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Niacin contents of cereal-milling products in food-composition databases need to be updated

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ABSTRACT

The niacin content of cereal raw materials reported in food-composition databases often differs considerably. One major reason for this discrepancy is the analytical method used for its measurement is that a significant part of the niacin in cereals exists in bound form. In this study, we compared the niacin content of some representative cereal raw materials analysed with a sensitive and validated ultra-high performance liquid chromatography–fluorescence method against the values found in five national food-composition databases. We used established extraction methods that are assumed to liberate niacin available for absorption (acid hydrolysis mimicking human digestion) or total niacin (strong acid–alkaline hydrolysis). The niacin content (mg/100 g dry weight) obtained with acid hydrolysis ranged from a low level in corn flour (0.26), white wheat flour (0.45) and oat flakes (0.48), to a higher level in wholegrain flours (rye: 0.79, barley: 0.99, wheat: 0.88), wheat bran (2.7) and wheat germ (2.7). The niacin content with the acid–alkaline hydrolysis, however, was 1.9–11-fold the value measured after extraction with acid hydrolysis. In general, the niacin content found in the databases is closer to the results obtained after the acid–alkaline extraction, suggesting that the niacin values reported in the databases may not reflect actual bioaccessible niacin but total niacin.

1. Introduction

Niacin is a B group vitamin, which is a part of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) involved in many biological redox reactions. By definition, niacin refers to nicotinic acid (pyridine-3-carboxylic acid, NA), nicotinamide (nicotinic acid amide, pyridine-3-carboxamido, NAM) and derivatives that exhibit the biological activity of NAM (Institute of Medicine, 1998; Nordic Nutrition Recommendations, 2012, 2013). The recommended dietary allowance (RDA) value of niacin for adult male and female is 16 and 14 mg of niacin equivalents, respectively (Institute of Medicine, 1998). The niacin equivalents also include the contribution of niacin by the amino acid tryptophan, with each 60 mg of tryptophan contributing 1 mg of niacin (Institute of Medicine, 1998). Niacin occurs mainly in bound forms with highly variable bioaccessibility rates; in animal tissues, mostly as NAM in NAD and NADP, and in plants as NA bound to peptides or polysaccharides (Combs, 1982a, 1982b; Eitenmiller et al., 2008; Koetz et al., 1979; Mason et al., 1973, 1971; van den Berg, 1997; Wall and Carpenter, 1988). In grains, for example, niacin is present as esters with polysaccharides (niacytin) or is bound to polypeptides and glycopeptides (niacinogenes) (Mason et al., 1973; van den Berg, 1997). In addition, the methylated derivative trigonelline (1-methylnicotinic acid) that functions as a plant hormone is found (Combs, 2012). Trigonelline is not, as such, bioavailable, but it is converted to NA by thermal processing. Niacin in corn was reported to be ca. 35% bioavailable whereas alkaline treatment frees bound NA efficiently (Carter and Carpenter, 1982a). The poor bioavailability of niacin led to the well-known niacin-deficiency disease, pellagra, in populations relying on corn as their staple food. Added NAM and NA in fortified cereal foods are highly available.

The predominant forms in animal-based foods, NAD and NADP, are effectively hydrolysed to release NAM for absorption (Institute of Medicine, 1998; Nordic Nutrition Recommendations, 2012, 2013). The bound structures in plant foods are poorly characterised. They have mainly been studied in corn and a few other grains (Carter and Carpenter, 1982a, 1982b; Eitenmiller et al., 2008; Koetz et al., 1979; Mason et al., 1973, 1971; van den Berg, 1997; Wall and Carpenter, 1988). In grains, for example, niacin is present as esters with polysaccharides (niacytin) or is bound to polypeptides and glycopeptides (niacinogenes) (Mason et al., 1973; van den Berg, 1997). In addition, the methylated derivative trigonelline (1-methylnicotinic acid) that functions as a plant hormone is found (Combs, 2012). Trigonelline is not, as such, bioavailable, but it is converted to NA by thermal processing. Niacin in corn was reported to be ca. 35% bioavailable whereas alkaline treatment frees bound NA efficiently (Carter and Carpenter, 1982a). The poor bioavailability of niacin led to the well-known niacin-deficiency disease, pellagra, in populations relying on corn as their staple food. Added NAM and NA in fortified cereal foods are highly available.

