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THE EFFECT OF ELECTRICAL DISCHARGE TREATMENT OF MILK WHEY ON PARTIAL CONVERSION OF LACTOSE INTO LACTOBIONIC ACID

O. Kochubei-Lytvynenko, Candidate of Sciences, Associate Professor, *E-mail*: okolit@email.ua
director of Educational-scientific institute of food technologies
National University of Food Technology, 68, Volodymyrska St., Kyiv, Ukraine, 01601

Abstract. The article considers the scientific aspects of probable partial transformation of lactose into lactobionic acid due to the electrical discharge dispersion of magnesium and manganese conductive granules in milk whey – a traditional lactose-containing raw material. The object of this study was milk whey, defatted and with casein dust particles removed, which was treated in a discharge chamber with a conductive layer of magnesium and/or manganese granules at (20±2)°C with 120–180 s of exposure. A UPLC-MS/MS analysis of milk whey was carried out using a TSQ Vantage chromatograph-mass spectrometer (ThermoFinnigan, USA) connected to a Dionex Ultimate 3000 liquid chromatograph. The changes have been studied taking place in pH and redox potential of milk whey after electrical discharge treatment, as compared to the original whey. It has been established that electrical discharge treatment with 180 s of exposure increases the Mg content in milk whey by approximately 4 times, and increases the Mn content by 3.5 times. The dispersed metal particles are found in nano- (about 30 nm) and microscale (100 nm to 10 μm). The UPLC-MS/MS analysis of the test samples of whey that underwent electrical discharge treatment allowed identifying a chromatographic peak [M–H]– =357 m/z related to lactobionic acid. The derivative lactose content increased by 2 times in the whey samples treated for 180 s in a reaction chamber with a conductive layer of Mg between the corresponding electrodes, as compared to the original whey, and by 4 times in the samples subsequently treated in the reaction chambers with a layer of Mg and Mn granules between the corresponding electrodes, with 120 s of exposure in each chamber. The way has been presented of solving the problem of complex, economically practical and environmentally safe processing of milk whey with the prospect of obtaining lactobionic acid, a biologically valuable derivative of lactose.

Key words: milk whey, lactobionic acid, electrical discharge, magnesium, manganese.

ВПЛИВ ЕЛЕКТРОІСКРОВОГО ОБРОБЛЕННЯ МОЛОЧНОЇ СИРОВАТКИ НА ПРОЦЕС ЧАСТКОВОГО ПЕРЕТВОРЕННЯ ЛАКТОЗИ В ЛАКТОБІОНОВУ КИСЛОТУ

О.В. Кочубей-Литвиненко, кандидат технічних наук, доцент, директор, *E-mail*: okolit@email.ua
Навчально-науковий інститут харчових технологій
Національного університету харчових технологій, вул. Володимирська 68, м. Київ, Україна, 01601

Анотація. У статті розглянуто наукові аспекти ймовірного часткового перетворення лактози у лактобіонову кислоту внаслідок електроіскрового диспергування струмопровідних гранул магнію і мангану в середовищі традиційної лактозовмісної сировини – молочної сироватки. Об'єктом дослідження була молочна сироватка, попередньо знежирена і очищена від частинок казеїнового пилу, яку обробляли в електророзрядній камері експериментальної установки зі струмопровідним прошарком гранул магнію або/і мангану за температури (20±2)°С з експозицією 120–180 с. Зразки молочної сироватки аналізували методом УльтраВЕРХ-МС/МС на аналітичній системі, що складалась з рідинного хроматографа DionexUltimate 3000 з'єднаного з мас-спектрометром TSQ Vantage (ThermoFinnigan, США). Вивчено зміну pH та окисно-відновного потенціалу молочної сироватки після електроіскрового оброблення порівняно з вихідною сироваткою. Встановлено, що за умови електроіскрового оброблення з експозицією 180 с, у молочній сироватці збільшується вміст Mg приблизно у 4 рази і Mn – у 3,5 рази. Диспергувані частинки металів знаходяться в нано- (близько 30 нм) і мікророзмірному діапазоні (від 100 нм до 10 мкм). Аналіз дослідних зразків молочної сироватки, обробленої електроіскровими розрядами, методом УльтраВЕРХ-МС/МС дозволив ідентифікувати по іону [M–H]– =357 m/z хроматографічний пік, що відноситься до лактобіонової кислоти. Доведено зростання вмісту похідної лактози в 2 рази в зразках молочної сироватки, оброблених протягом 180 с в реакційній камері зі струмопровідним шаром Mg між відповідними електродами, порівняно із вихідною сироваткою, та у 4 рази в дослідних зразках, послідовно оброблених в реакційних камерах з шаром гранул Mg між відповідними електродами та Mn за експозиції 120 с в кожній камері. Показано напрям вирішення проблеми комплексного, економічно доцільного і екологічно безпечного перероблення молочної сироватки з перспективою отримання біологічно цінної похідної лактози – лактобіонової кислоти.

