Vegetable relishes, high in β-carotene, increase the iron, zinc and β-carotene nutritive values from cereal porridges

Reference:

Kruger Johanita, Breynaert Annelies, Pieters Luc, Hermans Nina.- Vegetable relishes, high in β-carotene, increase the iron, zinc and β-carotene nutritive values from cereal porridges
International journal of food sciences and nutrition - ISSN 0963-7486 - (2017), p. 1-7
Full text (Publisher's DOI): https://doi.org/10.1080/09637486.2017.1360259
To cite this reference: http://hdl.handle.net/10067/1451300151162165141

Vegetable relishes, high in β-carotene, increase the iron, zinc and β-carotene nutritive values from cereal porridges

Johanita Kruger[a]*, Annelies Breynaert[b], Luc Pieters[c], Nina Hermans[b]

[a]Department of Food Science and Institute for Food, Nutrition and Well-being, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

[b]NatuRA– Laboratory of Nutrition and Functional Food Science, Department of Pharmaceutical Sciences, Campus Drie Eiken, Building A, Universiteitsplein 1, Wilrijk, 2610, Belgium (nina.hermans@uantwerp.be; annelies.breynaert@uantwerp.be)

[c]NatuRA – Laboratory of Pharmacognosy and Pharmaceutical Analysis, Department of Pharmaceutical Sciences, University of Antwerp, Campus Drie Eiken, Building A, Universiteitsplein 1, Wilrijk, 2610, Belgium (Luc.pieters@uantwerp.be)

*Corresponding author: johanita.kruger@up.ac.za, Tel: + 27 12 420-3238, Fax: +27 12 420 2839

Johanita Kruger[a]* (PhD): conception and design of the study, generation assembly, analysis and/or interpretation of data, and drafting and revision of the manuscript, approval of the final version of the manuscript

Annelies Breynaert[b] (PhD): generation of data, drafting and revision of the manuscript, approval of the final version of the manuscript

Luc Pieters[c] (PhD): conception and design of the study, approval of the final version of the manuscript

Nina Hermans[b]: generation of data, revision of the manuscript, approval of the final version of the manuscript

Key words: sorghum, maize, cowpea leaves, OFSP, inhibitors, bioaccessibility

Word count: 3350

Abstract

Iron, zinc and vitamin A deficiencies, are serious public health problems in sub-Saharan Africa, which can be alleviated by dietary diversification. The effects of adding cowpea leaf (CL) and orange-fleshed sweet potato (OFSP) relishes to sorghum and maize porridges on iron, zinc and β-carotene contents and bioaccessibilities were determined. Except for the high iron content of the CL relish (14.59 mg/100 g), the vegetable relishes had little effect on the iron bioaccessibility from the cereal porridges. Importantly, the addition of the CL relish increased the percentage and amount of bioaccessible zinc 2 and 3 fold, respectively. Addition of CL and OFSP relishes resulted in β-carotene contents of 10-13 mg/100 g. The β-carotene from the OFSP relish meals was double as bioaccessible than that from the CL relish meals. Addition of the vegetable relishes has real potential to improve especially the vitamin A and zinc nutritive value of cereal diets.

Abbreviations: AAS – atomic absorption spectroscopy, CL – cowpea leaves, FWS – fermented white sorghum, OFSP – Orange fleshed sweet potatoes, OM – orange maize, TS tannin sorghum, WM – white maize, WS – white sorghum

1. Introduction

Iron, zinc and vitamin A deficiencies are global public health problems, especially during the first 1000 days of life, from conception to a child’s second birthday, including the nutritional status of the pregnant and/or lactating mother. According to the Global Nutrition Report (International Food Policy Research Institute 2015), globally, 29% of women of reproductive age are anaemic, 15% of infants are born with a low birth weight and 164 million children under the age of 5 are stunted. These statistics are even worse in developing countries, for example 50% pregnant woman and about 40% of preschool children are estimated to be anaemic (World Health Organisation 2016). A recent pooled analysis of population based surveys found that the prevalence of vitamin A deficiency (VAD) in sub-Saharan Africa has increased significantly ($p \leq 0.05$) from 45% in 1991 to 48% in 2013 (Mason et al. 2014). This is of incredible consequence as the VAD in 138 other low and middle-income countries outside of sub-Saharan Africa, was estimated to have declined 10 percentage points, from 39 to 29%.