Sample preparation is always a crucial step in vitamin analysis. Ideally, only vitamin compounds that are available for absorption should be determined. The liberation and quantification of all NA in cereal foods leads to the overestimation of bioavailable niacin (later referred to as “free niacin”). Free niacin is often defined as the niacin that is extractable by heating or autoclaving with 0.1 M mineral acid (Ball, 2006). Acid hydrolysis may, however, liberate part of the bound...
NA and partly transform NAM into NA (Hepburn, 1971; Ndaw et al., 2002). As the obtained free niacin content depends on the severity of the hydrolysis conditions (time, temperature), the gradual degradation of bound NA (Hepburn, 1971). As an alternative, enzymatic hydrolysis with NAD glycohydrolase (or NADase) is recommended (EN 15652, 2009; Ndaw et al., 2002). In the European standard method (EN 15652, 2009), either acid hydrolysis with 0.1 M HCl (1 h, 100 °C) or enzymatic hydrolysis with NADase (from e.g. *Neurospora crassa*) is used, whereas to determine the total niacin, acid hydrolysis is followed by alkaline hydrolysis (5 M NaOH, 121 °C/1 h). Other options to determine the total niacin content are given in Table 1. An in-house reference sample (wholegrain wheat flour) was used to study the recovery of NA during the analysis.  

2.2. Cereal materials and sampling

Wholegrain flours (wheat, rye and barley), white wheat flour, maize flour, oat flakes, wheat bran and wheat germ produced by major food suppliers in Finland were collected following a stratified sampling procedure. The sampling plan is briefly explained. The eight chosen sample materials were purchased from ten retail stores in the Helsinki area representing the major food chains in Finland. Primary samples, one retail package (500–1000 g) of each item from each shop, were bought. Pooled samples of each item were obtained by combining equal amounts of the primary samples into the pool. Each pooled sample was divided into aliquots of 100–200 g, vacuum packed in plastic bags and stored at −20 °C until analysed. The moisture content of the samples was analysed using a gravimetric method (at 103 °C overnight drying), and the ash content using the AACC method 08–01 (AACC International, 2000). The results for the moisture content and the ash content are given in Table 1. An in-house reference sample (wholegrain wheat flour) was used to study the recovery of NA during the analysis.  

2.3. Extraction of niacin

Niacin was extracted from sample materials according to the EN 15652 method (2009) using acid hydrolysis to measure the free niacin and combining acid and alkaline hydrolysis to determine the total niacin content. Each cereal sample was extracted in triplicate.  

2.3.1. Acid extraction

Analytical samples (0.5–1 g) were vortexed with 25 mL of 0.1 M hydrochloric acid. The tubes were placed in a boiling water bath for 1 h with an occasional shaking of the tubes (2–3 times). After cooling on an ice bath, the pH of the extracts was adjusted to 4.5 with sodium acetate solution (2.5 M). The extracts were then transferred into 50-mL volumetric flasks and filled up to the mark with MilliQ water. The sample extracts were syringe-filtered (0.2 μm; Pall, MI, USA) into 2-mL UPLC vials (Waters, MA, USA) prior to the UHPLC analysis.  

