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(Original Article)

Reduction of the Microbiological Action During Sugar Beet Extraction with Focus on Nitrite Contamination

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Abstract

Nitrite is one of the harmful pollutants for both people and animals. The formation of nitrite during the manufacturing of beet sugar was investigated. The results indicated that the cossettes contained lower nitrite content (1.23±0.1 mg/kg DM). However, the nitrite mainly formed during the extraction and purification processes. Most of the nitrite was transferred to molasses, with the remaining portion returning to the crystallization process through the circulation of juices. The nitrite content of raw juice was lower than 10 mg/kg DM in the aerobic extraction system, and it was reduced by 50% during the liming and carbonation. In the tower extraction system for both factories F1 and F2, the nitrite content increased from 5.5 and 25 mg/kg DM in the raw juice to 298 and 247%, 284 and 238%, 716 and 1032%, and 307 and 881% in the thin juice, thick juice, raw sugar green, and molasses, respectively. A significant reduction in the nitrite content of raw and de-foamed juice was achieved by the suggested point (L3) for dosing the disinfectant B (hop β-acids). The effect of the disinfectant lasted for more than 6 h at normal nitrite levels of juice and up to 90 min at high nitrite levels. Furthermore, the nitrite contents were reduced in thin juice, thick juice, and molasses by 58, 48 and 30%, respectively. By optimizing the dosing procedure and point of disinfectant hop β-acids dosage, the microbial load on the extraction system and formation rate of nitrite could be reduced significantly.

Keywords: *Nitrite, Microbial load, Nitrification, Molasses quality*

Introduction

The microbial load during the extraction process of juice from sugar beet is one of the serious challenges facing manufacturers of beet sugar. In addition to the loss of sugar by microorganisms, the outputs may negatively affect, directly or indirectly, the various stages of manufacturing. The most important of these bacterial compounds are lactic acid, nitrite, dextran, and levan which affect directly or indirectly the sugar manufacturing (Hallaus *et al*., 1997; Abdel-Rahman *et al*.,

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2007). The presence of nitrite in both human and animal foods has been a concern during the past years as it is suspected to be toxic and carcinogenic. The Acceptable Daily Intake (ADI) of the nitrite anion for humans was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to be 0.07 mg/kg b.w. per day (World Health Organization, 2003). Nevertheless, the negative effects of nitrite on human health are not the focus in the sugar industry, since sugar as the main product is made up of almost pure sucrose and hence contains only insignificant amounts of non-sucrose materials.

In contrast non-sugar components of the mother liquor accumulate in the liquid phase remaining after crystallization. Also, nitrite plays an important role in the further processing. It has been verified that nitrite, which is accumulated in the molasses from the sugar processing, may hinder microbiological fermentation increasing the fermentation time (Glacet *et al*., 1985; Le Gutierrez, Martin Orelli, 1991). Some animals, especially the monogastric animals such as pigs, are very sensitive to nitrite intake because they do not have the mechanisms for their further conversion into ammonia because it is absorbed in the upper part of gastrointestinal tract (Baranova *et al*., 2000). In contrast, nitrate content is not dangerous for the monogastric animals as in ruminants because it is converted to nitrite in the end of digestive tracts (Cockburn *et al*., 2013; Waterlander *et al*., 2011). Since 2009, the nitrite content in animal feed has been restricted by the European Food Safety Authority and may no longer exceed 15 mg/kg, expressed as sodium nitrite with 12% moisture content (Alexander *et al*., 2009). Studies demonstrated that feed products from beet sugar manufacture contained a high level of nitrite. Hence, comprehensive understanding and knowledge are needed (Frenzel, 2016).

The presence of nitrate content in the root of sugar beets depends on nitrogen fertilizer rates during plant cultivation. The high level of nitrate in the sugar beet is considered a main source of nitrite formation by thermophilic bacteria during the extraction processes under the anaerobic condition (Hollaus *et al*., 1997; Waterlander *et al*., 2011; Emerstorfer *et al*., 2014). Given that the existence and activity of nitrifying bacteria is one source of nitrite formation, it is logical that eliminating such bacteria could be a way of reducing the nitrite level in the sugar processing. This could be achieved by using disinfectants, such as formaldehyde, carbamates, sulfur dioxide, hydrogen peroxide and so on (van der Poel *et al*., 2000). However, sugar manufacturers are opting for more natural selections like hop β-acids (under the trademark "BetaStab") and pinetree-derived rosin acids because of toxicity of these disinfectants (Pollach *et al*., 2002).

Other than direct elimination of the responsible bacteria, nitrite formation could also be prevented by minimizing the bacterial activity of degrading nitrate into nitrite. For example, this can be done through increasing the temperature beyond the optimal temperature of the bacteria. It is believed that the nitrate reduction in sugar beet juice is primarily caused by thermophilic bacteria from Thermus and Bacillus strains, such as *Bacillus stearothermophilus*, whose activity decreases significantly at temperatures above 75 °C (Hollaus *et al*., 1997). Besides, lowering the pH level of the sugar juice could also hinder the nitrite formation by

microorganisms. Establishing a pH value below 5.0 can dramatically reduce nitrate degradation by bacteria (Frenzel, 2014). Nevertheless, setting the pH below 5.0 is considered an impractical solution, because it may lead to a high sucrose loss due to sugar inversion. Moreover, an aerobic condition presumably favors the activity of nitrite forming bacteria, and hence an aeration of the system could be used as an approach to minimize the activity of the bacteria (Emerstorfer *et al*.,. 2014;). During the storage of beet, the climatic conditions affect the beet quality (Abdel-Rahman *et al*., 2007). Frost damaged beet deteriorate rapidly and they cause many problems during processing, such as increases of nitrate levels and activity of denitrifying bacteria leading to a high rate of nitrite (Wojtczak *et al.*, 2016). Therefore, the aim of this study was to evaluate the nitrite formation and determine its content during the beet sugar manufacturing process. Additionally, reduction of the nitrite content via applying optimized disinfectants dosing procedures and locations was investigated.

Materials and Methods

Samples and chemicals

All the samples were collected from Aarberg and Frauenfeld sugar factories during beet and thick juice campaign 2011 to 2015. Nitrite kits (REF 985 069, Test: 0-69, Nanocolor® Nitrite 2 and 4, Also Quantifix nitrate / nitrite test strips (semi-quantitative 10-500 mg/L NO3 / 1-80 mg/L NO2) were used. The instrument utilized to measure the nitrite contents is Nanocolor® UV/VIS Spectrophotometer, Quantofix® Relax Automated Reader. All of Nitrite kits and

Spectrophotometer were purchased from Macherey-Nagel GmbH & Co. KG, Düren, Germany. The disinfectants used in this study were disinfectant A (formalin) and disinfectant B (hop β- acids -under the trademark "BetaStab", from Beta Tec Hop Products Ltd, Great Britain).

