the market.

4.2.2.4 Economic impact

Usually, Lactogal purchases milk from producers at a minimum price of 0.30-0.32 € per liter (depending on the quality of the milk). Since paper splicing occurs every hour, strip splicing every 2 hours and Lactogal operates 24 hours a day, a total of 12 strip and 24 paper splices can occur per day in each machine. As only 6 machines can operate simultaneously, reusing the last two packages of paper and strip splicing, translates into a total of 432 packages that could be introduced in the market per day. This translates into a daily saving of 129.6 € or 47304 € per year. These values are increased when Edge machines are operating, since the last three

rejected packages from paper splices could be introduced in the market. If of the 6 machines operating, four are from the Edge format, this would result in the introduction of 528 packages per day in the market, which translate into a saving of 158.4 € per day or 57816 € annually.

Thus, the introduction of the last two packages rejected due to splicing (three for paper splices from Edge machines) in the market causes a high economic impact, making it possible to save thousands of euros in a period of 1 year.

4.2.3 Bacterial contamination analysis: Plate Count

For all suspect and positive samples, the traditional plate count method was performed, as well as for some negative samples to study the correlation between flow cytometry and standard plate count. Table 16 shows the results of the agar incorporation (aerobic and anaerobic research) and streaking methods, in relation with the D-Count® results. It should be noted that D-Count® results are in IBC/mL while plate count results are in CFU/mL, which makes a direct comparison between methods impossible.

Table 16 - D-Count®, plate count and pH results for positive, and suspect samples (highlighted).

Splice	Machine	D-Count® (IBC/mL)	Anaerobic microorganisms (CFU/mL)		Aerobic microorganisms (CFU/mL)		Streak	pH
			0,1 mL	1 mL	0,1 mL	1 mL		
Paper	602	227037	0	0	A	A	P	6.50
	607	123765	0	0	A	A	P	5.30
		891575	0	0	A	A	P	6.61
	619	20653	0	0	A	A	P	5.53
		60686	0	0	A	A	P	6.60
	620	806549	A	A	2.42x10 ³	A	P	4.51
	609	317	0	0	A	A	P	6.54
Strip	601	282	0	0	D	2.02x10 ²	P	5.58
	602	2318038	0	0	A	A	P	5.48
		189	0	0	0	0	N	6.68
	607	4836988	0	0	A	A	P	4.55
		168	0	0	0	0	N	6.69

*N - negative; P - positive; A - count over 300 CFU; D - count under 30 CFU.

For microbiological analysis, it should be noted that all positive samples in Table 16 showed damage in the package, while the suspect samples didn't. The damage in the positive samples caused a break in the aseptic conditions created during filling, since the packages had small pin-holes that allowed the contact of UHT milk with atmospheric oxygen. As such, the existence of pin holes in the packaging not only creates an aerobic environment (which is conducive to the development of aerobic microorganisms) but also allows the contamination of

milk by airborne contaminants. The latter may include bacteria, fungi and pollen. They are microscopic with diameters of 0.5 to 50 μm and can occur as droplets or solid particles, that can be easily translocated by air currents (Brandl et al., 2014).

All positive samples (>200 IBC/mL) showed growth of aerobic microorganisms in MPCA medium and positive streak, and only one sample showed growth of anaerobic microorganisms in RCA medium. In most samples it was not possible to calculate the value of CFU/mL, since the counts were greater than 300 CFU for the dilutions performed. Thus, to obtain these results, other dilutions should have been tested (10^{-2} , 10^{-3} and 10^{-4}). Also, the majority of positive samples (80%) demonstrated a pH below the authorized ranges, $6.55 < \text{pH} < 6.74$, for packages incubated at 30°C . The MPCA is a non-selective nutrient medium and allows good growth conditions for *B. subtilis*, *E. coli* and *S. aureus*, under aerobic incubation during 72 ± 3 hours at $30 \pm 1^\circ\text{C}$ (Liofilchem, 2015). While, RCA is an enriched non-selective medium and allows good growth conditions for *Clostridium perfringens*, *Clostridium sporogenes*, and *Pseudomonas aeruginosa*, under anaerobic incubation during 48 hours at $30 \pm 1^\circ\text{C}$ (Scharlau, 2021).

Kamei et al. (1991) studied UHT milk packages (filled by Tetra Pak's aseptic filling machine) with pin-holes made artificially in the polyethylene layer. After incubation (7 days at 30°C), the milk samples showed high viable counts of 3.7×10^8 and 2.8×10^8 CFU/mL and the microorganism most frequently isolated was *Lactobacillus spp.*, followed by *Streptococcus spp.* and *Enterobacteriaceae*. Also, after the bacteriological examination the pH was measured. All contaminated samples showed a decreased in pH, due to the presence of lactic acid bacteria (*Lactobacillus spp.* and *Streptococcus spp.*) which produce lactic acid from carbohydrates such as lactose, being able to grow rapidly in milk especially at temperatures above 20°C (Kamei et al., 1991; Robinson, 2002). In addition, Brandl et al. (2014) characterized airborne bacteria in a dairy facility and the highest particle loads were observed in areas where filling and storage took place. Also, a total of 25 bacterial genera were observed, with the majority belonging to the gram-positive genera *Bacillus* and *Staphylococcus*. Gram-negative bacteria from the *Pseudomonaceae* and *Enterobacteriaceae* families were also observed, although at low frequencies.

Thus, due to the low specificity of the plate count method, it is impossible to report which microorganisms are responsible for the contamination of positive samples. However, analyzing the literature it is possible to associate the contamination with airborne microorganisms, mainly from the *Bacillus* and *Staphylococcus* genera, and with lactic acid bacteria, coliforms, and mesophilic bacteria. The absence of anaerobic colonies in the majority of positive samples may be due to the incubation conditions used. As previously mentioned, RCA is a suitable medium for the cultivation of the genera *Clostridium*, which are thermophilic

microorganisms (optimal growth between 50 and 55 °C). As the incubation temperature used was 30 °C, it may have affected its growth and consequently detection.

Finally, the presence of aerobic and anaerobic microorganisms in 45 packages that obtained negative results on the D-Count® (range between 0-90 IBC/mL) were studied. For all of them, zero counts were obtained and there was no growth in the streaked plates. The same was true for the suspect samples (189 and 168 IBC/mL), in which zero counts were obtained. The reason why there are viable cell counts by flow cytometry and colonies are not detected, in the same rejected packages by the incorporation/striation method, could be due to the stress the cells are exposed to during the UHT treatment. As previously mentioned, cells can enter a physiological state called viable but not cultivable (VBNC) as a response to stress. These cells are living cells that have lost the ability to grow on suitable media and therefore, are detected by flow cytometry and not by traditional plate count methods. Gunasekera *et al.* (2002), studied a commercially available pasteurized milk and reported the presence of cells in a VBNC state, since the viable cell counts determined from colony forming unit (CFU) were significantly lower than direct viable counts. In addition, this variation may be due to the medium and incubation conditions employed that may not be specific for cell growth and/or the error associated with the measurement of the flow cytometry equipment (for example, a dead cell may contain residual esterase activity that allows its fluorescence and, consequently, its detection). The reason why there is no growth on the plates could also be indicative of the presence of heat resistant microorganisms since they have several restrictions for their growth in plate and are difficult to detect using the incorporation technique.

All negative and suspect packages under analysis were also evaluated for pH, recording values within the stipulated ranges ($6.55 < \text{pH} < 6.74$ for packages incubated at 30 °C).

4.3 Fat Content Assessment

As formerly mentioned, the composition of milk in fat is one of the most important factors for its quality and marketing. Also, cream (formed with about 35% fat) is a commercially valuable product and its price is significantly higher than milk. Therefore, it is important to study the existence of losses in fat content throughout the production process since fat has a considerable influence in the quality of the product and economic profitability.

Initially, it was necessary to understand which are the stages of the milk process that can be associated with a significant loss of fat. Product loss can happen throughout the entire process, but according to Tóth *et al.*, (2021) the most significant losses in raw milk are from the separation processes between cream and milk (decreaming), in which the milk loss is approximately 0.2% of the intake. In Lactogal, decreaming is carried out by a disc-bowl centrifugal clarifier and the standardization of fat is performed automatically by the direct in-

line standardization method (Figure 3 in the Context and state of art section). Thus, the decreaming and standardization process has been carefully designed and automated to avoid losses and produce a milk with compliant fat content. Therefore, changes in this system are almost impossible, leading to the need to analyse other causes of losses at different stages.

Other significant losses occur during line changes and cleaning of pipelines and tanks (Tóth et al., 2021). Most of the equipment used for handling milk is cleaned and disinfected by cleaning-in-place (CIP) systems, and it is usually performed by circulating hot water (Bylund, 2015). Since no water can remain in the pipelines and tanks, some milk will inevitably end up in the drain mixed with the water. These losses translate into fat losses, especially at stages where the milk has not yet undergone the homogenization process e.g., from transport trucks to raw milk tanks.

In addition, the UHT treatment and subsequent storage can lead to a decrease in milk fat content (Ajmal et al., 2018; Lu et al., 2019). As previously mentioned, fat is present in milk in small spherical globules comprising a core rich in triglycerides (TAG) enveloped in a tri-layer structure of proteins and phospholipids. However, in processed UHT milk, the size of fat globules decreases, and the structure of the membrane can be damaged or lost (Lu et al., 2019). This can result in lipolysis, which is the primary cause of milk fat deterioration, resulting in the release of FFA from TAG, and phospholipids (Ajmal et al., 2018). Additionally, some bacterial lipases can survive the orthodox UHT treatment, which leads to lipid oxidation of milk where FFA are broken down to oxidation products. This reaction is responsible for the formation of off-flavours and reduced shelf life of UHT milk (Ajmal et al., 2018; Lu et al., 2019). According to Ajmal et al., (2018), UHT treatment significantly affects the fatty acid profile of milk, as the concentration of short, medium and long-chain fatty acids decreased immediately after the UHT treatment. The heat treatment led to a loss of about 8,3% of short-chain fatty acids, 5,66% of medium-chain fatty acids and 5,32% of long-chain fatty acids. It also significantly changed the TAG profile since the UHT treatment decreased their content.

Thus, we can conclude that losses can occur at all stages of the process due to CIP system; however, UHT treatment and decreaming should be studied as potential stages of greater fat loss. To this end, the sequence of processes that gave rise to 5 final products (from different batches) of the Mimosa brand was analyzed, since this brand is the most sold and produced by Lactogal. The process scheme from one of the final products analyzed is present in Figure 11, while the remaining schemes are comprised in Annex IV. In Figure 11, it is possible to visualize not only the stages that gave rise to the final product, but also the percentage of milk fat at each stage as well as the volume used. It is important to remember that the traceability and volume information was taken from the SAP management program, and the fat percentages

were obtained by MilkoScan FT1® (except for milk trucks, where the fat percentage was also taken from SAP).

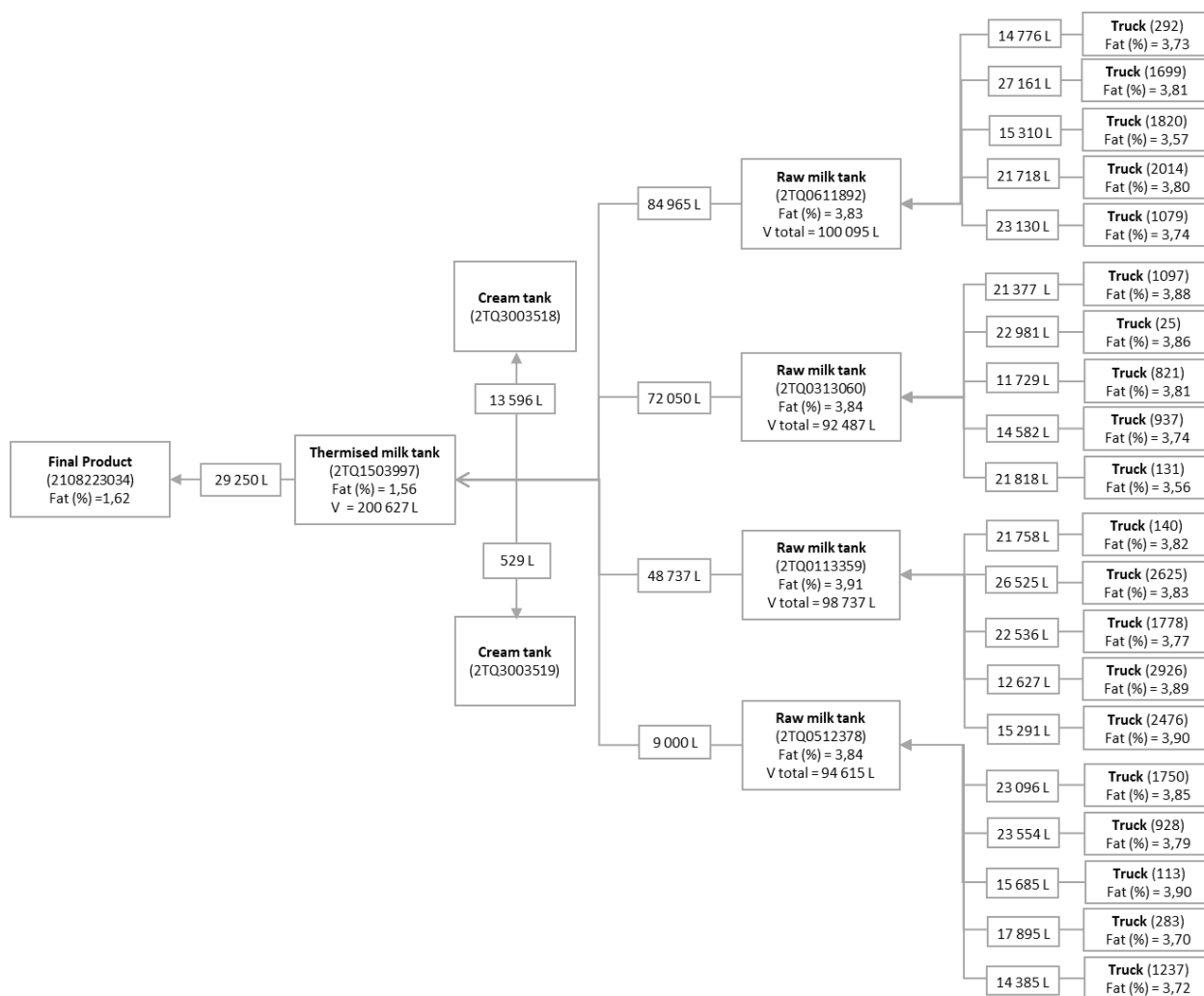


Figure 11 - Production scheme for the final product (Mimosa semi-skimmed).

To understand the sequence of samples taken, it is necessary to understand the process. First, the milk arrives at Lactogal in transport trucks. These trucks are identified by numbers and the milk from each truck is unloaded into a raw milk tank, which has a capacity of 100 000 L. When the tank reaches its maximum capacity, the tank batch is closed and identified with a number. The milk subsequently undergoes the de creaming and standardization step, where the stream of cream is stored in cream tanks and the standardized milk goes to the thermisation step, where it is stored in a thermised milk tank with a capacity of 200 000 L. Again, when the tank reaches its maximum capacity, the batch is closed and identified. From these tanks, the milk goes to the UHT treatment, which takes place in a closed circuit. Hence, it is only possible to handle the milk when it is in the form of the final product (packaged). Therefore, it is important to realize that several milk trucks can produce only one batch of raw milk, and that several batches of raw milk can produce one or two batches of thermised milk. The same reasoning applies for the final product, where more than one tank of thermised milk can give

rise to the same batch of product. Thus, dozens of transport trucks arrive each day and around 18 batches of raw milk and 8 batches of thermised milk are opened daily.

It is only possible to know the traceability of the final product, when its batch number is entered into the SAP system. Thus, until the final product is produced and identified, it is impossible to know which batches of thermised milk/raw milk/truck originated it. Therefore, it was necessary to take samples from all batches of thermised and raw milk for 3 days to ensure that, when the final product was analyzed on the 3rd day, there was information about the stages that originated it. No samples were taken from the cream tanks, but it is known that standardization of 35% cream is performed. Also, it was important to ensure that the composition analysis was performed in the same MilkoScan FT1[®] equipment, to guarantee the reliability of the results.

First, it is necessary to see if there was volume loss during the process. For this purpose, the Equation IV. 1, present in Annex IV, was used. As we can see from Figure 11, the only time that there was any milk loss was on the raw milk tank number 2TQ0611892, where 1130 L are missing. This value is easily explained due to the cleaning steps of the pipeline that connect the milk trucks to the raw milk tank. As mentioned earlier, in the CIP cleaning system, a stream of hot water is used to clean and disinfect the pipeline. Since no water can remain in the pipes, there must be a "push" step in which the water is replaced by milk. Therefore, the pipelines are never empty, that is, they are either filled with water (when cleaning) or with milk (when loading or waiting for processing). This exchange entails the loss of some milk for drainage, to ensure that the recovered milk does not have a higher content of water than the legal limit. Thus, we can conclude that the filling of tank number 2TQ0611892 occurred after a washing step, and that the lost volume (1130 L) remained in the pipeline after the "push" step and that some of that volume will be compensated in another batch, when the cleaning step occurs again. Hence, it is not strange that there are batches with more or less volume at the output than at the input.

Then, from the Equation IV. 2 represented in Annex IV, it was possible verify if there was any fat loss along the 3 stages: milk truck → raw milk tank; raw milk tank → thermised milk tank + cream tank; and thermised milk tank → final product. The values obtained are represented in Table 17.

Table 17 - MilkoScan FT1® and mass balance values regarding total fat (wt/wt).

