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DEVELOPMENT OF TECHNOLOGIES FOR THE SUSTAINABLE REUSE OF WHEY

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Abstract. In Spain, 1,726,000 tons of whey are generated annually. In particular, in Cantabria there is talk of a whey generation of 15,600 tons per year. Whey is considered highly polluting waste if it is dumped directly into the environment given its high content of organic matter. This project sought to develop new methods for the treatment and use of this waste. Separation of the solid fraction from fermented whey can be achieved sustainably and effectively with bentonite. On the other hand, in the composition of the acidified, clarified and sterilized liquid fraction (LCE), no presence of compounds of economic interest for the dairy industry was observed. On the other hand, opportunities were detected to convert it into value-added by-products for the agricultural and canning sector. In the case of agriculture, work was done to obtain a new biostimulant capable of providing minerals, proteins, pH regulation, etc. On the other hand, it is also considered that the food industry market for canned vegetables may be a target market that integrates this by-product as a covering liquid (preservative) and can replace vinegar.

Keywords: whey, vegetable preserves, biostimulant, lactic acid.

DESARROLLO DE TECNOLOGÍAS PARA LA REUTILIZACIÓN SOSTENIBLE DEL LACTOSUERO

Resumen. En España se generan 1.726.000 toneladas anuales de lactosuero. En particular, en Cantabria se habla de una generación de lactosuero de 15.600 toneladas al año. El lactosuero se considera un residuo altamente contaminante si se vierte directamente al medioambiente dado su contenido elevado en materia orgánica. Con este proyecto se buscaba desarrollar nuevos métodos para el tratamiento y aprovechamiento de este residuo. La separación de la fracción sólida del lactosuero fermentado se puede conseguir de forma sostenible y efectiva con bentonita. Por su parte, en la composición de la fracción líquida acidificada, clarificada y esterilizada (LCE), no se observaron presencia de compuestos de interés económico para la industria láctea. En cambio, sí se detectaron oportunidades para convertirlo en subproductos de valor añadido para el sector agrícola y conservero. En el caso de la agricultura, se trabajó en la obtención de un nuevo bioestimulante capaz de aportar minerales, proteínas, regulación del pH, etc. Por otro lado, también se considera que el mercado de la industria alimentaria de conservas vegetales puede suponer un mercado

objetivo que integre este subproducto como líquido de cobertura (conservador) pudiendo sustituir al vinagre.

Palabras clave: lactosuero, conservas vegetales, bioestimulante, ácido láctico

Introduction

The environmental challenge assumed by this project is framed in the management of Industrial Waste within the Waste Plan of Cantabria 2017-2023. The project is framed in the reduction of waste from the cheese industry.

Cheese production in Spain is around 500,000 tons per year (Sainz, 2002 and Martín P. 2021), while in the world it reaches the figure of 17 million tons (Martínez et al. 2020). During the process of coagulating milk to obtain cheese, large volumes of cheese whey are generated, which is the main waste given that for every 100 liters of milk that enter a cheese factory, 80 liters of whey may be produced or, in other words, between 9 and 12 liters of whey are generated for every kilo of cheese produced. This means that in the world a production of 190 million tons of whey is generated annually and in Spain 1,726,000 tons of whey are generated annually and up to 190 million tons in the world (Vázquez et al. 2019).

The aim is to develop new methods for the treatment and use of whey to convert it into two value-added by-products for the agricultural and canning sectors. In this case, the commercial opportunity is perceived to transfer the technologies arising from this project to these sectors and the cheese factories.

In the case of agriculture, the aim is to provide the market with a new biostimulant that also uses the water present in whey. This biostimulant market is still in its infancy (3.5% of arable land) but is growing at an annual rate of 12%, favored by the tendency to increase agricultural land.

On the other hand, the market of the canned vegetable food industry is also considered, which is a mature market, with around 1.5 million tons produced annually and a turnover of 7,000 million euros per year.

Finally, it is believed that the cheese production sector can also take advantage of these technologies given that certain cheese dairies can choose to adapt their production plants to generate added value by-products, with commercial interest for third parties. In this case, it is aimed at the cheese production sector, which has a very relevant specific weight in Spain and also in Cantabria, for it has 43 cheese dairies that are not taking economic advantage of the whey that is generated.

The aim of this research is to take advantage of the liquid fraction of the residual whey from the cheese industry either as a biostimulant liquid or as a covering liquid for vegetable preserves.

