Production of soft thermo-acid cheeses has a series of advantages compared to the production of hard rennet cheeses, namely: it requires less consumption of raw materials, the raw materials are not subjected to specific requirements in terms of cheese making. When they are produced, such technological operations as renneting the milk, clot cutting, setting the grain, maturing, are shortened. This significantly reduces the labor intensity of the technological process, and provides for the fast turnover of invested funds at smaller production costs [2].

1. Introduction

The development of healthy food trends has resulted in the increased popularity among consumers of thermo-acid cheeses such as Ricotta, Ricotone, Mascarpone, Adyheyskyy, etc [1]. Cheeses that are made by the thermal-acid settling of raw milk are characterized by good consumer properties, have high biological value and lower cost compared to hard cheeses.
That is the reason why interest in the technology of thermo-acid cheeses is not fading from both scientists and manufacturers.

The technology of Adyheysky cheese implies using, as an acid agent (coagulant), acidic whey. The process of its fermentation, before achieving a proper acidity (150...180 °T), can last up to 48 hours [3], which significantly increases the technological cycle duration, requires certain production areas for the preparation and storage of the coagulant. Advancements involving the intensification of thermo-acid cheese making, specifically preparation of milk whey-coagulant without the use of organic acids and food additives, are extremely rare. There are no data on the application of innovative methods for treatment of milk whey, which would aim at forming its target properties that could ensure the acceleration of fermentation when making thermo-acid cheese.

A promising direction to improve the technology of thermo-acid cheese is to find ways to intensify the production of acidic whey by creating in a fermented environment favorable conditions for cultivating lactic acid microflora. This can be achieved by enriching milk whey with mineral elements that act as a nutritious medium for microorganisms, contribute to the growth of biomass of lactic acid bacteria and intensify the process of acid formation. Such biologically valuable mineral elements for lactobacilli are magnesium and manganese. Therefore, it is a relevant task to study the impact of milk whey, enriched with these elements, on the process of fermentation and the quality of thermo-acid cheese.

2. Literature review and problem statement

Work [4] substantiated the technological aspects and modes of enrichment of the thermo-acid cheese mass with lactic acid microflora in order to improve the biological value of the product. The author paid attention to the selection of a fermenting culture to obtain a fermented environment, milk ripening before thermo-acid settling, to studying the influential fermentation factors on the properties of cheese mass, cheese yield, and its shelf life [5]. However, the issue of preparing acidic whey and reducing the duration of a given technological operation was not considered in works [4, 5] because the object of research was the technology of thermo-acid cheese, rather than coagulant.

The author of a study related to this issue [6] examined basic regularities in the thermo-acid settling of milk using various coagulants and developed a scientific base for the technology of soft cheese made from skimmed milk and whey cream. The study established the regularities and regimes of the thermo-acid clotting of milk proteins as coagulants of lactic, chloric, acetic acids and milk whey. Their influence on the degree of use of milk proteins, and the rheological and synergetic properties of clot were studied. The proposed approaches, along with increasing the biological value of cheese, made it possible to reduce the production cycle and receive a significant economic effect. However, the use of inorganic and organic acids, especially chloric acid, is unacceptable from the point of view of safety of the finished product. And the cost of these coagulants exceeds the cost and expenditures for making acidic whey.

Paper [7] reports a technique to increase the output of finished products by using, as a coagulant, a mixture of fruit and berry juices and whey. The paper’s authors proved that such cheeses are characterized by good organoleptic properties. The resulting product does exceed the product range of thermo-acid cheeses, but it cannot be attributed to traditional ones.

Study [8] gives a comparative analysis of the composition and properties of mozzarella cheese, made with the use of rennet enzyme and kiwi juice as a coagulant. It is shown that cheese output in case of application of kiwi juice is lower compared to the yield of cheese produced with a curd clot. However, the use of juice enriches the product with biologically valuable components and gives functional properties to the product. The proposed product extends a product range of cheeses for functional purposes, it does not require long time to prepare the coagulant, but its cost is higher than the use of acidic whey as a coagulant.

