Food Matrices: A Review of Critical Factors Impacting Nutritional Bioavailability

A Report from the Food Aid Quality Review

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Acronyms

a_w	Water Activity
BHA	Butylated Hydroxyanisole
BHT	butylated hydroxytoluene
BMI	Body Mass Index
BV	Biological Values
Ca	Calcium
CSB	Corn-Soy Blend
CSB +	Corn-Soy Blend Plus
CSB++	Corn-Soy Blend Plus Plus
CSM	Corn-Soy Milk
Cu	Copper
DCHA	Bureau for Democracy, Conflict and Humanitarian Assistance
DIAAS	Digestible Indispensable Amino Acid Score
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FAQR	Food Aid Quality Review
FBFs	Fortified Blended Foods
Fe	Iron
FePP	Ferric Pyrophosphate
FeSO4	Iron II Sulfate
FFA	Free Fatty Acids
FFP	Office of Food for Peace
GIT	Gastrointestinal tract
HEB	High-Energy Biscuit
HPP	High Pressure Processing
IAA	Individual Limiting Amino Acids
IGF-1	Insulin-Like Growth Factor 1
LNS	Lipid-Based Nutritional Supplement
MAM	Moderate Acute Malnutrition
MDFP	Micronized Dispersible Ferric Pyrophosphate
Mn	Manganese
MNP	Micronutrient Powder
MUAC	Mid-Upper Arm Circumference
MUFA	Monounsaturated Fatty Acids
NaFeEDTA	Ferric Sodium EDTA
NAL	National Agriculture Library

PDCAAS	Protein Digestibility Corrected Amino Acid Score
PER	Protein Efficiency Ratio
PG	Propyl Gallate
PUFA	Polyunsaturated Fatty Acids
RBV	Relative Bioavailability
RDA	Recommended Daily Allowance
RDS	Rapidly Digestible Starch
RNI	Recommended Nutrient Intake
RS	Resistant Starch
RUSF	Ready-to-Use Supplementary Food
RUTF	Ready-to-Use Therapeutic Food
SC	Supercereal
SC+	Supercereal Plus
SDS	Slowly Digestible Starch
TBHQ	Tertiary Butylated Hydoxyquinone
TIM	In vitro Gastrointestinal Model
USAID	United States Agency for International Development
UV	Ultraviolet
WFP	World Food Programme
WSB	Wheat-Soy Blend
WSM	Wheat-Soy Milk
Zn	Zinc
ZnO	Zinc Oxide
$ZnCO_3$	Zinc Carbonate
$ZnSO_4$	Zinc Sulfate

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1.Executive Summary

The bioavailability of nutrients from foods (how the foods get converted and used in the body) forms the crux of "nutritional efficiency." This is important when using food products to address issues associated with malnutrition. Food aid products are designed to provide defined amounts of energy and essential nutrients (in optimal forms) to undernourished populations in developing economies. It is therefore vital for product design to keep up with science and food technology where potential gains in nutrient bioavailability are concerned. Efforts to improve the bioavailability of food aid products could potentially offer enhanced efficacy and cost-effectiveness.

This report reviews the existing published and gray literature to determine possible opportunities for improvements to existing products. The review considers ingredients as well as production processes which have some potential to unlock nutrient availability to undernourished consumers.

The review finds various elements which affect the food matrix and thus the bioavailability of nutrients. These factors can be majorly divided into: i) ingredients; and ii) processing. The primary reason or the most common reason attributed to the factor affecting nutrient bioavailability is the presence of phytate and other antinutritional factors in the cereals and legumes which constitute a considerable share of food aid product portfolio. The antinutritional factors are present in raw as well as processed foods. The other factors related to ingredients which influence the food matrix and therefore influence nutrient bioavailability from food aid products are: protein quality, shelf life, micronutrient forms and nutrient interactions. For example, processing impacts and modifies the food matrix and milling removes most antinutritional factors present in grains and legumes. Extrusion processing increases the starch digestibility and contributes in preparing a less sticky porridge which has better chances of nutrient bioavailability. Additionally, there is a need to quantify and correlate the bioavailability of nutrients from food aid products in terms of in vitro and in vivo tests to present a cost-effective prediction of bioavailability from the different preparations of food aid products. Some non-food factors related to health and sanitation of the recipients of food aid was also found to play a critical role in nutrient absorption from the food matrices.

Further interaction with food aid industry stakeholders, commercial food technologies and operationally-informed nutritionists is warranted to discuss and prioritize options with a view to their adoption to enhance USAID's evolving food aid product portfolio.

2. Introduction

"Food" is defined here as a substance edible to humans, be it in solid or liquid form. Such food provides defined quantities of energy and other nutrients to the consumer. The bioavailability of nutrients in any food matrix becomes an important factor in determining the actual use of nutrients by the body.[1] Thus, the intrinsic availability of nutrients and the interactions among nutrients within a food matrix, determine the role that food plays in determining an individual's nutritional status.

Foods we consume come in variety of forms which contain different ratios of macro- and micronutrients, which are largely determined by the matrix of the food. The food matrix can be defined as "the nutrient and non-nutrient components of foods and their molecular relations to each other" (USDA NAL Glossary, 2015). Interactions among nutrients control the rate of digestive process and the absorption of individual elements (Troncoso and Aguilera, 2009; Zuniga and Tronscoso, 2012). Consequently, it is of utmost importance in food product research to develop a detailed understanding of the time-dependent transient changes in the structural aspects of food matrices from raw material harvesting, to product processing, to the point of breakdown during shelf life, consumption and final digestion. Understanding the mechanism of processing or breakdown and absorption or the bioavailability of nutrients from ingested food is an important aspect while designing functionally-superior foods rather than looking at foods from the nutritional content point of view.

Part of the work under the Food Aid Quality Review (FAQR) project, funded by the United States Agency for International Development (USAID), Office of Food for Peace (FFP), is to review the state of science on the role of food matrices in impacting the nutrient bioavailability. This review considers pathways of nutrient absorption and examines the effects of different food aid products in addressing undernutrition issues followed by understanding the gaps in food matrices–formulation or process-wise and suggesting ways to improve the nutritional efficiency (efficiency to absorb more nutrients from the food consumed) of these foods.

3. Methods

The literature review was conducted in two parts. First, basic information on absorption pathways and nutrient bioavailability was sought via databases. Second, studies relating specifically to food aid products were considered from 2000 to mid-2018. The three main search engines used were: Web of Science, Scopus and Google Scholar. The main keywords used were: food, nutrient bioavailability, fortified foods, food aid, nutrient interactions, processing and nutrition, food matrix(ices), and antinutritional factors. To further enhance and supplement the information accrued from the literature review, a one-day roundtable meeting was hosted with all experts and stakeholders–academia, industry, government agencies and non-profits to assess and discuss the current scenario of food aid as applied to nutrient bioavailability and the future pathways for improving the nutritional efficiency of food aid products. The science and practical aspects of food design, formulation and bioavailability was divided into key subthemes

[1] Bioavailability is defined here as the fraction of total nutrients which is absorbed by the body after its release from the food matrix.

with relevant presentations followed by discussions to individually address them and collectively could be used as a roadmap for improving the bioavailability of nutrients.

This review breaks down the topic of bioavailability in a way to sequentially understand the issues of bioavailability in foods in general and food aid products in particular. The understanding of pathways of macronutrient digestibility followed by the effects of processing on them and then examining micronutrient bioavailability sets the stage for nutrient bioavailability from food aid products. The review also looks at the bioavailability measurement techniques—both in vitro and in vivo—to understand the correlation between them. The review ends with a list of activities which needs to be undertaken to improve the nutrient bioavailability from food aid products.

4. Macronutrient Digestibility and Bioavailability

There are foods with different structures, such as fibrous structures like meat, encapsulated embryos like grains and legumes, complex fluids such as milk (Aguilera, 2005). Prepared or processed food structures are typically composites of different ingredients and varies greatly from its natural ingredient state. For example, bread has very different digestibility and nutritive properties compared to its individual ingredients like wheat flour, sugar, salt, oil, yeast, etc. The food undergoes time-dependent changes since it is processed from its raw state to its digestible state. Food structure/matrix directly affects the time the food gets to spend inside the mouth. For example, solid foods spend more time in the mouth because it must be broken down into pieces small enough to be swallowed. In comparison, liquid foods are almost immediately swallowed, which gives it very little time in the mouth as compared to solid foods which need to mix with saliva. This affects the breakdown of carbohydrates by salivary amylase.

