#### COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY



# What is food-to-food fortification? A working definition and framework for evaluation of efficiency and implementation of best practices

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#### **Abstract**

Food-to-food fortification (FtFF) is an emerging food-based strategy that can complement current strategies in the ongoing fight against micronutrient deficiencies, but it has not been defined or characterized. This review has proposed a working definition of FtFF. Comparison with other main food-based strategies clearly differentiates FtFF as an emerging strategy with the potential to address multiple micronutrient deficiencies simultaneously, with little dietary change required by consumers. A review of literature revealed that despite the limited number of studies (in vitro and in vivo), the diversity of food-based fortificants investigated and some contradictory data, there are promising fortificants, which have the potential to improve the amount of bioavailable iron, zinc, and provitamin A from starchy staple foods. These fortificants are typically fruits and vegetables, with high mineral as well as ascorbic acid and  $\beta$ -carotene contents. However, as the observed improvements in micronutrient bioavailability and status are relatively small, measuring the positive outcomes is more likely to be impactful only if the FtFF products are consumed as regular staples. Considering best practices in implementation of FtFF, raw material authentication and ingredient documentation are critical, especially as the contents of target micronutrients and bioavailability modulators as well as the microbiological quality of the plant-based fortificants can vary substantially. Also, as there are only few developed supply chains for plant-based fortificants, procurement of consistent materials may be problematic. This, however, provides the opportunity for value chain development, which can contribute towards the economic growth of communities, or hybrid approaches that leverage traditional premixes to standardize product micronutrient content.

#### KEYWORDS

 $\beta$ -carotene, essential minerals, hidden hunger, iron, micronutrient malnutrition, staple food, vitamin A, zinc

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#### 1 | INTRODUCTION

While substantial reductions in the worldwide prevalence of undernourishment (the percentage of the population without regular access to adequate calories) were made over the period 2005 to 2015, since 2015 there has been no real improvement, and the number of hungry people in fact increased to 822 million from 785 million in 2015 (FAO, IFAD, UNICEF, WFP, and WHO, 2019). The 2019 global hunger score was 20, with average scores as high as 29 for south Asia and 28 for sub-Saharan Africa (von Grebmer et al., 2019). Globally, there are 151 million stunted and 51 million wasted children (Development Initiatives, 2018), and most countries (88%) experience more than one form of malnutrition (double burden of malnutrition), based on childhood stunting (>20%), anemia in women of reproductive age (>20%), and overweight in adult women (>35%).

While the practice of compositing/blending of foods has been applied for centuries at a household level to enrich grain foods with key macronutrients, food-to-food fortification (FtFF) is now viewed as an emerging strategy which can complement current strategies in the fight against micronutrient deficiencies. In this context, FtFF is where micronutrient-dense foods are added to food recipes (household level) and importantly also food formulations at a commercial level to increase their micronutrient quality. It is a component of food-based strategies, which promote the production, access, and intake of micronutrientrich foods with the aim of enhancing the content and/or bioavailability of target nutrients, especially micronutrients (Ruel, 2001). The most commonly implemented foodbased strategies are conventional fortification, biofortification, and dietary diversification/modification. Food-based strategies such as biofortification, dietary diversification, and FtFF that encourage the utilization of local resources are increasingly being recognized as sustainable, as they promote self-reliance and create market opportunities for locally produced foods, thereby also contributing to economic growth (Burchi, Fanzo, & Frison, 2011).

The term FtFF has been used in numerous peer-reviewed publications, and the concept is currently in the process of being implemented by the Kenyan government (Farm Concern International and Kenyan Government, 2018). In a recent review, Chadare et al. (2019) described FtFF as an additional strategy to address the challenges and limitations of conventional food fortification. However, despite the term FTFF being in widespread use, to date a clear definition does not exist. This has resulted in a range of approaches involving differing techniques and objectives all being categorized as "FtFF." Another problem is the absence of an in-depth evaluation of the effectiveness of the strategy in increasing micronutrient bioavailability, status as well as growth, development, and

long-term health. Chadare et al. (2019) laid the ground-work for this by evaluating the effect of FtFF on macro - and micronutrient contents and sensory acceptability of food products.

With this in mind, the most critical questions to be asked are: what is FtFF? Is FtFF really an emerging food-based strategy in its own right, or would it fall under already existing strategies? Is there any evidence that FtFF is effective in improving micronutrient bioavailability or status? What is the best approach to develop and implement a FtFF solution? To answer these questions, this review will first evaluate the use of the term FtFF to date and then propose a working definition for further discussion within the scientific community. As defined in this review, FtFF will be compared to the main food-based strategies to determine if it really is an emerging strategy. Published research on the efficiency of FtFF in improving the iron, zinc, and vitamin A (including provitamin A) quality from starchy staple-based home recipes and commercial-type food formulations will be evaluated. The review will be limited to iron, zinc, and vitamin A and starchy staple food products, as deficiencies of these micronutrients are most prevalent (FAO et al., 2019), especially in the developing world, and the potential impact from food-to-food fortified staples are the highest (FAO/WHO, 2006). Lastly, from the literature and current experience from the USAID Feed the Future Innovation Lab for Food Processing and Post-harvest Handling (https://ag.purdue.edu/food-processing-innovationlab/) a "best practice" approach to FtFF with plant-based fortificants will be described. Here the review will focus specifically on plant-based fortificants in the context of the developing world, with focus on sub-Saharan Africa.

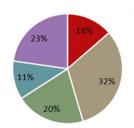
## 2 | THE USE OF THE TERM FtFF AND A PROPOSED DEFINITION

More than 50 documents that use the term FtFF were found, including original research articles, review articles, and institutional documents. The term "food-to-food fortification" was searched in Google Scholar, Pubmed, and Science Direct. and all original descriptions of the term which included two or more characteristics were evaluated, totaling 43 descriptions of FtFF (Supplementary Table S1).

The first published use of the term FtFF seems to have been by Underwood (1998), in a background paper in the "Prevention of Micronutrient Deficiencies: Tools for Policymakers and Public Health Workers" policy document (Horwitz et al., 1998). In this document on approaches to prevent or correct vitamin A deficiency, FtFF was described as the opportunity to maximize the potential for vitamin A retention in traditionally preserved vitamin A-rich products, which could then be used to fortify the

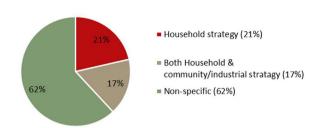


#### Type of strategy

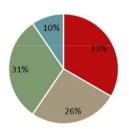


- Improved dietary diversification (14%)
- Improved food formulations (32%)
- Improve dietary diversification and food formulations (20%)
- Alternative to conventional fortification (11%)
- Non-specific (23%)

#### **b** Level at which FtFF is applied

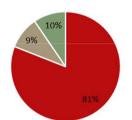


#### **Objective of FtFF**



- Improved micronutrient content (33%)
- Improved micronutrient bioavailability
- Improved micronutrient content and bioavailability (31%)
- Non specific (10%)

#### **Technique of FtFF**



- Addition of micronutrient rich foods (81%)
- Both addition of micronutrient rich food and removal of antinutrient rich foods (9%)
- Non-specific (10%)

#### **e** Vehicle of FtFF

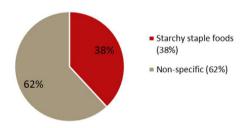


FIGURE 1 A summary of the major differences in the published descriptions of FtFF (based on 41 published articles, which described FtFF; see Supplementary Table 1 for citations)

vitamin A-poor diet during out of season periods. The example given was "mixing of staple foodstuffs—for example, mango with gruel—at the household level to enrich nutrient content" (Horwitz et al., 1998). Two recent published descriptions of the term were by Chadare et al. (2019) as "an approach that uses an interesting (contain useful amounts of micronutrients), available, and accessible local resource (plant or animal) to fortify another food" and by Kruger (2020) as "nutritionally enhanced staple food products produced through food-to-food fortification with micronutrient-dense fruit and vegetables." Furthermore, in the 20 years between the first and the most recent descriptions, there have been various different and sometimes contradictory descriptions and uses of the term FtFF.

The main differences in the descriptions can be summarized as follows (Figure 1):

· Type of strategy within which FtFF is placed: some describe it as synonymous with dietary diversification,

- others as a replacement for conventional fortification, and yet others as a method to improve existing and/or new food recipes/product formulation, or combinations of these.
- · Level at which FtFF is applied: restricted to household level or at both household and community/commercial level.
- · Objective of FtFF: to increase the nutrient content and/or nutrient bioavailability of foods.
- Technique of FtFF: involving only the addition of nutritious foods or also the removal of foods high in nutrient bioavailability inhibitors
- Vehicle for FtFF: some articles specify appropriate food vehicles for FtFF.

The first point on which all descriptions of FtFF are in agreement with is that this strategy focuses on leveraging nutrient-dense foods to improve the nutrient status of populations. The second is that the aim of FtFF is to

improve the micronutrient quality of the food. In fact, only one paper specifies that FtFF should be used to improve both the micro- and macronutrient value of food products (Tenagashaw et al., 2017).

The majority of the literature reviewed (52%) describes the FtFF strategy as one that seeks to improve the nutritional quality of existing and new food recipes/product formulations (Figure 1). Eighty-one percent propose that this should be done through the addition of nutrientdense foods, while only 9% also propose the removal of foods high in antinutrients. Twenty-one and 17% of the descriptions indicate that FtFF should be applied at household or household and community/industry level, respectively, while the majority of the descriptions did not specify (62%). Over half of the descriptions (57%) include improved micronutrient bioavailability as the main objective of FtFF, while only 33% of the descriptions limit it to improving only micronutrient content. Only 38% of the descriptions of FtFF specify the type food vehicle to be used and all of these specified starchy staple food products.

On the basis of the most common characteristics in these previous descriptions, FtFF can be defined as the addition of micronutrient-dense food/s to a recipe (household level) or food formulation (food industry level), or the replacement of micronutrient-poor/antinutrient-rich ingredients, to substantially increase the amount of bioavailable micronutrient/s, with the aim of improving the micronutrient status of populations where the intake of bioavailable micronutrients is inadequate.

## 3 | COMPARISON OF FtFF WITH OTHER FOOD-BASED STRATEGIES

With a working definition, the question is whether FtFF truly is an emerging strategy, or if it falls under another main food-based strategy. This can now be addressed by comparing FtFF with common definitions of biofortification, conventional fortification, and dietary diversification. While the different food-based strategies, including FtFF, have their own strengths and weaknesses, some of which will be highlighted in the text, FtFF should not be viewed as a replacement of any of the strategies, but rather as an additional tool to combat micronutrient deficiencies which can complement the existing strategies.

First, it is important to clearly differentiate between FtFF and food compositing/blending. As mentioned earlier, while these strategies can be viewed as similar, the primary aim of compositing or blending (commonly of cereals and pulses (e.g., corn (maize)–soy blends)), is to improve the protein quality (Fleige et al., 2010). Secondly, FtFF as a food-based strategy, has only recently become applica-

ble due to increased scientific knowledge of nutritional composition and compound interactions affecting nutrient bioavailability. This makes it possible to elevate a concept such as food compositing/blending from a household method, to a strategy which has the potential to be standardized, safe, and effective on a commercial scale.

#### 3.1 | Biofortification

The WHO defines biofortification as "the practice of deliberately increasing the content of an essential micronutrient, that is, vitamins and minerals (including trace elements) in a food crop through agronomic practices, conventional plant breeding, or modern biotechnology, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health" (WHO, 2019). The preliminary proposed definition by the CODEX Alimentarius Commission also specifies that biofortification can be focused on increased nutrient bioavailability and not necessarily just increased content (Codex, FAO & WHO, 2018). The different biofortification techniques (agronomic, conventional plant breeding, and transgenic techniques) have been reviewed elsewhere (Sharma, Aggarwal, & Kaur, 2017).

While both the biofortification and FtFF strategies require little changes to be made in the dietary habits of consumers, they do differ in development and implementation. The process of developing a biofortified crop through breeding (both conventional and through modern biotechnology) is time consuming and can take several years before it is made available to farmers and the public (Miller & Welch, 2013). The application of conventional breeding can be limited as it depends on the preexisting genetic variation within the plant as in the case of provitamin A in rice (Listman et al., 2019). Agronomic biofortification focuses on increasing the mineral concentration of plants through the supplementation of fertilizers and/or enhancing the solubility/mobility of soil minerals (Garg et al., 2018), but the application of this technique becomes challenging, when targeting micronutrients that are synthesized by the plant such as provitamin A carotenoids. Transgenic techniques, in addition to increasing the levels of multiple micronutrient levels, have also been leveraged to improve micronutrient bioavailability by increasing the activity of enzymes that can eliminate known inhibitors and/or silencing genes that promote their expression (Bhati et al., 2016). FtFF provides the option of easily increasing the content of multiple micronutrients in a staple food product, using a shorter development window as it is based on identification of local micronutrient-rich materials and is formulation based. Further, FtFF is met with fewer regulatory

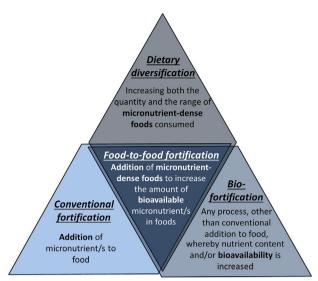


FIGURE 2 The relationship of FtFF to the objectives of the main food-based strategies (modified from Kruger, 2020)

challenges than transgenic biofortification and through use of local plants can be formulated to be well suited to consumer acceptance. Biofortification, however, has the advantage of a one-time investment with minimal cost to the target consumer. Comparatively, FtFF is reliant on the availability and price of the food fortificants, and therefore, includes a recurring cost of materials and the need to formulate more complex foods (see the section 10.1 Costs versus benefits).

Hence, FtFF is similar to biofortification as both strategies aim to improve the content and bioavailability of micronutrients (Figure 2). These strategies, however, differ fundamentally in that the technique is to increase micronutrient levels in food through formulation and during food preparation or processing, rather than by increasing plant micronutrient accumulation by genetic or agronomic factors. These strategies are however complementary, where biofortified crops, for example, orange-fleshed sweet potato (OFSP), can be used to fortify cereal products with micronutrients (provitamin A).