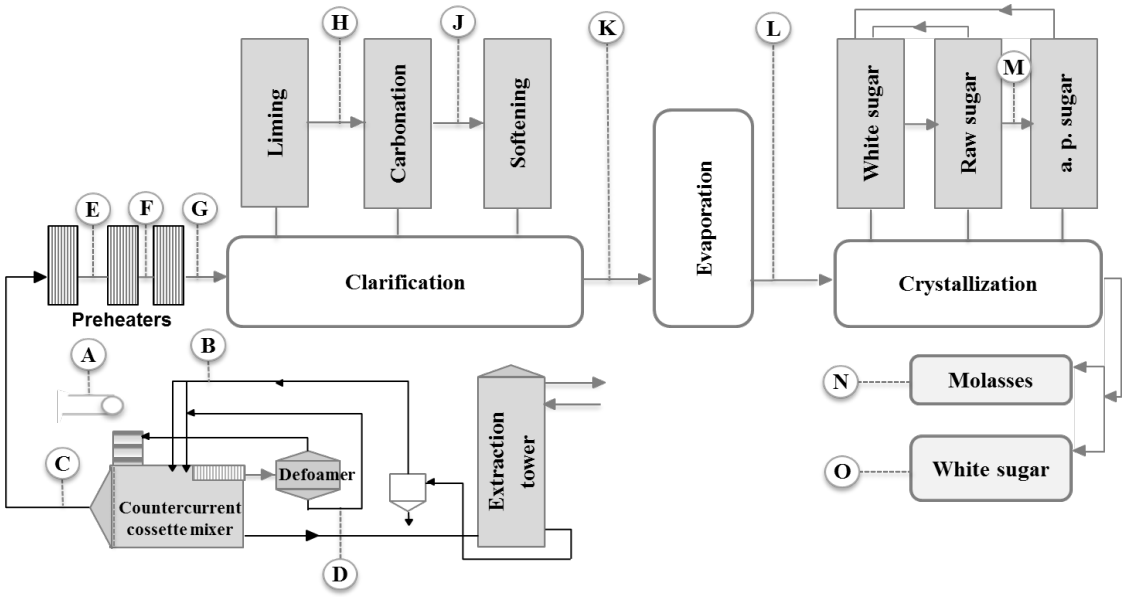

Figure 1. Flowchart of the manufacturing process for white sugar from fresh juice with indication of sampling points (BMA system)

Sampling points: Figure 1 shows the main stages of the beet sugar production process starting from fresh juice. Sampling points used for analyses are indicated.

A to O, The sampling points are as follows: A: cossette; B: recycling raw juice; C: mixture raw juice; D: defoamer raw juice; E: raw juice preheater1; F: raw juice preheater 2; G: raw juice preheater 3; H: juice after limitation; J: juice after carbonation (juice filters1 and 2); K: thin juice after softening; L: thick juice; M: raw sugar green (RSG); N: molasses; O: white sugar.

Nitrite determination

To determine the nitrite content in the sugar solutions, the samples should be prepared to suit the nitrite estimation method of the treated wastewater. 5-20 g sample (depend on its nitrite concentration) was transferred quantitatively by 25 mL of distilled water into 100 mL volumetric flask, and 4 mL of Carrez solution I (21.9 g of zinc acetate dehydrate with 3 mL of acetic acid were dissolved in water, and final volume was adjusted to 100 mL with water) and Carrez solution II (10.6 g of potassium hexacyanoferrate (II) trihydrate was dissolved in water, and the final volume was adjusted to 100 mL with water) as clarifier solutions (Rios-Rios KL, *et al*.,. 2018) were added and filling the volumetric flask with distilled water up to the mark. The sample solutions were filtered through Whatman filter paper and then by syringe filter 0.45 µm (cellulose acetate).

The determination of nitrite content of solutions containing low (< 0.465mg/L) or high (> 0.465mg/L) nitrite levels is according to German standard methods for the examination of water, wastewater, and sludge (DIN EN 26 777-D10).

Determination of lactic acid and glucose

Glucose and lactate were analyzed by Biosen C Line, EKF Diagnostic, an enzymatic-amperometric method that uses chip-sensor technology (Shimomura *et. al*., 2012). Measuring range: glucose 0.5 to 50 mmol/L (9–900 mg/dL) and lactate 0.5 to 40 mmol/L (5–360 mg/dL) imprecision: coefficient of variation ≤ 1.5% (12 mmol/L)."Biosen C-Line" as dual channel systems that can measure Lactate and Glucose at the same time. Undiluted sample should be filtered through 0.45 µm filter and 50 µl transferred by a 50 ml pipette into a transparent reaction vessel containing the solution and place it in the device.

Measurement of pH

The pH value was determined at room temperature using a pH-meter (Mettler-Toledo GmbH, Germany).

Determination of refractometric dry substance (°Brix)

Dry substance, which represented total soluble solid was measured as °Brix (%w/w) at 20°C by the Refractometer ATR W2Plus, Schmidt & Haensch.

Measurement of bacterial nitrite formation

The bacterial nitrite formation at 25, 35 and 45 °C for 0 to 6 h in raw juice was investigated. 100 ml raw juice sample is transferred to a sterile 100 ml glass bottle, and then closed well to provide anaerobic conditions. This repeats for all treatments. After the end of the experiment, samples are withdrawn to estimate nitrite as an indicator of the growth of nitrite-forming bacteria.

Disinfectant effect measurement

To study the disinfectant effect, total microbial count and lactic acid bacteria were tested. Samples (10 ml) were token and diluted by sterilized distilled water to 100 g. The serial dilutions of collected samples (10^{-1} to 10^{-6}) were made and 1 ml portions of the appropriate dilutions were pour-plated on MRS-Agar media (De Man *et al*., 1960). The cultivated microorganisms were incubated anaerobically for 72 h at 30±1 °C for enumeration of microorganisms.

Statistical analysis

The statistical analysis of data was performed using the program GraphPad Prism 6 with t-test and ANOVA method. Significance level of $P \leq 0.05$ was used to determine significant differences.

Results and Discussion

Nitrite levels during beet sugar manufacturing

The behavior of nitrite formation at the practical conditions of sugar manufacturing, three different extraction systems; tower extraction, RT-drum and DDS-trough in the European factories were stated. The most important problem that we have encountered to perform this work is that there are not internationally standardized and validated methods to estimate nitrite in the sugar solutions through the stages of sugar production. Therefore, a common method according to German standard methods for the examination of water, wastewater and sludge (DIN EN 26 777-D10) after making some modifications in preparation of samples for analysis was used as described in methods section (Frenzel, 2014).

