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Analytical Methods

The use of ionic chromatography in determining the contamination of sugar by-products by nitrite and nitrate

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ABSTRACT

In 2010, the Directive on undesirable substances in animal feed entered into force and for the first time was laid down a maximum limit for nitrite content in sugar industry feed materials such as molasses and beet pulp. Due to a lack of suitable analytical methods for nitrite determination, this study was developed with the aim to standardize a nitrite analytical method in by-products from sugar industry.

In this study high performance anion exchange chromatography with conductometric detection was used for determining nitrite and nitrate content of sugar by-products included in feed material.

The study confirms the usefulness of the applied ion chromatographic method for the evaluation of the content of nitrite and nitrate in sugar by-products.

The results showed that in many samples of beet pulp and molasses the content of "undesirable substances for animal feed" was below 15 mg kg^{-1} (expressed as sodium nitrate at 12% of moisture).

1. Introduction

Nitrates presents in sugar beets are taken up from soil by the fibrous root system to the leaves where there they are partly reduced to nitrites and finally to hydroxylamine and ammonia (Mahn, Hoffmann, & Märländer, 2002). The nitrate content in sugar beets is very variable and depends mainly on nitrogen fertilizer rates and climatic conditions during growth, whereas the nitrite content in sugar beets is found in small quantities (Hoffmann & Märländer, 2005). The reduction of nitrate to nitrite with the involvement of thermophilic bacteria occurs predominantly during the extraction process – in the extractor and cossette scalder (De Bruijn, Van der Poel, Heringa, & Van den Bliek, 1991; Emerstorfer, Bergwall, Hein, Bengtsson, & Jensen, 2014; Frenzel, 2014; Hollaus, Hein, Pollach, Scheberl, & Messner, 1997; Schiweck, Jeanteur-De Beukelaer, & Vogel, 1993; Waterlander, Puke, Bengtsson, & Frenzel, 2011). The course of this process depends mainly on: quality and chemical composition of raw material; number, activity and type of bacteria introduced to the extraction process with cossettes; type of the extraction system and extraction parameters. (Emerstorfer et al., 2014). This can influence the nitrite content in the extractor and finally the nitrite content in molasses which is undesirable because excessive levels of nitrite adversely affect animal health (Bąk, Antczak-Chrobot, & Wojtczak, 2016; Schiweck, Kozianowski, Anderlei, & Burba, 1994).

Molasses and beet pulp are valuable materials for the production of livestock feed: dairy cows, beef cattle, pigs and goats. Due to consumption of feed materials with excessive nitrite and nitrate contents, the nitrite poisoning in animals occurs very often (Baranova, Jackova, Mala, Burdova, & Zezula, 2000; Lewicki, Wiechetek, Souffrant, Karlik, & Garwacki, 1998; Mikoś, Antczak-Chrobot, & Wojtczak, 2015; The EFSA (European Food Safety Agency, 2009). In ruminants such as cattle, goats and sheep, as a result of the activity of rumen microorganisms nitrate are converted to nitrite and then to ammonia which is further transformed and used to form amino acids and proteins. However when high amounts of nitrate and/or a high intake of nitrite with feed takes place, this process is not in balance and the production of nitrite is higher than the nitrite conversation to ammonia. It leads to the formation of a large amount of nitrite, which enter the bloodstream (Cockburn et al., 2013; Lee & Beauchemin, 2014; Waterlander et al., 2011).

In monogastric animals such as pigs and horses, nitrite is absorbed in the upper part of gastrointestinal tract. These animals are very sensitive to nitrite intake because they do not have the mechanisms for their further conversion into ammonia (Baranova et al., 2000). The ingestion of feed containing high amounts of nitrites causes the absorption of these compounds into the bloodstream. While nitrate is converted to nitrite in the end of digestive tracts, where there is less absorption into the blood. Therefore, a high nitrate content in the feed is not so dangerous for the monogastric animals as in ruminants (Cockburn et al., 2013; Waterlander et al., 2011).

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Nitrite in the bloodstream changes hemoglobin to methaemoglobin which cannot carry oxygen. As a result, methemoglobinemia occurs. Acute poisoning can result in diarrhoea and vomiting, salivitation, noisy and difficult breathing, tremors, staggering and dark, chocolate-coloured blood. Chronical poisoning can lead to weight loss, greater susceptibility to infections, less milk production and miscarriages (Bruning-Fann & Kaneene, 1993; Lee & Beauchemin, 2014; Robson, 2007; Waterlander et al., 2011). Acute nitrite poisoning can result in increased heart rate, muscle tremors, vomiting and even death, whereas chronical poisoning can lead to weight loss, less milk production, greater susceptibility to infections, miscarriages (Robson, 2007). Therefore it is very important to limit the nitrite content of feed materials, because excessive levels of these compounds in feed adversary affect the animal health (Mikoś et al., 2015). Proper animal nutrition based on feed that guarantees high efficiency of livestock production while ensuring safety is currently one of the key subjects in the European Union.

