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EVALUATION OF ANALYTICAL METHODS OF MICROBIAL ACTIVITY DURING SUCROSE EXTRACTION FROM SUGAR BEET

S u m m a r y

The aim of our research was to verify selected methods to determine microbial activity during extraction process in term of speed, simplicity and accurancy of the analysis. Indirect methods – enzymatic determination of L-lactic acid, isotachophoretic determination of lactic acid, pH, as well nitrite amounts determination were verified. The results of these methods were compared with those of direct one – determination of bacteria counts (mesophilic and thermophilic bacteria). The determination of L-lactic acid by enzymatic device MICROZYM – L is very simple and rapid. But in same cases L-lactic acid does not indicate a true microbial situation in extractor. The determination of total lactic acid (L + D isomers) is more adequate for detection of contamination in extractor. The results of the nitrite amount determination did not confirm the sanitary situation found out by measurment of lactic acid, pH and bacteria counts in some cases.

Key words: microbial contamination, lactic acid, nitrite.

Introduction

The aim of the extraction process in sugar production is to extract the maximum amount of sucrose with the minimum amount of impurities.

Microbial infection in extractor connected with sugar decomposition causes unknown sugar losses and other serious problems. There are two major types of microorganisms according to their optima temperature [1, 14]. Mesophilic which grow at 15–45°C and thermophilic which grow at 45–80°C. Early identification of microbial state in extractor with following application of disinfectans has a great effect to decrease sucrose losses due to eliminate many problems during sugar production.

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L-lactic acid is the main product of microbial decomposition of sucrose in diffusion (raw) juice [2]. Several methods of lactic acid determination are used at present.

The determination of lactic acid (L- and D-) in juice and processing products by an enzymatic method are accepted in the sugar industry [5]. Reduced nicotinamid-adenine di-nucleotide (NADH), formed by oxidation of lactate, is measured photometrically by its characteristic absorbance at 340 nm. Another enzymatic method is the determination of L-lactic acid by device MICROZYM-L analyzer using enzymatic sensor [9]. The determination of non – sugars (anions and kations) by capillary isotachophoresis (ITP) is a new tool in sugar factory analysis. ITP is able to determine a wide range of quickly and simply ions, usually without sample pre-treatment [6, 7, 8, 10, 11]. HPLC and ionic HPLC are successfully used to determine some organic acids and inorganic anions in sugar factory products [12, 13, 20].

A certain bacteria thriving at higher temperatures reduce nitrates to nitrites. Because nitrates and not nitrites are only founded in soil, the reducing power of the bacteria to nitrites in the extractor can be used as a measure of their activity [14]. But the reduction of nitrates can be suppressed in presence of increased amount of oxygen during extraction [15].

Nitrites can be detected by capillary isotachophoresis [10, 11], by ionic HPLC [13, 20] as well as by photometric method [3].

The object of our investigation was to compare various methods of contamination in extractor – the enzymatic method of L-lactic acid determination, determination of total lactic acid by capillary ITP, determination pH and nitrites and determination of bacteria counts as a measure of microbial activity during the extraction of sucrose from sugar beet.

Material and methods

Samples

The juices were sampled from the 1st and 3rd chambers of the slope extractor in sugar factory Eastern Sugar Slovensko, a.s. Dunajská Streda during the campaign 2002. L-lactic acid (L-LA) and pH were measured in the factory´s laboratory. Further samples were sampled in sugar factory Trnavský cukrovar, a.s. Trnava, i.e. fresh cossettes (FC), juices from the 1st and 3rd chambers of the slope extractor, raw juice (RJ) and press water (PW) during the campaigns 2002, and 2003. Aliquote of juices was cooled, refrigerated and transported to the laboratory of faculty for bacteria counts determination and isotachophoretic analyses.

L-lactic acid was determinated by enzymatic device MICROZYM-L, which was provided by company Jako, s.r.o., Líbeznice, Czech Republic. It is an analyzer with enzymatic electrode, that measures L –lactic acid in mg/l [4].

