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Estimation of Iron and Zinc Bioavailability in Salad Formulations with Different Protein Sources

Assessing Nutritional Adequacy and the Impact of Different Protein Sources on Mineral Uptake

Master's thesis in Biotechnology

Elin Lagerroth

DEPARTMENT OF LIFE SCIENCE

CHALMERS UNIVERSITY OF TECHNOLOGY
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ELIN LAGERROTH

Supervisor: Inger-Cecilia Mayer Labba, Life Sciences
Examiner: Rikard Landberg, Life Sciences
In collaboration with Picadeli

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Department of Life Sciences
Division of Food and Nutrition
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone +46 31 772 1000

Cover: The three salad formulations composed from the Picadeli assortment according to the selected recipes in the report. Picture taken by Elin Lagerroth and edited by Gemini.

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Abstract

A plant-based dietary shift has the potential to reduce the climate impact of the food systems. However, this transition must not compromise the uptake of essential nutrients. Salad bars play a role in this transition, offering customizable and plant-rich convenience meals. Despite their popularity, there is limited knowledge regarding the bioavailability of essential minerals in these composed salad meals. This study estimated the bioavailability of iron and zinc in three salad compositions containing different protein sources (meat/fish, lacto-ovo, and plant-based) to determine if they meet the nutritional requirements of school lunches for teenage girls, who are highly susceptible to mineral deficiencies.

The salad components were analyzed for phytic acid, iron, and zinc content using high-performance ion chromatography (HPIC) and atomic absorption spectroscopy (AAS). Mineral bioavailability was subsequently estimated using established algorithms (Hallberg and Hulthén, Miller) and molar ratios. The results demonstrate that while the meat-based composition provides highly absorbable iron and zinc, the plant-based and lacto-ovo alternatives fail to meet the absorbable iron requirements for individuals with high physiological needs. This is potentially due to high concentrations of inhibitors such as phytic acid and calcium. Conversely, all salad compositions had high absorption efficiency of zinc. These findings suggest that further optimization is required for salad-based meals to be nutritionally adequate with regard to iron. Potential strategies include substituting meat analogs with fermented products like tempeh, which has a reduced phytic acid content, or utilizing non-soy-based alternatives. Furthermore, mineral bioavailability can be enhanced by moderating the intake of phytate-, calcium-, soy-, and egg-rich components, while increasing the presence of enhancers such as ascorbic acid or animal proteins.

Keywords: Iron bioavailability, zinc bioavailability, plant-based diets, phytic acid, Miller algorithm, Hallberg and Hulthén algorithm, atomic absorption spectroscopy, school meals, salad bars.

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Elin Lagerroth, Gothenburg, May 2026

List of Acronyms

Below is the list of acronyms that have been used throughout this thesis, listed in alphabetical order:

AAS	Atomic Absorption spectroscopy
CO2e	Carbon dioxide equivalents
CV	Coefficient of variation
DW	Dry weight
GHG	Global greenhouse gas
HPIC	High-Performance Ion Chromatography
IDA	Iron deficiency anemia
LCA	Life cycle assessment
MW	Molecular weight
NNR	Nordic Nutrition Recommendation
NRV	Nutrient Reference Value
PA	Phytic acid
PAL	Physical Activity Level
Phy:Fe	Phytate-to iron molar ratios
Phy:Zn	Phytate-to zinc molar ratios
RDI	Recommended daily intake
RI	Recommended intake
RPD	Relative Percent Difference
SLV	Swedish food agency

Nomenclature

Below is the nomenclature of parameters and variables that have been used throughout this thesis.

Parameters

σ	Standard deviation
μ	Average value of the replicates
A_{MAX}	Maximum zinc absorption (mmol/day)
K_T	Zinc transporter dissociation constant
K_P	Zinc-phytate dissociation constant
B_{Ca}	Calcium parameter (inhibitor factor)
B_{Pr}	Protein parameter (enhancer factor)
MW_{Phytate}	Molecular weight phytate
MW_{Iron}	Molecular weight iron
MW_{Zinc}	Molecular weight zinc

Variables

m_{dry}	The mass of the container and sample after freeze-drying.
m_{fresh}	The mass of the container and sample before freeze-drying
m_{tare}	The mass of the empty container
TAZ	Total absorbed zinc per day
TDZ	Total dietary zinc per day
TDP	Total dietary phytate per day
TDC	Total dietary calcium per day
$TDPr$	Total dietary protein per day

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1

Introduction

The global food system faces major challenges in meeting a protein demand predicted to double by 2050, while mitigating adverse environmental effects associated with industrial meat production [1]. As concerns regarding biodiversity loss, animal welfare, and climate change intensify, plant-based proteins have gained increasing interest, as they often have a lower climate impact and offer health benefits. Consequently, diets rich in plants are frequently proposed as a strategy for mitigating climate change. However, this dietary shift also introduces significant nutritional challenges, such as the lower bioavailability of essential micronutrients, particularly iron and zinc.

Phytate, which is naturally present in many plant-based ingredients, is known to inhibit the absorption of these minerals [2]. Improving the understanding and optimization of mineral bioavailability in plant-based meals is therefore critical to prevent nutritional deficiencies while simultaneously reducing the environmental impacts and meeting the growing protein demand. This makes bioavailability an important ethical aspect in the shift towards a more plant-based food system.

The convenience food sector plays a role in this transition. Salad bars offer a wide range of ingredients, including vegetables, grains, meats, plant-based proteins, dressings, and toppings, allowing consumers to compose personalized meals. Such assortments enable consumers to select meals that are predominantly plant-based. Picadeli is one example of a company operating within this segment, with a stated ambition to provide healthy and climate-conscious food choices. Despite the growing popularity of plant-rich convenience meals, there is limited knowledge regarding the bioavailability of essential minerals such as iron and zinc in composed salad meals.

In this context, this project aimed to estimate how different compositions in mixed salad meals influence iron and zinc bioavailability, in order to provide insights relevant to both human health and environmental sustainability. Salads inspired by a commercial salad bar context were used to evaluate the nutritional quality of three different salad compositions.

1.1 Aim and goals

The aim of the project was to estimate the bioavailability of iron and zinc in three selected composed salads containing different protein sources, and to develop evidence-based recommendations for enhancing mineral bioavailability in plant-rich salads. The project goal was to create three salad recipes with a maximum of 30 ingredients in total, one of which contains meat or fish, one of which contains lacto-ovo protein, and one that is entirely plant-based. Then analyze phytate, iron, and zinc contents

in the ingredients of the salads and estimate the bioavailability of iron and zinc from the entire salad meals.

1.2 Research questions

The project addressed the following research questions:

- For the target group, teenage girls, what percentile of the Nordic Nutrition Recommendation (NNR) 2023 requirement distribution is met by absorbed iron?
- Is it possible to reach the nutritional targets for a school meal for a teenage girl with the selected salads?
- How does the choice of protein source (animal-based, lacto-ovo, or plant-based) influence the predicted iron and zinc absorption in the full salad meals?
- To what extent can iron and zinc absorption be improved through realistic ingredient substitutions within a salad bar context?

1.3 Limitations

The project was limited to ingredients available within the Picadeli assortment and did not aim to represent the full diversity of salad bar meals. Ingredient selection was informed by sales data to ensure relevance.

To enable meaningful comparisons between protein sources, the salads were designed using a standardized meal structure; a common base of ingredients (vegetables and carbohydrate component) was constant across formulations, while the protein component varied between animal-based, lacto-ovo, and plant-based versions.

Portion sizes were defined as a school lunch suitable for a teenage girl according to the Swedish food agency (SLV) [3], which estimates a school lunch to be 30% of the daily nutritional intake. The meals had similar energy content per serving according to the NNR 2023 [4] by taking the average estimated energy intake of the age-groups 11-14 years and 15-17 years.

A part of the discussion of the nutritional quality focused on teenage girls as a target group. This is because they have a higher risk of iron deficiency and have higher iron requirements [5].

2

Theory

This chapter introduces the importance of this study, key information on the concepts of mineral nutrition and bioavailability, and the theory behind the methodologies. Nutritional requirement values are also presented.

2.1 Dietary shift

Food systems contribute to one-third of global greenhouse gas (GHG) emissions, identifying them as a major driver of climate change [6]. Consequently, lowering the emissions from food systems is essential for climate change mitigation. Specifically, meat production is associated with a high environmental impact [1]. As the global population grows and becomes increasingly wealthier, protein demand is projected to double by 2050, further exacerbating these adverse effects.

A potential solution to this problem has been suggested to be a plant-based dietary shift. There are different types of plant-based diets, where two common ones are lacto-ovo vegetarians who exclude all meat and fish, and strict vegetarians or vegans who exclude all animal products [7]. A life cycle assessment (LCA) study found that while a carnivore diet generates 1.83 t CO2e/person/year, vegan or vegetarian diets generate 0.89 and 1.37 t CO2e/person/year, respectively [8]. This indicates that plant-based diets have less of an impact on climate change.

Beyond environmental advantages, plant-based diets are suggested to offer health benefits, such as reduced risk of obesity and cardiovascular diseases [1]. Also, various types of plant proteins have demonstrated the capacity to replicate both the techno-functional and nutritional properties of conventional meat. However, plant-based diets are linked to a higher risk of nutritional deficiency and bone fractures compared to omnivore diets due to the low intake and uptake of essential micronutrients [7]. Consequently, a trade-off exists between nutrient bioavailability and environmental sustainability within this dietary shift strategy. A plant-based dietary shift could have the potential to reduce the climate footprint of the food system, provided that it is strategically balanced to optimize nutrient bioavailability and ensure overall nutrition adequacy [9].

2.2 Nutritional aspects of dietary iron and zinc

Iron, followed by zinc, is the most abundant micronutrient in the human body [10]. They are both essential minerals and are involved in many biological processes.

2.2.1 Dietary iron

Iron is an essential micronutrient for human life, as it plays vital roles in electron and oxygen transport, cell division and differentiation, and the regulation of gene expression [11]. The majority of iron in the body (70%) is bound to hemoglobin, which facilitates oxygen delivery throughout the body. Most iron turnover occurs endogenously, with 20-25 mg of iron being recycled daily from aged red blood cells [9]. However, adults lose 1-2 mg of iron per day, with higher numbers for menstruating women. Since the body lacks the capacity to synthesize iron, dietary sources are necessary.

Dietary iron consists of heme iron, found in meat and other animal products, and non-heme iron, which is found in both plant and animal sources [11]. Although heme iron represents only 10% of intake, its stable and high absorption rate of approximately 25% makes it highly bioavailable regardless of other dietary components [9]. Consequently, it accounts for approximately 40% of total intestinal iron absorption and is less sensitive to dietary conditions than non-heme iron [11]. In contrast, non-heme iron exists mainly as ferric iron (Fe3+), which is poorly soluble at intestinal pH. Dietary components, such as ascorbic acid, can help maintain iron solubility and promote reduction to ferrous iron (Fe2+), the form primarily absorbed by enterocytes. Thus, diet composition is crucial for optimizing non-heme iron absorption.

Iron deficiency is the most common nutritional deficiency, affecting up to a third of the world's population [11]. The most severe form of iron deficiency is called iron deficiency anemia (IDA) [12]. IDA induces, for example, negative effects on cognitive development, diminished quality of life, lack of productivity, and fatigue. The risk groups for iron deficiency are young children, menstruating females, pregnant women, and vegetarians [4].

Even if a food product contains iron, the absorption efficiency is only 5-20% [9]. The threshold of a food item to be considered a source of iron according to EU regulations is 2.1 mg/100 g, based on 15% of the iron Nutrient Reference Value (NRV) of 14 mg/day [13].

2.2.2 Dietary zinc

Zinc is an essential micronutrient with numerous crucial functions and is widely distributed throughout the human body [10]. It is required for gene expression, metalloenzyme activity, structural components of zinc fingers, and cellular processes such as differentiation, apoptosis, and proliferation.

An imbalanced zinc homeostasis can have severe consequences, such as depression, stroke, frequent infections, atherosclerosis, diabetes, breast cancer, and impaired fetal development [10]. Zinc deficiency is rare in the Nordic and Baltic countries, but people restricting animal products are at risk of suboptimal levels [4].

Zinc absorption depends a lot on body zinc homeostasis, meaning that zinc-deficient

individuals absorb more zinc than non-deficient ones [10]. From a mixed or vegetarian diet based on refined grains, the zinc bioavailability is approximately 26-34%. For a food item to be considered a source of zinc according to EU regulations, it needs to contain at least 1.5 mg/100 g, based on 15% of the zinc NRV of 10 mg/day [14].

2.2.3 NNR 2023 requirement distribution

The Nordic Nutrition Recommendations (NNR) 2023 presents scientific data on the health and planetary effects of food, advising on how to eat healthily and sustainably [4]. It contains recommended intake (RI) data for essential minerals, such as iron and zinc, for people in the Nordic and Baltic countries. For females aged 11-14, the RI of iron is 13 mg/day, and females aged 15-17 have an RI of 15 mg/day. For zinc, RI for females aged 11-14 is 10.8 mg/day, and RI for ages 15-17 is 12.2 mg/day. This is assuming a mixed diet of both animal products and vegetables with a phytic acid intake of around 600 mg/day. The upper limit of zinc intake is 25 mg/day, and 60 mg/day for iron.

Absorbable dietary iron requirements vary in a population due to physiological differences [15]. To ensure absorbable iron adequacy, requirements are evaluated both on average individual needs and extremes of population variance, using a percentile distribution. The 95th percentile defines the population with the highest iron requirements, and the median (50th percentile) represents the population with average to low iron requirements. For the group 11-14-year-old girls, the median requirement of absorbed iron is 1.47 mg/day, and the 95th percentile requirement is 1.90 mg/day. For 15-17-year-old girls, the median is 1.32 mg/day, and the 95th percentile requirement is 2.19 mg/day.

The population median activity level is a physical activity level (PAL) of 1.6, which corresponds to some increased physical activity levels and sedentary work [4]. Estimated daily energy requirements for 11-14-year-old girls at PAL 1.6 is 9.2 MJ, and 10.1 MJ for 15-17-year-olds. According to SLV, a school lunch represents 30% of the daily intake [3].

2.3 Bioavailability

Bioavailability of nutrients is generally defined as the fraction of a micronutrient in a given food that can be absorbed and utilized by the body [16]. This fraction differs from the actual mineral content in the food. The absorption of non-heme iron and zinc can be affected by enhancers and inhibitors present in the meal. In plant-based foods, phytic acid is an antinutrient that reduces the bioavailability of these micronutrients [17]. The processing of food and certain nutrients can also affect the bioavailability.

2.3.1 Phytic acid

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate) is a naturally occurring compound in plants, involved in the biosynthesis of cell-wall polysaccharides and growth regulators [18]. It is an intermediate in inositol phosphate metabolism and accumulates at the post-ripening stage of seed maturation, serving as the primary storage form of phosphorus in plants.

Due to its polyanionic nature, phytic acid has a high affinity for cationic minerals, such as iron (Fe), zinc (Zn), phosphorus (P), calcium (Ca), and magnesium (Mg)[18]. The chelation of these micronutrients by phytic acid forms insoluble phytate salts, visualized in Figure 2.1, significantly reducing their bioavailability. When ingesting plant-based food, the phytate complex passes through the intestine without absorption since the negatively charged complex cannot permeate the lipid bilayer of plasma membranes [19]. Phytic acid is therefore an antinutrient, reducing the nutritional value of plant-based foods [20]. The negative effects of phytic acid on non-heme iron absorption are dose-dependent and start already at 2-10mg phytic acid per meal [21]. The inability of monogastric animals to metabolize phytic acid is due to their insufficient level of phytate-degrading enzyme activity in the digestive tract [22]. The chelated minerals are released when phosphate groups are sequentially cleaved from phytate through phytase-catalyzed hydrolysis [19]. Phytases are usually found in microorganisms or plants.

