Determination of nickel in hydrogenated fats and selected chocolate bars in Czech Republic

Lucie Dohnalova [a], Pavel Bucek [b], Petr Vobornik [c], Vlastimil Dohnal [b],*

[a] *Department of Hygiene, Faculty of Military Health, University of Defence, Trebesska 1575, Hradec Kralove CZ-500 01, Czech Republic*
[b] *Department of Chemistry, Faculty of Science, University of Hradec Kralove, Rokitanskeho 62, Hradec Kralove CZ-500 03, Czech Republic*
[c] *Department of Informatics, Faculty of Science, University of Hradec Kralove, Rokitanskeho 62, Hradec Kralove CZ-500 03, Czech Republic*

A B S T R A C T

Nickel is a metal that can be present in products containing hardened edible oils, possibly as leftover catalyst from the vegetable oil hardening process. Nickel may cause toxic effects including the promotion of cancer and contact allergy. In this work, nickel content was determined in hydrogenated vegetable fats and confectionery products, made with these fats, available on the Czech market using newly developed method combining microwave digestion and graphite furnace AAS. While concentrations of 0.086 ± 0.014 mg.kg^{-1} or less were found in hydrogenated vegetable fats, the Ni content in confectionery products was significantly higher, varying between 0.742 ± 0.066 and 3.141 ± 0.217 mg.kg^{-1}. Based on an average consumer basket, daily intake of nickel from vegetable fats is at least twice as low as intake from confectionery products. Based on results, the levels of nickel in neither vegetable fats nor confectionery products, do not represent a significant health risk.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Nickel is a transition metal that in small doses has beneficial effects for animals and plants. It is known to fulfill essential functions as a micro-trace nutrient in the human body (Landsdown, 2014). However, nickel exposition may cause toxic effects including the promotion of cancer and contact allergy. Although the molecular mechanism of nickel carcinogenicity is not yet fully understood, interaction of Ni (II) ions with various cellular components, including proteins and DNA, is considered to be a key factor. Nickel itself is not carcinogenic, it acts more as a promoter than an inducer. Interactions with DNA are weaker than with RNA (Kaur & Dani, 2003) but promote DNA hypermethylation. In addition, nickel is known as a contact allergen, causing dermatitis. Exposure to nickel as low as 500 µg.day^{-1} (about 8 µg.kg^{-1} body weight.day^{-1}) have been reported to aggravate contact skin eczema in nickel sensitized individuals (Anonymous, 2006).

The toxicity of nickel and its compounds depends on bioavailability and fasting. Absorption of nickel in gastrointestinal tract is lowest for free Ni (II) ions but increases with complexation and solubility (Lodyga-Chruscinska, Sykula-Zajac, & Olejnik, 2012).

Unfortunately, data dealing with Ni speciation in foodstuffs are scarce or non-existent and, therefore it is not possible to estimate the bioavailability of nickel from margarines and hardened fats. Those studies that are available do not allow establishment of no-adverse-effect-level (NOAEL) or tolerable upper intake level for nickel and/or related species.

In the general population, the most important exposure route for nickel is food and drinking water. An average overall daily intake has been estimated at 0.1–0.3 mg (Schaumlöffel, 2012). Nickel concentration in water intended for human consumption in the European Union is 20 µg Ni.L^{-1} (EU Directive 98/83/EC), assuming consumption of 2 L of water per day. The most important natural food sources of nickel are dry beans, cocoa products, baking soda and some nuts (Solomons, Viteri, Shuler, & Nielsen, 1982), drinking water (Anonymous, 2015) and stainless steel food technologies and kitchenware (Scancar, Zuliani, & Milacic, 2013) or nickel based catalysts (Riya & Grant, 2015) used in fat hardening process. Thus, consumption of food with high nickel content and additional exposure from first-run drinking water and kitchen utensils could result in an intake higher than recommended (Anonymous, 2006).

Hydrogenated fats have become an important part of the human diet. They have replaced high-cholesterol butters (Lodyga-Chruscinska et al., 2012). They are also used in the food industry for products with more viscous or solid textures, such as various toppings or fillings in sweets and confectionery. Partially

* Corresponding author.
E-mail addresses: dohnalova.luc@gmail.com (L. Dohnalova), pavel.bucek@uhk.cz (P. Bucek), petr.vobornik@uhk.cz (P. Vobornik), vlastimil.dohnal@uhk.cz (V. Dohnal).

hydrogenated oils are used for the production of margarines (Misan, Estato, de Velasco, Spreafico, et al., 2015).

Due to the reaction of nickel catalyst and fat components, formation of lipophilic nickel salts also occurs, such as Ni(fatty acid)$_2$ salt. Together with nickel in colloidal form, these fat-soluble compounds can be present in final products (margarines, butters) at concentrations up to 3.40 mg.kg^{-1} of margarine (Anwar, Kazi, Jakharani, et al., 2003) and, thus, may represent significant dietary source of nickel. Analyses of Finnish margarines (Pihlaja, 1981) revealed differences in nickel content for margarines prepared by catalytic hydrogenation (0.38 ppm) while other margarines contained less than 0.2 ppm of nickel. In margarines analyzed by Anwar et al. (2003), levels of nickel varied from 0.41 to 3.40 mg. kg^{-1}. Kohiyama, Baba, and Maruyama (1993) discovered differences in nickel content among margarines in France (0.06 mg. kg^{-1} or less) and Spain (above 0.3 mg.kg^{-1} in three brands of Spanish products). Nickel content was studied by Noël et al. (2012) in the first and second French total diet study (TDS) where concentrations of nickel were 0.016 and 0.051 µg.g^{-1} in oils and fats and 0.135 and 0.825 µg.g^{-1} in sweeteners, honey and confectionery, respectively.