Ключові слова: молочна сироватка, лактобіонова кислота, електроіскрові розряди, магній, манган.

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Introduction. Formulation of the problem

Studying the directed impact on one of the main components of whey (lactose) in order to obtain its derivatives is an important world-class problem [1-2], which is given constant attention by the International Dairy Federation. The economic potential of lactose derivatives with a systematic list of more than 50 names [1] not only covers the costs of development

and production, but also compensates for all costs of raw materials.

Due to the unique physical, chemical, and biotechnological properties of lactose derivatives, particularly galactose, glucose-galactose syrups, lactulose, lactitol, lactobionic acid, etc., they are widely used in the production of functional foods and in medicine [1,3].

As of late, the attention of researchers around the world is drawn to lactobionic acid (4-O-β-D-galactopyranosyl-D-gluconic acid), one of the promising derivatives of lactose. It is a relatively new product that is obtained by lactose oxidation. It has significant potential as a promising biologically active compound.

Due to its antioxidant, chelating, and moisturizing properties, lactobionic acid (LBA) is used in medicine (particularly as a solution for organ transplantation) and in the production of cosmetics (anti-aging and moisturizing creams, lotions, etc.) [1,4-5]. LBA is a promising functional and technological ingredient for the production of food. It is known that its use contributes to the reduced duration of the fermentation of sour milk drinks and the ripening of cheese [6-9], supports the stability of gel structures [2,4], eliminates bitterness, improves the aroma and taste of fermented and dairy products [6,8,10], acts as a sweet acidifier [10], protects partially hydrogenated fats from oxidation [6,11], stabilizes functional drinks with essential minerals such as Fe, Ca, Cu [10], and exhibits antibacterial properties against pathogenic and conditionally pathogenic microorganisms [9].

In our country, lactobionic acid and technologies of obtaining and using it remain a little-studied issue. However, the commercial significance of lactobionic acid, which has recently grown rapidly in the world, has led to the development of new effective ways of obtaining and using it.

Analysis of recent research and publications

There are various ways of obtaining lactobionic acid, such as halogen oxidation of lactose, heterogeneous catalytic and enzymatic oxidation, etc. [9-10,12-16]. However, these methods are usually rather costly or require the use of toxic catalysts.

Electrochemical activation of lactose-containing raw materials has also aroused researchers' interest . It has been established that electrochemical activation, depending on the conditions created, can oxidize lactose to form lactobionic acid [10,17] or isomerize and transform it into lactulose [18-19].

A promising way of converting lactose into valuable derivative products is the electrical discharge dispersion of conductive metal granules in an aqueous medium. Today this technology is used to obtain hydrated or citrated nanoparticles of metals (nanoaquachelates) [20,21] which are used in medicine, veterinary medicine, agriculture, and food industry [22-25].

The optical emission spectroscopy of the plasma channel in the reaction zone has established that the process of electric erosion of metal granule surfaces is accompanied by sufficiently high temperatures that exceed both the melting point and the evaporation temperature of the corresponding metals [26]. Thus, for Cu, the temperature in the discharge channel is about 7.5×10^3–8.5×10^3 K, for Mg it is about 5×10^3–6.0×10^3 K, for Mn it is 3×10^3–4.5×10^3 K.

It is known that atmospheric pressure at temperatures of up to 3×10^3–4×10^3 K, when the water vapor dissociates almost completely (by 90%), facilitates the formation of atomic forms of oxygen (up to 30%) and hydrogen (up to 60%) that can dissolve in a crystalline lattice of the solid phase or form layers on its surface due to physical and chemical interaction. After reaching the plasma temperature in the discharge channel between 4×10^3 and 8×10^3 K, dissociation of the water vapor leads to the formation of molecules and ions O_2, H_2, O^-, H^+, OH^-, as well as the transition of the metal electrodes into the solution in the form of micro- and nanoparticles [26].

Thus, the conditions for the conversion of lactose into the derivatives are created in the zone of electrical discharge with dispersion of conductive metal granules in the medium of lactose-containing raw materials, particularly whey. Metal nanoparticles formed this way are likely to serve as catalysts for lactose oxidation [14,16].

The purpose of this work was to study the probable changes in whey lactose that occur during electrical discharge dispersion of Mg and Mn conductive granules in its medium.

The following tasks were set in order to achieve this goal:

1) to investigate physical and chemical properties of the treated whey;

2) to determine metal particle content in the treated whey;

3) to identify lactose transformation using a chromatographic mass-spectroscopic analysis;

4) to carry out quantitative mass-chromatographic determination of lactobionic acid in the test samples of milk whey.

Research Materials and Methods

The object of research is milk whey treated in a discharge chamber with a conductive layer of magnesium and/or manganese granules.

Before the treatment, ballast substances (fat and casein dust particles) had been removed from the milk whey by the centrifugal method (separation in a cream separator) at a temperature of 38±2°C. The whey flow was adjusted so that its fat content after separation did not exceed 0.1%.