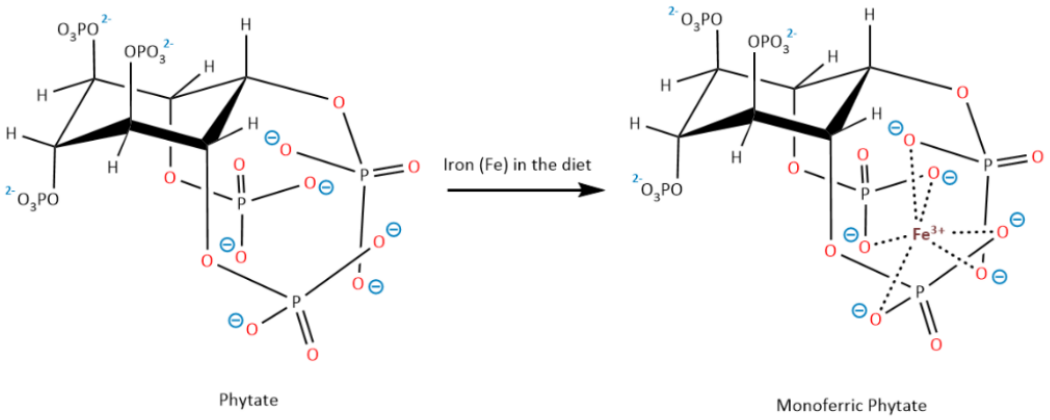

Figure 2.1: Schematic representation of phytate chelating one dietary iron molecule in physiological pH, creating monoferric phytate. Illustration by N. Storåker, adapted from [23]

2.3.2 Phytic acid and processing

Foods containing phytic acid are, for example, cereals, legumes, oilseeds, and nuts, where soy concentrates have the highest measured phytic acid content [22]. Phytic acid ranges per 100g are 390-680 mg in cooked soybeans, 140-310 mg/100 g in yellow peas, and 130-500 mg in cooked lentils [5]. To avoid inhibitory effects, nearly all phytic acid must be removed from these foods, as a single portion substantially exceeds the concentrations where mineral absorption begins to decline. This can

be done through the processing of the food. Cooking, soaking, and germination remove less than 10%, 5-15%, and 0-60%, respectively, meaning these methods are not sufficient to increase the non-heme absorption to a high efficiency.

To reduce phytic acid content to a low level, plant-intrinsic or microbial phytases need to be activated, or exogenous phytases added [19]. Fermentation lowers the pH, allowing optimal conditions for the endogenous phytases in the food to reduce the phytic acid up to 90-100% in certain cereals [5]. In sourdough fermentation, the long fermentation time and low pH activate phytases in cereal, resulting in a reduction of over 90%. Hydrothermal activation can create favourable conditions for intrinsic phytases, which can reduce the phytic acid by up to 90%. However, these methods are not effective enough for legumes since their endogenous phytase activity is lower, and the microorganism strains used in fermentation usually don't produce high levels of phytases themselves. To make it effective, exogenous phytases need to be added.

Tempeh is a product made from soybean fermentation by the mold *Rhizopus oligosporus* [5]. This process produces and activates phytases, which can reduce the phytic acid content by 60-80%. This increases the iron bioavailability considerably compared to non-fermented soybean food products. Due to its low phytic acid content and iron content close to the nutrition claim level, tempeh shows high potential as a soybean-based meat alternative compared to other meat substitutes [24].

Red and green lentils contain phytates, but red lentils often have lower concentrations. However, the content of nutrients and antinutrients varies substantially between cultivars, as shown in a study made on fava beans [25]. Certain processes, such as the extraction of proteins from vegetables, can accumulate phytic acid, leading to lower bioavailability of essential minerals in plant-based meat-analogs[26]. Meat analogs are often produced by concentrating the proteins via dry or wet fractionation, which simultaneously concentrates phytic acid [5]. Therefore, meat analogs often have poor bioavailability of minerals.

2.3.3 Dietary factors affecting mineral bioavailability

There are enhancing and inhibitory dietary factors that can affect the bioavailability of essential micronutrients such as iron and zinc, besides phytic acid. Some promoting factors to absorption of non-heme iron are ascorbic acid and animal tissues [5]. However, their effect is marginal if the inhibitor concentration is too high or the iron levels are too low. Ascorbic acid chelates the ferric iron (Fe^{3+}), which makes it soluble at higher pH, and promotes the reduction to ferrous iron (Fe^{2+}) so that it can be absorbed in the small intestine [11]. Animal tissues, such as meat, poultry, fish, and seafood, can increase absorption of non-heme iron via the "meat factor". A suggested mechanism is that it can bind the non-heme iron via cysteine and histidine residues to form soluble complexes, which increases the absorption [27].

Besides phytic acid, polyphenols and calcium are shown to inhibit iron uptake [11]. Polyphenols, which are present in vegetables, cereals, cocoa, etc, are assumed to have a similar mechanism to phytate. Calcium is shown to inhibit both non-heme and

heme iron. Soy and egg proteins can also reduce iron absorption [28]. This shows that many dietary factors besides phytic acid can have inhibitory effects.

Calcium also reduces the bioavailability of zinc by making the zinc-phytate complex stronger [10]. Proteins can positively affect zinc uptake, where animal proteins added to plant-based meals show substantially higher zinc bioavailability.

2.4 Laboratory methods

Laboratory methods are needed for determining phytate and mineral content in food samples, where sample preparations must be performed beforehand. Algorithms can then be used to calculate the absorbable iron and zinc.

2.4.1 Freeze drying

Freeze-drying is a method to remove water from food products via sublimation [29]. Free water in the food freezes under low temperatures, while water bound to the matrix does not. In the process, the solid water transforms directly into vapor and is released from the product surface. This is done through several steps, including freezing the product, primary drying, where sublimation takes place, and secondary drying to the final humidity. Primary and secondary drying takes place under reduced pressure with simultaneous heating.

2.4.2 Moisture content analysis

The moisture content of freeze-dried food can be measured using a moisture analyzer [30]. Residual moisture remains in the samples following freeze-drying [29], necessitating normalization of the data to ensure calculations are based on 100% dry product. The initial weight of the sample is measured, and a lamp is used to heat and dry the sample [30]. This is done until it reaches a constant weight, indicating that the moisture has evaporated. The difference between the initial and final weight is presented as the moisture content of the sample.

2.4.3 Phytic acid content analysis

High-performance ion chromatography (HPIC) can be used to measure phytate content [22]. It is a method for separating mixtures of ions, in which retention times are governed by the interactions of charged analytes with the stationary phase [31]. The chromatograph has an analytical column, which is designed to separate strongly negatively charged molecules, such as phytate. [22].

Extraction of phytic acid from food samples needs to be done to be able to analyze the phytate content with HPIC [22]. This can be done by mixing the sample with diluted hydrochloric acid under stirring to release the phytate from its complexes [32].

2.4.4 Iron and zinc content analysis

To analyze the iron and zinc content in foods, the organic fraction of the sample needs to be destroyed, to then use a suitable detection method of the selected minerals [33].

2.4.4.1 Microwave digestion

Microwave digestion is an acid-assisted method that solubilizes food samples into a clear solution suitable for analysis by techniques such as AAS [33]. The method uses an oxidizing agent and microwave-generated heat, where electromagnetic energy is converted into thermal energy through molecular movement within the liquid sample. This process occurs in closed vessels under high pressure, allowing temperatures to rapidly exceed the boiling point of the acids. Concentrated acids then destroy the organic matrix, leaving inorganic analytes in a measurable state. To ensure successful digestion and prevent mineral contamination, samples must be finely milled, and strict laboratory precautions are required.

2.4.4.2 Atomic absorption spectroscopy

To determine mineral concentrations in food, such as iron and zinc, atomic absorption spectroscopy (AAS) can be used [34]. Flame AAS, the most common type for analyzing trace metals, uses an acetylene-air flame to dissociate sample molecules into free atoms [35]. A hollow cathode lamp, coated with the specific analyte metal, emits light of characteristic wavelengths, which is then absorbed by the atoms in the flame. A monochromator isolates the desired wavelength before a detector measures the absorbance, which is proportional to the analyte's concentration. This method can detect metal concentrations as parts per billion.

2.4.5 Estimating bioavailability

The bioavailability of iron and zinc in meals can be estimated using various algorithms. These make it possible to modify diets to increase iron and zinc absorption.

2.4.5.1 Iron absorption algorithm

The Hallberg and Hulthén algorithm was developed to estimate the absorption and bioavailability of dietary iron [28]. It is based on the fact that the total iron bioavailability of a meal is the product of all enhancing and inhibiting factors, as well as iron status, and heme-and nonheme-iron contents. For each factor, there is a continuous function related to the amount of it in the meal. The factors taken into account are phytate, polyphenols, calcium, ascorbic acid, meat, fish and seafood, egg, soy protein, and alcohol. It was developed via a basal value that was multiplied by these factors influencing iron bioavailability. Also, equations were derived for the interactions between compounds for each factor in the meal.

2.4.5.2 Zinc absorption algorithm

The Miller algorithm can be used to estimate dietary zinc absorption and bioavailability [36]. Zinc absorption per day is modeled as a function of dietary zinc and

phytate ingested, since phytate has the greatest effect on zinc absorption. It also incorporates total calcium and protein, since calcium is a known inhibitor of zinc absorption, and proteins can enhance the bioavailability. Equation 2.1, Equation 2.2, and Table 2.1 define the Miller algorithm.

$$TAZ = 0.5\left(A_{sum} - \sqrt{A_{sum}^2 - 4 \cdot A_{MAX} \cdot TDZ \cdot (1 + B_{Pr} \cdot TDPr)}\right) \tag{2.1}$$

where A_{sum} represents the combined term for the saturable absorption components:

$$A_{sum} = K_T\left(1 + \frac{TDP(1 - B_{Ca} \cdot TDC)}{K_P}\right) + A_{MAX} + TDZ(1 + B_{Pr} \cdot TDPr) \tag{2.2}$$

Table 2.1: Constants and variables with descriptions for the Miller equation used to calculate the total absorbed zinc.

Parameter	**Value**	**Description**
A_{MAX}	0.084	Maximum zinc absorption (mmol/day)
K_T	0.069	Zinc transporter dissociation constant
K_P	0.44	Zinc-phytate dissociation constant
B_{Ca}	0.017	Calcium parameter (inhibitor factor)
B_{Pr}	0.012	Protein parameter (enhancer factor)
Variable	**Unit**	**Description**
TAZ	mmol/day	Total absorbed zinc per day
TDZ	mmol/day	Total dietary zinc per day
TDP	mmol/day	Total dietary phytate per day
TDC	mmol/day	Total dietary calcium per day
$TDPr$	g/day	Total dietary protein per day

2.4.5.3 Molar ratio

A simple way to assess the bioavailability of minerals is by calculating the molar ratio of phytic acid and the micronutrient [5]. This method does not include enhancing or other inhibiting factors, only producing a simplistic estimate of the micronutrient bioavailability. A molar ratio of phytate to iron below one is necessary for adequate iron bioavailability for meals not containing any iron absorption enhancers [37]. For meals containing enhancers, the molar ratio could instead be below 6 to counteract the inhibition of iron absorption. A molar ratio of phytate and zinc below 5 in diets corresponds to high zinc absorption efficiency, 5-10 is classified as moderate absorption efficiency, and diets above 15 have low absorption efficiency [38].

3

Methods

Estimating iron and zinc bioavailability in the salad components required analyzing each sample for phytate, iron, and zinc content. Consequently, the samples needed to undergo preparation. The data was then processed using algorithms.

3.1 Preparations

Salad components were selected, collected, freeze-dried, and milled using a coffee grinder in preparation for analysis. The moisture content for each sample was recorded, and the dry matter content was calculated.

3.1.1 Recipe determination

Three model salads were composed to reflect realistic choices within a commercial salad bar context. A constant standardized base of ingredients across formulations (vegetables and a carbohydrate base) was determined, which can be seen in Table 3.1. The protein component differed between the salads: (i) animal-based protein, (ii) lacto-ovo protein,(iii) plant-based protein. The chosen protein sources for each salad can be seen in Table 3.2. Ingredient selection was informed by availability within the Picadeli assortment and by sales data, but the formulations were intended as analytically comparable model meals rather than exact representations of individual consumer salads. The categories with the highest sales had the most ingredients.

Portion sizes were matched on energy content to equal a school lunch for teenage girls, aiming for 30% of the daily requirement according to SLV. Based on NNR 2023 data for females aged 11-14 and 15-17, the average energy, iron, zinc, and absorbable iron targets for each salad were calculated and can be found in Appendix B.1. Using Dietist.net, ingredient amounts were adjusted to meet the calculated energy target. The resulting amount of each ingredient in each salad is presented in Appendix B.2. Calcium and ascorbic acid levels were estimated from data of similar products available in the software, which can be found in Appendix B.3. The total nutritional value of each salad composition can be seen in Table 4.3.

Table 3.1: The selected standardized base of ingredients containing carbohydrates and vegetables from the Picadeli assortment. Each ingredient is part of a salad category, where the categories with the highest sales have multiple ingredients. The choice of ingredients in each category is based on sales data.

Category	Ingredient
Lettuce	• Lettuce mix • Green leaf mix
Vegetables	• Avocado • Diced cucumber • Cherry tomato • Mixed bell peppers
Seasoned vegetables	• Greek-ish salad
Pasta	• Pasta chili creamy • Pasta Bruchetta • Pasta Pesto
Grains/rice	• Couscous Moroccan style • Black Quinoa & Lentils
Toppings	• Salty roasted corn
Dressings	• Sriracha Mayo
Fruit	• Mango

Table 3.2: The selected protein sources for each salad composition (i) Meat/Fish, (ii) Lacto-ovo, and (iii) Plant-based. The proteins were chosen from the Picadeli assortment based on sales data and added to the salad (i, ii, or iii) depending on the type of protein source.

1. Meat/fish	**2. Lacto-ovo**	**3. Plant-based**
Diced chicken	Boiled peeled eggs	Veg. Teriyaki strips
Chicken meatballs	Creamy white cheese	Marinated soybeans
Shrimps	Mozzarella	Falafel

3.1.2 Sample preparations

Ingredients were collected from two different Picadeli restaurant locations at different times to capture batch-related variation. Different types of lentils and quinoa were bought from the grocery store, Willy's. The dry lentils and quinoa were boiled in water according to the instructions on the packaging. All products were weighed into containers and put in a -20°C freezer until completely frozen, and then in a -80°C freezer for one hour. Subsequently, the samples were placed in a freeze dryer for four days. The samples were then weighed again. The weight of the empty containers, and the containers with sample before and after freeze-drying, is found in Appendix B.4.1. The samples were milled with a coffee grinder and stored in a -20°C freezer until used for the experiments.

Dry matter content (%) in the products was calculated according to Equation 3.1. Due to container damage during processing, samples Chicken meatballs 1, Veg. teriyaki strips 1, Falafel 1, and Mozzarella 2 were excluded from dry matter content analysis. Consequently, dry matter values for these specific samples from the parallel batch were used for all subsequent calculations. The results of the dry matter calculations can be found in Appendix B.4.1, and an example calculation is found in Appendix B.4.2.

$$DW_{fresh}(\%) = \left(\frac{m_{dry} - m_{tare}}{m_{fresh} - m_{tare}} \right) \times 100 \tag{3.1}$$

- m_{dry} is the mass of the container and sample after freeze-drying.
- m_{fresh} is the mass of the container and sample before freeze-drying.
- m_{tare} is the mass of the empty container.

3.1.3 Moisture content analysis

Moisture content analysis was performed to determine the residual moisture in the freeze-dried samples. This allowed for the normalization of iron and zinc concentrations, ensuring results are reported per 100 g of completely dry product. Approximately 0.5 g of sample was weighed into an aluminum dish and analyzed using the automated moisture analyzer HE53 Mettler Toledo. The instrument heated the sample to a constant mass, and the moisture content (%) was recorded directly from the digital display. These values were then converted into dry weight fractions using equation 3.2.

$$DW_{dry} = \frac{100 - MC}{100} \tag{3.2}$$

Where:

- DW_{dry} is the dry weight fraction in the dried sample.

- MC is the moisture content measured by the moisture analyzer.

The resulting dry weight fractions for all samples are compiled in Appendix B.5. Samples Lettuce mix, Green leaf mix, Diced cucumber, and all samples of bell peppers did not have enough mass left in either batch to be analyzed. In later calculations, they were assumed to have a dry weight fraction of 1.

3.2 Phytic acid content analysis

Phytic acid concentration was determined by extraction and HPIC. Problems with the HPIC apparatus forced the use of previous data from similar products analyzed with HPIC.

3.2.1 Extraction

Extraction was done to later analyze the phytate content with HPIC. The standard operating procedure can be found in Appendix A.1. Approximately 0.5 g of prepared dry samples was weighed into centrifuge tubes, and 10 mL of 0.5 M HCl was added to each tube via a dispensing pump. The samples were then put on a rotating device for 5 hours in a cold-storage environment. Following extraction, the tubes were centrifuged. Samples with turbid supernatants were filtered using a syringe and filter into Eppendorf tubes.

3.2.2 High performance ion chromatography

HPIC was never completed due to problems with the apparatus. Bio-compatible LC-system from Jasco, Japan, was supposed to be used according to the standard operating procedure found in Appendix A.1. Standard solutions would be prepared from phytic acid dodecasodium salt hydrate and would range from 0.1mM to 0.8mM. An isocratic eluent of 80% 1 M HCl and 20% Milli-Q H_2O at a flow rate of 0.8 mL/min would have been used. A post-column reactor would be used to enable spectrophotometric detection by mixing the eluent with 0.1% $Fe(NO_3)_3 \cdot 9\ H_2O$ in a 2% solution of $HClO_4$.

