Milk solid deposition induced by an electric field. A preliminary study

Depósito de sólidos lácteos inducido por un campo eléctrico. Un estudio preliminar

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INFORMAN

Que el trabajo titulado "*Milk solid deposition induced by an electric field. A preliminary study"* ha sido realizado, bajo su supervisión, por Bernat Pérez Playà durante la realización del Máster en Calidad de los Alimentos de Origen Animal de la Universidad Autónoma de Barcelona, España.

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INDEX OF ABBREVIATIONS

α-La	α-Lactalbumin
ANOVA	Analysis of variance
β-Lg	β-Lactoglobulin
BSA	Bovine Serum Albumin
Ca	Calcium
d_{vs}, D_{d0}	Diameter of droplet
ε_0	Permittivity of vacuum
E	Energy consumption
EDX	X-ray energy scattering spectrometry
EHDA	Electrohydrodynamic atomization
h	Light intensity
H	Height
K	Electrical conductivity
LSD	Least significant difference
LSM	Least square means
MLR	Multiple linear regression
η	Viscosity
N	Number of revolutions
OD	Outside diameter
ρ	Density
P	phosphorus
p	Pressure nozzle
PEF	High voltage electrical pulses
PTFE	Polytetrafluoroethylene
q	Initial droplet charge
Q, $\dot{V}$	Flow rate
R	Disk diameter
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SMP	Skim milk powder
TS	Total solids
TS_i	Initial total solids
TS_f	Final total solids
TSI	Turbiscan Stability Index
t	Time
UHT	Ultra high temperature
w	Weight
Wa	Weight of water
WMP	Whole milk powder
WPNI	Whey protein nitrogen index
γ	Surface tension

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ABSTRACT

The main purpose of milk powder manufacturing is to transform raw milk, which is a perishable product, into a product with a shelf life of several years without substantial loss of quality. The successive heat treatments, traditionally used, cause damage to the main components, such as denaturation of whey proteins, changes in functional properties and a loss in nutritional value. In addition, the energy cost of milk powder processing is very high. Atomizers require hot air (> 175 °C) representing ~10% of the total energy consumption in the food industry. Electrospraying is proposed as an alternative method of depositing lactic solids without the application of heat. The important parameters for electrospray are basically four: solution viscosity, electric field, nozzle-collector distance and flow rate. In order to evaluate its technological potential, the degree of water extraction and the effect on proteins were analyzed at different concentration levels of initial milk solids and different electrospraying flow rates, keeping the voltage at 29 kV and the nozzle-collector distance at 10 cm. The Ca:P ratio was not altered in 24% milk. The diameter of the droplets was reduced by decreasing the flow rate and/or increasing the concentration of total solids. Finally, electrospraying, at a low flow rate, eliminated water significantly ($p < 0.05$) without producing any effect on the structure of proteins (i.e., caseins and whey proteins). In future, microbiological and enzymatic analyzes should be performed to stablish product shelf life.

Keywords: electrospraying, skim milk powder, non-heat treatment, flow rate, water extraction, milk droplet size, milk proteins.

RESUMEN

El objetivo principal de la fabricación de leche en polvo es transformar leche cruda, un producto perecedero, en un producto con una vida útil de varios años sin perder sustancialmente su calidad. Los sucesivos tratamientos térmicos, tradicionalmente usados, provocan daños en los componentes principales, como la desnaturalización de las proteínas del suero, cambios en las propiedades funcionales y pérdida de valor nutricional. Además, el coste energético del procesamiento de la leche en polvo es muy elevado. Los atomizadores requieren aire caliente (> 175 °C) que representa ~10% del consumo total de energía en la industria alimentaria. La electropulverización se propone como método alternativo para depositar sólidos lácteos sin la aplicación de calor. Los parámetros importantes para la electropulverización son básicamente cuatro: viscosidad del líquido, campo eléctrico, distancia boquilla-colector y caudal. Para evaluar su potencial tecnológico, se analizó el grado de extracción de agua y el efecto sobre las proteínas a diferentes niveles de concentración de sólidos lácteos iniciales y diferentes caudales de electropulverización, manteniendo el voltaje (29 kV) y la distancia boquilla-colector (10 cm). La relación Ca:P no se alteró en la leche al 24%. El diámetro de las gotas se redujo disminuyendo el caudal y/o aumentando la concentración de sólidos totales. Finalmente, la electropulverización, a un caudal bajo, eliminó el agua de forma significativa ($p < 0.05$) sin producir ningún efecto sobre la estructura de las proteínas (caseínas y proteínas de suero). En el futuro, se deben realizar análisis microbiológicos y enzimáticos para establecer la vida útil del producto.

Palabras clave: electropulverización, leche desnatada en polvo, tratamiento sin calor, caudal, extracción de agua, tamaño de gota de leche, proteínas de la leche.

1. Introduction

Milk production has been booming in recent centuries. Improvements in livestock feed and feeding, animal selection as well as in the field of genetics have resulted in a significant increase in production, in addition to a decrease of maintenance costs (Bach et al., 2020).

Raw milk is a fresh material very susceptible to being altered by microorganisms because it has an excellent composition of nutrients and elevated water activity, having a shelf life of 12 to 24 h (Lara-Aguilar and Alcaine, 2019). Current processing methods to ensure safety and extend shelf life are mainly thermal. Highlighting the extremes, pasteurization is capable of extending the shelf life from one to three weeks in refrigeration, while, more intense treatments such as UHT (Ultra High Temperature), extends it from three to six months at room temperature, with gelation due to aging being the main cause of deterioration (Muir, 2011; Rankin et al., 2020).

Almost complete extraction of water from food is the best form of conservation over time so as not to waste the surpluses produced (Almena et al., 2018). In the case of milk powder, the shelf life is extended to six months and 3 years, for whole and skim milk, respectively (Scott et al., 2002). Milk powder has nutritional value preservation. Additionally, it has an added value and a great advantage is obtained at the logistical level, because the extraction of water leads to a much lower storage volume, reducing both stockage and transportation costs (Bocci and Casas, 2013). For several decades, the process of obtaining milk powder has been improved, along with related research and science. Technical improvements have been made to obtain a product with a better nutritional profile, specific functional properties or superior hygienic conditions.

Current processing methods have high energy consumption, as a consequence of heat treatment operations such as pasteurization and the extraction of water by evaporation and atomization (Bylund, 1995). Furthermore, the use of successive heat treatments implies thermal damage to the product. Among them are nutritional loss, denaturation of whey proteins and changes in functional properties (Walstra et al., 2001). Electrospraying is proposed as a sustainable alternative due to potential lower energy consumption and advantages, such as avoiding thermal damage during processing (Anu and Anandharamakrishnan, 2014).

1.1. Objectives

The present study aimed at evaluating the application of an electrical field as an alternative method of extracting water in skim milk.

This objective was divided into three subobjectives: a) assessing the processing parameters for skim milk using the electrospraying method, b) determining the degree of water extraction from the process and, c) observing the effect of the electric field on milk proteins, both caseins and whey proteins.

1.2. Bibliographic review

1.2.1. Definition

The Codex Alimentarius, in the second edition of milk and dairy products of 2011, defines milk powder as the product obtained by removing the water from the milk. The fat and/or protein content may adjust only to meet the compositional requirements set forth in the section 3 of this Standard, by adding and/or removing milk constituents, so that the ratio between whey protein and the casein of milk used as raw material is kept.

The Annex 1 of the Council Directive (CE) 114/2001 defines totally dehydrated milk as “the solid product, where the water content does not exceed 5% by weight of the finished product, obtained directly by the removal of water from milk, from wholly or partly skim milk, from cream or from a mixture of these products”. Milk powder is classified depending on its fat content into four types. In the case of skim milk powder, the fat content by weight, must not exceed 1.5%.

1.2.2. Market study

International prices for dairy products, measured by the FAO Dairy Price Index, increased 3% more in 2019 compared to the previous year, when it fell by 4.6%. The increase in the first half of the year was due to the limitation of Oceania exports due to internal demand, since the climatic conditions induced poor grass quality and water shortages. High temperatures during the summer months also reduced grass quality and food availability in Europe, negatively affecting the expansion of milk production. Since June, export availability improved, especially in New Zealand, when milk production was activated (FAO, 2020).

In Figure 1, the evolution of the price of main dairy products can be observed. The skim milk powder or SMP registered the largest year-on-year increase (32.7%), followed by cheese (7.8%) and whole milk powder or WMP (3.8%), and a decrease in butter prices (-15.7%). Although SMP prices increased significantly, the average value remained below that of other dairy products (FAO, 2020).

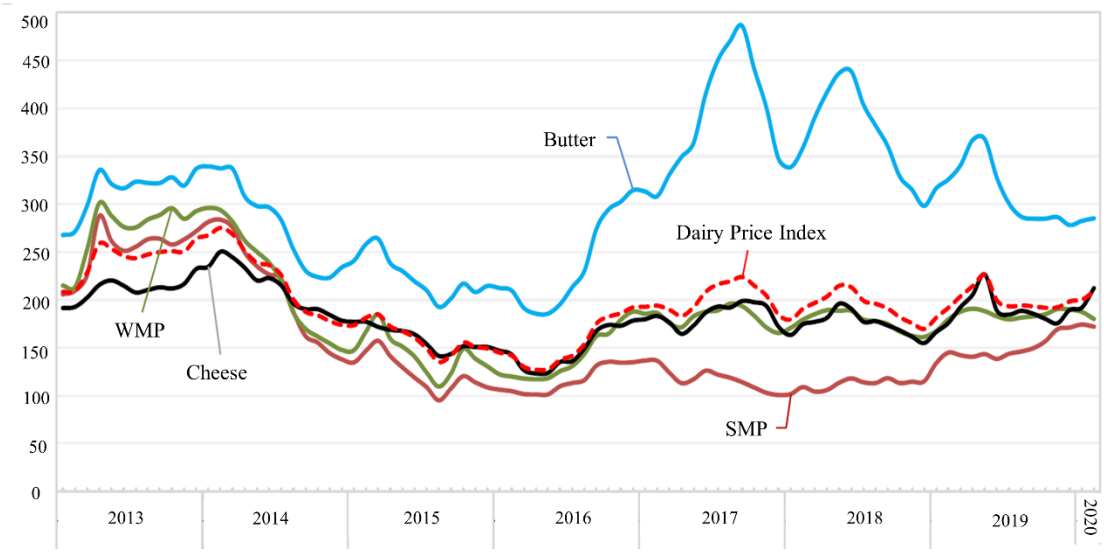

Figure 1. Evolution of the price indices for dairy products (FAO, 2020).

