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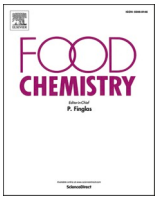

Bioaccessibility of thiamin, riboflavin, niacin, and folate in legume matrices

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ABSTRACT

Legumes are potential sources of B vitamins. Knowledge regarding the liberation of vitamins from legumes and their stability in digestion is scarce. Hence, we examined the thiamin, riboflavin, niacin, and folate contents and their *in vitro* bioaccessibility in dry, soaked, boiled, and germinated legume seeds. The contents in faba bean, lupin, and pea mainly retained in soaking (18 h, 8 °C) but decreased by 13–62 % in boiling (45 min). Germination (5 days, 15 °C) retained or increased the contents in faba bean and lupin seeds, and vitamins were synthesised in sprouts. The bioaccessibility of thiamin, riboflavin, and niacin was generally good (64–128 %), whereas the bioaccessibility of folate was lower (19–68 %) likely due to instability in digestion. Processing did not consistently enhance or diminish the bioaccessibility. The bioaccessible B vitamin contents varied among differently processed seeds, being the lowest in boiled seeds and the highest in dry seeds.

1. Introduction

Thiamin, riboflavin, niacin, and folate are water-soluble compounds that belong to B vitamins. Thiamin acts as a coenzyme in carbohydrate and amino acid metabolism, whereas riboflavin and niacin function in oxidation-reduction reactions (Blomhoff et al., 2023). Folate is an essential single-carbon donor, functioning in the methylation cycle and nucleic and amino acid metabolism.

Currently, meat and dairy products are important dietary sources of thiamin, riboflavin, and niacin (Blomhoff et al., 2023). Folate is obtained mainly from whole grain foods and green vegetables; however, according to Lemming and Pitsi (2022), dietary folate intake among adults is generally below the recommended levels in Nordic countries. Hence, a concern over the inadequate intake of B vitamins from a plant-based diet arises, as a transition towards a more sustainable food system is underway. Legumes are important protein sources in a plant-based diet, and their consumption is recommended due to their nutritional and environmental benefits (Blomhoff et al., 2023). Legume seeds and flours have notable thiamin, riboflavin, niacin, and folate content (Avezum et al., 2024; Liang et al., 2022; Siitonen et al., 2024; Zhang et al., 2021); thus, they are potential plant-based foods to provide B vitamins.

When assessing legumes as sources of B vitamins, the vitamin contents and their availability for utilisation in the body should be investigated. Bioavailability studies are expensive and laborious; hence, *in vitro* models, such as the standardised INFOGEST model by Minekus et al. (2014), have been developed in order to evaluate the bioaccessibility which can be defined as the proportion of a nutrient that is liberated from the food matrix and could potentially be absorbed. Accordingly, *in vitro* digestion models do not encompass the absorption of nutrients like *in vivo* bioavailability studies. However, results from the *in vitro* models have been reported to correlate with *in vivo* bioavailability; therefore, bioaccessibility could be a reliable indicator for the bioavailability of a nutrient (Bohn et al., 2018).

The bioaccessibility of thiamin and riboflavin in canned legumes and boiled green beans has been observed to vary considerably (Andac-Ozturk et al., 2022; Demir et al., 2023). Thiamin bioaccessibility ranged from 23 % to 96 % and riboflavin bioaccessibility from 44 % to 90 %. Niacin bioaccessibility in boiled green beans was found to be 82 % (Demir et al., 2023); however, niacin bioaccessibility has not been investigated in other legumes. Folate bioaccessibility (24–96 %) has been reported in various cereal matrices (Bationo et al., 2020; Liu et al., 2021; Liu et al., 2022a; Ringling & Rychlik, 2017). Nevertheless, to the best of our knowledge, only Liu et al. (2021) investigated folate bioaccessibility in a legume matrix; they reported a bioaccessibility of 63 % in faba bean flour. Overall, information regarding the bioaccessibility of niacin and folate in legumes—their liberation from the matrix and stability during digestion—is scarce. Moreover, only a few studies have

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investigated the bioaccessibility of thiamin and riboflavin in legumes, and the available data show a wide range.

Before the consumption of legumes, the dry seeds are usually soaked and then boiled; these processes may reduce the B vitamin contents due to leaching and degradation (Liang et al., 2022; Prodanov et al., 2004). In turn, legume germination is applied to improve the nutritional value by decreasing the content of antinutrients and harmful compounds, such as raffinose family oligosaccharides (RFOs) (Wei et al., 2022), enhancing the digestibility of proteins and starch (Alonso et al., 2000), and increasing the B vitamin contents (Prodanov et al., 1997; Zhang et al., 2021). B vitamins may occur in free form and for example protein-bound forms in foods (Konings et al., 2024; Ndaw et al., 2000); accordingly, soaking, boiling, and germination may alter the vitamin forms as well as modify the legume matrix and activity of enzyme inhibitors. Hence, the bioaccessibility of B vitamins in processed legumes may differ from that in dry seeds. However, the influence of processing on the bioaccessibility of B vitamins in legumes has been scarcely investigated. To our knowledge, the impact of soaking, boiling, and germination of legume seeds on the bioaccessibility of thiamin, riboflavin, niacin, and folate has not been previously reported. More research is required in order to assess the contribution of various legume foods to the intake of B vitamins.

The objective of this study was to investigate thiamin, riboflavin, niacin, and folate bioaccessibility in faba bean, lupin, and pea matrices. These legumes were selected as they thrive in boreal regions, such as Finland. We hypothesised that soaking, boiling, and germination could enhance the bioaccessibility of B vitamins. Therefore, we aimed to examine the impact of soaking and boiling of faba bean, lupin, and pea seeds on B vitamins and their bioaccessibility. We also aimed to evaluate how germination influences B vitamins and bioaccessibility in faba bean and lupin. The progress of germination was monitored by the RFO content in the seeds. Additionally, we evaluated the contribution of the bioaccessible B vitamin content in boiled legumes to recommended intakes, as they represented the edible form of legumes.

2. Materials and methods

2.1. Standards and enzymes

The standards—thiamin (hydrochloride, T4625, MW = 337.27), riboflavin, nicotinic acid (NA), nicotinamide (NAM), nicotinamide adenine dinucleotide (NAD) free acid, raffinose pentahydrate, and stachyose hydrate—were purchased from Sigma-Aldrich (St. Louis, MO, USA). Verbascose was obtained from Megazyme Ltd. (Bray, Ireland). The folate standards—(6*S*)-tetrahydrofolate (H_4folate, sodium salt), (6*S*)-5-methyltetrahydrofolate (5-CH_3-H_4folate, calcium salt), (6*R*,*S*)-5,10-methenyltetrahydrofolate (5,10-CH^+-H_4folate, hydrochloride), and (6*S*)-5-formyltetrahydrofolate (5-HCO-H_4folate, sodium salt)—were obtained from Merck & Cie (Schaffhausen, Switzerland). Folic acid (PGA) and 10-formylfolic acid (10-HCO-PGA) were obtained from Schircks Laboratories (Jona, Switzerland). The exact concentrations of thiamin, riboflavin, NA, NAM, and folate standard solutions were determined using respective spectrophotometric methods (Edelmann et al., 2012; EN 14122, 2014EN 14152, 2014; EN 15652, 2009). α-amylase (A9857), protease (P5147), pepsin (P7000), bile from bovine and ovine (B8381), chymotrypsin (C4129) and trypsin (T0303) were obtained from Sigma-Aldrich (St Louis, MO, USA); β-amylase was obtained from Megazyme Ltd. (Bray, Ireland); and Taka-Diastase (138.42 HUT/g) was obtained from Pfaltz and Bauer (Waterbury, CT, USA). Hog kidney conjugase was prepared in the laboratory and its activity was tested (Kariluoto et al., 2004). The activities of enzymes used in the *in vitro* protocol were determined according to Minekus et al. (2014).