Instead, phytic acid concentration data from previously made HPIC analyses were used. Simplifications were made, for example, data from plain pasta was used for the pasta mixes. An average concentration was calculated from multiple products for the samples Veg. teriyaki strips, Couscous moroccan, Black quinoa and lentils, and Falafel, since they contain multiple ingredients for which data were available. The data used for these samples can be seen in Appendix B.6. The phytic acid concentrations of each product are presented in Table 4.4.

3.3 Iron and zinc content analysis

Samples were digested using microwave digestion, and the iron and zinc content were analyzed with atomic absorption spectroscopy (AAS). The concentrations were

converted into mg per 100 g of ready product.

3.3.1 Microwave digestion

Microwave digestion was done to solubilize all minerals in the samples. The standard operating procedure can be found in Appendix A.2. The apparatus used was Milestone microwave laboratory system Ethos Plus and a laboratory terminal 800 controller equipped with MPR-300/12S.

Around 0.1 g of each sample was weighed into Teflon microwave digestion vessels in duplicates. Exact weights are found in Appendix B.7.1. The limited sample mass restricted some products to a single analysis. Digestion was performed by adding 7 mL Milli-Q H_2O, 1.75 mL of concentrated HNO_3, and 0.35 mL of concentrated HCl. The sealed vessels were loaded into the microwave digester and processed for 180°C for 20 minutes. Following a 4-hour cooling period, the resulting solutions were visually inspected for complete digestion, pooled with Milli-Q rinse water from the vessels, and diluted to a final volume of 10 mL. Between runs, all equipment was cleaned using distilled water, soap, and Milli-Q water, with Teflon components undergoing an overnight 0.25M HCl acid bath.

3.3.2 Atomic absorption spectroscopy

Atomic absorption spectrometry (AAS) was used to determine the iron and zinc content in the digested samples. It was done according to the standard operating procedure, which is available in Appendix A.3. The apparatus used was Agilent 240/280 Series spectrometer.

Calibration standards were prepared for iron and zinc via serial dilution of 50 mg/L stock solutions to a final volume of 20 mL. The standards for iron were prepared at concentrations of 4.0, 2.5, 1.25, and 0.625 mg/L. Zinc standards were prepared at 2.5, 1.25, 0.625, and 0.3125 mg/L. The required aliquot volumes were calculated based on equation 3.3. Example calculations can be found in Appendix B.7.2.

$$V_1 = \frac{C_2 \cdot V_2}{C_1} \tag{3.3}$$

- V_1 is the required aliquot volume
- C_1 is the previous concentration
- C_2 is the desired concentration
- V_2 is the final volume (20 mL)

The hollow cathode lamp, specific to the analyte, was activated 30-minutes before analysis. The ventilation system and acetylene gas were engaged, and the Spectra AA software package was configured with the relevant elemental parameters and calibration standards. Then the standards were run by turning on the flame and

dipping the tubing in order according to the program. The tubing was rinsed with Milli-Q water between every sample and standard. Following successful calibration, samples were analyzed. Analytical results were processed automatically by the software and can be seen in Appendix B.7.1.

The equipment generated mineral concentration in mg/L in the digested sample. Calculations were made to represent the mineral concentration in 100 g of fresh product (mg/100g), according to Equations 3.4, 3.5, and 3.6. An example calculation can be found in Appendix B.7.3, and the results are found in Appendix B.7.4. The mean mineral concentration for each sample was determined by first averaging the technical replicates, followed by a calculation of the grand mean across both batches to establish the final concentration in the fresh product, which is presented in Table 4.4.

$$\text{Unadjusted Fe (mg)/100 g DW} = \frac{C \times V}{m} \times 100 \tag{3.4}$$

Where:

- C: Concentration from equipment
- V: Total volume after digestion
- m: Mass of sample used for digestion

$$\text{Adjusted Fe (mg)/100g DW} = \text{Unadjusted Fe (mg)/100g DW} \times DW_{dry} \tag{3.5}$$

Where: DW_{dry}: Dry weight in the dried sample

$$\text{Fe (mg/100g product weight)} = \text{Adjusted Fe (mg)/100g DW} \times DW_{product} \tag{3.6}$$

Where: $DW_{product}$: Dry weight in product

The Relative percent difference (RPD) was used to calculate the variation between the replicates with Equation 3.7. The results can be seen in Appendix B.7.4. For samples exhibiting a high variation between replicates, an additional analytical run was performed, and the RPD was calculated again. For samples still exhibiting high RPD, the Coefficient of variation (CV) was calculated using all measured values using Equation 3.8. The new and old data and the calculated CV for these samples can be found in Appendix B.7.5. Example calculations of both RPD and CV are found in Appendix B.7.6.

$$RPD = \left(\frac{|R_1 - R_2|}{\frac{R_1 + R_2}{2}} \right) \times 100 \tag{3.7}$$

$$CV = \left(\frac{\sigma}{\mu}\right) \times 100 \tag{3.8}$$

- σ: Standard deviation
- μ: Average value of the replicates

3.4 Algorithms for estimating bioavailability

3.4.1 Iron absorption algorithm

The absorbable iron content in the three salad compositions was estimated using the Hallberg and Hultén algorithm. The percentage of uptake, defined by the absorbable mineral content divided by the total mineral content, was calculated. The results are presented in Table 4.1. The percentage of the NNR 2023 requirements met was also calculated using the requirements per meal calculated in Appendix B.1. The results of this can be seen in Table 4.2.

3.4.2 Zinc absorption algorithm

The Miller algorithm was used to estimate the absorbable zinc in the salad compositions. The equations 2.1 and 2.2 were used, with the parameters and variables in Table 2.1. The percentage of uptake was also calculated. Both of these results can be seen in Table 4.1. An example calculation can be found in Appendix B.8.

3.4.3 Molar ratios

The phytate-to-mineral molar ratio was calculated for samples with an iron content higher than 1.5 mg/100g and a zinc content higher than 1 mg/100g using Equation 3.9. The initial selection of ingredients for the molar ratio analysis was based on the criteria for a significant mineral food source (2.1 mg Fe/100g and 1.5 mg Zn/100g). However, to ensure a sufficiently large sample size for a meaningful comparative analysis, these thresholds were subsequently lowered. Samples that have mineral concentrations higher than the thresholds but do not contain any phytic acid are not included since the phytate-to-mineral ratio would be zero. Example calculations of the molar ratios can be found in Appendix B.9.1. The exact values of the molar ratios can be found in Appendix B.9.2, and visual representations are found in Figures 4.1 and 4.2.

$$\text{Phytate} - \text{mineral molar ratio} = \frac{\text{Intake of Phytate (mg)}/MW_{\text{Phytate}}}{\text{Intake of mineral (mg)}/MW_{\text{mineral}}} \tag{3.9}$$

4

Results

This section presents the most important results from the mineral analyses, portion optimizations, and bioavailability calculations.

4.1 Estimated absorbable iron and zinc contents and absorption efficiency in each salad composition

The estimated absorbable iron and zinc content for each meal, as well as the absorption efficiency, can be found in Table 4.1. The absorption efficiency is represented as a percentage (%) next to the absorbable iron and zinc concentration (mg/meal) in each of the three selected salads.

Table 4.1: Estimated absorbable iron and zinc concentrations (mg/meal) and the absorption efficiency (%) across the selected salad compositions. The percentage of the total iron and zinc content that is absorbable is stated in brackets () next to the absorbable mineral concentration.

Nutritional Value	**Meat & Fish**	**Lacto-ovo**	**Plant-based**
Absorbable iron (mg/meal)	1.19 (41.0%)	0.25 (6.6%)	0.42 (8.9%)
Absorbable zinc (mg/meal)	1.85 (54.5%)	1.95 (48.7%)	1.32 (36.6%)

The meat and fish composition contains the highest amount of estimated absorbable iron (1.19 mg/meal), followed by the plant-based salad (0.42 mg/meal), and lastly the lacto-ovo salad (0.25 mg/meal). The percentage increase from the lowest to the highest value is 376%. The iron absorption efficiency of each salad from highest to lowest is distributed in the same order as the absorbable iron concentrations, with 41.0%, 8.9%, and 6.6%, respectively. The meat-based salad has more than six times the iron absorption efficiency of the lacto-ovo composition.

The meat and fish salad also has the highest estimated zinc absorption efficiency at 54.5%, followed by the lacto-ovo composition at 48.7%, and then the plant-based composition at 36.6%. The percentage increase from the lowest to the highest value is 47.7%. All are above the approximate estimated standard absorption efficiency from a mixed or vegetarian diet (26-34%) [10]. The lacto-ovo composition has the highest absorbable zinc content at 1.95 mg/meal, followed by the meat and fish-based salad at 1.85 mg/meal, and lastly the plant-based salad at 1.32 mg/meal.

4.2 Achievement of NNR 2023 iron absorption percentiles across salad compositions

The extent to which the salad compositions meet the NNR 2023 iron absorption requirements for teenage girls' school lunches is presented in Table 4.2. The results are expressed as percentages, where a value exceeding 100% indicates that the percentile is met. The 95th percentile and median requirement distribution are evaluated. To provide a comprehensive overview, calculations were conducted for both the age groups 11-14 and 15-17. The results and calculations for the per-meal requirements used as reference are found in Appendix B.1.

Table 4.2: Percentile of the NNR2023 requirement met for a meal suited for a teenage girl. The percentiles are divided into the 95th percentile, which covers most teenage girls, even the group with the highest physiological needs, and the median, which represents the half with average to low iron requirements. The categories are based on the two age groups 11-14 and 15-17.

Percentile and Age group	Meat & Fish	Lacto-ovo	Plant-based
95th percentile: ages 11-14	208.8%	43.9%	73.7%
95th percentile: ages 15-17	180.3%	37.9%	63.6%
Median: ages 11-14	270.5%	56.8%	95.5%
Median: ages 15-17	297.5%	62.5%	105.0%

The meat and fish salad composition exceeds the absorbable iron requirements per meal for all target percentiles and age groups by at least 80%. In contrast, the lacto-ovo salad composition contains less than the required absorbable iron per meal in all categories and age groups, with at most 62.5% for the least demanding group, and 37.9% for the most demanding percentile and age group. The plant-based salad composition shows mixed results. While it provides sufficient absorbable iron to meet the median requirement for the 15-17 age group (105%), it remains insufficient for the 95th percentile and fails to reach any of the targets for the age group 11-14. However, it nearly reaches the median requirement for ages 11-14 (95.5%).

4.3 Nutritional values of the salad compositions

The nutritional values and the total weights of each composed salad are shown in Table 4.3. These are based on the portion sizes defined by the selected amounts of each ingredient, which are listed in Appendix B.2, and are optimized to meet the energy requirements for a teenage girl, as defined in Appendix B.1.

Table 4.3: Nutritional comparisons and total weights of the salad compositions made to match the energy requirement of a teenage girl. The Energy (kcal) is shown in bold. The nutritional values presented are the energy content in kJ and kcal, protein (g), Fat (g), Carbohydrates (g), Fiber (g), Ascorbic acid (mg), Iron (mg), Calcium (mg), Zinc (mg), and Phytic acid (mg).

Nutritional Value	Meat & Fish	Lacto-ovo	Plant-based
Total weight (g)	685	640	632
Energy (kJ)	2900	2906	2894
Energy (kcal)	**694**	**694**	**692**
Protein (g)	39.9	26.7	20.9
Fat (g)	30.8	39.9	33.0
Carbohydrates (g)	60.6	55.0	72.1
Fiber (g)	9.6	8.9	15.7
Ascorbic acid (mg)	183	166	166
Iron (mg)	2.9	3.8	4.7
Calcium (mg)	139	290	216
Zinc (mg)	3.4	4.0	3.6
Phytic acid (mg)	118	114	395

The energy content is roughly the same for all salads and aligns with the estimated energy requirements of a school lunch for a teenage girl. The plant-based salad contains more than three times as much phytic acid (395 mg) as the other two salads, which have similar phytic acid content (118 mg for the meat-based and 113 mg for the lacto-ovo-based). The salad containing meat has the most ascorbic acid (183 mg) and the least amount of calcium (139 mg), while the lacto-ovo composition has the most calcium (290 mg). Ascorbic acid content is similar for both the plant-based and lacto-ovo salad compositions. The salad with meat contains the least amount of iron (2.9 mg), followed by the lacto-ovo composition (3.8 mg), and the plant-based salad contains the most iron (4.7 mg). The salad with meat also has the lowest zinc values (3.4), followed by the plant-based salad (3.6 mg), and then the lacto-ovo salad (4.0).

Only the plant-based salad reaches the recommended total iron intake per meal for ages 11-17, and only the lacto-ovo composition contains the recommended total zinc intake per meal for ages 11-17. The recommended intakes per meal are calculated and presented in Appendix B.1.

4.4 Iron, zinc, and estimated phytic acid content

The average iron (Fe), zinc (Zn), and estimated phytic acid content of the tested products can be found in Table 4.4. The ranges of the concentrations can be seen for iron and zinc, which are shown in brackets () next to the average value. The raw data is found in Appendix B.7.1, which is transformed into concentrations per 100g product, as seen in Appendix B.7.4.

Table 4.4: Average Mineral and estimated Phytic Acid Content (mg) per 100g product. The ranges are presented in brackets () next to the average values from lowest to highest concentration measured for the iron and zinc.

Product ID	Fe (mg/100g)	Zn (mg/100g)	Phytic Acid* (mg/100g)
Lettuce mix	0.24 (0.22–0.26)	0.18 (0.17–0.18)	0.5
Green leaf mix	0.67 (0.66–0.69)	0.61 (0.60–0.63)	2
Avocado	0.28 (0.19–0.38)	0.90 (0.84–0.94)	1
Diced cucumber	0.12 (0.11–0.12)	0.11 (0.11–0.12)	1
Cherry tomato	0.24 (0.20–0.27)	0.16 (0.14–0.19)	2
Greek-ish salad	0.32 (0.23–0.43)	0.14 (0.14–0.15)	3.5
Pasta chili creamy	0.43 (0.34–0.59)	0.56 (0.45–0.67)	30.89
Pasta bruchetta	0.71 (0.59–0.87)	0.72 (0.64–0.77)	28.65
Pasta pesto	0.50 (0.48–0.53)	0.74 (0.73–0.76)	27.55
Couscous Moroccan	1.16 (1.06–1.23)	0.88 (0.81–0.95)	277.5
Black quinoa and lentils	2.12 (1.94–2.33)	1.62 (1.48–1.73)	60.13
Salty roasted corn	1.27 (1.09–1.38)	1.65 (1.58–1.82)	24
Sriracha mayo	0.23 (0.01–0.40)	0.10 (0.09–0.11)	0
Mango	0.10 (0.03–0.17)	0.08 (0.07–0.09)	1
Diced chicken	0.31 (0.20–0.47)	0.53 (0.49–0.57)	0
Chicken meatballs	0.77 (0.62–0.87)	0.90 (0.88–0.96)	8.2
Shrimps	0.07 (-0.03–0.15)	0.68 (0.64–0.70)	0
Boiled egg	1.76 (1.37–2.23)	1.24 (1.06–1.39)	0
Creamy white cheese	-0.10 (-0.17– -0.03)	1.14 (1.05–1.23)	0
Mozzarella	-0.01 (-0.12–0.07)	2.52 (2.39–2.65)	0
Veg. teriyaki strips	2.38 (2.28–2.51)	1.35 (1.28–1.43)	397.4
Marinated soybeans	2.01 (1.82–2.17)	1.03 (0.91–1.14)	207
Falafel	1.78 (1.71–1.91)	1.09 (1.03–1.22)	126.5
Red bell peppers	0.20 (0.18–0.22)	0.09 (0.09–0.09)	0.5
Green bell peppers	0.27 (0.22–0.30)	0.10 (0.92–0.10)	2
Yellow bell peppers	0.28 (0.28–0.29)	0.09 (0.09–0.09)	1
Red dried lentils	2.70 (2.69–2.71)	1.85 (1.85–1.85)	120
Organic dried red lentils	2.56 (2.45–2.66)	2.45 (2.40–2.50)	120
Red lentils (pre-boiled)	2.42 (2.37–2.47)	1.30 (1.30–1.31)	120
Organic dried green lentils	3.13 (3.00–3.26)	1.91 (1.85–1.97)	142
Green lentils (pre-boiled)	1.38 (1.25–1.52)	0.61 (0.58–0.65)	142
Organic red quinoa	1.65 (1.60–1.70)	2.12 (2.11–2.12)	0.25
Organic white quinoa	1.81 (1.68–1.94)	1.66 (1.63–1.68)	0.25

*estimated values from previous data of similar products

Negative values, as for creamy white cheese and mozzarella, indicate no iron content. Samples with an iron concentration over 2.1 mg/100g, which are considered sources of iron according to EU regulations, were Black quinoa and lentils, Veg. teriyaki strips, Red dried lentils, Organic dried red lentils, Red lentils (pre-boiled), and Organic dried green lentils. Samples with a zinc concentration over 1.5 mg/100g, which are

considered sources of zinc according to EU regulations, were Black quinoa and lentils, Red dried lentils, Organic dried red lentils, and Organic dried green lentils. The top three highest phytic acid contents were found in Veg. teriyaki strips (397.4 mg/100g), followed by Couscous Moroccan (277.5 mg/100g), and Marinated soybeans (207 mg/100g).