In 2019 there was an increase in exports of dairy products estimated at 76.7 million tons (in milk equivalents), which represents an increase of 1.0% compared to 2018. China, the Russian Federation, the Philippines and Indonesia reported an increase in imports, offset by the decrease in imports in Algeria, and United Arab Emirates, among others. Oceania and the European Union supplied most of the demand, while the United States exported in less quantity. Asia was the most powerful importing region in the world, where import increased 1.8%, exceeding 45 million tons. The other regions (Europe, North America, Central America, the Caribbean and Oceania) also saw an increase in exports, against the decrease in the regions of Africa and South America (FAO, 2020).

1.2.3. Milk powder manufacturing process

The main purpose of making milk powder is to transform raw milk, which is a perishable product, into a product with a shelf life of several years without substantial quality losses (Walstra et al., 2001). The process used is dehydration, which will have a direct effect on the appearance, physicochemical properties and nutritional value depending on the process

conditions (Sarria, 1998). The milk powder must present a humidity <5% for a correct storage (Scott et al., 2002).

There are several techniques to produce milk powder, but all of them share the same first steps, although in some cases there may be exceptions. First, a standardization of the starting milk is carried out. Next, a heat treatment is performed, considering whether the milk is skim or whole. If the product is to be made from whole milk, it is important to increase the temperature to 80-85 °C, achieving a negative peroxidase test, and above all, eliminating native lipolytic enzymes so that the product does not lose quality during storage due to enzymatic oxidation. In the case of skim milk, a mild pasteurization is applied to obtain a negative alkaline phosphatase test. It should be noted that this heat treatment will have a great impact on one of the most important quality aspects of milk powder, the whey protein nitrogen index or WPNI, which reports the amount of whey proteins that have not been denatured by the processing (Walstra et al., 2001).

After the heat treatment, a vacuum evaporation is carried out at 60-70 °C in order to remove part of the water without damaging the milk components. This procedure is advisable because it reduces the associated energy consumption and facilitates the subsequent dehydration process. For example, skim milk, with an initial content in total solids of about 8.7%, is evaporated until its total solids content is ~50%, by eliminating 90% of the initial water in the milk before it is spray dried (Early, 1998). Depending on the drying process, it will be necessary to homogenize either, whole or semi-skim milk. If the spraying technique is used to dehydrate milk, this step can be ignored, because the spray head causes the breakage of fat globules since it works at pressures close to that a homogenizer. It should be considered that the homogenization process with concentrated milk produces an increase in viscosity associated with the increase in the effective volume of the lipid fraction. As a result, if the drying process is carried out by spraying, a prior heating must be carried out to reduce viscosity before milk atomization (temperature is >80 and >175 °C for milk and hot air of atomization, respectively). The concentrate must not be kept hot for more than the minimum time, avoiding thickening due to aging and the development of microorganisms. Next, drying is carried out to further eliminate water until the desired humidity is reached. The dehydrated product is rapidly cooled and packaged. Occasionally a lecithination process can be applied

during drying, the purpose of which is to achieve instantization of the product (Walstra et al., 2006).

Currently, drying is done for the most part by spraying, but as an alternative freeze drying, foam drying, or roller dryer can be used (Walstra et al., 2006).

1.2.4. Effect of temperature on milk powder

The application of heat treatments during milk processing causes changes in milk components and physicochemical properties. The resulting effect will depend on the intensity of the treatment, usually the combination of temperature and time. The main components of milk that can be affected by the application of heat are caseins, whey proteins, enzymes, fat globules, lactose, vitamins, calcium and phosphates.

1.2.4.1. Caseins and whey proteins

Caseins and whey proteins represent 80 and 20% of the total milk protein, respectively. Caseins do not undergo denaturation phenomena during heat treatment because they have an open structure and a high proline content, which prevents the formation of helical structures (Deeth and Hartanto, 2009).

Whey protein are typically globular and present high levels of tertiary and quaternary structures; therefore, they are susceptible to denaturation by various agents, including heat (Donovan and Mulvihill, 1987; Anema et al., 2020). Normally, an intensity of treatment is sought that damages whey proteins the least, but the use of the product must be considered to determine which conditions are optimal. The degree of denaturation will determine the WPNI. At a fundamental level, protein denaturation is often defined as any noncovalent change to the secondary or tertiary structure of the protein molecule. From this denatured state, the protein can revert to its native state (refold) or interact with other components in the system (aggregate). The aggregation is due to exposed free sulfhydryl groups. These groups react with each other, giving rise to new disulfide bridges, forming between the whey proteins or with kappa-casein (Anema, 2020). Denaturation begins at 65 °C, but the effect occurs mainly when heating exceeds 80 °C. The effect of treatment is determined by the degree of denaturation of β-Lactoglobulin (β-Lg), since it represents approximately 50% of all whey proteins (Morr, 1985). Donovan and Mulvihill (1987) determined that the heat stability of

whey proteins follows this order: α-Lactalbumin (α-La) > β-Lg > Bovine Serum Albumin (BSA) > Immunoglobulin (Ig).

1.2.4.2. Calcium and phosphates

The solubility of calcium and phosphates, as well as calcium phosphate, is highly dependent on temperature. The solubility of calcium phosphate decreases with increasing temperature, therefore, during thermal treatment, precipitation of calcium phosphate is induced, triggering the compaction of the casein micelles. Contrarily, the decrease in temperature induces an increase in the concentration of soluble calcium and phosphate at the expense of colloidal calcium phosphate, weakening the casein micelle; a point can be reached that leads to the dissociation of the caseins that make up the micelle (Walstra and Jenness, 1984). At low heating temperatures, changes in ionic balance are reversible, but if heating is intense, reversibility is slow and incomplete (Fox et al., 2015).

1.2.5. Dehydration by electric field

1.2.5.1. History

The phenomenon of electrohydrodynamic atomization or EHDA, commonly called electrospray, was observed by William Gilbert in 1600 (Gilbert et al., 1600). In 1750, Jean-Antoine Nollet demonstrated that water flowing into a container would aerosolize if the container was electrified and closed in contact with electrified ground (Dumont and Cole, 2013). A century later, Lord Kelvin designed a configuration consisting of two nozzles connected to opposing collection tanks and showed that differences in charge between the dripping water and the nozzles instantly caused differences in the kilovolt scale and electrospray of the nozzles (Smith, 2000). The first patent for the EHDA configuration appeared in 1900 by John F. Cooley (Xie et al., 2014). In 1914, Zeleny conducted an ethanol electrospray experiment by photographing a cone-jet (Zeleny, 1917). In the 1960s, Taylor developed a mathematical description of the EHDA process, defining the "Taylor cone" that simulates the conical shape of the liquid phase in the presence of the electric field (Taylor, 1966). In the 1980s, Fenn and his colleagues conducted a series of experiments that allowed the introduction of dissolved analytes by electrospray into the gas phase for mass analysis (Fenn, 1989).

1.2.5.2. Theoretical basis

EHDA (electro refers to electrical energy; hydrodynamic refers to fluid dynamics; and atomization refers to converting liquid into fine droplets) is a technique that generates very fine droplets with a mono-dispersed size from a liquid under the influence of an electrical field (Xie et al., 2014). The energy of an intense electric field, on the order of $kV \cdot m^{-1}$, is used to achieve the separation of microdroplets. The main properties that the fluid must meet to be atomized are to have a representative electrical conductivity and low surface tension. These properties are important to have an adequate control of the particle size and the electrical intensity that circulates in the capillary (Monar and Redrován, 2017).

The microdroplets formed become dehydrated by traveling through the electric field (Figure 2). When the drop leaves the needle it becomes electrically charged, causing the accumulation of charges on its surface. This causes the electrostatic repulsion of the charges to counteract the surface tension of the liquid, provoking the deformation of the drop surface. This deformation continues until a critical point is reached where the electrostatic repulsion exceeds the surface tension, causing the evaporation of the solvent (Huang et al., 2003; Anu and Anandharamakrishnan, 2014; Landa, 2017).

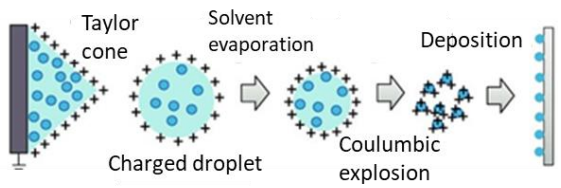

Figure 2. Electrospraying mechanism (Saallah and Lenggoro, 2018).

The degree of evaporation of the solvent will depend on many factors, among them, those intrinsic to the product and the instrumental ones. The type and concentration of the polymer and solvent used determine the properties of the solution, such as pH, conductivity, viscosity, and surface tension. Instrumental parameters include the applied electrical potential, the flow rate of the solution, the distance between the tip of the needle and the collector, and occasionally the nature of the collector material. In addition, the environmental conditions such as temperature, relative humidity and air speed in the process chamber affect the process (Anu and Anandharamakrishnan, 2014).

The basic EHDA setup consists of several major components: a syringe pump, a syringe, a metal needle that serves as a nozzle, a high-voltage power supply, and a grounded substrate that serves as a collector (Figure 3). Some EHDA configurations employ a closed chamber where the air/nitrogen flow transfers particles to the collecting filters. The use of the closed chamber reduces solvent evaporation and facilitates the formation of smaller particles with a smoother surface morphology (Xie et al., 2014; Anu and Anandharamakrishnan, 2014).

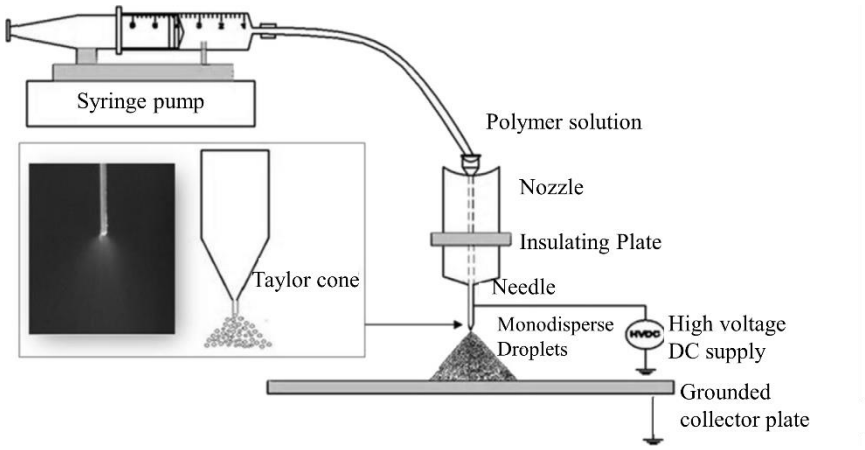

Figure 3. A typical electrospraying setup. Inset shows the digital image and representation of the electrospraying process (Anu and Anandharamakrishnan, 2014).