This is often because many households depend on monotonous cereal-based diets, low in vitamin A and high in mineral bioavailability inhibitors (phytate, tannins and other phenolics), for energy as well as micronutrients. Maize and sorghum are very important crops in sub-Sharan Africa. In 2013 the food supply in sub-Sharan Africa included 37 320 and 13 622 thousand tonnes of maize and sorghum cereal and food products, respectively, compared to 19 171 thousand tonnes of wheat (Food and Agricultural Organisation 2013). Under subsistence farmers and low socio-economic populations, affordable and sustainable dietary diversification is exceedingly important to alleviate malnutrition (Miller and Welch, 2013). Orange fleshed sweet potato (OFSP) is widely consumed throughout sub-Saharan Africa and even further promoted by the international potato centre and it’s partners as a tool to alleviate VAD (Hagenimana and Low 2000). Cowpea leaves and other dark green leafy vegetables are also consumed throughout sub-Saharan Africa and are often foraged by some of the most vulnerable populations (Singh et al. 2003). While CL (Uusiku et al. 2013) and orange OFSP (Vimala, et al. 2011) can play a major role in increasing the nutrient (iron, zinc and/or pro-vitamin A) contents of a cereal-based staple diet, there is limited to no information on the nutrient bioavailability from such meals.

This study evaluated the effect of adding CL and OFSP relishes on the mineral and vitamin A nutritive value of monotonous cereal diets by looking at the nutrient and bioavailability inhibitor contents and iron, zinc and β-carotene bioaccessibilities.

2. Materials and methods

2.1. Vegetable and cereal crops

The following cereals and vegetables were used in the study: White tan-plant sorghum (WS) (Macia, cultivated in Botswana), red tannin (type III) sorghum (TS) (hybrid PAN 3860,kindly donated by PANNAR Seeds, Klerksdorp, South Africa), white maize (WM) and pro-vitamin A biofortified (traditional breeding) orange maize (OM)(both kindly donated by the African Centre for Crop Improvement (ACCI), Pietermaritzburg, South Africa), young cowpea leaves (CL) (handpicked at the Ukulima Research Farm, Limpopo Province, South Africa) and orange-fleshed sweet potato (OFSP) (Bophelo, Kindly donated by the Agricultural Research Council (ARC), Roodeplaat, Vegetable and Ornamental Plants institute, Gauteng, South Africa).

2.2. Preparation of cereal porridges and vegetable relishes

To remove soil and dust contamination, all grains were quickly rinsed (<30 seconds) with distilled water and dried overnight at 25ºC. The cereals were then milled using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) fitted with a 500 μm opening screen. The normal thick porridges and traditional spontaneous fermented porridges were prepared according to Kruger et al. (2012).

The OFSP was boiled whole (30 mins), with the skin on, to minimise nutrients leaching into the boiling water. The OFSP was then cooled to room temperature, cut into approximately 1.5 cm^2 cubes. The Cowpea leaves were cooked in small amounts of water as described for boiled amaranth (Faber et al. 2010). As preparation for the analyses, after cooking, all samples were frozen at -20ºC, freeze-dried, crushed and again passed through a 500 μm screen to ensure homogenous samples. All samples were then stored at -4^{o}C in airtight and opaque containers to minimize degradation of β-carotene.

2.3. Meal compositions

The OFSP relish was added to each cereal porridge at a 1: 1 (db) porridge to relish ratio. The cowpea leaf relish was added to the cereal porridges at a lower 3: 2 (db) porridge to relish ratio.

2.4. Analyses

2.4.1. Chemicals and reagents

All chemicals and reagents were of analytical grade except nitric acid and hydrogen peroxide that were suprapur and the Zn and Fe standards were certipur.

