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Impact of postharvest storage and cooking time on mineral bioaccessibility in common beans

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Mineral (Mg, Ca, Fe and Zn) bioaccessibility in common beans was evaluated taking into consideration the common bean food chain from postharvest storage over processing (soaking and cooking) until consumption. Beans were stored at realistic tropical conditions (35 °C and 80% RH) which resulted in already significantly different cooking behaviour after 8 weeks compared to freshly harvested beans. Based on postcooking hardness, different storage times were selected: unstored, 8 and 20 weeks. Independently from storage condition, beans were soaked overnight and cooked for 30, 60 or 120 min. Both storage and cooking time decreased mineral bioaccessibility. Decreasing mineral bioaccessibility with storage time was proven to be the result of increasing mineral chelation of cell wall polymers (e.g. pectin). Additionally, we hypothesize that by cooking, mineral chelators become more accessible, e.g. through pectin solubilization phenomena, in turn capturing more free minerals leading to a reduced mineral bioaccessibility.

1. Introduction

One of the world's biggest challenges is feeding the growing world population efficiently and sustainably to meet the carrying capacity of the earth. Common beans are nutritious crops with a low carbon footprint, which have efficient land and water use.^{1,2} Moreover, as common beans are low in fat and high in proteins, complex carbohydrates, vitamins and minerals, they can contribute to a healthy lifestyle.² Especially their high amount of minerals is interesting, as Fe and Zn deficiencies are prevalent micronutrient deficiencies worldwide.³ However, since minerals should be present in a free state to enable their absorption in the small intestine, the mineral quality of a food product is not only determined by its mineral content but also by the mineral bioaccessibility and bioavailability.⁴ Different external factors, such as postharvest storage and processing, can influence the mineral bioaccessibility and bioavailability in common beans and therefore affect their mineral quality.

1.1. Effect of postharvest storage on mineral quality of common beans

Despite the fact that the common bean is an interesting food product in terms of nutritional profile and sustainability, it can develop the hard-to-cook (HTC) defect upon postharvest storage, resulting in retarded softening of beans during cooking. This HTC defect mainly occurs upon storage of beans in tropical conditions, at high relative humidity (above 50%) and high

temperature (above 30 °C).⁵ Unfortunately, these are relevant conditions in regions where common beans are important staple foods and long storage periods are often necessary to bridge the period between consecutive harvesting seasons. One of the factors that plays a role in softening of bean texture upon cooking is solubilisation of pectin in the cell wall middle lamella of the parenchyma cells. In HTC beans, solubilisation of pectin during cooking is limited.⁶ This could possibly be explained by the elevated relative humidity and temperature during postharvest storage, which can lead to enhanced molecular mobility. In addition, upon this kind of postharvest storage, membrane damage is believed to occur.⁷ As a consequence, diffusion of minerals from the cytoplasm to the cell wall is now assumed to no longer be hindered by the plasma membrane. Therefore, minerals can diffuse and crosslink pectin molecules in the middle lamella whereby insoluble pectates can be formed and cell-cell adhesion increases. As a result, pectin solubilisation during cooking is reduced and beans only soften if they are cooked for longer times.

Pectin is a complex heteropolysaccharide that is mainly present in the middle lamella of dicotyl plant tissues and consists generally of three structurally different regions including homogalacturonan (HG), which is a smooth region due to absence of side chains (neutral sugars) and two hairy regions, being rhamnogalacturonan (RG) I and II. HG and RG II have a backbone of α -1,4-linked galacturonic acid (GalA) residues which can be methylesterified.⁸ Pectin can interact with several divalent cations through its ionized (COO⁻), non-methylesterified GalA residues. This can result in the possible formation of a pectin-mineral network, the "egg-box" model.⁹ Therefore, the degree of pectin methoxylation (DM) will determine its polyanionic nature and consequently influence its mineral binding capacity.^{10,11}

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Phytic acid is another mineral chelator in common beans which is mainly located in the protein bodies of the cotyledons.¹² Phytic acid, or phytate when in salt form, consists of a myo-inositol molecule that can contain up to six phosphate groups. It is through these negatively charged groups that phytic acid can chelate minerals. Due to the presence of phytase, phytic acid can be hydrolysed resulting in the release of phosphate groups and in turn also its bound minerals.¹³ During postharvest storage at tropical conditions it is hypothesised that phytase becomes active and hydrolyses phytic acid.⁵ As a consequence, minerals can be released from phytic acid during postharvest storage. These released cations can then further crosslink pectin molecules in the middle lamella, hereby potentially contributing to prolonged cooking times in HTC beans.

It can be assumed that minerals can be displaced during postharvest storage, for example, from a free state to a bound state and vice versa, or they can be released from one binding agent (e.g. phytic acid) and then again chelated by another (e.g. pectin). Since minerals are believed to play an important role in the development of the HTC defect, the question arises how this HTC defect might influence the mineral bioaccessibility in common beans. It is hypothesized that postharvest storage conditions might affect mineral bioaccessibility in common beans. On the one hand, free minerals might become chelated to form insoluble and indigestible complexes which can lead to reduced mineral bioaccessibility. On the other hand, minerals can be released from indigestible mineral chelators (e.g. phytic acid) whereby mineral bioaccessibility can increase. Previously, it has been shown that mineral bioaccessibility in cowpeas can be reduced after development of the HTC defect (storage for 3 weeks at 42 °C and 67% RH).¹⁴ In the present study, the effect of postharvest storage time (35 °C and 80% RH) on mineral bioaccessibility in common beans was evaluated. Since the mineral binding capacity of phytic acid can be reduced during postharvest storage, the phytic acid content was measured as well as pectin content and degree of methoxylation. The latter is believed to be the structural characteristic of pectin which mainly influences its mineral binding capacity.¹⁵ Moreover, the effect of postharvest storage on the free mineral content and on the mineral content specifically bound to the cell wall fraction in the cotyledons was determined.