2.3.2. Acid–alkaline extraction

The samples (0.5–2 g) were vortexed with 25 mL of 0.1 M hydrochloric acid and heated for 1 h in a boiling water bath. The extracts were ordered from Sigma-Aldrich (Steinheim, Germany). NA and NAM (purity ≥ 99.5 %) were purchased from Sigma-Aldrich. The reference material BCR 431 (Brussels sprout powder) produced by the Institute for Reference Materials and Measurements (Geel, Belgium) was bought from Sigma-Aldrich. The water used in the experiments and analyses was produced by the MilliQ Plus system (0.22 μm, ≥ 18.2 MΩ cm, Millipore Corporation, Bedford, MA, USA).
were then transferred into Erlenmeyer flasks (250 mL). 20 mL of MilliQ water and 4 mL of 5 M sodium hydroxide were added and the flasks were autoclaved (121 °C; 1 h). After cooling, the pH of the extracts was adjusted to 4.5 first with concentrated and then with dilute (0.1 M) hydrochloric acid. The extracts were diluted to achieve 100 mL with MilliQ water and filtered into UPLC vials.

2.4. UHPLC analysis

2.4.1. UHPLC system

A Waters Acquity UPLC system (Milford, MA, USA) was used for the analysis. The niacin vitamers (NA and NAM) were separated with a normal phase silica (HSS) T3 column (2.1 × 150 mm, 1.8 μm) and fluorometrically (322 nm excitation and 380 nm emission wavelengths) derivatization was carried out in a post-column reactor (Section 2.4.2). The chromatographic separation was performed at 30 °C using an isocratic flow of the mobile phase (MP) (0.3 mL/min) consisting of an optimised concentration of copper sulphate (CuSO4) and H2O2 in a potassium phosphate buffer (70 mM of potassium dihydrogen phosphate; pH 4.5). The sample extracts were injected (10 μL) in duplicate. The run time was 15 min. The NA and NAM concentrations were calculated using external calibration curves (calibration range: 0.2–20 ng). The actual concentration of the NA and NAM standards was confirmed spectrophotometrically according to the EN 15,652 method (2009) at 420 nm and 410 nm for NA and NAM, respectively.

2.4.2. Post-column derivatization and optimisation of the mobile-phase composition

NA and NAM fluorochromes were formed in a post-column photochemical reactor (Beam Boost, Austria). The eluent flow from the column was exposed to a long-wavelength UV light (366 nm, 8 W) in a knitted PTEE reaction coil (1.59-mm o.d., 0.17-mm i.d. and 5-m length). The chromatographic eluent proposed by the CEN method (2009) (H2O2, 75 mM and CuSO4, 5 μM) was optimised with a Box–Behnken experimental design model (Centurio XVI Statgraphic, Virginia, USA). The test points in the Box–Behnken model were 10 mM, 120 mM and 225 mM for H2O2, and 1 μM, 5.5 μM and 10 μM for CuSO4. The concentration ranges for the CuSO4 and H2O2 that were examined varied from 1 to 1000 μM and from 7.4–600 mM, respectively (Table 2).

2.5. Method validation

The limit of detection (LOD) was measured as a signal-to-noise ratio of 3:1 and the limit of quantitation (LOQ) equals to 3-fold the lowest detected amount. Linearity was confirmed over a range of 0.2–1000 ng.

The recovery of the method was monitored by adding NA (15 μg) to an in-house reference sample (wholegrain wheat flour) in triplicate and the analysis was carried out on two separate days. The accuracy of the method was evaluated by calculating a Z-value for the obtained results for the certified reference material using the Horwitz equation (Nordic Committee on Food Analysis (NMKL), 2007).

\[ Z = \frac{(C_{\text{certif}} - C_{\text{meas}})}{\sqrt{C_{\text{certif}} \times f \times 0.01}} \]

Where, \( C_{\text{certif}} \) = certified value of reference material, \( C_{\text{meas}} \) = measured value of reference material, \( f \) = fraction factor (i.e., % = 0.01, ppm = 10^-6) and \( n \) = number of independent replicates.

2.6. Food-composition databases

The niacin content of the studied raw materials was compared against the values found in five food-composition databases of countries (Finland, Sweden, Denmark, the UK and USA) representing the Western world. The composition data was retrieved for the products that closely matched with the studied raw materials taking into account of the ash and moisture contents of the studied products (Table 1).