Total lactic acid (D+L isomers) was determined by capillary isotachophoresis [6, 7, 8]. Measurements were realised on the isotachophoretic analyzer ZKI 01 VILLA LABECO, Spišská Nová Ves with conductivity detector. Applied electrolyte systems of following composition: LE (leading electrolyte) – 10 mmol/dm^3 HCl, 0.1% MHEC, aminocaproic acid, pH 4.25, TE (terminating electrolyte) – 5 mmol/dm^3 caproic acid. The current in the preseparation column was 250 μA.

Nitrite concentrations (NO_2^-) were determined by photometric method with α-naphtylamine and sulphanilic acid [3].

pH was measured with the aid of digital pH meter RADELKIS with combinate pH electrode OP 0808.

Determination of bacteria counts was realised by pour plate technique with plate count agar (PCA) from diluted juices. Plates were incubated at 35°C for mesophiles and at 55°C for thermophiles per 48 hours. The results were reported as CFU/ml (colony forming unit) [19].

Results and discussion

Sugar factory Dunajská Streda (DS)

The results of L-lactic acid amounts and pH values indicated good sanitary situation in both extractors (A, B). This statement was consequently confirmed by microbial analysis of mesophilic and thermopilic counts. The values of bacteria counts did not reached the critical value of 10^4 CFU/ml [18]. Average values of bacteria counts – mesophiles (M), thermophiles (T), L-lactic acid, pH and nitrite concentration are presented in Table 1 and 2.

L-lactic acid amounts varied from 28 to 246 mg/l in the 1st chamber and from 33 to 367 mg/l in the 3rd chamber. Increased amounts of this acid were detected on 20.11.02 in both chambers of extractor B, pH varied 5.33-5.98; pH value of the feed water was about 5.1 during this day. The values of L-lactic acid 300–350 mg/l can be consider as limiting values indicating good sanitary situation in this factory. For example Austrian factories are satisfied with a maxima of 400 mg/l of lactic acid in the raw juice [16]. According to the information of the company Jako s.r.o, that supplies biocides SUCAZUR, values up to 250–300 mg/l L-lactic acid in raw juice and press water did not correspond to infection [9].

As for nitrites determination, increased amounts were measured on 20.11.02 and on 6.12.02 in the 1st chamber (13.81–27.27 mg/l) and (11.1–14.08 mg/l) in the 3rd

chamber. Higher values of nitrites (above 10 mg/l) indicated infection in extractor but according to the L-lactic acid increased infection was found out only on 20.11.02.

T a b l e 1

Average values of bacteria counts, pH, L-lactic acid and nitrite level in the 1st chamber of extractor (DS 2002).
Średnia liczba bakterii, pH i stężenie kwasu L-mlekowego oraz azotanów(III) w pierwszej komorze dyfuzora (DS 2002).

Date	16.10.02	23.10.02	13.11.02	20.11.02	6.12.02	Campaign averages
Extractor	**A**	B	A	B	B	**A+B**
CFU/ml – M	$1.32 \cdot 10^3$	$1.08 \cdot 10^3$	$1.13 \cdot 10^3$	$0.92 \cdot 10^3$	$1.15 \cdot 10^3$	$1.12 \cdot 10^3$
CFU/ml – T	$3.52 \cdot 10^2$	$4.94 \cdot 10^2$	$7.11 \cdot 10^2$	$5.77 \cdot 10^2$	$5.42 \cdot 10^2$	$5.36 \cdot 10^2$
L-LA [mg/l]	94.1	91.9	62.4	271.4	89.8	116.6
pH	6.02	6.12	6.03	5.90	6.32	6.07
NO_2^- [mg/l]	3.56	5.05	0.68	13.96	15.42	9.17

T a b l e 2

Average values of bacteria counts, pH, L-lactic acid and nitrite level in the 3rd chamber of extractor (DS 2002).
Średnia liczba bakterii, pH i stężenie kwasu L-mlekowego oraz azotanów(III) w trzeciej komorze dyfuzora (DS 2002).