It is known that an increase in the biomass of lactobacilli and the intensification of acid formation require mineral elements in an accessible form. Microbial cells require, to live and grow, macro-elements in large quantities: carbon, nitrogen, oxygen, hydrogen, phosphorus, sulfur, potassium, calcium, magnesium, as well as micro-elements: zinc, manganese, sodium, chlorine, molybdenum, ammonium, etc. [9, 10].

The milk whey is rich in the specified macro- and micro-elements [11], although the amount of magnesium and manganese is insufficient to nourish the microorganisms. Magnesium and manganese take part in the catalytic action of many single- and two-component enzymes [12]. These elements can activate and stabilize the effect of almost all enzymes of the yeast cell and stabilize the cell membrane [13]. Magnesium and manganese accelerate the increase in the biomass of microorganisms, as part of the nutrient environment, prevent the cells from autolysis and they are necessary for the normal course of metabolic processes. It is known that these elements are the growth factors for lactobacilli and yeast [13] and contribute to the managed process of carbohydrate catabolism, especially during alcohol fermentation, etc. [14].

In [15], it is shown that the enrichment of microorganisms’ strains Lactobacillus rhamnosus and Lactococcus lactis with magnesium ions using the pulse electric fields did not exert a negative effect on the structural and mechanical properties of dairy products. At the same time, there was a positive effect on the adhesive properties of the product and the viability of microorganisms even after freezing. The above confirms the expediency of enriching milk whey with magnesium and manganese in order to accelerate fermentation.

Replenishment of a deficit of mineral substances in food raw materials and finished products is typically achieved by the introduction of salts of inorganic acids. However, most known preparations poorly dissolve in water, have an unpleasant bitter taste, which can negatively affect the organoleptic properties of food products and is unacceptable in terms of food enrichment requirements [16]. In addition, magnesium and manganese in the inorganic compounds have low biological accessibility, while, as is known, the main features of bio-elements include their high digestibility and an appropriate form of existence in the body [16].

Studies in the field of nanotechnology prove that the factor of influence on the increase in biological activity of mineral elements is a particle size [17]. Modern scientific achievements open up wide avenues for the production and use of new forms of nanopreparations made from biogenic metals. These include the hydrated or citrated nanopar-
The aim of this study is to substantiate the appropriateness of the use of electrospark treatment of whey in a reactor chamber with a current-conductive layer of magnesium and/or manganese in order to intensify its fermentation and reduce the duration of the production cycle of thermo-acid cheeses.

To accomplish the aim, the following tasks have been set:

- to study in the comparative aspect the process of fermentation of whey, treated with electrospark discharges in a reaction chamber with a current-conductive layer of magnesium and/or manganese, and to justify the duration of electrospark treatment in order to ensure the intensification of fermentation of milk whey;

- to examine the influence of the obtained acid whey on the qualitative indicators of thermo-acid cheese the type of “Adyheyskyy”.

4. Materials and methods to study the effect of electrospark treatment on the fermentation of whey and quality of thermo-acid cheese

4.1. Examined objects and materials used in the experiment

We have chosen, as an object to investigate the fermentation process during whey-coagulant production, the milk whey received by the thermo-acid settling of cheeses, which is considered the most susceptible environment to cultivate lactic acid bacteria [23, 24].

Before fermentation, the whey was filtered, treated in an electrospark discharge chamber by one of the following variants:

1) with a conductive layer of manganese granules (hereinafter, sample 1);

2) with a conductive layer of magnesium granules (hereinafter, sample 2);

3) with a conductive layer of granules of magnesium and manganese (hereinafter, sample 3).

The electrospark treatment technique was implemented at an experimental laboratory installation. It is composed of a thyristor generator of discharge pulses, a reaction chamber with the magnesium or manganese electrode systems and a conductive layer of granules of corresponding metals, and a control unit.

The capacitor of 25 to 100 µmF was used as an energy accumulator.

Voltage supply to the main electrodes induced the passage of an electric current around a circle of freely located granules of metals under a mode of stochastic commutation. Using low-voltage and small interelectrode gaps makes it possible to enable modes under which up to 85% of all accumulated energy on the capacitor is used for the local heating of the surface of contacting granules and their dispersing [20].

The duration of electrospark treatment in the discharge chambers varied from 30 to 120 s.