4.5 Molar ratios

Figures 4.1 and 4.2 present the phytate-to-iron (Phy:Fe) and phytate-to-zinc (Phy:Zn) molar ratios for ingredients exceeding iron and zinc concentrations of 1.5 mg/100 g and 1 mg/100g, respectively. The raw data used for the figures are found in Appendix B.9.2. Samples considered a source of these minerals, meaning products having an iron concentration higher than 2.1 mg/100g, and a zinc concentration higher than 1.5 mg/100g, are denoted with an asterisk (*).

To evaluate micronutrient bioavailability, critical thresholds are indicated by horizontal lines. In Figure 4.1, a molar ratio of 1 (red line) represents the threshold for optimal iron bioavailability in the absence of enhancers, while a ratio of 6 (yellow line) is used for meals containing enhancers. Similarly, for zinc in Figure 4.2, molar ratios of 5 (red line) and 15 (yellow line) serve as benchmarks for high and low absorption efficiency, respectively.

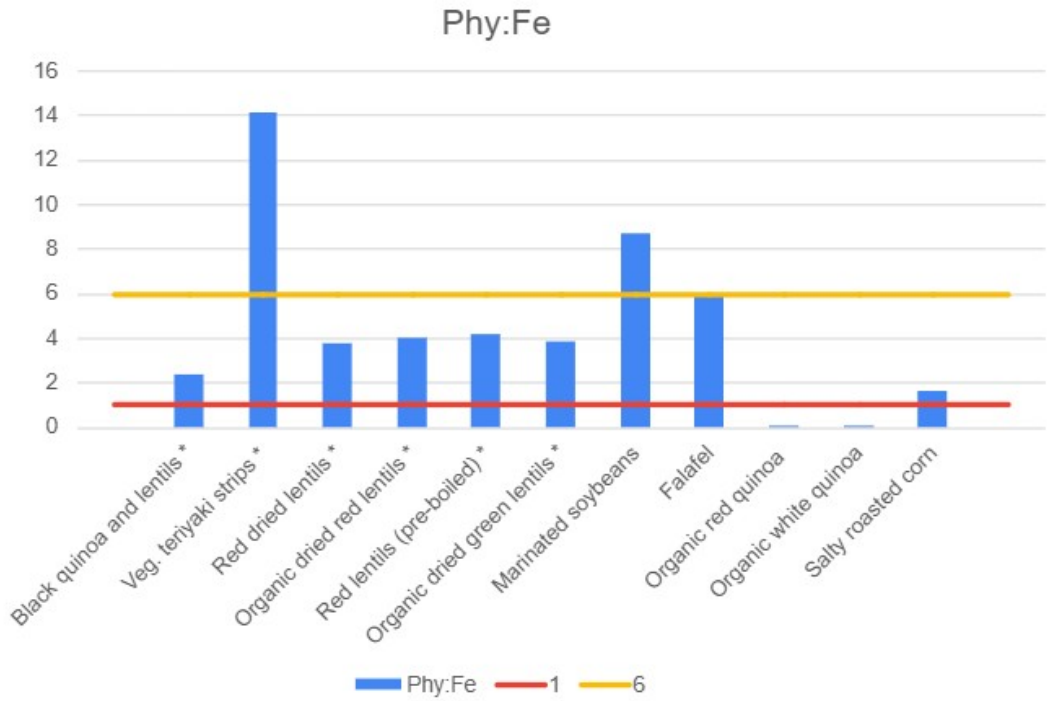

Figure 4.1: Phytate-to-iron molar ratios (Phy:Fe) for ingredients exceeding iron concentrations of 1.5 mg/100 g. Products having an iron concentration above 2.1 mg/100g, thus classified as a source of iron, are denoted with an asterisk (*). Thresholds for optimal iron bioavailability with enhancers (molar ratio 6) and without enhancers (molar ratio 1) are marked with a yellow and red horizontal line, respectively.

It can be seen in Figure 4.1 that all selected products except Organic red quinoa and Organic white quinoa have a higher phytate-to-iron molar ratio than 1. Veg. teriyaki strips and Marinated soybeans also have a higher ratio than 6, and Falafel is right below the upper threshold.

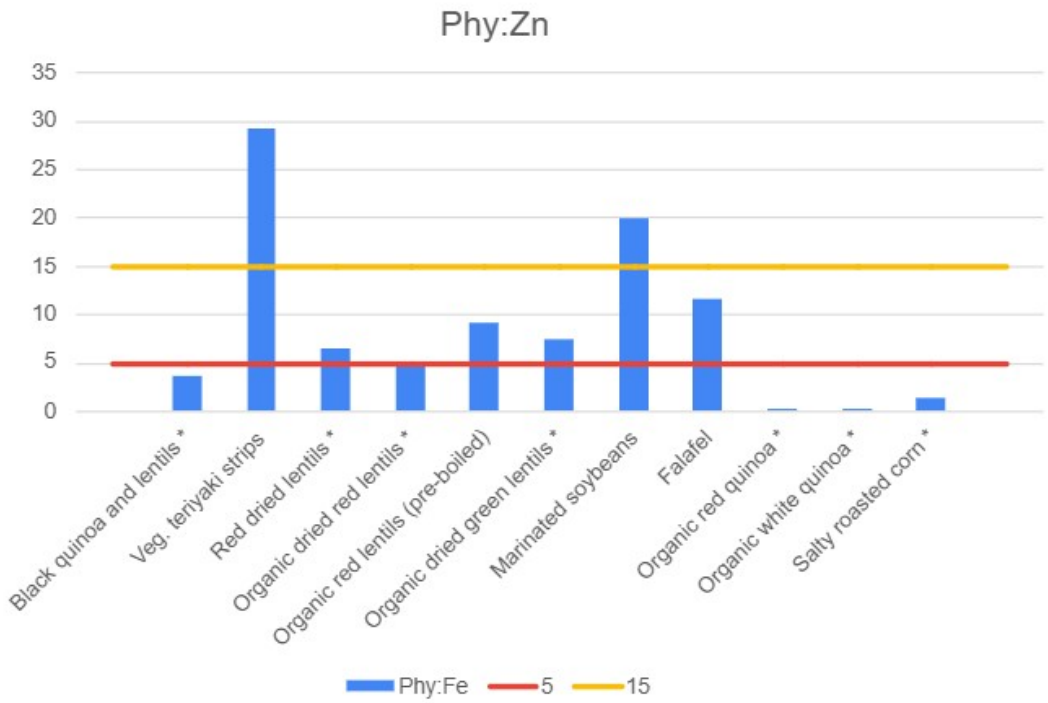

Figure 4.2: Phytate-to-zinc molar ratios (Phy:Zn) for ingredients exceeding zinc concentrations of 1 mg/100 g. Products having a zinc concentration above 1.5 mg/100g, thus classified as a source of zinc, are denoted with an asterisk (*). Thresholds for high zinc bioavailability (molar ratio 5) and low bioavailability (molar ratio 15) are marked with a red and yellow horizontal line, respectively.

In Figure 4.2, all samples except Black quinoa and lentils, Organic red quinoa, Organic white quinoa, and Salty roasted corn are above the threshold of 5 of phytate-to-zinc molar ratio. Veg. teriyaki strips and Marinated soybeans have a higher ratio than 15, and Falafel has the third highest ratio, but is below the higher threshold. Veg. teriyaki strips have both the highest phytate-to-iron and phytate-to-zinc molar ratios.

5

Discussion

This section aims to answer and discuss the research questions outlined in section 1.2. It starts with assessing the nutritional adequacy of the salad compositions in relation to the NNR 2023 requirements, and evaluating if it is possible to reach the nutritional targets for a school meal for a teenage girl with the selected salads. How the choice of protein source influences the mineral bioavailability in the meal is discussed. Realistic substitutions and improvements to the salad composition are suggested, and lastly, possible future improvements are presented.

5.1 Nutritional adequacy of salad compositions in relation to NNR 2023 requirements

Mineral analysis of the ingredients (Table 4.4) revealed that only two products within the Picadeli assortment qualify as sources of iron, and two products as sources of zinc, according to EU regulations. This was reflected in the relatively low total mineral content across the salad formulations, as seen in Table 4.3, and could suggest that the salads may not provide a substantial amount of iron and zinc.

The difference between the total mineral content and the actual estimated absorbable fraction is largest in the iron analysis. When evaluating the total mineral content (Table 4.3), only the plant-based salad exceeds the NNR 2023 iron intake target for a meal for 11-17 year old girls. However, when accounting for estimated iron absorption (Table 4.2), only the meat-based salad composition provides enough absorbable iron to meet the 95th percentile of the NNR 2023 requirement for girls in the age group 15-17, who have the highest iron demands. Specifically, this salad composition provides 80,3% more absorbable iron than the meal target, indicating that it is nutritionally adequate for most teenage girls. Furthermore, the surplus in this meal could potentially compensate for other meals of the day that fail to meet the absorbable iron requirements. It is therefore possible to reach the nutritional targets for a school meal suited for a teenage girl with this salad.

The remaining salad compositions do not meet the 95th percentile requirements of absorbable iron, as seen in Table 4.2. However, the plant-based salad meets the median requirement for absorbed iron per meal for the age group 15-17. This means that this salad covers the requirements for 15-17-year-old girls with median or lower iron needs, but would be insufficient for the upper half of the distribution. Consequently, the plant-based salad cannot be generally recommended as a nutritionally complete school lunch for all teenage girls. The lacto-ovo composition performed the poorest, and does not reach any of the NNR2023 absorbable iron targets as shown in Table 4.2. This means it is not generally suitable as a school meal for teenage

girls in terms of absorbable iron content. For individuals consuming the lacto-ovo or plant-based meals, other meals of the day would need to be significantly higher in iron to prevent deficiency.

In terms of zinc, all compositions met the recommended intake for 11-14-year-old girls, but only the lacto-ovo salad exceeded the target intake for 15-17-year-old girls, as seen in Table 4.3. However, the absorption efficiency of zinc in all salads, shown in Table 4.1, was higher than the standard estimates for mixed or vegetarian diets of 26-34% [10]. This high zinc bioavailability suggests that even the salads with lower total zinc content may provide adequate zinc intake. Therefore, all three salad compositions could potentially be considered suitable school lunch options in terms of zinc adequacy.

5.2 Protein source influence on mineral absorption

The results demonstrate that the choice of protein source influences the predicted iron and zinc bioavailability, as it sets the balance between mineral enhancers and inhibitors, and can be the deciding factor if a meal meets the nutritional requirements or not.

5.2.1 Meat and fish protein sources

As shown in Table 4.1, the meat and fish salad composition exhibited the highest estimated iron bioavailability. Despite containing the lowest total amount of iron, it was the only composition to exceed the 95th percentile requirements for both age groups, with over 80% more than the per-meal target (seen in Table 4.2).

This high iron absorption efficiency can be explained by several synergistic factors. First, the protein sources Diced chicken and Shrimps contain no phytic acid, and Chicken meatballs contain a very low amount, as shown in Table 4.4. Second, the inclusion of animal tissue provides the "meat factor", which is known to enhance the absorption of non-heme iron. Third, it contains the highest levels of ascorbic acid (Table 4.3), which also enhances non-heme iron bioavailability. These factors likely mitigated the inhibitory effects of the phytic acid from the other ingredients. Furthermore, this salad had the lowest calcium levels, which inhibits iron absorption (Table 4.3). These interactions combined likely provided the high iron bioavailability.

Regarding zinc, the meat and fish salad again showed the highest estimated absorption efficiency (Table 4.1). However, its total zinc content was lower than that of the other salad compositions, as presented in Table 4.3, and therefore it did not contain the highest amount of absorbable zinc. This is likely because the meat protein sources contain the lowest amount of zinc, compared to the lacto-ovo and plant-based options, shown in Table 4.4. The combination of the low levels of the inhibitory compounds phytate and calcium (Table 4.3), and the enhancing effect of animal proteins, could be the reason for the high zinc absorption efficiency. Therefore, choosing animal proteins could have a positive effect on zinc bioavailability.

5.2.2 Lacto-ovo protein sources

The lacto-ovo salad composition exhibited the lowest estimated iron bioavailability, as seen in Table 4.1, failing to meet any of the per-meal nutritional requirements for girls in either age group (Table 4.2). The absorption efficiency was similar to the plant-based composition, but since the lacto-ovo salad had lower total iron content, the amount of absorbable iron was lower. This is unexpected because the lacto-ovo protein sources do not contain any phytic acid, as seen in Table 4.4, meaning this salad composition contained the least amount of phytic acid (Table 4.3). Also, the total iron content was near the school meal iron target for the 11-14 age group, which suggests that it has the potential to have a high amount of absorbable iron.

The reason that it has such low estimated iron bioavailability is likely driven by the high calcium content in the cheese (Table 4.3), which is a known inhibitor of both heme and non-heme iron. Additionally, egg proteins also reduce iron absorption. Both of these factors in the same meal suggest a combination of the inhibitory effects, which outweigh the benefits of the low phytic acid levels. Therefore, choosing only lacto-ovo proteins may negatively affect the iron bioavailability of the meal unless cheese and egg portions are reduced or replaced. Even though the lacto-ovo salad contained less phytic acid than the meat-based salad, its iron absorption efficiency was six times less. This highlights the importance of enhancer and inhibitor interactions for iron uptake.

The lacto-ovo salad composition contained the most estimated absorbable zinc, but not the highest absorption efficiency, as seen in Table 4.1. A reason for the high absorbable zinc content could be that the lacto-ovo proteins all contain high amounts of zinc, with Mozzarella having the highest content out of all protein sources (Table 4.4). It also contains the least amount of phytic acid, as seen in Table 4.3, which has a great effect on zinc absorption. Furthermore, it contains animal proteins, which enhance zinc bioavailability. Although the high inhibitory calcium levels (Table 4.3) likely prevented this salad from reaching the highest efficiency, the amount of zinc may compensate for it. A salad containing lacto-ovo protein sources could therefore have positive effects on zinc absorption.

5.2.3 Plant-based protein sources

The plant-based salad contained the highest total estimated iron content yet provided the lowest nutritional value in terms of iron absorption. A low bioavailability is confirmed in Figure 4.1, where the plant-based proteins show the highest molar ratios, suggesting the lowest iron absorption efficiency of the tested products.

Looking at the absorbable iron in Table 4.2, the plant-based composition does not reach the iron target for a meal suited for most teenage girls, despite it containing the most total iron and exceeding that target value. The reduction could be explained by the strong chelating effects of the high amounts of phytic acid present in the plant-based proteins, as seen in Table 4.4. Also, the presence of soy proteins in Veg. teriyaki strips and Marinated soybeans, which are also known to inhibit iron

absorption, could be a reason for this. This shows that the plant-based protein sources contain high levels of iron, but very little of it is available for absorption. Therefore, choosing mainly these plant-based proteins in salad meals could negatively influence iron absorption.

The plant-based salad composition contained the lowest estimated zinc absorption efficiency and absorbable content (Table 4.1). Without animal protein enhancers and with inhibitory calcium and the high phytate-to-zinc molar ratios of the plant-based protein sources (Figure 4.2), the bioavailability was likely restricted. However, the absorption efficiency is higher than the standard estimates for mixed or vegetarian diets, suggesting that it contains enough zinc, and too little phytic acid and calcium to reduce the absorption too low.

5.3 Improvement opportunities through ingredient substitutions

The results indicate that realistic ingredient substitutions within a salad bar context can influence mineral bioavailability. The extent of the improvement depends on whether the ingredients' inhibitory properties can be mitigated by enhancers.

A critical finding is the big difference in phytic acid among the plant-based options. Veg. teriyaki strips contain almost double the phytic acid of Marinated soybeans and triple as much as Falafel, as seen in Table 4.4. As illustrated in Figures 4.1 and 4.2, Falafel is the only plant-based protein below the higher molar ratio thresholds for improvable bioavailability. This suggests that Falafel's mineral content is high enough, compared to the inhibitory load, which is low enough that the mineral absorption efficiency is not compromised beyond a point of recovery. Furthermore, Falafel does not contain soy protein, avoiding additional mineral inhibition. This makes Falafel a potentially good option for improved mineral bioavailability in plant-based salads. It could also be used in combination with enhancers, such as meat or ascorbic acid, to possibly get a salad with adequate bioavailability.