4a. Starch

Carbohydrates are the major source of energy to the body. Monosaccharides (glucose, fructose, etc.) are the building blocks of carbohydrates needed to form disaccharides (sucrose), oligosaccharides (maltodextrin) and polysaccharides (amylose, amylopectin, cellulose, etc.). Simple carbohydrates such as starch are easily digested and are bioavailable in a short time after consumption (Turgeon and Rioux, 2011). Complex carbohydrates must be broken down to their monosaccharide component by respective enzymes before they are absorbed in the small intestine. The ease of converting complex polysaccharides to monosaccharides is influenced by many factors which determine the bioavailability of carbohydrates. These factors include fiber content, enzyme inhibition, food matrix, etc. For example, certain phenolic compounds can inhibit the action of enzymes such as α-amylase and α-glucosidase (Williams et al., 2006).

Starch is the primary component of carbohydrate in foods. Starch digestibility largely depends on the physical state of the ingested starch. Other constituents of food matrix like proteins, lipids and polysaccharides also play a role in defining overall physicochemical characteristics of the digesta and final digestibility of starch (Singh et al., 2010).

Starch is generally composed of amylopectin (70-80%) and amylose (20-30%). Some amount of lipids (0.15-0.55%) are associated with cereal starches primarily with the amylose fraction. This can significantly reduce the swelling capacity of the starch

granules (Morrison and Azudin,1987). Gelatinization of starch is aided by heat, water, and/or shear, and it improves the digestibility of starch considerably due to transformation from a semi-crystalline structure to an amorphous structure. Digestibility pathway of starch is aided by enzymes which breakdown/hydrolyze starch into glucose after several steps.

The digestibility of amylopectin is more rapid than amylose (Hu et al., 2004). The shape and size of starch granules affect the hydrolysis of starch—for example, small starch granules of barley and wheat which hydrolyze faster than large granules (Lindeboom et al., 2004). However, during enzymatic hydrolysis, the surface characteristics of starch play an important role. The presence of "pin holes" or pores on the surface of starch granules of corn, sorghum, millets, wheat, rye and barley as compared to the smooth surface of starch granules of potatoes, rice, oat, tapioca, arrowroot, can make the "pin-holed" starch granules more susceptible to enzymatic hydrolysis (Fannon et al., 1992). The entry of amylases is facilitated through these pores and aid in digestion. Therefore, Dreher et al. (1984) reported higher digestibility of cereal starches over tuber and legume starches. Further, the enzymatic hydrolysis may be affected by the presence of non-starchy components like lipids and proteins on the starch granule. These block the absorption sites, limiting the binding sites for enzymes (Oates, 1997).

Hard-to-cook phenomena, or the need for longer cooking time to achieve the same amount of nutrition, is often observed in legumes and pulses stored at high temperatures and humidity. During these storage conditions, there are changes in the seed coat and cotyledons, making them impermeable to hydration (Yousif et al., 2007). Many times, even after removal of the seed coat, the cotyledons don't absorb water due to the formation of insoluble pectinates, lignification of the cell walls, tannin-protein interaction, and changes to the structure and functionality of cellular proteins and starch (Nasar-Abbas et al., 2008; Pirhayati et al., 2011). Storage affects the molecular structure of the legumes, thereby affecting the overall matrix. Hard-to-cook beans require more puncture force than standard beans (Aguilera, 2005) and because of this, will affect the digestibility of all nutrients.

Pulses have a thicker cell wall compared to cereals and because of this, impede the water penetration. This makes pulses one of the most slowly digestible starch sources. The cell walls of pulses can survive moist heat treatment as practiced in domestic cooking (Venn et al., 2006). A food matrix containing high fat content and soluble fibers will form a thicker chyme, leading to longer gastric emptying and prolonged absorption of glucose (Turgeon et al., 2011). Therefore, the type of starch granule and the effectiveness of its breakdown due to enzymes within the body determine the digestibility of starch.

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4b. Proteins

Proteins are also a source of energy to the body but they are not as good a source as carbohydrates. Proteins play a major role in other important body functions like muscle-building and supporting immune functions. Proteins are composed of smaller molecules

called amino acids. Protein digestibility occurs when the complex chain of amino acids is broken down into individual amino acids and is absorbed by the blood stream. In the acidic environment of the stomach the proteins are denatured. The enzymes pepsin and protease are activated and break down the peptide bonds of the protein molecule into smaller amino acid chains called polypeptides. Further digestion of polypeptides occurs in the small intestine (alkaline environment) where enzymes, trypsin, chymotrypsin, elastase and carboxypeptidase hydrolyze the protein molecule into amino acids. The broken-down amino acids pass through the intestinal lining through capillaries into villi located in the intestinal wall. The blood stream distributes these amino acids to different cells and tissues of the body which aid in the repair of cell structures. The action of enzymes is dependent on several factors such as type and amount of protein consumed, acidity of the food, presence of inhibitors, etc.

On an energy equivalent basis, protein is the most effective macronutrient found in food to provide satiety (Zuniga and Tronscoso, 2012). Any process/activity which changes the secondary, tertiary and quarternary of protein molecules without cleavage of the backbone peptide bonds constitutes "denaturation." Denaturation causes the proteins to lose their functionality but it is an important feature which aids digestibility and biological availability, etc. In addition to changes in physical state, denaturation affects susceptibility to pepsin and trypsin/chymotrypsin mixture during digestion (Troncoso and Aguilera, 2009).

The usefulness of proteins in the diet is dependent on the source of protein and the amino acid profile. The foods which can provide the nine essential amino acids are of higher quality than those which only supply a part of the essential amino acids. Protein sources from animals are of higher quality than plant-based proteins because they deliver all essential amino acids. Protein quality can be measured through a variety of ways like protein efficiency ratio (PER), biological value (BV), net protein utilization, protein digestibility corrected amino acid score (PDCAAS) (Hoffman and Falvo, 2004). In order to overcome the shortcomings of PDCAAS in rating the protein quality, Food and Agriculture Organization (FAO) (2013) introduced a new method known as digestible indispensable amino acid score (DIAAS). DIAAS was based on the fact that all amino acids are not absorbed equally. The absorption of amino acids at the end of the small intestine is measured and it provides the contribution of individual proteins consumed in a mixed diet. However, more data needs to be developed to fully implement this tool.

Boirie et al. (1997) conducted a study to assess the role of slow and fast dietary proteins based on the absorption speed of dietary amino acids on modulating postprandial protein accretion. The authors used leucine kinetics to study this hypothesis by using either whey protein (fast) or casein (slow). Whey protein is a soluble protein whereas casein clots into stomach and slows its gastric emptying. The results indicated that the speed of amino acid absorption after protein ingestion had a major impact on the postprandial metabolic response. The slowly-absorbed casein promotes postprandial protein deposition by an inhibition of protein breakdown without excessive increase in amino acid concentration, as opposed to a fast-dietary protein which stimulates protein synthesis but also oxidation. This impact of amino acid absorption speed on protein metabolism is true when proteins are given alone.

However, as with carbohydrates, this might be blunted in more complex meals which could affect gastric emptying (lipids) and/or insulin response (carbohydrate). This concept of slow and fast proteins could be applied to circumstances in which protein deposition must be improved (i.e., protein-energy malnutrition) and in which excessive protein intakes must be avoided (i.e. in the elderly, those with renal diseases).