### Table 2

<table>
<thead>
<tr>
<th>Mobile-phase (MP) number</th>
<th>H2O2 (mM)</th>
<th>CuSO4 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
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<td>14</td>
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</tbody>
</table>

3. Results and discussion

3.1. UHPLC method optimisation and its performance

3.1.1. Mobile-phase composition

The composition of copper(II) ions and H2O2 had a stronger effect on the response for NA compared to that for NAM (Fig. 1) but it did not affect the retention times of the vitamers (NA, 3.6 min; NAM, 10.6 min). The Box–Behnken surface response plot showed the highest response on NAM when the MP contained 300 mM H2O2 and 125 mM CuSO4 (MP12). This high concentration of CuSO4 decreased NA’s signal intensity. When the copper ion content was decreased to 25 μM, a good signal was also achieved for NA, thus suggesting a good compromise for both vitamers. The suggested amount of H2O2 in the MP, 300 mM, needed to be reduced to half (150 mM) due to the stability issue. The high H2O2 content caused the disproportionation of H2O2 into water and oxygen, forming gas bubbles to the eluent lines. Similarly, decreasing the CuSO4 concentration to 5 μM allowed for the better stability of the MP without significantly affecting the signal intensity of NA and NAM. Thus, the optimum concentrations of H2O2 and CuSO4 in the MP were found to be 150 mM and 5 mM (MP7), respectively, and this composition was used for the analysis of the cereal materials in this study.

3.1.2. Chromatographic separation and detection parameters

The retention times of the vitamers were consistent for both the standards and the sample extracts (Fig. 2). The NA peak was well separated from the interfering peaks, whereas minimal interference was observed for the NA peak when analysing the sample extracts. However, when the acid–alkaline-treated samples were analysed, the baseline separation of NA depended on the type of cereal material. For example, the NA peak was not clearly separated from the higher background peaks for wholegrain rye flour.

The LOD for NA and NAM was found to be 0.02 ng/injection and 0.01 ng/injection, respectively. The LOQ (3-fold the LOD) for NA and NAM was thus 0.06 ng/injection and 0.03 ng/injection, respectively. In terms of the cereal sample (for a 5-g sample), the LOQ for NA and NAM was in the range of 0.2–1000 ng, with consistently excellent linearity (average R² = 0.9985; RSD of < 3.5 %). The LOD values of the 0.05 ng/injection for NA and the 0.02 ng/injection for NAM with the HPLC method (EN 15652, 2009) were 2-fold the values obtained with
the current UHPLC method, and were achieved with a much bigger sample injection (100 μL) compared to the injection volume in the present study (10 μL).

3.1.3. Method performance

The average NA recovery rate spiked into the in-house reference material (for a spiking level of 15 μg NA/g; n = 6) and extracted with acid hydrolysis was very good (97 ± 8%), with an inter-day recovery rate (for two days) of 93.5% and 99.7%. The recovery study of only NA but not NAM was performed because a part of NAM could be converted into NA during acid hydrolysis, thus complicating the estimation of the accurate recovery of NAM. The niacin content of the reference material BCR 431 (Brussel sprout powder; 4.25 ± 0.35 mg/100 g) matched with the certified reference value (4.3 ± 0.3 mg/100 g) with a Z-score of 0.3, suggesting excellent method performance (Nordic Committee on Food Analysis (NMKL), 2007).