	Batch Number	(%) Fat obtained by MilkoScan FT1®	(%) Fat obtained by mass balance	Difference (%)
milk truck → raw milk tank	2TQ0611892	3.83±0.01	3.79	0.04
	2TQ0313060	3.84±0.01	3.77	0.07
	2TQ0113359	3.91±0.00	3.83	0.08
	2TQ0512378	3.84±0.01	3.80	0.04
raw milk tank → thermised milk tank + cream tank	2TQ1503997	1.56±0.02	1.65	-0.10
thermised milk tank → final product	2108223034	1.62±0.00	1.56	0.06

There was an apparent 'gain' of fat at all stages, except between raw milk tank → thermised milk tank + cream tank. These values are unrealistic as 'gaining' fat is impossible and can be easily explained due to the resolution of MilkoScan FT1® which is $\leq 1\%$ CV (Coefficient of Variation) (FOSS, 2021). Thus, all the differences obtained are within the resolution of the equipment, so we cannot conclude whether there really is a loss or gain of fat. However, there are other factors that can influence the results obtained by MilkoScan FT1®.

As previously mentioned, MilkoScan FT1® employs an indirect measuring principle which correlates absorption from chemical bonds to actual component concentrations by the FTIR method. In this equipment, fat can be measured by three different infrared channels: Fat A, Fat B and Fat C (FOSS, 2020). The two wavelengths for Fat A and B absorb energy from different parts of the fat molecule, while Fat B and C absorb energy from the same part of the fat molecule but from different wavelengths.

In the fat A channel, the absorption at 5.7 μm is due to stretching vibrations in C=O bonds of the carbonyl group (FOSS, 2020). This measurement is a measure of the number of fat molecules, regardless of the length and weight of the individual fatty acids. If the average chain length of the fatty acid is changed, the number of triglycerides molecules per unit weight will change too, and an error will occur in the results unless the change is compensated by re-calibration of the instrument (Di Marzo & Barbano, 2016). The composition of milk fat varies with season, region, cow and stage of lactation (Walker et al., 2013). The effect of this is that an instrument using the 5.7 μm channel must be re-calibrated when, for instant, going from winter to summer milk.

On the other hand, in fat B channel, the absorption at 3.5 μm is due to stretching vibrations of the saturated C-H bonds (FOSS, 2020). This measurement is therefore related to both the size and number of fat molecules in the sample. The number of C-H bonds increases substantially in proportion to the molecular size. Both -CH₃ and -CH₂ groups absorb infrared energy at 3.5 μm , but the C-H stretching is markedly reduced by the presence of double bonds

adjacent to these groups (FOSS, 2020). The absorption decreases as a function of the degree of saturation (the number of $-C=C$ bonds). However, the $3.5\ \mu\text{m}$ determination (Fat B) is less sensitive to variations in the refractive index in cow's milk than the $5.7\ \mu\text{m}$ determination (Fat A), as it reflects the variation in chain lengths (Di Marzo & Barbano, 2016). Another advantage of the $3.5\ \mu\text{m}$ wavelength is that the determination includes free fatty acids that may have formed during storage and rough treatment of the milk. These cannot be measured at $5.7\ \mu\text{m}$ (FOSS, 2020).

Finally, the fat C Channel absorption at $6.8\ \mu\text{m}$ is due to bending vibrations in the saturated C-H bond on the fatty acids chain (FOSS, 2020). This measurement is therefore related to both size and number of fat molecules in the sample, as fat B. However, only $-\text{CH}_2$ groups absorb infrared at $6.8\ \mu\text{m}$. The major difference between fat B and C is the absorbency level, which for fat C is approximately half of the fat B absorbency. The Fat C, wavelength is usually used in calibration for products with a very high fat content, such as high fat cream (FOSS, 2020). Thus, there are several factors that can influence the fat channels readings and consequently, the infrared results.

One of the factors is inefficient homogenization of milk. As previously mentioned, the fat in milk exists in the form of small globules. In inefficiently homogenised milk, these act as tiny lenses and reflect the light, which passes through the cuvette in MilkoScan FT1[®], causing the Christiansen light-scattering effect (Di Marzo & Barbano, 2016; FOSS, 2020). The infrared detector cannot distinguish between light lost by reflectance and light lost by absorbency, so it records the result as if more light has been absorbed than was actually the case. This causes a shift in the apparent wavelength of maximum absorption by the carbonyl and carbon-hydrogen groups to a longer wavelength (Di Marzo & Barbano, 2016). As a result, the accuracy of the determination of the concentration of total fat percentage is negatively affected. Di Marzo & Barbano (2016), studied the effect of homogenization on the accuracy and repeatability of FTIR values for milk fat and protein. Poor homogenization (increased fat particles size) affected fat tests the most, presenting lower results than well-homogenised milk, fat B by $-0,165\%$ and fat A $-0,074\%$. Thus, variations in the results obtained in MilkoScan FT1[®], especially in the steps prior to the homogenization process, may be due to the Christiansen light-scattering effect. However, this effect only justifies the decrease in the percentage of total fat, not the increase.

Other fact that influenced infrared results, is lipolysis (Longo et al., 2016). Milk contains enzymes called lipases, which are responsible for the catabolism of triglycerides into their constituent molecules: glycerol and free fatty acids. The Fat A reading will be affected by lipolysis, since the enzyme removes the ester bond that his wavelength is registering, which results in lower values regarding total fat percentage. However, the Fat B wavelength will not be affected and should therefore be used for raw milk samples, which have a high lipase

content, since they have not yet been subjected to heat treatment. Although UHT treatment inactivates most lipases in milk, there are several reports that psychrophiles, including *Pseudomonas spp.*, *Alcaligenes spp.* and *Flavobacterium spp.*, produces extremely heat resistant lipases which could survive the UHT treatment (Adams & Brawley, 1981; Tamime, 2009). Hence, this effect can be applied in any stage of the milk process. Longo et al., (2016), Quantified the effect of lipolysis, induced using *Pseudomonas fluorescens* lipases, on milk fat by FTIR (Combiscope® equipment). As a result, there was a significant reduction of milk fat (up to 27%) for the treatments with highest enzyme concentration.

We can conclude that the results obtained through MilkoScan FT1® cannot be used to identify fat losses during the process. Since, the differences obtained between the values recorded by MilkoScan FT1® and the mass balances, for total fat (%), are within the resolution of the instrument. Therefore, it is not possible to draw viable conclusions. There are other equipments on the market that allow quick quantification of total fat in milk samples and are more sensitive to its measurement, such as Lactoscan® (Lactoscan, 2021). This equipment allows the analysis of different types of milk (cow, sheep, buffalo, among others) and determines several parameters such as fat, SnF, protein, lactose, pH, and others. For the determination of total fat (%), Lactoscan® has a measuring range of 0.01-45.0%, with an accuracy of $\pm 0.06\%$ (Lactoscan, 2021). The use of this equipment could allow the identification of the stages with greatest fat losses, however less significant losses could fall under the accuracy range. Like MilkoScan FT1®, the method applied by Lactoscan® is the Fourier-transform infrared spectroscopy. Thus, in both equipment, it is important to consider that the FTIR method may register incorrect values due to factors such as incorrect homogenization or the existence of active lipases in the sample.

In addition, highly sensitive methods such as gas chromatography coupled to a mass spectrometer (GC-MS) could be used to identify and quantify the fatty acid and triglyceride content of milk samples (Ajmal et al., 2018; Amores & Virto, 2019). Thus, by using this method, it is possible to determine the composition in fatty acids and triglycerides, which is beneficial in determining milk quality, and their concentration, which allows the quantification of fat losses through the production process. Although this method is more complex and the equipment is expensive, it would be the most sensitive method in determining fat loss.

5 Conclusion

The main aim of the present study was to identify and evaluate possible milk losses in the packaging process, through the volume control of the final product and microbiological analysis of rejected packages during splicing, and in milk fat content, from reception to packaging.

In order to evaluate the filling process, the destructive control plan of the effective content of pre-packages dictated by the *Decreto-Lei n.º 310/91* (1991) was performed. In a total of 38 batches evaluated, all were in accordance with the control plan: individual sample volume was always greater than the lower specification limit, and the average batch volume was always higher than $\bar{x} \geq Q_n - 0.640 s$. Hence, it is possible to conclude that the filling process is according to the legislation, and in case of an external auditing, the process will be complying. In addition, to understand the behavior of the machines during filling, run charts were constructed from the weights of the final products. It was possible to verify a large oscillation and frequent crossing of the mean line of the data. These results may be due to poor calibration of the transverse sealing jaws, since if they present slight variations in their speed, it results in sequential final products with different weights. However, this hypothesis needs to be studied in more detail. As the volume of the final product is calculated through the values of density and tare, these values were evaluated. The average tare and density values obtained from the samples studied were very similar to the values used in Lactogal (present in the ACCEPT program). Thus, the tare and density values defined in ACCEPT can be used to calculate the volume accurately. Also, the volume calculated from the density and tare values (volumetric weight) was compared to the net volume. It was possible to verify that the average net volume was always lower than the volumetric weight for all machines, with the higher variation registered in the machine 612 (1 mL). This variation may be due to the measurement of the net volume, since there can be considerable losses if samples are not properly emptied. To identify possible machines that operate with overfilling, the average of the obtained volumes was analyzed. Although all machines had an average volume higher than the nominal (1000 or 200 mL), only the machines 601, 602, 607, 609 and 613 registered an overfilling (>1003 mL). However, in a more detailed analysis and based on the control charts taken from ACCEPT for the month under analysis, it was possible to verify that the average volume of the machines were lower than 1003 mL. Also, based on the maximum and minimum volume values recorded, it was possible to observe a large oscillation in the values. Thus, a reduction in the volume of these machines could result in final products outside the legal limits, so no reduction was implemented.

To assess the possibility of reducing the number of rejected packages during splicing, the microbiological quality of the packages was analyzed. Initially, the number of packages rejected by splicing was studied. A large number of non-conforming paper splices (rejection of more packages than established in the software) were verified on the machines 606 and 602. These events may have occurred due to poorly calibration of these machines, which should be studied in greater detail. On Edge machines, all paper splices were non-conforming. This can be due to the machine calibration but also the filling speed, as this changes during splicing. Strip splices were always compliant on all machines. Regarding the microbiological analysis, of the 676 studied packages, 10 presented a microbial load above 200 IBC/mL (positive) and 2 between 100-200 IBC/mL (suspect). The remaining samples obtained a negative result (<100 IBC/mL) which confirms their quality and possible commercialization. A damage analysis was carried out on the positive samples, and all showed pin-holes in the package, which leads to the conclusion that the contamination comes from packaging damage and airborne bacteria, not contamination during splicing. Thus, it is possible to conclude that the last two rejected packages (3 in the case of paper splicing on the Edge machine) can be used and introduced in the market which translates to saving 47304-57816 €/year. In addition, the positive samples were analyzed using the plate count method. All positive samples showed growth of aerobic microorganisms and positive streak, and only one sample showed growth of anaerobic microorganisms. However, it was not possible to calculate the value of CFU/mL in most samples, being necessary to study other dilutions (10^{-2} , 10^{-3} and 10^{-4}). Analysing the literature, it is possible to associate the contamination with airborne microorganisms, mainly from *Bacillus* and *Staphylococcus* genera, and with lactic acid bacteria, coliforms, and mesophilic bacteria. Also, the suspect samples and 45 negative samples (between 0-90 IBC/mL) were studied by the plate count method. For all of them, zero counts were obtained and there was no growth in the streaked plates. This may be due to cells being in the VBNC state, medium and incubation conditions used were not ideal, or errors associated with the D-Count® equipment.

Regarding the analysis of fat loss during the production process, it was not possible to draw plausible conclusions. After a study of the literature, the stages with the greatest fat loss were identified and evaluated: de creaming, CIP system and UHT treatment. It was found that in most of the evaluated stages there were gains in the total fat percentage, which is not realistic. This is due to the resolution of the equipment ($\leq 1\%$ CV), since all results obtained are within this range. Also, the FTIR principle can be influenced by several factors such as homogenization or enzyme action. Thus, it can be concluded that MilkoScan FT1® results cannot be used to accurately quantify fat loss.

6 Assessment of the work

6.1 Aims achieved

From the planned aims, it was possible to complete the analysis of milk losses in the packaging process. Regarding overfilling, despite all machines showed a filling volume higher than nominal, it was found that the filling process is oscillatory and that there is not enough overfilling to implement reductions. In addition, it was found that the number of rejected packages due to splicing is occurring in excess. Thus, it was proposed to introduce the last 2 packages for all machines from paper and strip splices, and the last 3 for paper splice in Edge machines, in the market. As to the aim of identifying fat losses throughout the production process, this was not possible due to the equipment used. Thus, to fulfil this objective it would be necessary to use and study other types of equipment or methods.

6.2 Limitations and future work

Regarding the filling machines, it is suggested to carry out a study on the influence of the transverse sealing jaws on the weight of the final product. This analysis would be important to understand the oscillatory behaviour of the machines and implement correction measures to standardize the process. In the analysis of rejected packages, it is suggested to analyse in greater detail the Edge machines, since they present a high number of rejected packages by splicing. Thus, it would be important to analyse microbiologically, through the D-Count® equipment, the sequence of packages rejected by this machine and study their microbiological load, to identify further reductions. Also, to prevent the packages from falling of a considerable height and to prevent package damage, it would be important to introduce a small mat at the exit of the machine. This change would also be important to sequence more easily the rejected packages. Also, it would be interesting to study the rejected packages from 0.2 L machines.

For the quantification of milk fat throughout the production process, one of the limiting factors was the equipment used since the coefficient of variation of MilkoScan FT1® is quite high. An alternative equipment available on the market is Lactoscan® which has an resolution of $\pm 0.06\%$ and has a fat measure range from 0.01% to 45% (Lactoscan, 2021). However, the use of this equipment entails an additional cost for Lactogal and despite having a CV significantly lower than MilkoScan FT1®, there may be values within that range. Another way would be to study the fatty acid and triglycerides profile of the samples at each stage. This would be possible in a future partnership between FEUP and Lactogal, in which the gas chromatography equipment could be used (Ajmal et al., 2018).

Also, it was recognized that during the cleaning steps (CIP) there are considerable losses of milk to the sewage. One of the future works could be to quantify these losses and propose possible reductions. For this purpose, samples should be taken before and after the CIP process in the different stages of production and the cryoscopy coefficient should be measured (above >520 mC means that the sample is 100% milk and is not adulterated with water). Thus, it would be possible to recognize whether unadulterated milk was being rejected and propose reductions in the CIP 'push' time, in order to save milk.

6.3 Final assessment

It was possible to propose a reduction in the number of rejected packages, which implies a great economic advantage to Lactogal since UHT milk and packaging losses are reduced. On a personal level, working at Lactogal and understanding the working methods within a large-scale industry, allowed me to acquire important knowledge for my professional future.

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Annex I - Procedure Considerations

D-Count®: Reagents, cleaning and calibration

The layout of the D-Count® equipment, as well as the reagents required for the analysis, are represented in Figure I. 1 and Table I. 1.

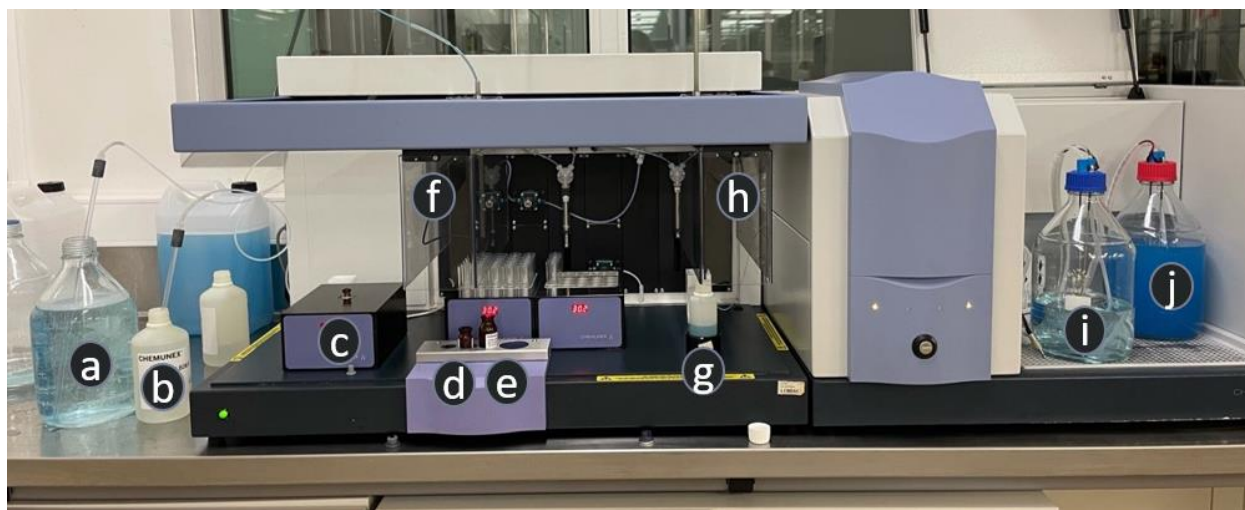


Figure I. 1 - Layout of the D-Count® equipment. The caption assigned to each letter is described throughout the text.

Table I. 1 - Reagents used in D-Count® provided by AES CHEMUNEX.