Dangin et al. (2001) built upon the above study and found that after the ingestion of "protein," meals of identical amino acid composition and nitrogen content—but of different digestion rates than fast meals—induce a strong, rapid, and transient increase of aminoacidemia, leucine flux, and oxidation. After slow meals, these parameters increased moderately but durably. Similar results were found by Willoughby et al. (2007) who saw that the ingestion of 20g of protein and amino acids one hour before and after 20 weeks of resistance training was more effective than carbohydrate placebo in up-regulating markers of muscle protein synthesis and improvement in muscle performance. Thus, protein quality and the rate of protein digestion within the body essentially determines the health outcomes from consuming the same. **The need to have a cost-effective balance between animal foods and plant foods in the design of foods for moderate malnutrition is important (Michaelsen et al., 2009). The food should be rich in minerals important for growth (e.g. phosphate and zinc) with high-quality protein and virtually no antinutrients. Options to incorporate cheaper animal-source foods should be explored, such as small fish which is eaten whole and thus have high nutrient content, as well as options like ingesting insects, snake, rodents and offal.**

4c. Lipids

Lipids or fats are a source of dense energy to the body. They are complex molecules composed of fatty acids and glycerol, with fatty acids being the source of energy. Lipids are classified according to the presence or absence of double bonds in the fatty acids– monounsaturated fatty acids (MUFA) having one double bond, polyunsaturated fatty acids (PUFA) with up to six double bonds and saturated fatty acids having no double bonds. Other ways to classify the lipids are based on the orientation of double bonds as *cis* or *trans* and based on n-3 or n-6, the position of first double bond from the fatty acid methyl end in PUFA (Orsavova et al., 2015).

Fat solubility is central to fat digestion and absorption. The hydrophobic nature of the lipids causes it to be insoluble in the aqueous environment of the digestive tract. In order to accomplish this, the large molecules of fat–the triglycerides (three fatty acids linked to glycerol and the major portion of dietary fat) are broken down into smaller droplets with the help of bile acids through a process called emulsification. These small droplets are further hydrolyzed into monoglycerides and free fatty acids by the action of pancreatic lipase. The liberated components of the triglycerides due to lipase action forms a complex with bile salts and phospholipids to form micelles. Micelles transport the poorly-soluble monoglycerides and fatty acids to the intestinal absorptive cells, which are called enterocytes.

Different types of lipids are ingested by humans, including triacylglycerols, cholesterol, oil-soluble vitamins, anti-oxidants and nutraceuticals which play an important role in growth and well-being. Lipid bioavailability depends on their molecular characteristics

and the food matrix they are consumed with (Wildman and Kelley, 2007). The supply of essential fatty acids to the body is primarily through triacylglycerols. The absorption and digestion of triacylglycerols is highly efficient with >95% of lipids consumed being utilized in healthy adults (Patton et al., 1985). Lipids can also be used to improve the bioavailability of hydrophobic components like β-carotene, lutein and lycopene.

During mastication of lipid-based foods, several changes occur to the food matrix inside the mouth—like change in pH, ionic strength, temperature, etc. It's also assumed that the lipids will be carried forward to the next steps of digestion in an emulsion system with a continuous aqueous phase. The structural organization of lipids within the "bolus" (swallowed material after ingestion and mastication) depends on the initial structure and properties of the ingested food, degree of mastication, quality of saliva and other physiological characteristics of the individual consuming the food. Long-chain saturated fatty acids such as 16:0 (palmitic acid) are not as well absorbed in the lumen as free fatty acids (FFA) because its melting point is above the body temperature and also because of its strong tendency to form insoluble calcium soap with divalent cations at the alkaline pH of the small intestine (Tomarelli et al., 1968). Infant formulas containing palm oil and its derivatives mixed with other vegetable oils to increase the content of 16:0 in infant formula up to the percentage found in human milk. However, formulas containing 50 percent of total fat as palm olein show lower fat and calcium absorption than infants fed a soy-based formula (Nelson et al., 1996).

The chain length of fatty acids and the number of double bonds influence fat absorption (Ramirez et al., 2001). The absorption of saturated long-chain fatty acids is lower than unsaturated long chain fatty acids (Ockner et al., 1972). In a laboratory rat study, Decker (1996) observed that when mixed micelles of saturated and unsaturated fatty acids were infused, saturated palmitic acid required a greater length of intestine for absorption as compared to unsaturated linoleic acid. Unsaturated fatty acids are hydrolyzed by gastric lipase in the stomach and absorbed there. They are also absorbed in the intestine and transported directly to the liver through the portal vein. With saturated long-chain fatty acids, an increasing proportion is absorbed into the lymphatic pathway and a decreasing proportion is absorbed through portal venous blood.

The micelle formation in the gastrointestinal tract is exclusively physical in nature. A deviation or change in the ratio of components (fatty acids, bile salts, etc.) required for micelles will alter their stability and functionality. Further, the addition of ingredients in food which compete with micelles on a solubility basis for nutrients and other important food components affects micelle formation. Therefore, the organic solubility of a wide variety of nutrients, phytochemicals, toxicants, drugs would potentially provide the basis for predicting which food constituent will interact with lipids and lipid-like materials, and potentially have their bioavailability altered (Beecher, 2000).

At birth, the infant digests the fat with the help of lipase contained in the breast milk and triacylglycerol lipase secretion from glands in the stomach. As the baby is weaned onto solid food, the major site of fat digestion shifts from the stomach to the duodenum. It has been estimated that gastric lipase is responsible for 25 percent of the partial triacylglycerol hydrolysis necessary for absorption to occur.

A single meal study by Schram et al. (2007) showed that the PUFA uptake from fish oil supplemented into yoghurt, fitness bars and in fish oil capsules depended on food matrices. It was fastest from yoghurt followed by fitness bars and then from capsules. The study concluded that due to the preformed emulsion system in yoghurt facilitating lipid absorption, the lipid uptake was fastest and the absorption from the solid food matrix (fitness bars) was lower than that from yoghurt, probably because of the slower release of lipids from the solid matrix and the reduced absorption rate. This study was different from several other studies (Higgins et al., 1999; Wallace et al., 2000) which reported no significant difference in bioavailability of (n-3) PUFA from different food matrices like fish oil in milkshake, in capsules, in bread, biscuits and soup. Schram et al. (2007) attributed the differences in their results to that of others due to the reporting of results in non-steady state as compared to steady-state results by others.

Another single meal crossover study by Ottestaad et al. (2016) was similar to other studies, Raatz et al. (2009), Garaiova et al. (2007), etc. in finding that emulsification of (n-3) PUFA increased its absorption. The matrices tested were enriched juice, yoghurt, oil capsules with oxidized or non-oxidized cod liver oil and high-oleic sunflower oil. The authors attributed this phenomenon to emulsification which helped bypass the normal physiological process of oil digestion by breaking down oil globules into smaller emulsion droplets. The authors explained that another theory could be that emulsification of oil reduced droplet size, causing them to be more dispersed and being affected more by pancreatic lipase activity. Oxidation of oil did not affect the absorption. Hence, the digestibility and absorption of lipids/fats is dependent on the chain length of fatty acids and presence of double bonds along with its ability to form micelles after the emulsification process initiated by the action of bile salts and phospholipids.

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5. Effect of Processing on Macronutrient Digestibility

The digestion process and absorption described above is impacted to a great extent by the processing of raw foods. Processing helps improve the nutritional quality, sensory attributes and shelf life of a food product. This step also brings about changes in the macronutrient profile of the food by changing its form and also creating some interactions between the macronutrients. The changes in the macronutrients are generally irreversible—like starch after gelatinization and proteins after denaturation—due to the impact of processing, which improves the digestibility but cannot return to the original state.

5a. Starch

To utilize the energy from starch sources found in the native state requires overcoming the protective plant tissue structure. Simple processing steps like milling, pounding and crushing have been used to increase the energy availability from plant tissues primarily from seeds. On the other hand, reduction in the tissue disruption lowers the starch

digestibility which is suited for situations which do not require all energy from starch (e.g. in cases with diabetes and obesity) (Venn and Mann, 2004).