1.2. Effect of processing on mineral quality of common beans

Soaking followed by cooking are the most commonly used processing steps for whole common beans to obtain a palatable solid food product.¹⁶ However, during soaking and cooking, the nutritional composition might change. For example, mineral/nutrient displacement can take place with these processes. Besides minerals being displaced between the different compartments in the bean (e.g. diffuse from cotyledon to seed coat and vice versa) or being leached in cooking and soaking media, minerals can also change from a free to a bound state. For example, minerals can be released from one chelator and captured by another. Moreover, free minerals can be chelated by molecules that become more accessible due to cooking¹⁷ (e.g. pectin solubilisation) and minerals that are bound to chelators can be released as a result of structural

changes that might occur during cooking¹⁸ (e.g. protein denaturation). Therefore, it was the objective to investigate the effect of cooking on mineral bioaccessibility as well. Along with this, the leaching of dry matter and minerals during soaking and cooking was determined.

It is thus hypothesized that both postharvest storage and processing can influence mineral bioaccessibility in common beans. In this study, mineral (Mg, Ca, Fe and Zn) bioaccessibility in common beans was evaluated taking into consideration the chain from postharvest storage and processing (soaking and cooking) until consumption. Therefore, beans were stored at realistic tropical conditions (35 °C and 80% RH) and based on cooking behaviour, three different storage times were selected. These beans were subsequently soaked overnight and cooked for different cooking time moments. Mineral (Mg, Ca, Fe and Zn) bioaccessibility in these samples was determined after *in vitro* simulation human digestion, including the oral, gastric and small intestinal phases. Mg and Ca were selected as essential macro elements, while Fe and Zn represent essential trace metals that exert important physiological functions in the human body.

2. Material and methods

First, freshly harvested common beans were stored at realistic tropical postharvest storage conditions to induce development of the HTC defect (section 2.3). Based on analysis of cooking behaviour (section 2.4), three different storage times were selected and these beans were then soaked and cooked. Mineral content in soaking and cooking water was analysed (section 2.5). Mineral bioaccessibility in soaked and cooked bean samples was subsequently determined (section 2.7). An overview of the experimental approach applied is shown in Figure 1.

Figure 1. Experimental approach applied for the creation of a range of relevant bean samples through variables of postharvest storage and processing.

2.1. Raw plant material

Freshly harvested Canadian wonder beans (*Phaseolus vulgaris* L.) were obtained from the Kenya Agricultural and Livestock Research Organization (KALRO). These beans were kept at -40 °C or stored at conditions aimed to induce biophysical changes as described in section 2.3. The beans that were immediately stored upon arrival at -40 °C represent the fresh sample.

2.2. Inducing the HTC defect

To induce development of the HTC defect, a part of the fresh beans was incubated at 35 °C and a relative humidity of 80%. To achieve and maintain this relative humidity the beans were stored above a saturated KCl solution. Every 4 weeks, up until 20 weeks, an amount of the beans was sampled and stored at -40 °C until further use.

2.3. Cooking profile

To assess whether the HTC defect was induced, the hardness evolution of beans during cooking at 95 °C was evaluated. Therefore, cooking profiles of fresh, 4, 8, 12, 16 and 20 weeks stored beans were generated by measuring their hardness as a function of cooking time.

First, beans were soaked in Milli-Q water (organic free, 18.2MΩcm resistance; Simplicity™ water purification system; Millipore, Billerica, USA) in a ratio of 1:5 (w/v) at 25 °C for 16 h. To thermally process the beans, at least ten soaked beans were cooked for different cooking times (between 5 min and 360 min) depending on the condition of material (fresh or stored), in Milli-Q water (1:5 w/v) at 95 °C. At the end of each predetermined processing time, samples were cooled in an ice bath until room temperature.

After cooling, bean seed coats were manually removed and seed cotyledons were isolated. Each bean contained two single cotyledons. Hardness of a cotyledon was determined by a compression test using a TA-X2i texture analyser, equipped with a 25 kg load cell and a cylindrical flat-head aluminium probe of diameter 25 mm (Stable Microsystems, UK). The maximum force necessary for compression to a 75% strain level of a cotyledon was defined as the hardness (N). For each cooking time, the hardness of at least 20 cotyledons was measured and the average hardness (H) was calculated where after the hardness evolution with cooking time for each storage time was evaluated by modelling the experimental data by a fractional conversion model (Equation 1). Hereby, two parameters, i.e., final hardness, H_f (N), and the rate constant of hardness degradation, k (min^{-1}), were estimated by non-linear regression (SAS version 9.3, SAS Institute, Inc., Cary, NC, USA). H_i represents the initial hardness (N) and corresponds to the hardness of soaked cotyledons.

$$H = H_f + (H_i - H_f) \times e^{-kt} \quad \text{Equation 1}$$

2.4. Processing of fresh and stored bean samples

Based on the results of the cooking profiles obtained (discussed in section 3.1), bean samples with three different storage times were selected: fresh beans, 8 weeks stored beans and 20 weeks

stored beans. For the following investigations, these beans were soaked in Milli-Q water in a ratio of 1:5 (w/v) at 25 °C for 16 h. Soaking water was removed and some of the soaked beans were dehulled. Whole soaked beans and/or cotyledons were then cooked for 30, 60 or 120 min as described in section 2.3. To obtain homogeneous, standardized and stable samples, cooking water was removed and soaked and cooked beans were ground (60 sec, A 10 basic mill, IKA, Germany) and lyophilized to create a dry powder which was then sieved (500 μm). Besides, the microstructures of the powders obtained were open cell structures for soaked beans and individual cells for cooked beans (data not shown) which have been reported to be microstructures that are created during the human mastication process.¹⁹ Therefore, it could be concluded that the grinding process applied is similar to human mastication. Soaking and cooking water was lyophilized as well and stored at -40 °C until further use, as well as powders of cooked whole beans and cotyledons.

