International Journal of Food Science and Biotechnology
2020; 5(2): 12-21
http://www.sciencepublishinggroup.com/j/ijfsb
doi: 10.11648/j.ijfsb.20200502.11
ISSN: 2578-9635 (Print); ISSN: 2578-9643 (Online)

Cultivation of *Aspergillus oryzae* and *Saccharomyces cerevisiae* in Whey for the Production of Single-celled Protein Intended for Feeding Cattle

Norma Angelica Santiesteban-Lopez, Teresa Gladys Ceron-Carrillo, Jose Luis Carmona-Silva, Juan Chavez-Medina

Faculty of Administration, Meritorious Autonomous University of Puebla, Puebla, Mexico

Email address:
asantiesteban2@hotmail.com (N. A. Santiesteban-Lopez), teresa.ceronco@gmail.com (T. G. Ceron-Carrillo), joselscs@coltlax.edu.mx (J. L. Carmona-Silva), chavez09@hotmail.com (J. Chavez-Medina)

To cite this article:
Norma Angelica Santiesteban-Lopez, Teresa Gladys Ceron-Carrillo, Jose Luis Carmona-Silva, Juan Chavez-Medina. Cultivation of *Aspergillus oryzae* and *Saccharomyces cerevisiae* in Whey for the Production of Single-celled Protein Intended for Feeding Cattle. *International Journal of Food Science and Biotechnology*. Vol. 5, No. 2, 2020, pp. 12-21. doi: 10.11648/j.ijfsb.20200502.11

Received: February, 17, 2020; **Accepted**: March 13, 2020; **Published**: MM DD, 2020

Abstract: The nutritional capacity of lactoserum allows the cultivation of microorganisms such as *Aspergillus oryzae* and *Saccharomyces cerevisiae*, which have probiotic properties for cattle. The objective of this work was to evaluate the use of whole lactoserum as a culture medium for the production of biomass of these microorganisms under different conditions and types of culture. The results obtained from the characterization of lactoserum indicate similarity between those reported by other authors for whey of different origins. An experimental design of three factors and two levels was carried out in a model system, where the temperature of pasteurization and/or sterilization was evaluated at 60 and 120° C respectively; initial pH of 4 and 7; as well as the use of yeast extract, 0 and 10 g/L. The best results were obtained at 60°C, pH=7 and in the absence of yeast extract for A. oryzae and in the presence of this for the cultivation of S. cerevisiae. These conditions were used for crops, obtaining maximum biomass values of 6.5 g/L for axenic crops of S. cerevisiae and A. oryzae, 4.80 g/L for mixed cultivation, 22.25 g/L and 15.0 g/L respectively; the latter highlighting the latter for allowing greater productivity.

Keywords: *Aspergillus oryzae*, *Saccharomyces cerevisiae*, Lactoserum, Biomass, Probiotics, Cattle, Protein

1. Introduction

Whey, whey or cheese whey is the liquid resulting from the coagulation of milk during cheese making. In general, there are 2 types of whey according to the cheese making process: acid whey and sweet whey, the latter corresponding to the highest-producing whey in Mexico [1]. This by-product of the milk industry is often used, in small quantities, for the feeding of different types of livestock and the rest is discarded in the effluents causing pollution to the environment [2, 3]. According to what is reported by Badui [4], for every 10 liters of milk, 8 to 9 kg of whey is produced and according to the Ministry of Agriculture, Livestock and Rural Development, Fisheries and Food (SAGARPA) during the year 2018 11,607,493,000 liters of milk and 342,000 tons of cheese per year were produced nationwide; Likewise, a production of 448,782,000 liters of milk was reported in the state of Puebla. Following that trend, in that year approximately 5 475 tons of cheese were produced in the state, which translates into an approximate production of 4.4 million liters of whey per year. Currently, whey production is a problem due to its high pollutant capacity, a consequence of its buffering properties of pH, proteins, lactose and other nutrients that make it an excellent culture medium for a wide variety of bacteria, yeasts and fungi. In the world there are different technologies for the reuse of whey, from dehydrated whey, flavored drinks, fermented products to protein concentrates. The present investigation focused on the production of single-cell protein of *Aspergillus oryzae* and *Saccharomyces cerevisiae* by fermentation of whole serum. Due to the high nutritional value of the unicellular protein and the probiotic effects attributed to some microorganisms, the use of fungi and yeasts as animal feed has become a topic of interest for the animal feed industry. Based on the above,

in this work the biomass production of both microorganisms was studied using the whey as a substrate, both in pure and mixed cultures.

2. Background

The dairy industry is in third place in terms of importance in the activities in the Mexican industrial branch. According to statistics provided by SAGARPA during 2016, 11,607,493,000 liters of milk were produced in the country, placing it in the ninth position worldwide. Regarding the current situation of the dairy sector in the state of Puebla, in the same year 448,782,000 liters were produced, placing the state in eighth place nationally [5]. Likewise, the production of different types of cheeses reached the figure of 342,000 national annual tons and 5,475 state tons [6]. Regarding livestock production, according to data provided by the National Institute of Statistics and Geography, in 2014, there were 28 415 337 head of cattle in the country of which 379,595 heads correspond to the state of Puebla and that place it in the 22nd place in the national ranking [7, 8]. Based on these figures, it is estimated that the national annual production of whey is of approximately 273.6 million liters and 4.4 million liters in the state of Puebla.

The Agrifood and Fisheries Information Service (SIAP) reports that, in Puebla, at the end of 2016 in the Cholula region, 120,765,000 liters of milk were produced, representing about 27% of state production. The main producing municipalities were San Matías Tlalancaleca, Ocoyucan, San Gregorio Atzompa and San Martín Texmelucan with productions greater than 9 thousand liters per year and concentrated about 10% of state production [9]. This information allows to locate in this area a niche of opportunity for the transformation of the whey into value-added products.

2.1. Milk Components

2.1.1. Carbohydrates

Lactose in bovine milk is found in concentrations between 4.5% and 5.2%. This is a disaccharide formed by a glycoside bond (1,4) between a galactose molecule and another of glucose. It is much less sweet than sucrose, and together with salts, it contributes to overall taste of milk. It is a reducing sugar prone to enzymatic hydrolysis by the enzyme ∝-galactosidase produced by a wide variety of microorganisms *(Lactococcus, Lactobacillus, Kluyveromyces, Candida, Aspergillus, Muc*or, etc.) and in the small intestine of mammals [4, 1].