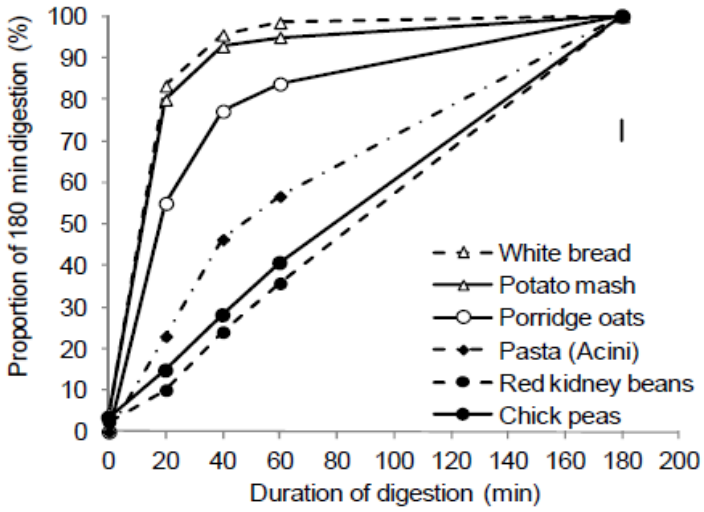

Figure 1. In vitro digestion patterns (percentage of carbohydrate available after 180 minutes of digestion) associated with different types of food structure.

Source: Mishra et al. 2012

Food processing disrupts the original cellular structure of the plant source to create a food matrix with secondary structure. The secondary structure affects the access of digestion medium to starch and thereby regulates starch digestibility. Food processing creates food with: 1) Open porous structures like leavened products–breads, cakes and puffed products created by steam expansion like breakfast cereals, many snack foods, and puffed rice; and 2) Dense low porosity structures like pasta. The high internal surface area and pre-cooked format of open structure foods makes them almost immediately accessible to amylase action. Thus, these foods have high digestibility, most of it being attributed to the rapidly-digestible starch (RDS), little of slowly digestible starch (SDS) and a low fraction of retrograded resistant starch (RS Type3).

On the other hand, food matrices with secondary structure of dense low porosity are made of hydrated dough and are formed into specific shapes under the use of force during processing. These products contain unaltered starch and must be boiled to cook. The effect of digestive enzymes on these types of products is much slower than that of open porous structure foods and are only as fast as the enzyme's ability to erode the superficial layers of food to expose the starch. Further, the surface area exposed to digestive enzymes control the rate of digestion of starch. The surface area of dense foods is therefore dependent on the particle geometry. Monro et al. (2011) suggested that as surface area of sphere depends on the square of the radius, even a small change in the particle size would greatly affect the digestion rates. There exists a potential to influence the digestion rates in these kinds of foods as long as a proportion of particles survive mastication. As dense foods are soft after cooking so that they can be partially swallowed

in intact state, which would help in retaining the influence of surface area on digestion rate. The continuous decrease in RDS and increase in inaccessible (resistant starch) with increasing particle size is very clear from Figure 2 below.

Figure 2: Influence of tissue structure on digestion revealed by the effect of cutting and crushing of cooked wheat kernels on in vitro digestion of starch.

Source: Mishra et al., 2012

> ***Depending on the process involved, the digestibility level of starch changes and some processes produce more digestible starch than others.***

Cooking starch in the presence of water and heat increases the rate of hydrolysis and causes starch to gelatinize and increases the starch digestibility. Between the extremes of raw starch and completely gelatinized starch, the digestibility can be regulated by controlling the degree of gelatinization. For example, rolled oats are prepared under restricted hydrating conditions and if consumed uncooked, they will have a lower starch digestibility as compared to that of fully-cooked oats in the form of porridge. The presence of other hydrating components in food products other than starch like non-starch polysaccharides in cell wall remnants, intrinsic and added gums, and sugars compete for water and may reduce gelatinization of starch during cooking (Pomeranz et al., 1977). This causes the digestion-inhibiting effect of native starch to continue.

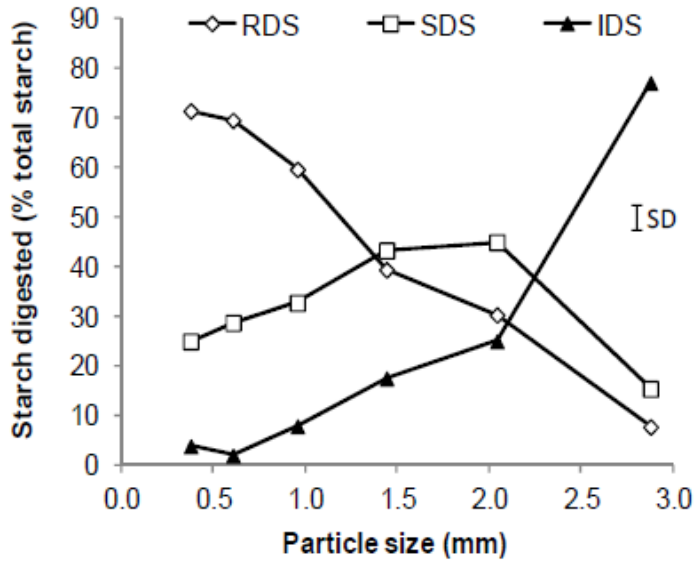

Figure 3. Effect of tissue structure on digestibility: Effect of particle size in chopped kernel fragments of the wheat cultivar 'Claire' on the in vitro digestibility of starch.

Source: Mishra et al., 2012

The rate of starch digestion depends on the form of starch–intact, dispersed and gelatinized or as retrograded starch. Intact starch granules are absorbed relatively slower and is dependent on the botanical origin of granules (Donald, 2004). Afterwards, the undigested carbohydrates—primarily non-starch polysaccharides—enter the colon. They are acted upon by colonic bacteria which break down the cell walls and provide carbohydrate substrate for fermentation. The short chain fatty acids produced from

fermentation are absorbed and provide up to 10 percent of dietary energy requirements for humans.

Table 1: Common Processes and Its Effect on Starch Digestibility

Processes	Actions	Effect(s) on food	References
Milling	Removes bran and germ Causes starch damage Increases the surface area of the raw material due to reduction in particle size	Improves starch digestibility	Kerr et al., 2000
Processes	**Actions**	**Effect(s) on food**	**References**
Cutting/chopping	Tissue damage on the outer surface	Improves starch digestibility moderately	Mishra et al., 2012
Cooking with excess water and heat	Hydrates, swells and breaks starch granules	High starch digestibility	Rehman and Shah, 2004
Baking	Cooking in dry heat	Improves starch digestibility considerably	Sterbova et al., 2016
Roasting	Cooking in dry heat with generally higher temperatures than baking	Improves starch digestibility but to a lower extent than baking	Sterbova et al., 2016
Extrusion (high temperature and pressure)	Breaks down starch molecules due to high temperature and shear	High in starch digestibility	Joseph, 2016
Extrusion (low temperature and shear)	Shapes the product and starch remains intact	Starch digestibility increases but is slow as compared to high temperature extrusion	Kim et al., 2008

Table 1 shows the effect of different processes on starch digestibility.

It is observed that depending on the process involved, the digestibility level of starch changes and some processes produce more digestible starch than others.

5b. Protein

The nutritional value of food proteins is determined by the amino acids profile and the capability of the body to absorb them. The 20 amino acids can be divided into four categories based on their nutritional importance: 1) Eight essential amino acids, which cannot be synthesized by our tissues; 2) Two semi-essential amino acids which are synthesized by our tissues from an essential amino acid; 3) several conditionally-essential amino acids which can be synthesized in our bodies but due to various physiological reasons, are produced at levels below the requirement; and 4) the non-essential amino acids, which are never indispensable.

Lysine, which is the most sensitive amino acid to heat, was used to estimate its loss and subsequent nutritional damage in milk proteins (Malec et al., 2002). They found that lysine losses are dependent on the water activity (a_w) and temperature. The activation energy or the threshold energy required to facilitate a chemical reaction required for lysine loss did not vary significantly between a_w of 0.52-0.98, but the activation energy values for a_w of 0.33 and 0.43 were found to be significantly higher than that between 0.52-0.98. This makes the control of storage temperature for dairy products, particularly dehydrated dairy products, very critical due to the temperature dependence on the reaction rate which is more important at lower a_w.

Aggregation or cross-linking of proteins due to processing or chemical changes causes the formation of high molecular weight polymers which affect the kinetics of protein digestion due to reduced availability of reaction sites to proteolysis (Zuniga and Tronscoso, 2012). Monoguiodi et al. (2011) reported that the digestibility of enzymatically cross-linked β-casein decreased as compared to native β-casein. Enzymatic hydrolysis of a lactalbumin reduces the quantitative bioavailability of the amino acids by 12 percent.