2.5. Mineral analysis

Bean powders, as well as lyophilized cooking and soaking water, were then analysed for Mg, Ca, Fe and Zn content by ICP-OES analysis as in Gwala et al.²⁰ 10 mg sample (in duplicate) was incinerated in a muffle furnace at 550 °C and the remaining ashes were dissolved in 10 mL 1% HNO₃ solution. After 24 h, the sample was filtered through a 0.45 μm filter (Chromafil® A-45/25, German) and stored at 4 °C until mineral analysis in triplicate by ICP-OES analysis (ICAP 7000 series, ThermoFisher Scientific). Mg and Ca were measured radially at 285 nm and 318 nm, respectively, while Fe and Zn were measured axially at 238 nm and 206 nm. Accuracy of quantification was evaluated by analysing standardized reference material: BCR-129 (Joint Research Centre, European Commission). This material was ashed and analysed as described above. The reported minerals were recovered within 10% of the certified values of the BCR-129 reference (Supplementary Table 1A). Calibration was performed using SPS-SW2, before each run and after measuring 20 samples. Matrix effects were excluded by spiking the sample with different concentrations of Zn (Supplementary Table 1B).

2.6. Mineral bioaccessibility of processed beans samples

The bean powders obtained were subjected, in duplicate, to *in vitro* simulation of the oral, gastric and small intestinal phase of human digestion according to the standardized INFOGEST *in vitro* digestion method²¹, but without addition of salivary amylase in the oral phase (this was believed to not affect mineral bioaccessibility values). Before the start of the oral phase, bean powders were rehydrated with Milli-Q water up to the same moisture level they had after soaking and cooking. After the intestinal phase, samples were heat shocked (100 °C, 5 min) to inactivate enzymes and cooled in an ice bath to room temperature. To separate the bioaccessible fraction from the inaccessible fraction, samples were centrifuged (5000 g, 15 min). Pellets, which represent the inaccessible fraction, were then lyophilized, weighed and their Mg, Ca, Fe and Zn content was determined by ICP-OES analysis as described in section 2.5.

ARTICLE

Food & Function

Bioaccessibility (BAC) of Mg, Ca, Fe and Zn was then calculated (Equation 2).

$$\text{BAC (\%)} = \frac{A_i - A_p}{A_i} * 100 \quad \text{Equation 2}$$

A_i and A_p represent the initial mineral amount in the processed bean sample (mg/g) and the mineral amount in the pellet after digestion (mg/g), respectively.

2.7. Characterization of selected raw samples

An overview of parameters that were characterized in fresh and stored, raw beans is shown in Figure 2.

Figure 2. Experimental approach applied for the characterization of fresh and stored raw beans.

For determination of moisture content, total mineral content, soluble mineral content, phytic acid content, pectin content and pectin degree of methoxylation, whole beans, cotyledons and/or seed coats of fresh and stored beans (8 and 20 weeks) were first ground by ball milling (2 times 30 sec at 25/s) to create a representative powder. In contrast to these parameters, the cell wall bound mineral fraction was not determined in powders, but in slices.

Moisture content. Moisture content of whole raw beans, cotyledons and seed coats was determined in duplicate by vacuum-drying.²² 200 mg of whole beans and cotyledon powder and 50 mg of seed coat powder was dried using a vacuum oven (UNIEQUIP 1445-2, Planegg, Germany), with sequential drying steps for 1 h at 0.8, 0.6 and 0.4 bar and for 30 min at 0.2 bar. The difference in weight before and after drying represents the moisture content.

Total Mg, Ca, Fe and Zn content. The amount of Mg, Ca, Fe and Zn in whole raw beans, cotyledons and seed coats was determined in duplicate by ICP-OES analysis as described in section 2.5.

Soluble Mg, Ca, Fe and Zn content. In order to determine the soluble mineral fraction in whole raw beans and cotyledons, approximately 50 mg sample (in duplicate) was dissolved in 10

mL Milli-Q water at 25 °C while stirring. After 2 h, this solution was centrifuged (15 min, 5000 g) and supernatant was collected and lyophilized. The amounts of Mg, Ca, Fe and Zn in this dried supernatant was determined by ICP-OES analysis after ashing as described in section 2.5.

Mg, Ca, Fe and Zn bound to cell wall fraction in cotyledons. First, the seed coat was manually removed from the selected raw bean samples. Subsequently, these cotyledons were cut into slices with a thickness of 40 µm by using a cryomicrotome (Reichert Technologies, NY, USA). By using this approach, only cotyledon slices could be obtained since seed coats were too thin to be able to properly slice them. Cell content was then removed by washing the slices with Milli-Q water in multiple steps until the wash water was transparent. Thereafter, the slices (which now mainly consisted of cell wall material) were dried overnight in at 40 °C. The weight of the dry matter content in the wash water was determined after lyophilisation. The Mg, Ca, Fe and Zn content of the slices was measured by ICP-OES analysis after ashing as described in section 2.5.

Phytic acid content. For determination of phytic acid content in whole raw beans and cotyledons the “Phytic Acid Assay Kit” (Megazyme) was used and analysis was performed in duplicate. This method is based on spectrophotometric analysis of free phosphorus before and after phytase and alkaline phosphatase treatment of the bean powder. Consequently, phytic acid content was estimated based on the difference in phosphorus content.

Pectin content and degree of methoxylation. Since pectin contains a backbone of galacturonic acid (GalA) residues, GalA content was determined as a measure for pectin content. In a first step, alcohol insoluble residue (AIR) was extracted. GalA in AIR was then quantified by using a colorimetric assay as well as the methanol content because the degree of methoxylation (DM) is defined as the molar ratio of methanol to GalA. In duplicate, cell wall content (AIR) was extracted from whole bean and cotyledon powders based on the method of Chigwedere et al.¹⁷. First, starch and proteins were removed by enzymatic hydrolysis before AIR was extracted with ethanol and acetone. In duplicate, AIR was hydrolysed with concentrated sulphuric acid as described by Ahmed and Labovitch²³. Thereafter, GalA content of the hydrolysed samples was quantified in triplicate by a colorimetric assay.²⁴ In duplicate, the ester-bonds of pectin in AIR were saponified with 2 M NaOH.²⁵ Subsequently, quantification of the released methanol was done in triplicate according a colorimetric assay.²⁶

2.8. Statistical analysis

The data obtained are presented as the average of at least two measurements ± standard error. Differences for a certain parameter were statistically analysed using one-way ANOVA combined with Tukey’s test for multiple comparison ($P < 0.05$) with JMP statistical software (JMP Pro 14, Cary, NC, USA).