Among the characteristics of the biostimulant liquid are being a source of minerals and proteins as well as having an acid pH that allows the bioavailability of other minerals. In addition, it allows the reduction of irrigation water expenditure and, therefore, of the water footprint.

In the case of the covering liquid (preservative), the proposal is to use the new by-product obtained from the whey for use in vegetable preserves, which could replace vinegar. This liquid should respond positively to heat resistance tests, be neutral in flavors and aromas and cost 30% less.

In order to achieve the research objectives, it was proposed to evaluate the fermentation conditions of residual whey from the cheese industry to increase its lactic acid content, to design an industrial processing line to separate the solid and liquid fractions of whey, to process and characterize the liquid fraction of whey enriched in lactic acid for its use in the canning industry, and to process and characterize the liquid fraction of whey enriched in lactic acid for its use as a biostimulant for agricultural crops.

Method

The objective of this research is to chemically and microbiologically characterize the whey to identify if there is any parameter that limits the subsequent development of fermentations.

The whey was supplied by a local cheese company that uses pasteurized cow's milk for the production of fresh cheese (sweet whey). Therefore, it was not possible to perform microbiological characterization from a sterilized product.

For the different trials the company provided 20 liters at two different times. The values shown in the rest of the article correspond to the overall average value.

For the chemical characterization, in addition to some parameters indicated in RD 140/2003 on water for food use (including those of the agri-food industry), other milk quality parameters were measured that could affect subsequent fermentation, such as hydrogen peroxide, the presence of which could be due to a health problem in the cow. The physicochemical parameters considered were:

- Cations and anions: ammonium, nitrite, nitrate, and phosphates.
- Minerals: nitrogen, calcium, potassium, iron, and phosphorus.
- Others: pH, Lactic Acid, and BOD (Biological Oxygen Demand)

The physicochemical methods applied were spectrophotometric, potentiometric, and volumetric. The following table shows in detail the distribution of the methods by parameters.

Table 1
Characteristics of the methods of analysis (I)

Parameter	Method of analysis	Observation
Ammonium	Spectrophotometric (λ = 690 nm)	Hypochlorite and phenol
Nitrite	Spectrophotometric (λ = 543 nm)	Sulphanilic acid and N-(1-naphthyl)-ethylenediamine dichlorohydrate
Nitrate	Spectrophotometric (λ = 220 nm)	2,6-dimethylphenol
Phosphate	Spectrophotometric (λ = 470 nm)	Vanadate-Molybdate
Chloride	Spectrophotometric (λ = 530 nm)	Mercury (II) thiocyanate
Nitrogen	Volumetric	Kjeldahl Method
Calcium	Spectrophotometric (λ = 570 nm)	Glyoxal-bis(2-hydroxyanil)
Potassium	Spectrophotometric (λ = 500 n)	Tetraphenyl borate
Iron	Spectrophotometric (λ = 510 nm)	Triazine and thioglycolate
Phosphorus	Spectrophotometric (λ = 400 nm)	Vanadate-Molybdate
Hydrogen peroxide	Spectrophotometric (λ = 528 nm)	Phenanthroline
pH	Potentiometry	-
Acidity	Volumetry	Valuation up to color change
BOD	Spectrophotometric (λ = 620 nm)	Ramirez's approach (1992)

Once the methods of analysis had been identified, the spectrophotometric methods were calibrated by determining:

- The linearity range, which will give information on the minimum and maximum concentration of the analyte that the determination method can be applied to (see Figure 1).
- The equation relating absorbance to concentration and its goodness of fit. Goodness of fits close to 1 were sought throughout because this implies that the relationship between concentration and chemical signal is of "high quality."
- The limit of detection and quantification. These were measured from a blank (distilled water) to which the same procedure was applied as if it were a whey sample. With these values, a mean value and deviation were determined, which were used to determine both limits by means of the following expressions (see Figure 1):
  - Detection limit: mean ± 3· deviation
  - Limit of quantification: mean ± 10· deviation

Different solutions were made at the same concentration of each analyte, and their real value was compared with the theoretical value. The ideal is the smallest possible error values. It was decided to set the maximum threshold value for the error to be less than 5%.