The electrical discharge treatment was carried out in a laboratory unit that consisted of a thyristor discharge pulse generator (pulse rate 0.2–2.0 kHz, inductance of the discharge circuit 1 μH), a discharge chamber with a magnesium or manganese electrode system and a conductive layer of granules of the correspond-

ing metals between the main electrodes, a control unit, and measuring and auxiliary devices. A capacitor with a capacity of 25–100 μF was used as a power storage device [25].

Voltage applied at the main electrodes caused the electric current flow in the circle of freely located granules of metals in a stochastic communication mode. The use of low voltage and small inter-electrode intervals made it possible to provide modes where up to 85% of all energy accumulated in the capacitor was used to heat locally the surface of the contact granules and to disperse them [21].

Treatment parameters in the reaction chamber filled with milk whey were as follows: the size of the discharge chamber 300 cm^3; exposition time 120–180 s; the temperature of milk whey 20±2°C.

The content of metallic elements in the whey samples was determined by means of inductively coupled plasma atomic emission spectrometry with an Optima 210 DV device (Perkin Elmer, USA) in the Laboratory of Analytical Chemistry and Monitoring of Toxic Substances at the Institute of Labor Medicine of the National Academy of Medical Sciences of Ukraine.

Particle size was determined with a particle dispersion analyzer Malvern Instruments Ltd. (UK), pH was measured with a pH-meter I-160 M, and oxidation-reducing potential was measured with a pH-meter with platinum electrodes EB-74.

The UPLC-MS/MS analysis was carried out with a TSQ Vantage chromatograph–mass spectrometer (ThermoFinnigan, USA) connected to a Dionex Ultimate 3000 liquid chromatograph. Separation was performed on a chromatographic column ThermoGold C18, 100×2.1 mm, with particle size 1.9 μm (ThermoFinnigan, USA). The column temperature was 40 °C, the volume of the injected sample 5 μL. Chromatographic conditions were as follows: flow rate 0.3 cm^3/min, mobile phase A – a 0.1% de-ionized aqueous formic acid solution, phase B – methanol containing 0.1% of formic acid. The mobile phase flow rate was 0.3 mL/min. The substances were separated in a gradient mode: 0 min, 5% B; 5 min, 95% B; 7.5 min, 95% B; column equilibration started using 5% B; solvent at 7.51 min. The total time of the analysis, including system stabilization before the injection of the next sample, was 10 min.

A mass spectroscopic analysis was carried out in a negative ion mode. Capillary voltage was 3.5 kV, electrospray temperature 370°C, argon pressure 0.2 Pa. The samples were analyzed in a selected reaction monitoring mode (SRM), using the following diagnostic transitions: m/z 359→89 (quantitative analysis), m/z 359→119, and m/z 359→159. The collision energies (CE) and the S-lens value were optimized to obtain the maximum intensity of diagnostic transitions.

Test samples of the treated whey were prepared before the chromatographic mass spectroscopic analysis as follows: cooled to 4 °C, centrifuged for 10 minutes at 10,000 rpm, and had 1 cm^3 of 0.5 % metaphosphoric acid added per 1 cm^3 of volume after filtering.

The lactobionic acid in the studied samples was identified by the retention time and by comparing the relative intensities of the MS/MS diagnostic transitions in the samples and in the standard solutions of lactobionic acid (Sigma-Aldrich) [27].

Results of the research and their discussion

The results of studying physical and chemical properties as well as magnesium and manganese content in the test samples of whey before and after electrical discharge treatment are presented in Table 1.

Table 1 – Properties of whey test samples (n = 3, p = 0.95)

Sample	pH	Redox potential, (-E), mV	Mg, mg/kg	Mn, mg/kg
1	4.18 ± 0.20	10.0±0.5	96.30±4.82	0.0280±0.0007
2	5.33 ± 0.20	195.0±8.0	382.40±14.12	0.0250±0.0007
3	5.45 ± 0.20	201.0±8.0	93.80±8.44	0.0960±0.0003
4	6.18± 0.20	280.0±9.5	282.40±14.12	0.0690±0.0005

Sample: 1 – milk whey (control); 2, 3 – milk whey treated in a reaction chamber with a layer of metal granules of magnesium and manganese respectively between the main electrodes (exposure 180 s); 4 – milk whey sequentially processed in the reaction chambers with a layer of magnesium (exposure 120 s) and manganese (exposure 120 s) granules between the main electrodes.

It has been established that electrical discharge treatment of whey, with 180 s of exposure, increases the content of magnesium by about 4 times, and manganese by 3.5 times.

In the course of the experiments, an increase in the pH and redox potential (that is an increase in antioxidant properties) was observed in the treated whey. This may indicate the process of $M \leftrightarrow M^{n+} + ne$ taking place in the system. Besides, it may mean a probable process of complex formation between metal ions and bioligands present in milk whey, as well as formation of substances with antioxidant (reducing) properties, such as lactobionic acid. Moreover, such properties become more noticeable with increased exposure.