Reagents	Function	Storage Conditions
ChemChrome V26 (c)	Labeling substrate	2-8 °C upon reception
ChemSol B26/1 (b)	Labeling buffer	Room temperature (18-28 °C)
CS26A	Mask auto-fluorescent particles and validation of analysis (presence of fluorescent beads in the solution)	2-8 °C upon reception
CS26B	Optimization of the labelling step	
Diluent II and CRS	The mix of these reagents is used as a reducing solution of the free fluorescein	Separately, at room temperature (18-28 °C)
Isored	Used with the reducing solution to prevent oxidation	
Cleaning 3	Cleaning solution of the total system	Room temperature (18-28 °C)
Cleaning 5 (g)	Used to clean and decontaminate the injection well and the flow cell between each analysis	
ChemSol S 50 X and ChemSol S1 Supplement	Used as wash liquid for the D-Count® SPU and as a sheath fluid for the D-Count® analyser	Room temperature (18-28 °C) for a maximum of 5 days after opening or reconstitution

Table I. 1 - Continuation.

Reagents	Function	Storage Conditions
Antifoam	Prevention of foam formation in the waste bottles	Room temperature (18-28 °C)
Cleaning 3	Cleaning solution of the total system	
Standard G	Used for daily control of the analyser	

Some reagents/solutions required preparation in the laboratory, namely: (a) adding 20 mL of ChemSol S 50X in 1 L of deionized water; (d) mixing the CSR reagent with Diluent II and adding 15 drops of Isored followed by homogenization; (e) mixing and homogenization of CS26B with CS26A; (i) adding 20 mL of ChemSol S 50X and 20 mL of ChemSol S1 supplement in 1 L of deionized water; and (j) addition of 15 drops of a stabilizing agent (Antifoam) to the sewer flask.

There are several cleaning steps during when using the D-Count®. In the preparation of the equipment for analysis, 3 cleaning cycles are carried out automatically:

- **Daily Rinse:** this cleaning step is carried out when the equipment is powered up, during the laser heating period, with the ChemSol S 50X solution, which allows the rinsing of the sample preparation unit (SPU) (f) and analyser (g).
- **Daily Clean:** this cleaning step is carried out when the equipment is powered down, with the Cleaning 3 solution, which allows the correct cleaning of the analyser and SPU.
- **Weekly Clean:** this cleaning step is carried out every week, with the Cleaning 3 solution, and allows the cleaning of the total system of the equipment.

Between the analysis of the several samples, the injection well and the flow cell are washed and decontaminated with Cleaning 5 solution, thus avoiding contamination.

The D-Count® calibration is performed automatically in a daily basis through a control solution: Standard G. This solution consists of a calibrated and homogenous suspension of micro latex balls. These balls, with bacteria-like size, are loaded with a fluorochrome. The analysis of the Standard G by the D-Count® allows the verification of the proper functioning of the system.

Plate Count: medium and methods

For the microbiological study of the rejected packages, the Milk Plate Count Agar (MPCA) culture medium (1) and Reinforced Clostridial Agar (RCA) culture medium (2), were used.

- (1) **MPCA:** Milk Plate Count Agar is a non-selective nutrient medium used for the enumeration of bacteria in milk and dairy products. The medium complies with the recommendations of the American Public Health Association (APHA), International Dairy Federation (IDF) and ISO 4833:2003 for the microbiological examination of milk and milk products. This medium was supplied by Liofilchem S.r.l. and allows good growth conditions for *B. subtilis*, *E. coli* and *S. aureus*, under aerobic incubation during 72 ± 3 hours at 30 ± 1 °C (Liofilchem, 2015).
- (2) **RCA:** Reinforced Clostridial Agar is an enriched non-selective medium suitable for the cultivation of Clostridia and other anaerobic and facultative bacteria. This medium was supplied by Scharlau and allows good growth conditions for *Clostridium perfringens*, *Clostridium sporogenes*, and *Pseudomonas aeruginosa*, under anaerobic incubation during 48 hours at 30 ± 1 °C (Scharlau, 2021).

The samples collected were inoculated in Petri dishes following the inoculation method by incorporation or by streaking. The latter is used to verify growth from a single colony, and the sample is inoculated in a plate following the movements exemplified in Figure I. 2.

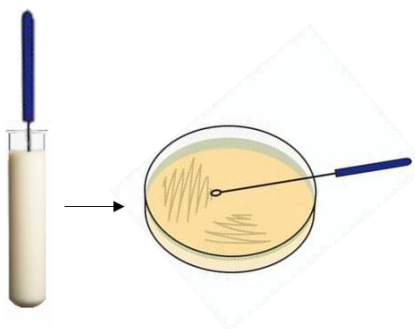


Figure I. 2 - Layout of the streaking inoculation method.

After the respective incubation time for each medium, the colony forming units (CFU) of each plate, that represent an estimated number of viable cells present in the sample, were counted. In order to be acceptable, the number of CFUs must be between 30 and 300, and the final result must be expressed in CFU/mL.

MilkoScan FT1®: Reagents, cleaning and calibration

The layout of the MilkoScan FT1® equipment, as well as the reagents required for the analysis, are represented in Figure I. 3 and Table I. 2.



Figure I. 3 - Layout of the MilkoScan FT1® equipment. The caption assigned to each letter is described throughout the text.

Table I. 2 - Reagents used in MilkoScan FT1® provided by FOSS.

Reagents	Function	Storage Conditions
S-470 Cleaning Agent (1)	Caustic cleaning solution	Room temperature (18-28 °C)
S-6060 Zero Liquid Concentrate (2)	Sets the 'zero' of the equipment and is a cleaning solution.	Room temperature (18-28 °C)
FTIR Equalizer Reagent	Calibration solution	2-8 °C upon reception

The S-470 and S-6060 reagents required previous preparation in the laboratory, by adding a bag of each reagent to 5 L of deionized water. The cleaning procedure of the equipment is carried out automatically with the cleaning solutions (1) and (2). The S-470 reagent, is a caustic cleaning solution that removes fat and proteins that accumulates in the analyser during daily use. This solution is used by the equipment 2 minutes after finishing the sample analysis or every 30 minutes when the equipment is being continually used. The S-6060 solution, is used automatically after each sample analysis, in order to wash the equipment and set the 'zero' with water, which is the signal used as reference.

The MilkoScan FT1® calibration is performed automatically with the FTIR Equalizer Reagent, to confirm that the analyser is operating in the desired spectrum, steadily and optimally, according to their specifications. The calibration is performed after the installation of the equipment, after any maintenance or repair operation, such as changing the cuvette or sensor and finally, after the analysis of every 5000 samples.

Annex II - Effective control of the final product

Quality control sheet



Controlo efectivo dos Pré-Embalados			
Produto / Product:	Primor Gordo	Data de Validade / Expiry Date:	22/09/2021
Lote/Batch:	2109223037	Máquina / Machine Code:	601
UF/Factory:	Modivas	Data de Análise / Date of Test:	26/03/2021
Densidade / Density (g/mL)	Balança / Scale	Temperatura / Temperature	
1,032	ACQ1	20	
Nº Amostras/ Nº of Samples:	Massa Bruta/Total weight (g)	Tara Média/Tare weight (g)	Conteúdo Efectivo/Net weight (mL)
1	1064,5	26,1	1006,7
2	1061,1	25,9	1003,4
3	1064,9	26,1	1007,1
4	1057,1	26,0	999,5
5	1065,2	26,2	1007,4
6	1060,1	26,2	1002,4
7	1064,2	26,2	1006,4
8	1060,2	26,0	1002,5
9	1062	26,1	1004,3
10	1061,4	26,1	1003,7
11	1063,5	26,1	1005,7
12	1058,1		1000,5
13	1062,6		1004,8
14	1059,8		1002,1
15	1064		1006,2
16	1057,8		1000,2
17	1063,8		1006,0
18	1059,4		1001,7
19	1063,7		1005,9
20	1059,6		1001,9
Quantidade Nominal / Nominal Quantity			1000
TRATAMENTO DE DADOS / DATA TREATMENT			
Erro Admissível p/ Defeito / Permissible error by default:			15
Quantidade Nominal - Erro Admissível para Defeito / Nominal Quantity - Permissible error by default:			985
Valor Máximo / Maximum value:			1007
Valor Mínimo / Minimum value:			1000
Desvio Padrão / Standard Deviation:			2,46
Nº Amostras / No. of Samples:			20
Factor K / Factor K:			0,640
Unidades abaixo ao erro admissível / Number of units below the permissible error:			0
Critério Legal = Quantidade Nominal - (Factor K x Desvio Padrão) / Legal Criteria = Nominal Quantity - (Factor K x Standard Deviation):			998
Média do Lote / Average Batch:			1004
CRITÉRIOS / CRITERIA:			
Conteúdos (Nº amostras abaixo do erro admissível) / Contents (Nº samples below the permissible error):			ACEITE / ACCEPTED
Média (Média > Critério Legal) / Average (Average > Legal Criteria):			ACEITE / ACCEPTED

Figure II. 1 - Sheet used for the destructive control.

Run Charts

- Base Machines

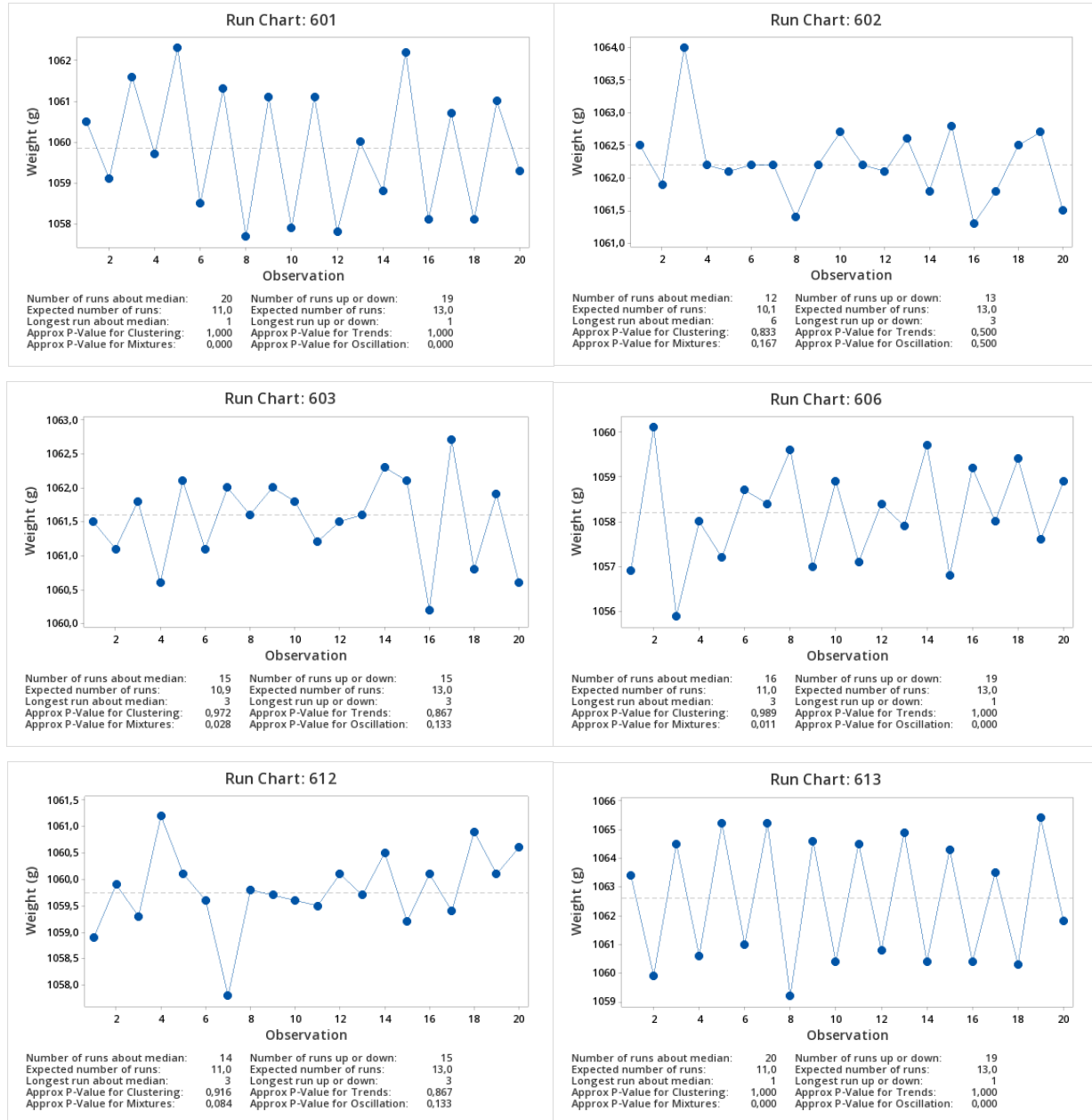


Figure II. 2 - Run Charts for Base machines.

- Edge Machines

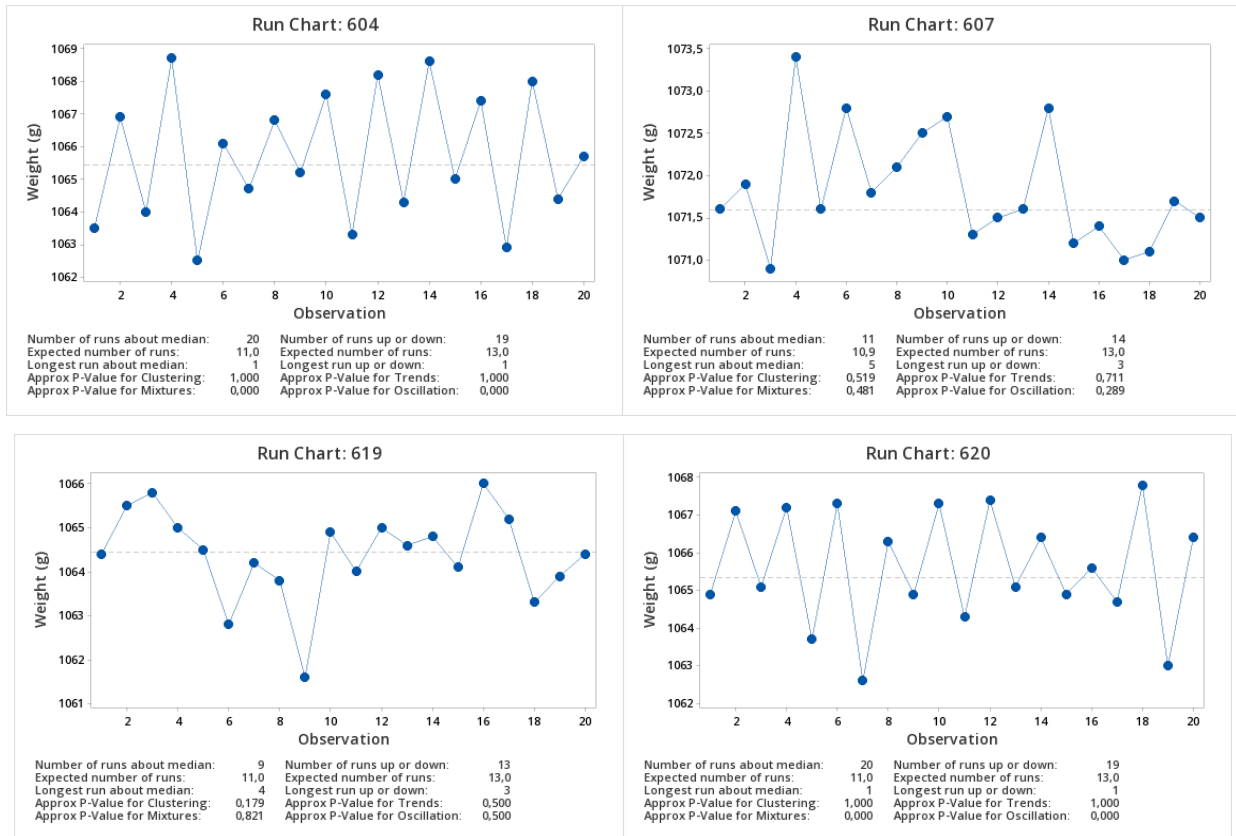


Figure II. 3 - Run Charts for Edge machines.

- Slim-Cap Machines

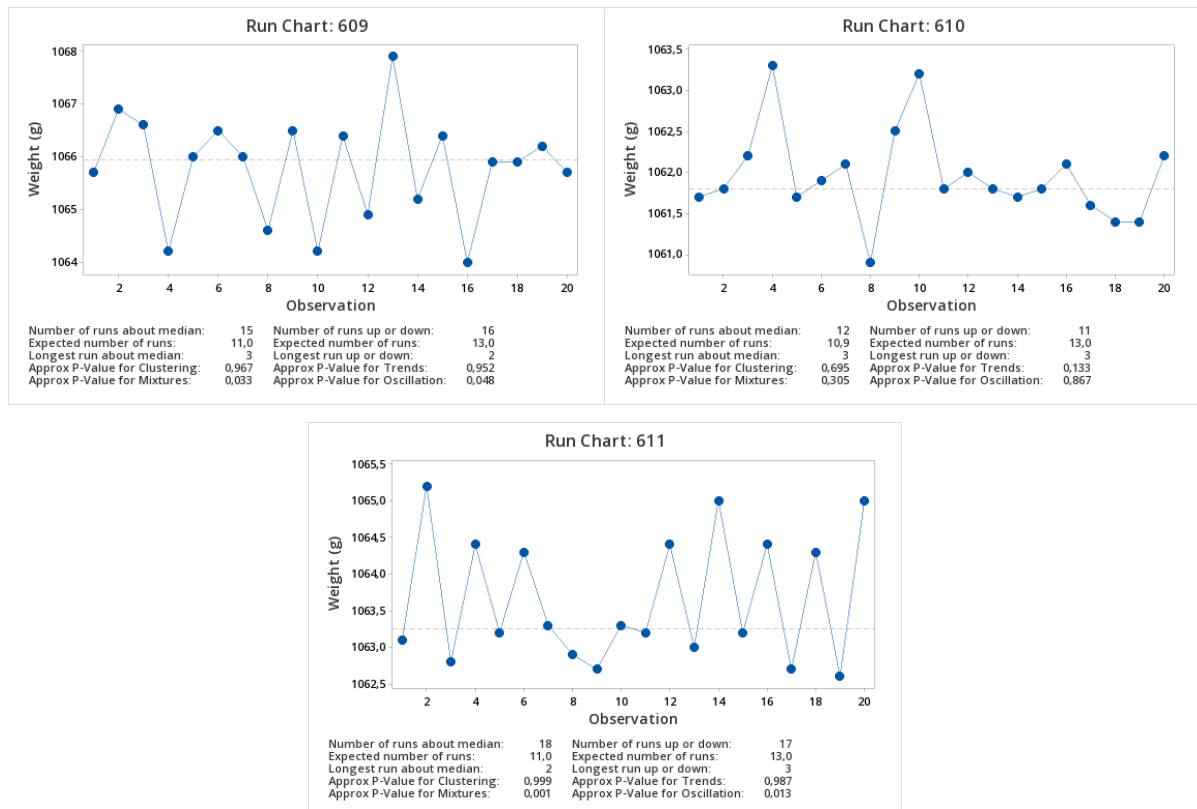


Figure II. 4 - Run Charts for Slim-Cap machines.