A study by Thorisdottir et al. (2014) in Iceland showed that consumption of animal protein–dairy, meat and seafood (≥ 12.2 percent of total energy)—in infancy (at 12 months) had higher accelerated growth in and higher Body Mass index (BMI) when they were six years old as compared to infants who consumed plant proteins (from cereals, fruits and vegetables). They said that this could be attributed to relatively higher proportions of essential amino acids in animal protein leading to increase in levels of serum of insulin-like growth factor 1 (IGF-1) which led to rapid weight gain early in life and higher BMI in childhood (Larnkjaer et al., 2012).

Previously, Agneta et al. (2013) had suggested that in the first two years of life, the mean protein intake should be 15 percent of energy as the upper limit. Animal proteins had stronger associations with growth as compared to plant proteins. They also suggested that protein intake between 15 to 20 percent of energy in early childhood could be associated with increased risk of being overweight later in life. They added that high intake of dairy foods in childhood could lead to increased risk of colorectal cancer due to higher IGF-1 activity in childhood than in adulthood. The acceleration in growth due to animal proteins (especially dairy proteins) could be beneficial in short-term but causes long-term resetting of the pituitary, resulting in lower long-term IGF-1 levels (Martin et al., 2005), leading to potential pathways for adult insulin resistance, cardiovascular diseases (Singhal et al., 2004; Singhal and Lucas, 2004) and carcinogenesis.

However, another study by Gunther et al. (2007) showed that higher animal proteins, cow milk and dairy products might be associated with and unfavorable body composition (chances of obesity) at 7 years of age, with ages between 5 and 6 years being the time of obesity rebound. Thus, ages 5 to 6 years might represent a second critical period of protein intake for subsequent body fatness. The authors also pointed out that the insulinotropic effect (increase in IGF-1) may be caused by whey protein but are not sure which milk compound stimulates IGF-1 secretion (Hoppe et al., 2006). Hoppe et al. (2004) found that milk intake was positively associated with IGF-1 growth and that increase in milk intake from 200 to 600 mL/d corresponded to 30 percent increase in circulating IGF-1.

In a study by Andersen et al. (2005) on combining resistance training (14 weeks) with timed ingestion of protein on muscle size and muscle strength, protein supplementation (protein 25g-16.6g whey protein, 2.8g casein, 2.8g egg white protein, and 2.8g L-glutamine) had a minor advantage over carbohydrate supplementation (25g of maltodextrin). Because of this, muscle size growth was found to be slightly higher with protein supplementation. **Therefore, processing induces positive (denaturation and random coil formation) and negative (some Maillard reaction products) changes in the protein digestibility. The overall effect on digestibility of processed proteins would be the net effect of the individual changes and also the source of protein.** The understanding of the mechanism of these changes would better aid in predicting the nutritional significance of proteins as affected by processing (Salazar-Villanea et al., 2016).

5c. Lipids/Fats/Oils

Lipids or oils (lipids that are liquid at ambient temperature) are used in food formulations and recipes for a wide variety of functions. They increase the energy density of foods, provide a silky texture, add to the taste and palatability of the food, add a moist and shiny appearance, provide crisp texture (when foods are fried in oil) and act as a carrier for fat-soluble vitamins. These uses of lipids are often challenging due to the oxidation of lipids. Oxidation of lipids cause a change in flavor, color and can make the product less acceptable. The fats break down when they are heated to a particular temperature called a smoke point. The smoke point for plant-based oils is higher (450°F/232°C) than that of animal fats (375°F/191°C) and the development of a sharp flavor at these temperatures make the products unacceptable (Professional Chef, 2006). Lipid oxidation occurs due to the reaction between unsaturated fats and oxygen. The process of oxidation is accelerated by the presence of transition metal ions, UV light, photosensitizers and certain enzymes (Erickson, 2002). The oxidation accelerators are called prooxidants and are generally present in food systems/matrices.

However, the use of antioxidants has been effective in retarding lipid oxidation. Some of the synthetic antioxidants like BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), TBHQ (tertiary butylated hydoxyquinone) are inexpensive and effective but are not considered "label friendly" (Chaiyasit et al., 2007). Many natural free radical scavengers such as catechins have additional health benefits (Yang et al., 2000). Other antioxidants consist of chelators (which bind the metal ions of the prooxidants) such as citric acid, phosphoric acid, ethylenediaminetetraacetic acid

(EDTA). When the ratio of EDTA:iron is less than 1, it acts as a prooxidant and as an antioxidant when the ratio is greater than 1 (Mahoney and Graf, 1986). A_w is one of the primary factors that determine the rate of deterioration of foods. The reactivity of food constituents in a heterogenous food matrix is influenced by its affinity for surrounding water and the competing influences of hydrophilic and hydrophobic groups (Lewicki, 2004). Environmental factors such as heat, pH, light, pressure, etc., affect the molecular state of water and thereby influencing its reactivities and functional properties. A_w affects lipid oxidation to a great extent. Oxidized lipids also oxidize fat-soluble vitamins and carotenoids At a_w=0, the lipid oxidation is rapid (e.g. in dehydrated foods) but it slows down and levels off at around a_w = 0.5. It has been observed that foods having a_w between 0.2 to 0.4 have the slowest oxidation rates (Chaiyasit et al., 2007). The solvent and mobilization properties of water become dominant at intermediate a_w of 0.55 to 0.85, causing lipid oxidation. Hence, the prevention of oil oxidation during processing and storage would be the leading factor which contributes to the beneficial effects of oil in the formula.

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6. Processes Used in Food Aid Products

Extrusion, baking, roasting and milling are the common processes used to make food aid products. The choice of process depends on the products that are manufactured, initial cost of equipment setup, and ease of operation.

6a. Extrusion

The extrusion process as related to food aid products is a low moisture process (18 to 22 percent). The pressure, temperature and shear inside the extruder barrel aid in gelatinizing and dextrinizing the starch thereby increasing the starch digestibility. The process also denatures the protein and improves protein digestibility. The high temperatures reduce the activity of lipid-breaking enzymes lipase and lipoxygenase and consequently helps in decreasing the factors favoring free fatty acid development and the oxidation of fatty acids (Alam et al., 2016). Some of the lipid oxidation in extrudates could be attributed to increased surface area of extrudates due to expansion (Rao and Artz, 1989) and exposure to light (Saxena and Chauhan, 1985; Hsieh et al., 1998). The starch and lipid interaction at temperatures above around 97°C through the amylose fraction of starch with lipids to form amylose-lipid complex created during extrusion retards radial expansion of extrudates and the rigid matrix has lower porosity, small cells and higher-bulk density. Because of this, it has a lower surface area, leading to lower chances of oxidation and a compact structure compared to expanded products. The compact structure would impact the overall digestibility and would be slower as compared to digestibility from expanded extrudates.

6b. Roasting and Baking

The roasting process involves cooking cereals and legumes separately and then cooling them before mixing them both in the right amounts prior to milling. WFP (2002) recommends the roasting of cereals at 140°C for 10 minutes and 15 minutes at 170°C for pulses and soybeans. Benefits of roasting include the activation of proteolytic enzymes and therefore improved protein digestibility (Mbah et al., 2012; Olanipekun et al., 2015) and destruction of surface microflora. Baking is used for the manufacture of high-energy biscuits. The high temperature of baking (180 to 200°C) and the biscuits being at very low moisture content have a long shelf life. The a_w levels of biscuits and RUTF are 0.3 and 0.4 respectively (Michaelsen et al., 2009) and does not support microbial growth, leading to long shelf life.

Dehulling and heat treatments in any or all of the processes are effective in reducing the fiber content and antinutritional factors and helps in improving the bioavailability of nutrients from these food matrices.

In properly-processed products and mixed diets which contain minimal amounts of residual antinutritional factors, the digestibility of protein is generally considered a good approximation for the bioavailability of most amino acids. However, in coarse cereals and grain legumes, and in those products which contain antinutritional factors (either present naturally or formed during processing), there are quite large differences (up to 81 percent) between the digestibility of protein and individual-limiting amino acids (IAA). Therefore, there may be a need to include corrections for the bioavailability of individual IAA and not just for the digestibility of protein in calculating PDCAAS values of such products. It is quite possible that the differences due to age in the bioavailability of individual amino acids may be even greater than the observed effect on the digestibility of protein in products containing antinutritional factors (Gilani et al., 2012).