In contrast, Veg. teriyaki strips have the highest molar ratios (Figure 4.1 & 4.2) and phytic acid concentrations (Table 4.4) of all tested products. This high concentration is likely a result of the protein isolation process used in the manufacturing of meat analogs, which concentrates phytic acid along with the protein. The high amounts of soy protein can also inhibit the non-heme iron absorption, further reducing the bioavailability. Since the molar ratios are so far above the thresholds for optimal absorption, the effect of enhancers would only be marginal, meaning the bioavailability of meals containing these could not be improved through the use of dietary enhancers alone. Consequently, Veg. teriyaki strips appear unsuitable as the main protein source in school meals to meet the nutritional targets for teenage girls. To reach the requirements, it would need to be limited or substituted with products having higher mineral bioavailability. Marinated soybeans also have a molar ratio above the thresholds for improvable mineral bioavailability since it contains high phytic acid. It also contains soy proteins that inhibit mineral bioavailability. Therefore, the same

actions could be taken for salads containing Marinated soybeans as for Veg. teriyaki strips to increase the mineral bioavailability.

To implement plant-based products without compromising mineral absorption, alternative soy products such as tempeh could be used. Tempeh is a plant-based product that has been suggested to have potentially better bioavailability since the fermentation in the tempeh production activates phytases that degrade the phytic acid. Substituting meat analogs, like Veg. teriyaki strips, who contains concentrated phytic acid, with tempeh could potentially increase the absorbable iron in plant-based salad meals to levels that align with the NNR 2023 requirements. Additionally, other processing could be used to limit the phytic acid content in plant-based protein sources, such as the addition of exogenous phytases, which could degrade the phytic acid content to increase mineral bioavailability.

5.4 Optimizing Black quinoa and lentils mix

The Black quinoa and lentils mix can be classified as a source of both iron and zinc according to EU regulations. Therefore, optimizing the type of lentils and quinoa, and their respective compositional ratios, presents an opportunity to enhance the bioavailable mineral fraction within the salad meals.

While the green lentils contain slightly higher phytic acid concentrations than the red lentils, the Organic dried green lentils analyzed in this study also have the highest total iron concentration (Table 4.4). This resulted in a phytate-to-iron molar ratio similar to that of the other lentils, as seen in Figure 4.1, neutralizing the inhibitory disadvantage. For zinc, however, the distinction is clearer. The green lentils has a slightly higher phytate-to-zinc molar ratio than the other dried lentils, illustrated in Figure 4.2, where Organic red dried lentils are below the lower threshold, representing high bioavailability. Thus, from a zinc-optimization perspective, red lentils appear superior.

Interestingly, the results also highlight the difference between the levels of processing. From Table 4.4, it can be seen that Green lentils (pre-boiled) fail to meet the criteria for being a source of iron and zinc, and Red lentils (pre-boiled) are not considered a source of zinc, whereas their dried counterparts are. This could mean that it is preferable to use dried lentils, subsequently boiled in-house, than to use packaged pre-boiled alternatives for preserving mineral content.

The tested quinoa varieties showed promising results regarding bioavailability. Although they narrowly missed the source of iron classification, their low phytic acid content resulted in very low molar ratios (Figures 4.1 & 4.2). This suggests that the minerals present in quinoa are highly bioavailable. Therefore, increasing the ratio of quinoa relative to lentils in the mix could increase the mineral bioavailability of the mix, subsequently increasing mineral absorption of whole salad meals.

However, it should be emphasized that nutrient and antinutrient content are subject to substantial variation between cultivars. The findings of this study provide a strong

indication of potential, but further analysis of multiple batches and cultivars of each product would be required to draw any definitive conclusions.

5.5 Future improvements

While the findings of this study provide a strong indication of mineral bioavailability in salad compositions, the limitations in the approach leave room for future research. A primary consideration is that the phytic acid concentrations used for the salad components are estimated based on values for similar products. To increase the strength of the conclusions and account for variations between products, it would be beneficial to test the actual phytic acid content in each specific product.

Furthermore, future studies could evaluate the nutritional impact of removing high-phytate components, such as Veg teriyaki strips, from the plant-based salad and replacing them with fermented products, such as tempeh. Measuring the mineral absorption of these compositions would explain to what extent this type of substitution can improve the bioavailability.

Finally, creating "hybrid" salads, which both contain plant-based and meat-based protein sources, presents a promising area for optimization. Comparing these with 100% plant-based or lacto-ovo salads and seeing how much the bioavailability improves would provide information on the minimum threshold of animal protein required to significantly enhance non-heme iron absorption through the "meat factor". This could be used to design nutritionally adequate meals.

6

Conclusion

Regarding absorbable iron, the meat and fish salad composition was the only meal to meet the 95th percentile of the NNR 2023 requirement for girls aged 11-17. This indicates that this composition reaches the nutritional targets for a school lunch and is suitable for most individuals in the target group. In contrast, the plant-based salad only met the median requirement for the 15-17 age group, meaning it is only adequate for the half of the population with average or lower iron needs. The lacto-ovo salad failed to meet any NNR 2023 absorbable iron targets. Consequently, while it may be possible to reach absorbable iron targets with the meat-based selection, plant-based and lacto-ovo salads might not currently be recommended as general school lunches for this demographic without further optimization. For zinc, the meat and fish composition has the highest estimated absorption efficiency, but the Lacto-ovo salad has a higher absorbable zinc content. The plant-based composition has the lowest zinc absorption. Due to the high zinc absorption efficiency across all meals, all three salads could be considered nutritionally suitable school lunch options in terms of zinc.

The choice of protein source likely influences predicted mineral absorption due to the varying presence of enhancers and inhibitors. Meat enhances both iron and zinc bioavailability, possibly through the "meat factor". Conversely, plant-based protein sources inhibited absorption, potentially due to high phytic acid concentrations. In the lacto-ovo composition, high calcium levels likely acted as an inhibitor for both minerals, though the presence of animal proteins in the cheese and eggs probably provided some enhancement of zinc absorption. Additionally, the results indicate that egg and soy proteins influence the mineral bioavailability negatively. The results suggest that the mineral bioavailability can be improved by moderating the intake of phytic acid, calcium, soy, and egg proteins, while increasing the presence of enhancers such as ascorbic acid or animal proteins.

Iron and zinc absorption could be improved by 376% and 47.7%, respectively, through realistic ingredient substitutions within a salad bar context. Substituting high-phytate soy-based meat analogs with fermented alternatives like tempeh offers higher bioavailability potential, as the fermentation activates phytases in the fermentation that degrade phytic acid. Among the plant-based options analyzed, Falafel showed the highest potential for improving bioavailability due to its lower inhibitory profile. Furthermore, optimization of the mineral-rich Black quinoa and lentils mix could increase the mineral content of whole meals. This can be achieved by increasing the ratio of quinoa and utilizing dried lentils rather than pre-boiled varieties. However, further analysis of different cultivars is required to determine the optimal lentil varieties.

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A

Appendix 1: Standard operating procedures

The standard operating procedures for the laboratory methods used to determine mineral and phytic acid concentrations in the samples. Procedures for phytic acid analysis by HPLC, microwave digestion, and atomic absorption spectroscopy are found in this chapter.

A.1 Standard operating procedure for Phytic acid analysis by HPLC

Phytic Acid analysis by HPLC (Rikard Fristedt 2019-01-29)

The software set the method to 0 in flow! Change back to: Upper derivatization-reagent mixing pump: 145 bar (0.4mL flow) Lower running pumps: 145 bar (0.8 mL flow)

1. Aim and principle: Analysis of Phytic acid (inositol hexakisphosphate (IP6), inositol polyphosphate, or phytate).

Sensitive method for determination of IP6 in various samples by HPLC coupled with UV-vis detection. Myo-inositols can be separated, using either a gradient or isocratic elution. In the case for the rapid IP6 analysis the isocratic elution is used, and the detection is done using ultra violet detection after post column reaction. The eluents are mixed with 0.1

2. Reagents: Deionized water purified by a Millipore (Bedford, MA) water system 18 HCl from Scharlau (Warning hazardous) Perchloric acid (HClO4) (Warning hazardous, extremely corrosive!) Methanol IronNitrate (Fe(NO3)3*9H2O)

3. Solutions: 1M HCl. Add 165 ml HCl into 1 liter Milli-Q in a 2liter volumetric flask, use the glass pipettes of 50ml and 15ml and adjust the volume with Milli-Q up to 2liter. Iron nitrate (the reagent for derivatization) for the transparent glass bottle placed on top of the LC together with the eluents. Add 1.8 g Iron-nitrate, found behind the scale in analytical lab, into 0.5 liter Milli-Q in a 1l volumetric flask. Then carefully add 30ml perchloric acid (HClO4), mix and finally adjust the volume up to 1liter. Methanol/Milli-Q in a 50:50 solution, this is used for the needle washing solution. Prepare by mixing equal volumes of methanol and Milli-Q. There is gas formation in the liquid, so swirl for a while before putting it back to avoid getting bubbles in the wash tubings. Fresh Milli-Q for one of the bottle on top of the LC.

Change for every new run. Also make sure to empty the big waste bottle (on the right in a big brown glass bottle) as well as the auto sampler waste (in a small plastic bottle attached to the side of the cupboard underneath the auto sampler).

4. Standard Solutions: QC Samples: Dinkelmjöl Fullkorn 190204. Always use these QC samples, the concentration is x umol/gram. Carefully document this number and enter into the QC samples list on the common folder. Standards range from 0.1mM to 0.8mM (0.1umol/mL to 0.8umol/mL) and are prepared from a carefully determined stock solution in water. Standards in this specific article (Carlsson, et al. 2001) were prepared from phytic acid dodecasodium salt hydrate from Aldrich chemicals Co Inc., which has shown lower degree of contamination from lower inositol phosphates (0.65. Experimental procedure:

BE careful to always spin/filter your sample before LC analysis. After extraction the samples contains a lot of particulate that can clog the system!

Swedish Annette Almgren 190101 - Jag extraherar ca 0.5 gram frystorkat (malt) prov (eller mjöl) i 10ml 0.5M HCl i 3timmar på magnetomröraren. Därefter för jag över 1ml till ett eppendorf och centrifugerar några minuter med 12000*g. Supernatanten ska vara klar. Klart att föra över till vial för HPLC. Buffert pH 5 har jag använt då jag inkuberat ca 1g tex mjöl med 10ml för att se om det finns fytas aktivitet kvar. Detta gör jag i 50 gradigt vatten bad i en E-kolv. Efter exempelvis 1timma sätter jag 30ml 0,5M HCl till detta och extraherar 3timmer. Centrifugering som ovan. English Sample extraction is done my weighing 0.5 g dry matter sample and mix (by magnetic stirring) with 10 - 20 ml of 0.5M HCl for 3h at R.T. Take 1 ml to eppendrof tube and spin for 5 minutes 12 000*g. The supernatant should be clear. Transfer to vial for HPLC analysis. From this extraction, different types of sample preparation can be done according to the needs, for example ultracentrifugation using a filtration unit with a certain weight limits to remove compounds below a certain size, or general centrifugation to remove insoluble solids before the analysis. Samples should not contain lots of insoluble solids since this may clog the system! For samples where extraction is not needed, such as cell-free culture supernatants, the acid extraction step is excluded and only a centrifugation or filtration is done to remove insoluble solids. The acid treatment may at some occasions modify the inositol phosphates by rearranging the phosphate groups.

For rapid analysis of IP6, an isocratic eluent at a flow rate of 0.8 ml/min, with 80% of 1M HCl and 20% Milli-Q H2O is used. The injection volume is 50μl, and the analysis time is only 7 minutes, but then also results in only analysis of IP6. Although the lower myo-inositols can be seen in the chromatogram, we only have standards for IP6, why also only this compound can be quantified. The eluents are mixed with 0.1% Fe(NO3)3*9H2O in a 2% HClO4 solution in a post column reactor. The combined flow rate is 1.2 ml/min. A mixing tee and a homemade reaction coil made of a crocheted Teflon tube (i.d 0.2mm, 4.5m) optimized with respect to reaction time and to avoid peak broadening, is applied to get enough reaction time and blending rate.

For sample treatment plastic materials were used if possible. The glassware, Teflon bombs, and Microcon YM-30 centrifugal filter (Millipore, Bedford, MA) is always acid-washed prior to use, due to observation of contamination. The utensils should be stored in closed containers to avoid airborne contamination.

6. Apparatus: Bio-compatible LC-system from Jasco. Password computer: 123456 Password ChromNav: Administrator Choose the project "phytic acid". Dionex™ CarboPac™ PA100 IC Columns. Column: DIONEX, CarboPac PA-100, 4*250 mm, 043055. Guard column: 043054.

Normal pressures: Upper derivatization-reagent mixing pump: 145 bar (0.4mL flow) Lower running pumps: 145 bar (0.8 mL flow)

7. Calculation: Calculations of peak area, elution times, regression equation and standard curves can be done with the software ChromNav on the chromatograph computer.

References: Carlsson, N. G., et al. 2001 Rapid analysis of inositol phosphates. Journal of Agricultural and Food Chemistry 49(4):1695-1701. Imanari, Toshio, et al. 1982 High-performance liquid chromatography of inorganic anions using Fe3+ as a detection reagent. Journal of Chromatography A 250(0):55-61. Phillippy, Brian Q., and John M. Bland 1988 Gradient ion chromatography of inositol phosphates. Analytical Biochemistry 175(1):162-166. Skoglund, E., N. G. Carlsson, and A. S. Sandberg 1997a Analysis of Inositol Mono- and Diphosphate Isomers Using High-Performance Ion Chromatography and Pulsed Amperometric Detection. Journal of Agricultural and Food Chemistry 45(12):4668-4673. 1997b Determination of Isomers of Inositol Mono- To Hexaphosphates in Selected Foods and Intestinal Contents Using High-Performance Ion Chromatography. Journal of Agricultural and Food Chemistry 45(2):431-436.

A.2 Standard operating procedure for microwave digestion

Microwave Digestion 1. Aim and principle: Samples are mixed with acid and then microwave digested in order to fully solubilize all minerals.

2. Reagents: Deionized water was purified by a Millipore (Bedford, MA) water system 18.2 M $\Omega \cdot$ cm or greater.

HNO3 (trace metal grade, use plastic tubes for aliquotes) HCl (trace metal grade, use plastic tubes for aliquotes)

3. Apparatus: Milestone microwave laboratory system Ethos Plus and a laboratory terminal 800 controller (Sorisole, Italy) equipped with MPR-300/12S (medium-pressure segmented rotor).

4. Experimental procedure:

- Use plastic materials to avoid possible contamination of e.g. zinc from glass ware (ref Annette). - Make sure the fume hood is clean before you start. Change lab mat if neccessary. - Make sure the teflon tubes are clean (no visual dirt or yellowness is seen). Otherwise make a microwave digestion with only acid and water to clean them. - Use fresh Milli-Q water (in plastic beaker) and clean pipette tips. - Use higher sample weight if you suspect low mineral concentrations

1. Weigh out 0.15-0.5 g of material (note exact weight) into Teflon microwave digestion vessels. 2. Add 7 mL of Milli-Q H2O, 1.75 mL of concentrated HNO3, and 0.35 mL of concentrated HCl into each Teflon vial with sample in it. 3. To vial in position 1 in the microwave digester you should always have sample. This is to get correct temperature control in case of exothermic reactions. 4. Each vial must be sealed and loaded properly into the microwave digester: a) Slip the Teflon tube into a protective tube jacket b) Put the pressure cap on the top of the tube c) Put the pressure cap seal (2 parts) on top of the pressure cap (make sure the small plastic part is not too flat, see "additional information" below) d) Put an extra plastic sleep over and around the pressure cap and protective tube jacket e) Load the sample into the microwave sample frame f) Tighten the screw on the microwave sample frame using the wrench in the fume hood until the wrench clicks. g) Load the microwave sample back into the microwave frame. h) Begin the process again with next sample, until complete. 5. Insert the microwave frame in the microwave digester (don't forget the thermometer) and turn on the equipment. Make sure the carusell can rotate. 6. Use the user profile to run the microwave digester, with the passcode 123. 7. Use an appropriate program, for example: run the samples at 180C for 20minutes of digestion (e.g. run "180 20 min", this includes 20 min ramp time). Ventilation will continue for 60 min after the program is finished to cool down the samples. 8. Wait until the temperature has decreased to 60C before you open the vessels. If the temperature is below 60C before the 60 min of ventilation finished, you can press stop and remove your samples. 9. Pour the liquid into a plastic tube. Make sure the digestion was successful, i.e. no particles are seen and the solution is clear and yellow. (If the tubes were not completely tight during digestion the liquid is almost transparent, which is usually fine.) Color will also disappear over time. 10. Rinse the teflon tube with Milli-Q-H2O and pool with the original sample, giving a total volume of 12 mL. 11. After run: clean all teflon parts according to point 5. The other parts should be cleaned if they were in contact with any spillage (use hot water and detergent).