3. Results and discussion

3.1. HTC development and selection of storage periods

Evolution of hardness with cooking time is an indicator of the extent of HTC development in pulses.⁶ Therefore, cooking profiles of fresh and stored beans were established and are shown in Figure 3.

Figure 3. Cooking profiles of common beans that were stored at 35 °C and 80% RH, soaked and cooked in Milli-Q water at 95 °C. Markers represent mean values of at least 20 hardness measurements from 20 cotyledons expressed as a ratio of the initial hardness (H/H_i) $\pm$ standard errors. Continuous lines are corresponding modelled data by the fractional conversion model (Equation 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Based on the experimental data, it can be observed that for each storage period, the hardness decreased as cooking progressed. However, for a certain cooking time, the hardness was found higher when storage time increased. Kinetic modelling of the hardness as a function of cooking time enables quantification of the rate of hardness degradation and final hardness values in relation to different storage periods. Therefore, the fractional conversion model (Equation 1) was used and kinetic parameters (H_f , final hardness and k , rate constant of hardness evolution) were estimated (Table 1).

Table 1. Parameter estimates $\pm$ standard errors for H_f -values (final hardness) and k -values (rate constant of hardness evolution). Experimental data were first normalized (H/H_i) and then modelled according to the fractional conversion model. Different lower case letters (a-b) indicate significant differences ($p < 0.05$) for a certain parameter estimate.

	H_f -value (N)	k -value (min^{-1})
Fresh	0.114 ± 0.010^b	0.0546 ± 0.0018^a
Stored (4 weeks)	0.188 ± 0.016^a	0.0551 ± 0.0029^a
Stored (8 weeks)	0.225 ± 0.018^a	0.0232 ± 0.0015^b
Stored (12 weeks)	0.224 ± 0.015^a	0.0174 ± 0.0009^b
Stored (16 weeks)	0.210 ± 0.010^a	0.0172 ± 0.0007^b
Stored (20 weeks)	0.243 ± 0.010^a	0.0168 ± 0.0007^b

After 8 weeks of storage, the k -value was significantly decreased from 0.0546 min^{-1} to 0.0232 min^{-1} compared to fresh beans. A further decrease was observed for beans stored for 8 up to 20 weeks, although not significant. A lower k -value indicates that a longer cooking time is required to reach the final

residual hardness. The lower k -value after at least 8 weeks of storage indicates that the cooking behaviour was significantly changed and therefore it was concluded that hard-to-cook (HTC) development occurred. Based on these results, three storage periods with distinct cooking behaviour were selected: fresh beans, 8 weeks stored beans and 20 weeks stored beans. Although no further significant difference between the cooking profile of 20 weeks stored beans and 8 weeks stored beans was observed, it might be possible that other characteristics could have changed during this extra 12 weeks of storage. Moreover, batches of beans are often stored for these periods between two harvesting seasons. Therefore, beans that were stored for 20 weeks were selected (next to fresh and 8 weeks stored beans) as well as part of the further experiments.

3.2. Mineral bioaccessibility

The bioaccessibility of Mg, Ca, Fe and Zn in fresh and stored (8 and 20 weeks) cotyledons that were soaked, whether or not followed by cooking for 30, 60 or 120 min, was determined and shown in Figure 4.

Effect of storage. Figure 4 shows that bioaccessibility of Ca, Fe and Zn significantly decreased with storage time in cotyledons for each processing condition and is thus affected by development of HTC. Considering all processing conditions, the decrease in Ca, Fe and Zn bioaccessibility between fresh and 20 weeks stored beans ranged respectively from ca. 22 to 24%; from ca. 23 to 27% and from ca. 16 to 24%. Similar findings were reported by Kruger et al.¹⁴, who reported a decrease in Ca, Fe and Zn bioaccessibility in cowpeas that were HTC (stored for 21 days at 42 °C and 67% RH) in comparison to fresh cowpeas. These results suggest that during postharvest storage these minerals dislocate from a free state to a bound state. For example, they might be chelated by indigestible compounds, such as cell wall polymers which will be further discussed in §3.3 (Figure 6).

While Ca, Fe and Zn bioaccessibility was decreased, it was observed that postharvest storage did not significantly affect Mg bioaccessibility. Minerals can bind to a chelator with a strength proportional to their atomic mass and electronegativity. Compared to Ca, Fe and Zn, Mg has a lower atomic mass and electronegative value. Therefore, it can be hypothesized that during storage, Ca, Fe and Zn will more easily be chelated than Mg. For example, previously, Mg-ions were shown to limitedly interact with pectin. They are poor pectin crosslinking agents.²⁷ Besides, the binding mechanism between pectin and Mg-ions is described to be different from binding between pectin and Ca-, Fe- and Zn-ions, being through polycondensation instead of formation of an “egg-box” model.¹⁰ This might also explain why Mg bioaccessibility was found to be higher than Ca, Fe and Zn bioaccessibility in all conditions.

Effect of cooking. Figure 4 shows that mineral bioaccessibility of all minerals decreased with increased cooking time, irrespective of the storage condition. For example, in fresh beans the mineral bioaccessibility decreased from ca. 71% to 58% for Mg, from ca. 55% to 37% for Ca, from ca. 66% to 41% for Fe and from

Figure 4. Mineral bioaccessibility (BAC) (%) of soaked and/or cooked cotyledons (cooking time: 30, 60 or 120 min). Bars represent average $\pm$ standard errors. Mineral bioaccessibility experiments were performed in duplicate. Different lower case letters (a-h) indicate significant differences ($p < 0.05$) between samples and cooking time for a certain mineral.

ca. 68% to 47% for Zn between soaking and 120 min cooking. Taking into consideration only one cooking time (30 min), Pereira de Oliveira et al.¹⁸ reported for several divalent minerals similar findings, namely that cooking can decrease the mineral bioaccessibility in beans. Various changes that can occur during cooking might influence mineral bioaccessibility. According to Pereira de Oliveira et al.¹⁸, proteins can undergo denaturation upon cooking which might affect their ion chelating capacity and consequently the mineral bioaccessibility. Chigwedere et al.¹⁷ reported that during cooking, pectin can be solubilized which might increase its mineral binding capacity since the molecule becomes more accessible. As a consequence of these possible phenomena, mineral bioaccessibility is decreased upon cooking.