2.1.2. Proteins

In general, there are two types of milk proteins: fat globule membrane proteins and skim milk proteins. Firsts are absorbed within a pellicle that surrounds the fatty blood cells and which are associated with some enzymatic functions, whose function is to release fat and, sometimes, this disrupt the properties of some milk by-products such as the solubility of powdered milk [10].

From all proteins contained in it, 80% corresponds to casein, and the remaining 20% corresponds to whey proteins. The latter are considered soluble even in acidic conditions, while casein coagulates at pH less than 4.6.

2.1.3. Lipids

Milk fat fraction is mainly form by triacylglycerides (96-98%), although there are other lipids such as diacylglycerides, mono acylglycerides, phospholipids, free fatty acids, cholesterol and traces of some hydrocarbons like esters of sterols. These compounds are found in emulsion form, and they are responsible for some properties of milk, such as taste, nutritional and caloric requirements. Furthermore, they are an excellent means of transport to vitamins and other fat-soluble compounds [4].

2.1.4. Vitamins and Mineral Salts

The main vitamins contained in milk are Rivoflavin (vitamin B2), vitamin A, vitamin D and thiamine (vitamin B1), although it also has the rest of the necessary vitamins in the human diet.

The fat-soluble vitamins are transported through chemical interactions with the lipids present, while the rest, travels through the water; therefore, water-soluble vitamins are found in whey obtained from cheese making (Table 1).

As far as salts are concerned, the main mineral in milk is calcium, followed by phosphorus, chlorides, potassium, sodium, magnesium and others such as iron and copper. Sodium and potassium chlorides are completely dissolved in the aqueous phase of milk, while calcium, magnesium and citrate phosphates form colloidal complexes with casein [10].

Table 1. *Origin bovine milk percentage composition.*

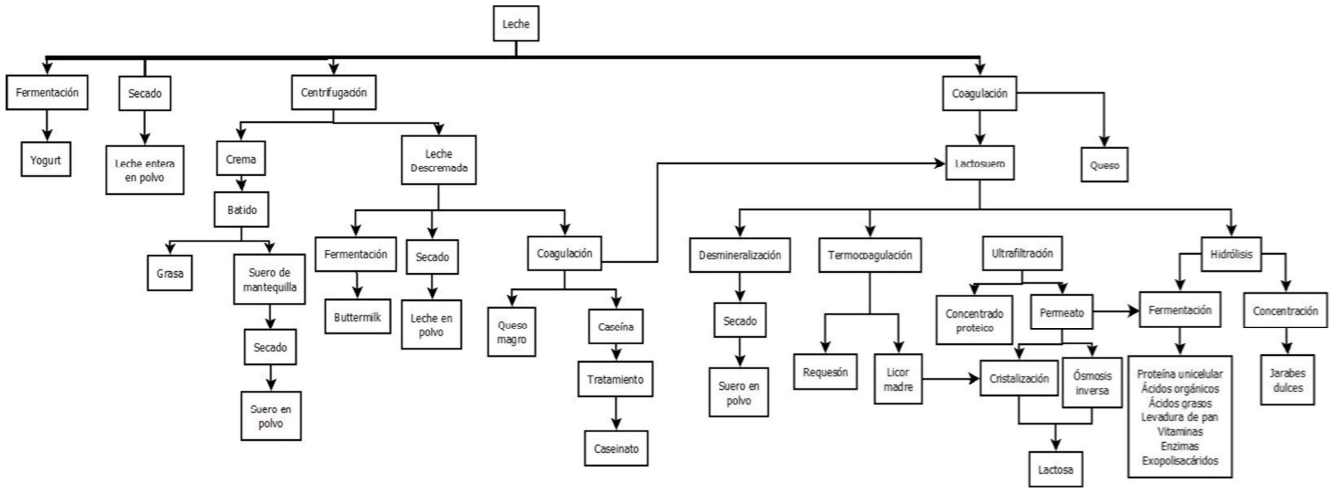
Component	Content (%)
Non oily solids	8.9
Fats	3.5
Lactose	4.9
Total protein	3.61
Sodium	0.057
Potassium	0.173
Calcium	0.12
Phosphorus	0.096
Magnesium	0.013
Vitamins	0.13

Source: Own elaboration.

2.2. Milk Derivatives

Currently, in Mexico and in much of the world, bovine milk is the most consumed and from it a large variety of dairy products are produced that result from different processes; whether by physical transformations that allow extending the shelf life of the milk itself or by chemical or biological induced transformations for the generation of milk derivatives. Physical transformations allow immediate consumption products such as powdered milk, sweetened condensed milk and evaporated milk [11]. Among the induced transformations are coagulation, whip and fermentation to obtain cheese, butter and yogurt, respectively.

Milk fermentation is a widespread practice throughout the world, it is a process in which a large variety of microorganisms producing ∝-galactosidase are involved. Some products obtained from fermentation are yogurt, jocoque (drink or dessert made from soured milk), kefir, Bulgarian and "Yakult" [1]. Generally, this process is accompanied by the addition of other ingredients or food additives, in order to improve its physicochemical properties. As a consequence of the biochemical transformations that happened in lactic cultures, they contain supplemental nutritional requirements such as B vitamins complex, an increase in the concentration of proteins and partially hydrolyzed lactose that are easily digested. Therewith, a diagram with the various processes to which milk and by-products are subjected, is shown in Figure 1 [1].



Source: Own elaboration.

Figure 1. *Milk by-products through different processes.*

2.3. Lasctoserum

2.3.1. Physicochemical and Biological Characteristics

During cheese making, proteins and fats are concentrated in a process called coagulation, then they are separated and compressed with the aim of giving consistency to cheese. The residue of this process is whey, which is considered a waste from the cheese industry. The fraction of milk that is transformed into cheese is only 10% to 20%, therefore, whey constitutes up to 90% of the total volume and contains up to 55% of the total nutrients of whole milk. Among the main components of the whey are: lactose, proteins, fats and mineral salts. In Table 2 [11] it can be observed that the serum is divided into two types according to its acidity but that in general there are no considerable differences in its chemical composition. As noted, the lactose and protein content is in high amounts, these confer a high organic content and a C/N ratio of intermediate value that make it a biodegradable substrate, in the same sense, this relationship is a fundamental factor in the development of any organism and will determine whether these nutrients will be consumed for the production of biomass or for the formation of biomolecules and other by-products of fermentation [12-14].