Figure 2. shows the changes in the nitrite content during beet sugar production by the laboratory pilot. The nitrite content of raw juice was lower 10 mg/kg DM and approximately about 50% of nitrite was lost during liming and carbonation processes. A trough-type extractor (DDS) system of the laboratory pilot which is aerobic extraction system was used (Figure 3). In this system, the extraction depends on effective heating of the incoming cossettes in extractor zone A; this can be reflected by a requirement that the temperatures of the juice and cossettes between zones A and B is sufficiently high (Urbaniec, 1989). The aerobic condition and the thermal treatment of cossettes and juice at short time decreased the nitrite formation during the extraction process.

During the evaporation and crystallization process, the nitrite accumulates as a non-sugar in the run off solution even up to molasses. The nitrite contents during the various stages presented in Figure 2a, were re-calculated at non-sugar weight

basis for comparison (Figure 2b). The results indicated that there are no significant differences between nitrite contents of thick juice and run off samples, which were calculated at non-sugar weight basis. Using pure CO_2, and anti-scales addition affected on the total soluble solid in the solution. The difference of nitrite levels between thin juice and thick juice in DDS system maybe because color and sediment materials as non-sugars are formed (without anti-scales addition) during the evaporation and crystallization processes which play a role on the nitrite calculation on dry matter bases or non-sugar bases.

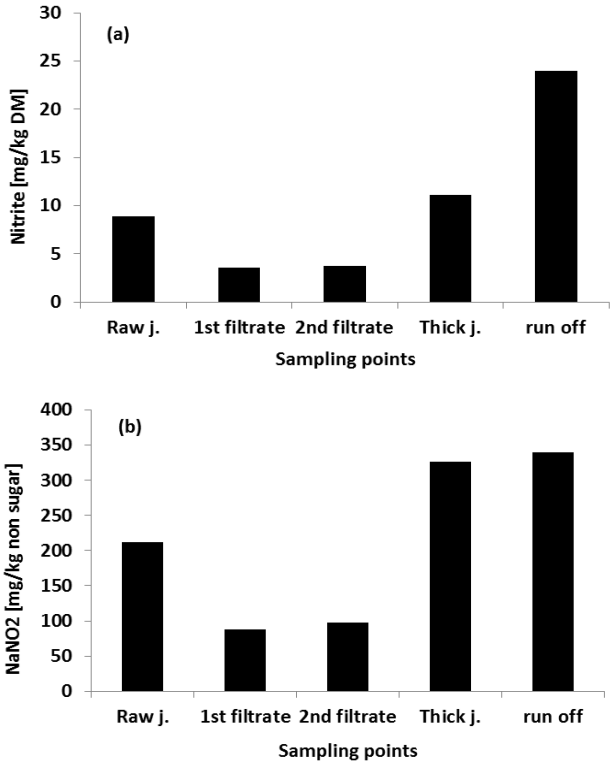

Figure 2. Changes in the nitrite content during laboratory beet sugar production (small pilot), calculated at dry mater basis (a) and non-sugar basis (b).

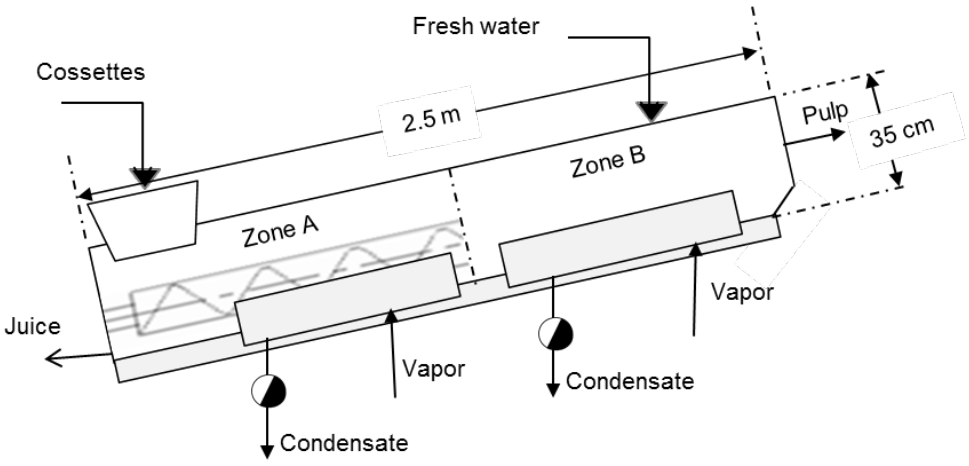

Figure 3. Trough-type extractor (DDS) of laboratory pilot.

In this study, BMA tower extract system for two factories was selected. Figure 4 shows the nitrite content during processing in two beet sugar factories, (F1) and (F2) during fresh beet campaign 2012. From the results, it has observed that the content of nitrite in the sugar juices vary from factory to another. It was low in the first factory, while it was very high in the second factory in the same year campaign 2012. The results for both factories F1 and F2, show that the nitrite content increased by about 298 and 247%, 284 and 238%, 716 and 1032% and 307 and 881 % of thin juice, thick juice, raw sugar green and molasses compared with raw juice (5.5 and 25 mg/kg DM), respectively. These results indicate that the nitrite formation was during the extraction and purification processes and did not record a significant change during the subsequent processes. The higher nitrite content in thick juice, raw sugar green, and molasses was due to the collection of non-sugar substances.

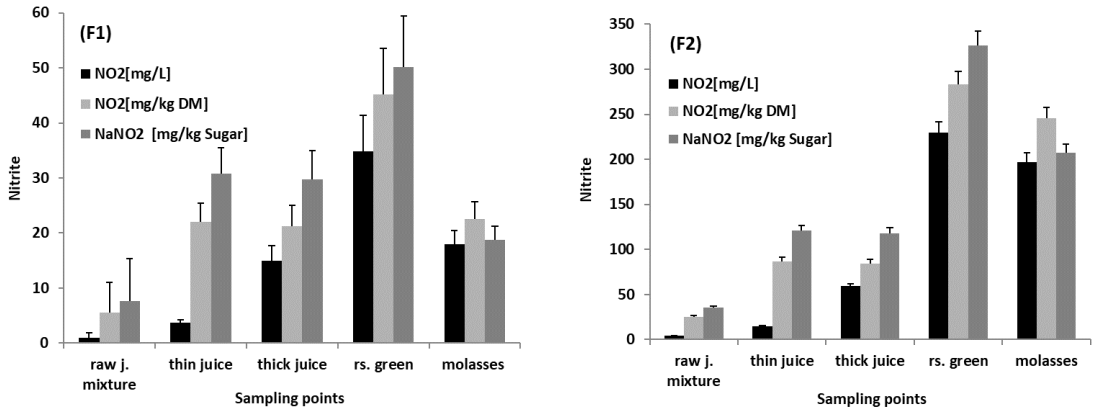

Figure 4. Changes in the nitrite content during beet sugar manufacturing of factory 1 (F1) and factory 2 (F2) as concentration in the liquid phase (mg/L) or fraction of dry substance (mg/kg DM) or sodium nitrite in the fraction of sugar content (mg/kg Sugar), juice samples: raw juice (sampling point C); thin juice (sampling point K); thick juice (sampling point L); raw sugar green (sampling point M); molasses (sampling point N), sampling points as indicated in Figure