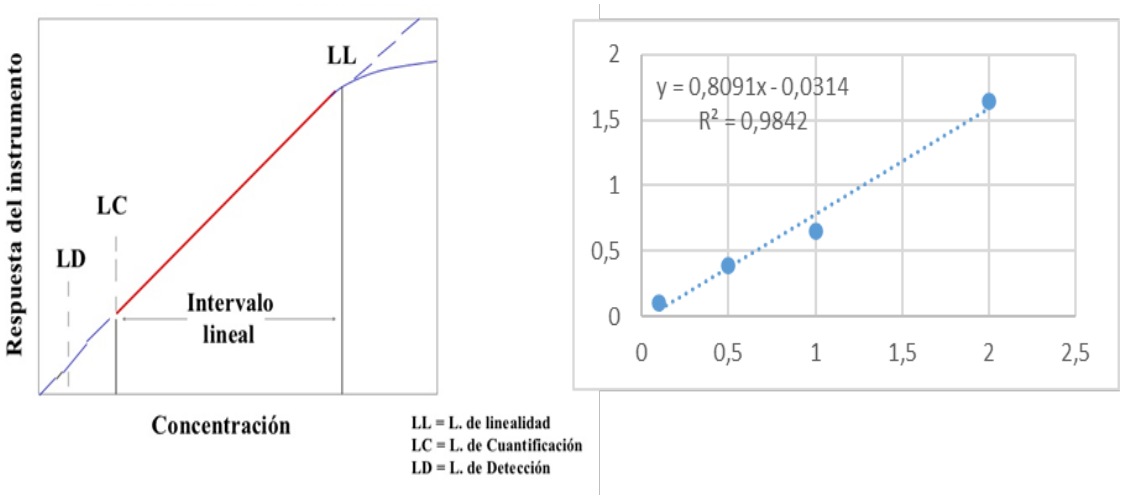

Figure 1. (A) scheme of calibration parameters, and (B) Nitrite curve obtained in the project.

Results

The following table shows the calibration values of the different parameters measured spectrophotometrically:

Table 2
Characteristics of the methods of analysis (II).

Parameter	Equation	Setting	Limits (mg/L)		Error (%)
Ammonium	y = 0.0362x + 1099	0,9996	1,01	3,50	2,24
Nitrite	y = 0,8091x - 0314	0,9842	0,11	0,15	3,42
Nitrate	y = 0,0014x - 0111	0,9836	154,6	398,8	0,87
Phosphate	y = 0.0217x + 0081	0,9981	0,17	0,89	1,38
Chloride	y = 0,0106x + 2429	0,9951	0,06	0,11	3,14
Calcium	y = 0.0075x	0,9893	3,42	7,22	1,55
Potassium	y = 0.0004x + 0095	0,9827	26,8	55,5	7,50
Iron	y = 0.2701x + 0.036	0,9987	0,05	0,12	1,01
Phosphorus	y = 0,4474x + 0383	0,9999	0,01	0,04	0,22
Hydrogen peroxide	y = 0,1717x + 7587	0,9739	0,01	0,02	1,69

It can be seen how the goodness of fits are close to 1 and with errors lower than 5%, except for potassium, which had an error of 7.5%. Several attempts were made to adjust the method, but it was not possible to reduce the error, so it was decided not to include this parameter in the successive analyses.

Once the characterization of the starting whey was carried out, the pH of the whey was reduced by means of a fermentation process that increases the lactic acid content. The fermentations were carried out with two bacteria widely used in the dairy industry:

- *L. acidophyllus*
- *E. faecium*

The following tables show the results of the fermentation test in terms of pH and lactic acid evolution. Day 0 corresponds to the day of inoculation, and thus to the initial pH and lactic acid value of the whey. These original pH values are within the range that most of the literature reviews consulted show for sweet whey (Villota et al 2015; Instituto Nacional de Tecnología Industrial - INTI, 2017).

The following tables show the data for both bacteria (with whey supplementation, expressed with the + symbol and without supplementation, expressed with the - symbol). For both bacteria, the pH was reduced more in the unsupplemented medium and between day 2 of fermentation and day 3, the decrease in pH was not as high as between day 1 and day 2. In addition, comparing between bacteria, the lowest pH was obtained for the case of *L. acidophilus*, so it was concluded that the characteristics of the fermentation for the objective sought in this project were inoculation with 2% of *L. acidophilus* directly on the whey and 48-72 hours of fermentation.

Table 3
pH evolution during fermentation (+: supplemented medium, -: unsupplemented medium).