Effect of seed coat: cotyledons vs. whole beans. The effect of postharvest storage and cooking on mineral bioaccessibility in whole beans shows similar trends as in cotyledons (Supplementary figure 1). Both parameters decreased mineral bioaccessibility in whole beans. However, the mineral bioaccessibility is lower in whole beans than in cotyledons, suggesting that the seed coat contains more and/or different mineral chelators. Therefore, raw cotyledon and whole beans samples were further characterized and results are discussed in section 3.3.

3.3. Characterization of raw fresh and stored bean samples

In order to explain the effect of postharvest storage on mineral bioaccessibility, raw and stored bean samples were characterized in terms of important mineral chelators (phytic acid and pectin) and total, soluble and cell wall bound mineral content was determined.

Moisture content. The moisture content in fresh, 8 weeks stored and 20 weeks stored raw whole beans was respectively 10.93 ± 0.01 , 13.32 ± 0.05 and $13.90 \pm 0.01\%$. This increase in moisture content with storage time was expected since beans were stored a relative humidity of 80%. This increase was also observed in cotyledon and seed coat fractions. Although the moisture content in stored raw beans is still quite low, the molecular mobility might have been increased during storage since moisture content was increased. Besides, under these conditions beans are stored above their glass transition temperature whereby the system is physicochemical unstable and molecular mobility is possible.²⁸

Table 2. Phytic acid content (mg/g dry sample), galacturonic acid (GalA) content (mg/g dry sample) and degree of methylesterification (DM) of pectin in the raw cotyledon fraction and in whole raw beans for different storage times. Values represent average $\pm$ standard errors. Different lower case letters (a-b) indicate significant differences ($p < 0.05$) for a parameter between storage times. n.d. is not determined.

	Storage time (weeks)	Phytic acid content (mg/g dry sample)	GalA content (mg/g dry sample)	DM (%)
Cotyledons	0	12.80 $\pm$ 0.29 ^a	23.9 $\pm$ 4.1 ^b	62.22 $\pm$ 7.91 ^a
	8	11.46 $\pm$ 0.24 ^b	n.d.	n.d.
	20	9.06 $\pm$ 0.12 ^c	21.0 $\pm$ 3.8 ^b	66.89 $\pm$ 3.99 ^a
Whole bean	0	12.00 $\pm$ 0.17 ^a	30.8 $\pm$ 1.3 ^a	38.89 $\pm$ 4.73 ^b
	8	10.46 $\pm$ 0.36 ^b	n.d.	n.d.
	20	8.18 $\pm$ 0.19 ^c	30.9 $\pm$ 2.4 ^a	38.21 $\pm$ 7.14 ^b

Phytic acid content. Total phytic acid content of cotyledons of raw beans and whole raw beans for different storage times is represented in Table 2. During storage, total phytic acid content was reduced indicating that during storage phytic acid is hydrolysed by endogenous phytase enzymes.^{5,14} If phytic acid is hydrolysed, its mineral binding capacity is reduced and therefore it is believed that minerals are released from phytic acid.^{13,29} As a consequence, it can be expected that the mineral bioaccessibility increases with storage time since phytic acid-cation complexes are broken down. However, it was found that mineral bioaccessibility decreased upon storage. Possibly, storage allows mineral reorganization between mineral chelators. For example, minerals which are released from phytic during storage are chelated by other molecules (e.g. pectin).

Pectin content and degree of methoxylation. Pectin is a polysaccharide which has a backbone of α -1,4-linked galacturonic acid (GalA) residues.⁸ Therefore, GalA content was determined as a measure for pectin content in raw whole bean and cotyledons samples of different storage times and is shown in Table 2. As expected, GalA content remained constant during storage but is significantly more present in whole beans compared to cotyledon fractions only. Besides, the degree of methoxylation (DM) of pectin was found to be lower in whole beans in comparison to cotyledon fractions only. As a consequence, the mineral binding capacity related to pectin of whole beans is believed to be higher since it contains more pectin and its DM is lower. In accordance to Chigwedere et al.¹⁷, the DM did not change with occurrence of the HTC defect, suggesting that changes in DM do not play a crucial role in HTC development. Although mineral bioaccessibility decreased with storage time, the intrinsic mineral binding capacity of beans, which is related to negatively charged groups of pectin, did not increase with storage time. This suggests that possibly postharvest storage at tropical conditions (high temperature and high relative humidities) enhances mineral mobility rather than causing a molecular change in pectin that increases its binding capacity. This increased mineral mobility might be caused by the increased moisture content during storage and cell membrane damage.⁷ Consequently, membrane damage is believed to allow for relocation of minerals from the cytoplasm to the cell wall. Moreover, as the intrinsic mineral binding capacity of pectin was found not to be affected by postharvest storage, it is suggested that in fresh, raw beans not all mineral

binding sites of pectin were fully occupied/saturated with minerals. Due to enhanced molecular mobility, because of increased moisture content along with membrane damage during storage, minerals from within the cell can now migrate to the cell wall and become chelated by available pectin binding sites (§3.3 Mg, Ca, Fe and Zn bound to cell wall fraction), explaining the decrease in mineral bioaccessibility (Figure 4).