- Slim-Leaf Machines

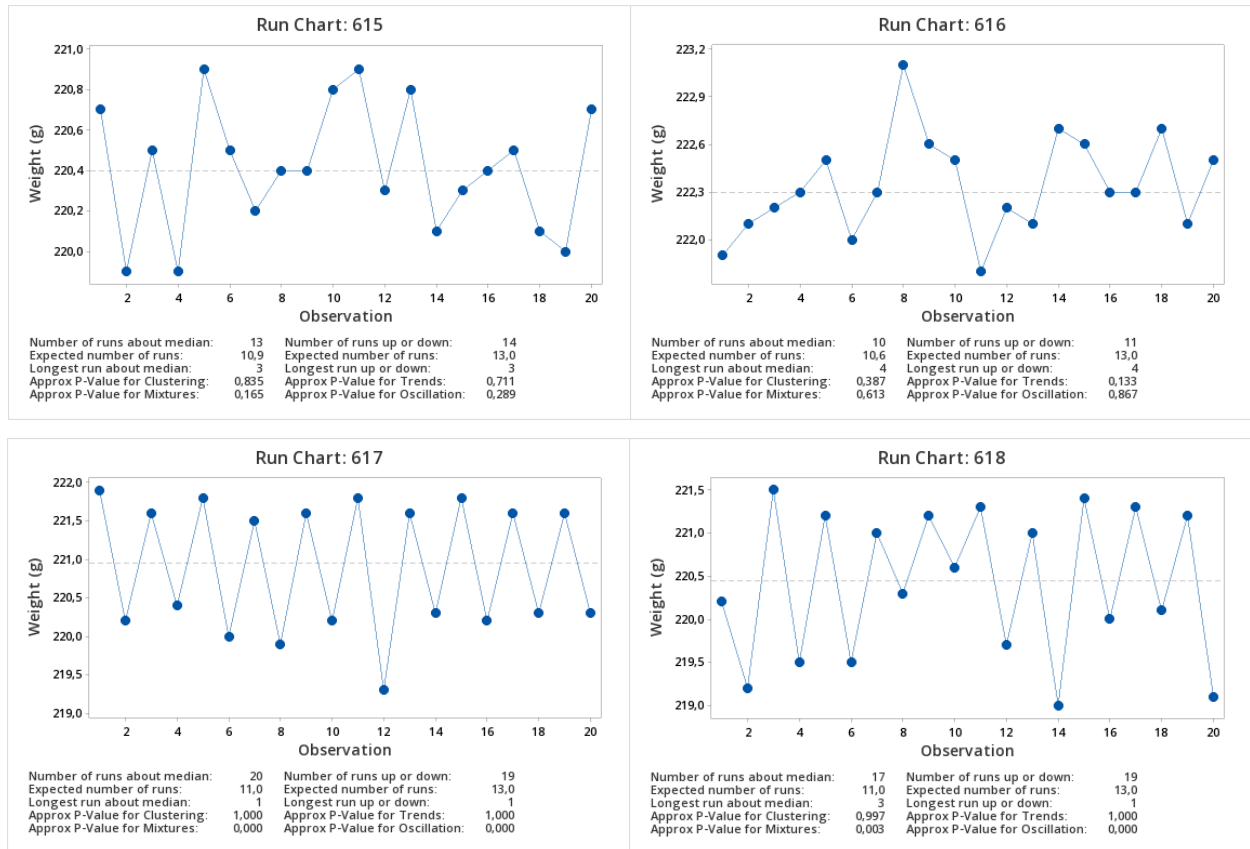


Figure II. 5 - Run Charts for Slim-Leaf machines.

Control Charts

In order to study the filling process of the machines: 602, 607, 609 and 613, the control charts regarding the volume data (retrieved from ACCEPT) were analysed. This program elaborates: average ($\bar{X}$), amplitude ($\bar{R}$) and standard deviation ($\bar{S}$) charts. Also, according to Montgomery (2013) it is essential to ensure that the construction of $\bar{X}$ and $\bar{R}$ control charts is made for data that present a normal distribution. This occurs because, in general, the control limits obtained from non-normal data are unreliable, making them inappropriate for the statistical process control. Thus, in addition to the control charts ($\bar{X}$, $\bar{R}$ and $\bar{S}$) for the machines under study (602, 607, 609 and 613), the normal distribution graphs were also retrieved from ACCEPT. In all machines, a normal distribution was verified, so the data can be used for statistical control. For a better visualization of the data, a histogram is also displayed.

- Machine 602

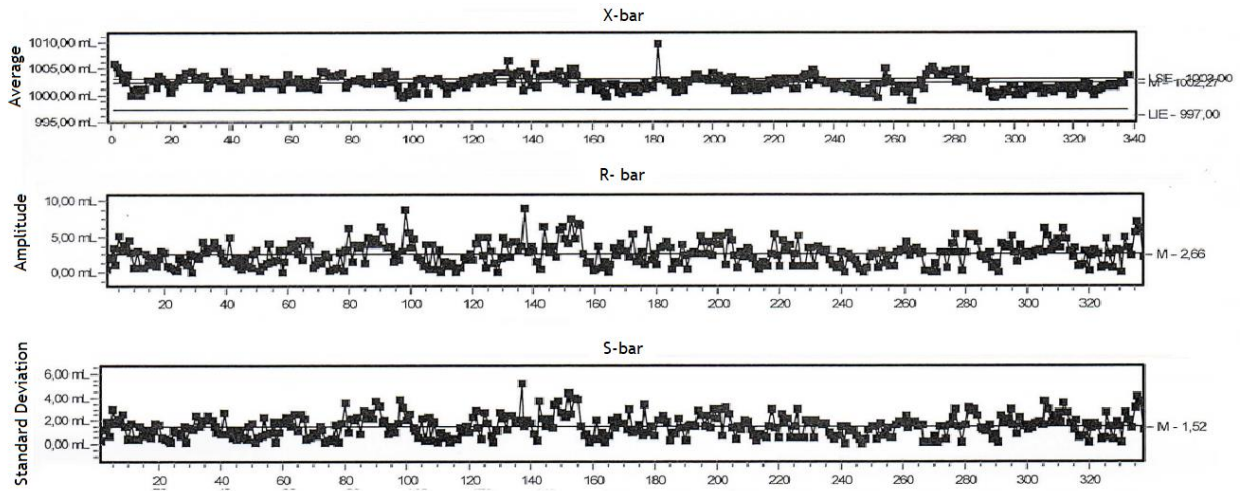


Figure II. 6 - Control chart: X-bar; R-bar and S-bar for machine 602.

Table II. 1 - Statistical analysis of the control chart from machine 602.

Specifications		Measured Values		Statistical Values	
Nominal	1000.0	$X_{\min}$	994.67	$\bar{X}$	1002.27
		$X_{\max}$	1011.61	Lower Control Limit (LCL)	1000.33
Lower Specification Limit (LSL)	997.0	$R_{\max}$	16.94	Upper Control Limit (UCL)	1004.21
		n_{total}	1352	$6s$	3.88
Upper Specification Limit (USL)	1003.0	$n < \text{LSL}$	3	$p < \text{LSL}$	0.00%
		$n > \text{USL}$	429	$p > \text{USL}$	28.57%
Tolerance	3.0	$\text{LSL} < n < \text{USL}$	920	$\text{LSL} < p < \text{USL}$	71.43%
TL1	985.0	$n < \text{TL1}$	0	$\bar{R}$	2.66
TL2	970.0	$n < \text{TL2}$	0	σ	1.29

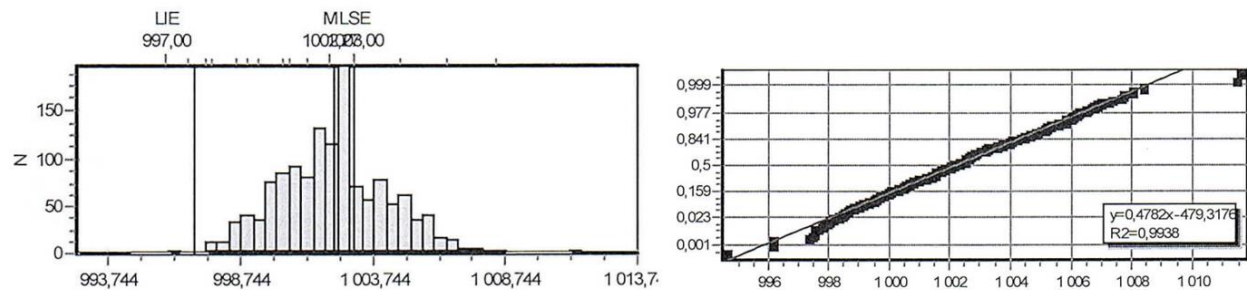


Figure II. 7 - Histogram and normal distribution of machine 602 data.

- Machine 607

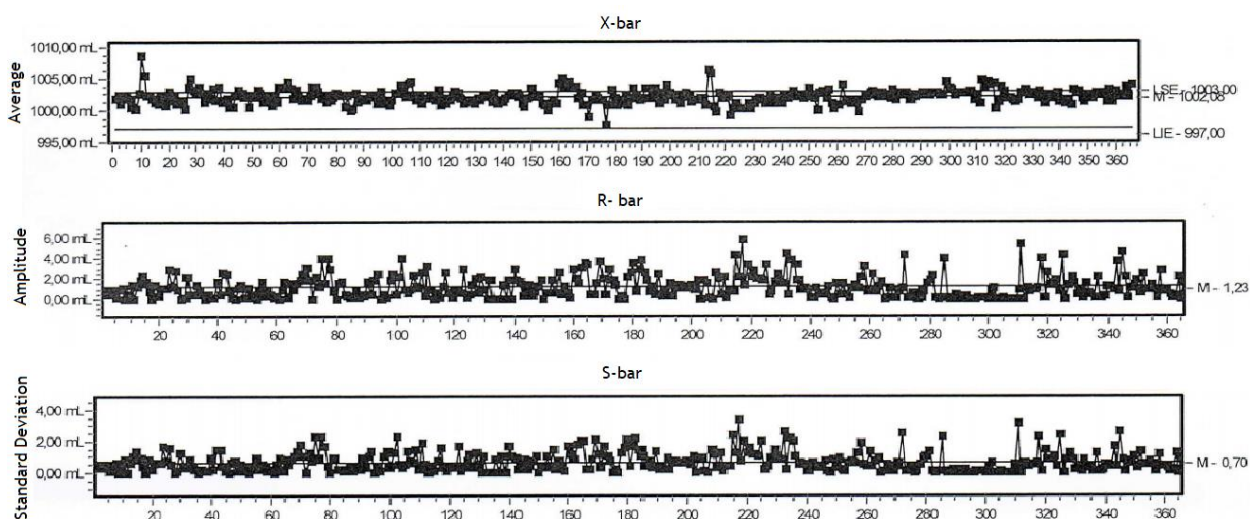


Figure II. 8 - Control chart: X-bar; R-bar and S-bar for machine 607.

Table II. 2 - Statistical analysis of the control chart from machine 607.

Specifications		Measured Values		Statistical Values	
Nominal	1000.0	X _{min}	996.81	$\bar{X}$	1002.08
		X _{max}	1009.10	Lower Control Limit (LCL)	1001.18
Lower Specification Limit (LSL)	997.0	R _{max}	12.29	Upper Control Limit (UCL)	1002.98
		n _{total}	1464	6s	1.80
Upper Specification Limit (USL)	1003.0	n < LSL	2	p < LSL	0.00%
		n > USL	281	p > USL	6.26%
Tolerance	3.0	LSL < n < USL	1181	LSL < p < USL	93.74%
TL1	985.0	n < TL1	0	$\bar{R}$	1.23
TL2	970.0	n < TL2	0	σ	0.60

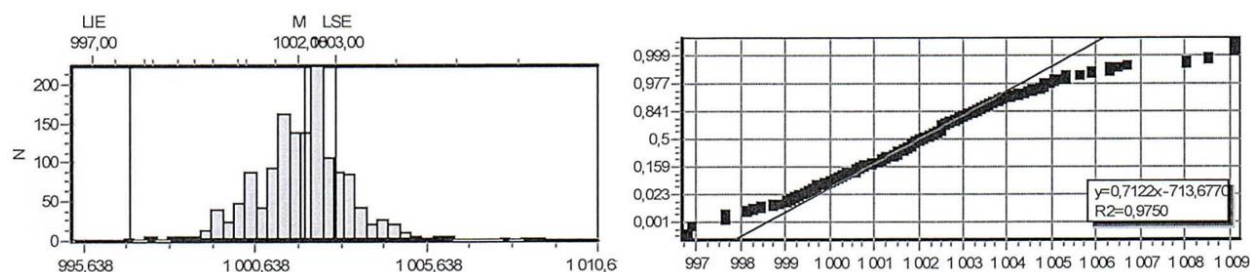


Figure II. 9 - Histogram and normal distribution of machine 607 data.

• Machine 609

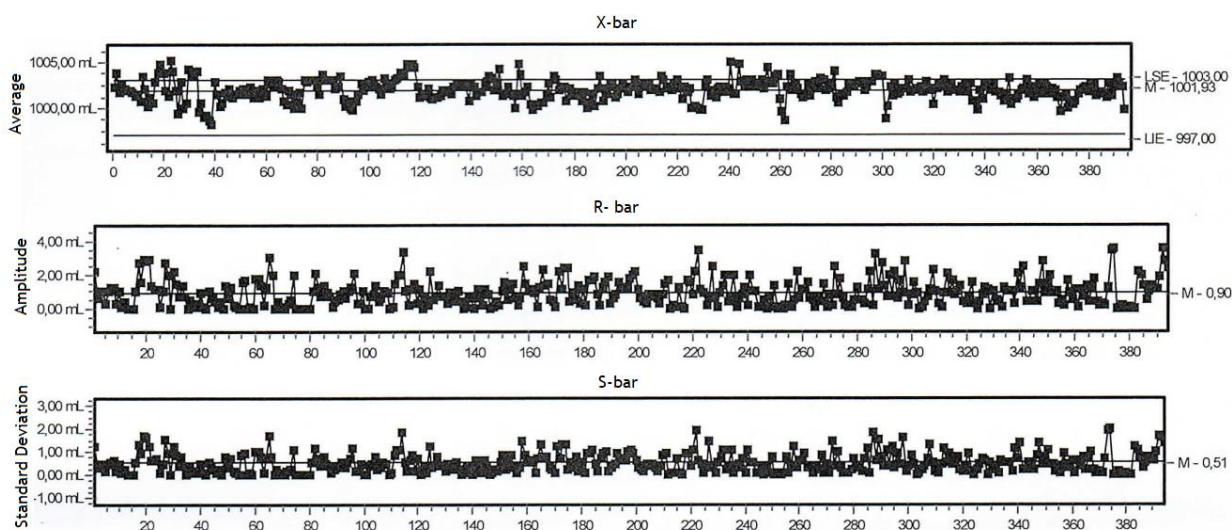


Figure II. 10 - Control chart: X-bar; R-bar and S-bar for machine 609.

Table II. 3 - Statistical analysis of the control chart from machine 609.