Table 2. *Lactoserum percentage composition.*

Component	Sweet whey	Acid whey
pH	5.9-6.6	4.5-4.6
Total solids (%)	6.4	6.5
Water (%)	93.6	93.5
Fats (%)	0.05-0.37	0.04-0.27
Protein (%)	0.6-1.0	0.6-0.8
Lactose (%)	4.6-5.2	4.4-4.6
Minerals (%)	0.5	0.8
Calcium (%)	0.043	0.12
Phosphorus (%)	0.04	0.065
Sodium (%)	0.05	0.05
Potassium (%)	0.16	0.16
Chloride (%)	0.11	0.11
Lactic acid (%)	0.05	0.05
Relationship C/N	16.36-23.84	17.94-21.9

Source: Own elaboration.

2.3.2. Alternatives for Lactoserum Reuse

Due to lactoserum is a highly polluting waste product, multiple applications have been studied in other areas than the dairy industry. Figure 1 shows some products obtained from whey using different procedures and several of them are described below. Whole whey can be used directly in liquid form as food for different types of farm animals, water can also be removed by evaporation and obtained a concentrated whey or dry it thoroughly and marketed in powder form. When the protein fraction is desired, it is advisable to subject lactoserum to ultrafiltration to separate the proteins from the rest of the solution and to maintain their properties. It is a high-cost process, but at the same time it generates a product of great added value.

2.4. Unicellular Protein Production

The generation of bacterial cells, filamentous fungi and yeasts by fermentation in different types of culture medium and under controlled conditions is what, in microbiology, is

known as biomass or single-celled protein. This name is derived from the fact that single-celled organisms have high protein proportions in relation to their size and the presence of other cellular components.

Furthermore, single-celled protein contains lipids, carbohydrates, vitamins and minerals; as well as essential amino acids, such as lysine or methionine, which are found in limited amounts in plant and animal tissues [15]. Single-celled protein production is born in response to two needs: counteracting the scarcity of protein sources for food and the use of wastes rich in fermentable sugars.

The presence of different types of carbohydrates in a large number of industrial by-products represents a great opportunity for the development of a wide range of microorganisms. Which may grow in such waste whether they require previous treatment (hydrolysis) or not. In most cases, the microorganisms selected to obtain protein are yeasts, due to they contain between 40-80% of true protein in their cells; such as *Kluyveromyces marxianus* [16]. Table 3 lists some species of yeast grown on different substrates and their respective protein percentages. *Candida utilis, Kluyveromyces fragilis and Saccharomyces cerevisiae* can be highlighted as the main producers of single-celled protein with production percentages around 50% of its dry weight.

Table 3. *Protein content of single-celled yeast proteins.*

Yeast	Substrate	Protein (% dry weight)
Cándida utilis	Sulfuric liquid	55
Hansenula	Methanol	50
Kluyveromyces fragilis	Whey	52
Saccharomyces cerevisiae	Molasses	53
Schwanniomyces castelli	Amylum	42

Source: Own elaboration.

3. Methodology

Whole whey was used as a growing medium for all fermentations, it was provided by a local cheese shop in the community of Santa María Tonantzintla, Puebla. Whey was characterized by proximal analysis, with the samples being analysed by triplicate.

3.1. Lactoserum Physicochemical Characterization

3.1.1. Protein Determination

Soluble protein concentration was carried out by the micro-Kjeldahl method, modified by NOM-155-SCFI-2003 [17]. 2.5 mL sample was placed in a Kjeldahl tube that was previously added 3 g of potassium sulphate and 0.25 g of pentahydrate copper sulphate, subsequently 5 mL of concentrated sulphuric acid were added. The tubes were placed in the digestion equipment with an initial temperature of 180-230°C for 30 min to avoid foaming, gradually increasing the temperature to 410-430°C; digestion stopped when the solution was clarified. Once the samples are digested, they are kept at rest until they reach room temperature and 20 mL of water and 15 mL of sodium hydroxide are added to 50%. In an Erlenmeyer flask, 50 mL of 2% boric acid and 5 drops of the Shiro Tashiro indicator were placed. Finally, the tube and flask are placed in the distillation unit and the receiving platform, respectively.

Distillation is carried out until 150 mL of solution is recovered in the receiving flask. The distillate was titled with 0.1 N hydrochloric acid and the percentage of proteins was calculated using the formula of Equation 1.

$$(V)\ (N)\ (0{,}014)\ (100)\ (6.38)\ \%\ \text{Protein} = M \qquad (1)$$

Where:
V=Volume spent on titration
N=Normality of hydrochloric acid
0.014=Nitrogen milliequivalent
6.38=Nitrogen-protein ratio factor in milk
M=Wet sample mass

3.1.2. Lactose

Lactose was quantified using the DNS method, using a 4 g/L lactose standard for the development of a calibration curve and 1:40 dilution of the sample [18]. The standard curve was drawn up according with to lay out in Table 4, which specifies the added volumes of standard solution, water and DNS.

Table 4. *Concentration of reagents in the standard lactose curve.*

Tube	Pattern solution (µL)	Water (µL)	DNS (µL)
1	0	250	250
2	50	200	250
3	100	150	250
4	150	100	250
5	200	50	250
6	250	0	250

Source: Own elaboration.

The samples were prepared identically to tube 6 of the standard curves, replacing the standard solution with the diluted sample. All tubes were boiled water bath for 5 min, when cooled they were added 1.25 mL of water and the absorbance was read at 540 nm. With the results obtained from the standard solution, a graph was drawn up and a linear regression equation was obtained, in which the result absorbance values of each sample were replaced in the independent variable to calculate the lactose concentrations (dependent variable).