6c. Macronutrient Bioavailability from Food Aid Products

The common food matrices in major processed food aid products are: i) flour-based matrices; and ii) lipid-based matrices.

The flour-based matrices are dry flour blends of cereals and legumes which are thermally processed by roasting or extrusion before being fortified with vitamin/mineral premixes and other ingredients which may enhance the nutritional profile of the blend. These products need to be cooked in boiling water before it can be consumed as a porridge. The most widely-used cereal/legume blend is corn-soy blend (CSB). Other products include wheat-soy blend (WSB), soy-fortified bulgur, etc.

Conversely, lipid-based matrices include lipid-based nutritional supplement (LNS), ready-to-use therapeutic food (RUTF) and ready-to-use supplementary food (RUSF). These products are primarily made from peanuts, milk powder and oil and have a higher nutrient and energy profile than fortified blended foods (FBFs) and require no cooking prior to consumption.

As can be observed, the major components of these food aid products are cereals, legumes and dairy-based products. The plant-based ingredients have antinutritional factors like phytic acid, tannins, trypsin inhibitors, etc. and their presence can affect the digestibility and bioavailability of nutrients from the food matrices. Phytic acid binds with

multivalent metal ions especially calcium, iron and zinc to form phytates (Lonnerdal, 2002; Abebe et al., 2007) and make them unavailable for absorption and utilization by the body. Even small amounts of phytate can affect iron absorption (Hurrell, 2004). Processes like degerming of corn, removing the hull of beans and exogenous phytase addition are some ways to reduce the phytic acid content in these blends.

Complementary foods made from unrefined cereals and legumes have been found to have the highest phytate concentration. In order to effectively overcome the effect of phytates, molar ratios of phytate: metal ion has been suggested and is taken care of while formulating the mineral premix for addition to food aid products. The molar ratios are phytate: iron <1 (Hurrell, 2004), phytate: zinc <15 (WHO, 2004), and phytate: calcium <0.17 (Umeta et al., 2005). Phytates also interact with proteins and impedes their digestion (Selle et al., 2012). There is lack of data on the bioavailability of nutrients from lipid-based matrices or energy dense matrices.

Roos et al. (2013) conducted a study to screen antinutritional compounds in complementary foods and food aid products for young and infants. They analyzed the products for phytates, polyphenols, and trypsin and chymotrypsin inhibitors. It was found in the study that only 2 out of the 11 FBFs had lower molar ratios for phytate: Fe <1, 5 of the FBFs and 4 out of 12 lipid-based exceeded the phytate: Ca molar ratio of <0.17, and 5 FBF samples and four lipid-based products exceeded the phytate: zinc (Zn) molar ratio of <15. This screening study points out the high phytate levels in cereal legume blends. Further, they observed a high variation in phytates from peanut-based products. Two similar peanut-based RUTFs had phytate contents of 1055 and 371 mg/100. **These variations in phytates in peanut-based lipid pastes should be looked into for causes either in the raw material or processing and improved guidelines should be drafted for manufacturers to lower the phytate content.** They found that rice is a recommended product in complementary foods from limiting antinutrients as the rice-based product had low or no measured antinutrients. Similar observation was found by Gibson et al. (2010) that foods with rice and no legumes had low phytate content, similar to that of foods based on starchy roots and tuber.

Webb et al. (2011) recommended that animal-based proteins particularly dairy-based proteins be a part of the FBF formulations. They recommended 18g of protein /100g of FBFs. The protein quality, as determined by PDCAAS for the common macro ingredients used in food aid products, are 0.35, 0.37 and 0.93 for corn, wheat and soy, respectively (Hoppe et al., 2008). They further reported that the blends of cereals and legumes complement the amino acid profile of the food and PDCAAS of CSB and WSB changes to 0.65 and 0.64, respectively. The addition of milk, as in CSM (corn-soy milk) or WSM (wheat-soy milk), further improves the PDCAAS to 0.81 and 0.76, respectively. Generally, the use of less-refined cereals and legumes make the plant-based proteins less digestible due to the presence of fiber and antinutritional factors. Additionally, the presence of trypsin and trypsin inhibitors (in legumes), tannins (cereals and legumes), phytates (cereals and oilseeds) and gossypol (cottonseed protein products) also affect the protein digestibility (Gilani et al., 2005).

Fat is another important component of fortified foods for providing energy. The FBFs have lower energy density (1kcal/g) than lipid-based foods (5 kcal/g) (Hoppe et al., 2008). Low intake of essential fatty acids–linoleic (omega-6) and alpha linolenic acid (omega-3)—along with a high ratio of them affect the growth, development and cognitive functions (Uauy et al., 2003; Hoffman et al., 2004; Davis and Prall, 2014) of the affected consumer. It has been reported that the 30 to 40 percent of the energy should be from fats with the right fatty acid composition for having food that is effective in preventing and treating MAM in young children (de Pee and Bloem, 2009). They further reported the ratios of omega-6 to omega-3 to be around six. FBFs are generally low in overall fat content and fatty acid levels.

A point to be considered is that in case of the addition of oil with high omega-3 content suitable antioxidants such as vitamin E, mixed tocopherols or rosemary derivatives should be added to prevent oxidation in the oils (Jacobsen et al., 2008). Ascorbyl palmitate was used as an antioxidant in omega-3 fortified (through flax oil) LNS and it was found that 0.02 percent of the antioxidant, 1.5 percent emulsifier (soy lecithin), and 4.9 percent of flaxseed oil proved to be the best combination after six months of accelerated storage (Gaur et al., 2017). During this period, the peroxide value (measure of oxidation in fats) raised to 7.75 mEq/kg fat which was below 10.0 mEq/kg fat, the threshold for perception of rancid flavors in oils (FAO/WHO, 1999). RUTFs have marginal amounts of α-linolenic acid delivered through soybean or rapeseed oil which results in the imbalance of essential fatty acids ratio in the product (Brenna et al., 2015). The use of soybean oil or a blend of oils that have favorable omega-3 content like canola oil should be considered (Hoppe et al., 2008) while formulating food aid products. In a short-term single meal study by Schram, et al. (2007), it was found that the bioavailability of omega-3 fatty acids was affected by food matrices. They found that it was more bioavailable from yogurt than from fitness bars or fish capsules after three to six hours of ingestion. The explanation was that it was easier for fatty acids to be more bioavailable from yogurt due to the existing preformed emulsions. Whereas, it takes longer for the fatty acids to be released from the matrix of fitness bars or gelatin capsules (for fish oil). Their result was contrary to the findings of Higgins et al. (1999) and Wallace et al. (2000) who found that there was no significant difference in the bioavailability of omega-3 fatty acids from different food matrices like milkshakes, bread, biscuits, soup and fish oil capsules. Difference in measurement time–unsteady state by Schram et al. (2007) and steady state by the others—could have been the possible explanations for this difference.

Lenters et al. (2013) found that RUSFs were more effective in treating moderate acute malnutrition (MAM) as compared to the standard CSB. Uauy et al. (2000) and Prentice and Paul (2000) reviewed the energy content supplied by fat in whole diet in developing countries and found that lower than 22 to 25 percent would have a negative effect on growth. This is one of the reasons that RUTF is more effective than CSB-based porridge (five times more energy than standard CSB).

Studies by Patel et al., 2005 and Matilsky et al., 2009 have shown that RUTF and lipid-based pastes have better recovery rates than CSB when fed to children 10 to 60 months and 6 to 60 months for eight weeks, respectively, in Malawi. They reported higher

weight, height and middle-upper arm circumference (MUAC) gains in children receiving RUTF as compared to CSB. Higher nutrient density and better nutrient bioavailability from a lipid-based matrix could be attributed for this result. Another reason being that the high volume of porridges compared to lipid-based supplements might have led to the incomplete consumption of porridges (Thakwalakwa et al., 2010). However, another study by Galpin et al. (2007) on feeding supplements (RUFs and CSB) to breastfed infants of 5.5 to 6.5 months with varying energy density (5.3 kcal/g for RUFS and 1.1 kcal/g for CSB) for one month showed significant improvement in weight gain in all groups but there was no significant difference among the different groups. Another study by Phuka et al. (2008) also showed that no significant differences were observed in overall changes in weight, length, MUAC, weight-for-age, weight-for-length, or height-for-length in infants aged 5.5 to 6.99 months fed with RUFs or fortified maize/soy flour for one year.