2.4.2. Phytate, total phenolics, mineral and β-carotene contents

Phytate content was determined using an indirect quantitative anion exchange method (Fruhbeck et al. 1995). Total phenolic content was determined using a modified FolinCiocalteu method (Kaluza et al. 1980). Tannin content was determined a modified vanillin assay (Price et al. 1978). The modification in both the total phenolic and tannin assays were that reagent blanks that corrected for the colour of the flour extracts were included. Dialysates and acid digested food (Hendrix et al. 1998) and retentates (Bosscher et al. 1998) samples were analysed for iron and zinc using Atomic Absorption Spectroscopy (AAS) analysis (Flame AAS, Perkin-Elmer, Analyst 400) as previously described (Bosscher et al. 1998; Hendrix et al. 1998). β-carotene from the food, retentate and dialysis samples was extracted and analysed as described by Hermans et al. (2014) using an Agilent 1260 HPLC system with colorimetric 8-channel detector (ESA 5600A Coularray Detector). Extraction and analyses was done under subdued light.

2.4.3. Iron, zinc and β-carotene bioaccessibilities

The experimental set up (gastric and small intestine) was based on an *in vitro* continuous flow dialysis model, described by Breynaert et al. (2015). Fat was added at 10% (w/w) to the food samples before digestion to ensure micelle formation during digestion. The intestinal phase was simulated using Amicon stirred cells equipped with a dialysis membrane with molecular weight cut-off of 1000 Da. Continuous removal of the dialysate mimics the one-way gastrointestinal absorption form the lumen to the mucosa. After the intestinal phase, both the retentate (non-bioaccessible compounds) and the dialysate (bioaccessible compounds) were stored at -80°C prior to analysis. The bioaccessibility of the element (Fe or Zn) was calculated from the amount of element that passed the dialysis membrane in proportion to the total elemental content of the original food sample. β-carotene bioaccessibility or micellization was determined by centrifuging the digest (stomach and intestinal digestion) (3500 g, 50 min) and then filtering through a 20 µm syringe filter to isolate smaller, bioaccessible micelles.

2.5. Statistical analyses

Statistical analyses were performed by using the Statistica 12 (StatSoft, Johannesburg, South Africa). One-way analysis of variance (ANOVA) and multifactorial ANOVA with Fisher's LSD Post-hoc test was applied to determine significant differences between specific means at a confidence level of 95% ($p \leq 0.05$).

3. Results and discussion

3.1. Nutrient and bioavailability inhibitor contents

The iron contents of the Orange fleshed potato relish (OFSP) relish, white sorghum (WS), fermented white sorghum (FWS), white maize (WM) and orange maize (OM) porridges were similar (2.20 ± 0.13 to 3.40 ± 0.12 mg/100 g), but significantly ($p \leq 0.05$) lower than that of the tannin sorghum (TS) porridge (5.46 ± 0.18 mg/100 g) (Table 1). The iron content of the cowpea leaf (CL) relish however, was approximately 3 to 6 times higher than that of the cereal porridges ($p \leq 0.05$). The OFSP had the lowest zinc content (0.89 ± 0.05 mg/100 g) ($p \leq 0.05$), while that of the cereal porridges were similar (2.05 ± 0.1 to 2.84 ± 0.08 mg/100 g). The CL relish had approximately 2 to 3 times higher zinc content compared to the cereal porridges. The β-carotene content of the OM porridge was substantially lower (0.1 ± 0.0 mg/100 g) than that of the OFSP and CL relishes (27.3 ± 3.9and 25.3 ± 0.8 mg/100 g, respectively).

Concerning the bioavailability inhibitor contents, fermentation resulted in a significant ($p \leq 0.05$), 59% phytate reduction in the WS (Table 2). The FWS and OFSP porridge had substantially lower phytate contents compared to the other dishes (492 ± 34 and 353 ± 31 mg/100 g, respectively). The phenolic content of the CL relish was the highest (5295 ± 65 mg/100 g), followed by that of the TS (1504 ± 36 mg/100g). Interestingly, the total phenolic content of the OFSP (692 ± 12 mg/100 g) was substantially higher than the non-tannin cereal porridges (133 ± 9 to 290 ± 14 mg/100 g). As expected, the tannin contents of all the dishes were negligibly low (109 ± 42-158 ± 47 mg/100 g), except for the CL relish (1021 ± 44 mg/100 g) and TS porridge (2382 ± 133 mg/100 g).