Specifications		Measured Values		Statistical Values	
Nominal	1000.0	$X_{\min}$	997.67	$\bar{X}$	1001.93
		$X_{\max}$	1006.30	Lower Control Limit (LCL)	1001.27
Lower Specification Limit (LSL)	997.0	$R_{\max}$	8.63	Upper Control Limit (UCL)	1002.59
Upper Specification Limit (USL)	1003.0	n_{total}	1576	$6s$	1.32
		$n < \text{LSL}$	0	$p < \text{LSL}$	0.00%
		$n > \text{USL}$	241	$p > \text{USL}$	0.75%
Tolerance	3.0	$\text{LSL} < n < \text{USL}$	1335	$\text{LSL} < p < \text{USL}$	99.25%
TL1	985.0	$n < \text{TL1}$	0	$\bar{R}$	0.90
TL2	970.0	$n < \text{TL2}$	0	σ	0.44

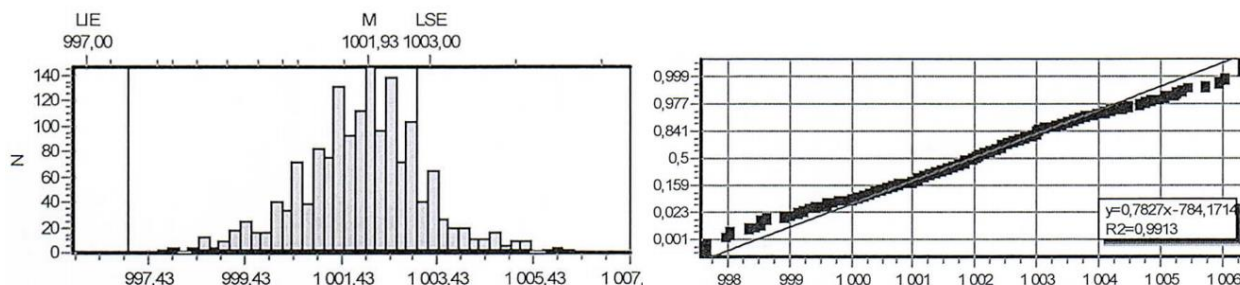


Figure II. 11 - Histogram and normal distribution of machine 609 data.

• Machine 613

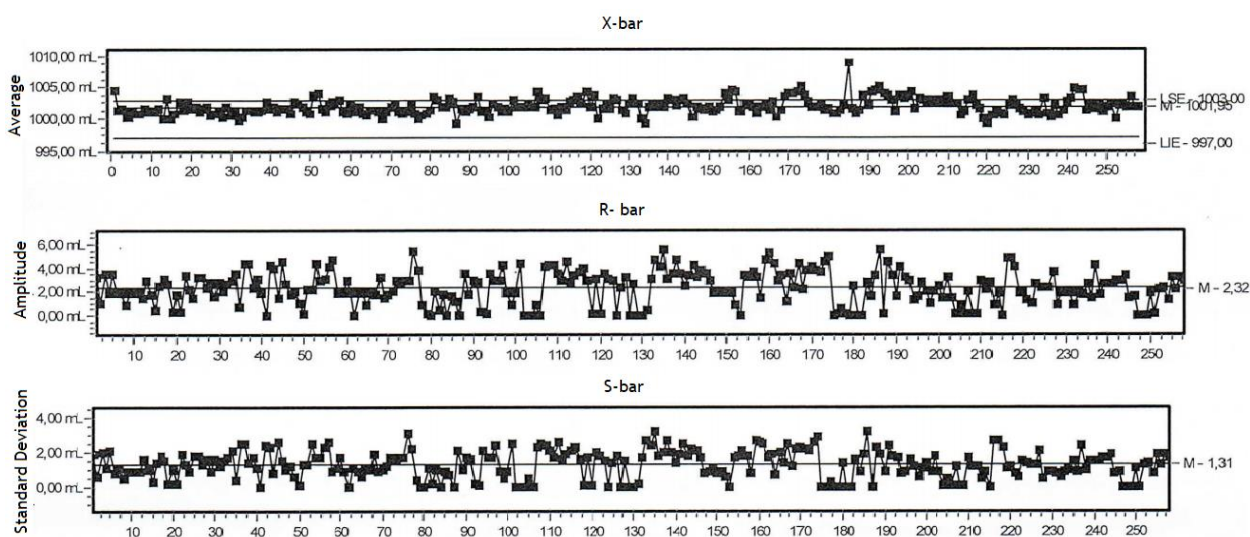


Figure II. 12 - Control chart: X-bar; R-bar and S-bar for machine 613.

Table II. 4 - Statistical analysis of the control chart from machine 613.

Specifications		Measured Values		Statistical Values	
Nominal	1000.0	$X_{\min}$	997.00	$\bar{X}$	1001.95
		$X_{\max}$	1010.56	Lower Control Limit (LCL)	1000.26
Lower Specification Limit (LSL)	997.0	$R_{\max}$	13.56	Upper Control Limit (UCL)	1003.64
		n_{total}	1032	$6s$	3.38
Upper Specification Limit (USL)	1003.0	$n < \text{LSL}$	0	$p < \text{LSL}$	0.00%
		$n > \text{USL}$	245	$p > \text{USL}$	17.64%
Tolerance	3.0	$\text{LSL} < n < \text{USL}$	787	$\text{LSL} < p < \text{USL}$	82.36%
TL1	985.0	$n < \text{TL1}$	0	$\bar{R}$	2.32
TL2	970.0	$n < \text{TL2}$	0	σ	1.13

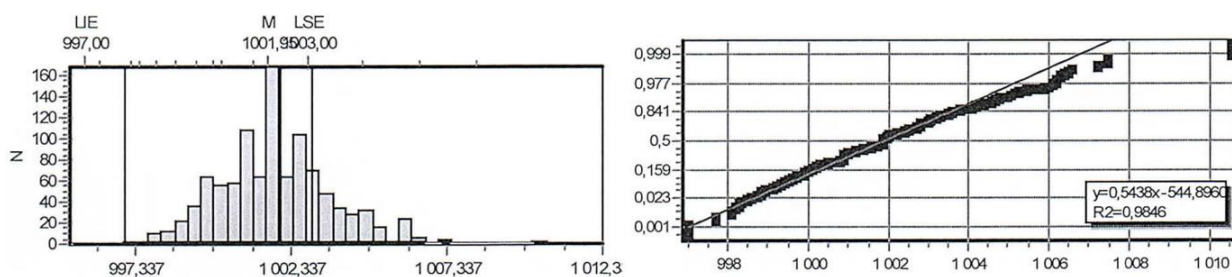


Figure II. 13 - Histogram and normal distribution of machine 613 data.

Annex III - Data regarding splices

Table III.1 represents the data related to the microbiological results of the rejected packages due to splicing. The samples that are highlighted were used for plate count method (aerobic and anaerobic research).

Table III. 1 - Data related to microbiological analysis of splices.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
22-03-2021	Slim-Cap	610	Agros SS	2111173010	15:02	5	Paper	30	6.68	30	6.68	-	-
23-03-2021	Edge	604	Mimosa SS	2109043036	10:36	12	Paper	0	6.68	0	6.68	30	6.66
23-03-2021	Edge	604	Mimosa SS	2109043036	10:45	6	Strip	0	6.68	0	6.68	-	-
23-03-2021	Edge	607	Mimosa SS	2109043036	11:02	12	Paper	0	6.67	30	6.67	10	6.67
23-03-2021	Edge	607	Mimosa SS	2109043036	12:25	12	Paper	10	6.69	0	6.69	10	6.68
23-03-2021	Edge	607	Mimosa SS	2109043036	10:14	6	Strip	4836988	4.55	20	6.68	-	-
23-03-2021	Edge	619	Mimosa SS	2109043039	12:45	12	Paper	10	6.68	10	6.68	10	6.68
23-03-2021	Edge	619	Mimosa SS	2109043039	12:15	6	Strip	20	6.68	30	6.68	-	-
23-03-2021	Slim-Cap	609	Agros SS	2109043038	09:55	5	Paper	0	6.68	0	6.67	-	-
23-03-2021	Slim-Cap	609	Agros SS	2109043038	11:10	5	Paper	0	6.68	30	6.68	-	-
23-03-2021	Slim-Cap	609	Agros SS	2109043038	12:15	5	Paper	0	6.68	20	6.69	-	-
23-03-2021	Slim-Cap	609	Agros SS	2109043038	11:25	3	Strip	10	6.68	0	6.68	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	09:46	5	Paper	30	6.68	10	6.68	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	10:52	5	Paper	10	6.67	20	6.67	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	12:01	5	Paper	20	6.68	10	6.67	-	-

*S - skimmed; SS - semi-skimmed; W - whole; EXP - exportation; Prof - professional; 1 - package closest to the splice; 3 - package further to the strip; N.D. - not determined.

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	12:01	5	Paper	20	6.68	10	6.67	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	13:05	5	Paper	20	6.68	30	6.68	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	10:35	3	Strip	10	6.67	0	6.68	-	-
23-03-2021	Slim-Cap	610	Mimosa SS EXP	2111183009	12:45	3	Strip	0	6.67	20	6.67	-	-
05-04-2021	Base	601	Primor W	2110023022	10:01	5	Paper	20	6.7	10	6.7	-	-
05-04-2021	Base	601	Primor W	2110023022	11:38	4	Paper	40	6.68	40	6.68	-	-
05-04-2021	Base	601	Primor W	2110023022	10:45	3	Strip	0	6.68	30	6.68	-	-
05-04-2021	Base	602	Primor W	2110023039	14:20	5	Paper	0	6.68	40	6.68	-	-
05-04-2021	Base	603	Primor W	2110023024	10:12	4	Paper	30	6.68	30	6.68	-	-
05-04-2021	Base	603	Primor W	2110023024	11:47	4	Paper	20	6.68	50	6.68	-	-
05-04-2021	Base	603	Primor W	2110023024	14:01	4	Paper	10	6.69	0	6.69	-	-
05-04-2021	Base	603	Primor W	2110023024	10:16	3	Strip	30	6.7	10	6.7	-	-
05-04-2021	Edge	604	Mimosa SS	2109173023	10:28	12	Paper	10	6.68	10	6.68	0	6.68
05-04-2021	Base	606	Gresso SS	2110023025	14:33	5	Paper	20	6.68	10	6.68	-	-
05-04-2021	Base	606	Gresso SS	2110023025	10:22	3	Strip	10	6.68	10	6.68	-	-
05-04-2021	Edge	607	Mimosa SS	2109173024	11:42	12	Paper	30	6.69	10	6.69	0	6.69
05-04-2021	Edge	607	Mimosa SS	2109173024	11:40	6	Strip	0	6.68	0	6.68	-	-
05-04-2021	Slim-Cap	610	Mimosa SS EXP	2112013008	10:39	3	Strip	20	6.69	0	6.69	-	-
05-04-2021	Slim-Cap	610	Mimosa SS EXP	2112013008	14:59	3	Strip	40	6.69	10	6.69	-	-
05-04-2021	Edge	619	Mimosa SS	2109173025	10:09	12	Paper	20	6.68	20	6.68	10	6.68
05-04-2021	Edge	619	Mimosa SS	2109173025	10:30	12	Paper	891575	6.61	20653	5.53	0	6.68
05-04-2021	Edge	619	Mimosa SS	2109173025	14:05	12	Paper	0	6.69	30	6.69	20	6.69

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
05-04-2021	Edge	619	Mimosa SS	2109173025	14:18	6	Strip	10	6.69	10	6.69	-	-
05-04-2021	Edge	620	Mimosa SS	2109173026	09:45	12	Paper	10	6.69	10	6.69	10	6.69
05-04-2021	Edge	620	Mimosa SS	2109173026	14:47	12	Paper	10	6.69	0	6.69	10	6.69
05-04-2021	Edge	620	Mimosa SS	2109173026	09:48	6	Strip	20	6.68	20	6.68	-	-
06-04-2021	Base	601	Primor W	2110033018	10:22	4	Paper	10	6.69	10	6.69	-	-
06-04-2021	Base	601	Primor W	2110033018	14:31	5	Paper	30	6.69	0	6.69	-	-
06-04-2021	Base	601	Primor W	2110033018	14:30	3	Strip	30	6.69	30	6.69	-	-
06-04-2021	Base	602	Primor W	2110033019	09:57	4	Paper	0	6.69	10	6.69	-	-
06-04-2021	Base	602	Primor W	2110033019	11:38	4	Paper	30	6.69	0	6.69	-	-
06-04-2021	Base	602	Primor W	2110033019	14:35	4	Paper	20	6.69	0	6.69	-	-
06-04-2021	Base	602	Primor W	2110033019	14:15	3	Strip	0	6.69	0	6.69	-	-
06-04-2021	Base	602	Primor W	2110033019	11:44	3	Strip	20	6.69	0	6.69	-	-
06-04-2021	Base	603	Primor W	2110033020	10:32	5	Paper	20	6.69	10	6.69	-	-
06-04-2021	Base	603	Primor W	2110033020	14:25	4	Paper	10	6.69	20	6.69	-	-
06-04-2021	Base	603	Primor W	2110033020	10:43	3	Strip	10	6.69	30	6.69	-	-
06-04-2021	Base	603	Primor W	2110033020	14:03	3	Strip	0	6.69	0	6.69	-	-
06-04-2021	Edge	604	Mimosa SS	2109183049	11:07	12	Paper	0	6.68	0	6.68	0	6.68
06-04-2021	Edge	604	Mimosa SS	2109183049	11:17	12	Paper	0	6.68	0	6.68	0	6.68
06-04-2021	Edge	604	Mimosa SS	2109183049	14:06	12	Paper	10	6.68	30	6.68	10	6.68
06-04-2021	Edge	604	Mimosa SS	2109183049	11:01	6	Strip	0	6.68	10	6.68	-	-
06-04-2021	Edge	607	Mimosa SS	2109183050	11:09	12	Paper	10	6.68	0	6.68	0	6.68
06-04-2021	Edge	607	Mimosa SS	2109183050	11:13	12	Paper	0	6.68	30	6.68	30	6.68

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
06-04-2021	Edge	607	Mimosa SS	2109183050	14:01	12	Paper	0	6.68	20	6.68	0	6.68
06-04-2021	Edge	607	Mimosa SS	2109183050	14:22	6	Strip	0	6.68	10	6.68	-	-
06-04-2021	Edge	607	Mimosa SS	2109183050	11:14	6	Strip	10	6.68	0	6.68	-	-
06-04-2021	Slim-Cap	609	Mimosa SS EXP	2112023006	11:28	5	Paper	20	6.68	10	6.68	-	-
06-04-2021	Slim-Cap	609	Mimosa SS EXP	2112023006	14:40	5	Paper	10	6.68	0	6.68	-	-
06-04-2021	Slim-Cap	609	Mimosa SS EXP	2112023006	13:58	3	Strip	0	6.69	0	6.69	-	-
12-04-2021	Base	601	Primor W	2110093030	11:04	4	Paper	0	6.69	0	6.69	-	-
12-04-2021	Base	601	Primor W	2110093030	14:02	4	Paper	10	6.69	0	6.69	-	-
12-04-2021	Base	601	Primor W	2110093030	15:17	4	Paper	20	6.69	10	6.69	-	-
12-04-2021	Base	601	Primor W	2110093030	14:59	3	Strip	10	6.69	10	6.69	-	-
12-04-2021	Base	602	Primor W	2110093031	11:35	4	Paper	30	6.70	10	6.7	-	-
12-04-2021	Base	602	Primor W	2110093031	14:07	4	Paper	10	6.70	79	6.7	-	-
12-04-2021	Base	602	Primor W	2110093031	11:33	3	Strip	10	6.70	30	6.7	-	-
12-04-2021	Base	603	Primor W	2110093032	11:31	4	Paper	10	6.70	10	6.7	-	-
12-04-2021	Base	603	Primor W	2110093032	15:10	4	Paper	10	6.70	10	6.7	-	-
12-04-2021	Base	603	Primor W	2110093032	15:25	3	Strip	10	6.70	60	6.7	-	-
12-04-2021	Slim-Cap	609	Agros SS	2110093030	15:23	5	Paper	79	6.67	50	6.67	-	-
12-04-2021	Base	606	Prado Verde SS	2109093017	10:58	4	Paper	0	6.68	0	6.68	-	-
12-04-2021	Base	606	Prado Verde SS	2109093017	14:41	5	Paper	10	6.68	0	6.68	-	-
12-04-2021	Base	606	Prado Verde SS	2109093017	11:46	3	Strip	10	6.68	20	6.68	-	-
12-04-2021	Edge	607	Mimosa SS	2109243024	11:38	12	Paper	10	6.68	0	6.68	N.D.	N.D.
12-04-2021	Edge	607	Mimosa SS	2109243024	14:19	12	Paper	30	6.68	0	6.68	0	6.68