Several studies show the superiority of RUTF over CSB (Ciliberto et al., 2005; Matilsky et al., 2009; Thakwalakwa et al., 2010) in treatment of MAM, although this trend has not always been true (Maleta et al., 2004). However, the difference in the matrices of the two types of food with more leftovers from FBFs as compared to RUTFs—which is easy to handle and has more energy density—adds to this effect. Recent changes in formulations of FBFs to enable better response/effect in addressing MAM issues have led to two different products–Corn-Soy Blend Plus Plus (CSB++) for children 6 to 24 months of age and Corn-Soy Blend Plus (CSB+) for children above 24 months (UNICEF, 2012). A study by LaGrone et al., (2012) has shown that developments in the FBF formulations have made them as effective as lipid-based pastes. The improved FBFs had a change in formulation by increasing the energy density through the addition of oil, sugar and dried skim milk, and a change in the micronutrient profile. Also, tighter specifications for aflatoxin and coliform control, and lower antinutrients through the inclusion of less soybeans and corn, and the use of dehulled soybeans.

Medoua et al. (2015) found that there was no significant difference in the recovery of children treated for MAM between improved (10 percent oil added) CSB+ (73 percent) and locally-produced RUSF (85 percent). However, the mean duration of treatment required to achieve recovery was 44 days in the RUSF group and 51 days in CSB+ group. This could have possibly been due to the need for the consumption of higher volume of CSB (or porridge consumption) due to it being less energy dense as compared to lipid-based food. Additionally, based upon the time required to recover, the cost of the product for treatment was relatively lower with CSB+ (3.48 €) as compared to RUSF (3.52 €).

Extruded rice-shaped kernels fortified with micronutrients used in rice fortification presents another type of matrix–a dense product that has easily digestible starch which forms the major part of extruded kernels. The most stable iron form for fortification was an insoluble iron source–ferric pyrophosphate (FePP). FePP addition caused only minimal thiamine loss and had acceptable sensory properties (Li et al., 2008). Other forms of iron like ferrous fumarate and ferric sodium EDTA (NaFeEDTA) promoted thiamine loss and caused rancidity, respectively. Microencapsulation of iron could be another way to prevent iron interaction with other micronutrients. At the end of 20 weeks of storage at 40°C and 100% RH, all of the vitamin B1 and ferrous iron were

retained when using encapsulated ferrous fumarate with soy stearin as a protective coating material (Li et al., 2008).

In addition, to expand the footprint of nutritious food to the general public as well as undernourished populations, fortification of staples has been underway. Wheat and maize flours, cooking oil, condiments and rice are all effective vehicles to carry nutrition.

7. Micronutrient Bioavailability

Vitamins can be divided into two categories based on their solubility–fat soluble vitamins (A, D, E, and K) and water-soluble vitamins (C and B vitamins). Minerals are inorganic substances that are not made by living things. The five major minerals and macrominerals in the human body are calcium, phosphorous, potassium, sodium and magnesium (Neilsen, 2014). Minerals are found naturally in soil and water. They are absorbed by plants and later, consumed by living beings. The importance of minerals in the body can be understood by the fact that they constitute 4 to 6 percent of the body weight. Of that, calcium constitutes almost one-half and phosphorous constitutes one-quarter. The remaining one-quarter is made up of all other minerals needed by the body (Truswell et al., 2018). Vitamins and minerals are both required by the body to perform many of its functions like macronutrient metabolism, nervous system function, wound healing, immune function, etc. Regardless of the total micronutrient content in a food product as estimated experimentally, only the biologically-available amounts have nutritional relevance.

The bioavailability of micronutrients is affected by intrinsic and extrinsic factors. Some extrinsic factors are: the micronutrients' physical form, their concentration in the food product, food matrix, etc. Intrinsic factors are: the age and health profile of individuals.

7a. Food Matrices and Micronutrient Bioavailability

Micronutrients are provided to the beneficiaries through different types of foods like staples (wheat, rice, oils), condiments (salt, sugar) and processed foods (FBFs, RUTFs, complementary foods, etc.). Different forms of micronutrients are used in these types of foods to suit the overall specific food matrix. Each form of micronutrient get absorbed in the body differently based on the overall bioavailability from the food matrix.

Iron (Fe)

The bioavailability of heme iron is more than that of non-heme iron because after the release of heme iron from the food matrix, the heme molecule forms a protective ring around the iron molecule which prevents it from interacting with other food components and from being absorbed by specific transport systems on the surface of gut cells (Shayeghi et al., 2005). However, non-heme iron is poorly soluble in intestinal conditions and due to its interaction with other food components, its bioavailability is not as high as that of non-heme iron (Hurrell and Elgi, 2010). Sulibursk a et al. (2011) studied in vitro iron bioavailability from commercially-available fortified and non-fortified food products. They found that the relative bioavailability (RBV) of iron (Fe) was higher in fortified food products. Even if RBV was low, higher Fe release could be achieved by adding more Fe to the product.

The effect of food matrices on the RBV of iron (ferric pyrophosphate) has been reported by Moretti et al. (2006). They studied the RBV of ferrous sulfate and micronized dispersible ferric pyrophosphate (MDFP) with and without ascorbic acid in wheat-milk infant cereal and in extruded fortified rice. They found that geometric mean iron absorption of MDFP was 2 percent and that of ferrous sulfate was 3.2 percent in wheat-milk infant cereal without ascorbic acid. Whereas, mean iron absorption in rice was 1.7 percent for MDFP and 11.6 percent for ferrous sulfate. The addition of ascorbic acid at a molar ratio of 4:1 to iron increased the iron absorptions to 5.8 percent (MDFP), 14.8 percent (ferrous sulfate) in infant cereal and to 3 percent (MDFP) and 12.6 percent (ferrous sulfate) in rice matrix. Another factor which affected iron absorption was the iron status of participants in the study. There was an inverse relation between iron status and iron absorption.

Mass fortification entails the addition of micronutrients to foods such as cereal staples, condiments and milk which is mostly consumed by general population. However, technical constraints limit the amount of bioavailable iron which can be incorporated into wheat and maize flour (Dary, 2002). Highly bioavailable iron compounds may affect the storage properties of the flour and in addition, change the color and taste of the food (Hurrell, 1999).

In general, the effect size or improvements in the health/nutrition status has been found in efficacy trials as compared to programs (Dewey and Afarwuah, 2008). The extent to which these improvements in nutritional status sustained has not been reported. Micronutrient powders (MNP) alone have also not shown impact on linear growth (De-Regil et al., 2011). This points to the fact that MNP alone may not be sufficient to stimulate linear growth as the levels of micronutrient being provided are not adequate (Piwoz et al., 2012). A review by Gibson et al. (2010) on the concentrations of phytate, iron, zinc and calcium in complementary foods found in low-income countries and its implications on bioavailability. They reported that phytate content was highest in complementary foods made from unrefined cereals and legumes (~600mg/100 g dry weight), followed by refined cereals (~100mg/100g dry weight) and then starchy roots and tubers (<20mg/100g dry weight). Mineral concentrations also followed the same trend. Frequently, commercial complementary foods are recommended to be prepared with milk but it was found that even after 100 percent phytate removal by use of commercial phytase, the Fe absorption was improved in cereal porridges made with water and not with milk (Hurrell et al., 2003)

A study of selected raw and prepared foods consumed in rural Sidama (Southern Ethiopia) by Abebe et al. (2007) showed that phytic acid was least in enset (a starchy food similar to banana) followed by fermented injera (a type of sourdough flatbread) made from teff (a fine grain). Enset and teff were rich sources of calcium. Unlike barley flour and corn bread, raw teff and injera teff showed contaminant iron from soil. The molar ratio of phytate: zinc and phytate: iron was least in fermented foods prepared from enset and teff. The molar ratios were higher in unleavened corn bread, kidney beans, sesame and niger seeds. Due to low content of phytate in fermented foods, the absorption of intrinsic non-heme iron, zinc and calcium will not be compromised.