A dispersion analysis of the whey samples has shown that the average size of whey particles before and after the treatment was not significantly different. However, since the effectiveness of the catalytic effect of metals on lactose oxidation may depend on the size of the metal particles dispersed in whey [28], it also proved necessary to establish the average hydrodynamic diameter of magnesium and manganese particles in colloidal solutions obtained by electrical discharge dispersion of the granules of corresponding metals in the aqueous medium (with treatment parameters similar to those of the whey). It has been determined that colloidal solutions of magnesium and manganese have na-

noscale (about 30 nm) and microscale (100 nm to 10 μm) particles. In this case, the average particle size in the colloidal solution was 118±5 nm for magnesium and 270±11 nm for manganese.

These results indicate the preconditions for a specific-purpose transformation of lactose into lactobionic acid in milk whey that underwent electrical discharge treatment.

To identify the products of lactose's probable transformation, a chromatographic mass-spectroscopic analysis of the whey samples before and after the electrical discharge treatment was performed.

Fig. 1 shows mass-chromatograms of a standard lactobionic acid solution, Fig. 2–5 show the test samples of the whey before and after electrical discharge treatment with magnesium and/or manganese electrode systems.

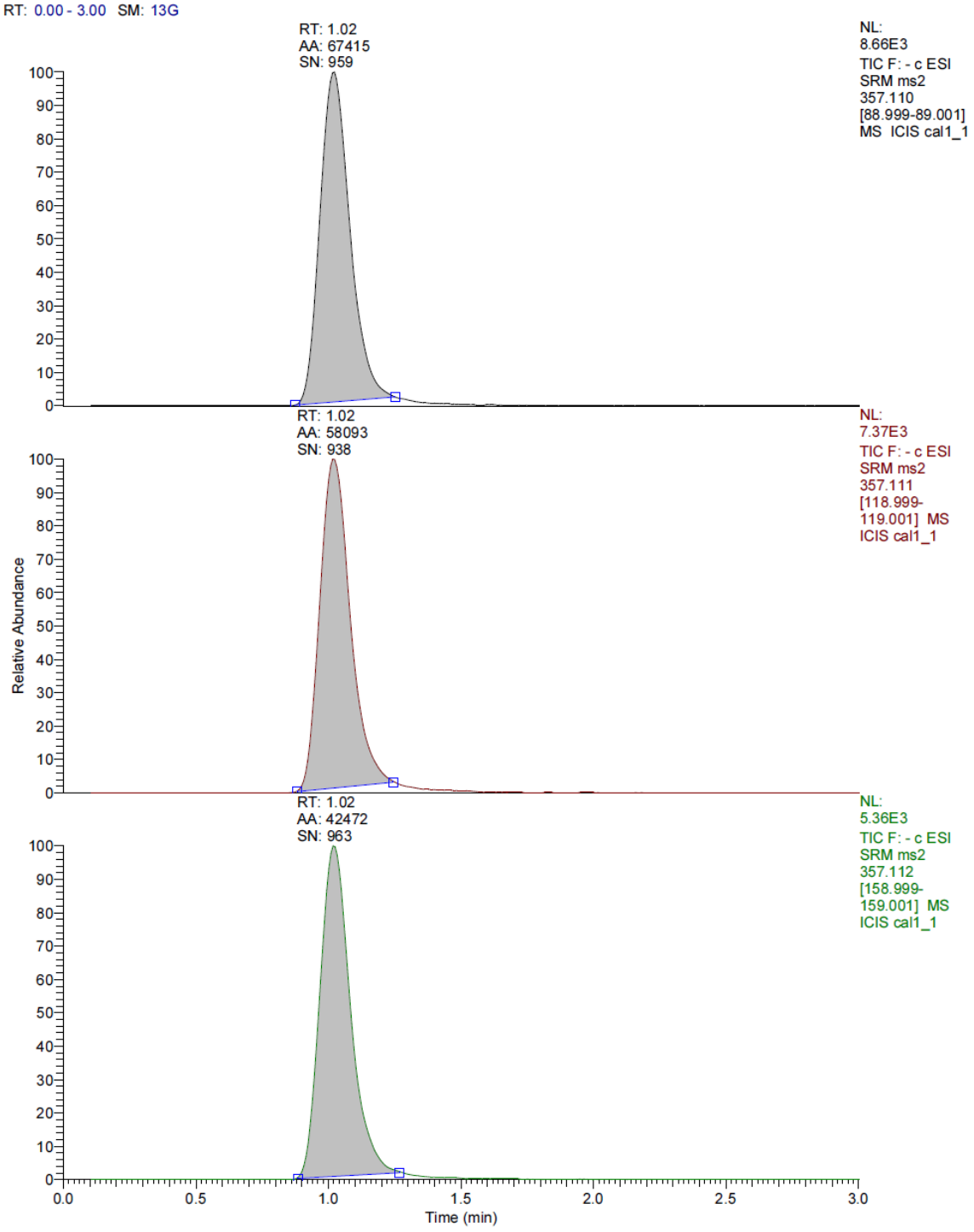

Fig. 1. Mass-fragmentogram of lactobionic acid standard solution in three MS/MS diagnostic transitions; c = 2.5 mg/dm^3