Fermentation	**Day 0**	**Day 1**	**Day 2**	**Day 3**
E. faecium +	4,93	4,46	4,33	4,3
E. faecium -	5,4	4,55	4,28	4,22
L. acidophilus +	6,21	4,2	4,09	4,03
L. acidophilus -	6,53	4,13	3,98	3,87

If the results obtained for acidity are analyzed, it is observed that there are almost zero values from day 0 (because it is a sweet whey), and that its content increases with the days of fermentation until it reaches 12.15 g/L in the case of unsupplemented *L. acidophilus.*

Once the fermentation conditions were identified, a comparison of the chemical composition of the original whey and the same whey fermented with both bacteria for 48 hours was carried out (see Table 4).

Table 4
Chemical composition of the original whey and after fermentation (mg/L).

Parameter	Original whey	*E. faecium*	*L. acidophilus*
Iron	9,25 ± 1,12	1,71 ± 0,37	2,55 ± 1,08
Calcium	85,36 ± 15,11	54,18 ± 13,59	63,06 ± 22,80
Phosphorus	14,86 ± 1,63	47,73 ± 2,25	47,99 ± 5,36
Ammonium	173,34 ± 19,29	439,22 ± 24,41	411,94 ± 51,27
Nitrite	3,08 ± 0,12	0,68 ± 0,11	1,04 ± 0,35
Phosphate	235,36 ± 16,05	151,03 ± 13,28	153,51 ± 16,93
Chloride	341,34 ± 37,20	242,33 ± 9,69	255,61 ± 12,09
Nitrogen (%)	0,14±0,00	0,17±0,01	0,17±0,01
Hydrogen peroxide	3,07 ± 1,99	-	-

The acidified whey obtained is a mixture of solids (including the remains of microorganisms) and water. The purpose was to separate both fractions so that the liquid part (of interest in this project) could be applied for the generation of a biostimulant liquid or a preservative liquid. For this, we proceeded to the development of two separation tests:

- Test 1: physical filtration by means of membrane and inert earth (such as celite, zeolite, or others of interest).
- Test 2: phase separation using fining agents frequently used in the food industry (bentonite, albumin, etc.).

Before carrying out the tests with a high volume of fermented whey, for the case of test 1, a small filtration rate test was carried out through laboratory filter paper of different thicknesses (similar to a membrane). This paper clogged very quickly, making it almost impossible to improve the process even by applying vacuum filtration. For this reason, the filter paper was replaced by a bed of celite, but it was observed that although initially the process occurred at an adequate rate; the celite bed was compacted preventing the passage of more whey. On the other hand, when trying to separate the fraction of solids from the whey that remained on the top of the celite bed, part of this soil was dragged so it was not possible to obtain a product of a certain purity.

Due to these difficulties, all efforts were focused on the use of clarifying agents such as bentonite and albumin. As a first step, dose-response tests were carried out using as a starting product the whey acidified by L. *acidophiluse* previously pasteurized to stop the fermentation process after 48 hours, destroying all the microbiological load that could cause any problem in the subsequent development of the task.

The dose-response assay was performed as follows. The same amount of this acidified and sterilized whey was placed in a collection of falcon tubes and increasing concentrations of albumin and bentonite were added in triplicate to each tube.

To evaluate the efficiency of one product with respect to the other, the loss of color was measured (see Figure 2) and the evolution of the yellow color at 420 nm, similar to how this color is measured in wine. The process lasted 24 hours and, as can be seen in Figure 2, there was no loss of color with albumin, quite the opposite to what was achieved with 5% bentonite.