#### 3.2 | Conventional fortification

The 1987 Codex General Principles for the Addition of Essential Nutrients to Foods (Codex Alimentarius Commission, 1987) define fortification/enrichment as "the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups."

FtFF is similar to conventional fortification in that both can be applied at point of use (household FtFF / micronu-

trient powders) and commercially. Two challenges with commercial conventional fortification highlighted in the FAO/WHO guidelines are the homogeneous distribution of the fortificant in the target food and evaluation of compliance, especially in regions where the production of staple food vehicles is carried out in locally established smaller enterprises instead of large industrial enterprises (FAO/WHO, 2006). As the amount of fortificant used in FtFF is greater (1 to 50%) (Chadare et al., 2019), it is practically easier to ensure homogeneous distribution of the nutrient dense fortificant within the food without the need for specialized dosing equipment (see the section 10.1 Costs versus benefits). Point of use fortification (e.g., home or school fortification) with micronutrient powders and micronutrient-dense foods circumvents many of the challenges of commercial fortification. Importantly, both fortification strategies are possible where households produce their own staple crop and do not benefit from commercial fortification. However, point of use fortification with micronutrient powders should go hand in hand with end user education to minimize the risk of high intake of nutrients especially vitamin A (WHO, 2016a).

However, compared to conventional fortification, the higher levels of food fortificants used may also cause significant changes in the sensory properties, which on the one hand make it easier to track fortification compliance but, on the other hand could also result in consumer acceptability issues. Another substantial benefit of FtFF, compared to conventional fortification, is that even if a fortificant is added to target specific micronutrient/s, it would also provide additional nutrients present in the plant fortificant (e.g., protein, other vitamins, and minerals or bioactive compounds), a strength it shares with dietary diversification.

Both FtFF and conventional fortification increase the micronutrient contents of formulated foods through the addition of micronutrients (Figure 2). However, FtFF differs fundamentally from conventional fortification in an approach in that the objective is to increase both micronutrient content and bioavailability in foods through addition/replacement of food ingredients, rather than increasing the content through addition of individual micronutrients/micronutrient mixes. However, the two strategies are highly complementary. In any particular food recipe or product formulation, the micronutrients could be provided by FtFF or conventional fortification or a combination of the two, a hybrid-type approach. This would depend on factors such the level of a particular micronutrient required, the availability, and cost of the food fortificant or micronutrient, consumer preferences, governmental policies, and a food company's marketing.

#### 3.3 | Dietary diversification

The WHO defines dietary diversification as increasing both the quantity and the range of micronutrient-rich foods consumed through diet selection and through traditional food preservation and processing methods (FAO/WHO, 2006). Dietary diversification has to be continuously supported, more so than with FtFF and conventional fortification, with nutrition education and dietary pattern assessments. Also, importantly, the success of dietary diversification depends on the availability of foods and the household's purchasing ability of a wide variety of food groups, including animal-based foods, in adequate amounts (Nair, Augustine, & Konapur, 2016). While FtFF could also increase the cost of the staple food, it should be focused on locally available and affordable micronutrient-rich plant foods, the cultivation of which would contribute to the economic growth of communities.

Both dietary diversification and FtFF increase the consumption of micronutrient-dense foods (Figure 2). However, FtFF differs from dietary diversification in that the objective is to improve the content and bioavailability of specific nutrient/s by using the technique of improving the food recipe/formulation, rather than to generally increase the diversity of food groups included in the diet (FAO/WHO, 2006). Having said that, all food-based strategies, including biofortification and conventional fortification, could be categorized under the broad description of dietary diversification. The techniques of these strategies, and we also propose FtFF, are, however, unique enough that they are classified as additional strategies to dietary diversification.

The above comparisons make it clear that while there are similarities in the objectives of the various food-based strategies, FtFF is in fact an emerging strategy with a technique used, not fully addressed by any of the current major strategies (Figure 2). In theory, FtFF has the capacity to improve the nutrient intake and absorption of multiple target micronutrients and then still provide more additional nutrients which are present in the plant fortificant. But are there any data available that support the efficiency of this strategy?

#### 4 | EVALUATION OF THE EFFICIENCY OF FtFF IN IMPROVING THE IRON, ZINC, AND VITAMIN A CONTENT AND BIOAVAILABILITY FROM STARCHY STAPLE FOODS

Iron, zinc, and vitamin A studies have been conducted using preclinical models including in vitro digestion and Caco-2 cell models to assess bioaccessibility and intestinal transport, animal bioavailability, and status studies and ultimately human clinical evaluation (see search criteria in Supplementary Figure 1). Where iron and zinc are concerned, the majority of the available data are currently derived from in vitro studies, whereas the majority of the available data on provitamin A fortification are derived from in vivo studies. Concerning the designs of the studies, in the Caco-2 and in vitro digestion studies, the food-based fortificants were mostly added to the starchy staple foods after processing. Studies designed in this way can in effect be considered either as FtFF or as dietary diversification, that is with the fortificant added to a dish (recipe/formulation) or as a side dish in the meal, respectively.

As summarized in Tables 1 to 4, a wide range of food-based fortificants have been investigated with the aim of improving the iron, zinc, and provitamin A contents and bioavailability from starchy staple foods. The main food groups investigated as single fortificants include other starchy foods, green leafy vegetables, other vegetables, fruits, flowers, nuts and oils, and animal products. While there are various studies where multiple food-based fortificants were included in a food product, these studies were not presented in the tables as it is difficult to isolate the effect of each fortificant. Selected examples of such studies are discussed in the following sections of the review.

#### 4.1 | Iron and zinc

In an effort to improve the iron and zinc contents and/or bioavailability of starchy staple foods with other micronutrient-rich starchy foods, the level of fortification has been high, at 30 to 50% (Table 1). An acute iron bioavailability study in adults, which involved grain amaranth fortification of sorghum porridge (Macharia-Mutie et al., 2012), and an intervention study of primary school-aged children, using cowpea-fortified wheat biscuits (Ayogu & Onah, 2018), did not clearly indicate improvement in iron bioavailability (Table 2). The lack of improvement in mineral bioavailability by fortification with a pseudocereal and legume is perhaps not surprising as they both have high contents of phytate, which inhibits the absorption of dietary minerals such as zinc, iron, calcium, magnesium, manganese, and copper through the formation of insoluble complexes (Kumar, Sinha, Makkar, & Becker, 2010).

With OFSP as a fortificant of sorghum products, two related rodent studies gave apparently contradictory results. In one study with Wistar rats, there was more than a doubling in hemoglobin maintenance, attributed to the  $\beta$ -carotene in the OFSP, which, by forming soluble complexes with iron, may counteract the inhibitory effect



TABLE 1 The effects of individual food fortificants on the iron and zinc, bioavailability inhibitor, and enhancer contents of cereal-based products, as well as in vitro iron and zinc bioaccessibility

	Reference	Kruger et al. (2018)	Kruger (2020)	Vilakati, Taylor, MacIntyre, and Kruger (2016)	Kruger et al. (2018)	(Continues)
o digestion ty assay se stated)	Outcome - zinc Ref	On average, the percentage and () amount of bioaccessible zinc increased by 96 and 12%, respectively	**Caco-2 cellular Kru uptake study On average, the percentage zinc bioaccessibility was increased by 28%, but the amount of bioaccessible zinc was not significantly affected	The percentage and Vila amount of bioaccessible zinc a was decreased by (64 and 33%, respectively	On average, the percentage and (2 amount of bioaccessible zinc was increased by 97 and 226%, respectively	
Bioaccessibility - In vitro digestion followed by a dialysability assay (**except where otherwise stated)	Outcome - iron Out	On average, there On a was no significant per change in the arr percentage and bi amount of in bioaccessible iron arrer	**Caco-2 cellular **Caco-2 cellular uptake study uptake study uptake study on average, the On a mount of bi bioaccessible zinc we increased by 86 and 117%, arr respectively we significant of the study	There was no The significant change are in percentage or bi amount of we bioaccessible iron. 64	On average, the percentage iron percentage iron per bioaccessibility an was decreased by bi 180%, but amount wo of bioaccessible 97 iron was not resignificantly affected	
c	Bioavailability Or	Beta-carotene: 27.3 Or	* 0	Ē	Beta-carotene: 25.3 Or	
Contents of minerals and bioavailability modulators in food fortificants (mg/100 g, db) [change in content of food product after FFFF, %]	Bioavailability I	Phytate: 353 I Total phenolics: 692 Tannins: 133	3 2.2 Phytate: 203, [293%] [463%] Total phenolics: 869, Tannins: ND	5.0 [23%] 2.8 [50%] Phytate: 1210 [34%]	Phytate: 721 Total phenolics: 5,295 Tannins: 1.021	
Contents of miner food fortificants (pood product after FtFF, %]	Iron Zinc	0.89	2.5 [293%] [463%]	[23%] 2.8 [50%]	6.9	
Z Q Q	Level of fortification <sup>a</sup> Ire	50% 2.2	3.3	30% 5.0	14.6	
	Food product	Various cereal porridges <sup>b</sup> and separately cooked OFSP	Conventionally fortified maize porridges <sup>b</sup> and separately cooked OFSP	Ready-to-eat sorghum porridge and separately dry cooked cowpea	Various cereal porridge <sup>b</sup> and separately cooked leaves	
	Food fortificant	Ipomoea batatas (orange- fleshed sweet potato, tuber)		Vigna unguiculata L. (cowpea, grain legume)	Vigna unguiculata (cowpea, Leaves)	
	Food groups	Starchy foods			Green leafy vegetables	

TABLE 1 (Continued)

		Reference	Kruger (2020)	Oluyimika et al. (2019)	n der Merwe et al. (2019)
		- Refe			n van det a et
	vitro digestion ability assay erwise stated)	Outcome - zinc	**Caco-2 cellular uptake study On average, the percentage zinc bioaccessibility was not changed but the amount of bioaccessible zinc increased by 295%	The percentage and amount of bioaccessible zinc was decreased by 22 and 24%, respectively	At lower fortification rates (10%), percentage and amounts of bioaccessible zinc were increased by 66 and 135%, but at higher rates (30%) percentage and amounts of bioaccessible zinc were decreased by 17% and unchanged, respectively
	Bioaccessibility - In vitro digestion followed by a dialysability assay (**except where otherwise stated)	Outcome - iron	**Caco-2 cellular uptake study On average, the percentage iron bioaccessibility was not changed but the amount of bioaccessible iron increased by 519%	There was no significant change in the percentage or amount of bioaccessible iron	At lower fortification At lower fortification van der Merwe rates (10%), rates (10%), et al. (2019) percentage and amounts of amounts of bioaccessible iron were increased by 56 and 149%, but at higher rates (30%) percentage and amounts of bioaccessible iron bioaccessible zinc were decreased by exercited amounts of bioaccessible iron bioaccessible zinc were decreased by 45% and increased 17% and by 54%, respectively rates (10%), et al. (2019)
	Contents of minerals and bioavailability modulators in Bioaccessibility - In vitro digestion food fortificants (mg/100 g, db) [change in content of followed by a dialysability assay food product after  [**except where otherwise stated]	Bioavailability enhancers		Ascorbic acid: 10 [increased from 0 to 1 mg/100 g], Citric acid: 2,017 [162%], Tartaric acid: 228 [-9%], Malic acid: 0 [-13%]	Organic acids: 2,255
	rals and bioavailab mg/100 g, db) [chí r	Bioavailability inhibitors	Phytate: 631 Total phenolics: 5,187 Tannins: 851		Phytate: 829 Tannins: ND, Total phenolics: 4,655
	of mine ficants ( uct after	Zinc	[276%]	2 [-3%]	2.3 [7 to 19%]
	Contents of miner food fortificants (r food product after FtFF, %]	Iron	29.3 [1%] 17.6	19.1 [19%] 2 [—3%]	58.4 [58 2 to 173%]
		f ation <sup>a</sup> I	2	Ŧ.	
		Level of fortification $^{\rm a}$	%00%	15%	10 to 30%
		Food product	Conventionally fortified maize porridges <sup>b</sup> and separately cooked leaves	Pearl millet porridge and raw dry powder	Pearl millet porridge and raw dry leaf powder
ì		Food fortificant		Moringa oleifera (moringa, leaves)	
		Food groups			

TABLE 1 (Continued)



	Reference	Gautam et al. (2010)		Gabaza et al. (2018)	Oluyimika et al. (2019)
vitro digestion ability assay erwise stated)	Outcome - zinc	Zinc bioaccessibility on average increased between 34 and 41% Zinc bioaccessibility on average increased between 4 and 8% Zinc bioaccessibility on average increased between 24 and 33% Zinc bioaccessibility	on average increased between 20 and 23%	Increased percentage Increased percentage of bioaccessible of bioaccessible iron on average by zinc on average by 78% 73%	There was no significant change in percentage or amount of bioaccessible zinc
Bioaccessibility - In vitro digestion followed by a dialysability assay (**except where otherwise stated)	Outcome - iron	Iron bioaccessibility on average increased between 7 and 16% Iron bioaccessibility on average increased between 15 and 23% Iron bioaccessibility on average increased between 28 and 45% Iron bioaccessibility	on average increased between 19 and 25%	Increased percentage of bioaccessible iron on average by 78%	The percentage and amount of bioaccessible iron was increased by 36 and 30%, respectively
Contents of minerals and bioavailability modulators in food fortificants (mg/100 g, db) [change in content of food product after FFFF, %]	Bioavailability enhancers				Ascorbic acid: 140 [increased from 0 to 18 mg/100 g], Citric acid: 3,345 [278%], Tartaric acid: ND [-13%], Malic acid: 210 [1%]
rals and bioavaila (mg/100 g, db) [ch r	Bioavailability inhibitors			Phytate: [-57%] Tannins: [193%], Soluble phenolics: [148%] Bound phenolics:	
Contents of miner food fortificants (a food product after FtFF, %]	n Zinc			%] [-21%]	3.7 [-4%] 0.9 [-5%]
Con foo foo FtF	Level of fortification <sup>a</sup> Iron	5 to 10% 15 to 30% 5 to 10% 15 to 30%		10% [-2%]	3.7.1
	Food product	Various cereals <sup>b</sup> 5 to 10% and garlic (raw and cooked together)  Various grain 15 to 30% legumes <sup>b</sup> and garlic (raw and cooked together)  Various cereals <sup>b</sup> 5 to 10% and onion (raw and cooked together)  Various grain 15 to 30% and onion (raw and cooked together)	legumes <sup>o</sup> and onion (raw and cooked together)	Various fermented raw cereal flours <sup>b</sup> and raw baobab fruit pulp	Pearl millet porridge and raw dried baobab pulp
	Food fortificant	Allium sativum (garlic, bulb)  Allium cepa (onion, bulb)		Adansonia digitata (baobab, fruit flesh)	
	Food groups	Other vegetables		Fruits	