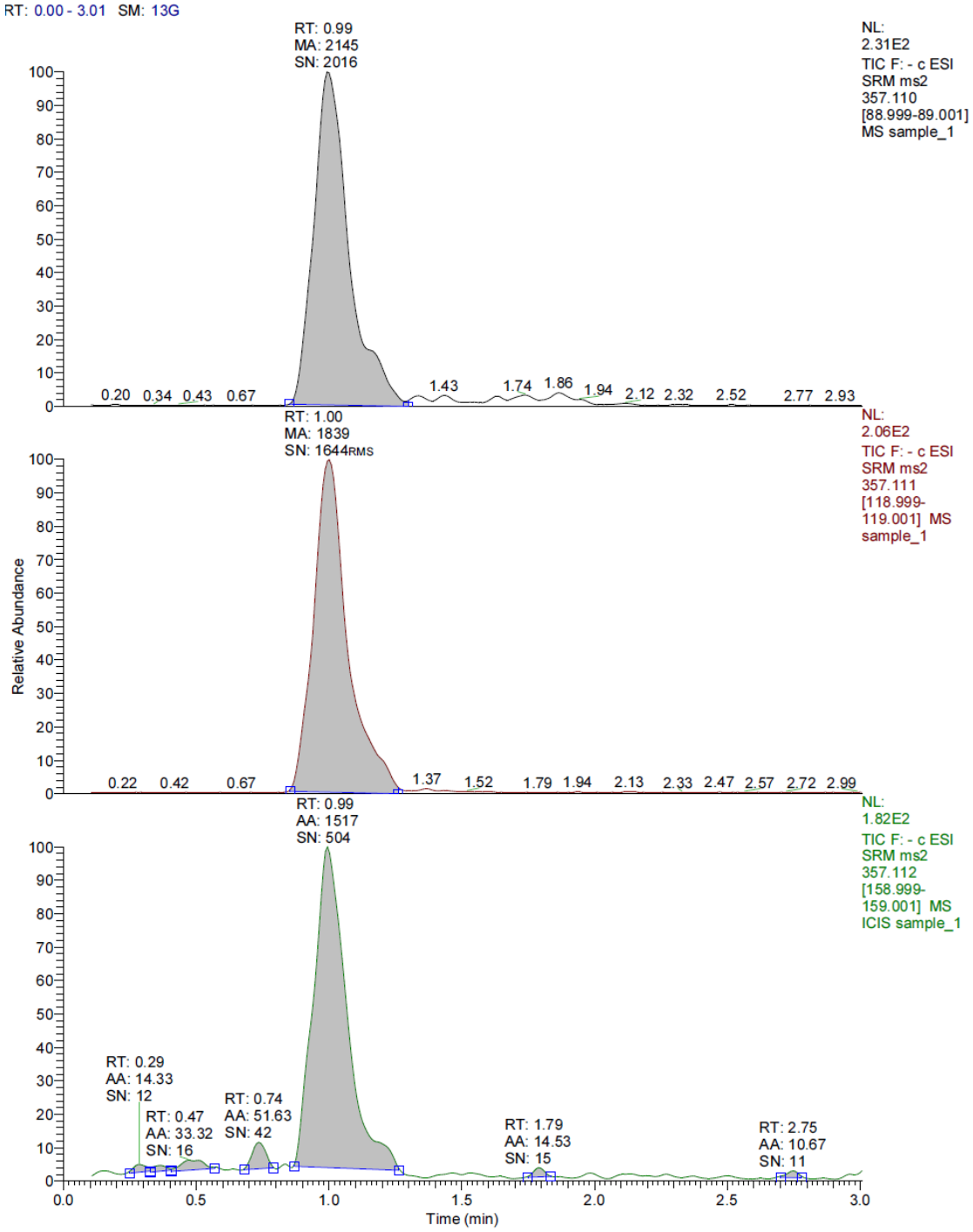

Fig. 2. Mass-fragmentogram of untreated whey in three MS/MS diagnostic transitions; c = 2.5 mg/dm^3

All mass fragmentograms on the quasimolecular ion [M-H]- = 357 m/z have the chromatographic peak related to lactobionic acid.

The conditions of quantitative mass-chromatographic determination of lactobionic acid are presented in Table 2.

Table 2 – Conditions of lactobionic acid quantitative determination

Retention time, min	Precursor ion, m/z	Product ion, m/z	Calibration graph equation	Detection limit, mg/dm^3	Quantitative determination limit, mg/dm^3
0.96	357 [M–H]	89	Y=(320±800)+(2632±117)·x R^2 = 0.998	0.37	1.2

The results of the quantitative determination of lactobionic acid in the test samples of whey before and after electrical discharge treatment are presented in Table 3.