3.1.3. Total Solids

Total solids were determined according to the methodology specified in NMX-F-527-1992 [19]. To that end was necessary to place porcelain capsules at constant weight and weigh them. In them were placed 40 mL sample, they were subjected to water bath in order to evaporate as much water as possible and then dried in stovetop at 110°C to constant weight. Each capsule was cooled in desiccator and weighed.

Equation 2 shows the formula with which the solids content was calculated in the sample.

$$(m)\ (100)\ \%\text{Total solids} = v \qquad (2)$$

Where: m=Dry sample mass in grams v=Sample volume

3.1.4. Humidity

Humidity content was made by weight difference, as indicated in NMX-F-083-1986 [20]. In constant and previously heavy weight porcelain capsules, 40 g of sample was placed and dried at 110°C on the stovetop until constant weight was reached. All capsules were transferred to the desiccator for 1 hour or until they reached room temperature and then weighed. Equation 3 shows how the humidity percentage calculation was performed.

$$(Pmh - Pms)\ (100)\ \%Humidity = M \quad (3)$$

Where:
Pmh=weight of the capsule with wet sample
Pms=Weight of capsule with dry sample
M=Wet sample mass

3.1.5. Ashes

The ash content was determined using the methodology described in NMX-F- 066-S-1978 [21]. The capsules with dry sample result of the determination of total solids were placed on an electric grill to burn the contents until no smoke was detached. Immediately, they were then placed inside a muffle furnace until the sample was fully calcined (800°C / 30 min). The capsules were allowed to cool inside the muffle furnace, then placed in the desiccator until they reached room temperature and weighed. The calculation of the ash content was performed using Equation 4.

$$(Pc - Pv)\ (100)\ \%\ Ashes = m \quad (4)$$

Where:
Pc=Weight of the capsule with ashes
Pv=Empty Capsule Weight
m=Dry sample mass

3.2. Lactoserum Physicochemical Characterization

The solution was titled with ammonium thiocionate 0.1 N until the appearance of a brick red precipitate. The chloride content is expressed as a percentage of sodium chloride, whose milliequivalent is 0.0585, as expressed in Equation 5.

$$(Vnp - Vta)\ (N)\ (0.0585)\ (100)\ \%Chlorides = M \quad (5)$$

Where:
Vnp=Silver Nitrate Volume
Vta=Spent volume of ammonium thiocyanate
N=Normality of the titling solution
M=Wet sample mass

3.2.1. pH

pH was measured by a Hanna-brand digital potentiometer previously calibrated to pH 4 and 7. The reading was made by direct introduction of the electrode into a beaker with the sample.

3.2.2. Lactic Acid

The percentage of lactic acid was quantified by the determination of acidity by volumetric method as established by the NMX-F-206-1986 standard [20]. In Erlenmeyer flasks were placed 10 g of sample, 100 mL of distilled water and 3 drops of phenolphthalein indicator. The mixture was titled with 0.1 N sodium hydroxide until persistent faint pink is observed for approximately 15 seconds.

The calculation was made using the formula of Equation 6, where, 90 is the equivalent of lactic acid.

$$(V)\ (N)\ (90)\ (100)\ \%Lactic\ Acid = M \quad (6)$$

Where:
V=Spent Volume of Sodium Hydroxide
N=Normality of the titling solution
M=Wet sample mass

3.2.3. Microorganisms

The filamentous fungus *Aspergillus oryzae* and the yeast *Saccharomyces cerevisiae* strain ATCC 9763 were used both provided by the Faculty of Chemical Sciences of UNAM. They were planted in potato-dextrose agar plates (PDAs), then in tubes with 10 mL of PDA broth, were incubated at 30°C, 72 hours and 28°C, 48 hours respectively and kept in refrigeration until use.

3.2.4 Lactoserum Pre-treatment

Lactoserum that would be sterilized at 120°C as part of heat treatment, was subjected to a process of prior deproteinization; because when exposed to high temperatures, the three-dimensional structure of proteins is deployed as a result of the rupture of hydrogen bridges between polypeptide chains and aggregates are formed that interfere with biomass determinations. The deproteinization was carried out by heating the whey to 100°C for 15 min and cooling sharply in the ice bath, then vacuum filtered with standard filter paper.

3.3. Experimental Design

The factorial design used for evaluation of growth of microorganisms in lactoserum was 3 factors and 2 levels, in other words, an experimental design 23. To this end, the following factors were taken into account: pasteurization or sterilization temperature, 60 and 120°C; pH, 4 and 7; yeast extract 0 and 10 g/L, as seen in Table 5.

Each treatment was performed in duplicate and the results obtained were analyzed using a variance analysis (ANOVA) through Minitab 17 statistical software in order to determine the most impact factors and which treatments are significantly different from each other.

Table 5. *Factorial design where X1 represents temperature (°C); X2, the addition of yeast extract (g/L) and X3, Ph.*

Treatment	X1: T	X2: EL	X3: T
1	+(12)	+(10)	+(7)
2	+(120)	+(10)	-(4)
3	+(120)	-(0)	-(4)
4	+(120)	-(0)	+(7)
5	-(60)	+(10)	-(4)
6	-(60)	+(10)	+(7)
7	-(60)	-(0)	+(7)
8	-(60)	-(0)	-(4)

Source: Own elaboration.

For *aspergillus oryzae* crops each flask with 100 mL of treated lactoserum was inoculated with 0.07 g/L biomass and grown for 72 hours at 30°C. In the case of crops of *Saccharomyces cerevisiae* each flask with 100 mL of treated lactoserum was inoculated with 0.07 g/L of biomass and grown for 48 hours at 28°C.

3.4. SCulture Medium

Lactoserum used was subjected to the conditions of the best treatment according to the results obtained in the previous experiments. These were 60°C temperature (pasteurization), pH of 7.0 and absence of yeast extract for *Aspergillus oryzae*; in contrast, the culture of *Saccharomyces cerevisiae* was performed under the same conditions, but adding 10 g/L of yeast extract to the substrate.

3.4.1. Inoculum

The initial inoculum was 10% of the total volume of the medium. It was prepared from test tubes with 10 ml of PDA broth incubated with each microorganism under the temperature and time conditions exposed above. Each tube was then poured into Erlenmeyer flasks with 200 ml of lactoserum for 72 hours, 28°C for *Aspergillus oryzae* and 48 hours, 30°C for *Saccharomyces cerevisiae*, both at 100 rpm.