2.2. Soaking, boiling, and germination of legume seeds

Dry faba bean (*Vicia faba*), lupin (*Lupinus angustifolius*), and pea (*Pisum sativum*) seeds were obtained from domestic suppliers (Vihreä Härkä Ltd., Kaarina, Finland; Koivunalhon luomutila, Lieto, Finland; Ylistalon tila, Perniö, Finland, respectively). Faba bean, lupin and pea were used in boiling experiments to mimic household cooking, and faba bean and lupin were also used in germination experiments to induce germination process in the seeds.

Legume seeds (150 g) were rinsed under tap water before and after soaking the seeds in MilliQ water (1,3 *w/v*) for 18 h at 8 °C. Sampling was performed from the soaked seeds; the remaining soaked seeds were boiled in a kettle (with a lid) in MilliQ water (1,4 w/v) for 45 min (Ferawati et al., 2019). Then, the sampling from boiled seeds and boiling water was conducted, and the samples were immediately frozen and stored at −20 °C. The B vitamin content in the soaked and boiled seeds was determined in duplicate from duplicate experiments ($n = 4$), whereas the dry seeds were analysed in triplicate ($n = 3$) and boiling water in duplicate ($n = 2$).

Prior to the germination experiment, seeds (500 g) were rinsed with water and then soaked in 0.1 % lactic acid in MilliQ water (1,3 w/v) for 18 h at 8 °C. Thereafter, the seeds were again rinsed with water and samples of soaked seeds were retrieved. The remaining soaked seeds were placed on filter papers in plastic containers. Based on preliminary germination tests, the seeds were germinated for five days at 15 °C in the dark with daily spraying of MilliQ water to maintain the humidity. The sampling of germinated seeds was performed each day after removing the sprouts. From day three, the sprouts were *ca.* 1–2 cm long, allowing sufficient material for sampling of sprouts. The seeds and sprouts were immediately frozen and stored at −20 °C. The B vitamin and RFO content in the soaked and germinated seeds were analysed in triplicate from duplicate germinations ($n = 6$). The B vitamin content in the sprouts was determined in duplicate from one germination experiment ($n = 2$) due to the small amount of sprout material.

Prior to extraction, the soaked, boiled, and germinated seeds and sprouts were let to thaw and homogenised with Knife Mill (Grindomix GM 200, Retsch GmbH, Haan, Germany). Dried seeds were milled through 0.5 mm sieve using Ultra Centrifugal Mill (ZM 200, Retsch GmbH, Haan, Germany).

2.3. In vitro digestion

The bioaccessible B vitamin content was assessed from digesta that was produced by applying the static INFOGEST *in vitro* digestion protocol of Minekus et al. (2014), with modifications. Lipase was excluded as it did not significantly impact on the bioaccessibility of B vitamin (folate) in an earlier study (Liu et al., 2022b). Additionally, α-amylase from *Aspergillus oryzae* was used instead of human salivary α-amylase and porcine pancreatic α-amylase. The activity of α-amylase was tested, and the amount of enzyme used in the digestion was adjusted to comply the activity proposed in the INFOGEST method, as for all the other digestion enzymes. Simulated salivary fluid (SSF, pH 7), simulated gastric fluid (SGF, pH 3), and simulated intestinal fluid (SIF, pH 7) were prepared as proposed in the INFOGEST protocol (Minekus et al., 2014).

The *in vitro* digestion consisted of oral, gastric, and intestinal phases, which were conducted in a shaking water bath at 37 °C. In the oral phase, 5 g of sample was mixed with SSF containing α-amylase (4 mL), then $CaCl_2$ and MilliQ water were added to obtain a volume of 10 mL. Subsequently, the mixture was incubated for 2 min. Gastric phase was initiated by adding SGF with pepsin (8 mL), and $CaCl_2$. The pH was adjusted to 3, and MilliQ water was added to attain a volume of 20 mL. Then, the mixture was incubated for 2 h. In the intestinal phase, SIF with bile acid (10 mL) and α-amylase (6 mL) were added; and the pH was raised to 7. Finally, $CaCl_2$, and trypsin and chymotrypsin solutions (1 mL) were added, the volume was set to 40 mL with MilliQ water, and the incubation was conducted for 2 h. To obtain the digesta, the mixture was centrifuged (12,900 *g* at 8 °C, 15 min). The digesta was stored at −20 °C until the B vitamin analyses. The *in vitro* digestion was conducted in duplicate from duplicate boiling experiments ($n = 4$) and in triplicate (n

= 3) for dry, soaked (0.1 % lactic acid), and germinated seeds.

The subsequent analyses of digesta required consideration as the INFOGEST method does not provide guidance for vitamin analysis methods after *in vitro* digestion. Moreover, certain intestinal enzymes (*e.g.*, phosphatases and NAD glycohydrolases) (Freese & Lysne, 2023) that convert vitamins into absorbable forms are not included in the standardised protocol. In this study, boiling was selected to inactivate digestion enzymes as the boiling step was also part of the vitamin extraction of initial samples. However, to minimise any further changes after *in vitro* digestion, combination of a high temperature and prolonged time was avoided whenever possible.

2.4. Thiamin and riboflavin analysis

2.4.1. Extraction of thiamin and riboflavin, and the pre-column derivatisation of thiamin

The extraction of thiamin and riboflavin in legume samples was performed by acid hydrolysis and enzymatic dephosphorylation (EN 14122, 2014; EN 14152, 2014). The simultaneous extraction of thiamin and riboflavin was reported in our previous study (Siitonen et al., 2024). In brief, the legume seed/sprouts sample (0.5–1 g) was mixed with 15 mL of 0.1 M HCl (pH 2) and boiled in a water bath for 60 min. Then, the extract (pH 4.5) was incubated with Taka-diastase (50 mg) and β-amylase (5 mg) for 18 h at 37 °C. Subsequently, the extract was centrifuged (8800 *g*, 10 min) and paper-filtered, and the final volume was set to 25 mL. For the analysis of riboflavin, *ca.* 1 mL of the extract was filtered through a 0.2-μm syringe filter.

Thiamin was converted to fluorescent thiochrome in a pre-column derivatisation step. The mixture of extract (1 mL) and alkaline potassium hexacyanoferrate solution (1 mL) was vortexed for 20 s and let to stand for 1 min. The reaction was stopped by adding 100 μL of sodium sulphite solution (100 mg/mL). Finally, the extract was filtered through a 0.2-μm syringe filter.

Faba bean extracts required a purification step prior to the derivatisation, as previously observed by Siitonen et al. (2024). Briefly, the extract (5 mL, pH 6) was purified using solid phase extraction (SPE) with weak cation exchange cartridge (Oasis WCX cartridge, 6 mL, 150 mg, Waters Corporation, Milford, MA, USA). Thiamin was eluted with 4 mL of methanol containing 2 % formic acid. Then, the eluate was evaporated under nitrogen flow and the residue was reconstituted in MilliQ water (pH 4.5).

To extract thiamin and riboflavin from digesta, 5 mL of digesta was mixed with 10 mL of 0.1 M HCl and boiled for 5 min. Thereafter, the extraction included incubation with Taka-diastase and β-amylase, SPE purification of faba bean extracts, and thiamin derivatisation.