3.2. ***Nutrient bioaccessibilities***

The total iron, zinc and β-carotene contents of the dishes and meals varied greatly (Table 1), which would make comparing only the percentage nutrient bioaccessibilities (%

bioaccessible nutrient of total nutrient content) insufficient. For this reason the discussion considers both the percentage and amount (mg bioaccessible nutrient/100 g food product) of bioaccessible nutrients of the different meals. This makes it is possible to get a good indication of the sum of the effects of differences in the total nutrient contents as well as the nutrient bioaccessibility inhibitors and enhancers (Kruger et al. 2015).

3.3. Iron and zinc bioaccessibilities

Fermentation of the WS (FWS) significantly ($p \leq 0.05$) increased the percentage, as well as, amount of bioaccessible zinc (LS mean, Table 2). It had, however, no significant ($p > 0.05$) effect on the iron bioaccessibility (Table 3). It is possible that the phytate content (Table 1) was not reduced enough to improve the iron bioaccessibility. The phytate:zinc and phytate:iron molar ratios, of the various meals with the FWS porridge, were 14 - 30 and 6 - 15, respectively. The critical levels for phytate:zinc, and phytate:iron molar ratios, above which the mineral bioaccessibility is seriously impaired are 15 (Saha, et al. 1994) and 1 (Hunt 2003), respectively. The phytate:zinc molar ratios were at the highest level only twice that of the critical limit, while the phytate:iron ratios were between 6 and 15 times that of the critical level.

In contrast, the zinc bioaccessibility from the TS porridge, while reduced, was not significantly ($p > 0.05$) lower than that of the WS (Table 2). However, the percentage and amount of bioaccessible iron from the TS porridge were significantly ($p \leq 0.05$) lower than that of the WS. The inhibitory effect of tannins and phenolics on zinc bioavailability has been found to be much less pronounced compared to that of iron (Santos-Buelga and Scalbert 2000). Tannins have been found to form insoluble complexes with both iron and zinc (Okuda et al. 1982) but, at acidic and neutral pH zinc's affinity for phenolics is very low (Santos-Buelga and Scalbert 2000). Tannins have also been found to reduce ferrous iron to ferric iron ($Fe^{2+} \rightarrow Fe^{3+} + e^-$) (Okuda et al. 1982), which further decreases iron bioavailability.

The overall iron and zinc bioaccessibilities (LS mean) (Tables 2 & 3) of the WM porridge were higher than the OM porridge. This could have been due to a combination of the lower phytate, phenolic and tannin contents in the WM. It could also have been due to the fact that the OM was a dent type (hard, corneous) maize kernel, while the WM was a floury (soft) type maize kernel, of which the general digestibility would be higher (Corona et al. 2006), possibly releasing more minerals from the matrix during digestion.

The addition of both the OFSP and CL relish almost doubled the percentage zinc bioaccessibility, compared to the cereal porridges alone (Table 2). However, only the addition of the CL relish significantly ($p \leq 0.05$) increased the amount of bioaccessible zinc, because the OFSP had substantially lower zinc content (approx. 2 to 3 times) compared to the cereal porridges (Table 1). This increase in zinc bioaccessibility is of huge importance as there are very few good plant sources of zinc, making adequate zinc intake, not even mentioning absorption, difficult when consuming a monotonous cereal-based diet.

There was an approximate 3 fold decrease in the percentage iron bioaccessibility after addition of the CL relish to the cereal porridges. However, the amount of bioaccessible iron did not vary from that of the porridge alone (Table 3), because of the substantially higher iron content of the CL compared the cereals (Table 1). The reduced percentage iron bioaccessibility (Table 3) was probably due to the substantially higher tannin and total phenolic contents of the CL relish (Table 1). Despite the lower phytate content of the OFSP (353 ± 31 mg/100 g) compared to the cereals (733 ± 39 to 1197 ± 77 mg/100 g) (Table 1), the addition of the OFSP had no significant ($p > 0.05$) effect on the iron bioaccessibility (Table 3).