Date	16.10.02	23.10.02	13.11.02	20.11.02	6.12.02	Campaign averages
Extractor	A	B	A	B	B	A+B
CFU/ml – M	$1.26 \cdot 10^3$	$1.12 \cdot 10^3$	$1.30 \cdot 10^3$	$1.11 \cdot 10^3$	$0.92 \cdot 10^3$	$1.10 \cdot 10^3$
CFU/ml – T	$3.76 \cdot 10^2$	$3.44 \cdot 10^2$	$4.84 \cdot 10^2$	$4.95 \cdot 10^2$	$4.80 \cdot 10^2$	$4.29 \cdot 10^2$
L-LA [mg/l]	72.7	98.7	58.5	294.7	209.3	141.7
pH	5.92	5.79	5.89	5.51	6.15	5.84
NO_2^- [mg/l]	4.87	2.11	0.31	8.93	10.63	5.79

Sugar factory Trnava (TA)

The measurements in this sugar factory were carried out to determine L-lactic acid, total lactic acid, pH and nitrite concentrations. Average values of L-lactic acid and pH measured during campaign in factory´s laboratory are given in the table 3.

To compare the results, decreasing amounts of L-lactic acid (raw juice, juices in the chambers of extractor) and subsequently increasing values of pH were measured during campaign 2003. In the campaign 2002 biocide SUCAZUR 1410 in average quantity of 16.7 ppm/day (12.4 ppm to the extractor and 4.3 ppm to the press water) and 23.7 ppm/day of formalin was applied.

In the campaign 2003 higher amounts of disinfectans i.e. SUCAZUR 1410 18 ppm/day, formalin 48 ppm/day were used. During this campaign biocide SUCAZUR 1451 in cold area (washed beet and fress cossettes) in average amount of 3 ppm /day was also used. Increased amounts of biocides had positive effect with regard to the L-lactic acid amount and pH.

T a b l e 3

Average values of L-lactic acid and pH in fresh cossettes (FC) and in juices of extraction process measured during campaigns 2002 and 2003 (TA).
Średnie stężenie kwasu L-mlekowego oraz pH świeżej krajanki i soku dyfuzyjnego podczas kampanii w latach 2002 i 2003 (TA).

Campaign	L – LA [mg/l] FC	L – LA [mg/l] RJ	pH RJ	L – LA [mg/l] "1"	pH "1"	L – LA [mg/l] "3"	pH "3"	L – LA [mg/l] PW	pH PW
2002	35.9	328.9	5.93	526.1	5.34	244.0	5.22	95.5	5.25
2003	81.1	200.5	6.15	311.1	5.82	178.9	5.73	121.7	5.43

In selected samples we have measured L-lactic acid, total lactic acid, pH and nitrites. The samples were sampled and refrigerated during the campaigns 2002 and 2003 in sugar factory Trnava. Measurements were realised in the laboratory of the faculty from refrigerated samples. The results are presented in tables 4 and 5.

T a b l e 4

Amounts of L-lactic, D+L-lactic acid, pH and nitrites in selected samples of extraction process (TA 2002).
Całkowita zawartość kwasu mlekowego (D+L) i jego izomeru L, oraz stężenie azotanów(III) w wybranych próbkach pobranych podczas procesu ekstrakcji (TA 2002).