2.4.2. UHPLC analysis of thiamin and riboflavin

Thiamin and riboflavin were determined using the ultra-high performance liquid chromatography (UHPLC) method, which was validated in our previous study (Siitonen et al., 2024). A UPLC system (Waters Corporation, Milford, MA, USA) consisted of a sample manager, binary solvent manager, column manager, and photodiode array (PDA) and fluorescence (FLR) detectors. Separation was performed on a BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters Corporation, Milford, MA, USA) at 30 °C separately for riboflavin and thiamin. The mobile phase was a mixture of 20 mM ammonium acetate in MilliQ water (70 %) and methanol (30 %), and the flow rate was 0.2 mL/min. Thiamin and riboflavin were detected using an FLR detector. For thiamin, the excitation wavelength was 365 nm and the emission wavelength was 435 nm. For riboflavin, the corresponding values were 432 nm (excitation) and 520 nm (emission). The retention time of thiamin was 2.9 min, and that of riboflavin was 3.6 min. Quantification was performed using external calibration curves (0.03–0.72 ng/injection for thiamin and 0.08–1.88 ng/injection for riboflavin). The thiamin content was expressed as thiamin chloride hydrochloride; however, the bioaccessible thiamin content was calculated as thiamin, using a conversion factor of 0.787, to enable comparison with the recommended dietary intake value.

2.5. Niacin analysis

2.5.1. Extraction of niacin

Acid hydrolysis was performed to extract the available niacin (EN 15652, 2009). In brief, the legume seed/sprouts sample (0.5–1 g) was mixed with 15 mL of 0.1 M HCl and boiled for 60 min. After cooling, the extract (pH 4.5) was centrifuged (8800 *g*, 10 min) and paper-filtered, and the final volume was adjusted to 25 mL. Finally, the extract was filtered through a 0.2-μm syringe filter.

To analyse niacin from the digesta, 5 mL of digesta was mixed with 10 mL of 0.1 M HCl and boiled for 60 min. Then, the extraction was continued as described earlier. We applied a 60-min boiling step to extract niacin from the digesta to compensate for the absence of intestinal phosphatases and NAD glycohydrolases necessary for the conversion of niacin into absorbable forms, NA and NAM (Freese & Lysne, 2023). Additionally, a short boiling step (5 min) was tested.

2.5.2. UHPLC analysis of niacin

Niacin was determined with a previously validated UHPLC method using a Waters Acquity UPLC system (see *2.4.2*) (Chamlagain et al., 2020) and expressed as the sum of niacin vitamers. NA and NAM were separated on an HSS T3 C18 column (1.8 μm, 2.1 × 150 mm, Waters Corporation, Milford, MA, USA) at 30 °C. The mobile phase consisted of potassium dihydrogen phosphate (70 mM), hydrogen peroxide (150 mM), and copper sulphate (5 μM) in MilliQ water, and the flow rate was 0.3 mL/min. To create fluorescent NA and NAM derivatives, a post-column derivatisation was applied in a knitted polytetrafluoroethylene (PFET) reaction coil (0.24 mm i.d., and 3 m in length) under UV light (366 nm, 8 W) with the presence of hydrogen peroxide and copper ions. FLR detection was conducted with an excitation wavelength of 322 nm and emission wavelength of 380 nm. The retention time of NA was 3.9 min, and that of NAM was 11.6 min. External calibration curves were used in the quantification (1.03–51.5 ng/injection for NA and 1.08–54.1 ng/injection for NAM).

To assess the difference in the bioaccessible niacin content after boiling the digesta for 5 min and 60 min, NAD was investigated from digesta samples. The identification of NAD was conducted with a Waters Acquity UPLC system equipped with quadrupole time-of-flight mass spectrometry (Q-TOF-MS) detector (Waters Synapt G2 Si). An HSS T3 C18 column (1.8 μm, 2.1 × 150 mm, Waters Corporation, Milford, MA, USA) was set at 40 °C. The mobile phase consisted of 20 mM ammonium formate in MilliQ water (A) and methanol (B). Gradient elution was performed within 10 min with a flow rate of 0.3 mL/min. The starting condition of the mobile phase was 99 % of A; thereafter the ratio of A was gradually decreased to 10 % in 7 min and kept for a 1 min, after which it was returned to the initial condition. The MS analysis was performed in the electrospray ionisation (ESI) positive mode. The MS conditions were as follows: capillary voltage 0.5 kV, cone voltage 40 V, source temperature 120 °C, desolvation temperature 500 °C, desolvation gas 1000 L/h, and nebuliser gas 6 bar. NAD was identified in sample extracts according to the retention time (4.28) and m/z of the NAD standard (664).

2.6. Folate analysis

2.6.1. Extraction, enzyme treatment, and purification of folate

Folate vitamers were extracted using tri-enzyme treatment (Edelmann et al., 2012). Briefly, the legume seed/sprouts sample (1–2 g) was mixed with 12 mL of buffer (50 mM CHES, 50 mM HEPES, 2 % sodium ascorbate and 10 mM 2-mercaptoethanol in MilliQ water, pH 7.85) and boiled for 10 min. After cooling, the extract (pH 4.9) was incubated with α-amylase (20 mg) and hog kidney conjugase (1 mL of enzyme solution) for 3 h at 37 °C. Subsequently, the extract (pH 7) was

incubated with protease (8 mg) for 60 min at 37 °C. The enzymes were inactivated by boiling for 5 min. After centrifugation (8800 *g*, 10 min), the extract was paper-filtered and the final volume was set to 25 mL. Then, the filtered extract (10–15 mL) was purified and concentrated by affinity chromatography using affinity agarose gel (Affi-Gel 10, Bio-Rad Laboratories, Richmond, CA, USA) coupled with folate-binding protein (Scripps Laboratories, San Diego, CA, USA).

The extraction of folate from digesta was performed as follows: 10 mL of digesta was mixed with 10 mL of extraction buffer and boiled for 5 min. Then, the extract (pH 4.9) was incubated with hog kidney conjugase (1 mL) for 3 h at 37 °C. Subsequently, the purification was performed as described earlier.

2.6.2. UHPLC analysis of folate

Folate vitamers—H_4folate, 5-CH_3-H_4folate, 5-HCO-H_4folate, PGA, 10-HCO-PGA, and 5,10-CH^+-H_4folate—were determined using a Waters Acquity UPLC system (see *2.4.2*) with the validated UHPLC method (Liu et al., 2022a). The folate content was determined as the sum of the monoglutamate forms of folate vitamers, which were separated on a Kinetex PS C18 column (2.6 μm, 2.1 × 150 mm, Phenomenex, Torrance, CA) at 30 °C. The mobile phase consisted of 0.7 % formic acid in MilliQ water and in acetonitrile, and the gradient elution was performed with a flow rate of 0.6 mL/min. PDA detection with a wavelength of 290 nm was used to detect PGA and 5-HCO-H_4folate and 360 nm to detect 5,10-CH^+-H_4folate. FLR detection with an excitation wavelength of 290 nm and emission wavelength of 356 nm was performed to detect H_4folate and 5-CH_3-H_4folate and an excitation wavelength of 360 nm and emission wavelength of 460 nm was performed to detect 10-HCO-PGA. The retention times for H_4folate, 5-CH_3-H_4folate, 5,10-CH^+-H_4folate, 10-HCO-PGA, 5-HCO-H_4folate, and PGA were 1.7 min, 2.1 min, 3.1 min, 3.7 min, 4.1 min and 4.4 min, respectively. Quantification was conducted using external calibration curves (0.08–1.44 ng/injection, except for 0.32–1.44 ng/injection for 5-HCO-H_4folate, and 0.16–1.44 ng/injection for 5,10-CH^+-H_4folate).