3.4.2. Crop Conditions

A 4 L bioreactor with a working volume of 2 L was used for the batch culture of *Aspergillus oryzae*. The medium was pasteurized inside the reactor for 20 min at 60°C. The inoculum was poured under aseptic conditions and the cultivation was carried out keeping the temperature at 30°C and aeration of 1 vvm. The cultivation of *Saccharomyces cerevisiae* was carried out under the same conditions; however, the temperature was adjusted to 28°C.

3.5. Mixed Cultivation

3.5.1. Inoculum

An inoculum was prepared for each microorganism under the same conditions exposed above, decreasing the volume of the flasks to 100 mL of substrate, so that together they increase a volume equal to 10% of the workload.

3.5.2. Inoculum and Cultivation Conditions

The inocules prepared for this crop were made as mentioned above. Initially, the cultivation of *Aspergillus oryzae* was carried out in a working volume of 500 mL of whole lactoserum at 30°C for 72 hours. Subsequently 300 mL of the substrate was transferred to the second reactor and inoculated with *Saccharomyces cerevisiae.* In the first reactor 300 mL of new substrate was added and successive additions were made every 48 hours after the media was removed from the second reactor. Samples were collected every 24 hours at the exit of the first reactor, the filtration system and the second reactor; each sample was determined biomass and sugars.

3.6. Biomass Determination

The biomass concentration was determined by gravimetric method. 5 mL was taken from each sample of the *S. cerevisae* crop were centrifuged at 5,500 rpm for 5 minutes in Falcon tubes at constant weight. Instantly, the supernatant was separated and reserved for further analysis, while the pill was centrifuged twice more with water and dried at 80°C to constant weight. For *A. oryzae* and mixed crops, 2 mL of each sample were taken, these were centrifuged at 16,000 rpm for 10 minutes in Eppendorf tubes at constant weight. Subsequently, the procedure already described for *S. cerevisiae* was followed. Once the display has completely dried, they were cooled and weighed. The calculation of the generated biomass was performed using the Equation, expressing itself in g of Biomass/L of substrate.

$$(Ptm - Ptv)\ \%Biomass = v \quad (7)$$

Where:
Ptm=Weight of tube with dry sample
Ptv=Weight of the empty tube

3.7. Lactose Determination

From the supernatant recovered from each sample, the quantification of residual lactose was performed using the reducing sugar technique; using a dilution factor of 1:25 for all samples from the *A. oryzae* culture and 1:10 for those corresponding to *S. cerevisiae*.

3.8. Characterization Physicochemical of The Single-Cell Protein

The residual broth of each culture with the biomass included in it was frozen and subsequently freeze-dried in order to obtain a product in the form of a powder on a dry basis and thus be able to quantify the different constituents present in it.

4. Results

4.1. Raw Material Characterization

Lactoserum chemical composition from Santa Maria Tonantzintla is summarized in Table 6 while comparing the results obtained in two other related works from Veracruz, Mexico [22] and Miranda [23].

Table 6. *Comparison of proximal chemical analysis of lactoserum.*

Component (%)	This work (%)	Miranda and col (2009) [23]	Hernández (2013) [22]
Lactose	3.63 ± 0.42	4.1	4.1
Protein	0.90 ± 0.16	0.94	2.54
pH	4.70 ± 0.01	4.22	4.77
Acidity	0.87 ± 0.003	0.32	0.33
Chloride	0.32 ± 0.01	—	—
Humidity	92.56 ± 1.11	—	94.79
Total solids	7.60 ± 1.15	6.4	5.21
Ashes	0.47 ± 0.02	—	0.49
Relationship C/N	12.72	13.68	5.059

Source: Own elaboration.

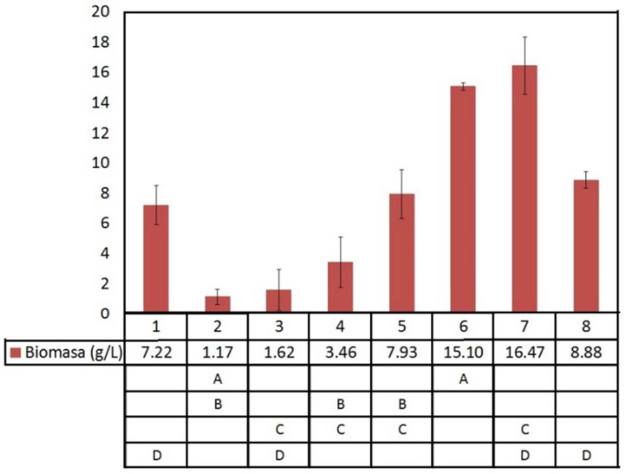
The lactose content reported in the other works differs slightly with what is determined in this work; however, the

results are kept in the same order of magnitude and there is no significant difference. The percentage of total protein is similar to that reported by Miranda [23] although low, compared to that exposed by Hernández [22]. Similar behaviors are shown in pH, humidity, total solids and ash determinations; where, although the results are close, small differences are observed. It should be noted that the chemical composition of the dairy industry's waste depends on a number of factors ranging from feeding and rearing of cattle to handling milk and final product [24].

4.2. Cultivation of Aspergillus Oryzae

Once the raw material was characterized, cultivations were made under the conditions specified in the methodology for factorial design. Figure 2 shows the responses (biomass production) obtained in each experiment. In this, it is observed that the treatment with the best response corresponds to treatment 7 of the experimental design (pH=7, T=60, EL=0) followed by treatment 6 (pH=4, T=120, EL=0); however, it is noted that there is not significant difference between two treatments since they share the same letter in the classification resulting from the statistical study. The selection criterion was therefore based on both economic aspects of both time and economic resources. Biomass production is similar in both culture because fungi of the genus *Aspergillus* have the ability to grow in acid pH as they are producting organisms of organic acids and withstand pH conditions between 4 and 6 [25]; thereby, a value of 0.44% of total protein and 4.85% lactose for deproteinized whey [26] has been reported, i.e. the main source of carbon is not affected by the deproteinization process and therefore does not limit the growth of the micro-organism as long as nitrogen is available, which restricts biomass production by reaching minimum concentrations [25, 27].