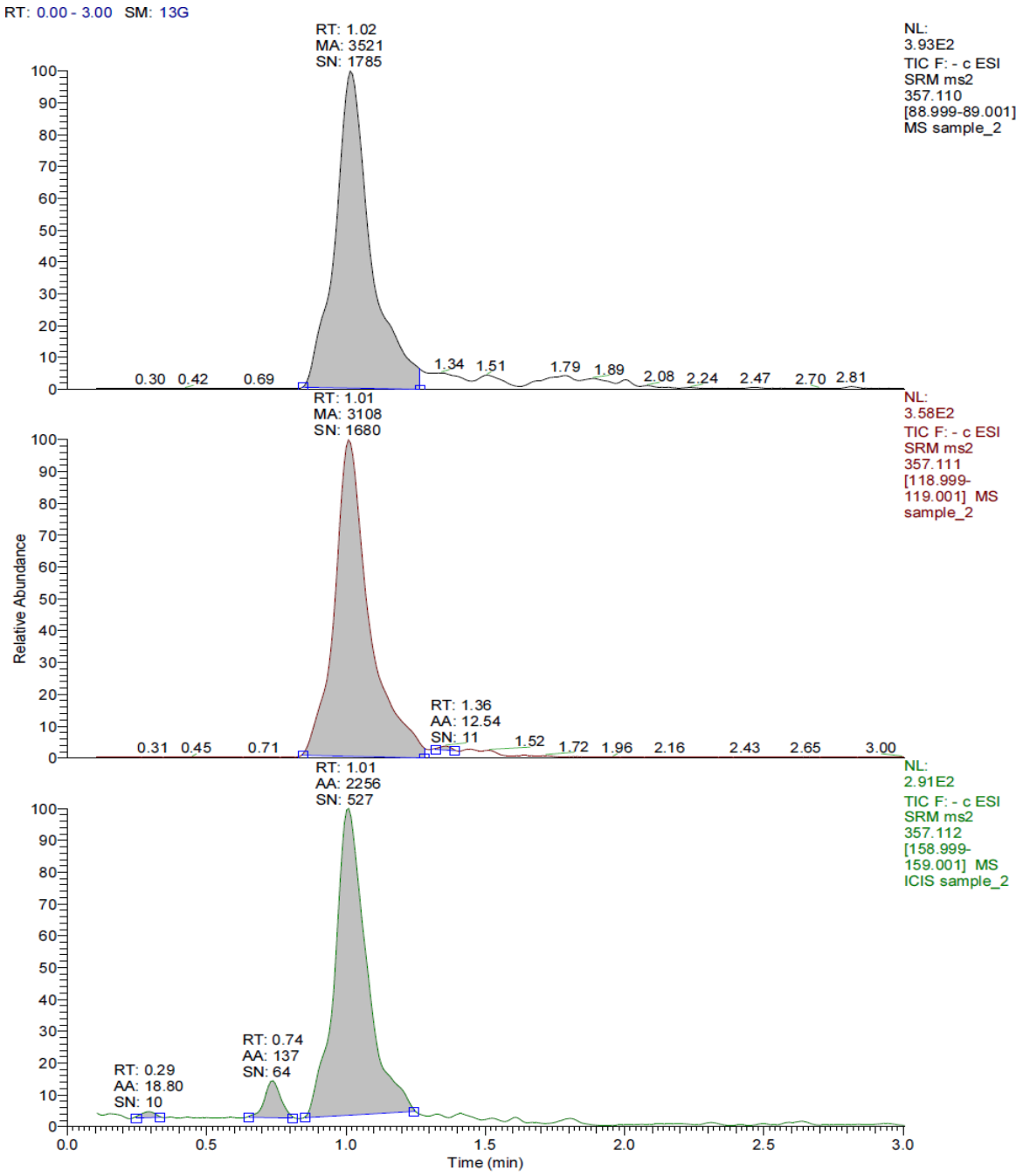

Fig. 3. Mass-fragmentogram of whey treated with electrical discharge in a reaction chamber with a magnesium electrode system (exposure 180 s) in three MS/MS diagnostic transitions; c = 2.5 mg/dm^3.

Table 3 – Lactobionic acid content in test samples of whey treated with electrical discharge (n = 3, p = 0.95)

Sample	Lactobionic acid content, mg/dm^3
1	0.61±0.25
2	1.12±0.25
3	0.57±0.25
4	2.32±0.24

Sample: 1 – milk whey (untreated); 2, 3 – milk whey treated in a reaction chamber with a layer of metal granules of magnesium and manganese respectively between the main electrodes (exposure 180 s); 4 – milk whey sequentially processed in the reaction chambers with a layer of magnesium (exposure 120 s) and manganese (exposure 120 s) granules between the main electrodes.