After identification of the main locations of nitrite formation during sugar manufacturing (see Figure 4), the nitrite content during the extraction and clarification processes of beet sugar juice was determined to illustrate causes of nitrite formation in these locations. Figure 5 shows the nitrite content in cossettes, circulation juice, raw mixture juice and raw juice preheater steps during BMA tower extraction system. The results indicated that cossette and circulation juice (tower juice) contain a small amount of nitrite (1.23±0.1 and 0.6±0.12 mg/kg DM). This is due to that the bacteria produces nitrite under the anaerobic conditions, and it does not need the nitrification process to get the oxygen in this case. These results are in a good agreement with Hoffmann & Märländer (2005) who reported very low nitrite content for sugar beets. On the other hand, Wojtczak *et al*., (2016) found that raw materials are not a direct source of nitrite, however, it can be a source of nitrates and denitrifying bacteria which convert nitrates to nitrites during the extraction processes.

Change of the climatic condition at the 2nd quarter of campaign, temperature decrease to below 0°C and heavy rains led to increase the rotten beets to 12.8±3.7%, consequently, lactic acid and nitrite formation during extraction process were increased to 877.5±85.9 and 31.35±14.7 mg/L, respectively (results are not shown). There are a little data on the changes in the levels of nitrates, nitrites and lactic acid in sugar beet during storage under controlled temperature conditions, and also, qualitative indicators of sugar beet damaged by frost during storage except those reported by Wojtczak *et al*., (2016). It is difficult to determine the changes in the indicators of quality depending on time and temperature, because the temperature distribution varies in different clamp zones (Jaggard *et al*., 1997).

Cossette mixer, 1st preheater and 2nd preheater were the main locations to nitrite formation where the optimization of nutritional and environmental factors. Aerobic condition, temperature and pH play a vital role in converting the nitrate to nitrite (nitrification) in sugar juices. Low pH inhibits the nitrite formation as shown in tower extraction (Hu *et al*., 2001). Nitrite formation while raw juice passes through preheaters may be due to the biofilm which produced by microorganisms where juice temperature ranged between 30-55°C.

Figure 6 illustrates the nitrite contents in the individual chambers of the pre-limer in factories A and B. The content of nitrite in the raw juice increased rapidly when entering the pre-limer. This increase may be due to microbial activity in the first chambers of the pre-limer and by the recycling of carbonation sludge. A little decrease (Factory A) or stable (Factory B) nitrite content which took place from chambers 3 and 4 onwards can be observed. High pH inhibits microbial activity. Also, it might have been a dilution effect by the recycled sludge. Moreover, it is not easy to fully explain this phenomenon (Frenzel 2016).

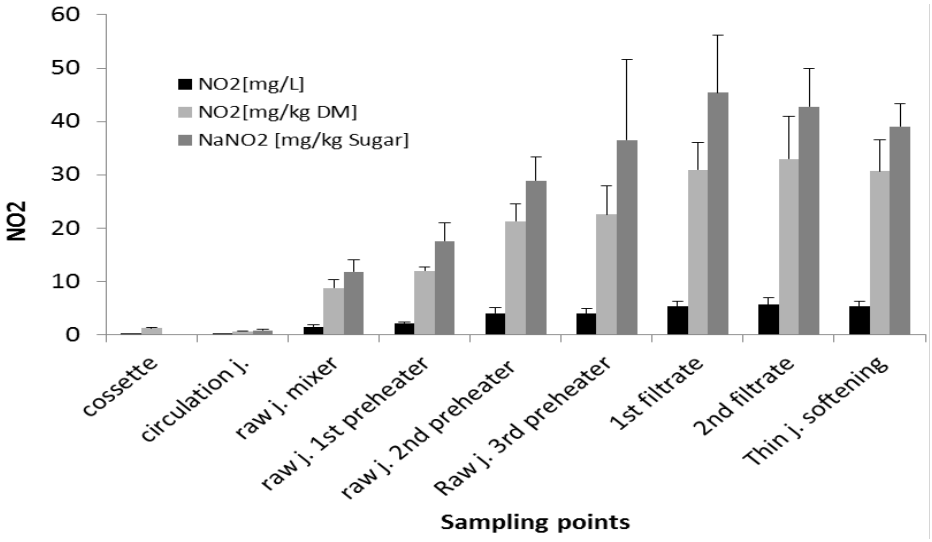

Figure 5. Changes in the nitrite content along the raw juice heater chain and juice purification during fresh juice campaign factory 1.

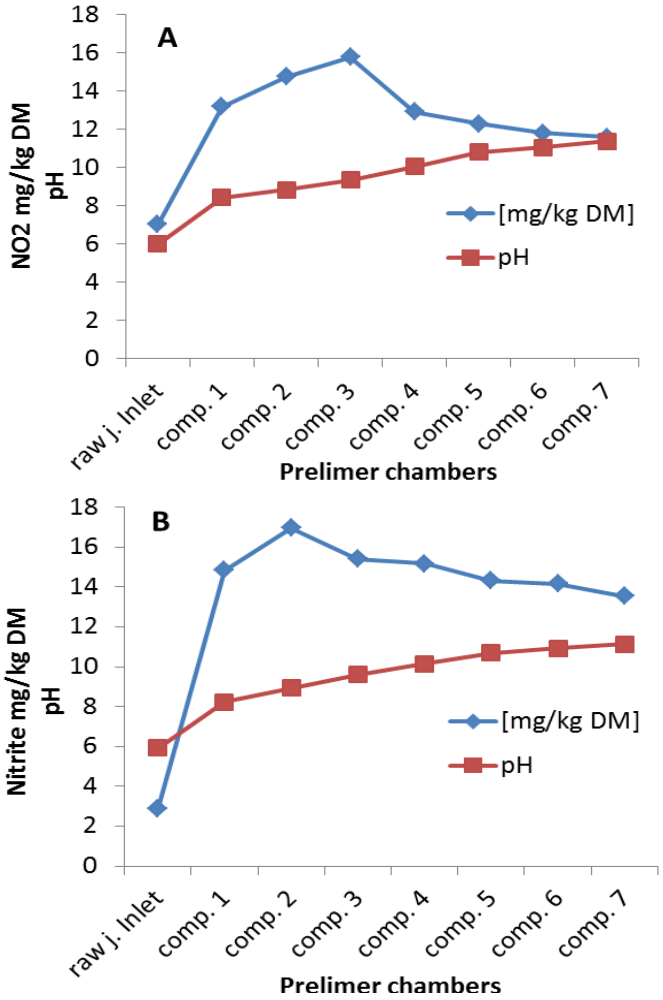

Figure 6. Nitrite formation during the pre-liming process of factory 1 (A) and Factory (B), fresh juice campaign.

