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CHAPTER 4

MICRONUTRIENT (PROVITAMIN A AND IRON/ZINC) RETENTION IN BIOFORTIFIED CROPS

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ABSTRACT

For biofortification to be successful, biofortified crops must demonstrate sufficient levels of retention of micronutrients after typical processing, storage, and cooking practices. Expected levels of retention at the breeding stage were verified experimentally. It was proven that the variety of biofortified crop, processing method, and micronutrient influence the level of retention. Provitamin A is best retained when the crops are boiled/steamed in water. Processing methods that are harsher on the food matrix (i.e. drying, frying, roasting) result in higher losses of provitamin A carotenoids. Degradation also occurs during the storage of dried products (e.g. from sweet potato, maize, cassava) at ambient temperature, and a short shelf life is a constraint that should be considered when foods are biofortified for provitamin A. Iron and zinc retention were high for common beans (*Phaseolus vulgaris*) and cowpeas *(Vigna unguiculata),* indicating that iron and zinc were mostly preserved during cooking (with/without soaking in water).

Key words: Retention, Carotenoid, Iron, Zinc, Biofortification, Processing, Storage, Degradation

INTRODUCTION

What Do We Mean by Retention?

During processing, micronutrients contained in biofortified foods can be lost due to chemical degradation (isomerisation and oxidation) and physical loss (for example, leaching of soluble solids into water). Measurement of micronutrient retention is an important aspect of research on biofortified foods, because a high loss of micronutrients in processing and cooking reduces the nutritional value of biofortified food. Micronutrient loss must be considered and breeding targets set appropriately so that biofortified foods will add sufficient micronutrients to the diet to have a nutritional impact.

Micronutrient retention in processed biofortified foods may be calculated as either apparent or true retention [1]. True retention measures the proportion of a micronutrient retained in processed food compared to the amount of micronutrient in the raw food. Apparent retention is calculated by dividing the micronutrient content after processing by the micronutrient content before processing. Apparent retention assumes that the amount of solids lost during processing are negligible and is expressed on a dry weight basis. The formulas for calculating apparent and true retention, using the example of carotenoid content, are found below.

$$Apparentretention(\%) = \frac{C}{C_0} \quad \text{(Equation 1)}$$

True retention is the recommended method for calculating micronutrient retention. True retention is expressed on a fresh weight basis. The basis is fresh food weight and not dry matter weight because it takes into account loss of physical mass (*i.e.* soluble solid losses) over the process. It is therefore more accurate than apparent retention:

$$True\ retention(\%) = \frac{mC}{m_0 C_0} \quad \text{(Equation 2)}$$

C: Carotenoid content of food (µg.g-1) at time t (e.g. after processing or after storage)
C_0: Carotenoid content of food (µg.g-1) at initial time (e.g. before processing or before storage)
m: mass of food at time t (g)
m_0: initial mass of food (g)

In processes where soluble loss is negligible (for example, in the case of drying or in storage of dried foodstuff), both formulas give equivalent results and the first formula is sufficient [2-4]. However, when soluble loss is more significant (e.g. cooking, blanching, chemical dipping, etc.), applying the second formula that takes into account soluble losses is recommended.

The final (after processing) micronutrient concentration in the biofortified food is also important to measure, because it assesses the nutritional value of the food as consumed.

PROVITAMIN A RETENTION IN BIOFORTFIED CROPS IN AFRICA

Sweet Potato

Sweet potato (*Ipomoea batatas*) is a major staple crop in sub-Saharan Africa [5]. Most of the production is consumed as human food, and therefore sweet potato plays an important role in food security in Africa. While annual world production has remained relatively constant at 100 million metric tons over the last 10 years, production in Africa has consistently risen and is now estimated at 20 million tons per year [6].

Most sweet potato found in sub-Saharan Africa contains very little provitamin A. Biofortified, vitamin A-rich orange sweet potato (OSP) has been promoted since 2006 by international organizations, including the CGIAR. Orange sweet potato has high provitamin A and beta-carotene content [3,7,8], which makes biofortified sweet potato a suitable crop to help address inadequate vitamin A intake.