Total Mg, Ca, Fe and Zn content. It was found that fresh whole common beans contain on average 2.1 mg Mg, 1.5 mg Ca, 69.3 μ g Fe and 22.8 μ g Zn per gram dry bean. As expected, no significant effect of storage time on total mineral content in the different fractions was observed (Supplementary figure 2). As a consequence, it can be concluded that there is no mineral translocation between seed coat and cotyledon during storage which can be explained by the fact that movement of minerals between these two elements is restricted.

In general, Mg and Ca are more abundantly present in common beans than Fe and Zn. These results are comparable to what has been reported in other studies.^{2,30} From the measured mineral content in cotyledon and seed coat (Supplementary figure 2), the mineral distribution over cotyledon and seed coat fractions was calculated, taking into consideration the determined w% of cotyledon and seed coat fractions on dry a basis (respectively, $\pm$ 91% and $\pm$ 9%). It was found that approximately 75%, 30%, 75% and 92% of respectively Mg, Ca, Fe and Zn is present in the cotyledon fraction. It can be noted that for the minerals investigated, Ca is more present in the seed coat while the other minerals are mainly found in the cotyledons. Ca is known to interact with pectin according to the “egg-box” model and more Ca can interact with pectin when DM is lower.¹⁵ Since it was hypothesized before that more pectin with a lower DM is present in the seed coat than in the cotyledon fraction, it can be deduced that from all the investigated minerals, Ca is probably interacting thoroughly with pectin in the seed coat.

Soluble Mg, Ca, Fe and Zn content. As can be observed in Figure 5, the soluble mineral content in cotyledons and whole beans is decreasing with storage time, except for Mg. This reduction indicates that during storage unbound minerals become more and more chelated by molecules to create insoluble complexes. For example, insoluble cation-pectin complexes, which have been proposed to be formed during development of the HTC

Figure 5. Soluble mineral content (%) in (A) cotyledon fractions of raw beans and (B) whole raw beans for different storage times. Bars represent average $\pm$ standard errors. Different lower case letters (a-c) indicate significant differences ($p < 0.05$) between samples for a certain mineral.

defect⁵, might be responsible for the reduction in soluble mineral content.

Minerals are believed to be able to migrate to available binding sites on pectin, which now came accessible due to membrane damage and increased moisture content, during storage (§3.3 Mg, Ca, Fe and Zn bound to cell wall fraction). It seems that the soluble Mg content is not affected by storage time and is probably related to the fact that Mg-ions are poor pectin crosslinking agents as described before (§3.2 effect of storage). This can also explain why Mg has a higher soluble mineral concentration compared to the other minerals. The reduction in soluble mineral content can explain why mineral bioaccessibility was decreased upon storage time. Moreover, soluble mineral content in whole beans was found to be lower than in cotyledons. This might indicate that the seed coat has a significant contribution to the observed mineral binding capacity. This is in accordance to what was observed for pectin content and DM: the seed coat fraction contains more pectin and has a lower DM. In addition, the seed coat can contain other and/or more mineral chelators than the cotyledon fraction, such as for example, polyphenols, which also can cause a lower soluble mineral fraction in whole beans.³¹

Mg, Ca, Fe and Zn bound to cell wall fraction in cotyledons. The previous results show that not all minerals are present in a free state in common beans and therefore a certain mineral fraction is chelated by, for example, cell wall polymers, phytic acid or polyphenols. Figure 6 represents the cell wall bound mineral fraction of the raw cotyledon samples for different storage times. Upon storage, the Ca, Fe and Zn content that is present in the cell wall material is increased, indicating that during storage, minerals are chelated by cell wall polymers, such as pectin. This increase in cell wall bound minerals can explain why the soluble mineral content and the mineral bioaccessibility was decreased with storage.

From these results it can be concluded that there is clearly mineral mobility within the cell. Moreover, according to the pectin-cation-phytate hypothesis, the pectin DM decreases and consequently its mineral binding capacity increases during postharvest storage. However, in this research, the average DM was found not to decrease with storage time but the cell wall bound mineral fraction did increase. As a consequence, it can be assumed that pectin already had available binding sites for minerals. Due to enhanced molecular mobility by increased moisture content and membrane damage, these available binding sites became accessible for free minerals. Similar to Mg bioaccessibility and free Mg fraction, the cell wall bound-Mg fraction was not affected by postharvest storage. When combining values of the soluble mineral fraction with the cell-wall bound mineral fraction it is clear that a certain mineral fraction is still bound to one or more other mineral chelators. Moreover, with increasing storage time, the sum of the soluble mineral fraction and the cell wall-bound mineral fraction is showing a small increasing trend for Ca, Fe and Zn. This might be due to the release of minerals from phytic acid which was found to be degraded during storage.

Figure 6. Cell wall bound mineral fraction (%) in cotyledons of raw beans for different storage times. Bars represent average $\pm$ standard errors. Different lower case letters (a-c) indicate significant differences ($p < 0.05$) between samples for a certain mineral.

Figure 7. Mineral distribution over soaking water, cooking water and the remaining bean fraction of fresh and stored (35°C and 85% R.H.) beans that were soaked and cooked up to 120 min. The absolute quantities and the associated standard deviations are represented in the Supplementary Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Mineral leaching during soaking and cooking

Mineral distribution over soaking water, cooking water and the remaining cooked bean fraction after soaking up to 120 min cooking of whole beans is shown in Figure 7. Based on the results for the fresh samples, it can be concluded that leaching is occurring during cooking rather than during soaking and that Mg is more sensitive to leaching during soaking and/or cooking than the other minerals studied. After soaking and 120 min cooking, only ca. 40% of Mg is retained in the bean fraction, while ca. 85% of Ca, ca. 80% of Fe and ca. 70% of Zn is still present. Moreover, Mg leached mainly during the first 30 min

of cooking. Leaching of Mg can be explained as this ion is believed to be the least interactive with low DM pectin given its low electronegativity and higher effective hydrated diameter²⁷ in comparison with Ca, Fe and Zn. Similar findings are reported by Chigwedere et al.¹⁷ The result that Mg is more sensitive than the other minerals to leaching into processing media during cooking of fresh beans, can also be observed in stored beans. In the end, postharvest storage did not affect the total amount of mineral that is leached after soaking and 120 min cooking. Additionally, no significant effect of storage time on total mineral content in whole beans, cotyledons and seed coats was observed. However, the longer the beans are stored, the more

minerals are already leaching into to soaking water while less minerals are subsequently leached during cooking. It was observed that leaching of overall dry matter during soaking was increased after longer storage (data not shown) which explains the increased minerals in the soaking water as well. This increased dry matter leaching during soaking of HTC beans in comparison to fresh beans, has been linked to membrane damage due to storage.⁷