The bacterial nitrite formation in the beet raw juice at 25, 35, and 45 °C for 0 to 6 h was studied (Figure 7). No nitrite formation was found at all the studied temperatures until the third hour of incubation time. At 35°C, the nitrite formation began to increase in the fourth hour of incubation time. On the other hand, no formation of nitrite at 25 and 45 °C treatment was observed. The results of this experiment explain the highest nitrite content ratios in the medium heat places (25-40), such as parts of the cossitte mixer and pre-heating the raw juice as well as the early stages of the pre-liming process. Although many researchers reported that the nitrite formed by thermophilic bacteria (Bergey, 1919). However practically, a decrease in the nitrite content of circulation juice which comes from the extraction tower was observed, where the temperature up to 70 °C as well as the availability of anaerobic conditions. Under the same conditions in extraction tower, an increase of lactic acid bacteria activity was observed. The mechanism of nitrite production may be at intermediate temperatures as was confirmed in Figure 7. The results indicated that, all bacterial activities stop at the beginning of the liming stage and the following stages during sugar manufacturing due to high temperatures and pH. The changes in the nitrite content during these processes probably due to redox

(reduction–oxidation reaction). The mechanism of these reactions should be investigated.

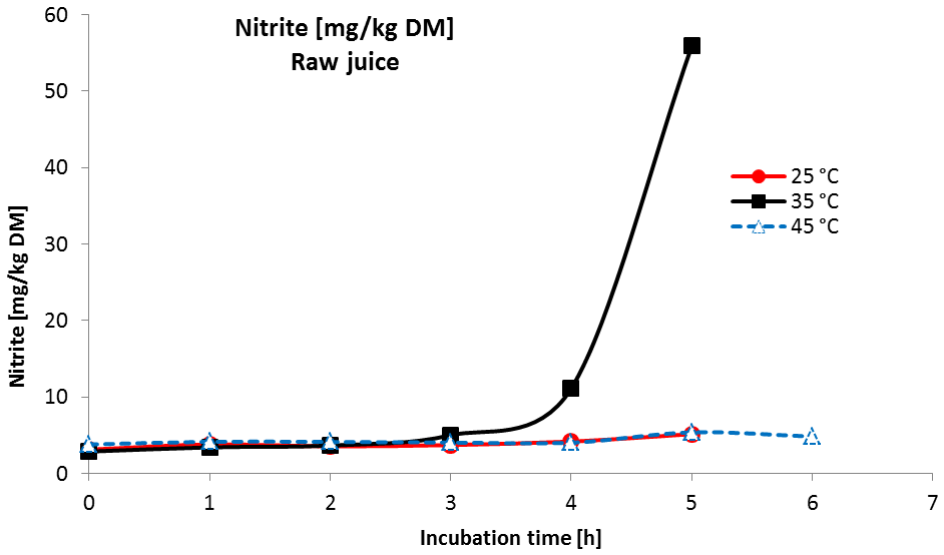

Figure 7. Bacterial nitrite formation in mixture raw juice under the laboratory conditions.

Nitrite reduction and mitigation

After identification of the most important locations of the bacterial nitrite formation, it is necessary to find an optimal method to inhibit the activity of these bacteria during the extraction process, additionally, to identify the dosage and location inject which has a highest impact of disinfectants. Figure 8 shows the common dosing locations (L1 and L2) and the suggested dosing location (L3). Generally, the location L1 is used for disinfectant injection to inhibit the biofilm on the sieve cossette mixer that caused by the microorganisms. On the other hand, the location L2 is used to optimize the pH in the extraction tower. Figure 9 shows the effect of dosages 20L/ 2h (Exp.1) and 10L/1h (Exp.2) in the location 1 on nitrite formation and a small difference was found between both treatments. Because the mixture raw juice takes a short time from the beginning of the disinfectant injection process to move to the preheating stages. Also, these treatments reduced the difference between nitrite contents before and after the preheaters. This indicates that the nitrite formation inters the cossette mixer was no affected by the disinfectant dosage in L1.

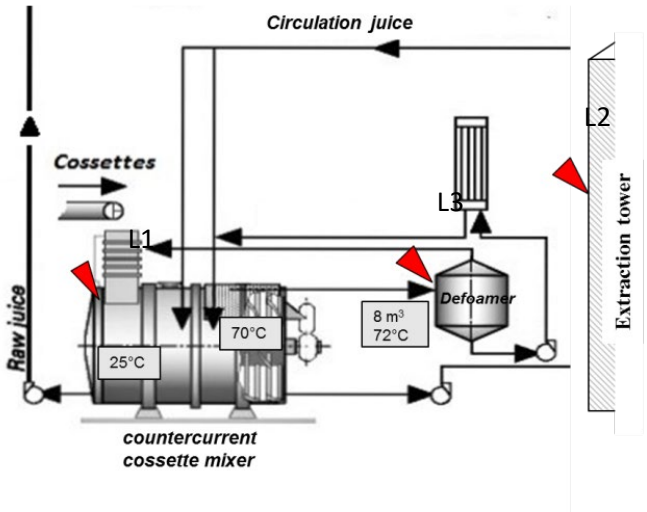

Figure 8. Dosing location during extraction processes (BMA tower system).

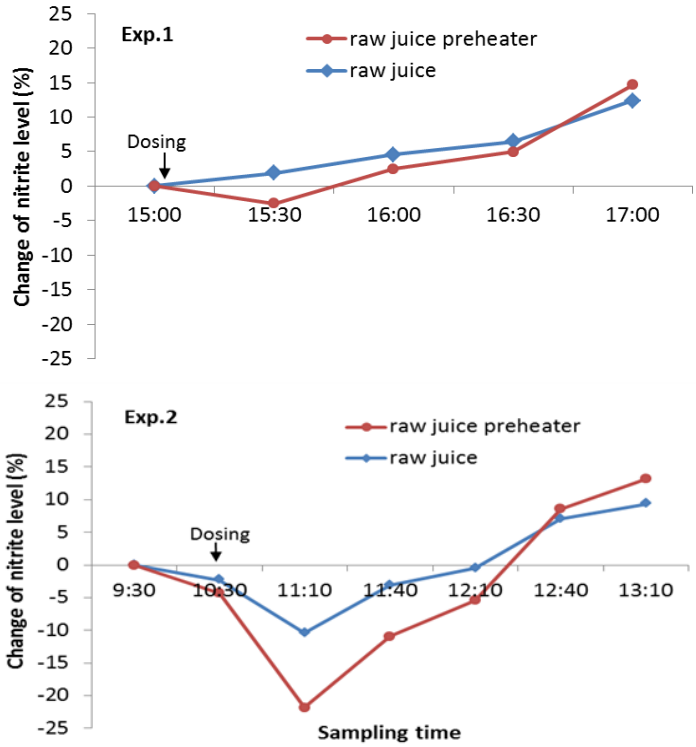

Figure 9. Effect of disinfectant B dosage L1, Exp. 1 = 20 L/2h, Exp. 2 = 10L/1h.