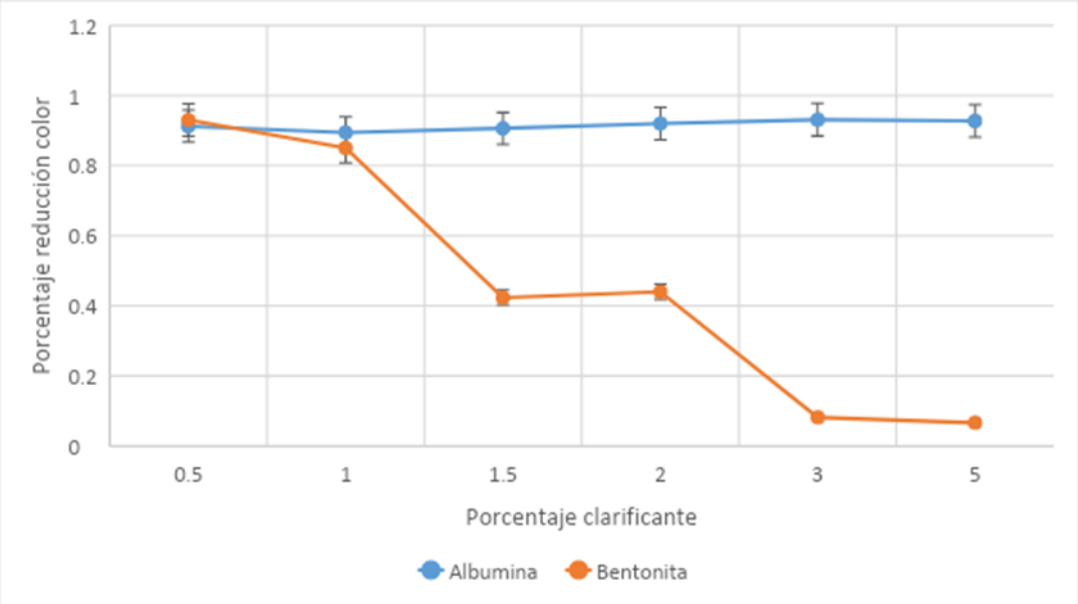

Figure 2. Loss of color after clarification.

The amount of precipitated product was also measured as a measure of the efficiency of the degree of separation (see Figure 3). It can be seen that in the case of bentonite, in addition to a greater decrease in color, there is also a higher percentage of solids with respect to albumin.

Figure 3. Estimation of the percentage of solids and liquids after clarification.

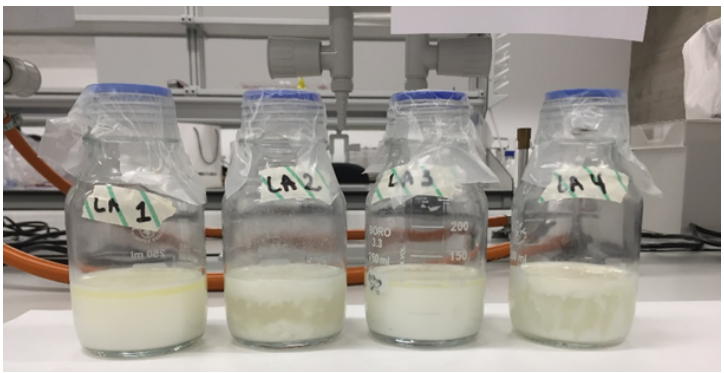

Figure 4. Detail of phase separation before clarification (upper image) and after clarification (lower image).

Once the clarified product was obtained, deodorization was carried out in order to eliminate the traces of lactic odor. For this purpose, the fermented whey was passed through a column of activated carbon, although it was observed that part of the activated carbon was dragged and darkened. Given this result, it is proposed that, for future occasions, the best option is to use skimmed whey. This whey skimming process is already common in some dairies to obtain the fat fraction needed to make whey butter. If this equipment is not available, another possible option would be to carry out sequential clarifications to eliminate as much protein as possible from the whey and the fat fraction would go with it.

Subsequently, an analysis was made of the results obtained previously and the uses to which the final product is going to be put: liquid for preserves and biostimulants. The proposal made is shown in the following figure, which includes some results of later tasks:

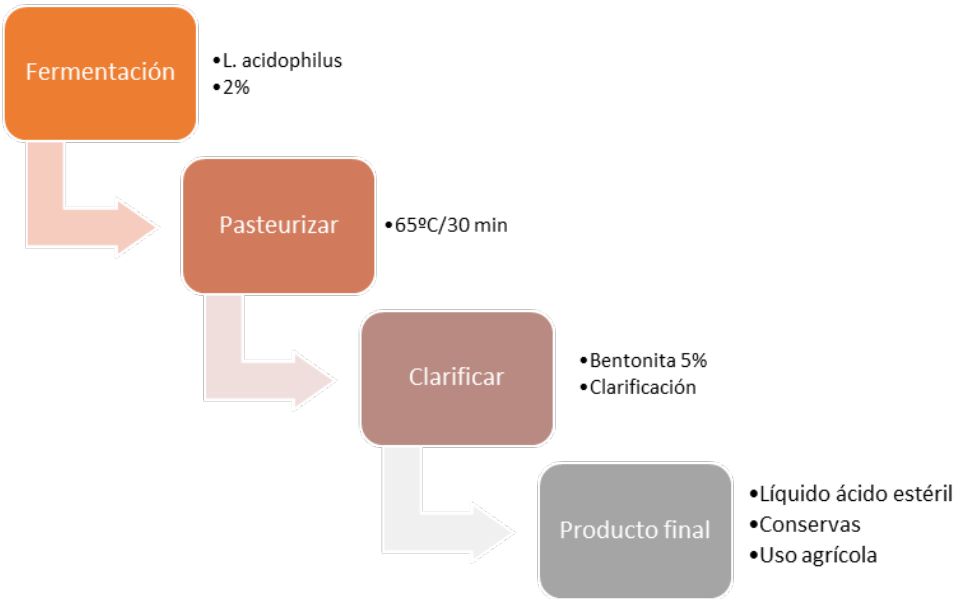

Figure 5. Scheme of whey treatment to obtain an acidified liquid fraction.

- STEP 1: Start with pasteurized or sterilized skimmed whey. In this way the fat fraction is eliminated from the beginning and the possible presence of microorganisms that may affect the subsequent fermentation.
- STEP 2: Ferment the whey with *L. acidophilus*, inoculating at a concentration of 2% for 48 hours. Fermentation can be stopped before that time depending on the final pH or lactic acid content.
- STEP 3: Stop fermentation by VAT or LT-LT pasteurization (65ºC for 30 minutes).
- STEP 4: Clarification with bentonite. Although in this project the effective bentonite concentration was 5% for 24 hours, the fact is that the clarification time and the percentage of the product to be used is carried out by means of a dose-response test because it depends on the degree of turbidity of the starting whey.

From this point on, the acidified, clarified, and sterilized whey (hereinafter called LCE) can be put to different uses:

As canning liquid: a double effect of the pH of the medium was observed. On the one hand, a negative effect by increasing the rate of decomposition of borage (product used for the test), but on the other hand, a lower oxidation of the same was observed, which is positive and suggests a different use than the one given in this project.

On the other hand, as a biostimulant: it was observed that it is necessary to raise the pH to avoid stress responses in plants. This can be done by stopping fermentation at pH values close to 5 and increasing the pH with some carbonate or hydroxide. In this case, it can never contain sodium because it would have a detrimental effect on the soil structure. In this project, the pH was increased with KOH but it could be of interest in the future to try other pH regulating agents.

The following table shows the chemical composition of LCE prior to its use as a preserving liquid and biostimulant.

Table 6
Chemical composition of the original whey after fermentation and after clarification (LCE) (mg/L).

PARAMETER	**Original whey**	***L. acidophilus***	**LCE**
Iron	9,25 ± 1,12	2,55 ± 1,08	0,40± 0,03
Calcium	85,36 ± 15,11	63,06 ± 22,80	45,27 ± 3,21
Phosphorus	14,86 ± 1,63	47,99 ± 5,36	22,75± 4,08
Ammonium	173,34 ± 19,29	411,94 ± 51,27	122,47 ± 52,40
Nitrite	3,08 ± 0,12	1,04 ± 0,35	0,05±0,03
Phosphate	235,36 ± 16,05	153,51 ± 16,93	91,89 ± 0
Chloride	341,34 ± 37,20	255,61 ± 12,09	183,93± 1,40
Nitrogen (%)	0,14±0,00	0,17±0,01	0,17±0,01
Hydrogen peroxide	3,07 ± 1,99	-	1,30 ± 0,44

It is observed that the chemical composition of the LCE with respect to the whey only acidified with *L. acipophilus* decreases, except for the case of nitrogen for which it remains constant. This may be due to the fact that bentonite is a clay with mineral retention capacity (Navarro Blaya and Navarro García, 2003) and when it precipitates

together with proteins and fat, it drags part of these compounds with it. In some cases, the losses are greater than 50% as in iron, phosphorus, ammonium, phosphate, and hydrogen peroxide.

A study was also carried out on the heat resistance and lifespan for its application in the canning industry. The thermoresistance study, aimed at determining which of the thermal sterilization systems currently used in the industry, is compatible with the characteristics of the new governing liquid. It also made it possible to validate whether the composition of the LCE can promote the development of microorganisms and whether the conventional thermal treatments are insufficient, so that some type of adjustment in terms of times and temperatures has to be considered. *St. Aureus* was selected to carry out this test. Three government liquids were also prepared: a standard one (water, sodium chloride, and acidified), LCE, and LCE supplemented with salt. It should be noted that the LCE is already acidified by the fermentation process itself.