	Reference	van der Merwe et al. (2019)	van der Merwe et al. (2019)	Gabaza et al. (2018)
itro digestion bility assay rwise stated)	Outcome - zinc	At lower fortification rates (10%), percentage and amounts of bioaccessible zinc were increased by 99 and 170%, but at higher rates (30%) percentage and amounts of bioaccessible zinc were increased by 115 and 257%, respectively	At lower fortification van der Merwe rates (10%), et al. (2019) percentage and amounts of bioaccessible zinc were increased by 44 and 106%, but at higher rates (30%) percentage and amounts of bioaccessible zinc were increased by 32 and 142%, respectively	Decreased percentage of bioaccessible zinc by 40%
Contents of minerals and bioavailability modulators in Bioaccessibility - In vitro digestion food fortificants (mg/100 g, db) [change in content of followed by a dialysability assay food product after (**except where otherwise stated) FFFF, %]	Outcome - iron	At lower fortification At lower fortification van der Merwe rates (10%), percentage and amounts of amounts of bioaccessible iron bioaccessible zinc were increased by were increased by were increased by 98 and 126%, but at higher rates (30%) percentage and amounts of amounts of bioaccessible iron bioaccessible zinc were increased by 159 and 267%, 115 and 257%, respectively rates (10%).	At lower fortification rates (10%), percentage and amounts of bioaccessible iron were increased by 42 and 107%, but at higher rates (30%) percentage and amounts of bioaccessible iron were increased by 55 and 269%, respectively	Increased percentage I of bioaccessible iron by 52%
Contents of minerals and bioavailability modulators in food fortificants (mg/100 g, db) [change in content of food product after FFFF, %]	Bioavailability enhancers	Organic acids: 3,695	Organic acids: 2,420	
rals and bioavailal mg/100 g, db) [ch r	Bioavailability inhibitors	Phytate: 321 Tannins: 2,286 Total phenolics: 2,255	Phytate: 4,833 Tannins - ND Total phenolics: 3,451	Phytate: [-54%] Tannins: [-63%] Soluble phenolics: [55%] Bound phenolics:
Contents of miner food fortificants (r food product after FtFF, %]	Iron Zinc	13.8 [14 1.2 [4 to to 41%] 10%]	47.1 [47 2.7 to [8 to 140%] 22%]	[56%] [160%]
	Level of fortification <sup>a</sup>	10 to 30%	10 to 30%	%01
	Food product	Pearl millet porridge and raw dried baobab pulp	Pearl millet porridge and raw dry calyx powder	Various fermented raw cereal flours <sup>b</sup> and dried Mopani worm powder
	Food fortificant		Hibiscus sabdariffa (rosella calyx)	Gonimbrasia belina (mopani worms, larvae)
	Food groups		Flowers	Animal products

[]: Values in square brackets are percentage change in iron, zinc, and bioavailability inhibitor or enhancer contents due to FtFF.

ND: Not detected.

"Percentage of replacement of starchy staple ingredient, db.

"Mean effect of fortification on the various cereal/legume products reported.



TABLE 2 The effects of individual food fortificants on the iron and zinc, bioavailability inhibitor, and enhancer contents of cereal-based products as well as in vivo iron and zinc bioavailability

יק	— Reference	Gomes et al. (2017)		Infante et al. (2017)		Macharia- Mutie et al. (2012)
Contents of minerals and bioavailability modulators in food fortificants (mg/100 g, db) [change in content of food product after FtFF, Iron and zinc bioavailability (animal and %]	Outcome	Increased the hemoglobin maintenance efficiency in rats by 125%	Did not affect the hemoglobin maintenance efficiency in rats	The hemoglobin regeneration efficiency was decreased by 21%	The hemoglobin regeneration efficiency was decreased by 3%	No significant change in hemoglobin, ferritin, or soluble transferrin receptor concentrations in iron deficient or noniron deficient adults compared to the control
Iron and zinc bioav human studies)	Type of study	Iron deple- tion/repletion study with male Wistar rats	Iron depletion/repletion study with male Wistar rats	Iron depletion/repletion study with male Wistar rats	Iron repletion study The hemoglobin with male Wistar regeneration rats efficiency was decreased by 3	Acute human iron bioavailability study (stable isotope)
vailability s (mg/100 g, db) duct after FtFF,	Bioavailability Bioavailability inhibitors enhancers			Carotenoids: [increased from 0 to 28.1 mg/100 g]	Carotenoids: [increased from 0 to 28.1 mg/100 g]	
Contents of minerals and bioavailability modulators in food fortificants (mg/100 g, db) [change in content of food product after FtFF, %]				Phytate: [-50%] Total phenolics: [14,456%]	Phytate: [-50%] Total phenolics: [17,510%]	
Contents of n modulators is [change in co	Iron Zinc	[-4%]	[-20%]	[-30%]	[-22%]	
	Level of fortification <sup>a</sup>	m 50%	%05	m 50%	1 50% v	n 70%
	Food product	Dry heated sorghum 50% and raw dried OFSP	Extruded sorghum and raw dried OFSP	Biscuits made from extruded sorghum and raw dried OFSP	Biscuits made from dry heated sorghum and raw dried OFSP	Porridge made from 70% maize and grain amaranth flour
	Food fortificant	Ipomoea batatas. (OFSP, tuber)				Amaranthus spp. (amaranth, pseudocereal grain)
огоауапаопи	Food groups	Starchy foods				

(2018)

serum ferritin of the

consumption, the

hemoglobin and

controlled trial in primary school

Single-blind

[-24%]

wheat cowpea grain flour

(cowpea, grain legume) intervention group who consumed the

children

biscuit was increased

cowpea fortified

by 15.5 and 28.3%

compared to the

control group at 5.0

respectively There was no

and 16.4%,

significant change in

TABLE 2 (Continued)	(Continued)								
				Contents modulatc [change i	Contents of minerals and bioavailability modulators in food fortificants (mg/100 g [change in content of food product after ]%]	Contents of minerals and bioavailability Iron and zinc bi modulators in food fortificants (mg/100 g, db) human studies) [change in content of food product after FtFF,	Iron and zinc bioa human studies)	Iron and zinc bioavailability (animal and human studies)	
Food groups	Food fortificant	Food product	Level of fortification <sup>a</sup>	Iron Z	Zinc Bioavailabil inhibitors	Iron Zinc Bioavailability Bioavailability Type of study inhibitors enhancers		Outcome	Reference
	Vigna unguiculat.	Agna unguiculat. Biscuits made from 40%	40%	[-60%] [44%]	44%]	Ascorbic acid -	Ascorbic acid - Randomized,	After 4 weeks of biscuit Ayogu and	yogu and

		Regula et al.	(2010)														
)	the serum zinc	Iron regeneration At 10% fortification,	there was no	significant difference	in the hemoglobin,	serum, or kidney	iron concentration.	Liver iron	concentration was	reduced by 30%. At	20% fortification,	there was no	significant difference	in the hemoglobin,	serum, liver -, or	kidney iron	concentration
		Iron regeneration	efficacy method in there was no	female Wistar rats													
		[3 to	7%]														
		rom 10 to 20%	ried														
		Ä	maize) and dried	Shitaki	mushroom	powder											
		Lentinula edodes	(shiitaki	mushrooms)													
		Other	vegetables														

[]: Values in square brackets are percentage change in iron, zinc, and bioavailability inhibitor or enhancer contents due to FtFF.OFSP: Orange-fleshed sweet potato.

\*\*Percentage of replacement of starchy staple ingredient, db.

(Continues)

margarine



TABLE 3 The effects of individual food fortificants on the provitamin A, bioavailability inhibitor, and enhancer contents of cereal-based products as well as in vitro provitamin A bioaccessibility

		Reference	Kruger et al. (2018)	Bechoff et al. (2011)	Chilungo, Muzhingi, Truong, and Allen (2019)
	Bioaccessibility: In vitro digestion model	Outcome	As original product contained no $\beta$ -carotene, bioaccessible $\beta$ -carotene content was significantly increased to 0.55 mg/100 g	Bioaccessibility of $\beta$ -carotene was higher from chapatti (73%) and mandazi (49%) as compared to porridge or from boiled OFSP root (10%)	Bioaccessibility of $\beta$ -carotene was higher from chapatti (83%) as compared to porridge (65%)  Sunflower oil resulted in the highest bioaccessibility (percentage and amount of bioaccessible values) of $\beta$ -carotene as compared to beef fat or
	bioavailability ght [change in	Bioavailability enhancers	Fat: 10% (type of lipid source not specified)	Oil in chapatti: 7.4% Oil in mandazi: 3.3%	Oil in chapatti only: 10% (sunflower oil, margarine, or beef fat)
	Content of provitamin A carotenoids and bioavailability modulators in food fortificants in dry weight [change in contents due to FtFF, %]	Bioavailability inhibitors	Iron: 2.2 mg/100 g		
	Content of provitamin A modulators in food forti contents due to FtFF, %]	Vitamin A orprovitamin A content	27.3 mg/100 g $\beta$ -carotene [increased from 0 to 13.7 mg/100 g]	Porridge, chapatti, mandazis: 0.9, 3.2, 3.3 mg β-carotene/100 g,° respectively	Porridge = 1.7 to 2.5 mg $\beta$ -carotene/100 g Chapatti = 1.6 to 5.1 $\beta$ -carotene/100 g
		Level of fortification <sup>a</sup>	%0%	30%	%0%
		Food product	Various porridge (fermented and thick porridge) <sup>b</sup> and separately cooked OFSP	Porridge = maize + 30% soybean + raw OFSP Chapatti = wheat + raw OFSP Mandazis = wheat + raw OFSP	Porridge = maize + 50% raw OFSP powder or puree OFSP Chapatti = wheat + raw OFSP powder or puree OFSP
		Food fortificant	Ipomoea batatas (OFSP, tuber)		
organo de la composition della		Food groups	Starchy foods		

(Continued) TABLE 3

				Content of provitamin A modulators in food forti contents due to FtFF, %]	Content of provitamin A carotenoids and bioavailability modulators in food fortificants in dry weight [change in contents due to FtFF, %]	oioavailability ght [change in	Bioaccessibility: In vitro digestion model	
Food groups	Food fortificant	Food product	Level of fortification <sup>a</sup>	Vitamin A orprovitamin A content	Bioavailability inhibitors	Bioavailability enhancers	Outcome	Reference
Green leafy vegetables	Vigna unguiculata (cowpea, leaves)	Various porridges (fermented and thick porridge) <sup>b</sup> and separately cooked cowpea	%04	25.3 mg/100 g $\beta$ -carotene [increased from 0 to 10.1 mg/100 g]	Iron: 14.6 mg/100 g	Fat: 10% (type of lipid source not specified)	While the addition of cowpea increased the bioaccessible $\beta$ -carotene content of the porridges from 0 to 0.19 mg/100 (on average), cowpea meals exhibited 2 to 4 times less bioaccessible $\beta$ -carotene as compared to the OFSP containing porridges	Kruger et al. (2018)
Other vegetables	Daucus carota (carrot, root)	Extruded together: 15% Pearl millet porridge and solar dried carrot Dry blend: Pearl millet porridge and solar dried carrot	15%	77.5 mg/100 g of total provitamin A <sup>d</sup> [increased from 0 to 11.6 mg/100 g]		Sunflower oil: 5% of porridge	Sunflower oil: 5% of The bioaccessible content Ndiaye porridge of $\beta$ -carotene was et al. increased to 283 $\mu$ g/100 (2020 g in the dry blend formulation  The addition of other nutrient-dense plant materials (moringa and baobab) at 5% of the dry mix increased the stability of carotenoids during the extrusion process	Ndiaye et al. (2020)

[]: Values in square brackets are percentage change in iron, zinc, and bioavailability inhibitor or enhancer contents due to FtFF.

Percentage of replacement of starchy staple ingredient, db.

Mean effect of fortification on the various cereal/legume products reported.

The values reported were on a fresh weight basis.

The values reported were on a fresh weight basis.

Total provitamin A carotenoids calculated as  $\beta$ -carotene equivalent = all-trans- $\beta$ C + 1/2( $\beta$ -cryp + all-*trans-* $\alpha$ C + cis- $\beta$ C).