In 2010, Directive 2010/6/EU entered into force (Directive 2010/6/EU), amending Annex 1 to Directive 2002/32/EC on undesirable substances in animal feed (Directive 2002/32/EC). The act for the first time laid down a maximum limit for nitrite in sugar industry feed materials such as molasses and pulp, which was set at 15 mg kg^{-1} expressed as sodium nitrite, relative to a feedingstuff with a moisture content of 12% (Directive 2002/32/EC). Following entry into force of the Directive, some sugar companies contacted the European Association of Sugar Producers to indicate that molasses produced in Europe mostly would not meet the new requirement. After conducting numerous discussions, it was decided to temporarily exclude sugar industry feed materials from the list of products subject to the limit (Waterlander et al., 2011). In 2013, Commission Regulation amended Annex I to Directive from 2010 and the feed materials from sugar industry were temporarily exempted from the maximum level for nitrite (Commission Regulation (EU) No 1275/2013). However, a condition was imposed that the sugar industry must collect further data and information on nitrite levels in molasses and pulp as well as identify the factors that might lead to the presence of these compounds in molasses and pulp (Summary record of the standing committee on the food chain and 20 July (2010) 655367; Summary record Summary record of the standing committee on the food chain and 22 February (2013) 208474; Waterlander et al., 2011). So far, published data highlight that molasses and beet pulp in most cases contain a significantly higher value of nitrites than the proposed limit (Waterlander et al., 2011).

The literature presents the legal background and nutritional consequences of limiting the nitrite content in feed (Mikoś et al., 2015; Suomi et al., 2016; The EFSA, 2009; Waterlander et al., 2011), but the challenge is to develop appropriate methods for detecting nitrite content in the feed materials.

The European Committee of Standardization (CEN) has elaborated a number of standards for the determination of nitrate and/or nitrite content in many products (vegetables and vegetable products, including vegetable containing food for babies and infants as well as in meat and meat products), but no specific methods for the determination of nitrite in feed were established (Waterlander et al., 2011).

A colorimetric method is the official method of analysis of the Association of Official Analytical Chemists (AOAC) for the determination of nitrate and nitrite in animal feed. In this method nitrate and nitrite are extracted with cadmium chloride and barium chloride solution, bulks of soluble proteins are precipitated in alkaline solution and the clarified solution is passed through a metallic cadmium column, reducing nitrate to nitrite. Nitrite is measured colorimetrically after a diazo-coupling reaction (Griess reaction) at 540 nm (Official methods of analysis of AOAC International, 1995). However, this method does not meet current analytical quality criteria, there is no available information on its performance, the reagents used in analytical procedure are toxicity.

Literature reports many alternative methods to determination of nitrite and nitrate content in different food products. Baião, dos, Conte-Junior, Paschoalin, and Alvares (2016) validated and evaluate the NO_3^- and NO_2^- contents in red beetroot in natura from different Brazilian regions by reversed-phase high-performance liquid chromatography (HPLC) method integrated with fluorescence detection. Chou, Chung, and Hwang (2003) developed a simple, rapid, precise and sensitive high performance liquid chromatography (HPLC) method using an UV detector for the determination of nitrate and nitrite amounts in vegetables. The determination and validation of nitrate and nitrite in vegetables by capillary electrophoresis with indirect detection was presented by Jimidar, Hartmann, Cousement, and Massart (1995). The major advantage of the capillary electrophoresis methods is possibilities to determine several anions simultaneously and quickly, what compensates for the disadvantage of having two methods in the capillary electrophoresis technique for the determination of NO_3^- and NO_2^- ions.

Ion chromatographic technique is well-known method for the determination of nitrite and nitrate content in meat products. Siu and Henshall (1998) presented the usefulness of ion chromatography with UV absorbance detection to evaluate of nitrite and nitrate in commercial samples of ham and salami. Iammarino, Di Taranto, and Cristino (2013) and Iammarino and Di Taranto (2014) presented an extensive report of nitrite and nitrate content determination of ion chromatographic in samples of leafy vegetables (spinach and lettuce), fresh meats (beef, pork, equine and chicken), cheese samples (unripened, ripened and Mozzarella cheese), shellfish (mussels and clams), animal feeds (for aquaculture, dairy cows, pets, cattle and veal).

Therefore, the authors decided to adopt the method of determination of nitrate and nitrite content of meat products (EN 12014–4:2005) and use high performance anion exchange chromatography for determining nitrate and nitrite content in sugar by-products. The literature reported that this standard method has a detection limit of 5 mg kg^{-1} and 10 mg kg^{-1} for nitrite and nitrate, respectively. In this data the ultraviolet detection used in EN 12014–4:2005 standard method was replaced by conductometric detection and its make it possible to detect and quantification an analytes at the level of μg L^{-1}.

In order to confirm the method reliability and its suitability for purpose of sugar by-products, a full validation of this method was developed and the most important validation parameters obtained are reported.

2. Materials and methods

2.1. Chemicals

Nitrite and nitrate standards (analytical grade) were purchased from Merck Co. (Darmstadt, Germany). The NaOH solution 50% v/v in water (eluent for ion chromatography) was purchased from Sigma-Aldrich® (Europe). Ultrapure water with resistivity 18 MΩ was used to dilution and as a mobile phase.

2.2. Instruments

Chromatographic analysis was performed with an ion chromatograph DIONEX ICS-3000 (Dionex®, USA) with a conductivity detector CD produced by the company DIONEX® (USA) and conductivity supressor ASRS- ULTRA II 4 mm (Dionex®, USA). Nitrite and nitrate were separated using the Ion Pac AS11-HC analytical column (4 μm, 4 × 250 mm, Dionex®, USA) and Ion Pac AS11-HC guard column (4 μm, 4 × 50 mm, Dionex®, USA). Chromatographic analysis was recorded with the use of the programme Chromeleon®, version 6.80 (Dionex®, USA).