1.2.5.3. Applications

This technology is widely studied in the fields of tissue engineering and regenerative medicine, nanostructured sensors, textile industry, materials science and chemistry, (Landa, 2017). However, the use of this technology in food processing remains unexplored. The possible food applications, in the field of study, are encapsulation, enzyme immobilization, food coating and the development of materials for filtration and active food packaging (Anu and Anandharamakrishnan, 2014). So far, only one study carried out in 2010 on the formation of chocolate fibers by electrospinning has shown that technique modifies texture and mouthfeel (Luo et al., 2011).

1.2.5.4. Potential advantages and disadvantages compared to heat treatments

The possible advantages that this technology could offer would be linked to the negative effects that heat treatment produces in the industrial methods mentioned in section 1.2.4. One of the great advantages provided by the EHDA technique is the decrease in denaturation of proteins and an increase in the porosity of the product (Anu and Anandharamakrishnan,

2014). As the effect on proteins would be reduced, one might think that the effect on the other components would be also less, but the lack of studies in this field does not allow us to confirm this.

Other potential advantages provided by this method could be the reduction in processing costs. This decrease would be given by the processing itself since there is no need to apply heat, compared to the current spraying where the air and the product reach at the beginning and the end of the operation 270 and 80 °C, respectively.

One of the possible disadvantages is that the EHDA equipment is not designed to process large volumes at an industrial level, although scalability seems very feasible. In 2019, the Bioinicia company (Valencia, Spain) announced the construction of a warehouse with a novel technique that combines high voltage and pneumatic pressure to obtain 1-3 $kg \cdot h^{-1}$ of dry product. It should be noted that it is specifically designed for encapsulation in many applications areas such as pharmaceuticals cosmetics, functional foods and nutraceuticals (Bioval, 2019).

One of the greatest drawbacks that could be found using this method would be in terms of hygiene and safety, due to the absence of a specific heat treatment. At a technical level, the closest treatment is high voltage electrical pulses (PEF), where electroporation of the membrane occurs, inactivating the bacteria present (Niu et al., 2020). A bactericidal effect of EHDA might be expected due to a similar principle but with two important differences. Firstly, PEF uses high voltage pulses ranging from 20 to 80 $kV \cdot cm^{-1}$, ~100 times higher than that applied by electrospraying. Secondly, the pulses are applied during a very short time (1-10 μs), while electrospraying is not pulsed. Thus, microbiological studies should be performed if electrospraying demonstrates useful performance as drying method. In the case of lacking a bactericidal effect on microorganisms, EHDA could be combined with other technologies, such as the application of ultraviolet light to obtain the appropriate degree of hygenization. Eventually, heat treatment to ensure adequate milk safety may not be eliminated from the overall process.

2. MATERIAL AND METHODS

The experimental study was carried out at the "Departament de Ciència Animal i dels Aliments" and the "Centre Nacional de Microelectrònica" (IMB-CNM-CSIC) at the Universitat Autònoma de Barcelona, between the months of February and October 2020.

2.1. Electrospray equipment and setup

The equipment used was the Fluidnatek LE-10 (Bioinicia, Valencia, Spain) (Figure 4.a). This equipment is located in the clean room of the CNM, where there is controlled atmosphere (positive pressure with respect to atmospheric pressure, relative humidity at 45% and the temperature at 20 °C). The equipment chamber is practically closed, having air tubes on two opposite sides and a small fan installed to ensure that the air is renewed and to avoid the accumulation of solvent vapor. The relative humidity is not controlled but is kept at that of the clean room (45%) through the renewal of the air in the chamber. To assemble the main components of the equipment, the manual and parts supplied by the manufacturer were used. The equipment was modified to incorporate the NE-4000 pump (New Era Pump Systems Inc., Farmingdale, United States) (Figure 4.b). There are several configurations for the ejection tip, depending on the liquid inputs and the number of tips. For the input of one or two liquids, the configuration would be Single or Coaxial Nozzle, respectively. Depending on the number of tips, you could have a Single-Phase Nozzle (1 tip) or Single-Phase 5-Nozzle (5 tips).

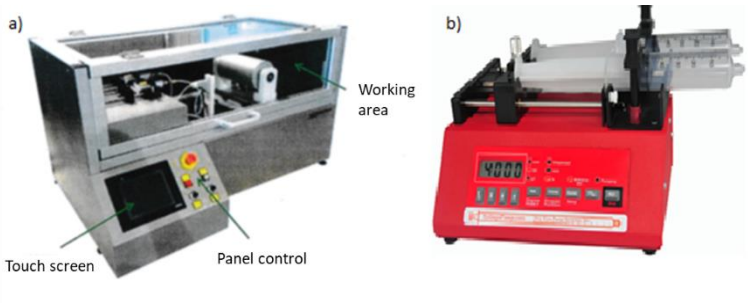

Figure 4. a) Fluidnatek Le-10; b) Pump NE-4000

Single-Phase Nozzle configuration was used. Firstly, a polytetrafluoroethylene pipe (or PTFE pipe) was introduced into the plastic raccord (1/16" OD: outside diameter) and fixed with a gasket (Figure 5.a). Then the plastic raccord was screwed into the aluminum part of the noddle with the blue gasket in between. Then, the capillary tube (1/16" OD with 40.9 cm

length) was inserted into the sleeve blue gasket (Figure 5.b). Finally, the stainless-steel raccord was screwed in the aluminum part of the nozzle, with a high voltage cable connected to the positive socket. The other end of the capillary tube was connected to a plastic needle tip.

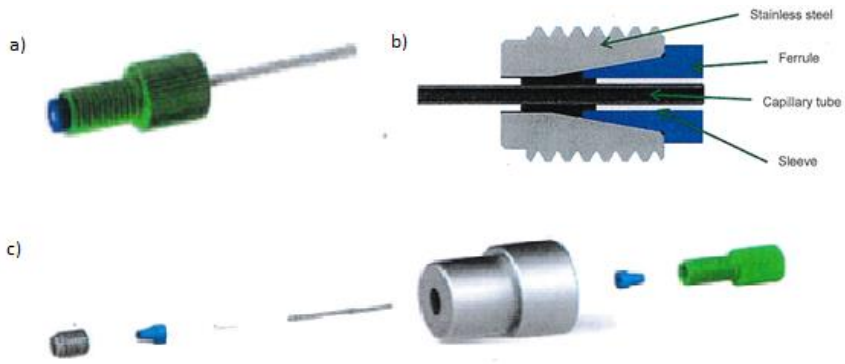

Figure 5. a) Raccord, ferrule and pipe assembling; b) Assembling of capillary tube; c) Exploded view of the Fluidnatek Single-Phase Nozzle.

In order to collect the processed product, a 200 × 200 mm stainless steel collector was used. This collector needed to be connected to the high voltage cable of the negative plug and to one of the set screws (Figure 6).

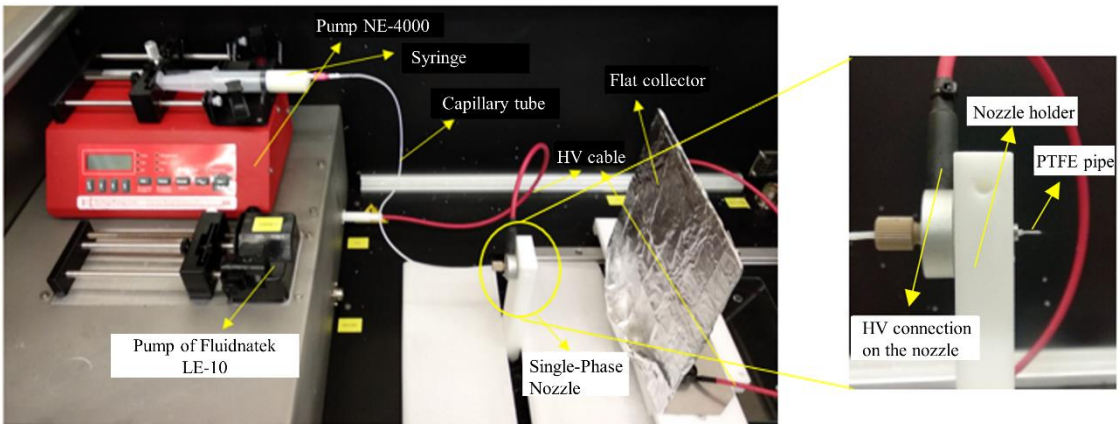

Figure 6. Assembling overview

During the initial tests, once the equipment was assembled, several problems arose that needed to be solved in order to successfully carry out the experiment without damaging the equipment.

The first problem was the appearance of electric discharge arcs when the equipment was turned on, because electricity prioritizes the path with low electrical resistance. In addition, it must be added that milk has a very low conductivity as compared to polymer solutions typically used for production of fiber or particles using electrospraying. This problem was solved by cleaning with Milli-Q water and drying every time before starting sample processing. At the beginning a steel tip was used and it constantly generated electric discharge arcs. Insulating material was used as a first approach to solve this problem but it did not prevent the formation of electric discharge arcs. So, the stainless-steel tip was replaced be a plastic one. Finally, electric discharge arcs were generated when using very small syringes (<12 ml), because the tip of the syringe was very close to the metallic areas of the equipment. Two types of syringes of 20 and 60 mL, having a diameter of 19.5 and 24.6 mm, respectively, were used.

The generation of electric arcs produced the following effects: the plastic parts through which the arc passed burned, it prevented the electric field from reaching the required voltage and, when it was generated at the injection tip, it caused an increase in temperature that dehydrated the milk, sealing the metal tip.

At the end of the experiment it was observed that it was very important to use the same syringes manufacturers since the pressure needed to move the plunger was very different depending on the brand and not so much on the volume.

2.2. Experimental design

In order to assess the potential of EHDA in obtaining milk powder, the main treatment variables were screened: the total solids of the reconstituted skim milk and the variables of the equipment used.

The concentration of milk solids was arbitrarily established as 12, 24 and 36%, to simulate milk and concentrated milk with concentration factor of 2 and 3, respectively. The analyses of their density, conductivity and stability allowed to detect that the 36% milk was unstable (see section 3.2.2.) and was replaced by 30% of total solids.