2.7. Raffinose family oligosaccharide analysis

RFO content in germinated seeds was investigated to monitor the progression of faba bean and lupin germinations. RFOs—including raffinose, stachyose and verbascose—were analysed according to the method described by Xu et al. (2017). Legume seed sample (0.1–0.2 g) in MilliQ water (4.5 mL) was vortexed for 1 min. Then, the extract was centrifuged (8800 *g*, 10 min) and boiled for 5 min. After cooling, the extract was filtered by centrifugation (10,000 rpm, 10 min) using Amicon ultra centrifugation filters (Merck KGaA, Darmstadt, Germany). Finally, the extract was diluted by mixing with MilliQ water and 2-deoxy-D-galactose solution (0.5 mg/mL). RFOs were determined using the high-performance anion exchange chromatography system (Waters Alliance 2690/5, Milford, USA) with pulse amperometric detection (Waters 2465, Milford, USA). Compounds were separated on a CarboPac PA1 column (4 × 250 mm; Dionex, Sunnyvale, US) at 22 °C with a flow rate of 1 mL/min. The gradient elution (60 min) was initiated with 2 mM NaOH, then the concentration was gradually increased to 200 mM within 38 min and kept for a 10 min, after which the condition was returned to 2 mM NaOH. A post-column addition of 300 mM NaOH was conducted with a flow rate of 0.3 mL/min. Quantification was performed using raffinose, stachyose, and verbascose (500–4000 ng/injection) as standards and 2-deoxy-D-galactose as an internal standard (500 ng/injection) to compensate for the intra- and interday variability in the detector response.

2.8. Calculations and statistical analysis

The B vitamin and RFO contents were expressed on a dry matter (DM) basis. The moisture content was determined using the oven-drying (130 °C) AACC 44-15A method (AACC, 2000). The bioaccessible B vitamin content was expressed on a fresh weight (FW) basis to evaluate the proportion of B vitamin that could be absorbed from a portion (100 g) of the legume seeds. A separate blank sample (5 mL of MilliQ water) was analysed for B vitamins to subtract the possible vitamins derived from the enzymes used in the extractions and *in vitro* digestion protocol. Bioaccessibility (%) was calculated by dividing the vitamin content in digesta (after digestion) by the content analysed from the initial sample (before digestion).

The differences in the B vitamin and RFO contents were assessed by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference *post hoc* test (IBM SPSS Statistics 22; IBM Corp., Armonk, NY, USA). To observe the differences in bioaccessibility between dry and processed seeds, a two-sample *t*-test was used. A *p*-value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. B vitamin contents in dry, soaked, and boiled seeds

The thiamin content in dry faba bean seeds was 3.2 μg/g DM, whereas an approximately two-fold higher content was determined in dry lupin and pea seeds (Fig. 1/**Table S1**). The riboflavin content was the lowest in pea (1.5 μg/g DM) and highest in faba bean (2.5 μg/g DM). The niacin content was lower in lupin seeds (14.9 μg/g DM) than in faba bean and pea seeds (20.1–22.8 μg/g DM). The predominant niacin vitamer in dry seeds was NAM, contributing 57–79 % to niacin content. The folate content was markedly higher in faba bean and lupin seeds (*ca.* 1570–1710 ng/g DM) than in pea seeds (195 ng/g DM). The major folate vitamers in dry seeds were 5-CH_3-H_4folate and 5-HCO-H_4folate, together contributing 63–83 % to folate content. The content of thiamin, riboflavin, niacin, and folate in dry seeds was similar as that in faba bean, lupin, and pea flours in our previous study (Siitonen et al., 2024).

The B vitamin contents generally retained or slightly decreased (8–22 %) in soaking (Fig. 1/**Table S1**), but a decrease of 39 % was observed in the folate content of pea seeds. Another exception was the 30 % higher niacin content in soaked lupin seeds than in dry lupin seeds. The ratio of NA and NAM retained in faba bean and lupin, but the proportion of NAM increased in pea. After soaking, the 5-CH_3-H_4folate content was slightly higher than its content in the respective dry seeds; thus, its contribution to folate content increased. The 5-HCO-H_4folate content decreased in all legume seeds, in fact it could not be detected in soaked pea seeds.

Similar to our results, thiamin content was stable in soaking (12 h, 22 °C) of common beans, whereas riboflavin content reduced by 18 % and niacin content by 13 % (Barampama & Simard, 1995). Moreover, Prodanov et al. (2004) observed a relatively good retention of thiamin and riboflavin in soaking (in water, 9 h, ambient temperature) of faba bean, chickpea, and lentils. They reported that the niacin content in faba bean retained but decreased in chickpea and lentils. In contrast to our finding of minor folate losses, Hefni and Witthöft (2014) and Liang et al. (2022) reported that soaking (12 h, 25–30 °C) of various legume species increased folate content (9–124 %). These studies performed soaking at higher temperatures than we used in the present experiment, which may have promoted *de novo* folate synthesis.

The thiamin, riboflavin, niacin, and folate content in boiled seeds was generally lower than that in the respective soaked seeds (Fig. 1/**Table S1**), with the only exception being the folate content in lupin, which was similar in soaked and boiled seeds. We observed a variation in the loss of B vitamins among different legume species. Accordingly, boiling caused the following reductions in the thiamin content: 15 % in faba bean, 25 % in lupin, and 41 % in pea. Riboflavin content reduced by 13 % in faba bean, 38 % in lupin, and 53 % in pea. In contrast, boiling caused a similar reduction (53–62 %) in the niacin content of legume seeds. The folate content reduced by 24 % in faba bean and 46 % in pea, whereas it retained in lupin.

Prodanov et al. (2004) reported similar losses of thiamin (24–62 %) and niacin content (32–72 %) due to soaking (9 h) and boiling (35 min)