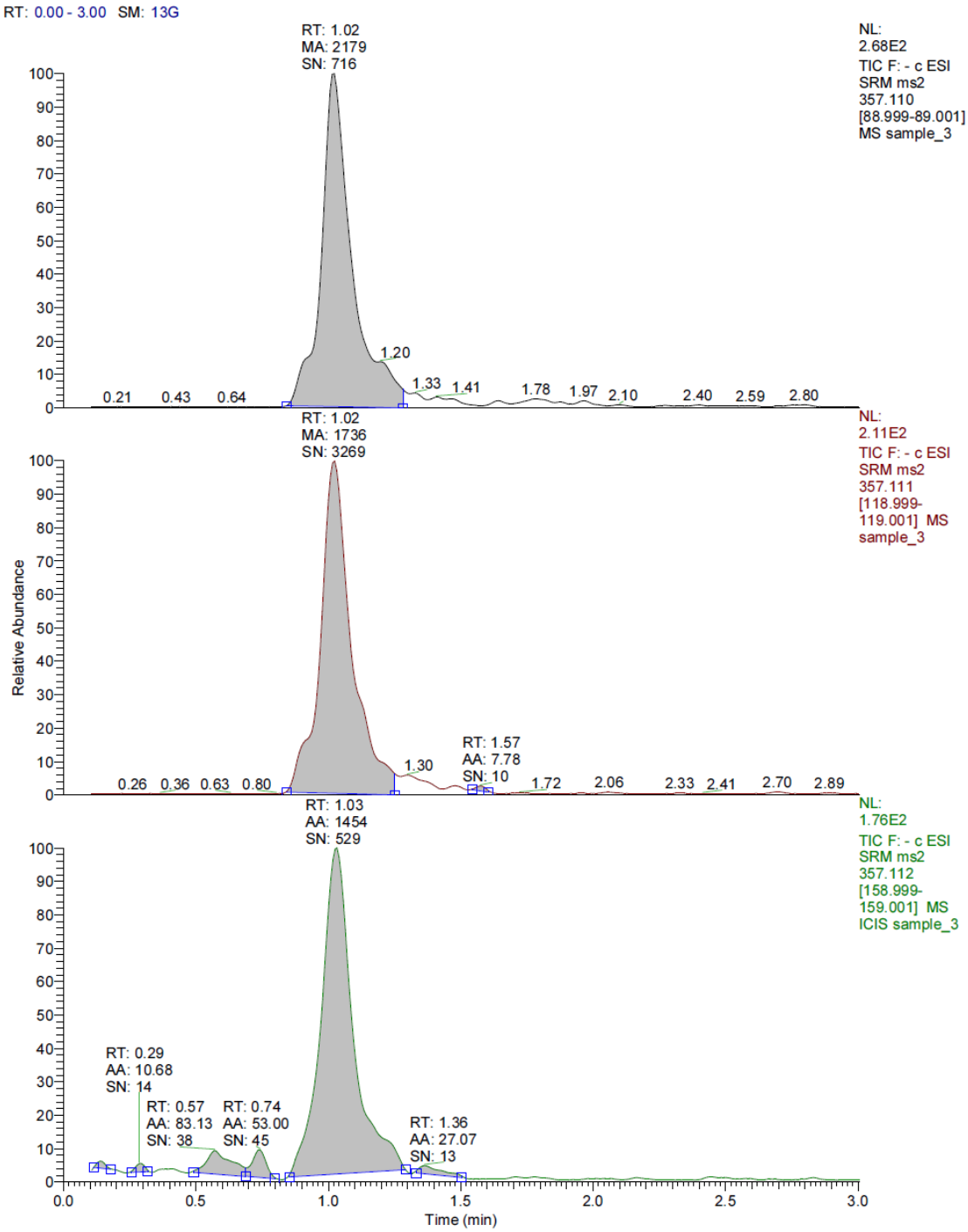

Fig. 4. Mass-fragmentogram of whey treated with electrical discharge in a reaction chamber with a manganese electrode system (exposure 180 s) in three MS/MS diagnostic transitions; c = 2.5 mg/dm^3.

It should be noted that a small amount of lactobionic acid was present in the original milk whey. After the treatment in the reaction chamber with electrodes and conductive layer of magnesium, its amount nearly doubled.

However, in the samples that underwent electrical discharge treatment in the reaction chamber with a layer of granulated manganese between the corresponding electrodes, the amount of lactobionic acid was the same as in the original whey.

It should be noted that the amount of lactobionic acid increased by almost 4 times in the samples sequentially treated in the reaction chambers with a layer of magnesium and manganese granules between the corresponding electrodes. It can be assumed that the magnesium particles accumulated in the system due to electrical discharge dispersion are likely to act as catalysts for lactose oxidation due to their electrochemical activity.

With a manganese electrode system employed, the plasma temperature in the discharge channel might not be sufficient to accumulate an adequate amount of atomic oxygen for lactose oxidation.