Regarding the equipment used, there were 3 adjustable variables: the distance between the injection tip and the collector (nozzle-collector; cm), the applied voltage (kV) and the flow rate ($mL \cdot min^{-1}$). In order to decide which conditions were the optimal for the experiment,

preliminary tests were carried out keeping the distance between the tip and the collector fixed at 10 cm. Regarding the applied voltage, the range given by the equipment was from 1 to 30 kV. During the initial tests, it was qualitatively observed that the higher the voltage, the ejected liquid came out with a more perpendicular angle towards the collecting plate and the more water removal was achieved. So, the standard voltage was set at 29 kV because 30 kV, which is the upper limit of the equipment, triggered some problems. Finally, for the flow rate, the initial tests started using very low flows around a few $\mu L \cdot h^{-1}$, but the fluidic resistance of milk provoked a percussion problem that blocked the flow. Due to this problem, the setup was modified, incorporating a pump with a higher flow rate, which was able to reach 10 $mL \cdot min^{-1}$. In addition to higher flow rates, larger syringes could be used, allowing an increase in sample throughput. A full screening (see **Annex**) was performed to select the optimal flow rates based on the study of the droplet size and the chemical elements of the obtained products. It started with a flow rate of 5 $mL \cdot min^{-1}$ and decreased to 0.2 $mL \cdot min^{-1}$ because no droplets were obtained. Subsequently, the flow rate was decrease to 0.005 $mL \cdot min^{-1}$ observing differences in the droplet size. But this flow rate was not selected because not enough product was obtained to perform the analyses. The selected flow rates at the end of the screening were 0.2 and 0.01 $mL \cdot min^{-1}$.

Once the initial tests had been completed, a non-randomized factorial experimental design was made with three milk reconstitution levels (12, 24 and 30%) and the two selected flow rates (0.2 and 0.01 $mL \cdot min^{-1}$). The field intensity and the distance between the tip and the collector were the same throughout the study to be able to observe the effect of the two studied factors on protein denaturation and total solids determination. The experiment was replicated twice, performing a total of 12 trials ($N = n \cdot a \cdot b = 2 \cdot 3 \cdot 2 = 12$).

2.3. Skim milk powder

For the study, commercial skim milk powder dehydrated by spray drying was used. It is a standard milk with a high microbiological and functional quality distributed by LACTALIS Ingredients (Bourgbarré, France). It has a WPNI ≥ 7, so it belongs to the “low heat” category. All the milk used came from the same 25 -kg bag.

The reconstitution of the milk was carried out individually with Milli-Q water (resistivity: 18 MΩ·cm) at 20 °C and the amount of added milk powder was adjusted to reconstitute it at 12,

24, 30 and 36% (*w*/*w*). Weighing of the milk powder and the water was done with an analytical balance (FV-220C, Gram Group, Barcelona, Spain). The reconstitution process was based on three stages: thirty minutes of stirring, thirty at undisturbed darkness, and five of sonication to eliminate small air bubbles that could affect the correct operation of the equipment. Leaving it undisturbed at darkness is necessary for the correct hydration and stabilization of the milk components, especially the casein micelles, and the darkness is needed to prevent the photooxidation. The beaker where the milk was reconstituted was kept closed, to avoid the evaporation of water due to the difference in relative humidity between the sample and the environment, minimizing the concentration of solids and evaporative cooling on the sample surface.

2.4. Analysis Methods

2.4.1. Density and conductivity

The density of reconstituted milk samples was estimated in triplicates with the help of an analytical balance (FV-220C, Gram Group, Barcelona, Spain) and volumetric flasks 25.00 ± 0.03 mL. Density (ρ) was calculated from weigh (w) and volume (v) as, $\rho = \frac{w}{v}$.

The conductivity of milk samples was measured in triplicates with a conductimeter (Sension+ 340 MM340, Hach Company, Loveland, United States).

2.4.2. Stability analysis

The stability analysis was performed with the Tubiscan (Lab expert, Formulaction, Toulouse, France), and the software Turbisoft Lab (version 2.3, Formulaction).

Turbiscan vials were filled up to 40 mm with a Pasteur pipette and the analysis was carried out a temperature of 20 °C. Two replicates were made for each sample.

Emulsion stability was characterized using the Turbiscan Stability Index (TSI), calculated as follows (Wang et al., 2018):

$$TSI = \sum_i \frac{\sum_h |scan_i(h) - scan_{i-1}(h)|}{H}$$

where $scan_i$ (h) is the light intensity of the *i*-th scan at a height of *h*, and *H* is the total height of the measured sample.

2.4.3. Reflected optical microscopy and particle size

A reflected optical microscope with visible light in transmission operation was used to carry out the droplet size analysis (Leica DM2700M, Leica Microsystems, Wetzlar, Germany). It has a built-in video camera (Moticam 10+ SB-09, Motic, Hong Kong, China). The image software used was Motic Image plus Glass slides of3.0. 20 × 20 × 1 mm and 22 mm radius spherical polished stainless-steel plates were used. Images were taken using a 2.5X ocular in order to observe the droplets. Four photos were taken for each glass slide: in the center and 2 cm to the right, left and top. For polished stainless-steel plates no picture was taken at the center. When the glass slides were being used to collect electrosprayed samples, the flow was very high, causing great accumulation and the formation of a big drop that dragged any other droplet down, thus images could not be taken in the lower section of the slide. This effect was reduced, but in some cases was still observed, by lowering the flow rate, which coincided with the change to polished stainless-steel discs, that will be explained in the next section.

All images were taken the day after production and later were analyzed using Image J (National Institutes of Health, latest version December 10, 2019).

2.4.4. Scanning electron microscopy and X-ray energy scattering spectrometry (SEM-EDX)

After being observed under the reflected optical microscope, the samples were completely dried in a desiccator connected to the vacuum line for thirty minutes. The samples were analyzed with Scanning Electron Microscope with Energy-dispersive X-ray spectroscopy (Auriga-40, Zeiss, Germany). The use of glass slides, being a non-conductive substrate, implied that only 10 keV could be used during the analysis. The substrate was changed to polished stainless-steel, to increase the energy to 20 keV, which improved the analysis. The focal distance between the milk samples and the electron beam was set at 3 mm. The analysis of the elements was carried out with the help of the SmartSEM software (Zeiss), and the sample elements observed were carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, potassium and calcium, as expected.

2.4.5. Total solids

To carry out the analysis, the sample was taken directly in weighed aluminum foil previously dehydrated for 2 h at 105 °C in a convection oven (UFB 400, Memmerk GmbH, Schwabach, Germany). As the flow rate used to electrospray the samples was very different, the time required to take enough sample was adjusted to 20 and 180 min, for the flow rates 0.2 and 0.01 mL·min^{-1}, respectively. The sample was stored in an airtight container and dehydrated at 105 °C for 24 h. The moisture content of the sample was calculated by the difference between the initial and final weight of the sample, from which the total solids were obtained. From the total solids, the Q factor, yield, wet yield and energy consumption indices were calculated.

Q factor is the ratio between the dry matter content of the concentrated product and that in the original material (Walstra et al., 2006). Q factor (Q) was calculated form initial total solids (TS_i, in %) and final total solids (TS_f, in %) as, $Q = \frac{TS_f}{TS_i}$.

The yield expressed as g of obtained total solids per 100 g of milk processed was calculated from the weight of the sample after drying it in a convection oven (w, in g) and the weight of processed milk (w0, in g) as, $Dry\ yield = \frac{100w}{w_0}$.

The wet yield expressed as g of obtained product per min of processing was calculated from the weight of the obtained product (w, in g) and the time of electrospraying process (t, in min) as, $Wet\ yield = \frac{w}{t}$.

The energy consumption was calculated using the formula:

$$E = tP\ /\ (Wa_0 - Wa_f)$$

Where E is energy consumption (kJ·g^{-1}), Wa_0 and Wa_f are the initial and final water content of the product (g), t is the time (s) and P is the maximum power of the equipment (kW).

2.4.6. SDS-PAGE

Both qualitative and quantitative analysis of the main proteins of the samples were carried out by SDS-PAGE analysis under reducing and non-reducing conditions using separating and stacking gels containing 14% or 4% acrylamide, respectively. The reducing conditions were achieved by the addition of a reducing reagent, β-mercaptoethanol, that breaks disulfide bridges. Reduced and non-reduced samples were mixed twice their volume with double-

strength buffer and centrifuged (Laemmli, 1970). Supernatants containing the protein without fat, were diluted with the same buffer to have a dilution 10^{-1} and 15 µL was loaded onto the gels. BSA (10 µg) and a mix of casein, α-la and β-lg (10 µg) were used as a standard and were run together with the samples. Gels were run at 200 V, stained using 0.1% (*w*/*v*) Coomassie Brillant Blue R250 in a 16:1:50 mixtures of methanol, acetic acid and distilled water. Distained gels were scanned with CanonScan (Lide 220, from Canon, Japon) and analyzed with GelAnalyzer software (version 19.1, created by Istvan Lazar Jr. and Istvan Lazar Sr.). The amount of sample was determined to obtain bands large enough for the whey proteins without reaching the total saturation of the casein bands, because the percentage of caseins is much higher than that of whey proteins.

2.4.7. Statistical analysis

Obtained data were processed and analysed using the Statgraphics Centurion software (version 18.1.13, Statgraphics Technologies Inc., The Plains, Virginia, United States).

The analysis of variance (ANOVA) is based on the comparison of variability of the dependent variables under study between and within different groups and allows to attribute the variability of said variables to the effect of certain explanatory variables (factors or effects). The ANOVA was performed using the simple Anova procedure, joining the two factors (total solids and flow rate) as one. LSD test was used for comparison of sample data. Evaluations were based on a significance level of $p < 0.05$.

Multiple linear regression (MLR) allows generating a linear model in which the value of the dependent variable or response is determined from a set of independent variables called predictors. The MLR was performed using multiple linear regression procedure. Analysis was carried out to estimate the value of total solids based on the two factors of the experiment (total solids and flow rate).

Pearson's correlation coefficients, which is an index that measures the degree of covariation between different linearly related quantitative variables, were determined using the multivariate Analysis procedure (correlations). The analysis was performed for all the dependent variables related to the efficiency of the treatment: total solids, yield and diameter of droplets.

The Shapiro-Wilk test was performed to determine if the droplet size distribution can be associated with a normal distribution with 95% confidence.

3. RESULTS

3.1. Preliminary flow rate screening at constant voltage

3.1.1. Droplet size

No significant correlation between the flow rate and the droplet size was observed when performing the screening, electrospraying at 29 kV and different flow rates skim milk powder reconstituted at 24% (*w/w*) (Figure 7a), although a certain trend could be observed. About 90% of the droplets had a size between 50 and 500 µm (Figure 7b). The pattern of the droplet size distribution was repeated in almost all individual analyses, irrespectively of the flow rate used. The Shapiro-Wilk test gave a *p*-value = $9.4 \cdot 10^{-16}$, confirming the normality of the data. Furthermore, the obtained data showed polydispersity, as observed by optical microscopy (Figure 8), since the sizes of the drops ranged from 50 to 700 µm. But previous studies have shown that when the electrospray conditions are kept constant, the droplet size is monodisperse, finding most droplets in a certain range (Anu and Anandharamakrishnan, 2014; Hernández, 2018). However, there were no relevant differences in mean droplet size due to small changes in the flow rate, e.g. from 0.005 to 0.01 $mL \cdot min^{-1}$, which is consistent with the bibliography.