Unsurprisingly, the type of processing influences the level of provitamin A retention in OSP. Different drying techniques produce varying levels of retention; it was reported that solar oven drying retained more provitamin A carotenoids than open-sun drying [9] (24% compared to 47% losses). Limiting the exposure to damaging ultraviolet radiation may explain these results. Similarly, Bechoff *et al.* [8] reported that two varieties, MGCL01 and Resisto, dried in the shade had negligible carotenoid losses (up to 4%) compared to those dried using solar or sun drying (up to 24%). Retention was also found to vary by variety; for example, the Ejumula variety loss in oven drying was only 12%.

Globally, the processing methods that result in the highest levels of retention are: boiling/steaming (80-90%) [3, 10], roasting, frying (70-80% [11] and sun/solar drying (60-80%) [3, 4, 8, 9, 11, 12]. Sun-drying resulted in the lowest levels of retention when tested in Uganda, Mozambique, and Tanzania. The results of retention varied by variety and some carotenoids were retained via solar/sun-drying, if carried out for a limited time and under good drying conditions (low humidity and high temperature) [4, 8].

Carotenoid degradation can also occur during storage, particularly for dried sweet potato [4, 8, 13, 14]. Provitamin A carotenoid degradation occurs during storage due to oxygen and temperature conditions that chemically oxidize the trans-beta-carotene [14]. The loss of carotenoids in dried orange sweet potato stored at ambient temperature was 70-80% after 4 months under field conditions in Uganda and Mozambique [4,8], a finding in agreement with the model prediction [14].

Dried sweet potato milled into flour can be substituted into processed cooked products, such as porridge, chapati, and mandazi that are very popular in Uganda. When these products were assessed, carotenoid retention during cooking was found to vary depending on the processors (69-97%), likely due to variability in processing conditions (such as temperature, time) [15].

In addition to the processing effect, retention was also dependent on the sweet potato variety used [4, 16]. Bechoff *et al.* [4] showed that varieties with higher dry matter had higher retention of carotenoids. However, high dry matter content varieties tended to be inversely linked to the carotenoid content, and therefore varieties with high carotenoid content tended to have lower carotenoid retention. Crop development work to increase the dry matter content in varieties with high carotenoid content is underway.

Maize

Maize (*Zea mays*) is a staple crop in several Sub Saharan African countries [17]. Because of the relatively low cost of production, this crop is commonly grown on subsistence farms where the incidence of micronutrient deficiency is higher. The main type of maize consumed in Africa is white maize, with a low level of provitamin A carotenoids [5]. In order to assess the potential impact of consuming vitamin A biofortified maize, it is important to examine carotenoid retention under typical storage and processing conditions.

Maize is typically grown in one season per year in Africa, which means that maize products are sometimes stored for several months before they are actually consumed. Consequently, storage of maize grain is an important factor affecting carotenoid retention. Burt *et al.* [18] reported that carotenoid retention in the whole grain of two maize genotypes was about 60% after 18 months storage at 25°C, and degradation of carotenoids in both genotypes occurred during the first 6 months of storage. Similarly, 58% carotenoid retention after 6 months of grain storage for four genotypes of maize was reported by Weber *et al.* [19]. However, Mugode *et al.* [20] found that carotenoid degradation in three biofortified hybrids of maize cultivated in Zambia was rapid during the first two weeks of storage of the grains. The grain of biofortified maize hybrids further degraded during storage, but at a slower rate, with a final retention of 40% after six months of storage. The differences reported across studies may be due to initial carotenoid concentration, genotype effect, storage method, or drying mechanisms before storage. Such factors have not been studied in-depth and further research is needed.

In rural communities, milled maize products are typically stored for a relatively short period of time and maize is milled as needed. In urban areas, however, freshly milled maize is less frequently consumed than industrially milled maize, which is typically stored for a longer time before consumption. The retention of micronutrients in maize meal during storage has not been well documented. However, preliminary data indicate that degradation may occur at a similar rate to whole grain.

The type of processing also influences the level of provitamin A retention in maize. In Africa, maize grains are typically processed into maize meal (mealie meal) to produce porridges or cooked dough, and grits (samp). In West Africa, maize is commonly fermented after wet milling [2].