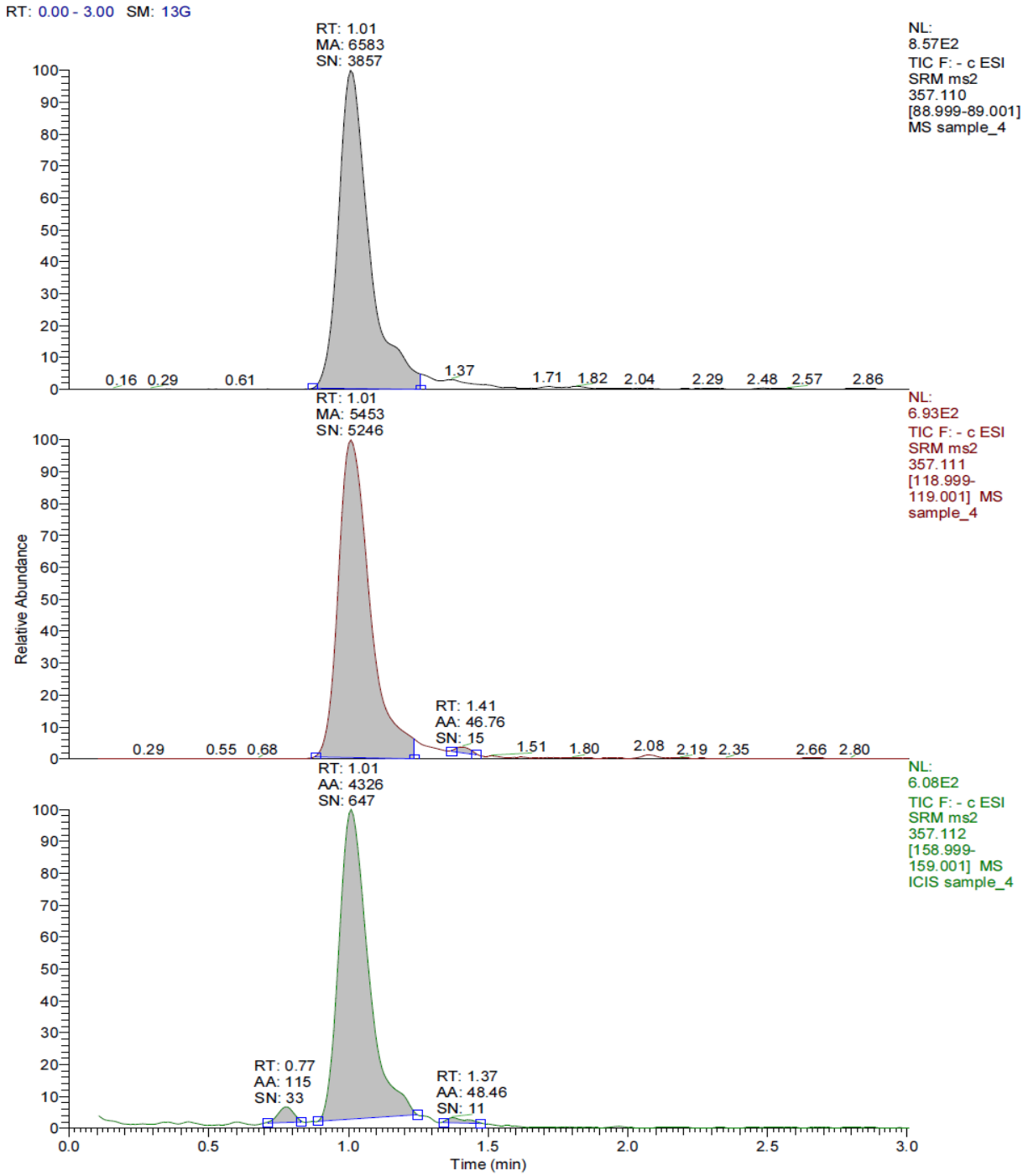

Fig. 5. Mass-fragmentogram of whey subsequently treated with electrical discharge in reaction chambers with magnesium and manganese electrode systems (exposure for each chamber 120 s) in three MS/MS diagnostic transitions; c = 2.5 mg/dm^3

Conclusion

1. Basing on the changes in physical and chemical parameters (pH and redox potential), the preconditions have been determined for the specific-purpose transformation of lactose into lactobionic acid by electrical discharge dispersion of magnesium and manganese conductive granules in whey.

2. It has been determined that electrical discharge treatment with 180 s of exposure increases the Mg content in whey by approximately 4 times, and the Mn content by 3.5 times. The dispersed metal particles

are found in nanoscale (about 30 nm) and microscale (100 nm to 10 μm).

3. A UPLC-MS/MS analysis of the whey samples treated with electrical discharge allowed identifying, by the ion [M-H]- = 357 m/z, the chromatographic peak related to lactobionic acid.

4. It has been proved that lactose derivative content increases 2 times in the whey samples treated for 180 s in a reaction chamber with a conductive layer of magnesium between the corresponding electrodes (compared to the original whey) and 4 times in the samples subsequently treated in reaction chambers with a layer of magnesium and manganese granules between the corresponding electrodes, with 120 s of exposure in each chamber.

5. Implementation of this area of scientific research opens up additional opportunities for solving the problem of complex, economically feasible, and environmentally safe processing of milk whey with a prospect of obtaining biologically valuable lactose derivatives.

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