4. Conclusions

During postharvest storage under tropical conditions (35°C and 80% R.H.), beans developed a significant different cooking behaviour already after 8 weeks of storage, expressed by an increased final residual hardness and longer cooking time required to reach particular softening levels. This behaviour was attributed to the hard-to-cook (HTC) development of pulses. Since minerals are believed to be involved in this HTC development phenomenon, it was hypothesized that postharvest storage-induced molecular changes might influence mineral bioaccessibility. It was observed that postharvest storage decreased Ca, Fe and Zn bioaccessibility. This decrease was proven to be attributed to minerals being more and more chelated by cell wall polymers. In addition, phytic acid (another important mineral chelator) content decreased during postharvest storage, as a consequence soluble mineral content is expected to increase and therefore also mineral bioaccessibility. Since mineral bioaccessibility was decreased, cell wall polymers (e.g. pectin) are believed to play the major role in affecting mineral bioaccessibility during postharvest storage. This study indicates that mineral translocation during postharvest storage is occurring: from a free state to a more bound state, or they can be released from one binding agent (e.g. phytic acid) and then again chelated by another (e.g. pectin). This increased mineral mobility is probably caused by increased moisture content during storage. Decrease in mineral bioaccessibility during cooking is believed to be attributed to chelation of free minerals by molecules that gain accessibility as a result of the cooking process (e.g. pectin solubilisation).

Mineral bioaccessibility was found lower in whole beans compared to cotyledons which could be related to higher pectin mineral binding capacity of whole beans since they contain relatively more pectin and their degree of methoxylation is lower compared to cotyledons. For the minerals investigated, it can be concluded that Mg shows different behaviour than Ca, Fe and Zn. Postharvest storage did not affect Mg bioaccessibility, soluble Mg content in whole beans and cotyledons and Mg content in cell wall fraction of cotyledons. Moreover, Mg was the most sensitive mineral to leach in soaking water and cooking water. All these observations could be linked to the fact that Mg-ions intrinsically possess characteristics that cause lower interacting properties with mineral chelators such as pectin. Mg-ions have a lower electronegativity and a higher effective hydrated diameter in comparison to Ca, Fe and Zn.

Overall, it was shown that, despite high mineral concentrations in beans, low mineral fractions are available for small intestinal absorption. Moreover, it can be concluded that postharvest storage has a negative effect on mineral bioaccessibility of common beans and unfortunately this cannot be counteracted by conventional processing. However, dehulling can lead to an improvement of mineral bioaccessibility.

In general, further *in vivo* validation is required with special focus on the relevance and importance of the large intestinal phase and the role of the gut microbiome. If it can be proven *in vivo* that minerals can be absorbed at this stage of digestion, it might be interesting to include this digestive phase in future experiments as well because pectin is fermented in the colon and the fermentation process is supposed to influence the pectin mineral binding capacity.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 J. E. B. de Souza and E. P. D. B. Ferreira, Improving sustainability of common bean production systems by co-inoculating rhizobia and azospirilla, *Agric. Ecosyst. Environ.*, 2017, **237**, 250–257.
- 2 I. Hayat, A. Ahmad, T. Masud, A. Ahmed and S. Bashir, Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): an overview., *Crit. Rev. Food Sci. Nutr.*, 2014, **54**, 580–592.
- 3 K. Platel and K. Srinivasan, Bioavailability of Micronutrients from Plant Foods: An Update, *Crit. Rev. Food Sci. Nutr.*, 2015, **56**, 1608–1619.
- 4 T. K. Basu and D. Donaldson, Intestinal absorption in health and disease: micronutrients, *Best Pract. Res. Clin. Gastroenterol.*, 2003, **17**, 957–979.
- 5 C. Reyes-Moreno and O. Parades-López, Hard-to-cook phenomenon in common beans — A review, *Crit. Rev. Food Sci. Nutr.*, 1993, **33**, 227–286.
- 6 C. M. Chigwedere, T. F. Olaoye, C. Kyomugasho, Z. J. Kermani, A. P. Pallares, A. M. Van Loey, T. Grauwet and M. E. Hendrickx, Mechanistic insight into softening of Canadian wonder common beans (*Phaseolus vulgaris*) during cooking, *Food Res. Int.*, 2018, **106**, 522–531.
- 7 J. C. RICHARDSON and D. W. STANLEY, Relationship of Loss of Membrane Functionality and Hard-to-Cook Defect in Aged Beans, *J. Food Sci.*, 1991, **56**, 590–591.
- 8 A. G. J. Voragen, G. J. Coenen, R. P. Verhoef and H. A. Schols, Pectin, a versatile polysaccharide present in plant