In this study, the location of defoamer addition (L3) was the suggested point to dose the disinfectant. That is for several reasons, including: a*)* the juice foam, which is pulled from the cossette mixer, is contaminated with a high number of microorganisms, particularly the thermophiles ones. These bacteria return to the cossette mixer again by the de-foamed juice which increases continuously its microbial load during the campaign. b*)* the capacity of the defoamer location is 8 cubic meters which helps to more spread efficiently of the disinfectant (10-20 L) in the cossette mixer. c*)* the effect of disinfectant will be not only on the cossette mixer but on both the extraction tower and after the out of juice to the preheating processes. The results of experiment 3 are shown in Figure 10. The results

obtained from both Experiments 3a and 3b has proven high effectiveness for the suggested dose location (L3). In the first case, the nitrite content of raw juice (<80 mg/kg DM) (Exp. 3a), the nitrite content was reduced dramatically after 30 min of the first dosage of disinfectant B. After the third dose, more than 90% of the nitrite content was reduced. In the second case, high nitrite content of raw mixture juice (>100 mg/kg DM) (Exp. 3b), the time rate of disinfectant dose became shorter (10L/h) than the normal case (10L/2h). Also, the results of Exp. 3b indicated a decrease of nitrite content by about 90% after the third dosage. Furthermore, lactic acid as a second parameter of microbial loading showed a decrease as a result of both treatments (Exp. 3a and Exp. 3b) by disinfectant B and dose location L3. The amounts of lactic acid reduced from 700 to 300 mg/L of both Exp. 3a and Exp. 3b, in other words, nearly 57% reduction of lactic acid during the extraction process of sugar beet. On the other hand, no effects on glucose content of raw juice were observed. This is probably due to that the glucose is a non-bacterial product.

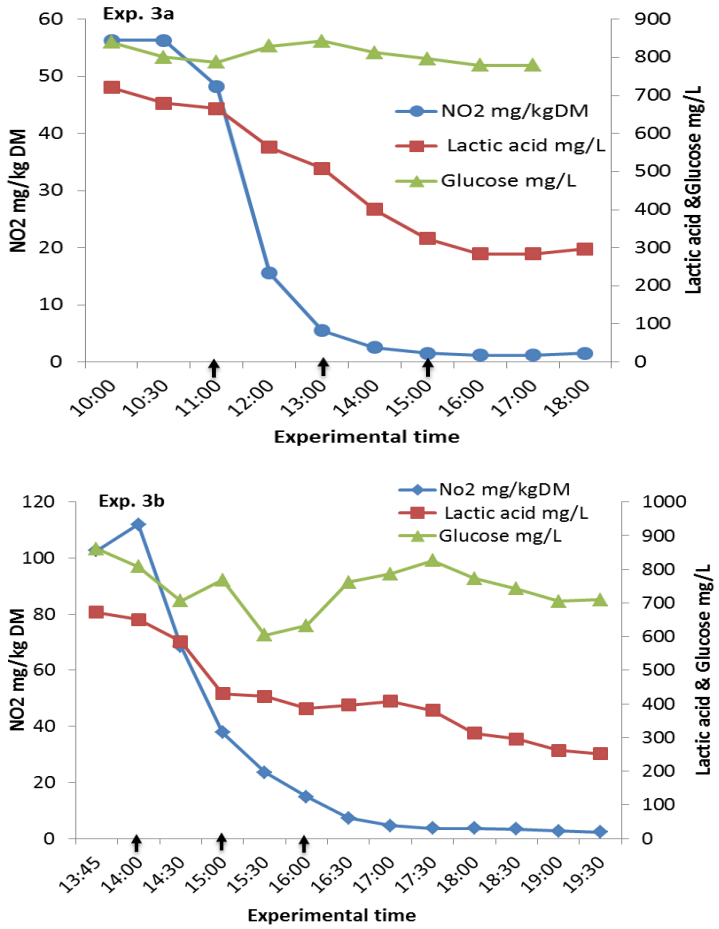

Figure 10. Effect of the dosage location L3, 10L/2h (Exp.3a) and 10L/h (Exp.3b), disinfectant B on the various microorganism parameters; Nitrite, Lactic acid and Glucose, (arrows indicate the time of disinfectant dosing).

The count of microorganisms in the collected samples, raw mixture juice and de-foamed raw juice (Exp. 3a, 10L/2h), after incubation on general agar (for all microorganisms) and agar (for lactic acid bacteria) at 30°C for 72 h was performed.

The average counts of total colonies on the general agar were $52.8*10^5$ and $40.53*10^2$ cfu/ml of raw juice and de-foamed raw juice, respectively. On the other hand, they were $33.6*10^5$ and 40 cfu/ml on the agar of lactic acid bacteria. These results indicated that total numbers of microorganisms reduced almost 99.92 and 99.99% of lactic acid bacteria after the addition of disinfectant B through the dosage location L3. This may be that the treatment by disinfectant during de-foaming process inhibited the growth of microorganism inside the cossette mixer and the most of extraction processes and consequently its productions during fresh beet campaign. Although the use of disinfectant B showed a strong effect on lactic acid bacteria, on the other hand in experiments 3, it showed a moderate effect on the estimated amount of lactic acid. This may be because of the disinfectant by injecting in L3 is greater in the cossette mixer and lower in the extraction tower. The most content of lactic acid in raw juices may be due to the formation of it in the extraction tower. It is essential not to reduce lactic acid to high levels to avoid damage of beet slices during the pressing process. These results are consistent with findings of Pollach, *et al*. (2002).

Determination of pH as parameter for microbial load of both raw mixture and circulation juices are illustrated in Figure 11. The results indicated the effectiveness of dosage disinfectant included cossette mixer and the extraction tower. However, the pH must not be higher than the required limit of cossette press processing.