Next, the treated milk whey was pasteurized, cooled, and fermented by pure cultures Lactobacillus acidophilus. The fermentation was carried out at temperature 40± 2 °C over 36 hours.

To prepare acidic whey, we chose pure Lactobacillus acidophilus cultures, characterized by a high level of acid formation (the limit of acid formation reaches above 300 °T), which is important for the treated whey with an elevated pH due to accumulation of particles of metals. At the same time, this culture is an active antagonist relative to foreign microbiota and can ensure safety of the finished product [4].

The control used was the fermented milk whey prepared without the involvement of electrospark treatment of raw materials (hereinafter, control).

4.2. Methods to study the properties of fermented whey and thermo-acid cheese

The organoleptic and physical-chemical parameters of milk whey and thermo-acid cheese were determined by standard methods.

Metal content in the samples of whey and cheese was determined at the laboratory of analytical chemistry and toxic substances monitoring DU “Institute of Labor Medicine by NAMS of Ukraine” by an atomic emission spectrometry with an inductive-bound plasma at the device Optima 210 DV (Perkin Elmer, USA).

The particle size was determined at the analyzer of particle dispersion Malvern Instruments Ltd., UK, with a detection angle of 173°, by a helium-neon laser He-Ne with a capacity of 4 mW at a wavelength of 633 nm. The samples were placed in disposable cuvettes made of polystyrene. All measurements in this study were carried out at 25 °C. To control the reproducibility of results, we performed at least three or five measurements for each sample. The size distribution in intensity units was derived from an analysis of correlation functions using a General Purpose algorithm in the software for the analyzer Zetasizer Software 6.20.

We determined pH at the universal ion meter I-160 M; the oxidation-reduction potential by a platinum electrode at the universal ion meter EV-74.

The number of lactic acid bacteria was determined at the laboratory of analytical chemistry and toxic substances monitoring DU “Institute of Labor Medicine by NAMS of Ukraine” by an atomic emission spectrometry with an inductive-bound plasma at the device Optima 210 DV (Perkin Elmer, USA).

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5. Results of studying the effect of electrospark treatment of whey on fermentation duration and cheese quality

It was established that under the condition of electrospark treatment with an exposure from 30 to 120 °C whey
demonstrates an increase in the content of magnesium and manganese depending on the duration of treatment. Thus, the magnesium content increases, on average, by 1.8 to 4 times, and the content of manganese by 1.5 to 3.8 times.

We noted the growth in pH and a change in the redox potential in the treated whey (increased antioxidant properties). This may testify to the progress of the $\text{M} \rightarrow \text{M}^{n+} + ne$ process in the system; as well as a possible complexation between metal ions and bioligands that are present in the milk whey. The change in pH and redox potential indicates the formation of substances possessing antioxidative (restorative) properties, for example lactobionic acid [25]. And with an increase in exposure such properties become more pronounced.

Dispersion analysis of whey samples has established that the average size of particles in whey was significantly different before and after treatment.

It is known that the efficiency of catalytic action of metals on the oxidation of lactose may depend on the size of the metal particles dispersed in whey [17]. Therefore, we additionally established the average hydrodynamic size of magnesium and manganese particles in aqueous solutions obtained from the electrospark treatment of similar whey parameters. It was established that colloidal solutions of magnesium and manganese had particles in a nano-(about 30 nm) and micro-dimension range (from 100 nm to 10 µm). In this case, the average particle size in the colloidal solution of magnesium was 118±5 nm, manganese 270±11 nm.

To establish the effect of electrospark treatment on the acid-forming properties of $\text{Lbc. acidophilus}$ we determined the titrated acidity of milk whey every 6 hours of fermentation at a temperature of 40±2 °C.

The dynamics of changes in the titrated acidity in the studied samples of fermented milk whey, depending on the initial parameters, are shown in Fig. 1–3.