Observing these results, it was possible to choose two flow rates. The flow rate of 0.2 $mL \cdot min^{-1}$ was chosen because it was the highest allowed by the device and showed a trend toward higher droplet diameters. The second level of flow rate selected was 0.01 $mL \cdot min^{-1}$, which generated smaller droplet size than 0.2 $mL \cdot min^{-1}$ and enough product was obtained in a reasonable time.

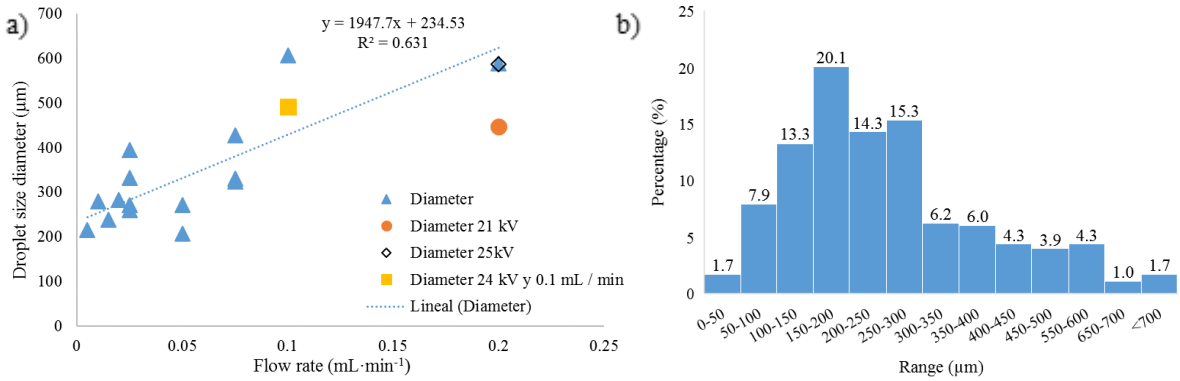

Figure 7. a) Relationship between droplet size and flow rate when processing skim milk reconstituted at 24%; b) Droplet size histogram of the entire screening for milk electrosprayed at 29 kV.

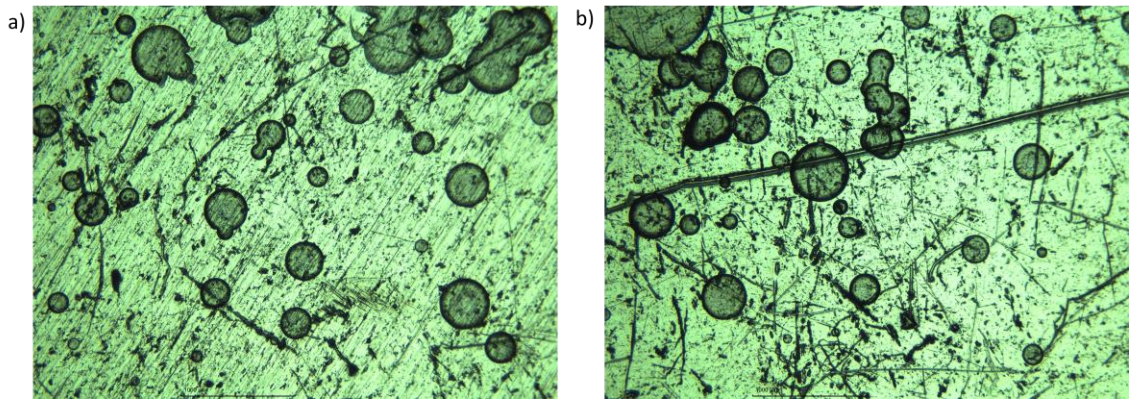

Figure 8. Optical microscopy images with ocular of 2.5X. Skim milk reconstituted at 24% electrosprayed at 29 kV and a flow rate of: a) 0.02 mL·min^{-1} and b) 0.015 mL·min^{-1}.

3.1.2. SEM-EDX

One of the big doubts at the beginning of the screening was whether the milk was being processed, since a separation of the milk solids and water components could occur, when applying an electric field to generate the electrospraying, hence, processing only the water. Another effect that could be expected was that the sample would "burn", during SEM measurement, due to the composition of the milk.

At first, the sample was taken on a glass slide, but the use of a non-conductive substrate did not allow the EDX analysis to be carried out in optimal conditions. It could only be raised up to 10 keV without burning the sample during the process, provoking the absence of spectra of elements such as calcium. For this reason, the glass slides were changed to polished stainless-steel, which allowed to rise the analysis to 20 keV and thus to observe the classic minerals of

milk such as calcium and phosphorus by EDX. In addition, having a conductive substrate, which allowed using 20 keV, made possible to obtain SEM images with higher resolution.

The obtained profile of elements was compared with that obtained by Guerrero (2017), confirming that a typical profile of milk was observed (Figure 9). The elements that are observed using this technique are those that have an atomic number greater than 3. Milk is composed mainly of water and organic compounds (proteins, sugars and fat) formed by hydrogen, carbon, oxygen and nitrogen, and minor minerals, highlighting calcium, phosphorus, magnesium and sulfur, as the most abundant. The appearance of the elements such as iron and chromium were due to the use of stainless-steel, so its presence in all spectra was expected.

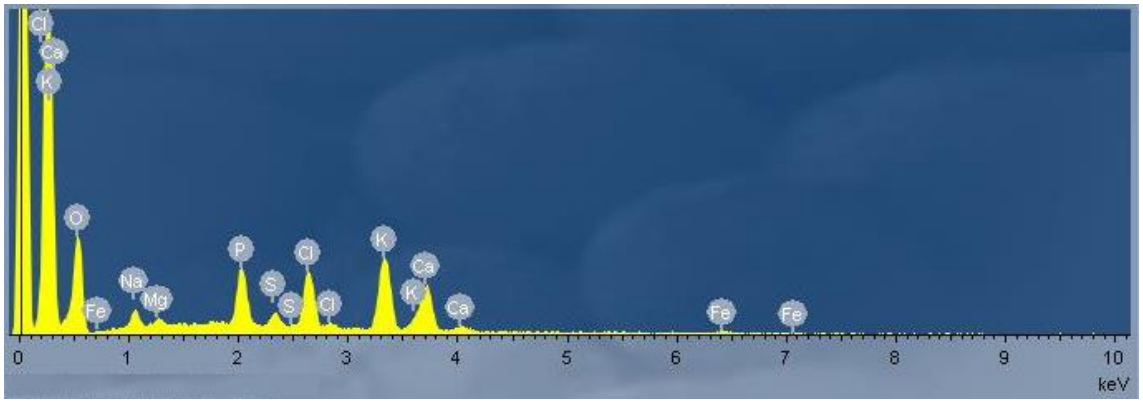

Figure 9. EDX spectrum at 3 mm and 20 KeV, from skim milk reconstituted at 24% and electrosprayed at 29 kV and a flow rate of 0.01 mL·min^{-1}.

With the results of this study, it was confirmed that the product atomic distribution was that of milk and that destabilization and burning of milk did not take place during electrospraying process. As the samples had to be completely evaporated using vacuum to perform the EDX-SEM analysis (SEM is performed under vacuum), this technique was not useful to evaluate the degree of water extraction by comparing oxygen between samples.

The Ca:P ratio is a good indicator of the heat treatment of milk because high temperatures shift the balance from soluble calcium phosphate to colloidal calcium phosphate, which is insoluble (Ramírez, 2009). No differences in the ratio were observed among the different flow rates analyzed (Figure 10), confirming that small changes in the flow rate of the treatment had no effect on the balance of these minerals. The average Ca:P ratio obtained (~0.778) was smaller than expected for whole milk (~0.9) (Walstra et al., 2006). The difference is very

small and can be associated with the displacement in the equilibrium of soluble Ca:P to insoluble Ca:P due to the loss of water and the heat treatment. More likely, even though using a low-heat skim milk powder, the balance of these minerals may have been affected by the heat treatment and the extraction of water.

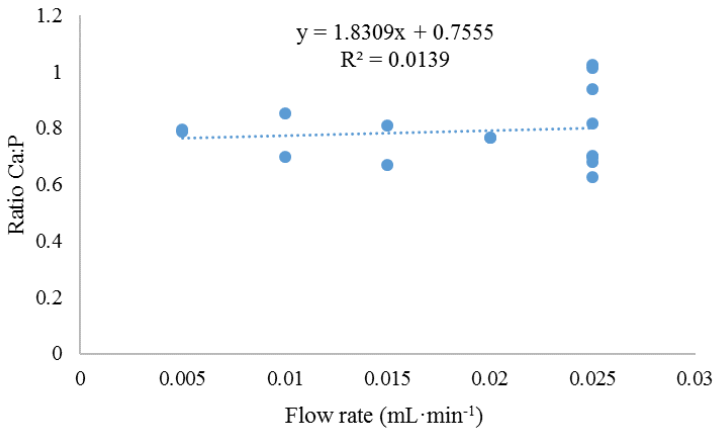

Figure 10. Ca:P ratio in milk spray dried at different flow rates.

3.2. Characterization of skim milk

3.2.1. Density and conductivity

When using capillary tubes with a very small diameter (1/16" OD), it must be verified that the liquid to be treated does not have a very high density that would not allow the flow of liquid through the capillary tube and PTFE pipe, thus collapsing the system by high fluidic resistance due to solution increased viscosity.

The density of the milk increases when the concentration of the solids-not-fat increases and decreases when the fat content increases (Walstra et al., 2001). In this preliminary phase, the skim milk with higher concentration of dry solids was set to skim milk reconstituted at 36% (*w*/*w*). Thus, it was necessary to verify that the increase in density (and associated viscosity) due to the increase in concentration did not lead to the problem mentioned above. The density can be estimated with Eqn. 1, where D, F and ρ^{20} are the dry-matter, fat content of the milk and density, respectively (Walstra et al., 2001).

$$D = 1.23F + \frac{260(\rho^{20} - 998)}{\rho^{20}} \qquad \text{Eqn. 1}$$

In Figure 11, the experimental density of the reconstituted milk is compared with the theoretical density estimated with the previous equation. The differences observed between theoretical and experimental values were quite small. The obtained results show that increasing the reconstitution concentration from 12 to 36% increased the density only by ~0.11 g·mL^{-1}.