- cell walls, *Struct. Chem.*, 2009, **20**, 263–275.
- 9 E. R. Morris, D. A. Powell, M. J. Gidley and D. A. Rees, Conformations and interactions of pectins. I. Polymorphism between gel and solid states of calcium polygalacturonate, *J. Mol. Biol.*, 1982, **155**, 507–516.
 - 10 M. Celus, C. Kyomugasho, A. M. Van Loey, T. Grauwet and M. E. Hendrickx, Influence of pectin structural properties on interactions with divalent cations and its associated functionalities, *Compr. Rev. Food Sci. Food Saf.*, 2018, **17**, 1576–1594.
 - 11 S. Rousseau, C. Kyomugasho, M. Celus, N. Yeasmen, M. E. Hendrickx and T. Grauwet, Zinc bioaccessibility is affected by the presence of calcium ions and degree of methylesterification in pectin-based model systems, *Food Hydrocoll.*, 2019, **90**, 206–215.
 - 12 S. Rousseau, C. Kyomugasho, M. Celus, M. E. G. Hendrickx and T. Grauwet, Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing foods and the perspectives for food processing, *Crit. Rev. Food Sci. Nutr.*, 2019, **60**, 826–843.
 - 13 V. Kumar, A. K. Sinha, H. P. S. Makkar and K. Becker, Dietary roles of phytate and phytase in human nutrition: A review, *Food Chem.*, 2010, **120**, 945–959.
 - 14 J. Kruger, R. Minnis-ndimba, C. Mtshali and A. Minnaar, Novel in situ evaluation of the role minerals play in the development of the hard-to-cook (HTC) defect of cowpeas and its effect on the in vitro mineral bioaccessibility, *Food Chem.*, 2015, **174**, 365–371.
 - 15 M. Celus, C. Kyomugasho, L. Salvia-Trujillo, J. Van Audenhove, A. M. Van Loey, T. Grauwet and M. E. Hendrickx, Interactions between citrus pectin and Zn²⁺ or Ca²⁺ and associated in vitro Zn²⁺ bioaccessibility as affected by degree of methylesterification and blockiness, *Food Hydrocoll.*, 2018, **79**, 319–330.
 - 16 M. Siddiq and M. A. Uebersax, *Dry Beans and Pulses*, John Wiley & Sons, Inc., 2013.
 - 17 C. M. Chigwedere, C. M. Nkonkola, S. Rai, C. Kyomugasho, Z. J. Kermani, A. P. Pallares, A. Van Loey, T. Grauwet and M. Hendrickx, Cotyledon pectin molecular interconversions explain pectin solubilization during cooking of common beans (*Phaseolus vulgaris*), *Food Res. Int.*, 2019, **116**, 462–470.
 - 18 A. Pereira de Oliveira, B. dos Santos Olivaira Mateo, A. M. Fioroto, P. Vitoriano de Oliveira and J. Naozuka, Effect of cooking on the bioaccessibility of essential elements in different varieties of beans (*Phaseolus vulgaris* L.), *J. Food Compos. Anal.*, 2018, **67**, 135–140.
 - 19 A. Pallares Pallares, B. Loosveldt, S. N. Karimi, M. Hendrickx and T. Grauwet, Effect of process-induced common bean hardness on structural properties of in vivo generated boluses and consequences for in vitro starch digestion kinetics, *Br. J. Nutr.*, 2019, **122**, 388–399.
 - 20 S. Gwala, C. Kyomugasho, I. Wainaina, S. Rousseau, M. Hendrickx and T. Grauwet, Ageing, dehulling and cooking of Bambara groundnuts: consequences for mineral retention and in vitro bioaccessibility, *Food Funct.*, 2020, **11**, 2509–2521.
 - 21 M. Minekus, M. Almgier, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carri, R. Bouteau, F. M. Corredig, D. Dupont, F. C. Dufour, L. Egger, M. Golding, L. S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Enard, I. Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. J. Wickham, W. Weitschies and A. Brodtkorb, A standardised static in vitro digestion method suitable for food – an international consensus, *Food Funct.*, 2014, **5**, 1113–1124.
 - 22 H. H. Nguyen, A. Shpigelman, S. Van Buggenhout, K. Moelants, H. Haest, O. Buysschaert, M. Hendrickx and A. Van Loey, The evolution of quality characteristics of mango piece after pasteurization and during shelf life in a mango juice drink, *Eur. Food Res. Technol.*, 2016, **242**, 703–712.
 - 23 E. A. R. Ahmed and J. M. Labavitch, A simplified method for accurate determination of cell wall uronide content, *J. Food Biochem.*, 1978, **1**, 361–365.
 - 24 N. Blumenkrantz and G. Asboe-Hansen, New method for quantitative determination of uronic acids, *Anal. Biochem.*, 1973, **54**, 484–489.
 - 25 A. Ng and K. W. Waldron, Effect of cooking and pre-cooking on cell-wall chemistry in relation to firmness of carrot tissues, *J. Sci. Food Agric.*, 1997, **73**, 503–512.
 - 26 J. A. Klavons and R. D. Bennett, Determination of methanol using alcohol oxidase and its application to methyl ester content of pectins, *J. Agric. Food Chem.*, 1986, **34**, 597–599.
 - 27 C. Kyomugasho, S. Gwala, S. Christiaens, Z. Jamsazzadeh Kermani, A. M. Van Loey, T. Grauwet and M. E. Hendrickx, Pectin nanostructure influences pectin-cation interactions and in vitro-bioaccessibility of Ca²⁺, Zn²⁺, Fe²⁺ and Mg²⁺ ions in model systems, *Food Hydrocoll.*, 2017, **62**, 299–310.
 - 28 C. M. Chigwedere, J. N. Humerez Flores, A. Panozzo, A. M. Van Loey and M. E. Hendrickx, Instability of common beans during storage causes hardening: The role of glass transition phenomena, *Food Res. Int.*, 2019, **121**, 506–513.
 - 29 Y. Luo and W. Xie, Effect of phytase treatment on iron bioavailability in faba bean (*Vicia faba* L.) flour, *Food Chem.*, 2012, **134**, 1251–1255.
 - 30 S. E. Suárez-Martínez, R. A. Ferriz-Martínez, R. Campos-Vega, J. E. Elton-Puente, K. de la Torre Carbot and T. García-Gasca, Bean seeds: leading nutraceutical source for human health, *CyTA J. Food*, 2016, **14**, 131–137.
 - 31 L. Bravo, Polyphenols : Chemistry , Dietary Sources , Metabolism , and Nutritional Significance, *Nutr. Rev.*, 1998, **56**, 317–333.

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Postharvest storage negatively influences common bean mineral bioaccessibility which cannot be counteracted by adapting conventional processing variables. However, dehulling can lead to an improvement of mineral bioaccessibility.