TABLE 4 The effects of individual food fortificants on the provitamin A, bioavailability inhibitor, and enhancer contents of cereal-based products as well as in vivo provitamin A bioavailability

				Vitamin A carotenoids a modulators in food forti contents due to FtFF, %]	Vitamin A carotenoids and bioavailability modulators in food fortificants) [change in contents due to FtFF, %]	ability nange in	Vitamin A carotenoid availability vivo bioavailability/vitamin A and provitamin A carotenoid status)	Vitamin A carotenoid availability (in vivo bioavailability/vitamin A and provitamin A carotenoid status)	
groups	Food groups Food fortificant	Food product	Level of fortification <sup>a</sup>	Vitamin A equivalent or provitamin A content	Bioavailability Bioavailability inhibitors enhancers	Bioavailability enhancers	Type of study	Outcome	Reference
vegetables	Moringa oleifera (moringa, leaves)	Various starchy 30 g/d (amount staples, of other legumes and ingredients wa dairy products* not specified) and moringa leaf powder		Content not provided			Twelve-week randomized control trial with preschool children	Significant increase in serum retinol but no significant differences between the control (from 0.64 to 0.73 \$\mu\text{mmol/L}\$, mean value) and moringa treatment (from 0.64 to 0.74 \$\mu\text{mmol/L}\$, mean value)	Zongo et al. (2018)
		Maize-based porridge, rice meal (waakye), or soup and moringa leaf powder	0.2 g leaf powder/kg body weight (amount of other ingredients was not specified)	Content not provided		Peanut butter (as Nine-week lipid source, randomiz amount not un-blind specified) controlle feeding the (3x/week children years of a	Nine-week randomized, un-blinded controlled feeding trial (3x/week) with children 5 to 12 years of age	Significant increases in Glover- serum retinol levels Amer (from 0.45 to 0.94 et al., µmol/L mean value) (2017) were observed after supplementation in the intervention group but not in the control group for those who were vitamin A deficient (serum retinol level < 0.70  µmol/L)	Glover- Amengor et al. (2017)

(Continued)

TABLE 4

(Continues)

equal amounts of synthetic  $\beta$ -carotene (7.2 mg)

leaves as compared to

were fed cassava

those who were fed

bioavailability were lower in rats which

, -, -, -, -, -, -, -, -, -, -, -, -, -,								
			Vitamin A carotenoids a modulators in food forti contents due to FtFF, %]	Vitamin A carotenoids and bioavailability modulators in food fortificants) [change in contents due to FtFF, %]	lability hange in	Vitamin A carotenoid availability vivo bioavailability/vitamin A and provitamin A carotenoid status)	Vitamin A carotenoid availability (in vivo bioavailability/vitamin A and provitamin A carotenoid status)	
Food groups Food fortificant	Food product	Level of fortification <sup>a</sup>	Vitamin A equivalent or provitamin A content	Bioavailability inhibitors	Bioavailability Bioavailability inhibitors enhancers	Type of study	Outcome	Reference
	AIN-93 rodent diet and moringa leaf powder	2.14%	94 nmol/g \$\beta\$-carotene [increased from 0 to 2.0 nmol \$\beta\$-carotene/g diet]	Cellulose: 5,870 mg/100 g to tt	6,000 mg/100 g	Four-week bioefficiency study with Mongolian gerbils	Gerbils that were fed moringa had significantly higher total liver retinol levels (0.74 µmol/liver, mean value) as compared to the negative control (0.35 µmol/liver, mean value), but no significant differences were observed in serum retinol levels	(2010)
Manihot esculenta (cassava, leaves)	AIN-93G diet and 1.95% cassava leaf powder	d 1.95%	[increased from 0 to 7.2 mg of \$\beta\$-carotene/kg diet]	2		Bioavailability study with vitamin A deficient male Wistar weanling rats (30 days depletion, 30 days repletion)	While vitamin A status de Almeida improved with cassava Siqueira, leaves (liver vitamin A Arruda, accumulation from 0 de to 259.2 µg, mean Vargas, value), both liver and de accumulation and Souza bioavailability were (2007)	de Almeida Siqueira, Arruda, de Vargas, and de Souza (2007)

the negative control (0.35  $\mu$ mol/liver, mean

significant differences

value), but no

were observed in

serum retinol levels

total liver retinol levels  $(0.78 \, \mu mol/liver, mean$ value) as compared to

gerbils

significantly higher

noid availability (in

y/vitamin A and

tenoid status)

TABLE 4 (Continued)	
Vitamin A carotenoids and bioavailability	ility Vitamin A caroteno
modulators in food fortificants) [change in	ge in vivo bioavailability
contents due to FtFF %]	provitamin A carot

Vitamin A

	d)	
	Reference	Soudy et al (2018)
	Outcome	The group which added Soudy et al. spirulina into the main dish had significantly higher serum retinol (1.26 $\mu$ mol/L, mean value) and $\beta$ -carotene concentrations (0.59 $\mu$ mol/L) as compared to the group that did not add spirulina (retinol: 1.03 $\mu$ mol/L, $\beta$ -carotene: 0.46 $\mu$ mol/L)
	Type of study	Observational study with healthy women
Bioavailability	enhancers	Peanut oil: 79 g/day
Bioavailability	inhibitors	Calcium: 177.8 mg/day Magnesium: 169.1 mg/day
provitamin A	content	10.8 mg of β-carotene/day [500%]
Level of	fortification <sup>a</sup>	%66·0 *
	Food product	Various starchy 0.99% staples, legumes and dairy products* and spirulina wafer
		$\begin{array}{cccccccccccccccccccccccccccccccccccc$

cyanobacterium)

Spirulina platensis

(spirulina,

Food groups Food fortificant

Four-week depletion/repletion Mongolian study with 6,000 mg/100 g Cotton seed oil: of feed Cellulose: 5,890 mg/100 g [increased from 0 to  $\beta$ -carotene 2.2 nmol 118 nmol/g AIN93 rodent diet1.86% and morielle leaf powder nightshade, leaves) (morielle or black Solanum nigrum

Ejoh et al. (2010)

Gerbils that were fed

morielle had

 $\beta$ -carotene/g diet]

TABLE 4 (Continued)

		Reference		Phorbee et al. (2013)	chmaelzle et al. (2014)
	Vitamin A carotenoid availability (in vivo bioavailability/vitamin A and provitamin A carotenoid status)	Outcome	Gerbils that were fed bitterleaf had significantly higher total liver retinol levels (0.77 \$\pmos months \text{mon}/\text{liver}\$, mean value) as compared to the negative control (0.35 \$\pmos/\text{mol}/\text{liver}\$, mean value), but no significant differences were observed for serum retinol levels	The liver $\beta$ -carotene level was 83% higher in the carrot fed rats as compared to the control (diet without the addition of carrots)	Total liver retinol levels Schmaelzle were significantly et al. higher in formulations (2014) fortified with carrot as compared to those which were fed vitamin A free diet.  The potato and banana treatment groups had the highest liver retinol stores
	Vitamin A carotenoid availabilit vivo bioavailability/vitamin A aı provitamin A carotenoid status)	Type of study	Four-week depletion/repletion study with Mongolian gerbils	Four-week bioavailability study using weanling albino rats	Four-week bioefficiency study using Mongolian gerbils
	ability ange in	Bioavailability enhancers	6,000 mg/100 g	Vegetable oil: 8 g/100 g	3,650 to 6,000 mg/100 of feed
	oids and bioavail   fortificants) [ch ?F, %]	Bioavailability inhibitors	Cellulose: 5,490 mg/100 g		Resistant starch in Cotton seed oil: maize, potato, 3,650 to 6,000 rice, banana, mg/100 of fee and carrot, respectively: 2.4, 1.2, 4.5, 4.8%
	Vitamin A carotenoids and bioavailability modulators in food fortificants) [change in contents due to FtFF, %]	Vitamin A equivalent or provitamin A content	18.7 nmol/g β-carotene [increased from 0 to 1.6 nmol β-carotene/g diet]	8.4 mg/100 g of total $\beta$ -carotene [increased from 0 to 0.05 mg/100 g of diet]	11.8 to 16.8 nmol \$\beta\$-carotene/g of feed 4.05 to 6.42 nmol \$\alpha\$-carotene/g of feed
		Level of fortification $^{a}$	%44%	%9.0	0.5%
		Food product	AIN93 rodent diet and bitterleaf powder	White cassava (carotenoid free) based meal and freeze-dried carrot	White maize meal, potato, rice, or banana* and freeze-dried carrot
(Communed)		Food groups Food fortificant	Vernonia calvoana (bitterleaf, leaves)	Daucus carota (carrot, root)	
+ TIOUT		Food groups		Other vegetables	

TABLE 4 (Continued)

uj.	 Reference	ntly al to oil lean ices ices	the palm Zeba,  ch Prével,  na Somé,  ase in and  vels Delisle  8 (2006)  alue)  and Lietz et al.  (2001)  vere  her in  up (0.5  .) as  tt  vels  ween
Vitamin A carotenoid availability (in vivo bioavailability/vitamin A and provitamin A carotenoid status)	Outcome	Gerbils that were fed kenaf had significantly higher total liver retinol levels (0.81 \$\mu\text{mol/liver}\$, mean value) as compared to the negative control (0.35 \$\mu\text{mol/liver}\$, mean value), but no significant differences were observed for serum retinol levels	Twelve-month The addition of red palm Zeba, clinical study oil to school lunch using pre- and meals resulted in a Son posttest design significant increase in and with preschool serum retinol levels Del children (from 0.82 to 0.98 (200 (3x/week $\mu$ mol/L, mean value) treatment)  Six-month clinical Plasma $\alpha$ -carotene and Lietz etrial with $\beta$ -carotene concentrations were pregnant women concentrations were oil was provided significantly higher in monthly red palm oil group (0.5 throughout the and 0.96 $\mu$ mol/L) as third trimester compared to the and the first 3 control group (0.0 and months 0.23 $\mu$ mol/L), but postpartum serum retinol levels
Vitamin A carotenoid availabilit vivo bioavailability/vitamin A a provitamin A carotenoid status)	Type of study		Twelve-month clinical study using pre- and posttest design with preschool children (3x/week treatment) Six-month clinical trial with pregnant women Oil was provided monthly throughout the third trimester and the first 3 months
ilability hange in	Bioavailability enhancers	Cotton seed oil: 6,000 mg/100 g	
Vitamin A carotenoids and bioavailability modulators in food fortificants) [change in contents due to FtFF, %]	Bioavailability inhibitors		<u>-</u>
Vitamin A carotenoids a modulators in food forti contents due to FtFF, %]	Vitamin A equivalent or provitamin A content	82.6 nmol/g β-carotene [increased from 0 to 2.0 nmol β-carotene/g diet]	15 mL (amounts 18 mg \beta-carotene\text{b} of other ingredients were not specified)  12 g/d (amount 2,034 \mug/day of total of other provitamin A ingredients was carotenoids\text{c} not specified)  not specified)
	Level of fortification $^{\mathrm{a}}$	iet2.44%	<b>p</b> r
	Food product	AIN93 rodent d and kenaf powder	School lunch meal (food products not specified) and red palm oil red palm oil staples and dark leafy green vegetables* and red palm oil
	Food groups Food fortificant	Hibiscus cannabinus AIN93 rodent diet2,44% (gabayidje or kenaf) and kenaf powder	Elaeis guineensis oil) Elaeis guineensis (palm, red palm fruit oil)
	Food groups	Flowers	Fruits

the control (1.14  $\mu$ mol/L) and red palm oil group (1.17  $\mu$ mol/L) at 3 months postpartum (Continues)

Comprehensive REVIEWS

TABLE 4 (Continued)

			Vitamin A carotenoids and bioavailability modulators in food fortificants) [change in contents due to FtFF, %]	noids and bioavail d fortificants) [ch FF, %]	ability iange in	Vitamin A carotenoid availability vivo bioavailability/vitamin A and provitamin A carotenoid status)	Vitamin A carotenoid availability (in vivo bioavailability/vitamin A and provitamin A carotenoid status)	
Food groups Food fortificant	Food product	Level of fortification <sup>a</sup>	Vitamin A equivalent or provitamin A content	Bioavailability inhibitors	Bioavailability enhancers	Type of study	Outcome	Reference
	Tortillas, beans, and dairy products* andred palm o	Tortillas, beans, 15 mL/treatment 15 mg and dairy day (amount of $\beta$ -cc products* other 6.8 m; andred palm oil ingredients was $\alpha$ -cc not specified)	rtillas, beans, 15 mL/treatment 15 mg and dairy day (amount of $\beta$ -carotene/meal products* other 6.8 mg andred palm oil ingredients was $\alpha$ -carotene/meal not specified)		8 g of fat (lipid source not specified)	Ten-day clinical trial using preand post-test design with pregnant women Six treatments over the 10-day period	Palm oil addition resulted in a twofold increase of serum $\alpha$ -carotene and $\beta$ -carotene concentrations as compared with 1.2-fold by $\beta$ -carotene supplements (15 mg of $\beta$ -carotene/treatment day)  Maternal serum retinol did not significantly increase with the addition of red palm oil (from 1.34 to 1.41 $\mu$ mol/L)	Canffeld et al. (2001)
	Wheat-based cookies (biscuits)	7.5 g (total amount of other ingredients was not specified)	35.5 mg/100 g of $\beta$ -carotene [increased from 0 s to 215 mg of $\beta$ -carotene/serving, 20% baking loss]		Vegetable oil: (amount not specified)	Controlled clinical trial with school-aged children (50 school days). Participants were provided with 6 fortified	Controlled clinical Red palm oil addition trial with resulted in a school-aged significant increase in children (50 plasma retinol (from school days). 0.75 to 2.17 $\mu$ mol/L) Participants were and $\beta$ -carotene provided with 6 concentration (from fortified 0.55 to 0.76 $\mu$ mol/L)	Ranjan, Passi, and Singh (2019)

[] : Values in square brackets are percentage change in iron, zinc, and bioavailability inhibitor or enhancer contents due to FtFF.

 $\ast$  Mean effect of fortification on the various cereal/legume/dairy products reported.

Percentage of replacement of starchy staple ingredient, db.

<sup>b</sup> One microgram retinol activity equivalents converted to 12  $\mu$ g all-*trans*  $\beta$ -carotene. <sup>c</sup> Total provitamin A carotenoids = the sum of all-trans isomers ( $\beta$ -carotene +  $\alpha$ -carotene +  $\beta$ -cryptoxanthin).



of, for example, phytate (Gomes et al., 2017). In the second study, also with Wistar rats, no effect was observed on hemoglobin maintenance, but no explanation was provided to account for this (Infante et al., 2017). In other studies, no significant change in bioaccessible iron with OSFP fortification was found when measured by the in vitro dialysability assay (Kruger, Breynaert, Pieters, & Hermans, 2018), whereas in a follow-up study an approximate doubling in both the percentage and amount of iron taken up by Caco-2 cells was found (Kruger, 2020) (Table 1). The cereals in the dialysability study were whole grain, while the maize in the Caco-2 cell culture work was refined, with a much lower phytate content. This indicates that nutrient and antinutrient contents of the starch base of the food product as well as the food-based fortificant must both be considered for successful implementation of FtFF. In contrast, with zinc, its percentage bioaccessibility/uptake was increased but the amount was not, when assayed by both dialysability (Kruger et al., 2018) and Caco-2 cell (Kruger et al., 2020) assays. This was probably due to the lower zinc content of the OFSP, compared to the cereals.