It was determined that it was characteristic of all the samples studied to demonstrate a natural growth of titrated acidity during fermentation. However, the samples enriched with mineral elements as a result of electrospark treatment over 30...60 s demonstrated a more intensive growth of titrated acidity ($\Delta T$) over time. Thus, in 24 hours of fermentation, $\Delta T$ in samples No. 1 amounted to 140 to 169 °T depending on the duration of treatment; in samples No. 2 – from 122 to 136 °T, in samples No. 3 – from 140 to 171 °T. Whereas in control, in 24 hours of fermentation, the titrated acidity increased only by 110 °T.

In samples No. 1 and 3, the start of fermentation occurred slower, and in samples No. 2 – 30...60 s after electrospark treatment.

It was established that the highest acid-forming activity was demonstrated by $\text{Lbc. acidophilus}$ in the whey that was treated in a discharge chamber with current-conductive Mn granules over 60 s, and in the whey enriched with both mineral elements.
In this case, it is necessary to emphasize the synergistic effect of magnesium and manganese on fermentation intensity due to a growth in the important nutrient micro- and macro elements for construction of components of living cells of lactobacilli in an accessible form.

A slower growth in acidity in the samples enriched with magnesium can be explained by the fact that whey, over 30...60 s of treatment, is enriched with Mg in quantities insufficient to cultivate *Lbc. acidophilus*. An increase in the time of treatment and, accordingly, the amount of the valuable microelement, leads to an increase in both the medium’s pH and the number of microparticles, which has a negative effect on the fermentation process.

It was found that the whey enriched with magnesium and/or manganese acquires proper acidity (150...170 °T) for the settling of proteins in thermo-acid cheeses making in 12...18 hours, whereas control – after 30 hours of fermentation.

The obtained results for the intensification of fermentation are confirmed by the study into the number of lactobacilli in the experimental samples of fermented whey (Table 1).

### Table 1

| Sample title                              | Quantity of lactobacilli, CFU/g, at fermentation duration, h |
|-------------------------------------------|---------------------------------------------------------------|
| Fermented whey                            | 1·10⁶                                                         |
| Fermented whey, enriched with Mn at treatment duration, seconds: 30 | 8·10⁶                                                         |
| Fermented whey, enriched with Mn at treatment duration, seconds: 60 | 4·10⁶                                                         |
| Fermented whey, enriched with Mg at treatment duration, seconds: 30 | 6·10⁶                                                         |
| Fermented whey, enriched with Mn and Mg at treatment duration, seconds: 30 (Mg)+30 (Mn) | 2·10⁶                                                         |
| Fermented whey, enriched with Mn and Mg at treatment duration, seconds: 60 (Mg)+30 (Mn) | 5·10⁶                                                         |
| Fermented whey, enriched with Mn and Mg at treatment duration, seconds: 30 (Mg)+60 (Mn) | 7·10⁶                                                         |
| Fermented whey, enriched with Mn and Mg at treatment duration, seconds: 60 (Mg)+60 (Mn) | 1·10⁷                                                         |

It should be noted that such a tendency to the intensification of fermentation in the production of whey-coagulant was observed when using milk whey from cottage cheese. Although at the initial stages of fermentation (0...6 hours) the acidity grew somewhat slower, which is objectively explained by the effect of elevated acidity of the starting whey (50...60 °T) on lactobacilli and is consistent with data by other researchers [23].

Taking into consideration the gained positive effect, our subsequent study addressed the influence of fermented whey, enriched with mineral elements, on the qualitative indicators of thermo-acid cheese.

For this purpose, we made experimental samples of cheese, the Adyheys’kky type, using the following:

- fermented milk whey, untreated (control);
- fermented whey, produced from milk whey, treated in a discharge chamber with a conductive layer of manganese granules over 60 s (sample 2);
- fermented whey, produced from milk whey, treated sequentially in a discharge chamber with a conductive layer of manganese granules over 30 s and with a conductive layer of manganese granules over 60 s (sample 3).

The acid whey (produced by one of the three variants) with the titrated acidity 150±5 °T was introduced at continuous stirring to the prepared, pasteurized at a temperature of 93...95 °C, normalized milk mixture in the amount of 8...10 %. Other technological operations matched the technology of Adyheys’kky cheese [3].

The results from analysis of the impact of the fermented whey, enriched with magnesium and/or manganese, on the technological process and the quality of produced samples of thermo-acid cheese, as compared to control, are given in Table 2.