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
12-04-2021	Edge	607	Mimosa SS	2109243024	15:16	12	Paper	20	6.68	0	6.68	0	6.68
12-04-2021	Edge	607	Mimosa SS	2109243024	13:54	6	Strip	50	6.68	10	6.68	-	-
12-04-2021	Slim-Cap	610	Agros SS	2109243027	10:22	6	Paper	20	6.68	0	6.68	-	-
12-04-2021	Slim-Cap	610	Agros SS	2109243027	14:39	5	Paper	0	6.68	20	6.69	-	-
12-04-2021	Slim-Cap	610	Agros SS	2109243027	11:26	5	Paper	40	6.69	10	6.69	-	-
12-04-2021	Edge	619	Mimosa SS	2109243025	10:19	12	Paper	0	6.68	N.D.	N.D.	10	6.68
12-04-2021	Edge	619	Mimosa SS	2109243025	11:15	12	Paper	0	6.68	60686	6.60	0	6.68
12-04-2021	Edge	619	Mimosa SS	2109243025	13:50	12	Paper	40	6.68	10	6.68	10	6.68
12-04-2021	Edge	619	Mimosa SS	2109243025	15:03	12	Paper	20	6.68	0	6.68	0	6.68
12-04-2021	Edge	619	Mimosa SS	2109243025	15:05	6	Strip	30	6.68	20	6.68	-	-
13-04-2021	Base	601	Primor W	2110103013	15:00	4	Paper	0	6.69	10	6.69	-	-
13-04-2021	Base	602	Primor W	2110103014	10:28	4	Paper	10	6.69	0	6.69	-	-
13-04-2021	Base	602	Primor W	2110103014	14:58	5	Paper	0	6.68	20	6.68	-	-
13-04-2021	Base	602	Primor W	2110103014	14:09	3	Strip	2318038	5.48	189	6.68	-	-
13-04-2021	Base	603	Primor W	2110103015	14:05	3	Strip	10	6.69	10	6.69	-	-
13-04-2021	Base	606	Grosso SS	2110103035	10:38	4	Paper	0	6.68	10	6.68	-	-
13-04-2021	Slim-Cap	610	Agros SS	2109253067	14:39	5	Paper	20	6.68	0	6.68	-	-
13-04-2021	Slim-Cap	610	Agros SS	2109253067	14:47	3	Strip	20	6.68	10	6.68	-	-
13-04-2021	Slim-Cap	611	Agros SS	2109253065	10:35	5	Paper	10	6.68	10	6.68	-	-
13-04-2021	Slim-Cap	611	Agros SS	2109253065	13:47	5	Paper	20	6.68	20	6.68	-	-
13-04-2021	Slim-Cap	611	Agros SS	2109253065	14:50	5	Paper	10	6.67	10	6.66	-	-
13-04-2021	Slim-Cap	611	Agros SS	2109253065	10:05	3	Strip	50	6.66	0	6.66	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
13-04-2021	Slim-Cap	611	Agros SS	2109253065	13:52	3	Strip	20	6.67	0	6.67	-	-
13-04-2021	Base	612	Grosso SS	2110103024	10:02	5	Paper	10	6.68	0	6.68	-	-
13-04-2021	Base	612	Grosso SS	2110103024	13:50	4	Paper	10	6.68	0	6.68	-	-
13-04-2021	Base	612	Grosso SS	2110103024	15:04	4	Paper	10	6.68	0	6.68	-	-
13-04-2021	Base	612	Grosso SS	2110103024	13:30	3	Strip	0	6.68	20	6.68	-	-
13-04-2021	Base	613	Grosso SS	2110103025	10:00	4	Paper	0	6.68	10	6.68	-	-
13-04-2021	Base	613	Grosso SS	2110103025	13:49	4	Paper	10	6.68	10	6.68	-	-
13-04-2021	Base	613	Grosso SS	2110103025	15:08	5	Paper	0	6.67	0	6.67	-	-
13-04-2021	Base	613	Grosso SS	2110103025	10:25	3	Strip	0	6.67	0	6.67	-	-
13-04-2021	Base	613	Grosso SS	2110103025	14:37	3	Strip	0	6.67	0	6.67	-	-
19-04-2021	Base	601	Primor W	2110163045	14:56	4	Paper	0	6.71	0	6.71	-	-
19-04-2021	Base	601	Primor W	2110163045	13:04	4	Paper	30	6.71	10	6.71	-	-
19-04-2021	Base	601	Primor W	2110163045	15:15	3	Strip	0	6.71	10	6.7	-	-
19-04-2021	Base	602	Primor W	2110163046	15:04	4	Paper	30	6.70	0	6.70	-	-
19-04-2021	Edge	604	Mimosa SS	2110013032	13:29	12	Paper	0	6.68	20	6.68	10	6.68
19-04-2021	Edge	604	Mimosa SS	2110013032	14:28	12	Paper	20	6.68	10	6.68	10	6.69
19-04-2021	Edge	604	Mimosa SS	2110013032	15:22	12	Paper	10	6.69	10	6.69	20	6.69
19-04-2021	Edge	604	Mimosa SS	2110013032	13:01	6	Strip	10	6.68	0	6.68	-	-
19-04-2021	Edge	604	Mimosa SS	2110013032	14:39	6	Strip	30	6.69	0	6.69	-	-
19-04-2021	Edge	607	Mimosa SS	2110013033	13:21	12	Paper	0	6.69	0	6.68	10	6.68
19-04-2021	Edge	607	Mimosa SS	2110013033	14:18	12	Paper	10	6.68	0	6.68	10	6.67
19-04-2021	Edge	607	Mimosa SS	2110013033	15:13	12	Paper	20	6.68	0	6.68	0	6.69

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
19-04-2021	Edge	607	Mimosa SS	2110013033	14:26	6	Strip	20	6.69	20	6.7	-	-
19-04-2021	Slim-Cap	609	Agros SS	2110013034	13:57	5	Paper	10	6.69	0	6.69	-	-
19-04-2021	Slim-Cap	609	Agros SS	2110013034	14:44	3	Strip	0	6.69	10	6.69	-	-
19-04-2021	Slim-Cap	611	Agros SS	2110013035	14:50	5	Paper	10	6.69	40	6.67	-	-
19-04-2021	Slim-Cap	611	Agros SS	2110013035	15:16	5	Paper	0	6.67	10	6.68	-	-
19-04-2021	Base	612	Primor W	2110163048	14:10	4	Paper	10	6.68	10	6.67	-	-
19-04-2021	Base	612	Primor W	2110163048	14:52	4	Paper	10	6.67	0	6.67	-	-
19-04-2021	Base	612	Primor W	2110163048	14:19	3	Strip	0	6.67	10	6.67	-	-
19-04-2021	Base	613	Primor W	2110163049	13:06	4	Paper	40	6.68	0	6.67	-	-
19-04-2021	Base	613	Primor W	2110163049	14:33	4	Paper	0	6.67	0	6.67	-	-
19-04-2021	Base	613	Primor W	2110163049	15:29	4	Paper	10	6.67	0	6.67	-	-
19-04-2021	Base	613	Primor W	2110163049	13:34	3	Strip	20	6.67	0	6.67	-	-
20-04-2021	Base	606	Grosso SS	2110173038	13:49	5	Paper	10	6.68	0	6.68	-	-
20-04-2021	Base	606	Grosso SS	2110173038	14:44	4	Paper	10	6.68	0	6.68	-	-
20-04-2021	Base	606	Grosso SS	2110173038	15:00	3	Strip	0	6.69	0	6.69	-	-
20-04-2021	Edge	604	Mimosa SS	2110023052	10:39	12	Paper	0	6.68	10	6.68	0	6.68
20-04-2021	Edge	604	Mimosa SS	2110023052	11:36	12	Paper	20	6.68	20	6.69	20	6.69
20-04-2021	Edge	604	Mimosa SS	2110023052	11:12	6	Strip	10	6.68	10	6.68	-	-
20-04-2021	Edge	607	Mimosa SS	2110023053	11:04	12	Paper	0	6.68	10	6.68	30	6.68
20-04-2021	Edge	607	Mimosa SS	2110023053	11:30	6	Strip	0	6.68	10	6.69	-	-
20-04-2021	Slim-Cap	609	Agros SS	2110023050	11:00	5	Paper	10	6.69	10	6.69	-	-
20-04-2021	Slim-Cap	610	Agros SS	2110023054	13:55	5	Paper	0	6.68	0	6.68	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
20-04-2021	Slim-Cap	610	Agros SS	2110023054	13:08	5	Paper	10	6.67	10	6.67	-	-
20-04-2021	Slim-Cap	610	Agros SS	2110023054	14:57	3	Strip	0	6.67	10	6.67	-	-
20-04-2021	Slim-Cap	611	Agros SS	2110023051	11:23	5	Paper	0	6.67	20	6.67	-	-
20-04-2021	Slim-Cap	611	Agros SS	2110023051	14:03	5	Paper	10	6.68	0	6.68	-	-
20-04-2021	Slim-Cap	611	Agros SS	2110023051	14:28	5	Paper	0	6.68	0	6.68	-	-
20-04-2021	Slim-Cap	611	Agros SS	2110023051	14:17	3	Strip	10	6.67	10	6.67	-	-
20-04-2021	Base	612	Grosso SS	2110173026	11:38	4	Paper	0	6.69	0	6.69	-	-
20-04-2021	Base	612	Grosso SS	2110173026	14:09	4	Paper	0	6.69	10	6.69	-	-
20-04-2021	Base	612	Grosso SS	2110173026	11:06	3	Strip	0	6.68	0	6.68	-	-
20-04-2021	Edge	620	Mimosa SS	2110023055	11:49	12	Paper	20	6.69	10	6.69	0	6.68
20-04-2021	Edge	620	Mimosa SS	2110023055	14:14	12	Paper	806549	4.51	0	6.68	10	6.68
20-04-2021	Edge	620	Mimosa SS	2110023055	14:58	12	Paper	10	6.68	0	6.68	0	6.68
20-04-2021	Edge	620	Mimosa SS	2110023055	11:24	6	Strip	0	6.68	0	6.68	-	-
20-04-2021	Edge	620	Mimosa SS	2110023055	14:46	6	Strip	0	6.69	10	6.69	-	-
26-04-2021	Base	601	Primor W	2110233022	14:26	4	Paper	30	6.69	10	6.69	-	-
26-04-2021	Base	601	Primor W	2110233022	14:18	3	Strip	30	6.69	20	6.69	-	-
26-04-2021	Base	602	Primor W	2110233023	14:05	4	Paper	10	6.69	0	6.69	-	-
26-04-2021	Base	602	Primor W	2110233023	14:02	3	Strip	0	6.70	10	6.70	-	-
26-04-2021	Base	603	Primor W	2110233024	13:02	4	Paper	10	6.70	0	6.70	-	-
26-04-2021	Base	603	Primor W	2110233024	14:23	5	Paper	0	6.70	0	6.69	-	-
26-04-2021	Base	603	Primor W	2110233024	13:44	3	Strip	40	6.69	20	6.69	-	-
26-04-2021	Base	606	Grosso S	2109243040	14:02	5	Paper	0	6.68	20	6.68	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
26-04-2021	Base	606	Grosso S	2109243040	14:23	4	Paper	0	6.68	0	6.68	-	-
26-04-2021	Base	606	Grosso S	2109243040	13:55	3	Strip	0	6.68	0	6.68	-	-
26-04-2021	Base	606	Grosso S	2109243040	15:25	3	Strip	10	6.67	0	6.67	-	-
26-04-2021	Edge	607	Mimosa S	2109233044	13:22	12	Paper	10	6.68	0	6.68	0	6.68
26-04-2021	Edge	607	Mimosa S	2109233044	14:21	12	Paper	0	6.68	20	6.68	0	6.68
26-04-2021	Edge	607	Mimosa S	2109233044	13:15	6	Strip	0	6.68	0	6.69	-	-
26-04-2021	Edge	607	Mimosa S	2109233044	14:48	6	Strip	10	6.69	20	6.68	-	-
26-04-2021	Slim-Cap	609	Mimosa SS EXP	2112223020	13:33	5	Paper	0	6.69	0	6.69	-	-
26-04-2021	Slim-Cap	609	Mimosa SS EXP	2112223020	14:39	5	Paper	20	6.69	20	6.69	-	-
26-04-2021	Slim-Cap	609	Mimosa SS EXP	2112223020	13:49	3	Strip	0	6.69	10	6.69	-	-
26-04-2021	Slim-Cap	611	Agros S	2109233046	13:03	5	Paper	20	6.69	0	6.68	-	-
26-04-2021	Slim-Cap	611	Agros S	2109233046	14:16	5	Paper	10	6.68	20	6.68	-	-
26-04-2021	Slim-Cap	611	Agros S	2109233046	14:20	3	Strip	0	6.68	10	6.68	-	-
26-04-2021	Edge	620	Mimosa SS	2110083026	13:02	12	Paper	10	6.68	10	6.68	0	6.68
26-04-2021	Edge	620	Mimosa SS	2110083026	14:07	12	Paper	0	6.68	0	6.68	0	6.68
26-04-2021	Edge	620	Mimosa SS	2110083026	15:21	12	Paper	10	6.68	10	6.68	0	6.68
26-04-2021	Edge	620	Mimosa SS	2110083026	14:45	6	Strip	10	6.68	0	6.68	-	-
27-04-2021	Base	601	Primor W	2110243017	13:43	4	Paper	10	6.69	0	6.69	-	-
27-04-2021	Base	601	Primor W	2110243017	13:08	3	Strip	282	5.58	0	6.69	-	-
27-04-2021	Base	601	Primor W	2110243017	14:34	3	Strip	0	6.69	40	6.69	-	-
27-04-2021	Base	602	Primor W	2110243018	13:44	4	Paper	227037	6.50	30	6.69	-	-
27-04-2021	Base	602	Primor W	2110243018	15:02	4	Paper	0	6.68	0	6.68	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
27-04-2021	Base	602	Primor W	2110243018	13:32	3	Strip	0	6.68	0	6.68	-	-
27-04-2021	Base	603	Primor W	2110243019	13:18	4	Paper	0	6.68	10	6.68	-	-
27-04-2021	Base	603	Primor W	2110243019	14:35	4	Paper	0	6.68	10	6.69	-	-
27-04-2021	Base	603	Primor W	2110243019	14:02	3	Strip	89	6.69	0	6.69	-	-
27-04-2021	Base	606	La Vaquera SS	2109243059	14:29	4	Paper	20	6.68	10	6.68	-	-
27-04-2021	Base	606	La Vaquera SS	2109243059	15:09	4	Paper	20	6.68	20	6.68	-	-
27-04-2021	Base	606	La Vaquera SS	2109243059	13:20	3	Strip	30	6.68	30	6.68	-	-
27-04-2021	Edge	607	Mimosa SS	2110093052	13:39	12	Paper	0	6.68	0	6.68	0	6.68
27-04-2021	Edge	607	Mimosa SS	2110093052	14:21	12	Paper	10	6.68	20	6.67	10	6.67
27-04-2021	Edge	607	Mimosa SS	2110093052	13:05	6	Strip	0	6.67	0	6.67	-	-
27-04-2021	Edge	607	Mimosa SS	2110093052	14:12	6	Strip	0	6.67	10	6.67	-	-
27-04-2021	Edge	619	Mimosa SS	2110093053	13:08	12	Paper	0	6.68	0	6.68	0	6.68
27-04-2021	Edge	619	Mimosa SS	2110093053	13:59	12	Paper	10	6.68	0	6.68	0	6.68
27-04-2021	Edge	619	Mimosa SS	2110093053	14:30	12	Paper	10	6.68	0	6.68	0	6.68
27-04-2021	Edge	619	Mimosa SS	2110093053	13:02	6	Strip	20	6.68	0	6.68	-	-
27-04-2021	Edge	619	Mimosa SS	2110093053	14:49	6	Strip	10	6.68	0	6.68	-	-
03-05-2021	Base	601	Primor W	2110303042	12:55	4	Paper	0	6.69	30	6.69	-	-
03-05-2021	Base	601	Primor W	2110303042	14:26	5	Paper	30	6.69	10	6.69	-	-
03-05-2021	Base	601	Primor W	2110303042	13:47	3	Strip	30	6.69	59	6.69	-	-
03-05-2021	Base	602	Primor W	2110303043	13:35	5	Paper	30	6.69	40	6.69	-	-
03-05-2021	Base	602	Primor W	2110303043	15:04	3	Strip	0	6.69	0	6.69	-	-
03-05-2021	Base	603	Primor W	2110303044	13:46	5	Paper	20	6.69	30	6.68	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
03-05-2021	Base	603	Primor W	2110303044	15:19	4	Paper	10	6.68	0	6.68	-	-
03-05-2021	Base	603	Primor W	2110303044	14:18	3	Strip	10	6.68	0	6.69	-	-
03-05-2021	Base	606	Agros SS Prof.	2109303051	14:00	4	Paper	0	6.68	0	6.68	-	-
03-05-2021	Base	606	Agros SS Prof.	2109303051	15:32	5	Paper	20	6.68	20	6.68	-	-
03-05-2021	Base	606	Agros SS Prof.	2109303051	14:29	3	Strip	10	6.68	20	6.68	-	-
03-05-2021	Edge	607	Mimosa SS	2110153033	12:54	12	Paper	N.D.	N.D.	20	6.68	0	6.68
03-05-2021	Edge	607	Mimosa SS	2110153033	14:04	12	Paper	123765	5.3	0	6.68	0	6.68
03-05-2021	Edge	607	Mimosa SS	2110153033	13:45	6	Strip	20	6.68	20	6.68	-	-
03-05-2021	Base	612	Grosso SS	2110303045	13:58	4	Paper	0	6.67	0	6.67	-	-
03-05-2021	Base	612	Grosso SS	2110303045	15:12	4	Paper	0	6.67	0	6.67	-	-
03-05-2021	Base	612	Grosso SS	2110303045	13:15	3	Strip	0	6.68	10	6.68	-	-
03-05-2021	Base	613	Grosso SS	2110303046	13:21	5	Paper	20	6.68	10	6.68	-	-
03-05-2021	Base	613	Grosso SS	2110303046	14:44	5	Paper	10	6.67	0	6.67	-	-
03-05-2021	Base	613	Grosso SS	2110303046	15:51	4	Paper	0	6.67	0	6.67	-	-
03-05-2021	Base	613	Grosso SS	2110303046	15:25	3	Strip	30	6.68	20	6.68	-	-
03-05-2021	Edge	620	Mimosa SS	2110153035	13:07	12	Paper	10	6.68	20	6.68	0	6.68
03-05-2021	Edge	620	Mimosa SS	2110153035	14:28	12	Paper	0	6.68	10	6.68	10	6.68
03-05-2021	Edge	620	Mimosa SS	2110153035	15:39	12	Paper	10	6.68	10	6.68	0	6.68
03-05-2021	Edge	620	Mimosa SS	2110153035	13:02	6	Strip	0	6.68	0	6.68	-	-
04-05-2021	Base	601	Primor W	2110313010	10:17	4	Paper	20	6.69	20	6.69	-	-
04-05-2021	Base	601	Primor W	2110313010	09:49	3	Strip	0	6.69	0	6.69	-	-
04-05-2021	Base	602	Primor W	2110313011	10:12	5	Paper	10	6.69	30	6.69	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
04-05-2021	Base	603	Primor W	2110313012	10:11	4	Paper	0	6.69	20	6.69	-	-
04-05-2021	Base	603	Primor W	2110313012	11:35	4	Paper	0	6.69	0	6.69	-	-
04-05-2021	Base	603	Primor W	2110313012	12:04	3	Strip	10	6.69	0	6.69	-	-
04-05-2021	Edge	604	Mimosa SS	2110163062	09:58	12	Paper	20	6.69	0	6.69	0	6.69
04-05-2021	Edge	604	Mimosa SS	2110163062	10:57	12	Paper	0	6.69	10	6.69	0	6.69
04-05-2021	Edge	604	Mimosa SS	2110163062	13:12	12	Paper	10	6.68	0	6.68	0	6.68
04-05-2021	Edge	604	Mimosa SS	2110163062	10:15	6	Strip	30	6.68	10	6.68	-	-
04-05-2021	Base	606	Agros SS Prof.	2110013047	09:47	5	Paper	0	6.68	10	6.68	-	-
04-05-2021	Base	606	Agros SS Prof.	2110013047	12:01	3	Strip	30	6.68	20	6.68	-	-
04-05-2021	Edge	607	Mimosa SS	2110163063	09:36	12	Paper	20	6.69	10	6.69	0	6.69
04-05-2021	Edge	607	Mimosa SS	2110163063	10:32	12	Paper	0	6.69	10	6.69	10	6.69
04-05-2021	Edge	607	Mimosa SS	2110163063	10:16	6	Strip	168	6.69	20	6.69	-	-
04-05-2021	Base	612	Grosso SS	2110313007	09:41	5	Paper	0	6.68	0	6.68	-	-
04-05-2021	Base	612	Grosso SS	2110313007	10:58	4	Paper	10	6.68	10	6.68	-	-
04-05-2021	Base	612	Grosso SS	2110313007	13:26	3	Strip	0	6.68	0	6.68	-	-
04-05-2021	Base	613	Grosso SS	2110313008	10:07	4	Paper	10	6.68	20	6.68	-	-
04-05-2021	Base	613	Grosso SS	2110313008	11:11	4	Paper	0	6.68	0	6.68	-	-
04-05-2021	Base	613	Grosso SS	2110313008	13:23	3	Strip	10	6.68	10	6.68	-	-
04-05-2021	Base	613	Grosso SS	2110313008	10:09	3	Strip	30	6.68	0	6.68	-	-
04-05-2021	Edge	620	Mimosa SS	2110163061	10:25	12	Paper	10	6.69	10	6.69	10	6.69
04-05-2021	Edge	620	Mimosa SS	2110163061	11:23	12	Paper	20	6.69	0	6.69	0	6.69
04-05-2021	Edge	620	Mimosa SS	2110163061	13:58	6	Strip	20	6.69	10	6.69	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
04-05-2021	Edge	620	Mimosa SS	2110163061	10:26	6	Strip	0	6.69	10	6.69	-	-
17-05-2021	Base	601	Grosso SS	2111133032	13:30	4	Paper	10	6.69	10	6.69	-	-
17-05-2021	Base	601	Grosso SS	2111133032	11:02	3	Strip	0	6.69	10	6.69	-	-
17-05-2021	Base	602	Grosso SS	2111133033	13:55	3	Strip	0	6.69	10	6.69	-	-
17-05-2021	Base	603	Grosso SS	2111133034	13:33	4	Paper	0	6.69	40	6.69	-	-
17-05-2021	Edge	604	Mimosa SS	2110293025	11:30	12	Paper	0	6.69	10	6.69	10	6.69
17-05-2021	Edge	604	Mimosa SS	2110293025	13:56	12	Paper	20	6.68	10	6.68	20	6.68
17-05-2021	Edge	604	Mimosa SS	2110293025	11:13	6	Strip	0	6.68	0	6.68	-	-
17-05-2021	Base	606	Agros SS Prof	2110143044	11:17	5	Paper	10	6.69	10	6.69	-	-
17-05-2021	Base	606	Agros SS Prof	2110143044	13:46	4	Paper	10	6.69	10	6.69	-	-
17-05-2021	Base	606	Agros SS Prof	2110143044	11:10	3	Strip	0	6.69	20	6.69	-	-
17-05-2021	Edge	607	Mimosa SS	2110293022	10:57	12	Paper	10	6.69	0	6.69	20	6.69
17-05-2021	Edge	607	Mimosa SS	2110293022	11:54	12	Paper	10	6.69	0	6.69	10	6.69
17-05-2021	Edge	607	Mimosa SS	2110293022	13:51	12	Paper	20	6.68	0	6.68	10	6.68
17-05-2021	Edge	607	Mimosa SS	2110293022	13:45	6	Strip	20	6.68	0	6.68	-	-
17-05-2021	Slim-Cap	609	Mimosa SS EXP	2201123009	10:55	5	Paper	10	6.68	10	6.68	-	-
17-05-2021	Slim-Cap	609	Mimosa SS EXP	2201123009	14:04	5	Paper	20	6.68	317	6.54	-	-
17-05-2021	Slim-Cap	609	Mimosa SS EXP	2201123009	11:19	3	Strip	20	6.68	10	6.68	-	-
17-05-2021	Slim-Cap	609	Mimosa SS EXP	2201123009	14:05	3	Strip	40	6.68	10	6.68	-	-
17-05-2021	Slim-Cap	610	Mimosa SS EXP	2201123010	11:34	5	Paper	10	6.68	10	6.68	-	-
17-05-2021	Slim-Cap	610	Mimosa SS EXP	2201123010	13:36	5	Paper	0	6.68	0	6.68	-	-
17-05-2021	Edge	619	Mimosa SS	2110293023	11:15	12	Paper	10	6.69	30	6.69	10	6.69