Average values (2002)	RJ	1. chamber	3. chamber	PW
L-lactic acid L – LA [mg/l]	369.3	635.1	188.0	40.0
Total lactic acid (D+L)–LA [mg/l]	463.3	717.0	312.8	148.1
Ratio L-LA / (D+L-LA) [%]	79.7	88.6	60.1	27.0
pH	5.9	5.3	5.2	5.3
Nitrites [mg/l]	35.20	29.60	0.11	0.13

The results indicate increased amounts of D-lactic acid. In the campaign 2002 high values of D-lactic acid were found out in the press water and subsequently in the

3rd chamber of extractor, where the press water is returned to the extraction process. The press water was not heated /sterilised/ enough and according to the investigation of American researchers [17] increased amounts of D-lactic acid were caused by *Leuconostoc mesenteroides* that produced almost exclusively D-lactic acid. The results of total lactic acid measured in 2003 indicated infection in the whole extractor, on the other hand L-lactic acid, nitrite amounts and pH /except press water/ indicated good sanitary situation.

Table 5

Amounts of L-lactic, D+L-lactic acid, pH and nitrites in selected samples of extraction process (TA 2003).
Całkowita zawartość kwasu mlekowego (D+L) i jego izomeru L oraz stężenie azotanów(III) w wybranych próbkach pobranych podczas procesu ekstrakcii (TA 2003).

Average values (2003)	RJ	1. chamber	3. chamber	PW
L-lactic acid L – LA [mg/l]	182.6	204.3	80.5	88.1
Total lactic acid (D+L)–LA [mg/l]	568.5	887.1	719.4	325.3
Ratio L-LA / (D+L-LA) [%]	32.1	23.0	11.2	27.1
pH	6.2	5.7	5.6	5.3
Nitrites [mg/l]	0.56	0.52	0.11	0.16

As for nitrite amounts determination – according to the results measured in 2002, increased values that indicated contamination were found out in the juice end of extractor, while in the water their amounts indicated good sanitary state.

Nitrite amounts measured in 2003 indicated good sanitary situation in the whole extraction process. These results were not confirmed by previous results of lactic acid high values in the whole extractor.

Conclusion

1. Determination of L-lactic acid by enzymatic device MICROZYM-L is simple and fast. It must be realised together with measurement of pH. The extraction process must be, however realised in the way which avoids production of D-lactic acid as the major metabolite (e.g. by strict following the temperature conditions in extractor, sterilisation of press water etc.).
2. Determination of nitrite amounts can be used as an informative method. It must be taken into consideration that some microorganism can further reduce nitrites to

ammonia eventually to nitrogen and nitrite is only intermediate product. In this case the negative result does not mean good microbial state.

Literature

[1] Bretschneider, R.: Technologie cukru. SNTL, Alfa, Praha 1980.

[2] de Bruijn, J.M., van der Poel, P.W., Heringa, R., van den Bliek, M.: Sugar degradation in sugar beet extraction – a model study. Zuckerind., 1991, **8 (116)**, 729-732.

[3] Cox, L., Tveit, M.: Der Nitritgehalt von Diffusionssäften als Mass zur Bewertung der Mikrobentätigkeit. Zucker, 1964, **22 (17)**, 625-627.

[4] Durant, H.G.: Microzym–L. L–lactate and/or D–glukose biosensory analyzer. Operating Instructions. Biotrade, Toulouse, France, 1999.

[5] ICUMSA, International Commission for Uniform Methods of Sugar Analysis, Method Book 1994, Method GS8/4/6-13. Bartens, Berlin.

[6] Karovičová, J., Kohajdová, Z., Šimko, P., Lukáčová, D.: Using of capillary isotachophoresis for determination of biogenic amines and D-isocitric acid in food products. Nahrung/ Food, 2003, **3 (47)**, 188-190.

[7] Karovičová, J., Kohajdová, Z., Hybenová, E.: Using of multivariate analysis for evaluation of lactic acid fermented cabbage juices. Chem. Pap., 2002, **4 (56)**, 267-274.

[8] Karovičová, J., Kohajdová, Z., Greif, G.: The use of PCA, CA, FA for evaluation of vegetable juices processed by lactic acid fermentation. Czech J. Food Sci., 2002, **4 (20)**, 135-143.