Research into FtFF with green leafy vegetables has been mostly limited to studies of cowpea plant leaf and moringa tree leaf fortification of cereal porridges, with only dialysability and Caco-2 assays having been performed (Table 1). With cowpea leaf fortification of cereal porridges, the percentage of iron bioaccessibility as measured by the dialysability assay was greatly reduced but the amount of bioaccessible iron was not affected (Kruger et al., 2018). However, in follow-up work using the Caco-2 assay, both the percentage of iron and zinc bioaccessibility was not affected (Kruger, 2020). According to a consensus statement from a HarvestPlus Expert Consultation, when in vitro assays are used to estimate iron and zinc bioavailability, the direction of the effects is of more significance than the magnitude measured (Fairweather-Tait et al., 2005). On this basis, it seems that the cowpea leaves did not positively affect iron and zinc bioaccessibility. This was likely due to their high content of tannins, other phenolics, and phytate (Kruger et al., 2018). The polyphenols in plants, especially the condensed tannins, can strongly decrease iron and to a lesser extent zinc absorption (Cercamondi, Egli, Zeder, & Hurrell, 2014; Fairweather-Tait & Hurrell, 1996). Phenolics can form insoluble complexes with the iron and zinc, but the structure of the specific polyphenol will dictate the extent of inhibition (Hart, Tako, Kochian, & Glahn, 2015). However, due to the fact that the cowpea leaves contained much more of these minerals compared to the cereal porridges, when fortified with cowpea leaves, the amounts of bioaccessible iron and zinc were increased several folds.

A few other types of vegetables have been investigated: garlic, onion, and shitaki mushroom. With garlic and

onion fortification of wet cooked cereals and legumes, iron and zinc bioaccessibility were slightly improved (Gautam, Platel, & Srinivasan, 2010) (Table 1). It was suggested that these improvements were due to their high content of sulfur compounds. Fortification of maize biscuits with shitaki mushroom powder, which has a high iron content, was found to have a positive effect on iron status in a feeding trial with iron-depleted rats, similar to that of iron gluconate fortification (Regula, Kreipcio, & Staniek, 2010).

Research on fruits as fortificant has been more limited. Only baobab fruit pulp has been studied, with only in vitro dialysability assays being performed (Table 1). However, the three studies (Gabaza, Shumoy, Muchuweti, Vandamme, & Raes, 2018; Oluyimika, Kruger, White, & Taylor, 2019; van der Merwe, Kruger, Ferruzzi, Duodu, & Taylor, 2019), by two different groups, revealed a generally consistent improvement in iron bioaccessibility from various cereal-based porridges. There was also some indication of improved zinc bioaccessibility. These improvements were attributed to its high content of citric acid and ascorbic acid. These organic acids are well-known promoters of iron absorption (Lönnerdal, 2000).

With flowers, only rosella (*Hibiscus*) calyx powder has been studied (van der Merwe et al., 2019) (Table 1). Its inclusion substantially increased iron and zinc bioaccessibility, probably as a result of a combination of the relatively high content of these minerals and organic acids in rosella calyx.

As stated, there have also been a few studies where the effect of multiple food-based fortificants on iron and zinc bioavailability were investigated. Despite the scientific difficulty of establishing which fortificant(s) actually affected mineral bioavailability, in practice, FtFF with multiple foodstuffs is likely to be the most efficacious. This is both a sensory quality standpoint where flavors can be optimized and also a formulation standpoint where the optimal contents of various micronutrients, and importantly, contents of bioavailability enhancers and inhibitors can be obtained from various food-based fortificant groups. A few in vitro studies (e.g. Gannon, Glahn, & Mehta, 2019; Lung'aho & Glahn, 2009) and one rat model study (Helbig, Buchweitz, & Gigante, 2008) have been performed, with varying results. Two human studies where multiple foodstuffs were investigated in the form of FtFF were found. In an interventions study with children aged 6 to 23 months, a traditional pearl millet porridge complementary food was fortified with a combination of common beans, peanuts, sorghum, and soumbala (fermented Parkia biglobosa [leguminous tree] seed) (Ouédraogo et al., 2010). After 22 weeks, the infants' blood hemoglobin concentration was significantly increased (p < .001). Of note, was that additional conventional mineral fortification of the porridge did not further improve blood hemoglobin

levels. The positive effect of the multiple food-based fortificants was attributed to diversification of micronutrient sources, reduction of antinutritional factors by the processing that the food-based fortificants were subjected to, and the very high iron content of the soumbala (70 mg/100 g) which was several times more than that of the other plant food components. Siqueira et al. (2003) investigated the effectiveness of fortifying foods with a "multimixture," composed of toasted wheat bran, cassava leaf powder, and eggshell, alongside other nonfood-based actions (e.g. nutritional education) to improve children's health. They found that the whole intervention improved the blood parameters and reduced anemia in the children, but this was independent of multimixtures fortification. However, the group that consumed these fortified foods had significantly increased height-for-age z-scores compared to the control group.

As a consequence of the limited number of studies to date, combined with the diversity of food-based fortificants investigated and some contradictory data, it is evident that the current body of research on iron and zinc FtFF of starchy staple foods lacks sufficient data to make general conclusions about its efficacy. The possible exceptions are fortification with ascorbic acid— and citric acid—rich fruits and  $\beta$ -carotene-rich plant foods where positive effects have been observed.

#### 4.2 | Vitamin A

As shown in Tables 3 and 4, target vitamin A fortificants of various food group types have been added into starchy staple food products comprising single or multiple ingredients, including cereals, legumes, and some dairy products. In addition to the target fortificants, it is important to note that most of the studies evaluating the bioaccessibility and bioavailability of provitamin A reported the use of additional lipid fortification (e.g., vegetable oil, cotton seed oil, and sunflower oil) to facilitate the intestinal micellarization and subsequent absorption of the provitamin A carotenoids.

OFSP has been the most commonly used starchy fortificant and can contribute substantially towards the recommended dietary allowance (RDA) of vitamin A, primarily in the form of  $\beta$ -carotene (Table 3). The effect of OFSP fortification on the bioaccessibility of  $\beta$ -carotene has been evaluated in three different in vitro studies. The addition of OFSP into different wheat- and maize-based food products was shown to provide sufficient amounts of bioaccessible provitamin A carotenoids to achieve 20 to 100% of the RDA for children 1 to 3 years of age (Bechoff et al., 2011). Similarly, a study by Kruger et al. (2018) demonstrated that the incorporation OFSP in different cereal-based porridge

formulations resulted in a significant content of bioaccessible  $\beta$ -carotene. The bioaccessible  $\beta$ -carotene content of porridge formulations fortified with OFSP was also significantly higher than those fortified with cowpea despite the similar  $\beta$ -carotene contents of the two food-based fortificants. This was attributed to the higher content of minerals in cowpea, which have been shown to react with carotenoids (Polyakov, Focsan, Bowman, & Kispert, 2010) and/or inhibit the formation of micelles by precipitating bile salts and also triglycerides and free fatty acids (Corte-Real et al., 2016). While some studies have demonstrated the efficacy of OFSP (Low et al., 2007; van Jaarsveld et al., 2005) and other starchy foods such as provitamin A cassava roots (Phorbee, Olayiwola, & Sanni, 2013) in improving provitamin A content and bioavailability, they were used as partial or complete replacement of the same staple ( $\beta$ -carotene free) crop, which is biofortification rather than FtFF.

Green leafy vegetables have also been widely investigated as food-based fortificants of vitamin A in in vivo studies (Table 4). The fortification of maize-based meal with moringa leaf powder has been shown to significantly improve liver vitamin A stores in Mongolian gerbils with no differences in serum retinol levels between the control and treatment group (Ejoh, Dever, Mills, & Tanumihardjo, 2010). This was explained by homeostatic regulation of serum retinol concentrations. The same study reported a similar increase in liver vitamin A levels with the addition of other green leafy-type vegetables, including morielle (black nightshade) and African bitterleaf, kenaf, and spirulina. In agreement with this animal study, the addition of moringa leaf powder into different starchy-based food preparations resulted in a significant improvement in vitamin A status in children aged 5 to 12 years as compared to the control group which did not receive the moringafortified dishes (Glover-Amengor, Aryeetey, Owusu, Afari, & Nyarko, 2017). Also, Soudy et al. (2018) reported that the incorporation of spirulina powder into daily meal preparations containing various starchy foods, legumes, and dairy products resulted in a significant increase in serum retinol and  $\beta$ -carotene levels in healthy women.

There are few studies involving the use of other vegetables in the FtFF approach. The incorporation of carrots into pearl millet-based porridge formulations has been shown to substantially improve the provitamin A carotenoid content as well as the bioaccessible carotenoid content in vitro (Debelo et al., 2019; Ndiaye, Martinez, Hamaker, Campanella, & Ferruzzi, 2020) (Table 3). However, the addition of other nutrient-dense plant foods such as baobab fruit pulp at high levels (25% of dry mix) has been shown to reduce carotenoid bioaccessibility from carrots in these porridge formulations (Debelo et al., 2019). In apparent contrast, the co-extrusion of pearl mil-



let with carrots and low amounts of baobab (5% of dry mix) has been shown to stabilize carotenoids to thermal degradation and thereby increase their bioaccessibility (Ndiaye et al., 2020). The authors suggested that this was due to the high ascorbic acid content of baobab fruit. These findings highlight that nutrient interactions in product formulations as well as the impact of different foodprocessing technologies play a key role in determining the ultimate efficacy of target fortificants. In agreement with the findings of these in vitro studies, the fortification of different starchy feeds with carrots has been shown to significantly improve storage of vitamin A in the liver of Mongolian gerbils (Schmaelzle et al., 2014) (Table 4). Similarly, the fortification of cassava-based meal with carrot powder was shown to increase liver  $\beta$ -carotene level by 83% as compared to the control in weanling Wister rats (Phorbee et al., 2013).

In regard to carotenoid-rich oils, red palm oil is one of the most commonly used food-based fortificants that has been shown to improve both vitamin A content and bioavailability in vivo (Table 4). The fortification of wheatbased cookies (biscuits) with red palm oil was shown to significantly increase plasma retinol and  $\beta$ -carotene concentrations in school-aged children (Ranjan et al., 2019). Similarly, the consumption of various meals fortified with red palm has been shown to increase serum  $\beta$ -carotene concentrations by twofold as compared with 1.2-fold increase by equal amounts of  $\beta$ -carotene (90 mg) supplement (Canfield, Kaminsky, Taren, Shaw, & Sander, 2001). The study also reported that the increase in the  $\beta$ -carotene content in breast milk was higher in the palm oil group (2.5-fold) as compared to the supplement group (1.6-fold). Lietz et al. (2001) also reported similar improvements in plasma and breast milk  $\alpha$ -and  $\beta$ -carotene concentrations with the addition of red palm oil.

While most nuts are known to contain low amounts of provitamin A carotenoids (Alasalvar & Bolling, 2015), their high lipid content can play an important role in facilitating provitamin A carotenoid absorption and potentially impact metabolism. For example, a randomized clinical trial using a stable isotope technique showed that the fortification of cooked kale with peanut butter resulted in a significant increase in the bioavailability of  $\beta$ -carotene and its subsequent conversion into vitamin A in preschool children (Muzhingi, Yeum, Bermudez, Tang, & Siwela, 2017). Other studies have also reported the use of peanut butter to facilitate carotenoid absorption from different food products (Solon et al., 2000; van Jaarsveld et al., 2005).

The simultaneous addition of multiple strategically chosen food-based fortificants has the potential to not only improve vitamin A bioavailability but also provide other nutrients including vitamin E, protein, and fatty acids for addressing multiple macro- and micronutrient defi-

ciencies. However, the inclusion of multiple fortificants must be considered carefully and potentially even screened using an applicable model as the potential for antagonistic effects within the food components exists that modulate the release of provitamin A carotenoids from their matrices. As demonstrated by Dhuique-Mayer et al. (2018), who found that the  $\beta$ -carotene bioaccessibility from formulations containing both OFSP and pumpkin was considerably lower as compared to the  $\beta$ -carotene bioaccessibility from OFSP or pumpkin alone. Similarly, the bioaccessibility of  $\beta$ -carotene from carotenoid-rich banana-based products was reported to be lower with the addition of amaranth leaves as compared to the formulations without the green leafy vegetable (Ekesa et al., 2012). These studies indicate that the combination of certain food matrices may not provide the optimal environment for provitamin A micellarization and ultimate bioavailability.

Studies evaluating the potential of vitamin A food-based fortificants are more promising than those for iron and zinc. This is partially due to the fact that, unlike with the minerals, the starchy staple foods normally contain negligible amounts of  $\beta$ -carotene or other carotenoids before fortification. This means that the fortification always results in a substantial increase in the amount of bioavailable  $\beta$ -carotene. Studies using OFSP and red palm oil are especially encouraging as most of studies reported improved vitamin A status or bioavailability with their addition.

#### 5 | CONSIDERATION OF BEST PRACTICES IN IMPLEMENTATION OF PLANT-BASED FtFF STRATEGIES

As evidence continues to emerge on the potential effectiveness for FtFF strategies, consideration and ultimate selection of local nutrient-dense plant materials for use in FtFF should rely on well-known materials that have a documented history of use and track record of human consumption. While alignment with local regulation remains a key requirement, potential plant-based fortificants should meet the criteria of generally recognized as safe or otherwise conform to safety assessment for botanical ingredients, as outlined by EFSA and FDA (Abdel-Rahman et al., 2011; German Federal Institute for Risk Assessment, 2018). The existing international regulatory frameworks for botanical ingredients in foods and dietary supplements, while not complete or in complete alignment (Low, Wong, Yap, De Haan, & Rietjens, 2017), should serve as a starting point for consideration of appropriate use and safety of any ingredient in consideration for FtFF. This includes the concepts of (1) botanical identity, (2) information on the agronomic and manufacturing processes, (3) chemical and microbiological profiles, (4) setting specifications for target nutrients and proposed usage levels, (5) compatibility with food application and stability to processing and storage, and if available (6) any toxicological data on the dietary or supplemental use of the intended material (Low et al., 2017).