### Table 2

| Indicator | Sample 1 (control) | Sample 2 | Sample 3 |
|-----------|--------------------|----------|----------|
| Fermentation duration to achieve acidity 150±5 °T, hours | 32±6.5 | 32±2.0 | 32±3.5 |
| Mg content in fermented whey, mg/kg | 96.3±4.8 | - | +2.0 times |
| Mn content in fermented whey, µg/kg | 2.8±0.1 | +3.0 times | +3.0 times |
| Ca content in fermented whey, µg/kg | 853±10.2 | - | - |

 Progress of thermo-acid settling of protein

| Losses of protein, in whey after settling, % | 26.7 ± 1.0 | - | -6.4 % |
| Manganese content in whey after settling, mg/kg | 67.7±1.5 | - | +20.2 % |
| Manganese content in whey after settling, µg/kg | 1.4±0.05 | +2.3 times | +2.0 times |
| Calcium content in whey after settling, µg/kg | 32.7±6.5 | - | - |

 Finished product

| Organoleptic indicators | According to the acting standards | - | Slight discoloration of cheese mass (barely noticeable gray tint) |
|-------------------------|----------------------------------|---|-------------------|
| Physical-chemical indicators: Mass share of moisture, % | 58±0.1 | - | - |
| Mg content, mg/kg | 112.8±3.1 | - | +64 % |
| Mn content, mg/kg | 0.51±0.01 | +35 % | +42 % |
| Ca content, µg/kg | 2765±55.0 | - | - |
| Yields of cheese from 1 kg of milk, g | 110±0.5 | +18 % | +6.5 % |

Microbiological indicators | According to acting standards |
|---------------------------|------------------------------|

Note: “+” indicator grew; “−” – indicator decreased; “−” – no pronounced change
Thus, the results of research into the growth of acidity and the number of lactobacilli in the experimental samples of whey showed the possibility of a decrease in fermentation duration almost by 2 times as a result of the following:

1) preliminary treatment of milk whey before fermentation by electrospark discharges in a reaction chamber with a conductive layer of manganese granules over 60 s;
2) treatment of milk whey before fermentation in a reaction chamber with a conductive layer of magnesium granules over 30 s and further treatment in the chamber with a conductive layer of manganese granules over 30...60 s.

It is noted that the use of acidic whey, produced from the raw materials enriched with magnesium and manganese, in the technology of thermo-acid cheese contributes to its enrichment with valuable mineral elements. It also provides for a more complete use of the protein potential of milk and, consequently, a growth of the thermo-acid cheese yield by 1.8...6.5 %.

6. Discussion of results from studying the influence of electrospark treatment of milk whey on the process of its fermentation and the quality of thermo-acid cheese the type of “Adyheys'ky’

The data obtained are consistent with the results from works [1, 14, 27] and indicate the positive effect of enriching the fermentation medium with magnesium and manganese on the intensification of biotechnological processes. In particular, this can be explained by the increased concentration of magnesium with respect to calcium, since, as it is known, these two metals are antagonists in biochemical functions [28]. Thus, in control, the ratio of Mg:Ca was 1:9.1, whereas the milk whey that was consistently treated with electrospark discharges in reaction chambers with magnesium and manganese electrode systems demonstrated the ratio of 1:4.7.

The Mg and Mn transition from the fermented whey to protein clot is due to their involvement in the protein deposition process. In this case, Mg, which in an electrochemical series occupies a position close to calcium, acts as structure-forming bridges to bind proteins similar to the type of calcium bridges according to the scheme -R-Mg-HPO$_4$-Mg-R- or –R-Mg-HPO$_4$-Mg-HPO$_4$-Mg-R [29, 30]. This statement is confirmed by the reduction (although not significant) of protein losses in the milk whey and an increase in cheese yield. Slight changes in the amount of Ca in the experimental samples suggests that Mg does not replace calcium, but is an additional factor contributing to the completeness of the use of protein substances.

The role of Mn for binding protein substances is unlikely. Its insignificant growth in the cheese clot is explained by its involvement in forming the cheese mass together with plasma (fermented by whey) enriched with this element.