[9] Kopecký, J., Dandár, A., Bubník, Z., Pawlowski, B.: Microbiology Control of Sucrose Extraction, Incrustation Control of Evaporation and Continuous Ionic Separation Process ISEP. In: CITS, Madrid, 2003, pp. 173-179.

[10] Kvasnička, F., Parkin, G., Harvey, C.: Capillary isotachophoresis as a new tool in sugar factory analysis. Int. Sugar J. 1993, **95 (1139),** 451- 458.

[11] Kvasnička, F., Blatný, P.: Application of capillary isotachophoresis and capillary zone electrophoresis to the determination of inorganic ions in food and feed samples. J. Chromatogr. A, 1999, **(834),** 419-431.

[12] Lodi, S., Rossin, G.: Determination of some organic acids in sugar factory products. J. Chromatogr. A, 1995, **(706),** 375-383.

[13] Magne, V., Mathlouthi, M., Robilland, B.: Determination of some organic acids and inorganic anions in beet sugar by ionic HPLC. Food Chemistry, 1998 **61 (4),** 449-453.

[14] McGinnis, R.A.: Beet –Sugar Technology. Beet Sugar Development Foundation, Fort Collins 1982.

[15] Nickish, A., Mauch, W.: Das Redoxpotential als Kriterium zur Früherkennung von Infektionen in Extraktionsanlagen. Zuckerind., 1981, **6 (106)**, 521-528.

[16] Pollach, G., Hein, W., Beddie, D.: Application of hop β-acids and rosin acids in the sugar industry. Zuckerind., 2002, **12 (127)**, 921-930.

[17] Samaraweera, I. S. et al: Is L –lactic acid a true indicator of microbial infection ? Int.Sugar J., 1995, **97 (1162)**, 566-570.

[18] Smolík, J.: Detekce mikrobiální kontaminace v potravinářství a její možnosti pri těžení šťávy II. Listy cukrov. a řep., 1996, **9 (112)**, 267-271.

[19] STN ISO 4833. Microbiology – General guidance for enumeration of micro-organisms – Colony count technique at 30 °C, 1997.

[20] Trifiró, A., Saccani, G., Gherardi, S., Vicini, E., Spotti, E., Previdi, M.P., Ndagijimana, M., Cavalli, S., Reschiotto, C.: Use of ion chromatography for monitoring microbial spoilage in the fruit juice industry. J. Chromatogr. A, 1997, **(770),** 243-252.

OCENA METOD BADANIA AKTYWNOŚCI MIKROBIOLOGICZNEJ PODCZAS EKSTRAKCJI SACHAROZY Z BURAKÓW CUKROWYCH

S t r e s z c z e n i e

W pracy dokonano oceny wybranych metod służących do określania poziomu aktywności mikrobiologicznej podczas ekstrakcji sacharozy z buraków cukrowych. Jako kryterium przydatności badanych metod przyjęto łatwość ich stosowania, szybkość oraz dokładność. Stosowano pośrednie metody polegające na: enzymatycznym oznaczeniu kwasu L-mlekowego, izokataforetycznym badaniu stężenia kwasu mlekowego oraz na oznaczeniu azotanów(III). Wyniki uzyskane przy stosowaniu powyższych metod porównano z rezultatami bezpośredniego oznaczania poziomu bakterii mezo- i termofilnych. Jakkolwiek oznaczenie kwasu L-mlekowego metodą enzymatyczną w aparacie MICROZYM-L jest bardzo proste i szybkie, to jednak w niektórych przypadkach jego zawartość nie odzwierciedla rzeczywistej sytuacji mikrobiologicznej w ekstraktorze. Podobnie poziom azotanów(III) nie reprezentuje dobrze stanu sanitarnego podczas badanego procesu. Oznaczenie natomiast obu izomerów kwasu mlekowego (L+D) okazało się tu najlepszym wskaźnikiem zanieczyszczenia mikrobiologicznego.

Słowa kluczowe: zanieczyszczenia mikrobiologiczne, kwas mlekowy, azotany(III)