## 5.1 | Raw material authentication and ingredient documentation

Leveraging local nutrient-dense plant materials as micronutrient sources in staple food fortification requires a robust strategy including authentication of the raw material in order to ensure quality and mitigate potential contamination or adulteration of the intended fortifying ingredient. Considering the nature of ingredients and the proposed use in FtFF strategies, authentication approaches used for botanical supplements can be applied. Such approaches set out in the literature vary widely, from simple macroscopic and sample tracking evaluations to genetic fingerprinting and advanced metabolomic profiling (Smillie & Khan, 2010). Minimum best practices should include a basic macroscopic/microscopic taxonomic identification and documentation of the plant material at its source. This would include key information used in botanical identification such as (1) identification (family, genus, and species of plant), (2) part of the plant used, (3) geographic origin, (4) growing condition, (5) wild or cultivated, (6) cultivation practice and phytosanitary measures (i.e., use levels of use for pesticides), and (7) postharvest handling or process (Kroes & Walker, 2004). Such documentation when combined with chemical profiling of key micronutrients and other characteristic compounds (i.e., secondary metabolites) can provide a reasonable level of authentication and can reduce the potential risk associated with poor quality material or raise the potential for detection of adulterated material (Simon et al., 2017).

Plant materials applied in FtFF are likely to be used as either whole fresh materials, dried materials, or fractions of plants (leaf, flowering, or other edible portions), or in the form of water extracts or dried extracts that followed traditional preparation methods (e.g., steeping or brewing). Considering the need to process these ingredients, it is critical to consider their extent of susceptibility to processing and storage conditions that could deteriorate both nutritive value and quality. The nature of postharvest and processing methods used in generation of the ingredient (i.e., drying, natural fermentation, physical separation, etc.) must be well documented. Taking the example of provitamin Arich carrots or mango, processing steps used in generation of a final ingredient could be as simple as slicing/grating

to increase surface area followed by drying (by solar or gas/electric drier), milling, and packaging. In this case, a choice of the drying method (solar or gas) could increase the exposure of carotenoid-rich materials to heat and solar energy thereby altering recovery of valuable provitamin A carotenoids and other sensitive micronutrients (Oliveira, Brandão, & Silva, 2016). Also, the final stability of target provitamin A carotenoids in dried products must be considered as these compounds are sensitive to oxidation that can be facilitated under conditions of enhanced light, heat, and moisture (Colle, Lemmens, Knockaert, Van Loey, & Hendrickx, 2016).

## 5.2 | Practical consideration of nutritional factors impacting plant-based fortificant choice

To achieve the objective of FtFF (increased content of bioavailable micronutrient/s), the plant-based fortificant must generally have notably higher levels of the desired micronutrient/s and/or bioavailability enhancers and/or lower levels of antinutrients, compared to the unfortified food product. Moreover, it should be able to be incorporated into a food product at levels as low as 1%, and no more than 50% (Chadare et al., 2019) and still substantially increase the content of the bioavailable micronutrient/s in the product.

Regarding target levels for fortification, with conventional fortification, the WHO recommends using the estimated average requirements (EAR) cut-point method to set target micronutrient levels (FAO/WHO, 2006). The aim of the EAR cut-point method, which is based on the usual micronutrient intake of a population subgroup and their micronutrient requirements, is to shift the distribution of intakes upwards so that only a small proportion (2 to 3%) is at risk of an inadequate intake of that micronutrient. This approach has been taken with biofortification of crop foodstuffs where the target micronutrient levels have been set to meet the requirements of nonpregnant, nonlactating women and 4 to 6 year-old children, based on their existing food consumption patterns (Bouis & Saltzman, 2017). However, this approach is probably too complex to be applied to FtFF by a village or small-scale processor. This is on account of the fact that the level of incorporation of a plant-based fortificant into an individual food product will be constrained by factors such as its impact on the sensory acceptability and cost of the product. The impact of these factors will be both fortificant and food product specific. Hence, a better approach will be to initially compile databases at the level of the desired micronutrients in available plant-based fortificants themselves in terms of their contribution alone to the EAR of



the at-risk groups. From these data, food product-specific information could then be determined. As an example, preliminary data on micronutrient contents of potentially valuable fortificants (which were not already reviewed in Tables 1 to 4) are presented in Table 5, including (in square brackets) the dry weight of the fortificant required to meet 25% the daily requirement of iron, zinc, and vitamin A of women of childbearing age.

## 5.3 | Challenges with identifying potential plant-based fortificants

#### 5.3.1 | Micronutrient contents

A major drawback of plant-based fortificants is that the levels of micronutrients can vary considerably. For example, from a small survey of six different plant-based fortificant products from suppliers in Senegal and South Africa (Table 6), it is clear that substantial variations in target micronutrient contents of the same botanical material from different sources occur. In the case of baobab, iron content differed by up to eightfold between products obtained in Senegal and South Africa from either small or large commercial enterprises. This variability is not unique to these examples provided in Table 6, as the micronutrient contents of most potential fortificants identified in Table 5 also varied greatly.

It is, however, difficult to pinpoint the source of the variation. Genotype and cultivation environment can strongly influence micronutrient levels. In the case of cassava, for example, the iron content of the leaves was found to vary fivefold among four cultivars and threefold across two environments in Mozambique, with highly significant two-way interaction (p < .001) (Burns et al., 2012). The zinc content of provitamin A cassava tubers was found to vary twofold across 13 environments in Uganda (Esuma, Kawuki, Herselman, & Labuschagne, 2016). Also, the total carotenoid content of the cassava tubers was found to vary twofold among 13 cultivars and twofold across 13 the environments, with highly significant two-way interaction (p < .001).

Harvesting, processing, and storage can also have an effect on the micronutrient content of the plant-based fortificants. Where the minerals are concerned, these activities would most likely result in increased mineral contents due to contamination from soil, dust, and/or processing equipment. Where the vitamins are concerned, these activities would most likely result in decreased vitamin contents, depending on the duration, and extent of exposure to factors such as oxygen, heat, sunlight, etc. The challenge with contamination minerals, especially iron, is that it is unclear to what extent they are bioavailable (Harvey,

Dexter, & Darnton-Hill, 2000). While it is possible to estimate the extent of soil contamination by analyzing the aluminum and vanadium content in the samples, together with the other elements (Joy et al., 2015), isolating contamination from processing is practically only possible by analyzing the mineral content of the food before and after processing.

Overall this presents significant challenges for standardization and consistency of final products, especially when conformational analysis of materials is not conducted. This supports the notion that as a practical consideration specifying and confirming micronutrient levels in a quality assurance program must be part of any plan to leverage natural plant foods or their ingredients in a fortification strategy.

## 5.3.2 | Controlling for bioavailability inhibitor contents

Another potential drawback of plant-based fortificants is that although they generally contain substantial levels of vitamins and minerals, they often also contain significant levels of compounds that can inhibit micronutrient absorption, commonly referred to as antinutrients or antinutritional factors (Akande et al., 2010; Gemede & Ratta, 2014).

The major plant antinutrients which inhibit mineral absorption are phytic acid, tannins and other polyphenols, and oxalic acid. Phytic acid, or phytate (inositol hexakisphosphate) its salt, is the principal storage form of phosphorus in many plant tissues, especially grains (Kumar et al., 2010) but also green leafy vegetables (Uusiku, Oelofse, Duodu, Bester, & Faber, 2010). Phytate binds the divalent ions of essential minerals, notably iron, zinc, and calcium, greatly reducing their absorption (Kumar et al., 2010). Oxalic acid [(COOH)<sub>2</sub>)] is widely distributed in plant foods, especially leafy vegetables (Noonan & Savage, 1999) and is a powerful inhibitor of calcium absorption as a result of the formation of insoluble calcium salts (Amalraj & Pius, 2015). Plant materials can also be high in divalent minerals such as calcium as well as dietary fiber-type components including, pectin, other gels, cellulose, and lignan that can have a general inhibitory effect on carotenoid bioaccessibility and potentially bioavailability (Corte-Real et al., 2017; Kruger, Stuetz, & Frank, 2019; La Frano et al., 2014; Tomas, Sagdic, Çatalkaya, Kahveci, & Capanoglu, 2018).

When selecting a plant-based fortificant, the relative levels of antinutrients versus micronutrients should be evaluated and compared to the unfortified starchy staple food, to obtain an estimate of potential effect on micronutrient bioavailability (Table 5).

Micronutrient and bioavailability modulator contents of especially promising food fortificants compared to various staple cereal foods TABLE 5

		Contonte					
		Contents					
	Examples of staple				Potential bioavailability	Potential bioavailability	
Food group	cereal foods which could be fortified	Iron (mg/100 g, db)	Zinc (mg/100 g, db)	$\beta$ -carotene (mg/100 g, db)	inhibitors (mg/100 g, db)	enhancers (mg/100 g, db)	References
Common staples	səld						
Cereal staple foods	Refined maize meal (yellow)	1 to 2 <sup>1</sup> [300 to 321 g]	<1 <sup>1</sup> [541 g]	<1 <sup>1</sup> [21 to 105 kg]	Dietary fiber: 2 to $6\%^1$	Fat: 1 to $5\%^1$ AA: ND <sup>1</sup>	1. USDA (2020)
	White rice (not fortified)	2 <sup>1</sup> [281 g]	$1^1$ [167 g]	$ND^1$	Dietary fiber: $3\%^1$	Fat: $1\%^1$ AA: ND <sup>1</sup>	
	White bread flour (not fortified)	$1^1$ [500 g]	$1^{1}$ [235 g]	$<1^1$ [>100 kg]	Dietary fiber: $2\%^1$	Fat: $2\%^1$ AA: ND <sup>1</sup>	
Potential fortificants	ificants						
Starchy foods	Cassia obtusifolia (senna (sicklepod), grain legume)	9 to 31 <sup>1,3</sup> , [14 to 53 g]	10 to 30 <sup>1:3,4</sup> [7 to 20 g]	13 <sup>2a</sup> [16 g]	Tannins: 160 to 600 mg/100 g <sup>1,2,4</sup> Total free phenols: 220 to 660 mg/100 g <sup>1, U</sup> Crude fiber: 7 to 11% <sup>1,2,3,4</sup> Phytate: 240 mg/100 g <sup>2</sup>	Fat: .4 to 7%1.3.4 AA: 11.88 mg/ 100 g <sup>2</sup>	Vijayakumari,     Siddhuraju, and     Janardhanan (1993)     Ingweye, Kalio, Ubua,     and Umoren (2010)     Vadivel and     Janardhanan (2002)     Vadivel and     Janardhanan (2002)
	Phaseolus lunatus (lima beans, grain legume)	16 to 25 <sup>1,2</sup> [18 to 28 g]	3 to 13 <sup>1.2</sup> [16 to 67 g]	<1 <sup>4</sup> [1.6 kg]	Tannins: 150 to 640 mg/100 g <sup>1,3</sup> Total free phenols: 260 to 340 mg/100 g <sup>1</sup> Crude fiber: 4.0 to $5.3\%$ <sup>12</sup>	Fat:2 to 5% <sup>1,2</sup>	<ol> <li>Vijayakumari et al.         <ul> <li>(1993)</li> <li>Oshodi and Adeladun</li> <li>(1993)</li> </ul> </li> <li>Fagbenro et al. (2010)</li> <li>USDA (2020)</li> </ol>
	Cicer arietinum (chickpea, grain legume)	5 to 7 <sup>1</sup> [67 to 96 g]	4 to 7 <sup>1</sup> [27 to 54 g]	<1 <sup>1</sup> [0.5 to 1.8 kg]	Total phenolics: 1,080 mg GA/100g <sup>2</sup> Phytate: 1,210 mg/100 g <sup>2</sup> Total fiber: 15% <sup>2</sup>	Fat: 5% <sup>2</sup>	Thavarajah (2012)     Sreerama, Sashikala,     Pratape, and Singh     (2012)
							(Continues)



TABLE 5 (Continued)

		Contents					
Food group	Examples of staple cereal foods which could be fortified	Iron (mg/100 g, db)	Zinc (mg/100 g, db)	β-carotene (mg/100 g, db)	Potential bioavailability inhibitors (mg/100 g, db)	Potential bioavailability enhancers (mg/100 g, db)	References
	Vigna subterranea (bambara groundnut, grain legume)	1 to 13 <sup>2,3</sup> [34 to 321 g]	2 to 8 <sup>2:3</sup> [26 to 133 g]	1¹ [420 g]	Crude fiber: 7 to 37%!:3 Phytate: 220 to 325 mg/100g <sup>3</sup>	Fat: 7 to 13% <sup>1.3</sup>	<ol> <li>Honi, Mukisa, and Mongi (2018)</li> <li>Amarteifio et al. (2006)</li> <li>Mitchikpe et al. (2008)</li> </ol>
	Manihot esculenta (provitamin A cassava, root)	2 to 4 <sup>3</sup> [118 to 265 g]	<1 <sup>3</sup> [1.0 to 1.1 kg]	2 to 4 <sup>2</sup> [57 to 111 g]	Dietary fiber: 2 to $4\%^{1.3}$ Phytate: 95 to 136 mg/100 g <sup>1</sup>	Fat: 0 to 1% <sup>1,3</sup> AA: 31 to 68 mg/100 g <sup>3</sup>	Aragón, Ceballos,     Dufour, and Ferruzzi     (2018)     Charles, Sriroth, and     Huang (2005)     Stadlmayr et al. (2010)
Green leafy vegeta- bles	Cucurbita pepo (pumpkin, leaves)	21 to 64 <sup>1,2,3</sup> [7 to 22 g]	5 to 12 <sup>1,3</sup> [17 to 38 g]	17 to 21 <sup>1,2</sup> [10 to 12 g]	Dietary fiber: 17 to 21%1.2 Tannins: 161 mg/100 g <sup>2</sup> Phytate: 99 mg/100 g	Fat: 1 to 6% <sup>1.2</sup> AA: 79 mg/100 g <sup>3</sup>	<ol> <li>Van Jaarsveld et al. (2014)</li> <li>Fadupin, Osuoji, and Ariyo (2014)</li> <li>Ifon and Bassir (1979)</li> </ol>
	Abelmoschus esculentus (okra, leaves)	4 to 111 <sup>1.3</sup> [4 to 118 g]	5 <sup>1</sup> [40 g]	2 to 21 <sup>2,3</sup> [10 to 100 g]	Crude fiber: 11 to $51\%^{1,3}$ Phytate: $ND^1$	Fat: 3 to 10% <sup>1,3</sup>	<ol> <li>Mitchikpe et al. (2008)</li> <li>Djuikwo et al. (2011)</li> <li>Gemede &amp; Ratta. (2014)</li> </ol>
	Ceratotheca sesamoides (false sesame, leaves)	128 to 146 <sup>1,3</sup> [3 to 4 g]	4 to 5 <sup>1,3</sup> [40 to 50 g]	13 to 18² [12 to 16 g]	Crude fiber: 6 to 51% <sup>1,3</sup>	Fat: 2 to $4\%^{1,3}$ AA: 1 mg/100 g <sup>3</sup>	<ol> <li>Mitchikpe et al. (2008)</li> <li>Djuikwo et al. (2011)</li> <li>Stadlmayr et al. (2010)</li> </ol>