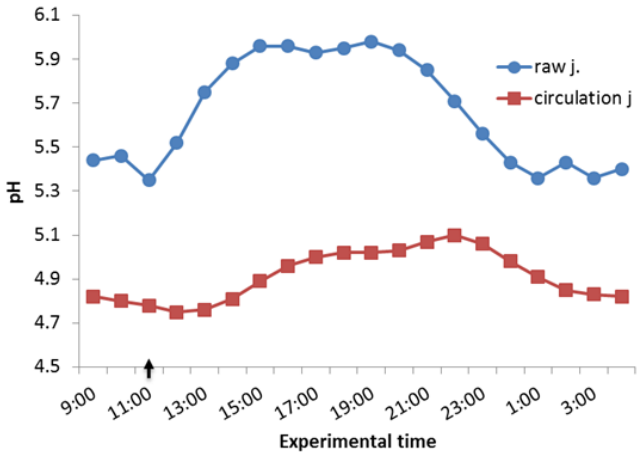

Figure 11. Effect of disinfectant B by dosage location L3 on the pH of raw mixture and circulation juices (arrow indicates the time of disinfectant dosing).

To determine the optimum dose of disinfectant dosage, experiments 4a and 4b were performed. The result of Exp. 4 in Figure 12 showed a significant difference in nitrite content between of the cossette mixer and de-foamed juices, 45 and 75 mg/kg DM, respectively. This difference may be due to the large numbers of bacteria collected inside the antifoam place. The results also show that the effect of disinfectant continued for about 6 h when adding one dose through suggested dose location in the normal nitrite levels case (Exp. 4a), then the nitrite tended to increase faster in the de-foamed raw juice than raw mixture juice. On the

other hand, the disinfectant activity continued for about 90 min in the high nitrite levels case (Exp. 4b). Therefore, the disinfectant doses should be at short periods.

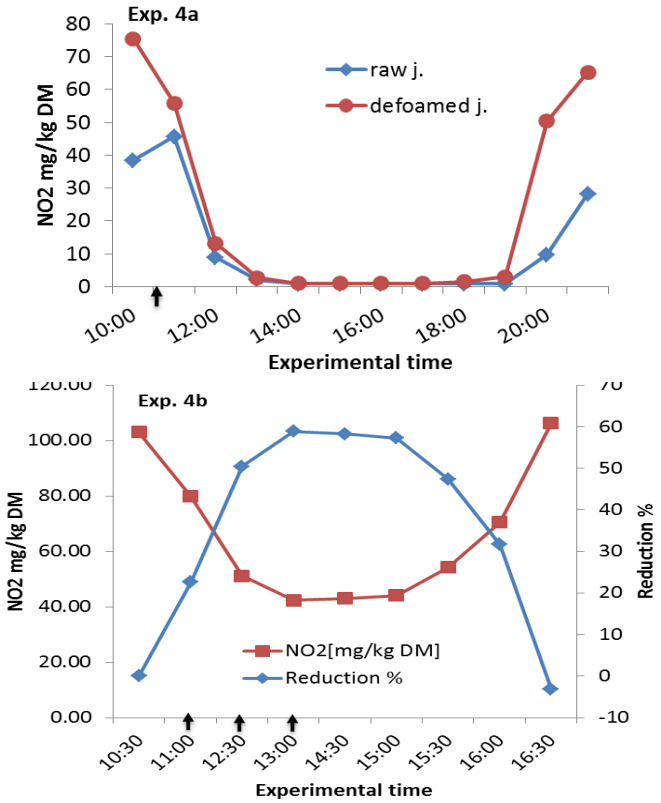

Figure 12. Effect of dosing time, L3, 20L one time (Exp.4a) and three times 10L/2h (Exp.4b), disinfectant B, dosing location L3 on the various microorganism parameters; Nitrite, Lactic acid and Glucose, Arrows indicate the time of disinfectant dosing.

The effect of the disinfectant dose on the nitrite content during the manufacturing processes was evaluated (Figure 13). A dramatic decrease in the nitrite content of the extracted juice was found, but there was a noticeable increase in nitrite content of thin juice after the purification process. Nitrite contents were reduced in raw juice, thin juice, thick juice and molasses by 90, 58, 48, 30 %, respectively.

Despite the obvious positive effect of these treatments on the nitrite contents in juice and molasses, the nitrite formation during the subsequent stages of the extraction processes should be investigated. When the same results of nitrite in Figure 13 were calculated based on the weight of non-sugar, the nitrite content was reduced during the crystallization processes (Figure 14). The degradation of nitrite may be attributed to the transformation of the nitrite compound into other compounds under the influence of high temperature for a long time. Also, available information regarding nitrite reaction and transformation during sugar manufacturing is limited.

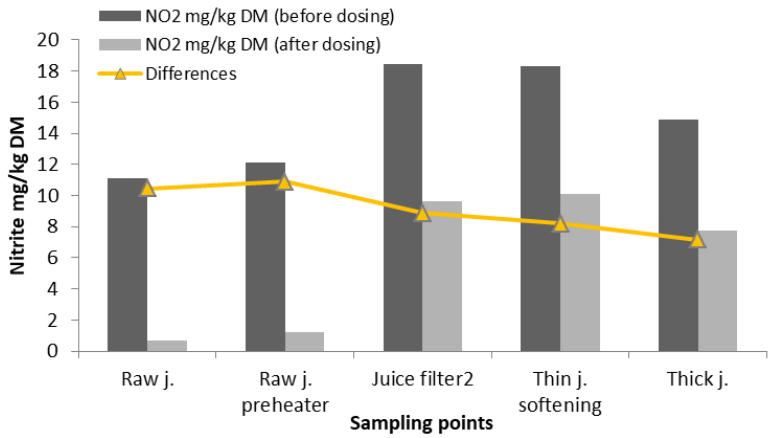

Figure 13. The nitrite content before and after the disinfectant dose during the manufacturing processes.

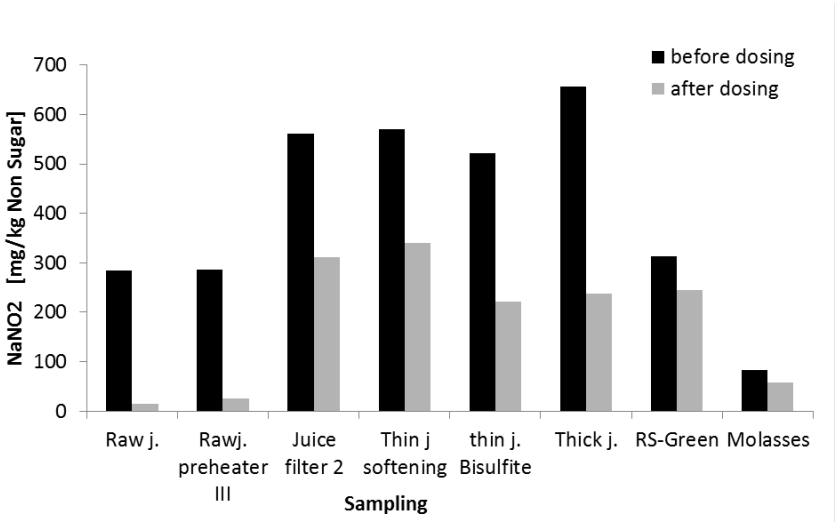

Figure 14. The nitrite content before and after the disinfectant dose during the manufacturing processes (based on the weight of non-sugar).