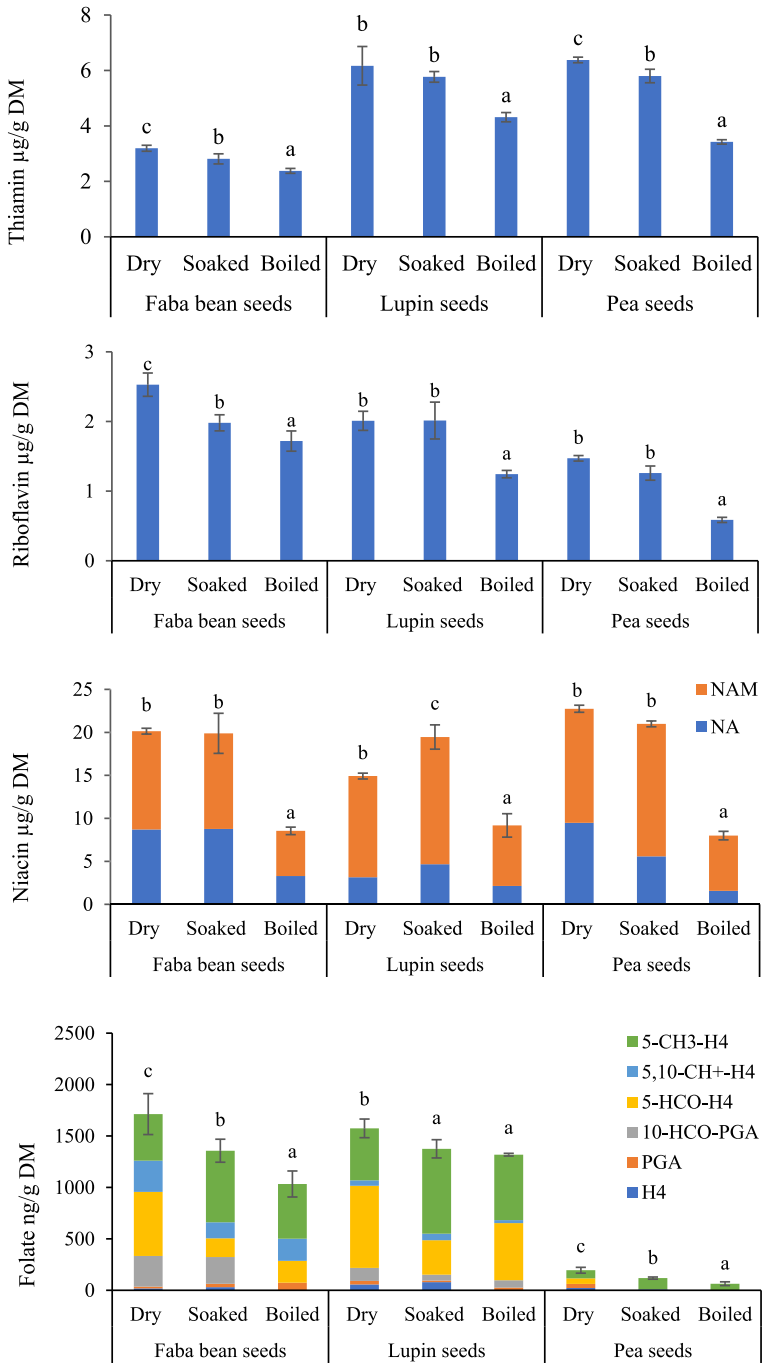

Fig. 1. Thiamin, riboflavin, niacin, and folate content on a dry matter basis (µg/g DM; ng/g DM for folate) in dry, soaked (MilliQ water), and boiled faba bean, lupin, and pea seeds. Error bars represent the standard deviation of analytical replicates from duplicate soaking and boiling trials ($n = 4$; except for dry seeds, $n = 3$). For niacin and folate, the error bars represent the standard deviation of total content. Different letters in each legume species indicate significant differences ($p < 0.05$) in vitamin content.

of faba bean, chickpea, and lentils. They observed a good retention of riboflavin in faba bean and lentils, but a reduction of 52 % in chickpea. Folate losses of 15–42 % due to boiling (20–50 min) have been reported for various legume species (Ferawati et al., 2019; Liang et al., 2022). Additionally, *ca.* 50 % losses have been discovered in the boiling of chickpea and pea for 2 h (Dang et al., 2000).

Degradation and leaching are possible reasons for vitamin losses in boiling. To evaluate the degree of leaching, we determined the B vitamin contents in the water used for boiling faba bean, lupin, and pea. The B vitamin contents were as follows: thiamin 0.1–0.3 µg/mL, riboflavin 0.1–0.2 µg/mL, niacin 1.5–2 µg/mL, and folate 7–66 ng/mL. The residual volume of water after the boiling was 350–370 mL. In general, leaching covered approximately 50 % of the vitamin losses. The minor riboflavin loss in the boiling of faba bean seeds was almost entirely due to leaching. Additionally, leaching completely caused the losses of thiamin, riboflavin, and niacin in the boiling of lupin seeds. The leaching of folate into the boiling medium was earlier observed in the boiling of different legume species (Dang et al., 2000; Liang et al., 2022). Nevertheless, Liang et al. (2022) assessed that degradation was also a prominent reason for folate losses. Overall, in this study, B vitamin losses during boiling were due to leaching and, in certain cases, also degradation.

3.2. Germination

3.2.1. RFO content in seeds during germination

Germination progressed similarly in faba bean and lupin, as evident by the gradual growth of sprouts. Additionally, gradually decreased RFO content in both legume species proved that the germination was progressing (**Fig. S1**). RFOs most likely degraded due to endogenous α-galactosidase activity (Xu et al., 2017). In three- and four-day germinated faba bean seeds, only 1 % of RFOs were left; after five germination days, the compounds were not observed. In contrast, after five days of germination of lupin seeds, 25 % of the initial RFO content was still remaining. Wei et al. (2022) also observed remarkable or even total reduction of RFOs in the germination of faba bean varieties. Similarly, Avezum et al. (2024) reported that the germination of lentils and cowpea decreased its RFO content.

3.2.2. B vitamin contents in seeds and sprouts during germination

The germination experiment began with the soaking of legume seeds in 0.1 % lactic acid solution, which either retained or caused reduction (12–25 %) in the B vitamin contents, except for a slightly higher niacin content in soaked lupin seeds than that in dry lupin seeds (Fig. 2/ **Table S2**). As shown in Fig. 2, the impact of germination on the B vitamin contents varied depending on the B vitamin and legume species. In general, the B vitamin contents increased gradually during the germination period, and the increase was mainly higher in lupin seeds than in faba bean seeds. The riboflavin, niacin, and folate contents increased by 17–114 % during the five days of germination, except for the decreased (10 %) folate content in the faba bean seeds. Further, the thiamin content was lower (24 %) in the five-day germinated faba bean seeds than in the respective dry seeds, whereas thiamin content retained in the germination of lupin. The highest increase was observed in the niacin content of lupin seeds, although the niacin content did not significantly increase anymore after three days of germination. The NAM content increased in the germination of both legume species. The gradual increase of 5-CH_3-H_4folate content in lupin seeds was the major reason for the increased folate content; nevertheless, 5-HCO-H_4folate content decreased simultaneously during the germination. The increase in riboflavin, niacin, and folate content may be due to the *de novo* synthesis of vitamins during germination (Avezum et al., 2022). In contrast, leaching into the soaking water was a plausible reason for the decreased thiamin and folate content in the germinated faba bean seeds.

Similar to our results, the varying impacts of legume germination on the B vitamin contents have been reported in literature. Highly variable increases (16–320 %) in the thiamin, riboflavin, niacin, and folate content were observed in the germination of various legume species (Avezum et al., 2024; Prodanov et al., 1997; Sierra & Vidal-Valverde, 1999; Zhang et al., 2021). Consistent with our observations, the retention or minor reduction in thiamin content (up to 28 %) was also reported during the germination of faba bean (Prodanov et al., 1997), pea (Sierra & Vidal-Valverde, 1999), cowpea (Avezum et al., 2024), and lentils (Zhang et al., 2021). Moreover, Ferawati et al. (2019) observed no major increase in the folate content of faba bean, and Avezum et al. (2024) even observed a reduction in the folate content of lentils in certain germination conditions. Similar to our results, an increase of the 5-CH_3-H_4folate content and a reduction of the 5-HCO-H_4folate content have been reported in the germination of legumes (Avezum et al., 2024; Coffigniez et al., 2021; Hefni & Witthöft, 2014; Zhang et al., 2021). Coffigniez et al. (2021) suggested that 5-HCO-H_4folate was converted to 5-CH_3-H_4folate due to germination, which possibly also occurred during the germination of lupin seeds in the present study.

The markedly variable results among germination studies may be due to different germination conditions, such as time and temperature as well as varying legume species and varieties. Accordingly, Avezum et al. (2024) observed that different parameters and their combinations influenced the thiamin and folate content in germinated legumes, and the impact varied depending on the legume species. We observed a different influence of germination on the B vitamin content in faba bean and lupin, and this was also reported for the folate content in the germination of pea, faba bean, and white bean by Ferawati et al. (2019). It should also be noted that we determined the contents in germinated seeds without sprouts, which may differ from other studies.