5. Cleaning instructions after use: a) Clean the teflon parts properly with a soft brush after use using hot water and detergent. Rinse in Milli-Q-water. b) Put all teflon parts in acid bath (0.25 M HCl) over night (or shorter). c) Put all teflon parts to a Milli-Q-bath over night. d) Rinse in Milli-Q-water and let all parts dry on a clean place. e) Put all teflon parts in the plastic box with cover for clean parts (in the drawer under the fume hood). f) All teflon vessels should be microwave digestied (with only acid and water) now and then to make sure they are properly cleaned. If they still look dirty/strange, buy new.

6. Additional information: a) Stronger acid concentrations is OK to use if atomic

absorption is to be used for subsequent mineral analysis. b) The small plastic cover used on top can become too thin to ensure proper closure. This can be tested by a special tool with a slot found in the "hidden" box on top of the microwave digester. c) Admin log in: 123456 d) Hydrogen peroxide (30%) can be added to facilitate digestion. e) Read Milestone Application Book (in SOP folder) for recommended sample amounts and acid proportions for different sample types.

A.3 Standard operating procedure for Atomic Absorbance Flame Spectroscopy

Atomic Absorbance Flame Spectroscopy

1. Aim and principle: Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions.

2. Reagents: Depending on the workup you do for the samples. Microwave digestion or acid hydrolysis can be used. Acetylene gas, very flammable!

3. Solutions: Depending on the workup you do for the samples. Microwave digestion or acid hydrolysis can be used to solubilize the sample. Only use chemicals with high purity to avoid contaminations (trace element grade).

4. Standard Solutions: A separate standard curve is prepared for each mineral you will analyse (Mn, Cu, Zn, Pb, Fe, Pd, Mg etc). Standards should be of ion chromatography grade or ASS grade. Standards are usually supplied at a concentration of 1000 ppm (1000 mg/L). Dilute standards (in plastic tubes) by weight with Milli-Q (it might be optimal to use Milli-Q mixed with a small amount of nitric acid so the dilution is not changing the pH). Suggested concentrations (in mg/L) for making the standard curve is given by the Agilent AAS program.

5. Reference samples: Reference diets with certified mineral content is found in the -20 freezer in the analytical lab. Certificate is in a file in the research engineers' office.

6. Pretreatment of samples

Acid hydrolysis of protein using concentrated HCl: 1. Add 4 mL Milli-Q-H20 and 4mL pure HCl to each tube. (This equates to 6M HCl solution. (Pure HCl should be processed in the fume hood using gloves and safety goggles). 2. Tubes should be well sealed, shaken to disperse the sample within the solution, and incubated at 110°C for 24hours. 3. Carefully move the 8 mL of sample from a glass tube to a plastic 15mL tube. Rinse the glass tube with 2 mL of Milli-Q-H2O to bring the total sample volume up to 10 mL.

Microwave digestion:

1. Follow ”SOP Microwave Digestion”. To fit your sample into the range of the standard curve you may have to dilute your samples or concentrate them (flush with nitrogen with appropriate filter to avoid contamination) before AAS analysis. Example from insects: Manganese - Termites 1:50d or 1:100d, other insects No dilution used. Zinc - 1:3d used for all insects Iron - No dilution through to 1:3d copper - No dilution, through to 1:3d Magnesium - 1:20d through to 1:100d depending on concentrations

7. Experimental procedure: OBS: Remember to turn the lamp on 30-60 min before you start using the instrument. The lamp needs this time to warm up and give reliable measurments.

Always optimize the lamp used and remember to check so the right lamp is being selected. Adjust the two screws at the mount of the lamp until you get a value as close to 1.0 as possible.

1) Press ON to start the AAS 2) Press Lamp ON 3) Turn on the ventilation (above the instrument) 4) Open the acetylene gas tube in the gas niche in the corridor and open the regulator on the wall in the lab. Check that the acetylene pressure is above 7 bar, otherwise ask the research engineers to order a new tube. 5) Turn on the lamp 6) Fill up Milli-Q-water in the container. Adjust the flow with the screw (use 5-6 mL/min) 7) Turn on the computer. 8) Open the program Spectra AA on the computer 9) Press Worksheet New Add methods Tick “CookBook” and choose which element to analyse, e.g. Mn. 10) Press Edit Methods tick Manual under Sampling Mode. Check/change Lamp Position under Optical (e.g. Mn in position 1 and Zn in position 2). Under Standards you will find suggested standard concentrations and you can change these. Under Calibration you can set how often the standard curve should be recalibrated, resloped etc. 11) Under Analysis press Optimize and choose the element. Press Optimize Lamps and OK (should be as close to 1.0 as possible) 12) Click Optimize signal to make an instrument zero while still aspirating distilled water (Press Inst zero). 13) Burner position can be optimaized by aspirating e.g. a 5 ppm Cu solution. Turn black screws vertically and horizontally and adjust flame conditions (metal slides) to give the highest signal. Glass bead position can be adjused (with beige screw) to maximize signal and minimize noise. End optimization window by pressing OK.

The standards (including Instrument Zero = solvent) will automatically be run first so no need to put in acquisition table. Write in the samples you want to analyse or keep track of the order yourself. Each injection will use around 500 µL.

Make a standard curve and run your samples: 1) Turn on the flame, hold the button a few seconds. 2) Press Start and dip the tubing in the order suggested by the instrument (zero, std 1, std 2. . .). Continue with your samples. Dilute if necessary. 3) Export the result to Excel or note it in your lab book. Results are given as mg/mL in the tube. Dilutions made during digestion has to be included in the postprocessing step.

Closing procedure: 1. At the end of the day run aspirate with blank solution (distilled

water) for 5 min to clean out residues from the nebulizer. 2. Turn off flame and wait some minutes until burner has cooled down. Remove the burner and rinse with 500 mL of blank solution through the top of the spray chamber. 3. Empty liquid trap (waste bottle). 4. Turn off the gas (both on the wall in the lab and in the gas niche).

Note! Sodium can be determined using emission! Read more in the manuals.

8. Apparatus:

Agilent 240/280 Series AA Spectrometers Important: Remember to check the liquid level in the Liquid trap (see below). Improper use of the liquid trap can create explosion hazards, fire hazards, and toxic vapor hazards that can result in death or serious personal injury. The liquid trap interlock is incorporated to minimize the possibility of attempting to operate the instrument with an empty trap or with the drain tube missing. Never interfere with this interlock. Never attempt to bypass this interlock. Always fill the liquid trap with the same solvent that is being used for your samples. The trap is designed to provide a liquid seal under all normal conditions with solutions having a specific gravity greater than 0.75. Never use a solution or solvent having a specific gravity lower than 0.75, otherwise the liquid seal can be breached. This can create a flashback and create an explosion hazard or a fire hazard.

When you're analytical program has been completed, or at the end of the working day, always empty, clean, and refill the liquid trap.

9. Calculation: Done in the setup. Automatic by the software after the standards have been run.

References: https://www.agilent.com/cs/library/usermanuals/public/1547.pdf

10. Changing to and using the Graphite oven (added by Isabel 2025-03-31): Start by making sure that the AA is and gas is off before changing to the graphite oven! It is alright to have the lamp switched on, but everything else should be switched off.

1. Start by taking off the chimney. 2. Take off the lid 3. Remove the water flask 4. It will then look like this, then remove the metal part by lifting it up:

5. Then unscrew the black buttons that are located on the right side of the white part until they are loose, and pull and wiggle the white part loose. Then place the part upright since it contains water and has two holes on each side.

6. These are the parts that have been removed:

7. Now attach the graphite oven part. You can find it in the compartment which is shown to the left. Tighten the screw on the right side bottom. Add some things.

8. Place the ventilator so that it is located directly above the center of the graphite oven part, as seen in the left picture. Make sure that the ventilation tubing is connected as in the right picture. Add some things.

9. Now attach the auto sampler by hooking it onto the screws. Add some things.

10. Attach the auto samplers' cables to the graphite oven.

11. The setup should then look like this. Add the beaker so that the waste water can be collected. Make sure that the bottle with MQ water is full.

12. Turn on the water cooling and make sure that there is no need for refilling the water (or glycol). Which type of water and glycol?

13. Turn on the argon gas flow. 14. Now you can turn on the power switch to the graphite oven and the AA.

Calibration of the auto sampler.

15. Open Will be added 16. If the needle does not go into the beaker, try to bend the tip of the tube a bit. 17. If the tip does not go into the hole, move the screws while lifting the head up as to not damage it. Try to put it back down and see if it goes into the hole. If not, continue until it does. THEN REPEAT this procedure until it the entire procedure goes smoothly.

B

Appendix 2: Data, results and example calculations

The raw data from the analyses and calculated partial results used to produce the final results presented in Chapter 4 are found in this section. There are also example calculations explaining the calculation process of the results.

B.1 Calculation of the nutritional requirements per meal for teenage girls

Targets for energy, iron, and zinc intake per meal were established by applying a 30% factor, representing a standard school lunch contribution (SLV), to the daily requirements defined in the NNR 2023. This included the total mineral concentration recommended intakes (RI) per meal, and targets for absorbed iron per meal for the two target age groups of girls (11-14 and 15-17 years).

B.1.1 Energy requirement for a single meal

The daily energy intakes for the age groups are 9.2 MJ, and 10.1 MJ, respectively. The two age groups were merged to get the average result for the age span 11-17, and a 30% factor was applied to get the per-meal energy target. The conversion factor 1 MJ $\approx$ 239 kcal was used to represent the results in kcal.

1. Average Daily Energy Requirement

Based on the values for the two age ranges ($E_1 = 9.2$ MJ and $E_2 = 10.1$ MJ):

$$E_{avg} = \frac{E_1 + E_2}{2} = \frac{9.2 + 10.1}{2} = 9.65\,\text{MJ} \tag{B.1}$$

2. Energy Requirement for Each Salad (30%)

The requirement per meal (E_{meal}) was estimated as 30% of the average daily requirement:

$$E_{meal} = E_{avg} \cdot 0.30 = 9.65 \cdot 0.30 = 2.895\,\text{MJ} \tag{B.2}$$

3. Conversion to Kilocalories

To convert the energy from Megajoules (MJ) to kilocalories (kcal), the conversion factor 1 MJ $\approx$ 239 kcal was used:

$$E_{kcal} = 2.895 \cdot 239 \approx 692\,\text{kcal} \tag{B.3}$$

Result: The estimated target energy content for each salad was determined to be approximately 2.90 MJ (692 kcal).

B.1.2 Calculation of Recommended Mineral Intake for a School Lunch

To determine the target nutritional values for the salads, the required absorbed intake of iron and zinc was calculated for a teenage girl, based on the requirements for age groups 11-14 and 15-17 years. A school lunch was estimated to provide 30% of the total daily requirement.

1. Calculation for absorbable Iron (Fe)

The absorbable iron targets are calculated for different percentiles. The 95th percentile represents most teenage girls, including the ones with highest physiological needs. The median value represents the half with average to low absorbable iron demands. The median targets for absorbable iron for the lower and higher age groups is 1.47 mg/day and 1.32 mg/day, respectively. The 95th percentile for the age groups is 1.90 mg/day and 2.19 mg/day.

- **Median absorbed iron target for a school lunch for 11-14 year old girls**

$$Fe50_{11-14years} = 1.47 \cdot 0.30 = 0.44\text{mg} \tag{B.4}$$

- **95th percentile absorbed iron target for a school lunch for 11-14 year old girls**

$$Fe95_{11-14years} = 1.90 \cdot 0.30 = 0.57\text{mg} \tag{B.5}$$

- **Median absorbed iron target for a school lunch for 15-17 year old girls**

$$Fe50_{15-17years} = 1.32 \cdot 0.30 = 0.40\text{mg} \tag{B.6}$$

- **95th percentile absorbed iron target for a school lunch for 15-17 year old girls**

$$Fe95_{15-17years} = 2.19 \cdot 0.30 = 0.66\text{mg} \tag{B.7}$$

2. Recommended Intake (RI) Calculation of Total Iron (Fe) per meal

The RDI for iron for the two age groups is 13 mg/day and 15 mg/day, respectively.

- **Total iron target for a school lunch for 11-14 year old girls**

$$Fetot_{11-14years} = 13 \cdot 0.30 = 3.9\text{mg} \tag{B.8}$$

- **Total iron target for a school lunch for 15-17 year old girls**

$$Fetot_{15-17years} = 15 \cdot 0.30 = 4.5\text{mg} \tag{B.9}$$

3. Recommended intake (RI) Calculation of Total Zinc (Zn) per meal

The RDI for zinc for the two age groups is 10.8 mg/day and 12.2 mg/day, respectively.

- **Zinc target for a school lunch for 11-14 year old girls**

$$Zn_{11-14years} = 10.8 \cdot 0.30 = 3.24\text{mg} \tag{B.10}$$

- **Zinc target for a school lunch for 15-17 year old girls**

$$Zn_{15-17years} = 12.2 \cdot 0.30 = 3.66\text{mg} \tag{B.11}$$

Summary: Based on these calculations, the 95th percentile target of absorbed iron per salad was set to 0.57 mg for 11-14 year old girls and 0.66 mg for 15-17 year old girls. The median absorbed iron target per salad was set to 0.44 mg for 11-14 year old girls and 0.40 mg for 15-17 year old girls. The total recommended iron intake per meal was 3.9 mg for 11-14 year olds, and 4.5 mg for 15-17 year olds. The zinc requirement per meal was set to 3.24 for 11-14 year old girls, and 3.66 for 15-17 year old girls.

B.2 Portion sizes

The common base of ingredients and the amounts (g) of each in all three salad compositions to define one portion are found in Table B.1. The compositions contained different protein sources, and the amount (g) of each protein ingredient in one portion of each of the three salad compositions is found in Table B.2. The amounts were regulated to match the energy requirement for a teenage girl's school lunch.

Table B.1: Ingredient weights for the common base used in all three salad compositions. These ingredients were combined with protein sources of one category, and the amounts of each ingredient were regulated to match the energy requirements for a teenage girl's school lunch.

Ingredient	**Weight (g)**
Lettuce mix	60
Green leaf mix	25
Diced cucumber	50
Cherry tomato	50
Avocado	35
Greek-ish salad	30
Couscous Moroccan style	25
Black quinoa and lentils	25
Pasta chili	25
Pasta bruschetta	25
Pasta pesto	25
Red bell peppers	25
Green bell peppers	25
Yellow bell peppers	25
Mango	35
Salty roasted corn	10
Sriracha mayo	15

Table B.2: Unique ingredient weights for the Meat and Fish, Lacto-ovo, and Plant-based salad compositions. Each composition contained three unique protein sources. These ingredients were combined with the common base, and the amounts of each ingredient were regulated to match the energy requirements for a teenage girl's school lunch.

Ingredient	**Meat and Fish (g)**	**Lacto-ovo (g)**	**Plant-based (g)**
Diced chicken	70		
Chicken meatballs	55		
Shrimps	50		
Boiled egg		80	
Creamy white cheese		30	
Mozzarella		20	
Falafel			47
Marinated soybeans			40
Veg. teriyaki strips			35

B.3 Calcium and ascorbic acid concentrations

Calcium and Ascorbic acid content for each product was estimated based on data from similar products given by Dietist.net. The estimations of each product are found in Table B.3.

Table B.3: Estimated calcium and Ascorbic acid Content (mg/100g) per Product

Product ID	Calcium (mg/100g)	Ascorbic acid (mg/100g)
Lettuce mix	34.5	8.5
Green leaf mix	116.67	28.9
Avocado	14	3.3
Diced cucumber	17	10.1
Cherry tomato	11	33.7
Greek-ish salad	13.48	56.14
Pasta chili creamy	22	0
Pasta bruchetta	10	0
Pasta pesto	10	0
Couscous morroccan	42.5	0
Black quinoa and lentils	12	0
Salty roasted corn	6	5.7
Sriracha mayo	34	0
Mango	15	26
Diced chicken	5	0
Chicken meatballs	6.4	31
Shrimps	36	0
Boiled egg	52	0
Creamy white cheese	663	0
Mozzarella	306	0
Veg. teriyaki strips	85	0.1
Marinated soybeans	73	0
Falafel	91	0.2
Red bell peppers	8	144
Green bell peppers	10	109
Yellow bell peppers	7	163
Red dried lentils	10	0
Organic dried red lentils	10	0
Organic red lentils (pre-boiled)	25	0
Organic dried green lentils	26	0
Organic green lentils (pre-boiled)	43	0
Organic red quinoa	14	0
Organic white quinoa	14	0

B.4 Dry matter analyses

The raw data of product weights before and after freeze drying, as well as the calculation of the dry matter percentage of the products, with its results are found in this section.

B.4.1 Dry matter analysis data and results

The weights of the empty containers used for each sample, the weights of the containers with sample before and after freeze drying, and the calculated dry matter percentage are presented in Table B.4. An example calculation of the dry matter percentage can be found in Section B.4.2.