	(commaca)						
		Contents					
Food	Examples of staple cereal foods which could be fortified	Iron (mg/100 g, db)	Zinc (mg/100 g, db)	β-carotene (mg/100 g, db)	Potential bioavailability inhibitors (mg/100 g, db)	Potential bioavailability enhancers (mg/100 g, db)	References
	Basella alba (Ceylon spinach, leaves)	37 to 60 <sup>1,4</sup> [8 to 12 g]	4 to 19 <sup>1,4</sup> [11 to 47 g]	13 to 27 <sup>2,4</sup> [8 to 16 g]	Dietary fiber: 32 to 63% 3.4 Phytate: 41 mg/100 g <sup>3</sup> Tannins: 221 mg/100	Fat: 3 to 16% <sup>1,4</sup>	<ol> <li>Akanfe (2013)</li> <li>Djuikwo et al. (2011)</li> <li>Amalraj and Pius (2015)</li> <li>Kumar, Manoj, and Giridhar (2015)</li> </ol>
	Corchorus olitorius (jute mallow, leaves)	18 to 23 <sup>15</sup> [20 to 25 g]	2.8 to 5 <sup>1</sup> [40 to 71 g]	10 to 28 <sup>2, 3, 5</sup> [8 to 21 g]	Dietary fiber: 21 to 53% <sup>3,5</sup> Phytate: 60 mg/100 g <sup>4</sup> Tannins:1450 mg/100 g <sup>4</sup> Oxalate: 1700 mg/100 g <sup>4</sup>	Fat: 1 to 7% 3.5	<ol> <li>Ndlovu and Afolayan (2008)</li> <li>Traoré, Parkouda, Guissou, Traoré, and Savadogo (2019)</li> <li>Traoré et al. (2017)</li> <li>Ifemeje, Egbuna, Eziokwudiaso, and Eziokwudiaso, and Ezebuo (2014)</li> <li>Van Jaarsveld et al. (2014)</li> </ol>
	Momordica cochinchinensis (Gac, fruit)	1 <sup>1</sup> [346 g]	1 <sup>1</sup> [182 g]	21 to 60 <sup>2</sup> [4 to 10 g]	Dietary fiber: $35.2\%^1$	Fat: 4% <sup>1</sup>	Pinthong, Judprasong,     Tangsuphoom,     Jittinandana, and     Nakngamanong (2019)     Müller-Maatsch et al.     (2017)
Fruits	Carica papaya (papaya, fruit)	1 to 5 <sup>1,3</sup> [96 to 643 g]	0 to 1 <sup>1,3</sup> [143 to 500 g]	1 to 3 <sup>1,3</sup> [78 to 350 g]	Dietary fiber: 2 to $19\%^{2.3}$ Phytate: 0.5 to 12.5 mg/100 g <sup>2</sup> Oxalate: 3,860 mg/100 g <sup>2</sup> Tannins: 570 to 750 mg/100 g <sup>2</sup>	Fat: 1% <sup>2, 3</sup> AA: 59 to 407 mg/100 g <sup>1,3</sup>	<ol> <li>Wall (2006)</li> <li>Ali, Devarajan, Waly, Essa, and Rahman (2011)</li> <li>Stadlmayr et al. (2010)</li> </ol>

TABLE 5 (Continued)

TABLE 5 (Continued)



	References	<ol> <li>Perpétuo and Salgado (2003)</li> <li>Arscott, Howe, Davis, and Tanumihardjo (2010)</li> <li>Mamiro et al. (2007)</li> <li>Stadlmayr et al. (2010)</li> </ol>	Okello, Okullo, Eilu,     Nyeko, and Obua (2017)     Djuikwo et al. (2011)     Garcia-Amezquita,     Tejada-Ortigoza,     Heredia-Olea,     Serna-Saldívar, and     Welti-Chanes (2018)     Stadlmayr et al. (2010)	<ol> <li>Romelle, Rani, and Manohar (2016)</li> <li>Souza et al. (2016)</li> </ol>	<ol> <li>Stadlmayr et al. (2010)</li> <li>Petropoulos, Fernandes, Barros, and Ferreira (2018)</li> </ol>	1. Knoblich, Anderson, and Latshaw (2005) 2. Navarro-González, García-Valverde, García-Alonso, and Periago (2011) 3. Peng, Zhang, and Ye (2008)
	Potential bioavailability enhancers (mg/100 g, db)	Fat: 0 to 1% <sup>1,4</sup> AA: 35 to 107 mg/100 g <sup>3,4</sup>	Fat: 0 to 1% <sup>3,4</sup> AA: 10 to 36 <sup>4</sup>	Fat: 2 to 4% <sup>1,2</sup>	AA: 29 <sup>1</sup> Organic acids: 473 to 2,247 $mg/100 g^2$ Fat: 0 to 4 <sup>1,2</sup>	Fat: $6\%^2$ AA: 131 to 298 mg/100 g <sup>3</sup>
	Potential bioavailability inhibitors (mg/100 g, db)	Dietary fiber: 2 to 13% <sup>1,4</sup> Tannins: 430 mg/100 g <sup>3</sup>	Dietary fiber: 8.4 to 13.3% <sup>3.4</sup>	Crude fiber: 9 to 15% <sup>1,2</sup> Phytate: 1,990 mg/100 g <sup>1</sup> Total phenolics: 1,420 mg/100 g <sup>1</sup>	Dietary fiber: $1\%^1$	Dietary fiber: 86% <sup>2</sup> Total phenolics: 158 mg/100 g <sup>2</sup>
	eta-carotene (mg/100 g, db)	2 to 4 <sup>2.4</sup> [50 to 95 g]	0 to 8 <sup>2,4</sup> [28 to 525 g]		0 to 2 <sup>1,2</sup> [100 to 2100 g]	29 <sup>1</sup> [7 g]
	Zinc (mg/100 g, db)	1 <sup>1,4</sup> [333 g]	3 to 8 <sup>1,4</sup> [25 to 69 g]	1 to 7 <sup>1,2</sup> [31 to 222 g]	2 to 3 <sup>1,2</sup> [67 to 95 g]	1 to 3 <sup>1,2</sup> [71 to 400 g]
Contents	Iron (mg/100 g, db)	1 to <sup>3,4</sup> [173 to 450 g]	2 to 38 <sup>1,4</sup> [12 to 205 g]	3 to 26 <sup>1,2</sup> [18 to 161 g]	1 to 5 <sup>1,2</sup> [90 to 900 g]	2 to 8 <sup>1,2</sup> [59 to 265 g]
	Examples of staple cereal foods which could be fortified	Mangifera indica (mango, fruit)	Tamarindus indica (tamarind, fruit)	Ananas comosus (pineapple, peel)	Abelmoschus esculentus (okra, fruit)	Solanum lycopersicum (tomato, peel)
	Food group					

(Continues)

(Continued) TABLE 5

		Contents					
Food	Examples of staple cereal foods which could be fortified	Iron (mg/100 g, db)	Zinc (mg/100 g, db)	β-carotene (mg/100 g, db)	Potential bioavailability inhibitors (mg/100 g, db)	Potential bioavailability enhancers (mg/100 g, db)	References
Other veg- etables	Cucurbita maxima (pumpkin, flesh)	1 to 3 <sup>1</sup> [167 to 450 g]	1 <sup>1</sup> [154 to 250 g]	3 to 19 <sup>1</sup> [11 to 81 g]	Crude fiber: 6 to 7%²	Fat: 3% <sup>2</sup> AA: 49 to 842 mg/100 g <sup>1</sup>	<ol> <li>Kulczyński and Gramza-Michałowska (2019)</li> <li>Kulaitienė et al. (2014)</li> </ol>
	Solanum melongena (eggplant)	1 to 39 <sup>2,3</sup> [12 to 500 g]	2 to 11 <sup>2,3</sup> [11 to 154 g]	0 to 23 <sup>1,2</sup> [9 to 488 g]	Dietary fiber: 3 to $52\%^{2,3,4}$ Total phenolics: 1350 to 1750 mg/100 g <sup>3</sup> Tannins: 4 to 161 mg/100 g <sup>3</sup>	Fat: 0 to 1% <sup>2,3</sup> AA: 6 to 314 mg/100 g <sup>2</sup>	<ol> <li>Djuikwo et al. (2011)</li> <li>Stadlmayr et al. (2010)</li> <li>Guillermo et al. (2014)</li> <li>USDA (2020)</li> </ol>
Flowers	Hibiscus sabdariffa (roselle, calyx)	16 <sup>1</sup> [28 g]	3 <sup>1</sup> [II g]	14 to 18 <sup>2</sup> [12 to 15 g]	Dietary fiber: 3.6%³	Fat: <1%³ AA: 17 mg/100 g³	<ol> <li>Djuikwo et al. (2011)</li> <li>Jung, Kim, and Joo (2013)</li> <li>Singh, Khan, and Hailemariam (2017)</li> <li>Stadlmayr et al. (2010)</li> </ol>
	Colocasia esculenta(taro, flowers)	30¹ [15g]	8 <sup>1</sup> [11 g]		Oxalic acid: $0.17$ $mg/100 g^1$ Fiber $20\%^1$	Fat: 5% <sup>1</sup>	1. Ejoh, Mbiapo, and Fokou (1996)
Seeds	Helianthus annuus (sunflower, seeds)	4 to 60 <sup>1,2</sup> [73 to 118 g]	3 to 7 <sup>1,2</sup> [11 to 29 g]	<1 <sup>2</sup> [7 kg]	Dietary fiber: 7 to 14%²	Fat: 48 to 54%² AA: 1.4 mg/100 g²	<ol> <li>Cabrera, Lloris,</li> <li>Gimenez, Olalla, and</li> <li>Lopez (2003)</li> <li>USDA (2020)</li> </ol>
	Pentaclethra 5 to 6 <sup>1,3</sup> macrophylla [12 to 28 g (African oil bean, seed)	5 to 6 <sup>1,3</sup> [12 to 28 g]	1 <sup>1</sup> [12 to 28 g]	<1 <sup>2</sup> [31 kg]	Crude fiber: 2 to 5% <sup>1</sup> Phytate: 1,800 to 2,110 mg/100 g <sup>1,2</sup> Tannins: 200 to 380 mg/100 g <sup>1,2</sup> Oxalate: 10 to 279 mg/100 g <sup>1,2</sup>	Fat: 39 to 54% <sup>1,2</sup> AA: 9.5 mg/100 g <sup>2</sup>	<ol> <li>Enujiugha and Akanbi (2005)</li> <li>Fungo et al. (2015)</li> <li>USDA (2020)</li> </ol>



<sup>&</sup>lt;sup>a</sup> Converted from 1 IU vitamin A = 0.3 μg retinol, 1 IU = 0.6 μg β-carotene.

[]: The amounts of fortificant (db) required to provide 25% of the iron, zinc, and vitamin A requirements of healthy women of childbearing age is also included (vitamin A - 700 μg retinol activity equivalents or 8.4 mg β-carotene/day, iron 18 mg/day and zinc 8 mg/day).

ND: not detected.

TABLE 6 Iron and zinc content and microbial load of some dried food fortificant powders (as is basis)

Food fortificant				Total microbial			
powder	Sourcea	Iron (mg/100 g)	Zinc (mg/100 g)	activity (cfu/g)	Coliforms (cfu/g)	Yeasts (cfu/g)	Molds (cfu/g)
Moringa leaf	SME Senegal	49.4	2.1	$6.1 \times 10^4$	$ND^{\mathrm{b}}$	ND	$1.8 \times 10^{2}$
	SME Senegal	42.9	1.9	$6.1 \times 10^3$	ND	ND	$1.4 \times 10^{2}$
	LCE South Africa	104.5	2.5	$8.9 \times 10^2$	$5.6 \times 10^2$	$1.0\times10^{1}$	ND
	LCE South Africa	100.9	2.1	$8.3 \times 10^3$	ND	ND	ND
	SME South Africa	31.4	0.3	$1.9 \times 10^4$	$1.8\times10^4$	ND	$4.0 \times 10^1$
Carrot	SME Senegal	2.6	1.9	$3.5\times10^3$	$7.0 \times 10^2$	ND	$5.0 \times 10^1$
	LCE South Africa	10.4	5.4	$1.2 \times 10^4$	$1.0 \times 10^{2}$	ND	$7.0 \times 10^1$
Baobab fruit	SME Senegal	10.7	1.8	$4.3 \times 10^3$	ND	ND	ND
	LCE South Africa	1.5	6:0	$1.0 \times 10^1$	ND	ND	ND
	LCE South Africa	2.3	0.7	$1.2 \times 10^4$	ND	ND	ND
Mango	SME Senegal	1.0	9.0	$2.0 \times 10^1$	ND	ND	$1.0\times10^{1}$
	LCE South Africa	3.4	9.0	ND	ND	ND	ND
Papaya	SME Senegal	1.9	0.5	$4.1\times10^2$	ND	ND	$6.3 \times 10^{2}$
Roselle calyx	SME Senegal	54.9	2.5	$16 \times 10^2$	ND	ND	ND

## 5.3.3 | Inclusion of bioavailability enhancer contents

While the presence of antinutrients is a real concern, certain plant-based fortificants contain enhancers of micronutrient absorption. The most notable and widely distributed enhancers of mineral absorption in plant foods are organic acids, especially ascorbic acid and citric acid (Gibson, Perlas, & Hotz, 2006). The mechanisms involve reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> and chelation of the minerals, which maintains them in a soluble and absorbable form (Iyengar, Pullakhandam, & Nair, 2010; Lönnerdal, 2000). Lipids are required for the absorption of  $\beta$ -carotene and other carotenoids through the formation of mixed micelles (Yeum & Russell, 2002). As such, it is critical to include dietary fat in the meal or with the intended FtFF application when possible. Selection of type of lipid will be dependent on common local sources. However, in general while triacylglcerides rich in monounsaturated fatty acid may be more effective in promoting carotenoid micellarization and absorption (Failla, Chitchumronchokchai, Ferruzzi, Goltz, & Campbell, 2014; Huo, Ferruzzi, Schwartz, & Failla, 2007), it is more critical to consider the amount of fat in a meal to promote sufficient absorption (Goltz, Campbell, Chitchumroonchokchai, Failla, & Ferruzzi, 2012).