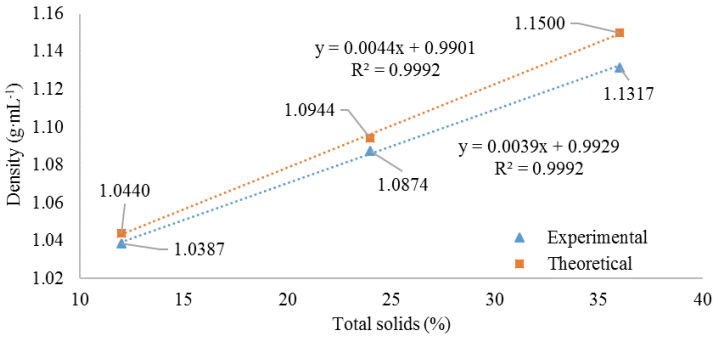

Figure 11. Experimental and theoretical density

Electrical conductivity is an important parameter for the electrospraying technique. The electrical conductivity must be high enough to allow the free charges found inside the droplet to migrate to the liquid-air interface (Rebollo, 2015). The phenomena of deformation by the action of the electric field are mainly due to the electrification of the liquid drops (Marginean et al., 2004). As a result, electrical conductivity has a direct effect on droplet diameter induce by electrospraying, with higher electrical conductivity producing a smaller droplet size (Fernandez, 2007; Hernández, 2018).

In Figure 12, the experimentally electrical conductivity of milks, measured with conductimeter, can be observed. Walstra et al. (2001) and Fox et al. (2015) highlighted that the conductivity of milk is approximately 0.5 S·m^{-1} at 25 °C. This value is similar to that obtained for 12% milk, which is the only conductivity that could be compared with the literature. But it must be taken into consideration that skim milk powder was reconstituted with Milli-Q water, while the referred conductivity value corresponds to raw cow's whole milk. Fat always tends to decrease electrical conductivity and Milli-Q water has a conductivity much lower than distilled or tap water. These effects may explain the variation in electrical conductivity. It increases with increasing concentration due to the concomitant increase in mineral concentration, such as ionic calcium and sodium, among others. A linear

increase would be expectable, but there comes a point where the increase in calcium was compensated by an increase in colloidal calcium phosphate (i.e., extra ionic calcium becomes insoluble in the form of colloidal calcium phosphate), producing a loss of linearity (Walstra et al., 2006). Likely as a result, according to Henningsson et al. (2005), conductivity of milk at constant temperature is proportional to milk concentration, but fitting a power law, as it can be observed in our experimental results (Figure 12).

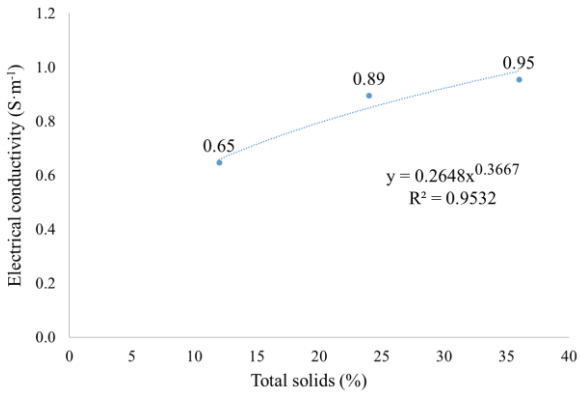

Figure 12. Experimental electrical conductivity

When a potential difference is applied between the nozzle and the collector, the liquid become unstable and evolves from a rounded shape to a conical structure called a Taylor cone (Hernández, 2018). Milk is not highly conductive but is considered a sufficiently conductive liquid due to its electrolytes, it can be used as a solution for the electrospraying technique.

3.2.2. Stability

During the density and conductivity analyzes, it was observed that the 36% milk presented precipitates. After leaving the samples at 4 °C for 24 h, precipitation increased. Since the processing time to obtain samples was estimated of 3 hours for the samples with the lowest flow rate, the appearance of precipitate in a shorter time was an inconvenience.

If used, the processed milk would have presented a variation in concentration during processing due to the formation of precipitate in the syringe of the equipment. Furthermore, with the flow rate of 0.01 $mL \cdot min^{-1}$, there was a risk of precipitation in the capillary tube, blocking milk flow. And if the precipitate would form in the PTFE pipe, where the electric field is generated, there was a risk of burning the product and damage the equipment.

Stability analyzes were performed for milk reconstituted at 12, 24 and 36%. Table 1 shows the different Turbiscan Stability Indexes (TSI), which measures the destabilization kinetics at a certain time (Wang et al., 2018) and can be measured globally or in different areas of the Turbiscan® vial (low, medium and high). In the lower zone, it was observed that after one hour of analysis there were significant differences between the concentration of 36% compared to the other two concentrations. In all milk samples evaluated, a very slight increase in turbidity could be seen in the lower part of the vials, mainly due to gravity, since they were kept undisturbed during the whole analysis ($0 < TSI < 0.2$). For 36% milk, the sharp TSI increase showed the reconstitution process was not suitable for such a high concentration.

Table 1. Average bottom Turbiscan Stability Indexes (TSI) for skim milk powder reconstituted at 12, 24 and 36% (*w*/*w*).

Reconstituted skim milk (%)	TSI (5 min) (a.u.·cm^{-1})	TSI (10 min) (a.u.·cm^{-1})	TSI (20 min) (a.u.·cm^{-1})
12	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.05 ± 0.07^{b}
24	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
36	0.20 ± 0.00^{a}	0.40 ± 0.00^{a}	0.85 ± 0.07^{a}

Number of replicates, n = 2; Number of observations, N = 6; Mean value ± s.d.; $^{a\text{-}d}$ values per column without common superscripts were significantly different ($p < 0.05$).; Values without common superscripts were significantly different ($p < 0.05$).

In an additional test where stability was measured, simultaneously, with Turbiscan and by direct eye observation, it was observed that when the increase in light backscattering (absorbance, ABS) of the lower zone was greater than 3-4%, precipitation could be observed in the vial. In the experiment, measurements were taken every minute to have the light backscatter profile. Five minutes after starting the analysis, the ABS value for 36% milk in the lower part already exceeded the ABS values of 12 and 24% milks at 4 h. After 10 min, the ABS reached more than 5% and the outer vial presented precipitation (data not shown).

With these results, the 36% milk was discarded with the reconstitution procedure that was being carried out. Subsequently, stability tests were carried out modifying the reconstitution process. If the shaking was carried out at a temperature of 45 °C for 1 h, rather than room temperature for 30 min, and resting under darkness also increased to 1 h, the profile obtained for 36% was very similar to 12 or 24% milk (data not shown). But this meant changing the

reconstitution process in the middle of the screening, so stability tests were performed with 30% milk without modifying the reconstitution process. The 30% milk presented a stability very similar to the concentrations of 12 and 24% (data not shown), and as a result, it was decided to reconstitute skim milk powder at 30% instead of 36%.

3.3. Final product characterization

3.3.1. Milk electrospraying performance: water removal, concentration factor, yield and energy consumption

In Table 2, the analysis of variance of factors related to the electrospraying dehydration efficiency can be observed. For the total solids, significant differences ($p < 0.05$) were observed between the samples, except for 24 and 30% of milk samples at flow rate of 0.2 $mL \cdot min^{-1}$. At flow rate of 0.2 $mL \cdot min^{-1}$, total solids did not vary compared with the initial total solids of the milk. Thus, no water extraction was obtained with a flow rate of 0.2 $mL \cdot min^{-1}$. However, when lowering the flow rate to 0.01 $mL \cdot min^{-1}$, there was a significant ($p < 0.05$) water extraction.

Table 2. Total solids, Q factor and droplet diameter of electrosprayed milk samples reconstituted at 12, 24, and 30% processed at two different flow rates.

Flow rate ($mL \cdot min^{-1}$)	Milk reconstitution (%)	Total solids (g TS/100 g product)	Q factor[1] (dimensionless)	Droplet diameter (µm)
0.20	12	12.32 ± 0.06^{e}	1.055 ± 0.007^{c}	377 ± 74^{a}
	24	23.55 ± 0.78^{d}	1.015 ± 0.035^{c}	$269 \pm 64^{a,b}$
	30	28.94 ± 0.30^{d}	0.995 ± 0.007^{c}	156 ± 155^{b}
0.01	12	36.77 ± 4.62^{c}	3.160 ± 0.396^{a}	$283 \pm 161^{a,b}$
	24	49.66 ± 4.55^{b}	2.130 ± 0.198^{b}	169 ± 60^{b}
	30	61.88 ± 1.93^{a}	2.125 ± 0.064^{b}	148 ± 39^{b}

Number of replicates, n = 2; Number of observations, N = 12; Mean value ± s.d.; $^{a-d}$ values per column without common superscripts were significantly different ($p < 0.05$). [1] Ratio between the dry matter content of the concentrated product and that in the original milk (Walstra et al., 2006).

It was observed that both the flow rate and the initial concentration influenced the total solids of the final electrosprayed milk. The following model, obtained from the multiple regression analysis, is proposed to estimate the final total solids for the flow rate of 0.01$mL \cdot min^{-1}$:

$$TS_f\,(\%) = 25.882 + 1.137 \cdot TS_i + 146.474 \cdot \dot{V} \qquad \text{Eqn.2}$$

where TS_f, TS_i and $\dot{V}$ are final and initial total solids (%) and volumetric flow rate ($mL \cdot min^{-1}$), respectively. The model is significant ($p < 0.05$) and presents a high determination coefficient, $R^2 = 97.09$, explaining 97% of total solids variation observed in the final product.

Observing the amount of total solids, the product could not be defined as powdered milk since the maximum humidity should be around 5% and the lowest moisture content value obtained was ~38%. Thus, the current conditions allowed to obtain concentrated skim milk. Using eqn. 2, and assuming that linearity is maintained, the initial solids concentration required to obtain a maximum humidity of 5%, by electrospraying at flow rate of 0.01 $mL \cdot min^{-1}$, could be estimated to be ~58%. Additionally, if milk with 12% solids is used, it is estimated that the volumetric flow rate required for a final product with a maximum moisture content of 5% is 0.38 $mL \cdot min^{-1}$. This result would be difficult to achieve with the current equipment design, since its maximum voltage is not enough, it has a horizontal configuration and a low distance between nozzle and collector. Industrial equipment that combines a higher electric field, a vertical nozzle configuration that takes advantage of gravity, and a greater distance between the nozzle and the collector could counteract this limitation.

Since no water extraction occurred at a flow of 0.2 $mL \cdot min^{-1}$, the Q factor at this flow rate was ~1 regardless of the initial concentration. However, for the flow rate of 0.01 $mL \cdot min^{-1}$, the Q factors were 3, 2 and 2 for the milk reconstituted at 12, 24 and 30%, respectively (Table 2). The Q LSM value for milk reconstituted at 12% was significantly higher than those reconstituted at 24 and 30% milk. This inversely proportional relationship was explained because the increase in concentration affects milk properties such as viscosity, electrical conductivity and surface tension of the liquid, which have a direct effect on the water extraction, and will be further elaborated along with the droplet size.