Table B.4: Dry matter analysis raw data and results in percentage. Weights are presented for the empty containers and the containers containing the sample before and after freeze-drying. The numbers "1" and "2" in the "Product ID" column denote whether the product was from the first or second batch collected.

Product ID	Container empty	Before drying	After drying	DW_{fresh} (%)
Lettuce mix 1	8.7025	28.3286	9.6937	5.0504
Green leaf mix 1	8.7149	18.8378	9.5469	8.2190
Avocado 1	8.7222	57.5184	22.2018	27.6243
Diced cucumber 1	8.7372	48.5297	10.5068	4.4471
Cherry tomato 1	8.6477	72.7133	12.8483	6.5567
Greek-ish salad 1	8.8063	65.0259	14.6959	10.4761
Pasta chili creamy 1	8.6737	59.7190	30.8852	43.5133
Pasta bruchetta 1	8.5854	47.4504	24.2678	40.3510
Pasta pesto 1	8.7541	45.2312	22.9089	38.8046
Couscous Moroccan style 1	8.6884	62.2912	28.0739	36.1651
Black quinoa and lentils 1	8.8153	74.1951	35.6317	41.0163
Salty roasted corn 1	8.7230	36.4293	36.3727	99.7957
Sriracha mayo 1	8.7472	54.8020	27.8647	41.5103
Mango 1	8.7137	72.3540	21.2163	19.6457
Diced chicken 1	8.6377	73.3914	24.1664	23.9812
Chicken meatballs 1				
Shrimps 1	8.7627	62.3490	18.8639	18.8503
Boiled egg 1	8.7605	93.7175	29.5860	24.5130
Creamy white cheese 1	8.6979	53.0492	29.1248	46.0570
Mozzarella 1	8.7854	87.3054	37.3912	36.4312
Veg. teriyaki strips 1				
Marinated soybeans 1	8.7366	48.4250	20.8752	30.5848
Falafel 1				
Red bell peppers 1	8.7019	63.3456	13.1049	8.0577
Green bell peppers 1	8.7019	63.3456	13.1049	8.0577
Yellow bell peppers 1	8.7019	63.3456	13.1049	8.0577
Lettuce mix 2	8.6387	31.3816	9.8675	5.4030

Product ID	Container empty	Before drying	After drying	$DW_{fresh}(\%)$
Green leaf mix 2	8.6764	28.6141	10.3577	8.4328
Avocado 2	8.7204	77.3778	28.4114	28.6801
Diced cucumber 2	8.6914	69.3841	11.5381	4.6903
Cherry tomato 2	8.6349	82.6900	14.0443	7.3046
Greek-ish salad 2	8.7716	74.4605	16.4248	11.6507
Pasta chili creamy 2	8.5628	102.6080	46.4695	40.3069
Pasta bruchetta 2	8.6472	72.1200	35.0028	41.5227
Pasta pesto 2	8.7615	86.7425	45.8161	47.5175
Couscous Moroccan style 2	8.5726	83.3813	36.2031	36.9349
Black quinoa and lentils 2	8.6740	76.3474	37.4586	42.5346
Salty roasted corn 2	8.7053	48.3194	48.2236	99.7582
Sriracha mayo 2	8.7008	65.0003	32.1099	41.5796
Mango 2	8.7130	94.2977	25.9793	20.1745
Diced chicken 2	8.7721	74.8407	24.6305	24.0029
Chicken meatballs 2	8.6428	73.7504	33.6577	38.4209
Shrimps 2	8.6385	71.2518	20.5594	19.0389
Boiled egg 2	8.5689	82.7804	26.8557	24.6415
Creamy white cheese 2	8.6536	66.9688	34.8872	44.9859
Mozzarella 2				
Veg. teriyaki strips 2	8.7062	82.4159	39.2868	41.4879
Marinated soybeans 2	8.6827	100.1370	36.6747	30.6076
Falafel 2	8.6379	71.6843	39.5807	49.0794
Red bell peppers 2	8.6891	75.3658	14.0035	7.9704
Green bell peppers 2	8.6891	75.3658	14.0035	7.9704
Yellow bell peppers 2	8.6891	75.3658	14.0035	7.9704
Red dried lentils	8.6989	71.4573	35.0741	42.0266
Organic dried red lentils	8.7665	64.6864	36.2472	49.1430
Organic red lentils (pre-boiled)	8.6913	69.9324	24.3602	25.5856
Organic dried green lentils	8.6922	70.3692	34.5642	41.9476
Green lentils (pre-boiled)	8.6999	97.2679	30.9907	25.1680
Organic red quinoa	8.6810	39.7715	25.8683	55.2815
Organic white quinoa	8.6719	40.5022	24.9140	51.0272

B.4.2 Example calculation of dry matter percentage

The Dry Matter (DW_{fresh}) percentage was calculated using the following equation:

$$DW_{fresh}(\%) = \left(\frac{m_{dry} - m_{tare}}{m_{fresh} - m_{tare}} \right) \times 100 \tag{B.12}$$

Below is an example calculation using the data for *Lettuce mix 1*:

1. Experimental Data:

- Mass of empty container (m_{tare}): 8.7025 g

- Mass of container + fresh sample (m_{fresh}): 28.3286 g
- Mass of container + dry sample (m_{dry}): 9.6937 g

2. Substitution and Calculation:

First, calculate the net mass of the dry matter (numerator):

$$m_{dry} - m_{tare} = 9.6937\,\text{g} - 8.7025\,\text{g} = 0.9912\,\text{g} \tag{B.13}$$

Next, calculate the net mass of the fresh sample (denominator):

$$m_{fresh} - m_{tare} = 28.3286\,\text{g} - 8.7025\,\text{g} = 19.6261\,\text{g} \tag{B.14}$$

Finally, calculate the percentage:

$$DW_{fresh}(\%) = \left(\frac{0.9912\,\text{g}}{19.6261\,\text{g}}\right) \times 100 \approx 5.0504\% \tag{B.15}$$

B.5 Dry weight fractions of the freeze-dried samples

The calculated dry weight fractions (DW_{dry}) of all freeze-dried samples can be found in Table B.5. Results from measurements in the moisture analyzer were transformed via equation 3.2 to get the dry weight fractions. Not all samples had enough mass left from the sample preparation and could therefore not be measured in the moisture analyzer. In later calculations, the dry weight fraction of 1 was used for these samples.

Table B.5: Dry weight fractions (DW_{dry}) of the freeze-dried samples developed from values given by measurements in the moisture analyzer. The samples without a value did not have enough sample left from the preparations to put into the moisture analyzer. The numbers "1" and "2" in the "Product ID" column denote whether the product was from the first or second batch collected.

Product ID	DW_{dry}
Lettuce mix 1	
Green leaf mix 1	
Avocado 1	0.9966
Diced cucumber 1	
Cherry tomato 1	0.9506
Greek-ish salad 1	0.9638
Pasta chili creamy 1	0.988
Pasta bruchetta 1	0.9807

Product ID	DW_{dry}
Pasta pesto 1	0.9885
Couscous morroccan 1	0.9761
Black quinoa and lentils 1	0.9891
Salty roasted corn 1	0.9875
Sriracha mayo 1	0.9971
Mango 1	0.9624
Diced chicken 1	0.9827
Chicken meatballs 1	0.9952
Shrimps 1	0.9854
Boiled egg 1	0.9904
Creamy white cheese 1	0.9916
Mozzarella 1	0.9891
Veg. teriyaki strips 1	0.981
Marinated soybeans 1	0.9788
Falafel 1	0.9705
Red bell peppers 1	
Green bell peppers 1	
Yellow bell peppers 1	
Lettuce mix 2	
Green leaf mix 2	
Avocado 2	0.9964
Diced cucumber 2	
Cherry tomato 2	0.9543
Greek-ish salad 2	0.9674
Pasta chili creamy 2	0.9984
Pasta bruchetta 2	0.9848
Pasta pesto 2	0.9904
Couscous morroccan 2	0.9780
Black quinoa and lentils 2	0.9846
Salty roasted corn 2	0.9808
Sriracha mayo 2	0.9963
Mango 2	0.9543
Diced chicken 2	0.9870
Chicken meatballs 2	0.9885
Shrimps 2	0.9838
Boiled egg 2	0.9906
Creamy white cheese 2	0.9932
Mozzarella 2	0.9867
Veg. teriyaki strips 2	0.9767
Marinated soybeans 2	0.9743
Falafel 2	0.9892
Red bell peppers 2	
Green bell peppers 2	
Yellow bell peppers 2	

Product ID	DW_{dry}
Red dried lentils	0.9871
Organic dried red lentils	0.9881
Organic red lentils (pre-boiled)	0.9834
Organic dried green lentils	0.9825
Organic green lentils (pre-boiled)	0.8558
Organic red quinoa	0.9825
Organic white quinoa	0.9880

B.6 Phytic acid concentration estimation

The average phytic acid concentration of similar products that were used to estimate the phytic acid content in the products having multiple ingredients, for which data were available, is found in Table B.6.

Table B.6: Phytic Acid Content estimated from data from similar products. The Product components and their respective phytic acid content, on which the average phytic acid content was based, are presented.

Product ID	**Avg PA (mg/100g)**	**PA (mg/100g)**	**Product Component**
Veg. teriyaki strips	397.4	588	Anamma vegoprins
		359	Krispig Vegoschnitzel ICA
		416	Sojafärs ICA
		163	Köttfria köttbullar Dafgårds
		302	Vegoschnitzel Garant
		345	Plant-based burger Hälsans kök
		442	Formbar färs Anamma
		393	Nuggets Hälsans kök
		366	Vegoburgare Anamma
		367	Vegokorv Anamma
		736	Sensational Sausage Hälsans kök
		233	Soynuggets Hälsans Kök
		338	Balls, Oumph
		580	Sojafärs
		333	Plant based balls Hälsans kök
Couscous moroccan	277.5	415	Couscous
		140	Kikärta
Black quinoa & lentils	60.1	0.25	Quinoa
		120	Röda linser
Falafel	126.5	146	Falafel Findus
		107	Falafel ICA

B.7 Iron and zinc content analysis

The raw data used and produced in the iron and zinc content analysis are presented in this section. The calculation of standards used in the Atomic Absorbance spectroscopy (AAS), the calculation of mineral concentration per 100g product weight, and precision calculations are presented.

B.7.1 Sample weights and measured mineral concentrations

The sample weights used for the microwave digestion (g) and resulting concentrations of iron and zinc from the AAS (mg/L) can be found in Table B.7. Duplicates are shown as two rows in the marked sections of the product ID. In some cases, only one batch of that sample was run due to a low sample mass. Therefore, only mineral concentrations from one batch represent the sample, instead of two batches.

Table B.7: Sample weights (g) used in the microwave digestion and Iron and Zinc Analysis Results from the AAS (mg/L). Duplicates are presented in two rows inside the section dedicated to that product ID. Some samples did not have enough mass to be analyzed, represented by empty rows

Product ID	Sample (g)	Iron (mg/L)	Zinc (mg/L)
Lettuce mix 1			
	0.1000	0.8050	0.7669
Green leaf mix 1	0.0608	0.5090	0.4420
	0.1173	0.0860	0.3825
Avocado 1	0.1077	0.0740	0.3694
Diced cucumber 1			
	0.1043	0.3980	0.2342
Cherry tomato 1	0.1012	0.4420	0.2241
	0.1015	0.2340	0.1359
Greek-ish salad 1	0.1005	0.2260	0.1368
	0.1113	0.1540	0.1640
Pasta chili creamy 1	0.1299	0.1350	0.2035
	0.1043	0.2300	0.1987
Pasta bruchetta 1	0.1027	0.1910	0.1963
	0.1012	0.1320	0.1959
Pasta pesto 1	0.1072	0.1490	0.2072
	0.1152	0.3460	0.2635
Couscous morroccan 1	0.1024	0.3570	0.2461
	0.1006	0.4820	0.3677
Black quinoa and lentils 1	0.1092	0.5780	0.4213
	0.1110	0.1540	0.1781
Salty roasted corn 1	0.1789	0.2500	0.3295
	0.1109	0.0720	0.0237

Product ID	Sample (g)	Iron (mg/L)	Zinc (mg/L)
Sriracha mayo 1	0.1528	0.1490	0.0395
	0.1053	0.0170	0.0401
Mango 1	0.1038	0.0420	0.0397
	0.1292	0.2590	0.2917
Diced chicken 1	0.1077	0.1650	0.2605
	0.1010	0.2300	0.2328
Chicken meatballs 1	0.1431	0.3180	0.3311
	0.1044	0.0030	0.3881
Shrimps 1	0.1053	-0.0180	0.3926
	0.1043	0.8860	0.5847
Boiled egg 1	0.1084	0.9940	0.6212
	0.1068	-0.0190	0.2800
Creamy white cheese 1	0.1054	-0.0260	0.2842
	0.1052	0.0210	0.7585
Mozzarella 1	0.1005	0.0110	0.7378
	0.1009	0.6230	0.3437
Veg. teriyaki strips 1	0.1030	0.6190	0.3619
	0.1452	0.9670	0.5533
Marinated soybeans 1	0.1032	0.6290	0.3906
	0.1007	0.4040	0.2227
Falafel 1	0.1134	0.4310	0.2452
Red bell peppers 1			
Green bell peppers 1	0.1010	0.3750	0.1279
Yellow bell peppers 1			
	0.1019	0.4210	0.3213
Lettuce mix 2	0.1014	0.4840	0.3425
Green leaf mix 2			
	0.1043	0.1380	0.3290
Avocado 2	0.1032	0.1210	0.3027
	0.1350	0.3580	0.3178
Diced cucumber 2	0.1035	0.2380	0.2623
	0.1014	0.3440	0.2738
Cherry tomato 2	0.1030	0.2980	0.2714
	0.1025	0.3940	0.1331
Greek-ish salad 2	0.1009	0.3580	0.1269
	0.1294	0.1090	0.1435
Pasta chili creamy 2	0.1099	0.0950	0.1363
	0.1024	0.1630	0.1614
Pasta bruchetta 2	0.1074	0.1540	0.1917
	0.1020	0.1050	0.1649

Product ID	Sample (g)	Iron (mg/L)	Zinc (mg/L)
Pasta pesto 2	0.1005	0.1070	0.1564
	0.1017	0.3300	0.2662
Couscous morroccan 2	0.1044	0.3430	0.2631
	0.1121	0.6230	0.4509
Black quinoa and lentils 2	0.1045	0.5190	0.4316
	0.1064	0.1340	0.1770
Salty roasted corn 2	0.1051	0.1170	0.1703
	0.1168	0.0690	0.0281
Sriracha mayo 2	0.1092	0.0020	0.0245
	0.1008	0.0740	0.0395
Mango 2	0.1060	0.0910	0.0507
	0.1039	0.0920	0.2390
Diced chicken 2	0.1105	0.0930	0.2283
	0.1105	0.1810	0.2505
Chicken meatballs 2	0.1088	0.2150	0.2755
	0.1014	0.0820	0.3449
Shrimps 2	0.1012	0.0820	0.3783
	0.1050	0.5930	0.4844
Boiled egg 2	0.1035	0.5810	0.4504
	0.1022	-0.0390	0.2402
Creamy white cheese 2	0.1132	-0.0080	0.2706
	0.1039	-0.0330	0.6917
Mozzarella 2	0.1205	-0.0140	0.8189
	0.1072	0.6020	0.3384
Veg. teriyaki strips 2	0.1014	0.5720	0.3280
	0.1105	0.8030	0.3447
Marinated soybeans 2	0.1074	0.7380	0.3261
	0.1015	0.3580	0.2549
Falafel 2	0.1033	0.3630	0.2223
	0.1033	0.2800	0.1184
Red bell peppers 2	0.1029	0.2280	0.1139
	0.1320	0.3580	0.1531
Green bell peppers 2	0.1113	0.3690	0.1405
	0.1033	0.3770	0.1189
Yellow bell peppers 2	0.1043	0.3630	0.1154
	0.1028	0.6710	0.4588
Red dried lentils	0.1066	0.6900	0.4754
	0.1010	0.5530	0.5207
Organic dried red lentils	0.1101	0.5560	0.5436
	0.1065	1.0460	0.5536
Organic red lentils (pre-boiled)	0.1051	0.9880	0.5437
	0.1320	0.9610	0.5928
Organic dried green lentils	0.1039	0.8220	0.4971
	0.1005	0.7070	0.3018

Product ID	Sample (g)	Iron (mg/L)	Zinc (mg/L)
Organic green lentils (pre-boiled)	0.1130	0.6570	0.3030
Organic red quinoa	0.1020	0.3190	0.3958
	0.1036	0.3060	0.4050
Organic white quinoa	0.1115	0.4290	0.3717
	0.1028	0.3420	0.3328

B.7.2 Example calculation of standards for AAS

Standards with known mineral concentrations were used to create calibration curves for the AAS to determine mineral concentrations in the samples. The starting concentration was 50 mg/L, which was diluted to create the selected concentrations. The volumes required for the serial dilution of iron and zinc standards were calculated using the equation B.16.

$$V_1 = \frac{C_2 \cdot V_2}{C_1} \tag{B.16}$$

Example: Preparation of 4.0 mg/L standard from 50 mg/L stock solution

Parameters:

- C_1 (Stock concentration) = 50 mg/L
- C_2 (Desired concentration) = 4.0 mg/L
- V_2 (Final volume) = 20 mL

Calculation:

$$\begin{aligned} V_1 &= \frac{4.0\,\text{mg/L} \cdot 20\,\text{mL}}{50\,\text{mg/L}} \\ V_1 &= \frac{80}{50}\,\text{mL} \\ V_1 &= 1.6\,\text{mL} \end{aligned} \tag{B.17}$$

Result: To prepare a standard of 4.0 mg/L, 1.6 mL of the 50 mg/L stock solution was transferred to a Falcon tube and diluted with milliQ water to a final volume of 20 mL (adding 18.4 mL of water).