The B vitamin contents were high in sprouts separated from the seeds that were germinated for three, four, and five days (Fig. 3). In fact, the contents in sprouts were 2–12-fold higher than those in the respective germinated seeds. The greatest differences between sprouts and seeds were found in the riboflavin content of faba bean and in the folate content of faba bean and lupin. The variation in the B vitamin contents of sprouts at different time points was relatively minor, but a marked difference in the B vitamin contents was observed between faba bean and lupin sprouts. The thiamin content was two–three-fold higher and

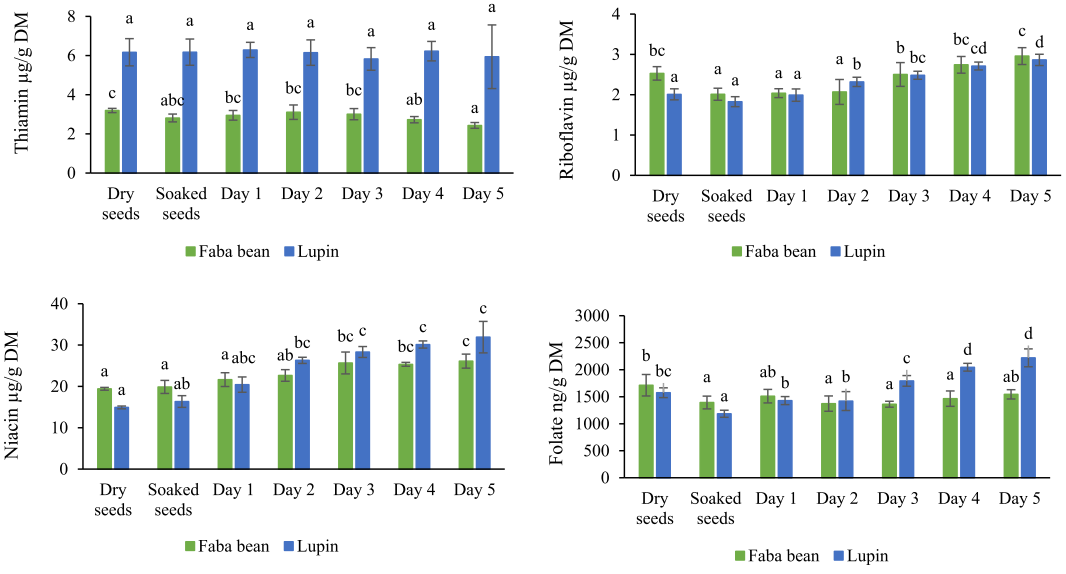

Fig. 2. Thiamin, riboflavin, niacin, and folate content on a dry matter basis (µg/g DM; ng/g DM for folate) in dry, soaked (0.1 % lactic acid), and germinated (1–5 days) faba bean and lupin seeds. Error bars represent the standard deviation of analytical replicates from duplicate germination trials ($n = 6$; except for dry seeds, $n = 3$). Different letters in faba bean and lupin seeds, separately, indicate a significant difference ($p < 0.05$) in vitamin content.

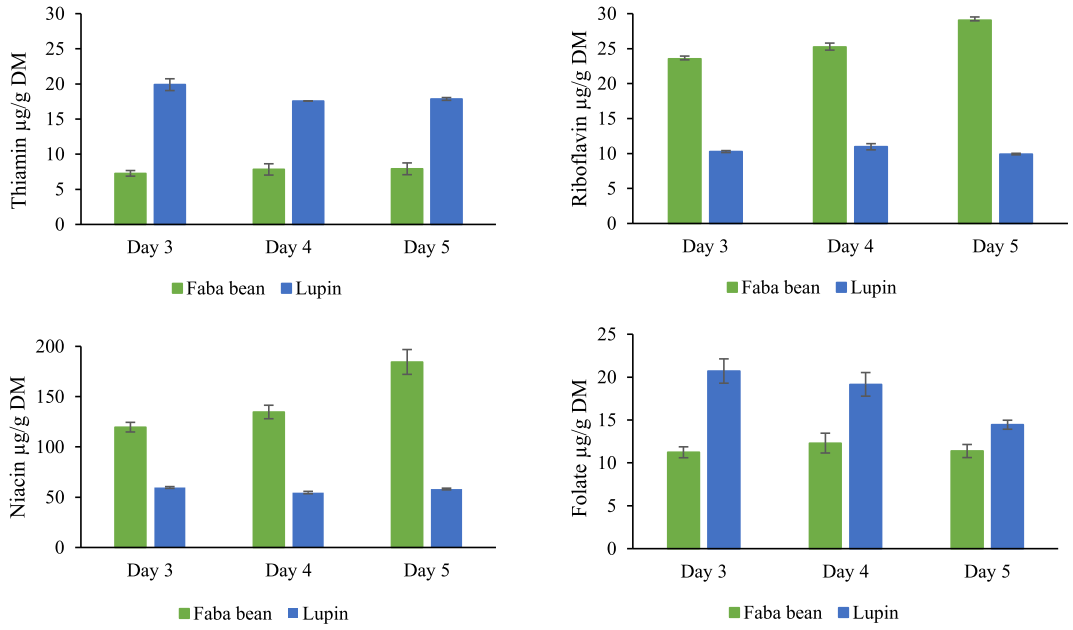

Fig. 3. Thiamin, riboflavin, niacin, and folate content on a dry matter basis (µg/g DM) in sprouts separated from three, four, and five days germinated faba bean and lupin seeds. Content in sprouts determined as analytical duplicate ($n = 2$) from one germination experiment. Error bars represent the range of averages.

the folate content was up to two-fold higher in lupin sprouts (*ca.* 18–20 µg/g DM of thiamin and *ca.* 14–21 µg/g DM of folate) than in the corresponding faba bean sprouts (*ca.* 7–8 µg/g DM of thiamin and *ca.* 11–12 µg/g DM of folate). The predominant folate vitamer was 5-CH_3-H_4folate: its proportion was approximately 50 % in faba bean sprouts and even *ca.* 90 % in lupin sprouts. H_4folate, 5-HCO- H_4folate, 5,10-CH^+-H_4folate, and 10-HCO-PGA were also observed in sprouts. In contrast with the thiamin and folate content, faba bean sprouts had two–three-fold higher riboflavin and niacin content (*ca.* 24–29 µg/g DM of riboflavin and 120–184 µg/g DM of niacin) than those of

Table 1
Bioaccessible thiamin, riboflavin, niacin, and folate content (µg/100 g on fresh weight basis), and bioaccessibility (%) in dry ($n = 3$), soaked ($n = 4$ in water and $n = 3$ in 0.1 % lactic acid), boiled ($n = 4$), and germinated ($n = 3$) legume seeds. Values are expressed as mean ± standard deviation. The bioaccessibility of B vitamins in soaked, boiled, or germinated seeds that differed significantly from respective dry seeds are marked with * ($p < 0.05$).

Legume seeds	Thiamin		Riboflavin		Niacin		Folate	
	Bioaccessible[a] (µg/100 g FW)	Bioaccessibility (%)	Bioaccessible (µg/100 g FW)	Bioaccessibility (%)	Bioaccessible (µg/100 g FW)	Bioaccessibility (%)	Bioaccessible (µg/100 g FW)	Bioaccessibility (%)
Faba bean								
Dry seeds	157 ± 1	73 ± 0	196 ± 13	90 ± 6	1958 ± 102	113 ± 6	74 ± 12	50 ± 8
Soaked (in water)	91 ± 6	76 ± 5	103 ± 11	96 ± 10	699 ± 27	65 ± 3*	25 ± 2	34 ± 3*
Boiled[b]	51 ± 3	78 ± 4	59 ± 2	99 ± 4	336 ± 24	113 ± 8	17 ± 1	53 ± 3
Soaked (in lactic acid)	104 ± 2	82 ± 1*	121 ± 11	107 ± 10	802 ± 7	96 ± 1*	53 ± 5	68 ± 6*
Germinated (3 days)[c]	78 ± 6	66 ± 5	125 ± 8	105 ± 6	1114 ± 39	96 ± 3*	36 ± 3	56 ± 4
Lupin								
Dry seeds	349 ± 4	77 ± 1	195 ± 10	105 ± 8	1339 ± 193	97 ± 14	55 ± 2	38 ± 1
Soaked (in water)	121 ± 6	64 ± 3*	105 ± 6	126 ± 7*	756 ± 17	94 ± 2	15 ± 1	27 ± 2*
Boiled[b]	114 ± 3	93 ± 3*	52 ± 4	116 ± 10	357 ± 30	107 ± 9	13 ± 2	28 ± 5
Soaked (in lactic acid)	153 ± 12	67 ± 5	95 ± 4	120 ± 5	582 ± 62	84 ± 9	16 ± 2	31 ± 4
Germinated (3 days)[c]	125 ± 3	65 ± 2*	127 ± 6	128 ± 6*	905 ± 72	75 ± 6	17 ± 1	24 ± 2*
Pea								
Dry seeds	391 ± 4	91 ± 1	151 ± 10	120 ± 8	1771 ± 20	91 ± 1	2.9 ± 0.9	19 ± 5
Soaked (in water)	220 ± 14	103 ± 7*	76 ± 9	116 ± 15	986 ± 131	97 ± 13	1.7 ± 0.9	30 ± 15
Boiled[b]	61 ± 2	85 ± 4*	17 ± 2	109 ± 15	161 ± 22	90 ± 12	n.d	n.d