Once *the St. Aureus* strains were reactivated, we proceeded to inoculate the three previous types of government liquid, and we determined if the pathogen grew effectively or if, on the contrary, the characteristics of the three liquids had inhibited its development, which could lead us to consider positive results when in fact they are not.

The following table shows that the inoculation with *St. Aureus* was effective, and that the acid character or salt concentration did not inhibit the growth of the bacteria, so the results obtained are only a consequence of the effect of the combination of temperature and time.

Table 7
St. Aureus count (cfu/100 µL) in the three types of government fluid.

Replica	**LCE**	**LCE+NaCl**	**Standard**
1	$9{,}5\text{-}10^3$	$2{,}5\text{-}10^3$	$1{,}05\text{-}10^4$
2	$9\text{-}10^3$	$7{,}5\text{-}10^3$	$1{,}55\text{-}10^4$
3	$1{,}25\text{-}10^4$	$1{,}35\text{-}10^4$	$1{,}65\text{-}10^4$

For the thermoresistance test, three types of thermal treatments were used in the previous government liquids (confirmed the existence of *St. Aureus*): VAT or LT-LT pasteurization (65ºC/30 min), HT-ST pasteurization (72ºC/15 s), and classic sterilization (120ºC/20 min).

Both for this case and the previous one, specific chromogenic medium was used for *St. Aureus*, whose colonies are shown as pink/purple dots. The green colonies correspond to *St. Epidermidis,* which is not considered a pathogen.

At the end of the thermal treatments, it was observed that the load of this bacterium was practically null in the two pasteurization systems and null in the sterilization one. No color formation was observed as a consequence of caramelization reactions of the possible residual lactose, which indicates that this sugar was practically transformed into lactic acid. Neither was the appearance of undesirable odors observed. With all this, it was possible to confirm that whatever the heat treatment system used by a canning company, LCE can be used.

The other test that was carried out was the study of the shelf life of the canned products with LCE as the governing liquid, comparing it with the standard governing liquid. It was decided not to use LCE supplemented with sodium chloride because its composition of salts would make the information redundant.

Three temperatures were chosen for the shelf-life study: 5 ºC, 20 ºC, and 5 ºC (with a humidity of 60%). For the first case, the preserves were kept refrigerated; the second case, at room temperature; and for the third case, a thermal cabinet with humidity control was used.

The preserves were made with borage, a typical vegetable from Aragon that was interesting for two reasons. Its green color helped to disguise the initial color of the LCE and, in addition, it slightly reduced the milky smell that could not be eliminated during processing.

Figure 6. Borage preserves in LCE (left) and control (right).

Figure 6 shows the slight initial turbidity of the canned water with LCE compared to the control. The test lasted 14 days, and the parameter chosen for monitoring was turbidity measured at 420 nm as is done for drinking water, and because it is a variable that would make the canned product lose commercial value.

Table 8
Values of the turbidity of the governing liquid on the different days considered.

Temperature	Time (days)	Control	LCE
5ºC	0	0,0583	0,0779
	4	0,0746	0,1051
	7	0,2869	0,1105
	10	0,3142	0,1676
	14	0,4722	0,2563
55ºC and 60%. humidity	0	0,0583	0,0779
	4	0,4209	0,4040
	7	0,4975	0,4068
	10	0,6175	0,6175
	14	0,6813	0,6321

Table 8 shows how the turbidity increases are greater in the control than in the LCE for the case of temperature at 5 ºC, while at 55 ºC the values are relatively similar. In addition, it is observed that the values at 55 ºC are higher than at 5 ºC. This last phenomenon is due to the fact that at a higher temperature the biochemical processes are

accelerated, which can be linked to a greater decomposition of the borage due to the effect of the lactic acid, and thus to a greater turbidity.