The extraction of water could be due to many factors that act simultaneously either during the electrospraying or, less probable, at the deposited product. During the electrospraying process, when the water molecules are ionized with enough electrical energy to overcome the surface tension of the droplet, the removal of the solvent occurred.

Once the product is deposited on the plate collector, dehydration could be due to:

- The difference in humidity between the environment (~45%) and the sample. This may cause evaporative cooling on the surface of the sample if samples reach the collector with water activity higher than 0.45. Note that, in any case, this is a surface phenomenon at the temperature and pressure used during electrospraying. As a result, water activity testing is recommendable in future experiments.
- The presence of a constant electric field over time due to the potential difference established between the tip and the plate collector. It must be borne in mind that, in order to collect enough sample for all the analyses, the obtained product stayed for a longer time on the collector when a flow rate of 0.01 mL min^{-1} was used (3 h as compared to 20 min for rates of 0.2 mL min^{-1}). However, it was noticeable that the final product obtained with the flow rate of 0.01 mL·min^{-1} at the time of depositing on the collector, showed a high viscosity compared with that obtained at 0.2 mL·min^{-1}, which undoubtedly suggest that a significant water extraction occurred before the product reached the collector. Additionally, since with a flow rate of 0.2 mL·min^{-1} almost no extraction of water occurred, it can be hypothesized that evaporation effect on the collector was likely minimal. Thus, a future experiment keeping the duration of electrospraying constant will be needed to confirm the hypothesis.

The decrease in flow rate might have favored an increase in the electrical load on the droplet formed, which would facilitate the extraction of water (Santos et al., 2017). This is due to the fact that the volume treated will be lower at the electrospraying effective section. The drops formed will be subjected to a greater electric field gradient, since a constant electrical energy is applied. The initial droplet charge can be calculated, if charges are uniformly distributed (Zhou and Biswas, 2020) as follows:

$$q_{d0} = 1.36(\rho\varepsilon_0 Q^2)^{1/2} \qquad \text{Eqn.3}$$

where ρ is density of the atomized liquid, Q is flow rate and $\mathcal{E}_0$ is the permittivity of vacuum.

Regarding the diameter of the droplets, it was observed that there were significant differences ($p < 0.05$) depending on the flow rate and the initial total solids (Table 2). According to Hernández (2018), the increase in the initial concentration of total solids triggers an increase of viscosity and superficial tension, which favors the formation of smaller droplets.

In spray drying the size of the droplets depends on the equipment used. Walstra et al. (2006) estimated the average diameter of droplets by atomization from various parameters. Eqn. 4 and 5 are designed for spinning disk and nozzle atomization, respectively, which are the typical configurations for spray drying.

$$d_{vs} = constant\ (Q\eta/\rho N^2 R)^{0.25} \quad \text{Eqn. 4}$$

$$d_{vs} = constant\ (Q\eta/p)^{0.33} \quad \text{Eqn. 5}$$

where Q is feed capacity, η is viscosity and ρ is density of the atomized liquid, N is number of revolutions per second of the disk, and R is disk diameter (disk atomization), and p is the pressure in the liquid before the pressure nozzle (nozzle atomization).

In electric field-induced atomization, the size of the droplets can be estimated with eqn. 6 (Zhou and Biswas, 2020):

$$D_{d0} = (\rho\varepsilon_0 Q^3/K\gamma)^{1/6} \quad \text{Eqn. 6}$$

where γ is surface tension, K is electrical conductivity, ε_0 is the permittivity of vacuum. The only similarity between the two models is that the increase in volumetric flow rate, which in the case of electrospraying is elevated to the power of three, induces a larger droplet. In Table 2, it can be observed that for the same concentration there is a slight decrease in droplet size with flow rate, which agrees with the theoretical equation.

On the other hand, at a theoretical level, an increase in the $\frac{\text{surface}}{\text{volum}}$ ratio of the drops provokes an increase in dehydration (Walstra et al., 2006; Santos et al., 2017) as vapor is removed from the drop by its surface. This effect is clearly observed in spray drying but, unexpectedly, in electrospraying, the decrease in droplet size did not lead to greater dehydration (Table 2). The Q factor was significantly higher in milk reconstituted at 12% with the flow rate of 0.01 mL·min^{-1}, even when it provoked larger droplets than treatments with higher solid concentration, at this flow rate. Surface tension is considered one of the most important dehydration effects during electrospraying (Xie et al., 2014). During electrospraying, water is expected to evaporate into the air when molecules are having enough electrical energy (depending on potential difference applied and drop electrical conductivity) to break free of the surface tension (Figure 13), but milk surface tension does not change much with increasing concentration at temperatures below 60 °C (Williams et al., 2005). On the other hand, a decrease in drop vapor pressure with increasing solids concentration should reduce

water evaporation, while solids concentration increases the electrical conductivity of the drop. From all these, it is clear that the rate of evaporation during electrospraying seems to result from a rather complicated relationship between potential difference, droplet size, surface tension and water vaper pressure. Note that drop size depends on flow rate, density, electrical conductivity and surface tension, while the last three depend on solids concentration.

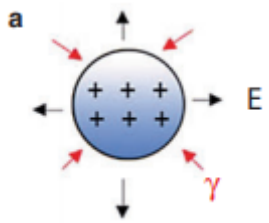

Figure 13. Electrospraying phenomenon. Relation between electrostatic repulsive forces and surface tension of the liquid (Alghoraibe and Alomari, 2018).

The dry yield refers to the total solids mass of the collected final product expressed in g per 100 mL of initial milk and provides information of losses during processing, while the wet yield provides information on the mass flow rate of the product collection (Table 3).

For the yield, there were no significant differences ($p \geq 0.05$) among samples generated at different flow rate but with the same initial solid content (Table 3). But it could be established that for the flow rate of 0.2 mL·min^{-1}, independently of the initial solid content, the amount of recovered solids was slightly smaller than the initial one. The cause of these small losses could be associated with the impact of drops against the collector at higher speed, which might have caused them to rebound towards the walls of the equipment, triggering lower dry yields.

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Table 3. Dry and wet yields and energy consumption of milk samples with different initial solid concentration, electrosprayed at two different flow rates.

Flow rate ($mL \cdot min^{-1}$)	Milk reconstitution (%)	Dry yield (g TS/100 g milk)	Wet yield ($mg \cdot min^{-1}$)	Energy consumption ($kJ \cdot g^{-1}$)
0.2	12%	10.540 ± 0.877^{c}	177.78 ± 15.63^{b}	-
	24%	22.435 ± 0.912^{b}	207.17 ± 1.58^{a}	-
	30%	27.541 ± 1.089^{a}	211.34 ± 10.49^{a}	-
0.01	12%	12.089 ± 0.806^{c}	3.46 ± 0.66^{c}	$11{,}053.6 \pm 1264.4^{b}$
	24%	23.935 ± 0.657^{b}	5.27 ± 0.62^{c}	$14{,}140.6 \pm 444.5^{a}$
	30%	29.574 ± 0.572^{a}	5.31 ± 0.06^{c}	$13{,}577.7 \pm 291.8^{a}$

Number of replicates, n = 2; Number of observations, N = 12; Mean value ± s.d.; a-d values per column without common superscripts were significantly different ($p < 0.05$).

Considering the wet yield in minutes, at the flow rate of 0.2 $mL \cdot min^{-1}$, significant differences ($p < 0.05$) were observed due to the initial concentration of the milk. As the solids increased, the amount of product obtained per minute increased. But as previously stated, there was not evaporation in these samples. However, at the flow rate of 0.01 $mL \cdot min^{-1}$, no significant differences ($p \geq 0.05$) were observed in the amount of product obtained per minute. It can be noted that 12% milk had a relatively lower value, but not significant, than the other two concentrations, for which almost the same amount of product was obtained. The observed trend was similar to that of 0.2 $mL \cdot min^{-1}$ flow rate.

Energy consumption expressed as kJ per gram of water extracted in the process was calculated (Table 3). For the flow rate of 0.2 $mL \cdot min^{-1}$, where the extraction was practically zero, the calculations were not done. At a flow rate of 0.01 $mL \cdot min^{-1}$, it could be observed that the same amount of energy was able to remove more water when the concentration of total solid decreased. Which agrees with the idea previously mentioned that concentration of solids makes it more difficult to remove water.

According to Bylund (1995) an industrial two-stage atomizer operating with hot air at 200 °C produces 1420 $kg \cdot h^{-1}$ of powder (3.5% moisture content) with an energy consumption of 20 kW-h (22 kW-h if the hot air is at 230 °C).The electrospraying equipment used in the present work has an approximate consumption of 1.32 kW-h. Since the equipment works with very low flow rates, the operating time to evaporate 1 kg of water would be extremely long.

However, it needs to be considered that the equipment used is not at industrial scale, Consumption will need to be compared with an industrial scale equipment electrosprayer. The company Bioinicia has patented a new technique, called Electrospraying Assisted by Pressurized Gas for the encapsulation of functional and/or nutraceutical ingredients. Getting to process about 3 $kg \cdot h^{-1}$ of dry powder. Then, it seems feasible to adapted/optimized the technology for milk dehydration to process flow rates of $kg \cdot h^{-1}$. In this equipment the voltage could be far higher than 29 kV (used in this study). This would cause an increase of ionization energy that would allow the water molecules to break free of the surface tension and exit the droplets formed during electrospray more efficiently. The increase in the intensity of the electric field would be associated with an increase in energy consumption. Another point is the distance between the tip and the collector. It is possible that an increase in this distance would allow the increase of flow rate maintaining the same dehydration or even improving it. . In that sense, it must be considered that the horizontal arrangement makes the force of gravity play a paper against, causing the product to make a parabola, limiting the distance between the nozzle and the collector plate. To counteract this effect, a higher voltage is required. But there are arrangements where the tip-collector configuration is in a vertical position. And finally, only one nozzle has been used for electrospraying, there are setup where up to 5 nozzles can be used at the same time, increasing the processing flow while maintaining the same energy consumption. There are also systems with the rotary collector, which helps with dehydration. It is based on the fact that the droplets are affected by the flow of air. In addition, a thinner layer of drops is created, making it more difficult for water to remain inside the layer of drops.