In a study by Guatam, Platel and Srinivasan (2010), they evaluated the effect of carrot and amaranth leaf addition on the iron and zinc bioaccessibilties from rice and sorghum. They found that the addition of both vegetables increased the percentage iron and zinc bioaccessibilities. They also observed similar increases in the iron and zinc bioacessibilities when just pure β-carotene was added to the cereals, indicating the carotenoid as the enhancer in the vegetable products.

It should also be noted that there was significant ($p \leq 0.001$ to 0.05) interactions between the type of cereal porridge and vegetable relish on the mineral bioaccessibility. So while the main effect of the relish addition was discussed here, the effect of the vegetable addition to different porridges will not always be the same. This highlights the need for more research in this area to improve the understanding on the complex nature of mineral availability from different crops and meals.

3.4. β-carotene bioaccessibility

The percentage β-carotene bioaccessibility from the various porridge meals varied between 1.58 ± 0.95% for the FWS and 2.85 ± 0.58% for the TS porridge meal (Table 4). While the percentage β-carotene bioaccessibility from the meals with the OFSP and CL relishes did not differ significantly ($p>0.05$) (2.15 ± 0.88 to 2.33 ± 0.73%), the amount of bioaccessible β-carotene from the OFSP meals (0.55 ± 0.24 mg/100 g) was more than double that of from the CL meals (0.19 ± 0.12 mg/100 g). The percentage bioaccessible β -carotene was calculated as the micellarized β -carotene in relation to the total β -carotene in the food sample after *in vitro* digestion. Despite the similar β-carotene contents of the relishes (Table 2), after digestion with the cereal porridges, the CL relish meals contained 2-4 times less β-carotene compared to the OFSP relish meals. This could have been due to higher β-carotene degradation during digestion due to the high iron content of the CL relish. Ferric iron has been found to reversibly react with carotenoids to form a cation radical ($\text{Car} + \text{Fe}^{3+} \leftrightarrow \text{Car}\bullet^{+} + \text{Fe}^{2+}$), which can again react with ferric iron to from a dication ($\text{Car}\bullet^{+} + \text{Fe}3^{+} \rightarrow \text{Car}^{2+} + \text{Fe}2+$) (Boon et al., 2010). Ferric iron has also been found to act as a catalyst of auto-oxidation and thermal degradation of β-carotene.

While CL relish addition substantially increases the zinc and β-carotene contents of cereal porridge meals, most important is enhanced zinc bioaccessibility. OFSP relish is however a better source of β-carotene, with not only high contents like CL, but also higher β-carotene bioaccessibility. The vegetable relishes do not have overall substantial effects on the iron nutritive value of the cereal porridge meals.

Overall the addition of OFSP and CL relish to monotonous cereal-based diets has the potential to substantially improve especially zinc and vitamin A nutritive value of monotonous cereal-based diets.

4. Aknowledgements

The DST-NRF Centre of Excellence in Food Security, South Africa is gratefully acknowledged for financial support. *Eurosa Plus* is gratefully acknowledged for supporting J Kruger at Antwerpen University. The funding bodies did not have any involvement in the research planning, conduct or publication.

Conflict of Interest: The authors declare that they have no conflict of interest.

5. Referencing

Afsana K, Shiga K, Ishizuka S, Hara H. 2004. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. Biosci Biotechnol Biochem, 68: 584-592.

Boon CS, McClements DJ, Weiss J, Decker E.A. 2010. Factors influencing the chemical stability of carotenoids in foods. Crit Rev Food Sci Nutr. 50: 515-532.

Bosscher D, Lu Z,, Van Cauwenbergh, R, Van Caillie-Bertrand M, Robberecht H, Deelstra H. 2001. A method for in vitro determination of calcium, iron, and zinc availability from first-age infant formula and human milk. Int J Food Sci Nutr. 32: 54-58.

Breynaert A, Bosscher D, Kahnt A, Claeys M, Cos P, Pieters L, Hermans N. 2015. Development and Validation of an in vitro Experimental GastroIntestinal Dialysis Model with Colon Phase to Study the Availability and Colonic Metabolisation of Polyphenolic Compounds. Planta Medica. 81: 1075-1083.