3.2. Niacin content of cereal materials

The eight different types of cereal materials were analysed with the optimised UHPLC method after extraction with acid hydrolysis and acid–alkaline hydrolysis. The average niacin content (sum of NA and NAM) of these products is shown in Fig. 3. The total niacin content, determined using acid hydrolysis, was the lowest in corn flour (0.26 mg/100 g dry weight (dw)), while it ranged in other flours from 0.45 (white wheat flour) to 0.99 mg/100 g dw (wholemeal barley flour). The niacin content levels in wheat bran and germ were higher and close to each other (ca. 2.7 mg/100 g dw). NAM accounted for 8–64% of the total niacin content (Fig. 4). The contribution of NAM in the total niacin content was the highest in wheat germ (64%) and the lowest in refined flours (white wheat flour, 8% and corn flour, 14%). A similar proportion (ca. 25%) of the niacin content in wheat bran and wholegrain wheat flour exists as NAM. However, it should be noted that acid hydrolysis partly changes NAM to NA (EN 15652, 2009; Ndaw et al., 2002).

After acid–alkaline hydrolysis, a clearly much higher niacin content was found, especially in bran-rich grain products (Fig. 3). For wholemeal rye flour, the niacin content, however, was quite similar. For other samples, the niacin content ranged from 1.9-fold (wheat germ) to 11-fold (wheat bran) when compared with the results from the acid hydrolysis alone. For different wheat products, the following values were found: white wheat flour 3.8-fold, wholemeal wheat flour 4.5-fold, wheat germ 1.9-fold and wheat bran 11.2-fold. Thus, in germ, a bigger part of the total niacin content is available, whereas it is available to a lesser extent in the fibre-rich fractions of the grains. The niacin content seemed to be positively correlated with the

![Fig. 1. Response of NA and NAM with the mobile phase (MP) containing different concentrations of CuSO₄ and H₂O₂ (Table 1). The best combination of CuSO₄ and H₂O₂ (MP7 = 5 μM CuSO₄ and 150 mM H₂O₂) used in this study is highlighted. The response with MP numbers 9 (MP9) and 10 (MP10) was poor due to the instability of the MPs.](image)

![Fig. 2. Example chromatograms showing the separation of nicotinic acid (NA) and nicotinamide (NAM) from a niacin standard and an extract of wheat bran after acid hydrolysis.](image)
ash content of the grain products \( r > 0.90, p < 0.0015; \) Fig. 5), with a higher niacin content in the wholegrain flours and wheat bran than in the flours with less fibre-rich fractions (Hegedüs et al., 1985).

### 3.3. Comparing the niacin content with the database values

The average niacin content in the database values for the eight cereal products included in this study were closer to the results after the acid–alkaline hydrolysis (Table 3) and in some cases (wholemeal barley flour and corn flour) they were even higher than the niacin values obtained from acid–alkali extractions. In most of the cases, a clear explanation whether the values in the databases are based on actual analyses or are estimates based on other data (so-called best estimates) is not given. The method of analysis can only be found in some of the databases. This can be reflected in the wide variation in the database values for some cereal materials (Table 3): e.g., wholemeal rye flour (0.2–4.3 mg/100 g fresh weight (fw)), wheat bran (13.6–29.6 mg/100 g fw) and corn flour (1.0–5.7 mg/100 g fw).

It is also interesting to note that many of the database values for the niacin content are based on analyses that were carried out much earlier on, and the results are often based on analytical methods that are less specific to the niacin vitamers than current methods are (Carter and Carpenter, 1982b; Hepburn, 1971; Kirkland, 2014; Koetz et al., 1979; Ndolo et al., 2015). For example, the niacin values in the UK database are based on the data from Holland et al. (1988). Similarly, the niacin values in the Danish food-composition database for some of the food types included in this study are from the 1950s (Lieck, 1954) or the 1970s (Paul and Southgate, 1978). For a reliable estimation of the niacin intake from the diet, the food-composition databases need to be updated with data obtained using extraction and analysis methods that better reflect the bioavailable niacin content. One such method could be the use of the UHPLC–FLR (after post-column derivatisation) method proposed in this study or a UHPLC-MS (Ndolo et al., 2015). In the food-composition databases, niacin content obtained by acid–alkaline

![Fig. 3.](image-url) Total niacin content of the eight cereal products analysed after extraction with acid hydrolysis and acid–alkali hydrolysis (n = 3). The error bars represent the standard deviations. WG = whole grain.