3.3.2. Protein analysis

Electrophoresis in polyacrylamide gels under denaturing conditions (SDS-PAGE) is a technique based on the separation of denatured proteins in gels with a polyacrylamide matrix (Laemmli, 1970). Proteins are subjected to an electric field in such a way that separate proportionally to their mass. The samples are previously denatured by heat with presence of SDS, a denaturing detergent of the tertiary structure of proteins that does not affect disulfide bridges or covalent bonds. In some cases, reducing conditions are used with the addition of β-mercaptoethanol in order to break the disulfide bridges formed from exposed free sulfhydryl groups upon denaturation between whey proteins or with kappa-casein (Anema,

2020). During electrophoresis, since SDS confers a net negative charge proportional to its mass, all the proteins migrate towards the anode and the separation is proportional to the mass of the protein, but independent of its charge, since they all have the same charge per unit mass. Any protein can be determined by comparison with a known standard (Brunelle and Green, 2014).

In Figure 14, two gels, non- and reducing conditions, obtained from the protein analysis are shown. With the help of the standard proteins, it was possible to identify which band corresponded to each protein. The same bands were obtained for all the samples and the only variation corresponded to their intensity and thickness. This was so because the protein concentration is different in each case and is directly related to the elimination of water. As expected, the intensity and thickness of the bands increased as the percentage of solids increased in milk reconstituted at 12, 24 and 30%.

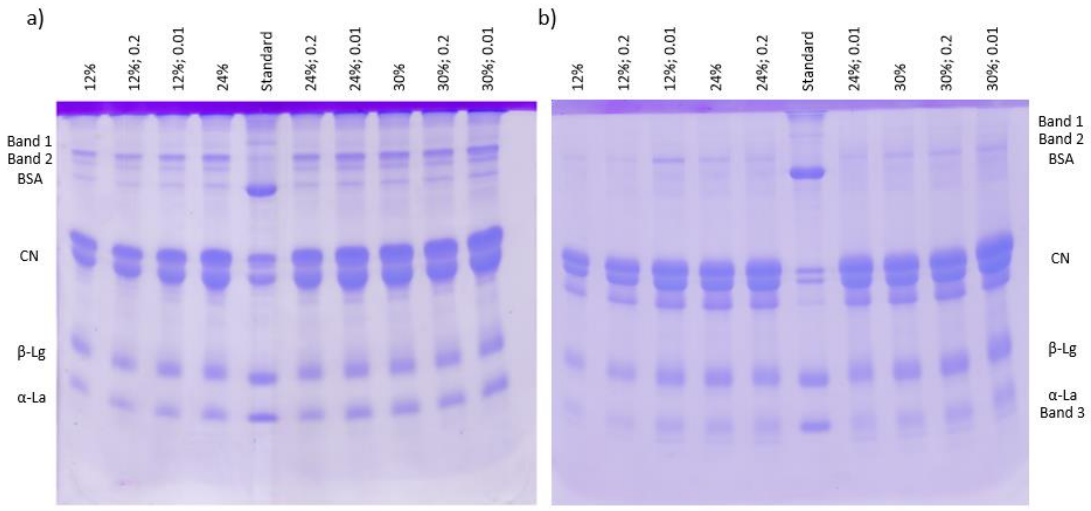

Figure 14. Polyacrylamide gels for milk reconstituted at 12, 24 and 30% and flow rates of 0.2 and 0.01 mL·min^{-1}. a) Non-reducing conditions; b) Reducing conditions. BSA: Bovine serum albumin; CN: casein; β-Lg: β-lactoglobulin; α-La: α-lactalbumin.

In between the stacking and running non-reducing gels, some material got stuck for all samples (Figure 14a); these proteins or aggregates had a molecular weight too high to run on the gel, for this reason they remained anchored. They could be associated with proteins of the membrane of the fat globule or with proteins linked together by disulfide bridges, as they mostly disappear in the gel under reduced conditions (Figure 14b). Band 3, which only appears under reducing conditions and shows a molecular weight lower than α-La could be

associated with gamma caseins, degradation products of caseins (Eskin and Goff, 2013). In heat treatments where protein denaturation occurs, at a fundamental level, protein denaturation is often defined as any noncovalent change to the secondary or tertiary structure of the protein molecule, whey proteins do not appear in their proper place on the polyacrylamide gel because they form aggregates between them through disulfide bridges (Anema, 2020). For this reason, they remain in the top part of the gel in non-reduced conditions; and when reducing them with β-mercaptoethanol, new bands appear or the intensity increases significantly, which correspond to denatured whey proteins (Jovanovic et al., 2007).

In order to evaluate the effect of electrospraying on the main milk proteins, their proportion was compared with that of the initial milk (Table 4). There were no significant difference ($p \geq 0.05$) between the final product and initial milk under either non- and reducing conditions. So, it can be established that electrospraying did not alter the secondary or tertiary structure of milk proteins.

Table 4. Percentages of proteins identified for electrosprayed product and initial milk.

	BSA (%)	**Casein (%)**	**β-Lg (%)**	**α-La (%)**
Non-reduced				
Final product	1.14 ± 0.35^{a}	73.26 ± 1.41^{a}	9.02 ± 0.39^{b}	6.26 ± 0.16^{a}
Initial milk	1.16 ± 0.32^{a}	74.47 ± 1.44^{a}	9.76 ± 0.11^{b}	6.41 ± 0.16^{a}
Reduced				
Final product	1.04 ± 0.01^{a}	71.23 ± 2.15^{a}	14.99 ± 1.35^{a}	5.91 ± 0.59^{a}
Initial milk	1.10 ± 0.19^{a}	71.45 ± 2.68^{a}	15.46 ± 1.56^{a}	5.86 ± 0.26^{a}

Number of replicates, n = 2; Number of observations, N = 12; Mean value ± s.d.; a-d values per column without common superscripts were significantly different ($p < 0.05$).

Significant differences ($p < 0.05$) appeared only for β-Lg between the non-reducing and reducing conditions. This might be due to the heat treatment used to obtain the starting milk powder, although being a “low heat” grade. The most important finding of the protein analysis performed at the present work was that electrospraying did not affect the integrity of the milk proteins, thus it is assumed that this technology would have a minimal impact on other milk compounds with nutritional value.

4. Conclusions

The alternative method of applying an electrical field for the extraction of water in reconstituted milk has been evaluated giving promising results. This method, widely used in microelectronics and materials science, is commonly called electrospraying. At sufficiently low flow rates with a field intensity of 29 kV and a nozzle-collector distance of 10 cm, electrospraying induces a very significant extraction of water. Milk reached a greater concentration factor when initial milk solids concentration was smaller, giving an inversely proportional relationship. This effect could be explained by the effect of increased solids concentration on physicochemical properties of the solution, preventing the evaporation of the solvent. Subsequent electrospraying studies should consider analyzing other parameters, such as surface tension, in order to evaluate their role on water extraction.

The optimal conditions to obtain milk powder have not yet been found, where the moisture content of the product should not be higher than 5%. Being a preliminary study, all possible processing parameters options, both for the equipment and the initial milk, have not yet been explored. Future studies should focus in improving the processing conditions, not only to attain milk powder with 5% moisture content, but also to increase the throughput of the technology. As dehydration has been partial, it might be the case that, we are dealing with a method for obtaining concentrated milk.

This work shows how electrospraying did not affect the structure of milk proteins, i.e., caseins and whey proteins. However, further studies are needed to evaluate the effect on their functionality or on the enzymes and other compounds activity such as lactose and fat globules. One of the advantages of this novel technology is that it does not apply high temperatures to the milk to concentrate it. This means that microbiological studies must be carried out in order to evaluate the hygenization capacity of this technology, although could be combined with alternative technologies such as ultraviolet light to ensure food safety.

Finally, it should be noted that current flow rates used in this wok are not suitable for the large-scale processing that the food industry is used to work with, which seems a problem, but the scalability of the process appears to be feasible since industrial equipment for non-food applications already exist and are being used for instance in the textile sector.

5. Bibliography

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ANNEX

Table 1. List of experiments performed and EHDA conditions.

				CONDITIONS OF EHDA		
SAMPLE	Conc. (%)	Characterization	Analysis	Voltage (kV)	Flow rate (ml min^{-1})	Distance (cm)
1	12	Physicochemical	Density			
2	24	Physicochemical	Density			
3	36	Physicochemical	Density			
4	12	Physicochemical	Conductivity			
5	24	Physicochemical	Conductivity			
6	36	Physicochemical	Conductivity			
7	24	Screening	Visual and subjective	15 to 23	5	10
8	24	Screening	Visual and subjective	14	1	10
9	24	Screening	Visual and subjective	14 to 15	2	10
10	24	Screening	Visual and subjective	15	2.5	10
11	24	Screening	Visual and subjective	15	3	10
12	24	Screening	Size bubble / SEM-EDX	21	0.2	10
13	24	Screening	Size bubble / SEM-EDX	25	0.2	10

				Conditions of EHDA		
SAMPLE	Conc. (%)	Characterization	Analysis	Voltage (kV)	Flow rate (ml min^{-1})	Distance (cm)
14	24	Screening	Size bubble / SEM-EDX	30	0.2	10
15	24	Screening	Size bubble / SEM-EDX	24	0.1	10
16	24	Screening	Size bubble / SEM-EDX	30	0.1	10
17	24	Screening	Size bubble / SEM-EDX	30	0.075	10
18	24	Screening	Size bubble / SEM-EDX	29	0.075	10
19	24	Screening	Size bubble / SEM-EDX	29	0.075	10
20	24	Screening	Size bubble / SEM-EDX	29	0.05	10
21	24	Screening	Size bubble / SEM-EDX	29	0.025	10
22	24	Screening	Size bubble / SEM-EDX	29	0.025	10
23	24	Screening	Size bubble / SEM-EDX	29	0.025	10
24	24	Screening	Size bubble / SEM-EDX	29	0.025	10
25	12	Physicochemical	Stability (Turbiscan)			
26	24	Physicochemical	Stability (Turbiscan)			
27	36	Physicochemical	Stability (Turbiscan)			
28	12	Physicochemical	Stability (Turbiscan)			
29	24	Physicochemical	Stability (Turbiscan)			

				Conditions of EHDA		
SAMPLE	Conc. (%)	Characterization	Analysis	Voltage (kV)	Flow rate (ml min^{-1})	Distance (cm)
30	36	Physicochemical	Stability (Turbiscan)			
31	36	Physicochemical	Stability (Turbiscan)			
32	36	Physicochemical	Stability (Turbiscan)			
33	30	Physicochemical	Stability (Turbiscan)			
34	30	Physicochemical	Stability (Turbiscan)			
35	24	Screening	Size bubble / SEM-EDX	29	0.02	10
36	24	Screening	Size bubble / SEM-EDX	29	0.015	10
37	24	Screening	Size bubble / SEM-EDX	29	0.01	10
38	24	Screening	Size bubble / SEM-EDX	29	0.005	10
39	12	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10
40	24	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10
41	30	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10
42	12	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10
43	24	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10
44	30	Experimental	SDS-page / Total solids / Size bubble	29	0.2 and 0.01	10