The criterion to consider in determining treatment 7 as suitable for batch culture was that it allows the use of whole lactoserum without deproteinizing, since pasteurization at 60°C does not require prior deproteinization of the substrate; therefore, it does not require pretreatment or generate waste.



Source: own elaboration.

Figure 2. *Biomass production A. oryzae in each treatment.*

Table 7 groups the effects of the three factors on biomass production resulting from ANOVA. Those values of P (Probability) lesser than 0.05 represent a significant effect on the response, which in this case represents the biomass produced. Thus, it is observed that the pH and temperature factors have a significant effect on biomass production, but not the addition of yeast extract. In contrast, it is observed that both double and triple interactions do not generate relevant effects on the response. Several authors have studied the influence of different sources of carbon and nitrogen, as well as their relationship, on the production of biomass and metabolites, Torres et al., [28]. report that the carbon source is the main factor involved in the production of fungal biomass when it is in concentrations close to 50 g/L, but not when the sugar concentration is low and nitrogen becomes the limiting element [28].

In that regarde, yeast extract is a source of nitrogen rather than carbon and since lactoserum itself contains organic nitrogen from casein and a mixture of amino acids, this element does not limit the growth of the fungus as long as it exists lactose; therefore, the addition of yeast extract is not a factor that significantly affects biomass production.

Table 7. *Analysis of variance for Biomass of A. oryzae.*

Effects	SC sec	GL	MC ajust	F	P
pH	229.788	1	229.788	35.300	0.000*
T	577.091	1	577.091	88.654	0.000*
EL	2.409	1	2.409	0.370	0.547
T*pH	17.508	1	17.508	2.690	0.111
pH*EL	12.570	1	12.570	1.931	0.175
T*EL	10.926	1	10.926	1.678	0.205
T*pH*EL	6.733	1	6.733	1.034	0.317
Error	156.228	24	6.510		
Total	1013.253	31	32.686		

*P<0.05, IC=95%.
Source: Own elaboration.

Changes that occur when each factor changes from its lowest value to its high value were also evaluated; this helps us to know the time when the best production is obtained according to the individual valuation of each factor (Table 7). Table 7 shows that biomass production increases by the lowest temperature value in contrast to the best response when pH is at 7. However, the effect corresponding to yeast extract did not have a significant effect between low and high value; a small increase in the response is seen when 10 g/L is added but is not comparable to the effect on the other factors, which is consistent with that concluded from the ANOVA.

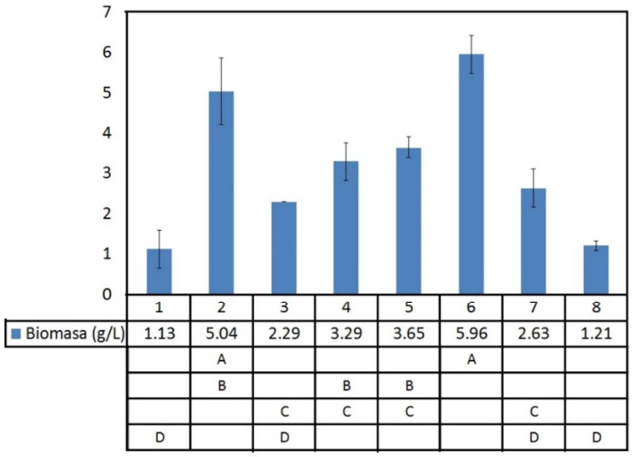
4.3. Saccharomyces Cerevisiae Culture

S. cerevisae cultures were carried out under the conditions indicated in the experimental design of section 3.3.3. Results obtained for the production of yeast biomass are shown in Figure 2. Treatment 6 (pH=7, T=60, EL=10) allowed higher biomass production of *S. cerevisiae,* however, no significant difference is observed with treatment 2 (pH=4, T=120, EL=10) but as it was in case of *A. oryzae*, this treatment requires the removal of lactoserum protein by heat treatment which generates waste and greater cost of time and effort. In

this case a phenomenon similar to that observed in *A. oryzae* culture is observed in that, even if treatment 2 has no observed protein growth, again it is due to the fact that biomass production initially depends on the carbon source.

Since yeast is unable to break down lactose, its growth depends on the added glucose in the yeast extract. [29]. they performed *S. cerevisiae* cultures in lactoserum without pH control because it has pH regulation capabilities and yeast resists adverse acidic conditions; therefore, it is able to reproduce even at pH close to 4 [29].

Figure 3 also shows letter classification of treatments that are significantly different from each other, so that treatments that share some letters are the same with each other. It is therefore confirmed that there are no significant differences between treatments 2 and 6 that showed the best responses, which is why the selection of the treatment to be used depended on the economic factors. With the results obtained from the experimental design, ANOVA was carried out to evaluate the effect of each factor on biomass production. Table 8 shows that factors with significant effect on response ($P<0.05$) are the interaction between temperature-pH and yeast extract-temperature.



Source: own elaboration.

Figure 3. *Biomass production of S. cerevisiae in each treatment.*

Table 8. *Analysis of variance for S. cerevisiae biomass.*

Effects	SC sec	GL	MC ajust	F	P
pH	0.163	1	0.163	0.789	0.388
T	0.715	1	0.715	3.455	0.083
EL	0.184	1	0.184	0.889	0.361
T*pH	11.028	1	11.028	53.249	0.000*
pH*EL	0.544	1	0.544	2.626	0.126
T*EL	28.578	1	28.578	137.991	0.000*
T*pH*EL	0.024	1	0.024	0.115	0.739
Error	1.657	8	0.207	-	-
Total	42.893	15	2.860	-	-

Source: Own elaboration.

In order to validate the results obtained in this work, a bibliographic review was carried out to make comparisons and obtain some conclusions.

5. Discussion

Table 9 shows some related works and the contrast of their results with those presented in this paper. It should be noted that in most cases the microorganism, substrate and duration of the culture were different from those used in this research, however, they are close references that allow for a comparative analysis.