n.d not detected.
[a] Thiamin content converted from thiamin chloride hydrochloride content with factor 0.787.
[b] Legume seeds soaked in water prior to boiling.
[c] Legume seeds soaked in 0.1 % lactic acid solution prior to germination.

corresponding lupin sprouts (*ca.* 10–11 μg/g DM of riboflavin and *ca.* 55–60 μg/g DM of niacin). NAM was the major niacin vitamer in most of the sprouts, contributing *ca.* 70 % to the niacin content. Similar to the high B vitamin contents found in legume sprouts, Kariluoto et al. (2006) reported major folate content in rye sprouts, where 5-CH_3-H_4folate was the most abundant vitamer.

Avezum et al. (2022) suggested that germinated legumes can be utilised in the production of innovative foods with enhanced nutritive value. From a nutritional aspect, we observed a desired reduction of RFOs in germination, but only a moderate increase in certain B vitamin contents in germinated seeds. We observed that B vitamins were majorly synthesised and accumulated in sprouts, thus, nutritional benefits could be obtained if the sprouts were utilised. Nevertheless, in the industrial germination process, the sprouts may not be formed if the germination time is short or they might be discarded in the subsequent processing steps. Overall, we observed that germination did not induce major losses in B vitamins, which is favourable considering germination applications in the food industry.

3.3. Bioaccessibility of B vitamins in legume seeds

The bioaccessibility of thiamin, riboflavin, and niacin in dry, soaked, boiled, and germinated seeds ranged from 64 % to 128 % (Table 1). Hence, thiamin, riboflavin, and niacin were mainly well liberated from legume matrices in the *in vitro* digestion and they were generally stable in those conditions. In contrast, folate bioaccessibility (19–68 %) was generally lower than that of other B vitamins. The bioaccessibility of B vitamins was similar in different legume species.

No consistent trend was observed in the bioaccessibility of the B vitamins that could be attributed to processing. Soaking, boiling, and germination potentially enhance the liberation of B vitamins from the legume matrix during digestion due to the altered state of macromolecules and the activities of enzyme inhibitors. In fact, germination has been observed to improve nutrient availability—for example, protein and starch digestibility (Alonso et al., 2000). Additionally, the stability of vitamins in digestion conditions could be affected if the composition of minor components changes due to processing. Overall, processing could impact vitamin bioaccessibility, but the present results indicated that soaking, boiling, and germination did not consistently enhance or decrease the bioaccessibility of B vitamins in legume seeds.

Thiamin bioaccessibility varied from 64 % to 103 % in dry, soaked, boiled, and germinated legume seeds. In general, riboflavin bioaccessibility in legume seeds was higher than that of other B vitamins. Riboflavin bioaccessibility was 90–107 % in faba bean. Interestingly, bioaccessibility was consistently over 100 % in lupin and pea (105–128 %). Riboflavin may occur in protein-bound forms in foods (Ndaw et al., 2000); thus, the *in vitro* digestion with its enzymes—especially pepsin, trypsin, and chymotrypsin—may have released riboflavin from the legume matrix more effectively compared to the extraction in vitamin analysis applied in this study.

Previously, *in vitro* digestion of B vitamins in the legume matrix has been investigated only in a few studies. Thiamin bioaccessibility in canned and cooked legumes was generally found to be 72–96 %, but lower bioaccessibility was observed for canned red lentils (23 %) and cooked green lentils (*ca.* 50 %) (Andac-Ozturk et al., 2022; Demir et al., 2023; Kesik et al., 2022). Riboflavin bioaccessibility varied from 40 % to 90 % in canned and cooked legumes (Andac-Ozturk et al., 2022; Demir et al., 2023; Kesik et al., 2023). These results are mainly in agreement with our findings; however, in certain cases, the bioaccessibility of thiamin and riboflavin was lower than that determined in the present study. However, it should be noted that in these previous studies an extensive heat-extraction was conducted for digesta, which differs from the short boiling step performed in our study. Additionally, there were differences in the *in vitro* digestion protocol.

In this study, niacin bioaccessibility was 65–113 % in legume matrices. Both NA and NAM were determined in the digesta of various legume seeds. Niacin bioaccessibility was the lowest in faba bean seeds soaked in MilliQ water and the highest in dry faba bean seeds. To our knowledge, only Demir et al. (2023) examined niacin bioaccessibility in a legume matrix. In accordance with our findings, they observed a bioaccessibility of 82 % for niacin in boiled green beans.

Folate bioaccessibility was highly variable in legume seeds (19–68 %). Additionally, folate was not observed in the digesta of boiled pea seeds. The predominant folate vitamers in digesta were consistently 5-CH_3-H_4folate and 10-HCO-PGA. Nevertheless, the 5-CH_3-H_4folate content was lower after *in vitro* digestion; thus, the bioaccessibility of this vitamer was low. In contrast, as the 10-HCO-PGA content was higher in the digesta than in the respective legume seeds, the stability and release of this oxidised vitamer during *in vitro* digestion was excellent. PGA was observed only in a few digesta, and H_4folate and 5,10-CH^+-H_4folate were not detected after *in vitro* digestion. In general, 5-HCO-H_4folate was not observed in the digesta; therefore, this vitamer—which is abundant in legume seeds—was not bioaccessible. Overall, it can be concluded that 5-CH_3-H_4folate and 5-HCO-H_4folate were the crucial folate vitamers responsible for low folate bioaccessibility.

To our knowledge, only one study reported folate bioaccessibility for legume matrix. Liu et al. (2021) observed slightly higher folate bioaccessibility in faba bean flour (63 %) than what we determined in dry faba bean seeds (50 %). Variable and, in certain cases, poor folate bioaccessibility was also reported for cereal-based matrices: 24–81 % in fermented foods (Bationo et al., 2020), 42–67 % in cereal flours (Liu et al., 2021), 44–96 % in wheat-based breads (Liu et al., 2022a), and *ca.* 30 % in wheat germ (Ringling & Rychlik, 2017). In comparison with our results, Liu et al. (2021) observed a more variable folate vitamer distribution in the digesta of faba bean flour. In addition to 5-CH_3-H_4folate and 10-HCO-PGA, they determined marked 5-HCO-H_4folate and 5,10-CH^+-H_4folate content. Similar to our observation, Liu et al. (2021) reported that the 10-HCO-PGA content increased in the digestion of faba bean flour. They also discovered that an addition of ascorbic acid (100 μmol/mL) in the gastric phase of the *in vitro* digestion led to higher folate content in digesta. Additionally, Liu et al. (2022a) reported a higher recovery for 5-CH_3-H_4folate when nitrogen flushing was included in the *in vitro* digestion protocol. These findings indicate that folate vitamers were prone to oxidation during the *in vitro* digestion. Ringling and Rychlik (2017) also reported that the stability of folate affected its bioaccessibility. Thus, a plausible explanation for low folate bioaccessibility is not only the incomplete liberation of folate from legume matrices but also, importantly, the poor stability during the *in vitro* digestion. Hence, the stability of a vitamin in digestion conditions needs to be considered as an integral factor in bioaccessibility.