Thus, it has been proven that the electrospark treatment of milk whey before fermentation reduces the time required to produce whey-coagulant by several times. Consequently, the duration of the technological process of thermo-acid cheese production decreases and their cost is reduced.

The use of fermented whey, pre-treated with electrospark discharges in reaction chambers with a conductive layer of magnesium and manganese, in the technology of thermo-acid cheeses contributes to a slight increase in cheese yield and its enrichment with valuable mineral elements.

Given the above, it is a priority to use in the technology of preparing whey-coagulant the milk whey, consistently treated in discharge chambers with magnesium and manganese electrode systems. Note, however, that the results obtained do not rule out the appropriateness of the use of whey enriched with manganese through the electrospark treatment aimed to intensify the fermentation of whey.

Structural diagram of acid milk whey production involving a technique of electrospark enrichment of the raw material with with magnesium and/or manganese is shown in Fig. 4.

![Structural diagram of acid whey production for thermo-acid settling of proteins involving the electrospark treatment of raw materials](image)

---

Fig. 4. Structural diagram of acid whey production for thermo-acid settling of proteins involving the electrospark treatment of raw materials
The devised acid whey production technology does not limit its use only to making thermo-acid cheeses. Possible directions for treating the fermented whey produced through electrospark discharges are shown in Fig. 5.

![Diagram](image)

**Fig. 5. Ways of using fermented whey produced through electrospark discharges**

The technological process of acidic whey production should be implemented in the following order:
1) prepare whey;
2) warm it up to a temperature of 35…40 °C;
3) separate to remove fat;
4) treat the fat-free milk whey with electrospark discharges at an experimental technological complex in a reaction chamber with a conductive layer of magnesium and/or manganese granules. The treatment parameters are: capacitor charging voltage is 80…100 V; capacitor capacity is 100 µmF; interelectrode spacing is 0.01–0.1 mm; temperature is 25±5 °C. Recommended duration of treatment in the discharge chamber with a conductive layer of Mn; 60 s in a discharge chamber with a conductive layer of Mg is 30 s and further treatment in the chamber with a conductive layer of Mn; 60 s and/or 60 s in a discharge chamber with a conductive layer of Mg is 30 s and/or 60 s in a discharge chamber with a conductive layer of Mn;
5) pasteurize at a temperature of 80±2 °C;
6) cooling to the fermentation temperature;
7) ferment and ripen with pure cultures *Lactobacillus acidophilus* (if necessary, it is possible to use pure cultures *Lbc. Bulgaricum, Lbc. Helveticum*) until reaching acidity of not less than 150…170 °T.

When using in the production of whey-coagulant the milk whey from sour milk cheese after its collection it is immediately directed to processing, it is not recommended to store it.

One can note the following advantages of the proposed acid whey-coagulant technology for thermo-acid cheeses:
- it substantially shortens the production cycle;
- it improves the utilization factor of technological equipment and resources for it;
- it does not require significant space for the introduction of an innovative electro-physical technique; on the contrary, it leads to a decrease in the number of tanks due to a reduction of fermentation time, and therefore a decrease in production areas for them;
- it provides for a more complete use of the protein potential of milk and, consequently, for a growth in the thermo-acid cheese yield by 1.8…6.5 %.
- it promotes the enrichment of dairy-protein products with valuable mineral elements and, consequently, it improves their biological value.

### 7. Conclusions

1. Based on the results from experimental laboratory studies, we have established a decrease in the fermentation duration of milk whey, treated with electrospark discharges, by almost two times, under the following conditions:
   - treatment of whey, before fermentation, with electrospark discharges in a reaction chamber with a conductive layer of manganese granules over 60 s;
   - treatment of milk whey, before fermentation, in a reaction chamber with a conductive layer of magnesium granules over 30 s and further treatment in the chamber with a conductive layer of manganese granules over 30…60 s.

2. It was established that the use of whey, produced from the raw materials enriched with magnesium and manganese, as a coagulant, in the technology of thermo-acid cheese contributes to enriching it with valuable mineral elements. The protein potential of milk is used in this case somewhat better, which contributes to an increase in the yield of thermo-acid cheese by 1.8…6.5 %.

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