Various techniques have been employed in the determination of nickel and other metals in edible oils and fats. Typically, atomic absorption spectrometry with flame atomization (Nunes et al., 2011; Tokay & Bağdat, 2016) or graphite furnace (Brkljača, Giljanović, & Prkić, 2013; Cabrera-Vique, Bouzas, & Oliveras-López, 2012; Ieggli, Bohrer, Do Nascimento, & De Carvalho, 2011; Matos Reyes & Campos, 2006; Zhang, 2012; Zhu, Fan, Wang, Qu, & Yao, 2011) and inductively coupled plasma (de Souza, Mathias, da Silveira, & Aucélio, 2005; Savio et al., 2014) are the most widely preferred detection methods. Samples are introduced directly (Matos Reyes & Campos, 2006), after dilution (Cabrera-Vique et al., 2012), solubilization (Savio et al., 2014) or emulsification with detergents (Benzo, Marcano, Gomez, et al., 2002; de Souza et al., 2005; Ieggli et al., 2011; Nunes et al., 2011). Also, nickel has been extracted from oils using chelating agents (Anwar et al., 2003; Baran & Yaşar, 2012; Tokay & Bağdat, 2016). Overall, the most common process in sample preparation is digestion in a muffle furnace (Brkljača et al., 2013). Recently, microwave digestion has been introduced (Zhang, 2012; Zhu et al., 2011). Apart from the speed of the process, advantages of closed vessel microwave-assisted mineralization include avoiding sample tarnishing and sample handling, calibration, etc. in a hydrophobic environment (as required for analyses according to ISO 8294).

In this work, the evaluation of nickel content in hardened fats and selected types of confectioneries containing these fats and available on the Czech market during 2014, was performed. A new method was developed, based on a combination of closed vessel microwave digestion and atomic absorption spectrometry with a graphite furnace. Results were compared with previous studies and, additionally, discussed with reference to the standardized consumer basket for the Czech Republic.

2. Materials and methods

2.1. Chemicals and solutions

Analytical or AAS purity chemicals were used for preparation of all solutions. For microwave decomposition of samples, concentrated nitric acid and 30% hydrogen peroxide of p.a. + grade were used (both supplied by Analytika Prague, Czech Republic).

Water of purity Type I (18.2 M.cm at 25 °C) (MilliQ, Merck, Darmstadt, Germany) was used for solution preparation and sample dilutions. All the materials used were made of plastic and com-

ponents were soaked for 24 h before use and thoroughly washed with 5% nitric acid.

The standard solution of nickel, ASTASOL®, was obtained from Analytika, s.r.o. (Prague, Czech Republic) as solutions with certified concentrations 1.000 ± 0.005 g.L^{-1} at 20 °C in 2% nitric acid and it was prepared from metal of 99.99% purity.

2.2. Samples and sample preparation

Various brands of vegetable fats and confectionery of various trade marks were bought in supermarkets in Hradec Kralove (Czech Republic) during the summer of 2014. Due to changes in fat processing, the number of hardened fats available on market was dramatically lower than previously (Lodyga-Chruscinska et al., 2012), and the work was extended to include selected types of confectioneries. The highest amounts of hydrogenated/hardened fats were expected in the cheapest products. The selection of confectionery was based on the declared content of hydrogenated fats declared in the ingredients list for products or their parts if the contents were divided. The control samples (C and D) did not contain hardened fats and were chosen as those most popular in recent customer surveys. In confectionery products that consisted of different materials (e.g. chocolate bars containing biscuit and stuffing or glazing), the first step was mechanical separation (with plastic spatula) of the different materials, which were analyzed separately. An overview of the products analyzed is presented in Table 1.

Analyzed vegetable fats contained palm oil and hydrogenated fats in various ratios. Only one of the vegetable fat samples contained a major portion of hydrogenated fat according to the manufacturer's declaration. The law requires ingredients to be listed in the order of amount. All of the confectionery samples declared chocolate matter with vegetable fats (thus, were not chocolate in the term of legislative requirements).

Sample preparation was adapted from Perkin-Elmer Application note (Sarojam, 2009). Samples of all materials (vegetable fats, bars, toppings and stuffing) were prepared by weighing approximately 0.5 g into the quartz reaction vessel and adding 3 mL of concentrated nitric acid and 1 mL of 30% hydrogen peroxide. Vessels were closed and placed in a 8SXQ80 rotor of Multiwave 3000 microwave system (Anton Paar, Graz, Austria) for microwave-assisted mineralization. The temperature program from Perkin-Elmer Application note (Sarojam, 2009) was simplified: applied power 800 W, ramp 2 min and hold for 15 min. After mineralization, the samples were cooled in Multiwave 3000 for an additional hour. Data log downloaded from Multiwave 3000 showed that 800 W of applied microwave power was enough for complete mineralization. Later during the cycle, the microwave control system limits the power applied according to prevailing pressure and temperature values. To our knowledge, and from monitoring data logs, for given sample amounts (approx. 0.5 g and 4 mL of HNO$_3$/H$_2$O$_2$ mixture per vessel) the applied power never exceeded 850 W.

Mineralized samples (homogenous transparent solutions) were transferred into 10 mL volumetric flasks and volume made up to 10 mL with 5% nitric acid.

2.3. Measurement

Mineralized samples were analyzed using an electrothermal atomization – atomic absorption spectrometry (model AAnalyst 400, Perkin Elmer, Waltham, Massachusetts, USA). Argon gas was used for the graphite furnace wash (Argon 5.1, SIAD, Braňany, Czech Republic). Data were collected and evaluated using WinLab 32 AA software by Perkin Elmer (Waltham, Massachusetts, USA). Temperature program and spectrometer settings were adopted from Lodyga-Chruscinska et al. (2012) and P-E Application note