3.4. Evaluation of niacin analysis of digesta

A short boiling step (5 min) and subsequent enzyme treatments were appropriate in the extraction of thiamin and riboflavin as well as folate from digesta. In contrast, a 5-min boiling step was not sufficient for niacin extraction, as indicated by a bioaccessibility of only 31 % for dry faba bean seeds and 47 % for dry lupin seeds. These values were remarkedly lower than those obtained with a 60-min boiling step. As niacin may occur in various forms in foods, NA and NAM must be released from these derivatives, *e.g.*, from NAD, during the analysis (Konings et al., 2024).

To further investigate the reason for varying niacin bioaccessibility between 5 min and 60 min boiled digesta, we analysed NAD in digesta using the UHPLC method with Q-TOF-MS. The retention time (4.28) and *m/z* of NAD standard (664) were used to identify the compound from sample extracts. NAD was detected from 5 min digesta, whereas it was not observed in 60 min digesta, thereby indicating that NAD degraded in the 60 min boiling. Hence, NAM was liberated from NAD and potentially also from other compounds such as nicotinamide adenine dinucleotide phosphate, nicotinamide mononucleotide, and nicotinamide riboside. These various niacin derivatives can be utilised in the body as niacin is

released by intestinal enzymes *e.g.*, phosphatases and NAD glycohydrolases (Freese & Lysne, 2023). Nevertheless, these enzymes are not included in the INFOGEST *in vitro* digestion model; thus, to enable the conversion of the compounds to NA and NAM, a 60-min boiling step was required for analysis of niacin from digesta in this study.

3.5. Bioaccessible B vitamin contents and contribution of boiled legume seeds to recommended B vitamin intake

The B vitamin bioaccessibility in certain legumes may be high but the bioaccessible B vitamin content may be low or *vice versa*. Therefore, both factors are crucial in assessing legumes as sources of B vitamins. In this study, the bioaccessible B vitamin content was expressed in FW to enable the evaluation of the proportion of B vitamin that could be absorbed from a portion (100 g) of the legume seeds. We observed a major variability in the bioaccessible B vitamin contents (μg/100 g FW): the highest content was consistently found in dry legume seeds and the lowest in boiled legume seeds (Table 1). In fact, the bioaccessible B vitamin content was 3–11-fold higher in dry seeds than in respective boiled seeds. The content in dry, soaked, boiled, and germinated seeds was 51–391 μg/100 g FW for thiamin (content converted to thiamin with factor 0.787), 17–196 μg/100 g FW for riboflavin, 161–1960 μg/100 g FW for niacin, and 13–74 μg/100 g FW for folate. An exception was the bioaccessible folate content in dry and soaked pea seeds which was only *ca.* 2–3 μg/100 g FW. The variation in bioaccessible B vitamin content (in FW) among different seeds was most likely due to vitamin losses from processing and the higher water content in soaked, boiled, and germinated seeds than that of dry seeds. The bioaccessible contents in DM are shown in **Table S3**.

In this study, the boiled legume seeds represented the edible legume products; hence, we evaluated the contribution of bioaccessible B vitamin content in boiled faba bean, lupin, and pea seeds to the respective recommended vitamin intake obtained from the Nordic Nutrition Recommendations 2023 (Blomhoff et al., 2023). A portion of 100 g of boiled legume seeds (FW) could contribute 5–13 % to recommended thiamin intake (thiamin content converted to thiamin with factor 0.787) (Table 2). The same proportion of boiled legume seeds could contribute only 1–4 % to recommended riboflavin intake and 1–3 % to niacin intake (no tryptophan included). Boiled faba bean and lupin seeds could cover 4–5 % of the recommended folate intake.

We observed a marked loss of vitamins in boiling due to leaching. The boiling time in this study was relatively long, and the vitamin retention in the seeds could be better with a shorter boiling time. If the boiling medium was consumed, such as in soups, the leached vitamins could still contribute to intake and provide nutritional value. On the other hand, leaching of vitamins could be avoided with a different cooking method, such as steaming. In summary, thiamin, riboflavin, and niacin were well bioaccessible in boiled legume seeds; however, their contribution to the recommended intake was only moderate or even low. The folate bioaccessibility in legume seeds was generally low but considering the bioaccessible folate content, the consumption of faba bean and lupin could still provide folate from diet.

Table 2
Contribution (%) of bioaccessible thiamin, riboflavin, niacin, and folate content (μg/100 g on fresh weight basis) in boiled legume seeds to the recommended vitamin intake[a].

Boiled legume	Thiamin[b] (%)	Riboflavin (%)	Niacin (%)	Folate (%)
Faba bean	5–6	4	2	5
Lupin	10–13	3	2–3	4
Pea	6–7	1	1	–

[a] Nordic Nutrition Recommendations 2023: thiamin 0.9–1.1 mg/day, riboflavin 1.6 mg/day, niacin 14–18 mg/day, and folate 330 μg/day.
[b] Factor 0.787 was used to convert thiamin chloride hydrochloride content to thiamin, and contributions were calculated from that.

4. Conclusion

We observed that the soaking of faba bean, lupin, and pea seeds did not result in major changes in the B vitamin contents but boiling caused vitamin losses majorly due to leaching. The B vitamin contents mainly retained or increased in the germination of faba bean and lupin seeds. We also observed that B vitamins were synthesised and accumulated in high contents in sprouts during germination.

A short boiling step (5 min) after the INFOGEST *in vitro* digestion was appropriate in the analysis of thiamin, riboflavin, and folate in digesta. In contrast, an extensive boiling step (60 min) was required to release niacin from its derivatives.

This study provided novel insights into the bioaccessibility of thiamin, riboflavin, niacin, and folate in various legume seeds. The results contribute to a deeper understanding of the release of these vitamins from processed legume matrices and their stability during simulated digestion. The bioaccessibility of B vitamins was similar among different legume species, and processing did not consistently impair or improve the bioaccessibility in legume matrices. Overall, the thiamin, riboflavin, and niacin bioaccessibility was good in dry, soaked, boiled, and germinated legume seeds; however, the bioaccessibility of folate was lower than that of other B vitamins likely due to its low stability during *in vitro* digestion. Hence, we emphasise the importance of considering not only the release of vitamins from the food matrix but also their stability in digestion conditions.

In contrast to similar bioaccessibility, the bioaccessible B vitamin content in FW varied greatly among dry, soaked, boiled, and germinated legume seeds. The content of bioaccessible thiamin, riboflavin, niacin, and folate was the lowest in boiled legume seeds and the highest in dry seeds. Overall, based on the results of *in vitro* digestion, boiled legume seeds could have up to a moderate contribution to the recommended intake of B vitamins.

CRediT authorship contribution statement

Aino Siitonen: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Minnamari Edelmann:** Writing – review & editing, Supervision, Conceptualization. **Miikka Olin:** Writing – review & editing, Supervision, Investigation. **Kirsi Jouppila:** Writing – review & editing, Supervision, Conceptualization. **Vieno Piironen:** Writing – review & editing, Supervision, Conceptualization. **Susanna Kariluoto:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.145564.

Data availability

Data will be made available on request.

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