Corona L, Owens FN, Zinn RA. 2006. Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. J Anim Sci. 84: 3020-3031.

Faber M, Van Jaarsveld P, Wenhold F, Van Rensburg J. 2010. African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. South Afr J Clin Nutr. 23: 30-38.

FAO: Food Supply - Crops Primary Equivalents. 2013. Rome: Food and Agricultural Organisation; [accessed 2017 Jul 13]. http://www.fao.org/faostat/en/#data/CC.

Fruhbeck G, Alonso R, Marzo F, Santidrian S. 1995. A modified method for the indirect quantitative analysis of phytate in foodstuffs. Anal Biochem. 2252: 206-212.

Gautam S, Platel K, Srinivasan K. 2010. Influence of β-carotene-rich vegetables on the bioaccessibility of zinc and iron from food grains. Food chem. 122: 668-672.

Hagenimana V, Low J. (2000). Potential of orange-fleshed sweet potatoes for raising vitamin A intake in Africa. Food Nutr Bul. 21: 414-418.

Hendrix P, Van Cauwenbergh R, Robberecht H, Deelstra H. 1998. Daily dietary zinc intake in Belgium measured using duplicate portion sampling. Zeitschrift für Z. Lebensm. Unters. Forsch A. 206: 222-227.

Hermans N, Cos P, Berghe DV, Vlietinck A J, de Bruyne T. 2005. Method development and validation for monitoring in vivo oxidative stress: Evaluation of lipid peroxidation and fat-soluble vitamin status by HPLC in rat plasma. J Chrom B. 822: 33-39.

Hunt JR. 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr. 78: 633S-639S.

International Food Policy Research Institute (IFPRI). 2015. Global Nutrition Report 2015: Actions and accountability to advance nutrition and sustainable development. Washington DC.

Kaluza WZ, McGrath R M, Roberts TC, Schroeder HH. 1980. Separation of phenolics of Sorghum bicolor L. Moench grain. J Agri Food Chem. 28: 1191-1196.

Kruger J, Mongwaketse T, Faber M, van der Hoeven M, Smuts CM. 2015. Potential contribution of African green leafy vegetables and maize porridge composite meals to iron and zinc nutrition. Nutr. 31: 1117-1123.

Kruger J, Taylor JR, Oelofse A. 2012. Effects of reducing phytate content in sorghum through genetic modification and fermentation on in vitro iron availability in whole grain porridges. Food Chem. 131, 220-224.

Mason J, Greiner T, Shrimpton R, Sanders D, Yukich J. 2014. Vitamin A policies need rethinking. Int J Epidemiol. 44: 283-292.

Miller DD, Welch RM. 2013. Food system strategies for preventing micronutrient malnutrition. Food Pol. 42: 115-128.

Okuda T, Mori K, Shiota M, Ida K. 1982. Reduction of heavy metal ions and solubilization of precipitates by tannins, Yakugaku Zasshi. 102: 735–742.

Price ML, Van Scoyoc S, Butler LG. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agri Food Chem. 26, 1214-1218.

Saha PR, Weaver CM, Mason AC. 1994. Mineral bioavailability in rats from intrinsically labelled whole wheat flour of various phytate levels. J Agri Food Chem. 42: 2531-2535.

Santos-Buelga C, Scalbert A. 2000. Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. J Sci Food Agric. 80: 1094-1117.

Singh BB, Ajeigbe HA, Tarawali SA, Fernandez-Rivera S, Abubakar M. 2003. Improving the production and utilization of cowpea as food and fodder. Field Crop Res. 84: 169-177.

Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J. Food Comp. Anal. 236: 499-509.

Vimala B, Nambisan B, Hariprakash B. 2011. Retention of carotenoids in orange-fleshed sweet potato during processing. J. Food Sci. Tech. 484: 520-524.

WHO: Micronutrient Deficiencies. 2016. Geneva: World Health Organisation. [accessed Oct 2016] www.who.int/nutrition/topics/ida/en/.