With respect to the possible nutritional biostimulant effect in plant products for application in the agricultural industry, it was analyzed how the composition of the biostimulant varied throughout the development of the crop and the response of the plant. The test that was proposed for this consisted of adjusting the pH to 5.5, with the addition of potassium hydroxide to the LCE since at these values there could be toxicity problems due to the possible aluminum in the medium. With this information as a starting point, the subsequent trials involved the comparison between LCE without modifying the pH, LCE with the pH adjusted and water as a control. It was observed that the plants irrigated with LCE without adjusting the pH presented symptoms of stress that could be due to the toxicity of aluminum or for developing in acid medium, in addition to the plants entered a stage of non-recovery. This forced to shorten the development of the task. For the test, lettuce seedlings were sown on organic substrate. Water was applied directly at a dose of 30 ml/pot every 4 days until it was considered that the trial should end due to the state of the plants irrigated directly with LCE.

On the other hand, it was observed that the plants irrigated with LCE with adjusted pH responded very positively, which confirmed the biostimulant effect of the liquid fraction of the whey for agronomic purposes. This statement is not only based on observation but also on the mean values of the parameters analyzed in the seedlings.

Figure 7. Developmental stages of lettuce seedlings irrigated with LCE without pH adjustment (upper left); with water (upper right); and with LCE with pH adjusted (lower left); and a comparison between them (lower right).

Table 9
Composition of some nutritional parameters of lettuce seedlings.

Sample	Weight (g)	Humidity (%)	Ash (%)	Ascorbic acid (mg/100g)	Nitrogen (%)	Calcium (mg/100g)	Phosphorus (mg/100g)
Control	2,89±0,85	94,58±0,42	0,85±0,31	11,70±1,28	0,17±0,02	1,72±0,58	2,93±0,09
LCE (without pH adjustment)	5,07±1,31	91,41±0,34	1,47±0,85	7,77±1,11	0,27±0,01	0,58±0,14	2,78±1,07
LCE (pH adjusted)	2,20±0,73	91,75±0,74	0,77±0,38	12,31±1,42	0,14±0,01	1,05±0,81	2,55±0,27

Although at this stage it is not possible to compare these results with those of the bibliography since the plants had not reached commercial maturity, which is the point where they are harvested and their chemical composition is determined, it is necessary to highlight the following results:

On the one hand, it was observed that the mineral content (determined through the ash content) was higher in plants irrigated with LCE of adjusted pH. This suggests a higher utilization of minerals with respect to the other cases, especially the control. Although the latter had higher values of Ca and P. The difference is in the nitrogen content, which was much higher in the case of the pH-adjusted LCE. On the other hand, the ascorbic acid content was lower for the pH-adjusted LCE. This, far from being a negative aspect, is an indicator that the plant was in a situation of lower stress since ascorbic acid (vitamin C), like any other antioxidant compound, is synthesized in greater quantity by plants if they are stressed. These results confirm that the liquid fraction of whey, after processing, can have a positive agronomic use.

Discussion and conclusions

Based on the results of this project, it can be concluded that among the acid lactic acid bacteria compared to carry out the fermentation of sweet whey from the production of fresh cow's cheese, *L. acidophilus* produced the best result when inoculated at a concentration of 2% and without the requirement of nutritional supplementation. Fermentation and lactic acid production occurred in 48 hours.

On the other hand, the separation of the solid fraction of fermented whey from the liquid fraction can be effectively achieved with bentonite. It is necessary to carry out dosage tests to adjust the exact amount of this clay to be used. In addition, it has been observed that the use of bentonite also implies that the minerals are distributed between the solid and liquid fractions of the fermented whey. The separation of the solid fraction (rich in protein and fats and minerals) from the liquid fraction (water and minerals) through the use of bentonite affirms that this methodology is sustainable (in environmental and economic terms as no chemical processes are used) and easy to apply by small and medium-sized cheese dairies.

Another thing that has been observed is that in the composition of the acidified, clarified, and sterilized liquid fraction (LCE), no compounds of economic interest for the cheese factory were observed, such as protein or fat, and its composition allows it to be

discharged without producing an environmental problem as it lacks lactose, fat, and protein (compounds responsible for its BOD).

As far as the use of LCE as a governing liquid for vegetable preserves is concerned, it has been shown as a potential agent capable of being compatible with the usual thermal treatment systems present in cheese factories (pasteurization and sterilization). In addition, LCE as a liquid of government for canned vegetables, has been shown as a potential agent able to inhibit the enzymatic oxidation processes in borage, possibly due to polyphenol oxidase. Finally, LCE as a component for the formulation of plant biostimulants has been shown as a potential agent able to improve the response of the plant after a process of pH adjustment towards neutral values.

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