![Fig. 4.](image-url) Proportion of nicotinic acid (NA) and nicotinamide (NAM) in eight cereal products analysed after the acid extraction. WG = whole grain.
extraction should be avoided. If such data is given, the information should be provided clearly so that these values can be distinguishable from the bioavailable niacin content. A thorough update of the niacin content of the food materials included in the food-composition databases is, therefore, recommended. Like for niacin, there is also a need for updating the databases for other vitamins using specific and reliable analytical methods.

**Table 3**

Database values for the niacin content of eight cereal-milling products (mg/100 g fw). The average and range of the values with the number of databases (n) included in the calculations are given. For comparison, the average niacin contents from this study (measured with acid hydrolysis and acid-alkaline hydrolysis) are also given (mg/100 g fw).

<table>
<thead>
<tr>
<th>Country database</th>
<th>WG rye flour</th>
<th>WG barley flour</th>
<th>Oat flakes</th>
<th>Wheat bran</th>
<th>Wheat germ</th>
<th>White wheat flour</th>
<th>WG wheat flour</th>
<th>Corn flour</th>
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</thead>
<tbody>
<tr>
<td>Finland(^a)</td>
<td>0.2</td>
<td>5.5</td>
<td>0.8</td>
<td>23.0</td>
<td>4.2</td>
<td>0.9</td>
<td>5.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Denmark(^b)</td>
<td>1.7</td>
<td>5.7</td>
<td>0.8</td>
<td>29.6</td>
<td>5.8</td>
<td>1.3</td>
<td>5.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Sweden(^c)</td>
<td>1.0</td>
<td>5.7</td>
<td>0.9</td>
<td>23.0</td>
<td>3.8</td>
<td>1.2</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>USA(^d)</td>
<td>4.3</td>
<td>6.3</td>
<td>na</td>
<td>13.6</td>
<td>6.8</td>
<td>1.2</td>
<td>5.0</td>
<td>1.9</td>
</tr>
<tr>
<td>UK(^e)</td>
<td>1.0</td>
<td>na</td>
<td>na</td>
<td>29.6</td>
<td>4.5</td>
<td>1.7</td>
<td>4.0</td>
<td>na</td>
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<tr>
<td>Average</td>
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<td>0.8</td>
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<td>Range</td>
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<td>0.8–0.9</td>
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<td>3.8–6.8</td>
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<tr>
<td>Acid hydrolysis</td>
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<td>0.4</td>
<td>0.8</td>
<td>0.2</td>
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<tr>
<td>Acid-alkaline hydrolysis</td>
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<td>27.1</td>
<td>4.7</td>
<td>1.5</td>
<td>3.5</td>
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</table>

WG = whole grain.
na = data not available in the country's database.

4. Conclusion

In this study, we compared the niacin content of representative cereal raw materials, analysed with a selective and sensitive UHPLC–FLR detection method, with the values reported in five major food-composition databases. Often, the niacin content reported in the food-composition databases is close to the content obtained with an acid–alkali extraction method, which clearly overestimates the bioavailable niacin content in cereal raw materials. A thorough update of the niacin content in the databases with values obtained using mild extraction methods (e.g. acid hydrolysis) and analyses employing the modern LC-based methods (e.g. UHPLC) is needed to enable accurate estimations of the bioavailable niacin content in food products.

CRediT authorship contribution statement

Bhawani Chamlagain: Supervision, Formal analysis, Writing - original draft, Writing - review & editing. Saija Rautio: Conceptualization, Writing - review & editing. Velimatti Ollilainen: Supervision, Writing - review & editing. Vieno Piironen: Resources, Supervision, Conceptualization, Writing - review & editing.

Declaration of Competing Interest

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Nordic Committee on Food Analysis (NMKL), 2007. Evaluation of method bias using certified reference materials. NMKL Procedure No. 9, 2nd ed..