During both milling and processing, biofortified maize hybrids showed good levels of retention of provitamin A carotenoids when using apparent retention values [21]. The retention values were measured between 105 and 137% in maize meal and samp, respectively. This could be due to the higher concentrations of carotenoids in the endosperm compared to the germ and pericarp, which are mostly removed during milling. For cooked products, the retention varies depending on the type of cooking. For traditional cooked dough (Phutu) the retention of provitamin A carotenoids was between 107 and 118% as found by Pillay *et al.* [21]. Fried products generally presented a higher degree of degradation, whereas boiled products presented better retention.

Cassava

Cassava originated in South America and is a staple for nearly 800 million people in Africa, Latin America, and Asia, mainly in developing countries [22]. In Nigeria, the leading cassava producer, production is estimated at 50 million tons [22]. Biofortified vitamin A cassava (“yellow cassava”) has been developed to address vitamin A deficiency and is currently promoted in Nigeria. Cassava roots can be boiled or steamed for consumption, but in Africa are usually processed into different types of flour. For example, the most popular way to consume cassava in Nigeria is gari, a fermented and roasted food product made from cassava roots.

Drying is used for the production of flour. A study that compared three types of drying found that the highest beta-carotene retention was obtained by oven drying (72%), followed by shade drying (59%), and sun drying (38%) [23].

Bechoff *et al.* [24] assessed the degradation of carotenoids during storage for Nigerian gari made from yellow biofortified cassava. A mathematical model (Arrhenius model) was applied to predict the degradation of carotenoids in the product. According to the model, gari with an initial beta-carotene content of 11 ppm had a retention of 40% after 2 months at an ambient temperature of 25°C and constant humidity. These findings align with the previous study carried out by Chavez *et al.* [23], though the sun-dried samples used in that study had lower initial (38%) and final retention values (24% after storage).

Flour, including High Quality Cassava Flour (HQCF), is being promoted by the Nigerian government as a substitute for wheat flour in bakery products [22]. Studies were carried out by Oliveira et al [25] on carotenoid retention in flour made from yellow cassava. The retention in high quality cassava flour was low and decreased with storage time. After 12 days of storage, total carotenoid retention varied from 5-16%, and there were no detectable carotenoids after 26 days. These results, in accordance with Bechoff *et al.* [24], demonstrate that the retention of carotenoids in dried yellow cassava products (flour, gari) during storage may be a constraint.

Boiled cassava roots retain 56 to 100% of provitamin A content [5]. Gari, the most popular method of cassava processing in Nigeria, is both roasted and fermented. It appears that the final provitamin A content in gari is largely a function of the intensity and duration of roasting, with values ranging from 10% retention in gari roasted at 195°C for 20 minutes to 63% retention for gari roasted at 165°C for 10 minutes [26]. Retention also varied based on the cassava variety used, demonstrating a strong genotype effect on levels of retention.

IRON/ZINC RETENTION IN BIOFORTIFIED CROPS

Common Beans (*Phaseolus vulgaris*) and Cowpea (*Vigna unguiculata*) Retention

Beans are a staple food and the major source of iron for populations in Eastern Africa and Latin America [27]. According to Corrêa [28] and Corrêa *et al.* [29], common beans (*Phaseolus vulgaris*) are consumed by individuals of all social levels, both in urban and rural areas, and are a source of good quality protein, fiber and carbohydrates. The cowpea (*Vigna unguiculata*) is also an excellent source of iron and zinc [30]. Mineral retention during processing and cooking is relatively high for common beans and cowpea. Biofortified iron beans are being grown in several countries in Africa, while biofortified cowpea for Africa is still under development.

Common Beans

Iron retention after cooking is close to 100% for beans as consumed in Rwanda, as beans are not pre-soaked before cooking and none of the water used for cooking is discarded. Retention studies were carried out by the Kigali Institute of Science and Technology (KIST) in 2012. Results indicate that approximately 98% of the iron in raw beans is retained through the traditional cooking process in Rwanda.