When selecting a plant-based fortificant, the relative levels of enhancers to micronutrients should also be evaluated (Table 5). The minimum molar ratios of ascorbic acid and citric acid for optimal iron absorption are >4:1 and 100:1, respectively (Hunt, 2005; Teucher, Olivares, & Cori, 2004), although lower ratios may bring about some enhancement (Teucher et al., 2004). Even a relatively low quantity of additional plant oils in the diet, 3 to 5 g per meal, will enhance  $\beta$ -carotene absorption from green leafy vegetables (Goltz et al., 2012; Jayarajan, Reddy, & Mohanram, 1980).

## 5.4 | Potentially valuable plant-based fortificants

In addition to those fortificants highlighted earlier (Tables 1 to 4), Table 5 summarizes promising yet underutilized food sources that are common in sub-Saharan Africa and/or Asia. Possible plant-based fortificants included contain substantial amounts of iron, zinc, and/or provitamin A and/or desired bioavailability modulators and are compared to three common starchy staple foods (Table 5).

It is evident that green leafy vegetables in general contain substantially greater amounts of iron, zinc, and  $\beta$ -carotene as compared to the starchy staple foods (Table 5). Green leafy vegetables, however, also contain

high amounts of inhibitors, including tannins, total phenolics, oxalates, and fiber. Concerning vitamin A fortificants, the goal is a balance between high  $\beta$ -carotene content and lower contents of inhibitors including divalent minerals and fiber, which would make jute mallow and okra leaves ideal vitamin A fortificants due to their relatively higher  $\beta$ -carotene and lower divalent mineral and fiber contents. Small weights of these leaves (8 to 100 g) can provide 25% of iron, zinc, and vitamin A requirements of women of childbearing age. Where iron and zinc fortificants are concerned, the aim would be high contents of both iron and zinc as well as bioavailability enhancers (provitamin A and ascorbic acid) and lower contents of inhibitors (especially phytates and tannins). An example of a fortificant with valuable potential is pumpkin leaves, which at 38 g could provide at least 25% of iron, zinc, and vitamin A requirements of women of childbearing age and it contains low levels of dietary fiber and tannins and high levels of ascorbic acid.

The fruits/fruit parts identified are promising as they contain high contents of both  $\beta$ -carotene and ascorbic acid, while the levels of inhibitors are lower compared to the green leafy vegetables (Table 5). Some of the fruits even contain substantial amounts of iron and zinc (tamarind pulp and pineapple peel). The fruit fortificants also provide the opportunity for the use of various waste products as fortificants, for example, pineapple and tomato peel. However, while these levels of iron, zinc, and  $\beta$ -carotene from the different food groups are favorable, their efficacy in improving the bioavailable content of target micronutrients in FtFF efforts is yet to be investigated.

## 5.5 | Microbiological quality and target safety levels

As plant-based fortificants need to comprise a significant proportion of the final food product, they must comply with accepted international food safety standards in terms of limits on heavy metals, pesticide residues, and hygiene especially microbiological contaminants and toxins. In setting target safety levels, a good starting point is the Codex Alimentarius standards for fresh fruit and vegetables (Codex Alimentarius, 2007), dried fruit such as apricots (Codex Alimentarius, 1981), and the Codex Code of Practice for Spices and Dried Aromatic Herbs (Codex Alimentarius, 2014). As of 2020, Codex standards for spices and dried herbs are only still under development. However, of relevance is that the Kenya Bureau of Standards has drafted a Codex type standard for dehydrated vegetables (KEBS, 2018).

When dealing with natural plant materials, considering their potential for microbial contamination,



achieving strict hygiene limits can be challenging as many processors still depend on traditional processing technologies such as open-air solar drying. In Table 6, the two products with the highest microbial counts were moringa leaf powder and carrot powder, with some samples having elevated counts of coliforms, suggesting possible fecal contamination. Such contamination is not entirely surprising since tree leaves and similar plant parts are directly exposed to the environment for a period of weeks during cultivation and the products are generally open air solar dried. The microbial contamination of carrot powder, a thermally dried processed product, could be due to postprocessing contamination in combination to the fact that it is a low acid food. Some microbiological contamination is acceptable when the food products containing the plant-based fortificants will be subject to thermal processing before consumption. However, one of the moringa powder products had coliform levels  $>10^4$ cfu/g, which would be clearly unacceptable.

Concerning heavy metals, there is some indication, although very incomplete, that some species of potential plant-based fortificant leafy vegetables may pose greater risk than others, when they are cultivated on contaminated soils (Nabulo, Young, & Black, 2010). For example, the hazard quotient (HQ $_{\rm M}$ ) for cadmium for *Solanum aethiopicum* (Ethiopian nightshade) was some 16 times higher than of *Vigna unguiculata* (cowpea).

While the potential to exceed upper intake limits of any micronutrient from FtFF is minimal relative to synthetic fortification or supplementation, a concern remains the potential for certain plant ingredients to contain naturally occurring toxic substances. For example, bitter varieties of cassava can contain dangerously high levels of cyanogenic glycosides, which are present in all the plant tissues (Burns et al., 2012). Other natural toxins include saponins, which have been found in some African green leafy vegetables (Uusiku et al., 2010) and pyrrolizidine alkaloids which occur in particular species of many plant families (Dolan, Matulka, & Burdock, 2010). In view of the fact that plant-based fortificants would be consumed as a significant component of staple food products, caution would be the best policy when evaluating a novel plant-based fortificant as a potential food ingredient and that these aspects should be properly evaluated prior to use.

#### 5.5.1 | Costs versus benefits

A potential challenge with FtFF, especially in low socioeconomic populations, is the cost implication when relatively inexpensive staple flours and foods are fortified with more expensive micronutrient-rich food-fortificants. Recent research in Kenya has shown that FtFF of an instant cereal porridge flour with 20% dried carrot and baobab fruit would currently increase the net cost of the product by approximately 28% on account of the higher costs of these ingredients (De Groote et al., 2020). However, this and related research in Senegal revealed that consumers are willing to pay a significant premium for these products fortified with fruits and vegetables, an additional 12% in Kenya and 4 to 9% in Senegal (De Groote et al., 2018, 2020), which would significantly mitigate the additional cost

Food fortification is, however, not a simple economic issue of the additional premium that consumers are willing to pay for what they perceive as being the benefits versus the cost to the food manufacturer. Fortification programs are usually a governmental initiative aimed at attaining improved health and other societal benefits. With regard to health benefits, Horton (2006) calculated the cost of conventional fortification in terms of DALY (cost per disability adjusted life-year) saved. In current (2020) US\$, the cost of vitamin A fortification in Africa per DALY saved would be approximately \$42. In terms of economic benefits, data from the FAO/WHO (FAO/WHO, 2006) indicate that the cost: benefit ratio of conventional-type iron fortification for a lower middle income country, as measured by improved manual labor productivity, is 1:8. However, the feasibility of small-scale fortification programs in developing countries to adopt conventional fortification has been identified as a significant remaining challenge (WHO, 2016b). This is due, in part, to its dependence on imported vitamin and mineral premix ingredients and because of its requirement for specialized dosing equipment, both of which directly impact cost and limit adoption to larger-scale producers. Because FtFF does not require highly specialized equipment, it can, in contrast to conventional fortification, be readily implemented at a smaller scale.

FtFF also has the potential to yield additional economic benefits, particularly in developing countries. In sub-Saharan Africa, losses during postharvest, processing and distribution account for 35% of the region's fruit and vegetable crop (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011). Concentration and drying, especially of edible substandard produce and by-products into shelf-stable plant fortificants, would expand fruit and vegetable processing enterprises and substantially contribute to a reduction in these losses. By-products such as pumpkin and okra leaves and pineapple and tomato peel are promising fortificants (Table 5), which would have smaller cost implications compared to the premium products (carrot and baobab powder) included in the calculations of De Groote et al. (2020). In time, the expansion of plant food fortificant production should serve to bring down the cost of the fortificants and also generate a food ingredient value chain domestically. While future research studies and FtFF adoption by entrepreneurs will determine its market potential, the economic case for FtFF in developing countries is increasing.

## 5.6 | Supply chain and product development considerations

Regarding the supply chain of fortification ingredients, traditional fortification relies on well-established supply chains that provides custom premix solutions, which target micronutrient levels with appropriate overages accounting for losses of sensitive vitamins (i.e., vitamins A and E) during processing and over the product's intended shelf life (FAO/WHO, 2006). While many of these micronutrient ingredients and even the complete premixes are imported by developing countries, they remain consistent and generally cost effective, when implementable. By comparison, plant-based fortificants can and should be obtained locally through the agricultural supply chain. This contributes both to nutritional goals and to the development of new agronomic value centers. However, there are some inherent problems, including inconsistent availability of high-quality raw materials from which to manufacture the plant-based fortificant. For example, while numerous micronutrient-dense plants have been identified in sub-Saharan Africa, only a few truly have an evolved supply chain suitable for use as plant-based fortificants. For example, moringa cultivation and application has expanded extensively in sub-Saharan Africa (Sagona, Chirwa, & Sajidu, 2019). However, as geographic, seasonal, agronomic, or other environmental factors can lead to variation in the micronutrient content and quality. As such, raw material quality assurance systems must be robust to ensure appropriate quality and safety targets are achieved.

In addition to raw material supply, the development of manufactured food products must include consideration of the impacts these new plant-based ingredients. From a product quality perspective, impacts on sensory attributes are to be expected. Traditional iron fortification of cereal products is well known to impact on color and taste, even when formulated at relatively low levels (Habeych, van Kogelenberg, Sagalowicz, Michel, & Galaffu, 2016). The higher usage rates and complexity of plant-based fortificants would be expected to have greater impacts. For example, highly pigmented plant-based fortificants such as moringa and roselle (hibiscus), both excellent sources of iron and zinc when used at 10% of cereal formulations impart significant green and pink colors to finished formulations (Figure 3). These ingredients also have characteristic flavors that may or may not be appreciated by



FIGURE 3 The effects of various FtFFs on the visual appearance of pearl millet porridges. (a) Plain pearl millet, (b) fortified with carrot and papaya and baobab, (c) fortified with hibiscus and baobab, and (d) fortified with moringa, carrot, mango, and baobab

consumers. Also, significant dilution of starch-rich cereal products with plant-based fortificants will impact the rheological properties of the finished product, apparent to consumers as altered mouthfeel. These sensorial aspects were covered in the review by Chadare et al. (2019). As an example, Mounjouenpou et al. (2018) found that in Cameroon rice cookies fortified with baobab fruit pulp were optimally acceptable at a 20% inclusion level. In contrast, Boateng, Nortey, Ohemeng, Asante, and Steiner-Asiedu (2019) in a review of research from across Africa into the consumer acceptability of complementary foods fortified with moringa leaf powder concluded that their acceptability declined above 10% inclusion. Taken together, it is critical to consider the nature of the products that have been targeted for food-to-fortification and to assess the consumer acceptability of the food-to-food fortification in those products. Experience from iron fortification programs has shown that a decision tree approach comprising internal screening by "experts," followed by descriptive and affective (consumer) sensory panels is the best way to ensure product acceptability.

#### 6 | CONCLUSIONS

FtFF is an emerging food-based strategy that can be defined as the addition of micronutrient-dense food/s to a recipe (household level) or food formulation (food industry level), or the replacement of micronutrient-poor/antinutrient-rich ingredients, to substantially increase the amount of bioavailable micronutrient/s, with the



aim of improving the micronutrient status of populations where the intake of bioavailable micronutrients is inadequate. FtFF should be rooted in the increased scientific knowledge of nutritional composition and compound interactions affecting nutrient bioavailability which makes the standardized, safe and effective implementation of this food-based strategy possible on a commercial scale. Despite the limited number of studies, the wide diversity of food-based fortificants currently applied and some contradictory findings, there are some promising fortificants that have been shown to improve the amount of bioavailable iron and zinc and provitamin A from starchy staple foods. However, as the observed improvements in micronutrient bioavailability are relatively small, a sustained positive impact on micronutrient status is only likely with longterm regular consumption as staple foods. Consequently, in vivo studies of extended duration are warranted in order to determine the full potential of promising food-based fortificants and FtFF strategies. Issues such as the plantbased fortificant supply chain, final product quality, and potential cost implications present significant challenges in implementation of FtFF, especially in countries lacking a sophisticated food-manufacturing sector. Nevertheless, it provides unique opportunities for the use of currently wasted by-products and for development of local food value chains that can contribute to community economic development in addition to its nutritional benefits. Hybrid fortification which leverages synthetic micronutrient premixes to standardize product micronutrient levels may also prove to be attractive complementary strategies to address some of the drawbacks of FtFF. Further important aspects of FtFF which were not covered in this review, but should be considered in future work include (1) the potential of FtFF to address the double burden of malnutrition, both undernutrition (micronutrient deficiencies) as well as overnutrition (overnutrition and its associated diseases like type-2 diabetes and cardiovascular disease), on account of the high content of bioactive compounds as well as micronutrients in many plant food-based fortificants; and (2) the potential socioeconomic impact of FtFF on developing communities where supply chain development takes place.

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#### **AUTHOR CONTRIBUTIONS**

JK contributed majorly to concept and design, review of the literature, manuscript preparation, and scientific editing. JRNT contributed to review of the literature, manuscript preparation, and scientific editing. MGF contributed to manuscript preparation and scientific editing. HD contributed to the literature searches and provided minor summaries for manuscript preparation.

#### CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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#### SUPPORTING INFORMATION

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