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
17-05-2021	Edge	619	Mimosa SS	2110293023	14:00	12	Paper	50	6.68	10	6.68	10	6.68
17-05-2021	Edge	619	Mimosa SS	2110293023	11:46	6	Strip	20	6.68	10	6.68	-	-
17-05-2021	Edge	619	Mimosa SS	2110293023	13:24	6	Strip	0	6.68	10	6.68	-	-
18-05-2021	Base	601	La Vaquera SS	2110153065	09:41	4	Paper	10	6.69	10	6.69	-	-
18-05-2021	Base	601	La Vaquera SS	2110153065	10:57	5	Paper	30	6.69	40	6.69	-	-
18-05-2021	Base	601	La Vaquera SS	2110153065	10:58	3	Strip	20	6.69	10	6.69	-	-
18-05-2021	Base	602	Grosso SS	2111143021	09:42	5	Paper	0	6.69	20	6.69	-	-
18-05-2021	Base	602	Grosso SS	2111143021	09:40	3	Strip	20	6.69	10	6.69	-	-
18-05-2021	Base	603	Grosso SS	2111143022	10:28	4	Paper	20	6.69	10	6.69	-	-
18-05-2021	Base	603	Grosso SS	2111143022	10:37	3	Strip	10	6.69	10	6.68	-	-
18-05-2021	Edge	604	Mimosa SS	2110303074	09:55	12	Paper	10	6.68	10	6.68	0	6.68
18-05-2021	Edge	604	Mimosa SS	2110303074	10:29	12	Paper	10	6.68	10	6.68	10	6.68
18-05-2021	Edge	604	Mimosa SS	2110303074	11:11	6	Strip	20	6.68	30	6.68	-	-
18-05-2021	Base	606	Agros SS Prof	2110153038	10:27	5	Paper	10	6.68	0	6.68	-	-
18-05-2021	Base	606	Agros SS Prof	2110153038	10:00	3	Strip	10	6.69	20	6.69	-	-
18-05-2021	Edge	607	Mimosa SS	2110303071	10:07	12	Paper	10	6.68	0	6.68	0	6.68
18-05-2021	Edge	607	Mimosa SS	2110303071	11:07	12	Paper	0	6.68	10	6.68	30	6.68
18-05-2021	Edge	607	Mimosa SS	2110303071	10:47	6	Strip	20	6.68	10	6.68	-	-
18-05-2021	Slim-Cap	609	Mimosa W EXP	2201133007	09:44	5	Paper	20	6.68	10	6.68	-	-
18-05-2021	Slim-Cap	609	Mimosa W EXP	2201133007	10:20	5	Paper	0	6.67	10	6.67	-	-
18-05-2021	Slim-Cap	609	Mimosa W EXP	2201133007	11:00	5	Paper	30	6.67	20	6.67	-	-
18-05-2021	Slim-Cap	609	Mimosa W EXP	2201133007	11:02	3	Strip	10	6.67	10	6.67	-	-

Table III. 1 - Continuation.

Production date	Type	Machine	Product	Batch	Time	Nr of Rejected Packages	Splice	1		2		3	
								D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH	D-Count (IBC/mL)	pH
18-05-2021	Edge	619	Mimosa SS	2110303072	09:43	12	Paper	30	6.67	0	6.67	30	6.67
18-05-2021	Edge	619	Mimosa SS	2110303072	10:39	12	Paper	20	6.68	0	6.68	30	6.68
18-05-2021	Edge	619	Mimosa SS	2110303072	10:03	6	Strip	0	6.68	10	6.68	-	-
18-05-2021	Edge	619	Mimosa SS	2110303072	11:23	6	Strip	10	6.68	0	6.68	-	-

Annex IV - Fat content schemes

In order to assess fat losses throughout the milk production process, it was necessary to monitor the percentage of milk fat from its arrival (transport trucks) to its consumption (final product). Thus, 5 final products of the Mimosa brand (brand with the highest production and number of sales at Lactogal) were studied and, with the SAP management program, their traceability until milk reception was identified.

Therefore, with the total fat percentages obtained by MilkoScan FT1[®], for the final product, thermised and raw milk, and taken from the SAP program for the transportation trucks, it was possible to build the following schemes considering the volume used at each stage (Figure IV. 1, IV. 2, IV. 3 and IV. 4). Due to the complexity of the scheme, the volume and percentage of milk fat from milk trucks are shown in a table (Table IV. 1, IV. 3, IV. 5, and IV. 7). The process scheme regarding the product 2108223034 it's represented in the Results and Discussion section.

To verify if there were losses regarding volume and fat content, the following equations were used, respectively:

$$\sum V_{out} = \sum V_{in} \quad \text{Equation IV. 1}$$

$$\sum (V_{out} \times x_{out}) = \sum (V_{in} \times x_{in}) \quad \text{Equation IV. 2}$$

Where V_{in} and V_{out} represents the volume, in liters, at entry and exit, respectively; and x_{in} and x_{out} the fat fraction, in percentage, at entry and exit, respectively. These equations were applied between the stages: milk truck → raw milk tank; raw milk tank → thermised milk tank + cream tank, and thermised milk tank → final product. The values obtained from Equation IV. 2 are represented in the Table IV. 2, IV. 4, IV. 6 and IV. 8.

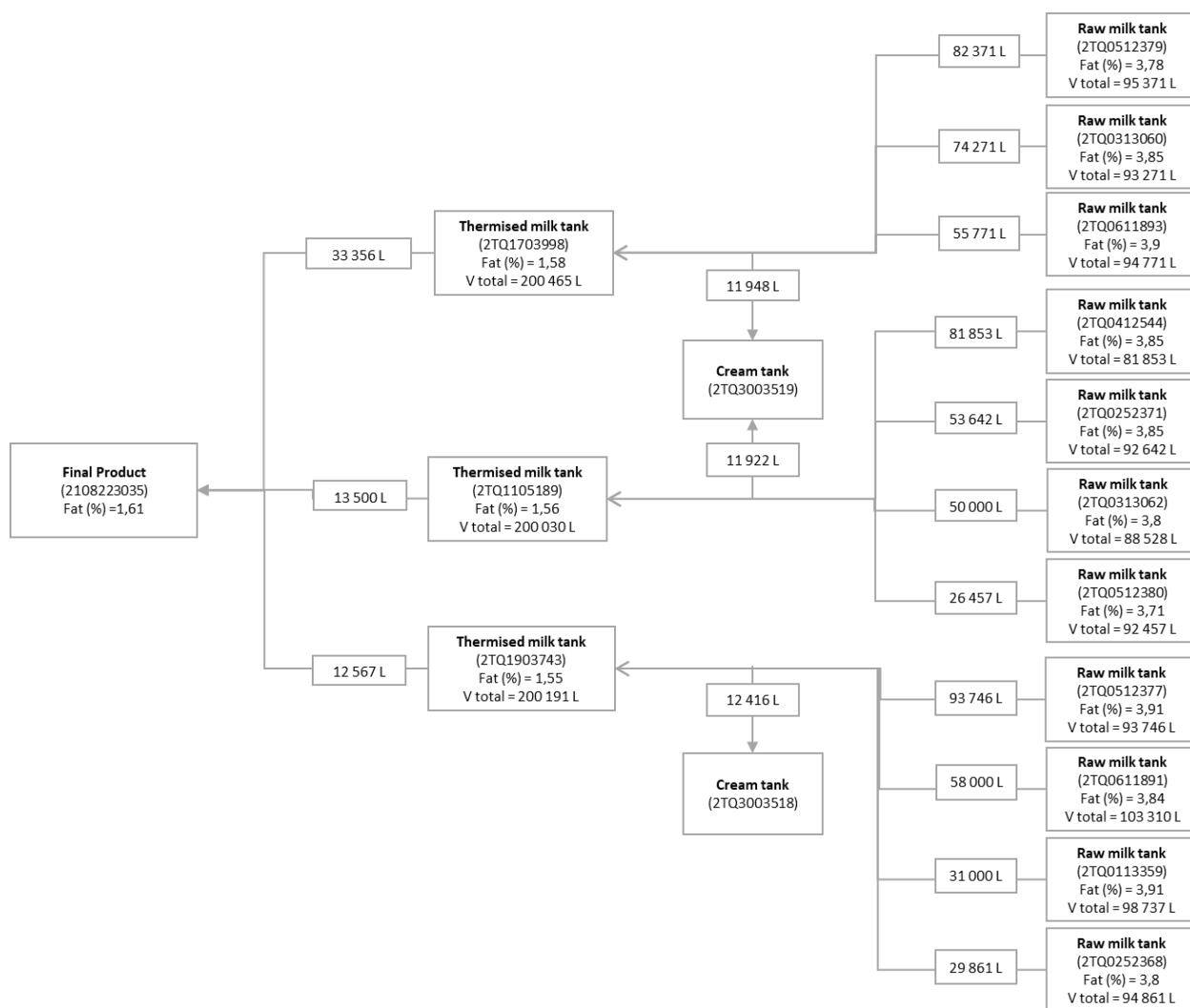


Figure IV. 1 - Production scheme for the final product: 2108223035.

Table IV. 1 - Volume and percentage of milk fat from the transport trucks that produced the final product: 2108223035.

Raw Milk Tank	Milk Truck	Volume (L)	Fat (%)
2TQ0512379	423	22422	3.54
	478	20705	3.84
	2935	14565	3.83
	2494	14824	3.56
	1033	8750	3.67
	177	9541	3.73
	876	4564	3.7
2TQ0113361	4205	22053	3.56
	830	18769	3.96
	812	15218	3.58
	1024	15074	3.74
	2801	22157	3.81
2TQ0611893	1501	26451	3.90
	1705	23583	3.86

Table IV. 1 - Continuation.

Raw Milk Tank	Milk Truck	Volume (L)	Fat (%)
2TQ0611893	210	22671	3.76
	511	22066	3.69
2TQ0412544	1857	22277	3.55
	1200	15130	3.86
	441	23062	3.80
	575	21384	3.82
2TQ0252371	3040	23630	3.96
	1440	26009	3.94
	1060	20277	3.76
	1796	22726	3.78
2TQ0313062	1316	15549	3.98
	238	15770	3.74
	548	13228	3.83
	247	22130	3.83
	52	21851	3.58
2TQ0512380	195	19788	3.63
	566	21364	3.82
	1732	23643	3.66
	1334	14623	3.76
	98	13039	3.57
2TQ0512377	1954	15793	3.85
	2050	22477	3.72
	1769	23384	3.91
	3305	15238	3.97
	2917	13898	3.84
2TQ0611891	70	14348	3.84
	1194	20667	3.72
	2421	23331	3.60
	1291	23185	3.85
	3004	21779	3.86
2TQ0113359	140	21758	3.82
	2625	26525	3.83
	1778	22536	3.77
	2926	12627	3.89
	2476	15291	3.90
2TQ0252368	1884	15587	3.78
	1714	23399	3.61
	469	16265	3.74
	168	15646	3.70
	1802	23964	3.80

Table IV. 2 - Difference between MilkoScan FT1® and masse balance values regarding total fat (%) for the final product: 2108223035.