Table 9. *Comparison of biomass production on different substrates.*

Microorganism	Culture medium	Additional salts	Time	Scale	Biomass (g/L)	Rx (g/Lh)	Author (s)
A. niger	Whole lactoserum	Ammonium sulfate and monobasic potassium phosphate	6 days	Flask 47 mL	29.05	0.201	[25]
	Deproteinized and hydrolyzed lactoserum	Ammonium sulfate and monobasic potassium phosphate	6 days	Flask 47 mL	20.36	0.141	
	Whole lactoserum	Ammonium diacid phosphate	8 days	Flask 100 mL	8.75	0.045	[30]
	Hydrolyzed lactoserum	Ammonium diacid phosphate	8 days	Flask 100 mL	15.21	0.079	
A. oryzae	Fat free milk	—	4 days	Flask 35 mL	43.64	0.454	[31]
A. oryzae	Whole lactoserum	—	72 hourss	Flask 50 mL	15.39	0.213	This work
S. cerevisiae	Whole lactoserum and yeast extrac	—	48 hours	Flask 50 mL	5.96	0.124	

Microorganism	Culture medium	Additional salts	Time	Scale	Biomass (g/L)	Rx (g/Lh)	Author (s)
S. cerevisiae	Deproteinized and hydrolyzed lactoserum	—	24 hours	Reactor 700 mL	7.8	0.325	[29]
S. cerevisiae y K. lactis en cultivo mixto	Deproteinized lactoserum	Sulfato de amonio	48 hours	Reactor 1L	22.38	0.466	[32]
A. niger y S. cerevisiae en cultivo por etapas	Potato starch	—	205.5 hours	Reactor Stage 1: 1L Stage 2: 500 mL	81.2	0.395	[33]
S. cerevisiae	Whole lactoserum and yeast extrac	—	48 hours	Reactor 3L	6.5	0.135	This work
A. oryzae	Whole lactoserum	—	72 hours	Reactor 3L	6.5	0.09	
S. cerevisiae y A. oryzae en cultivo mixto	Whole lactoserum	—	72 hours	Reactor 3L	4.8	0.066	
A. oryzae y S. cerevisiae en cultivo por etapas	Whole lactoserum	—	10 days	Reactor stage 1: 500 mL stage 2: 300 mL	22.25 15.0	0.271 0.166	

*P<0.05, IC=95%.
Source: Own elaboration.

[25] As well as used *Aspergillus niger* for whole whey, hydrolyzed and/or deproteinized serum cultures; this filament fungus belongs to the same species of *A. oryzae,* so similar results would be expected.

However, results reported by López et al. [25] are higher than 20 g/L, in cultures whose duration was to 6 days and with previous hydrolysis of lactose that allows to microorganism to concentrate its energy on reproductive functions and not in metabolic functions. A similar situation is presented in the results reported by Leal, in which case, biomass production in whole whey after 8 days is approximately 2 g/L higher than results obtained in this work in 3 days of culturing.

On the other hand, when is compared the volumetric speed of biomass generation, it is observed that Lopez et al. [25] report a slightly higher value for *A. niger* in deproteinized and hydrolyzed lactoserum than reported in this work; furthermore, Leal et al. [30] report lower productivity values than reported in this work. It should be noted that both references were added with salts and the work volume was smaller than that used for fermentations in this work, so, as noted in the results obtained from the previous treatments, in small volumes the microorganism produces greater amounts of biomass.

Cortés-Sánchez et al. [31] report a production of 12.25 g/L of *A. oryzae* in fat-free milk after 4 days of cultivation; i.e., it reports twice as much production as reported in this work; however, the work volume was 35 mL. The cultivation of *S. cerevisiae* made by Pisano et el. [29] was carried out in deproteinized and hydrolyzed lactoserum obtaining a production of 7.8 g/L of biomass in 24 hours, unlike the 6.5 g/L produced in this work after 48 hours. Although the cultivation carried out in this research showed a higher substrate consumption than 90%, the yeast did not grow as expected; however, in this work the substrate treatment performed by Pisano et al was not performed [29]. As regards mixed crops, no references of the *S. cerevisiae* and *A. orizae* culture were found. However, Moeni et al [32] cultivated a culture with two types of yeast: *S. cerevisiae and Kluyveromyces lactis,* the latter is a producer of ∝-galactosidase and is able to hydrolyzing lactose. Both yeasts produced 22.38 g/L of biomass in 48 hours growing in deproteinized lactoserum as a substrate, a yield 4 times higher than obtained in this work; it should be noted that no salts were added in this work. Finally, as regards multistage crops, it is observed that in stage 1 the accumulated biomass production was 22.25 g/L while in stage 2 it was 15 g/L; 37.25 g/L in total. This result turned out to be twice as minor as reported by Tellez et al. [33] however, the substrate used was not the same, as shown in Table 9.

6. Conclusions

The results obtained from lactoserum characterization were similar to those reported by other authors, especially in lactose concentrations (3.63%) protein (0.90%) which are within the ranges reported in the literature. The ANOVA study carried out on the different treatments for the cultivation of *A. oryzae* and *S. cerevisiae* showed that the best conditions for the cultivation of both microorganisms in whole whey are: pH=7 and pasteurization at 60°C. Although for yeast case was necessary to add yeast extract to the substrate. Both microorganisms are able to consume the sugars contained in lactoserum and, in turn, produce biomass; obtaining maximum biomass values of 6.5 g/L for axenic cultures of *S. cerevisiae* and *A. oryzae*, 4.80 g/L for mixed cultivation, The microorganism that develops best in whole lactoserum is *A. oryzae* in both axénic and staged cultivation, highlighting the latter for allowing greater productivity. The protein content of final product of the culture of *S. cerevisiae* (63%), *A. oryzae* (41%) mixed (32%) obtained is similar to what was reported in the literature (30%-54%).

References

[1] García M. y López A. (1999). Biotecnología Alimentaria. Limusa.

[2] Ávila R., Cárdenas A., y Medina A. (2000). Tratamiento del lactosuero utilizando la técnica de electrodiálisis. Interciencia, vol. 25, no. 2, pp. 80–84.

[3] Ramírez J. (2015). Diseño de procesos en industria láctea: Transformación de lactosuero. Libros Editorial UNIMAR.