To get information about the nitrite formation during the crystallization process, a separate experiment in the laboratory evaporation crystallization unit under controlled conditions was performed. The results in Figure 15a indicate a decrease in the nitrite content from thick juice to massecuite of about 18±1.86%. On the other hand, the nitrite content increased about 41.55±0.71% in run off as a collected non-sugar materials. For the identification of nitrite loss during crystallization process, the results in Figure 15a were re-calculated at non-sugar weight basis as shown in Figure 15b. From the results, no change was found in nitrite content after centrifugation process of massecuite. Most of nitrite content was found in the run off solution and a very little amount was found in the sugar. The difference between nitrite content (calculated at non-sugar weight basis) of thick juice and massecuite maybe due to the formation of colored materials as impurities during the crystallization process.

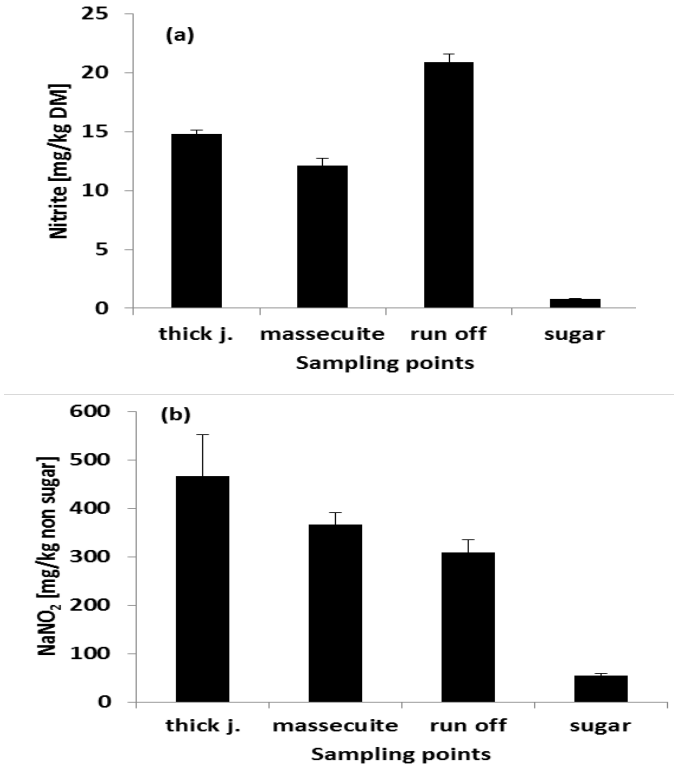

Figure 15. Nitrite development during the laboratory evaporation crystallization, calculated at dry mater basis (a) and non-sugar basis (b)

Conclusion

This study focused on determining the nitrite content during different stages of beet sugar production. The obtained results indicated that formation of bacterial nitrite mainly occurred during the extraction process. The nitrite formation was influenced by many factors such as climatic conditions, beet quality, beets grown under drought stress "nitrate levels", the technical setup of the extraction system [aerobic and anaerobic conditions], disinfectants "dosing and injection points", extraction conditions, temperature and pH. Cossettes were free of nitrite, but maybe it is the source of nitrate and bacteria that form the nitrite. Nitrite accumulates during the evaporation and crystallization processes and the bulk of them goes into the molasses and the rest goes back to the crystallization process during the circulation of the sugar solutions. Also, the results revealed that the optimum temperature for nitrite formation in raw beet juice by bacteria under anaerobic conditions was 35 °C and these conditions are quite similar to the beet cossettes mixing station. Furthermore, injecting the dose of disinfectant (hop β-acids) at the suggested point reduced the nitrite content in raw juice by about 90% and in molasses by 30%. Further studies are needed to investigate the chemical behaviour of nitrite during the various manufacturing stages of beet sugar production in order to minimize the nitrite content in the molasses as much as possible.

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خفض التأثير الميكروبي أثناء استخلاص بنجر السكر مع التركيز على التلوث بالنتريت

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الملخص

يعتبر مركب النتريت من الملوثات الضـارة للإنسـان والحيوان. تتبعت هذه الدراسـة تكوين النتريت أثناء عمليات تصنيع السكر. أظهرت النتائج أن شرائح البنجر احتوت كمية منخفضـة من النتريت (1.23 ±0.1 mg/kg DM)، لكن النتريت تَكَوَّنَ بشكل أساسي أثناء عمليات الاستخلاص والتنقية، ثم تراكم خلال عمليتي التبخر والتبلور. يذهب الجزء الأكبر من النتريت المتكون إلى المولاس والجزء الآخر يعود إلى عملية التبلور أثناء تدوير محاليل السكر. أشارت النتائج إلى أنه في نظام الاستخلاص الهوائي، كان محتوى النتريت في العصير الخام أقل من 10 مجم / كجم مادة جافة، وانخفض بنسبة 50٪ أثناء عمليات التجيير والكربنة. من ناحية أخرى، في نظام الاستخلاص البرجي لكلا المصـــنّعين F1 و F2، زاد محتوى النتريت من 5.5 و 25 مجم / كجم مادة جافة في العصير الخام إلى 298 و 247٪، 284 و 238٪، 716 و 1032٪، 307 و 881٪ في العصير الرائق والعصير المركز والسكر الخام الأخضر والمولاس على التوالي. كما ان استخدام النقطة المقترحة لحقن المطهر L3 أدى الى انخفاض معنوي في مستوى النتريت في العصير الخام وكذلك العصير المعامل بمضـاد الرغوة وصـلت الى ما يقرب من 90%. حيث استمر مفعول المطهر لأكثر من 6 ســـاعات عند معدلات النتريت الطبيعية بالعصـــير والي 90 دقيقة عند معدلات النتريت العالية. إضافة إلى ذلك، انخفضت محتويات النتريت في العصير الرائق والعصير الكثيف والمولاس بنسبة 58، 48، 30٪ على التوالي. من خلال تحديد الجرعة المثلى من المطهر (hop β-acids) واختيار الموقع المناســـب لإضـــافتها، يمكن تقليل الحمل الميكروبي وكمية النتريت المتكون أثناء عمليات استخلاص البنجر وتصنيع السكر بشكل معنوي.

الكلمات الدالة: *النتريت، الحمل الميكروبي، النترتة، جودة المولاس*