	Batch Number	(%) Fat obtained by MilkoScan FT1®	(%) Fat obtained by mass balance	Difference (%)
milk truck → raw milk tank	2TQ0512379	3.78±0.00	3.69	-0.09
	2TQ0113361	3.85±0.01	3.73	0.18
	2TQ0611893	3.90±0.01	3.81	0.09
	2TQ0412544	3.85±0.02	3.75	0.10
	2TQ0252371	3.85±0.00	3.87	-0.02
	2TQ0313062	3.80±0.00	3.78	0.02
	2TQ0512380	3.71±0.01	3.69	0.02
	2TQ0512377	3.91±0.00	3.73	0.18
	2TQ0611891	3.84±0.01	3.77	0.07
	2TQ0113359	3.91±0.00	3.83	0.08
	2TQ0252368	3.80±0.01	3.72	0.08
raw milk tank → thermised milk tank + cream tank	2TQ1703998	1.58±0.01	1.67	-0.09
	2TQ1105189	1.56±0.01	1.66	-0.10
	2TQ1903743	1.55±0.00	1.64	-0.08
thermised milk tank → final product	2108223035	1.61±0.01	1.57	0.04

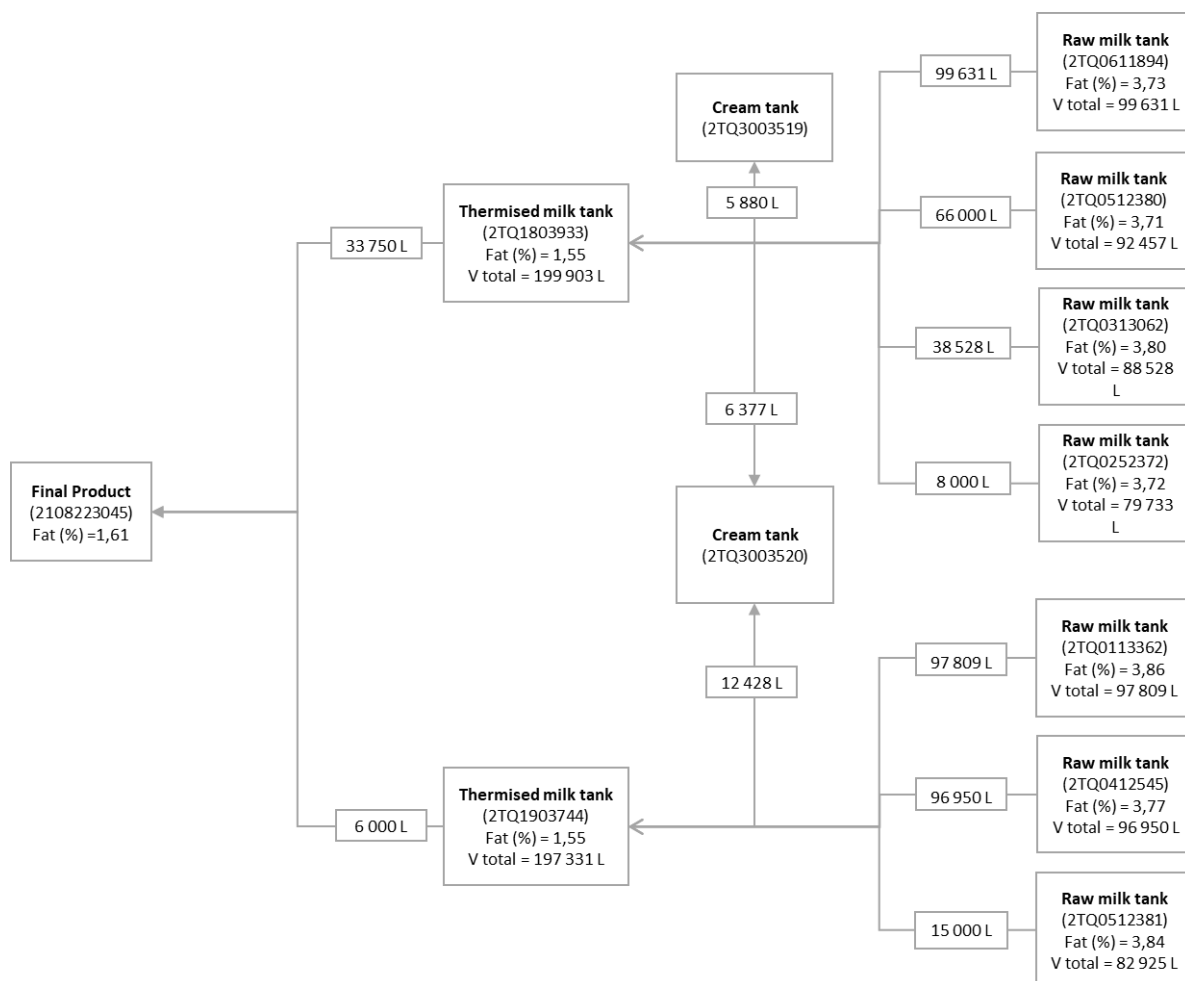


Figure IV. 2 - Production scheme for the final product: 2108223045.

Table IV. 3 - Volume and percentage of milk fat from the transport trucks that produced the final product: 2108223045.

Raw Milk Tank	Trunk Number	Volume (L)	Fat (%)
2TQ0611894	2944	14600	3.84
	122	26622	3.78
	89	20626	3.42
	919	23152	3.84
	1927	14631	3.95
2TQ0512380	195	19788	3.63
	566	21364	3.82
	1732	23643	3.66
	1334	14623	3.76
	98	13039	3.57
2TQ0313062	1316	15549	3.98
	238	15770	3.74
	548	13228	3.83
	247	22130	3.83
	52	21851	3.58
2TQ0252372	2379	23476	3.65
	2005	14966	3.72
	1486	15293	3.69
	1246	25998	3.85
2TQ0113362	2573	24054	3.71
	1990	7270	3.87
	1538	22034	4.00
	450	21498	3.88
	317	22953	3.78
2TQ0412545	751	22189	3.94
	1875	22564	3.65
	1264	14451	3.71
	1459	15025	3.83
	43	22721	3.68
2TQ0512381	1042	21846	3.88
	1741	22457	3.72
	539	15809	3.81
	1176	22813	3.90

Table IV. 4 - Difference between MilkoScan FT1® and masse balance values regarding total fat (%) for the final product: 2108223045.

	Batch Number	(%) Fat obtained by MilkoScan FT1®	(%) Fat obtained by mass balance	Difference (%)
milk truck → raw milk tank	2TQ0611894	3.73±0.00	3.75	-0.02
	2TQ0512380	3.71±0.01	3.69	0.02
	2TQ0313062	3.80±0.01	3.78	0.02
	2TQ0252372	3.72±0.02	3.74	-0.02
	2TQ0113362	3.86±0.00	3.84	0.02
	2TQ0412545	3.77±0.00	3.76	0.01
	2TQ0512381	3.84±0.01	3.83	0.01
raw milk tank → thermised milk tank + cream tank	2TQ1803933	1.55±0.01	1.51	0.04
	2TQ1903744	1.55±0.00	1.54	0.01
thermised milk tank → final product	2108223045	1.61±0.01	1.55	0.06

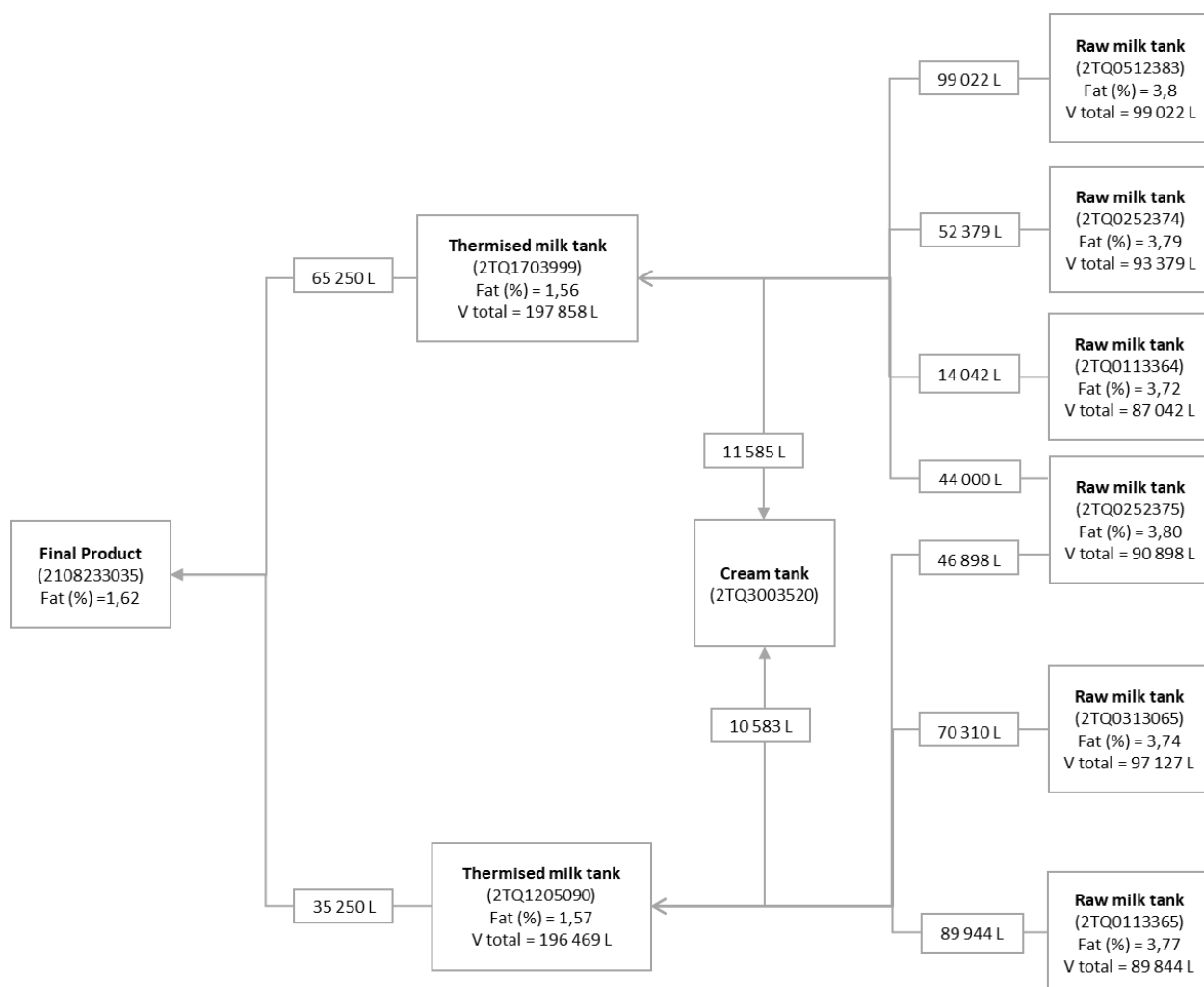


Figure IV. 3 - Production scheme for the final product: 2108233035.

Table IV. 5 - Volume and percentage of milk fat from the transport trucks that produced the final product: 21088233035.

Raw Milk Tank	Milk Truck	Volume (L)	Fat (%)
2TQ0512383	955	15841	3.79
	1778	22020	3.83
	2625	26337	3.86
	4002	20373	3.70
	70	14451	3.89
2TQ0252374	502	22173	3.77
	3235	25718	3.87
	229	22316	3.92
	61	23172	3.68
2TQ0113364	168	15351	3.72
	2023	15548	3.70
	469	16323	3.77
	1802	23934	3.87
	1884	15886	3.77
2TQ0252375	1097	21589	3.91
	779	21676	3.76
	821	11789	3.83
	937	14294	3.79
	885	21550	3.80
2T10313065	1820	15372	3.55
	1079	23120	3.77
	2351	22163	3.88
	1237	14354	3.75
	4201	22118	3.7
2TQ0113365	25	23031	3.81
	131	21863	3.58
	2014	21697	3.81
	1750	23253	3.84

Table IV. 6 - Difference between MilkoScan FT1® and masse balance values regarding total fat (%) for the final product: 2108233035.

	Batch Number	(%) Fat obtained by MilkoScan FT1®	(%) Fat obtained by mass balance	Difference (%)
milk truck → raw milk tank	2TQ0512383	3.80±0.00	3.81	-0.01
	2TQ0252374	3.79±0.01	3.81	-0.02
	2TQ0113364	3.72±0.01	3.78	-0.06
	2TQ0252375	3.80±0.02	3.82	-0.02
	2TQ0313065	3.74±0.00	3.74	0.00
	2TQ0113365	3.77±0.00	3.76	0.01
raw milk tank → thermised milk tank + cream tank	2TQ1703999	1.56±0.01	1.67	-0.11
	2TQ1205090	1.57±0.00	1.81	-0.24
thermised milk tank → final product	2108233035	1.62±0.01	1.56	0.06

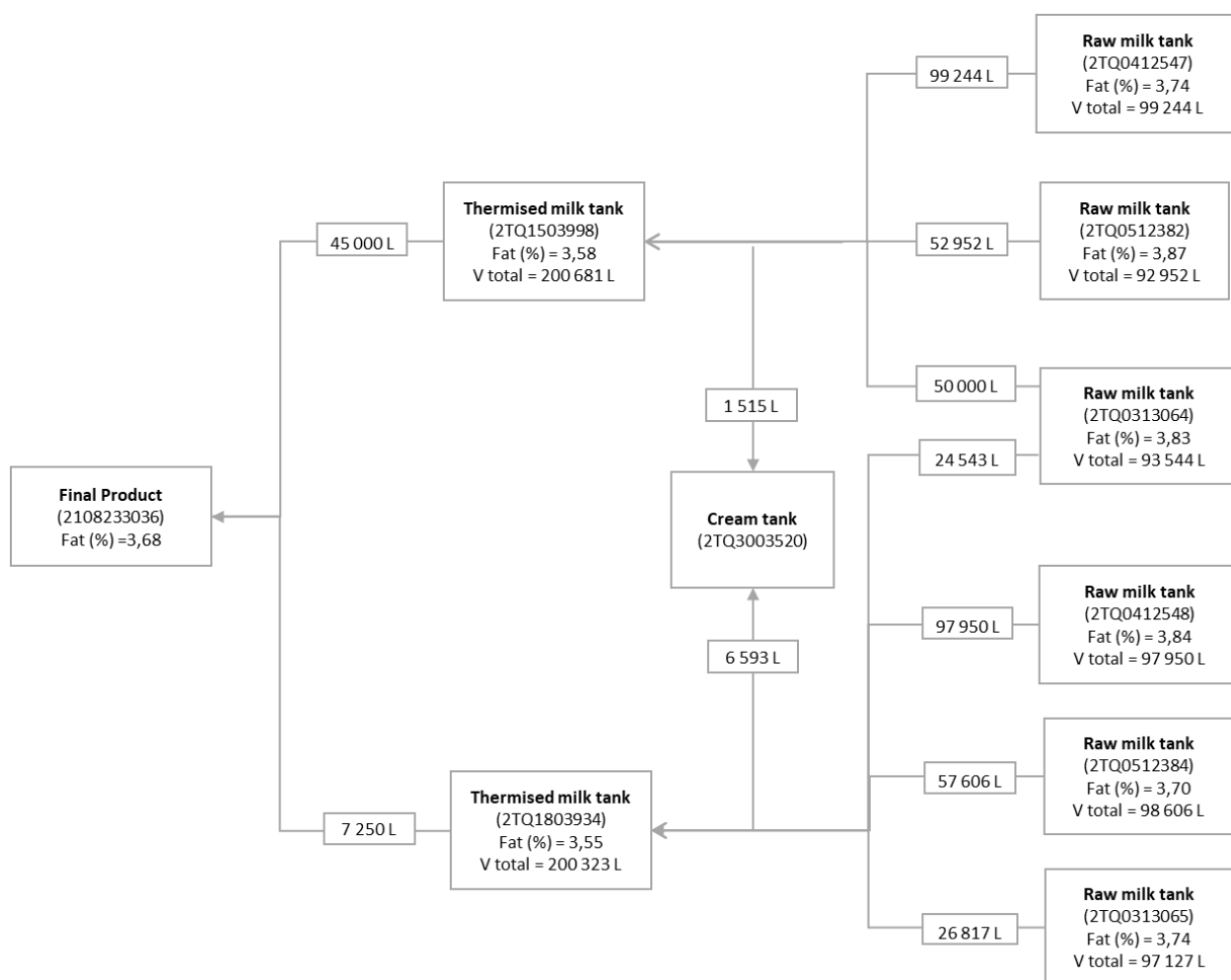


Figure IV. 4 - Production scheme for the final product: 2108233036.

Table IV. 7 - Volume and percentage of milk fat from the transport trucks that produced the final product: 21088233036.

Raw Milk Tank	Milk Truck	Volume (L)	Fat (%)
2TQ0412547	2917	13891	3.80
	788	15286	3.70
	706	25705	3.69
	1343	22269	3.63
	186	22093	3.85
2TQ0512382	496	22491	3.93
	900	23189	3.86
	201	23386	3.81
	760	23886	3.94
2TQ0313064	2926	12688	3.96
	487	15667	3.80
	3004	21634	3.86
	1291	23251	3.92
	1194	20304	3.78
2TQ0412548	113	15338	3.91
	1699	26806	3.94
	292	14641	3.72
	283	17540	3.7
	928	23625	3.78
2TQ0512384	858	21528	3.51
	797	23401	3.66
	1468	15117	3.83
	432	22697	3.66
	1389	15863	3.95
2TQ0313065	1820	15372	3.55
	1079	23120	3.77
	2351	22163	3.88
	1237	14354	3.75
	4201	22118	3.7

Table IV. 8 - Difference between MilkoScan FT1® and masse balance values regarding total fat (%) for the final product: 21088233036.

	Batch Number	(%) Fat obtained by MilkoScan FT1®	(%) Fat obtained by mass balance	Difference (%)
milk truck → raw milk tank	2TQ0412547	3.74±0.00	3.73	0.01
	2TQ0512382	3.87±0.01	3.88	-0.01
	2TQ0313064	3.83±0.01	3.86	-0.03
	2TQ0412548	3.84±0.02	3.82	0.02
	2TQ0512384	3.70±0.00	3.70	0.00
	2TQ0313065	3.74±0.00	3.74	0.00
raw milk tank → thermised milk tank + cream tank	2TQ1503998	3.58±0.01	3.60	-0.02
	2TQ1803934	3.55±0.00	3.59	-0.04
thermised milk tank → final product	2108233036	3.68±0.01	3.58	0.10