B.7.3 Example calculation of iron and zinc content per 100 g product weight

The mineral concentration per total product weight (mg/100g) was calculated to represent the nutrient density of the ready-to-eat samples. Below is an example calculation using the data for *Avocado 1* replica 1 for iron concentration.

Parameters:

- C: Concentration from equipment = 0.086 mg/L
- V: Total volume after digestion = 0.01 L
- m: Mass of sample used for digestion = 0.1173 g
- DW_{dry}: Dry weight in the dried sample = 0.9966
- DW_{fresh}: Dry weight in product = 0.2762 g

Step 1: Determine Iron concentration in the digested sample without adjusting for the moisture in the dried sample

The concentration of iron (mg/100 g) present in the digested solution without adjusting for the moisture in the dried sample is:

$$\text{Unadjusted Fe (mg)/100 g DW} = \frac{C \times V}{m} \times 100 = \frac{0.086\,\text{mg/L} \times 0.01\,\text{L}}{0.1173g} \times 100 = 0.7332\,\text{mg/100 g} \tag{B.18}$$

Step 2: Adjusting the iron concentration for the moisture content in the dried sample

The dried samples contained some moisture. To get the iron concentration in the fully dry sample, this moisture had to be taken into account. The concentration of iron (mg/100 g) present in the digested solution when adjusting for the moisture content in the dried sample is:

$$\begin{aligned}\text{Adjusted Fe (mg)/100g DW} &= \text{Unadjusted Fe (mg)/100g DW} \times DW_{dry} = \\ &= 0.7332\,\text{mg/100g} \times 0.9966 = 0.7307\,\text{mg/100g}\end{aligned} \tag{B.19}$$

Step 3: Standardize to 100g of product weight

To express the result in the unit of mg/100g of product weight:

$$\begin{aligned}\text{Fe (mg/100g product weight)} &= \text{Adjusted Fe (mg)/100g DW} \times DW_{fresh} = \\ &= 0.7307\,\text{mg/g} \times 0.2762 = 0.2018\,\text{mg/100g}\end{aligned} \tag{B.20}$$

Result: The iron content for *Avocado 1* replica 1 is 0.2018 mg/100g product weight.

B.7.4 Iron and zinc concentration per 100 g product weight and precision calculations

The iron and zinc concentration results from the AAS were transformed from mg/L to mg/100g product weight, and the results can be seen in Table B.8. An example of the calculations can be found in B.7.3. The results from the precision calculations

of the Relative Percent Difference (RPD %) are also presented in Table B.8. An example calculation of the RPD is found in B.7.6. If there were only one replica, the RPD could not be calculated.

Table B.8: Calculated iron (Fe) and zinc (Zn) concentrations per 100 g product weight (mg/100g) for each sample analyzed in the AAS and calculated precision for replicates (RPD %). The RPD was not calculated for samples with only one replica.

	Iron (Fe)		**Zinc (Zn)**	
Product ID	**mg/100g**	**RPD (%)**	**mg/100g**	**RPD (%)**
	0.0505		0.0505	
Lettuce mix 1				
	0.6616	3.92	0.6303	5.35
Green leaf mix 1	0.6881		0.5975	
	0.2018	6.49	0.8977	5.05
Avocado 1	0.1892		0.9443	
	0.0445		0.0445	
Diced cucumber 1				
	0.2378	13.48	0.1400	1.39
Cherry tomato 1	0.2722		0.1380	
	0.2328	2.49	0.1352	1.65
Greek-ish salad 1	0.2271		0.1374	
	0.5948	28.43	0.6335	6.12
Pasta chili creamy 1	0.4468		0.6735	
	0.8726	16.99	0.7679	1.51
Pasta bruchetta 1	0.7360		0.7564	
	0.5003	6.35	0.7425	0.15
Pasta pesto 1	0.5332		0.7414	
	1.0602	14.88	0.8074	4.95
Couscous morroccan 1	1.2307		0.8484	
	1.9438	9.95	1.4828	5.40
Black quinoa and lentils 1	2.1473		1.5652	
	1.3672	0.72	1.5812	13.77
Salty roasted corn 1	1.3771		1.8151	
	0.2687	40.13	0.0885	18.98
Sriracha mayo 1	0.4036		0.1070	
	0.0305	85.92	0.0720	0.43
Mango 1	0.0765		0.0723	
	0.4724	26.73	0.5321	6.89
Diced chicken 1	0.3610		0.5700	
	0.8707	2.44	0.8813	0.38
Chicken meatballs 1	0.8497		0.8847	
	0.0053	280.83	0.6905	0.29
Shrimps 1	-0.0318		0.6926	

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Table B.8 – continued from previous page

Product ID	Iron (Fe) mg/100g	Iron (Fe) RPD (%)	Zinc (Zn) mg/100g	Zinc (Zn) RPD (%)
	2.0623	7.64	1.3610	2.20
Boiled egg 1	2.2262		1.3913	
	-0.0812	32.40	1.1973	2.81
Creamy white cheese 1	-0.1127		1.2314	
	0.0719	58.35	2.5981	1.80
Mozzarella 1	0.0394		2.6454	
	2.5130	2.70	1.3864	3.10
Veg. teriyaki strips 1	2.4459		1.4300	
	1.9937	8.86	1.1408	0.68
Marinated soybeans 1	1.8246		1.1331	
	1.9109	5.41	1.0534	2.25
Falafel 1	1.8103		1.0299	
	0.2232	14.41	0.1704	6.84
Lettuce mix 2	0.2579		0.1825	
	0.3781	12.07	0.9014	7.27
Avocado 2	0.3351		0.8382	
	0.1244	14.23	0.1104	7.37
Diced cucumber 2	0.1079		0.1189	
	0.2365	15.89	0.1882	2.45
Cherry tomato 2	0.2017		0.1837	
	0.4332	8.00	0.1464	3.20
Greek-ish salad 2	0.3999		0.1418	
	0.3390	2.59	0.4463	11.17
Pasta chili creamy 2	0.3479		0.4991	
	0.6509	10.44	0.6445	12.42
Pasta bruchetta 2	0.5863		0.7299	
	0.4845	3.37	0.7608	3.81
Pasta pesto 2	0.5011		0.7324	
	1.1721	1.24	0.9455	2.62
Couscous morroccan 2	1.1868		0.9210	
	2.3275	11.23	1.6845	2.65
Black quinoa and lentils 2	2.0800		1.7297	
	1.2322	12.32	1.6276	2.63
Salty roasted corn 2	1.0892		1.5854	
	0.2447	187.97	0.1000	7.00
Sriracha mayo 2	0.0076		0.0930	
	0.1413	15.62	0.0754	19.87
Mango 2	0.1653		0.0921	
	0.2098	5.08	0.5450	10.73
Diced chicken 2	0.1994		0.4895	

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Table B.8 – continued from previous page

Product ID	Iron (Fe) mg/100g	Iron (Fe) RPD (%)	Zinc (Zn) mg/100g	Zinc (Zn) RPD (%)
	0.6221	18.71	0.8610	11.05
Chicken meatballs 2	0.7505		0.9617	
	0.1515	0.20	0.6371	9.43
Shrimps 2	0.1518		0.7002	
	1.3786	0.61	1.1261	5.84
Boiled egg 2	1.3703		1.0622	
	-0.1705	137.51	1.0501	1.68
Creamy white cheese 2	-0.0316		1.0679	
	-0.1142	92.87	2.3931	2.06
Mozzarella 2	-0.0418		2.4429	
	2.2755	0.45	1.2791	2.44
Veg. teriyaki strips 2	2.2858		1.3107	
	2.1671	5.59	0.9303	2.70
Marinated soybeans 2	2.0492		0.9055	
	1.7124	0.37	1.2192	15.41
Falafel 2	1.7060		1.0448	
	0.2160	20.09	0.0914	3.49
Red bell peppers 2	0.1766		0.0882	
	0.2162	20.02	0.0924	8.46
Green bell peppers 2	0.2642		0.1006	
	0.2909	4.75	0.0917	3.95
Yellow bell peppers 2	0.2774		0.0882	
	2.7078	0.84	1.8515	0.08
Red dried lentils- Eldorado	2.6852		1.8501	
	2.6587	8.08	2.5034	4.32
Organic dried red lentils	2.4522		2.3975	
	2.4712	4.38	1.3079	0.48
Organic red lentils (pre-boiled)	2.3653		1.3016	
	3.0005	8.31	1.8509	6.33
Organic dried green lentils	3.2606		1.9718	
	1.5152	19.00	0.6468	11.31
Organic green lentils (pre-boiled)	1.2523		0.5775	
	1.6986	5.72	2.1076	0.74
Organic red quinoa	1.6043		2.1233	
	1.9397	14.52	1.6806	2.93
Organic white quinoa	1.6772		1.6321	

B.7.5 CV calculations for samples with high RPD

Some samples had an RPD over 20%. These samples were rerun, transformed into Fe/100g product weight, and the RPD for the rerun values was calculated. The Coefficient of variation (CV) was calculated according to Equation 3.8, and the

results are seen in Table B.9. A result under 20% represents good variation, which is met for all samples, with Sriracha mayo 1 being slightly over, but was accepted anyway.

Table B.9: Iron per 100 g product weight for the new and old measurements, represented in rows in the sections of the product ID. The concentrations of the old measurements are presented in the first two rows, and the values for the new measurements are presented in the last two rows of the section. Outliers are removed. The RPD for the old and new measurements is presented. CV calculations are presented in the last column.

Product ID	**Fe/100g product weight**	**RPD (%)**	**CV (%)**
			14.70
	0.4964		
	0.5948	28.43	
Pasta chili creamy 1	0.4468		
			20.87
	0.3142		
	0.2687	40.13	
Sriracha mayo 1	0.4036		
	0.3685	25.68	15.15
	0.4770		
	0.4724	26.73	
Diced chicken 1	0.3610		
	0.2508	21.08	14.58
	0.2029		
	0.2160	20.09	
Red bell peppers 2	0.1766		
	0.2240	28.08	15.03
	0.2972		
	0.2162	20.02	
Green bell peppers 2	0.2642		

B.7.6 Example Calculation: RPD and CV for variation between replicates

Two replicas were made to analyze the mineral concentrations. To ensure reliable data and low variation between replicates, the Relative Percent Difference (RPD) was calculated for all samples. RPD for the iron analysis of *Avocado 1* was calculated using the iron concentrations per product weight for the two replicates ($R_1 = 0.2018$ and $R_2 = 0.1892$ mg/100g):

$$\begin{aligned} RPD &= \left(\frac{|R_1 - R_2|}{\frac{R_1+R_2}{2}} \right) \times 100 \\ &= \left(\frac{|0.2018 - 0.1892|}{\frac{0.2018+0.1892}{2}} \right) \times 100 \\ &= \left(\frac{0.0126}{0.1955} \right) \times 100 \\ &= 6.45\% \end{aligned} \tag{B.21}$$

A result under 20% indicates good precision between the replicates for this sample.

In cases where the RPD was higher than 20, such as *Diced chicken 1*, both replicas were run in the AAS again. The Coefficient of Variation (CV) was used to determine the relative precision across multiple replicates of *Diced chicken 1*. The calculation was based on the mean ($\mu = 0.4197$) and the standard deviation ($\sigma = 0.0636$) of the iron concentration per product weight:

$$\begin{aligned} CV &= \left(\frac{\sigma}{\mu} \right) \times 100 \\ &= \left(\frac{0.0636}{0.4197} \right) \times 100 \\ &= 15.1504\% \end{aligned} \tag{B.22}$$

B.8 Miller algorithm example calculation

This section contains an example calculation of the total absorbed zinc (TAZ) and the absorption efficiency (%) for the Meat/fish salad composition using the Miller equation.

Input Values

The raw nutritional data for one serving of the Meat/fish salad is:

- Phytate: 118 mg
- Calcium: 139 mg
- Zinc: 3.4 mg
- Protein (TDPr): 39.9 g

2. Molar Conversions

The values are converted to millimoles (mmol) using the respective molecular weights:

- $TDP = 118/660.04 \approx 0.1788$ mmol
- $TDC = 139/40.08 \approx 3.4681$ mmol
- $TDZ = 3.4/65.38 \approx 0.0520$ mmol

3. Calculation of Model Terms

Using the constants $K_T = 0.069$, $K_P = 0.44$, $A_{MAX} = 0.084$, $B_{Ca} = 0.017$, and $B_{Pr} = 0.012$:

Phytate Term (H):

$$H = 0.069 \cdot \left(1 + \frac{0.1788 \cdot (1 - 0.017 \cdot 3.4681)}{0.44}\right) \approx 0.0954 \tag{B.23}$$

Protein Term (I):

$$I = 0.0520 \cdot (1 + 0.012 \cdot 39.9) \approx 0.0769 \tag{B.24}$$

Combined Sum (A_{sum}):

$$A_{sum} = H + 0.084 + I = 0.0954 + 0.084 + 0.0769 = 0.2563 \tag{B.25}$$

4. Final TAZ Calculation

The final absorbed zinc in mmol is calculated:

$$TAZ_{mmol} = 0.5 \cdot \left(0.2563 - \sqrt{0.2563^2 - 4 \cdot 0.084 \cdot 0.0769}\right) \approx 0.0283 \text{ mmol} \tag{B.26}$$

Conversion back to mg:

$$TAZ_{mg} = 0.0283 \cdot 65.38 \approx 1.85 \text{ mg} \tag{B.27}$$

Absorption Efficiency (%):

$$\text{Efficiency} = \left(\frac{1.85}{3.4}\right) \cdot 100 \approx 54.5\% \tag{B.28}$$

B.9 Molar ratios

Molar ratios were calculated for products containing more than 1 mg/100g zinc and 1.5 mg/100g iron. The results are found in Table B.10. An example calculation of phytate-to-iron molar ratio for *Black quinoa and lentils* is found in B.8.

B.9.1 Example Calculation: Phytate-to-Iron Molar Ratio

The molar ratio for *Black quinoa and lentils* was calculated in three steps using the concentrations (mg/100g) and molecular weights (MW).

1. Calculation of Moles of Phytate

Using $PA = 60.125$ mg/100g and $MW_{\text{Phytate}} = 660.03$ g/mol:

$$\text{Mol PA} = \frac{\text{PA (mg)}/1000}{MW_{\text{Phytate}}} = \frac{60.125/1000}{660.03} \approx 9.11 \times 10^{-5}\,\text{mol} \tag{B.29}$$

2. Calculation of Moles of Iron (Fe)

Using $Fe = 2.1246$ mg/100g and $MW_{\text{Fe}} = 55.845$ g/mol:

$$\text{Mol Fe} = \frac{\text{Fe (mg)}/1000}{MW_{\text{Fe}}} = \frac{2.1246/1000}{55.845} \approx 3.80 \times 10^{-5}\,\text{mol} \tag{B.30}$$

3. Calculation of Molar Ratio (Phy:Fe)

The ratio is determined by dividing the moles of phytate by the moles of iron:

$$\text{Phy:Fe Ratio} = \frac{\text{Mol PA}}{\text{Mol Fe}} = \frac{9.11 \times 10^{-5}}{3.80 \times 10^{-5}} \approx 2.39 \tag{B.31}$$

B.9.2 Calculated phytate-to-mineral molar ratios

Table B.10: Phytate-to-Mineral Molar Ratios

Product	Phy:Fe	Phy:Zn
Black quinoa and lentils	2.39	3.68
Veg. teriyaki strips	14.12	29.11
Red dried lentils	3.76	6.42
Organic dried red lentils	3.97	4.85
Red lentils (pre-boiled)	4.20	9.11
Organic dried green lentils	3.84	7.36
Marinated soybeans	8.72	19.95
Falafel	5.99	11.52
Organic red quinoa	0.01	0.01
Organic white quinoa	0.01	0.01
Salty roasted corn	1.60	1.44

DEPARTMENT OF LIFE SCIENCES
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