[4] Badui S. (2006). Química de los alimentos. Pearson.

[5] SAGARPA. (2018). Boletín trimestral sobre la producción de leche de bobino. Recuperado el 08 de 06 de 2019, Secretaria de Agricultura y Desarrollo Rural: https://www.gob.mx/agricultura.

[6] Olguín I. (2017). Productores poblanos comercializan 15 toneladas de queso. [Online]. Available: http://archivo.unionpuebla.mx/articulo/2014/04/20/economia/otros- municipios/productores-poblanos-comercializan-15-toneladas-de-que.

[7] INEGI. (2014). Encuesta nacional agropecuaria 2014. Instituto Nacional de Estadística y Geografía, Tech.

[8] INEGI. (2016). Anuario estadístico y geográfico de puebla 2016. Instituto Nacional de Estadística y Geografía, Tech.

[9] SIAP. (2017). Gobierno de mexico. Recuperado el 12 de 06 de 2019, de Servicio de Información Agroalimentaria y Pesquera: https://ube.siap.gob.mx/cierreagricola/.

[10] Zavala J. (2005). Aspectos nutricionales y tecnológicos de la leche. Dirección General de Promoción Agraria.

[11] Marcelín M. y Vélez J. (2012). Características fisicoquímicas de sueros de queso dulce y ácido producidos en el combinado de queso de bayamo. Rev Cub Aliment Nutr, vol. 19, no. 1, pp. 21–25.

[12] Ambientum-InfoSald. (2015). Suelos y residuos: Relación carbono-nitrógeno. Green Tal S. A., Tech. Rep.

[13] Colón I. (2016). Producción de enzimas por cultivo en medio sólido usando subproductos agroalimentarios. Master's thesis, Universidad Autónoma Agraria Antonio Narro, 2016.

[14] López A., Luna, Buenrostro J., Hernández R., Huerta S., Escalona H., Aguilar C. y Prado L. (2016). Efecto del ph, temperatura y fuente de proteína y carbohidratos en la producción de proteasa por yarrowia lipolytica en cultivo sólido. Revista Mexicana de Ingeniería Química, vol. 15, pp. 57–67.

[15] Satyanarayana U. (2013). Biotechnology. Books and Allied.

[16] Ramírez J. (2012). Aprovechamiento industrial de lactosuero mediante procesos fermentativos. Revista Especializada de Ingeniería de Procesos en Alimentos y Biomateriales, vol. 6, no. 1, pp. 69–83, 2012.

[17] NOM-155-SCFI-2003. (2003). Leche, fórmula láctea y producto lácteo combinado: Denominaciones, especificaciones fisicoquímicas, información comercial y métodos de prueba. Secretaría de Economía.

[18] W. B. Corporation. (1972). Enzymes, enzyme reagents and reacted biochemical. Wort- hington Biochemical Corporation.

[19] NMX-F-527-1992. (1992). Alimentos: Determinación de sólidos totales, sólidos disueltos y sólidos en suspensión en agua. Secretaría de Economía.

[20] NMX-F-083-1986. (1986). Alimentos: Determinación de humedad en productos alimenticios. Secretaría de Economía, 1986.

[21] NMX-F-066-S-1978. (1978). Alimentos: Determinación de cenizas en alimentos. Secretaría de Economía.

[22] Hernández R. (2013). Caracterización fisicoquímica de un producto tipo cajeta elaborado a partir del suero dulce de quesería. Master's thesis, Facultad de Ciencias Químicas de la Universidad Veracruzana.

[23] Miranda O. (2009). Características físico-químicas de sueros de queso dulce y ácido producidos en el combinado de queso de bayamo. Rev Cub Aliment Nutr, vol. 19, no. 1, pp. 21–25.

[24] Loaiza M. (2011). Aprovechamiento del suero de leche para la elaboración de una bebida funcional. Master's thesis, Facultad de Ingeniería y Ciencias Agropecuarias de la Universidad de las Américas.

[25] López C., Zuluaga A., Herrera S., Ruiz A. y Medina V., (2006). Producción de Ácido cítrico con aspergillus niger nrrl 2270 a partir de suero de leche. Dyna, vol. 73, no. 150, pp. 39–57.

[26] Franchi O. (2010). Suero de leche, propiedades y usos. Quesos Maitenes de Occa.

[27] Anupama y P. Ravindra. (2001). Studies on production of single cell protein by aspergillus niger in solid state fermentation of rice brain. Brazilian Archives of Biology and Technology, vol. 44, no. 1, pp. 79–88.

[28] A. M. Torres, J. C. Quintero y L. Atehortua. (2011). Efecto de nutrientes sobre la produc- ción de biomasa del hongo medicinal ganoderma lucidum. Rev. Colomb. Biotecnol., vol. 13, pp. 103–109.

[29] Pisano I., Agrimi G., Grosso G., Concetta M., Ricci M, y Palmieri L., (2015). Improved *saccharomyces cerevisiae* growth on cheese whey by controlling enzymatic lactose hydrolysis. Chemical Engineering Transactions, vol. 1, no. 43, pp. 637–642.

[30] Leal D., Pico Y., Castro J., Guerra J. y Castro G. (2011). Producción de ácido cítrico a partir de suero lácteo entero e hidrolizado con aspergillus niger, por vía fermentativa. Alimentos Hoy, vol. 19, no. 19, pp. 32–38.

[31] Cortés A., Guadarrama L., Díaz M. (2006). Producción de biomasa a partir de *aspergillus oryzae* en cultivo sumergido. Biotecnia, vol. XVI, no. 3, pp. 11–16.

[32] Moeini H, Nahvi I y Tavassoli M. (2004). Improvement of scp production and bod removal of whey with mixed yeast culture. Electronic journal of biotechnology, vol. 7, no. 3, pp. 06–07.

[33] Téllez A., Alvarez G. y Roa A. (1997). Diseño y puesta en marcha de un "sistema semicontinuo en dos etapas: hidrólisis fermentación" para la producción de etanol a partir de almidón de papa usando simultáneamente aspergillus nigery *saccharomyces cerevisiae*. Revista Colombiana de Química, vol. 26, no. 2, pp. 1–10.