Similar results were obtained in experiments conducted by Brazilian collaborators [31]. Studies were carried out on retention of iron and zinc in both the cooked beans *Phaseolus* and *Vigna*. Six common bean (*Phaseolus)* cultivars were cultivated and cooked using four different methods: in a regular pot, in a pressure pan, with and without previous soaking. Iron content was assessed in both the bean grain and the associated broth. Pressure cooking and previous soaking diminished iron content in the bean grain, while increasing it in the broth. Regardless of cooking method, zinc was concentrated in the cooked bean.

Cowpea

Studies of iron and zinc retention after cooking were conducted for biofortified cowpea by Pereira *et al.* [30] and found that iron and zinc retention is high for all cooking methods. Three biofortified cultivars (BRS Xiquexique, Tumucumaque and Aracê) were cooked in common and pressure pans, with and without previous soaking. The varieties showing the highest iron retention were in BRS Aracê with previous soaking and cooked in a common pan (99.80 ± 2.2 %) and in the BRS Tumucumaque (98.17±1.3 %) without previous soaking cooked in a pressure cooker (Table 4.1).

The zinc retention in cowpea beans from cultivar BRS Xiquexique cooked with and without soaking in a common pan and in a pressure cooker presented significant differences ($p < 0.05$) among them varying from 86.17% ± 2.6 to 92.02% ±2.6 (without soaking) and 90.20% ± 2.1 to 97.22% ±2.9 (with soaking). However, the highest zinc retention was found 99.73% ±0.3 in BRS Tumucumaque cooked in a pressure cooker without soaking (Table 4.2).

HarvestPlus and its partners have also assessed iron and zinc retention in traditional African cowpea preparations. Iron and zinc retention was measured for three types of cowpea preparations common in Nigeria (boiled, deep fried paste [akara] and steamed paste [moin moin]). Boiled preparations retained the most iron (58%), followed by moin moin (27%) and akara (23%). The hull is removed when processing cowpea into akara and moin moin, and some micronutrients are lost.

CONCLUSION

The type of biofortified crop, processing method, and micronutrient influences the level of retention. Provitamin A was best retained when the crops (sweet potato, cassava) were boiled/steamed in water.

When processing methods used were harsher on the food matrix (drying or frying), more provitamin A carotenoids were lost and carotenoid retention was significantly diminished. Furthermore, high degradation occurred during storage of dried products (sweet potato, maize, cassava) at ambient temperature, and this constraint should be considered in the promotion programs for biofortified crops.

The iron and zinc retention were high for the cultivars of common beans (*Phaseolus vulgaris*) and cowpeas *(Vigna unguiculata)*, indicating that all cooking methods applied (with/without soaking in water) preserved this micronutrient.

Existing results on levels of micronutrient retention suggest that biofortification can benefit nutritionally vulnerable population groups in sub-Saharan Africa, who traditionally cultivate staple foods such as common beans, cassava, sweet potato, maize, and others.

Table 4.1: Iron retention in cowpea cultivars after cooked in different cooking methods

Cultivars	Iron retention (%)			
	Pressure cooker		Common pan	
	Without soaking	With soaking	Without soaking	With soaking
BRS Xiquexique	92.35±3.0^{a}	91.57±1.3^{b}	93.86±2.1^{a}	98.22±1.2^{b}
BRS Tumucumaque	98.17±1.3^{b}	97.05±1.6^{b}	87.10±3.8^{a}	94.58±1.1^{b}
BRS Aracê	92.54±0.6^{a}	92.85±1.2^{a}	94.08±0.2^{a}	99.80±0.3^{b}

Different letters in the same column differ significantly at 5% of probability.

Table 4.2: Zinc retention in cowpea cultivars after cooked in different cooking methods

Cultivars	Zinc retention (%)			
	Pressure cooker		Common pan	
	Without soaking	**With soaking**	**Without soaking**	**With soaking**
BRS Xiquexique	92.02±2.6^{a}	97.22±2.9^{a}	86.17±2.6^{a}	90.20±2.1^{a}
BRS Tumucumaque	99.73±0.3^{b}	99.05±1.2^{b}	87.21±3.6^{a}	96.41±0.9^{b}
BRS Aracê	99.18±1.1^{b}	96.34±1.6^{c}	96.54±0.7^{b}	98.64±0.4^{b}

Different letters in the same column differ significantly at 5% of probability.

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