A lipid-based nutritional supplement (LNS) has been recommended by Chaparro and Dewey (2009) in emergency settings where the beneficiaries are completely dependent on food aid. They noted that a typical cereal-based ration provided during emergencies does not meet the nutritional needs of the infants, young children, pregnant and lactating women. It only provided < 75 percent of the recommended intake for several micronutrients for certain age/physiologic groups. Also, it contained lower than recommended levels of fats and essential fatty acids. A 20g dose of LNS should be able to provide one RDA and is more effective than CSB because it can be directly consumed and it doesn't have the effect of antinutritional factors.

Effect of Dephytinization on Micronutrients

An evaluation of soy isolate-based infant formula (phytic acid 300mg/kg) and dephytinzed soy isolate formula (<6mg/kg phytic acid) was conducted by Davidsson et al. (2004). The stable isotope technique was used on 72h fecal excretion to determine non-absorbed stable isotopes zinc (Zn), iron (Fe), copper (Cu) and calcium (Ca). The chemical balance method was used to assess manganese (Mn), Zn, Cu and Ca in nine infants (69 to 191 days old). The results showed Zn absorption to be significantly greater after dephytinization. No other statistically-significant results for other minerals was observed. This indicated that dephytinization did not have a significant effect on absorption values of nutrients and soy protein isolate can be used in infant formula. Similarly, Egli et al. (2004) studied the effect of dephytinization of cereal-based complementary foods made from wheat and soy on apparent zinc and copper absorption. The dephytinized complementary food had (0.03mg/g phytic acid) and the other containing native phytic acid (4mg/g). The stable isotope method was used on nine adults and they found significant increment in apparent zinc absorption from the dephytinized formula but there was no significant difference in Cu absorptions. Thus, dephytinization helped in zinc absorption whereas, phytic acid did not inhibit the absorption of copper in humans.

Riboflavin

The rate of intestinal absorption of vitamins affects the bioavailability which again depends on the chemical form and physical state of the vitamin in the food matrix. The absorption of a riboflavin supplement taken with a meal was about 60 percent as compared to 15 percent when taken on an empty stomach (van den Berg, 1993). The low solubility of riboflavin (vitamin B2) and the limited capacity of intestinal absorption probably account for the lack of toxicity. Roe et al. (1978) concluded from their study that dietary fiber sources enhanced riboflavin absorption, probably by slowing the passage of chyme in the intestine and thereby increasing the duration of vitamin exposure to absorption sites.

Vitamin A

In a study by Kim et al. (2000) on cornflakes added with retinyl palmitate as itself or as a part of vitamin mixture (A, B1, B6, B12, C, and D) found that 90 percent of the retinyl palmitate was lost after six to eight weeks of storage at ambient or elevated temperatures. However, they found that the loss of retinyl palmitate was 30 to 40 percent in samples that had the vitamin mixture stored at ambient temperature. The presence of other vitamins reduced the losses from retinyl palmitate at ambient

temperatures and not at elevated temperatures. Vitamin A is absorbed with an efficiency of 70 to 90 percent compared to that of 20 to 50 percent from provitamins (National Research Council, 1989). The location of carotenoids inside the food matrix also affects the bioavailability. In a study on Indonesian school children (de Pee et al., 1998), the level of serum β-carotene in relation to ingested β-carotene was five to six times higher for fruit meals (oranges) than for vegetable meals (dark green leafy vegetables and carrots). The difference in bioavailability could be attributed to the location of carotenoids which in photosynthetic plants was located inside chloroplasts whereas it was found in chromoplasts in nonphotosynthetic tissues.

Vitamin E

The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix (Jeanes, et al., 2004). In the study conducted by Jeanes et al. on eight healthy volunteers fed with different meals containing different levels and sources of fat along with 150mg α-tocopheryl acetate. It was found that higher fat toast and butter meal (17.5g fat) had significantly higher ^{2}H-labelled α-tocopherol concentration as compared to cereal with full-fat milk (17.5g fat), low-fat cereal (2.7g fat) and water (0g fat).

Thiamin

Thiamin, as thiamin diphosphate (also known as thiamin pyrophosphate), serves as a coenzyme for enzymes involved in carbohydrate and amino acid metabolism. It is practically nontoxic when administered orally owing to its limited absorption and rapid excretion of excess amounts. In cereal grains, the thiamin is unevenly distributed, being relatively low in the starchy endosperm and high in the germ. Thiamin in most foods tested is either highly or totally available for absorption and utilization by humans (Gregory, 1997). Roth-Maier et al. (1999) compared the animal products with the plant products and showed on average a nearly equal prececal digestibility of thiamin (87.3 percent versus 83.5 percent). Ranhotra et al. (1985) found that the bioavailability of thiamin in whole wheat bread was slightly higher than that thiamin-restored white bread. Similarly, Yu and Kies (1993) found no significant difference in bioavailability of thiamin to humans from wet or dry-milled coarse and fine ground maize bran added to bread to provide 20g fiber per day.

Niacin

Some plant-derived foods contain niacin in chemically-bound forms that result in their bioavailabilities being low. In mature cereal grains, for example, as much as 70 percent of the niacin may be biologically unavailable after conventional cooking. The availability of vitamin B6 from whole wheat bread was only 5 to 10 percent less than that from white bread supplemented with vitamin B6 (Leklem et al., 1980), and the addition of wheat bran (15 g/day) to human diets resulted in only a minor decrease (maximum of 17 percent) in availability of the vitamin (Lindberg et al., 1983). However, synthetic niacin like nicotinic acid, niacinamide etc. are readily bioavailable (Mackay et al., 2012).

Biotin

Biotin (vitamin B7) requirement in humans is met by dietary supply and endogenous microbial synthesis in the gut. Biotin toxicity in humans has not been reported and is presumably low. Biotin is present in all natural foodstuffs, but the content of even the richest sources is very low when compared with the content of most other water-

soluble vitamins. Liver, eggs, soy beans and peanuts are particularly rich sources of the vitamin. Other good sources include yeast, wheat bran, oatmeal and some vegetables. Muscle meats, fish, dairy products and cereals contain smaller amounts but are important contributors to the dietary intake. Biotin in foods exists as the free vitamin and as protein-bound forms in variable proportions. Biotin is not commonly used in fortified foods, apart from infant formulas. Growth assays have shown that biotin in wheat is largely unavailable to the chick, in contrast to biotin in maize (corn) which is almost completely available (Frigg, 1976; Frigg, 1984). The bioavailability of biotin in the human diet awaits investigation. Most, if not all, of the biotin content of human milk is in the free form and because of this, is likely to be completely bioavailable to the infant (Heard et al., 1987).

Folate

A deficiency in folate leads to a lack of adequate DNA replication and consequent impaired cell division, especially in the hemopoietic tissue of the bone marrow and the epithelial cells of the gastrointestinal tract. Meat, fish and poultry are poor or moderate sources compared to plant products. Liver, all types of fortified breakfast cereals, cooked dried beans, asparagus, spinach, broccoli and avocado provide the highest amounts of folate per average serving. The bioavailability of folate in a wide variety of foods is incomplete and highly variable. It can be affected by dietary constituents or physiological conditions influencing: (1) the rate or extent of intestinal deconjugation of polyglutamyl folates; and (2) intestinal absorption. Bioavailability can be a major determinant of nutritional status when folate intakes are in the marginal range. Colman et al. (1975) compared the efficiency of absorption of folic acid from fortified whole wheat bread, rice, maize meal (porridge), and an aqueous solution of folic acid in human subjects. They found that the absorption efficiencies of the rice and maize were similar (ca. 55 percent), relative to the response observed with aqueous folic acid, while the absorption efficiencies of the bread were lower (ca. 30 percent). Finglas et al. (2002) reported that bioavailability of folic acid added as fortificant to cereal-based foods (white bread and bran flakes) when compared to folic acid capsules was 0.71 and 0.37 respectively indicating that some cereal-based vehicles may inhibit fortificant absorption.

Vitamin B12

Vitamin B12 (Cobalamins) deficiency has a drastic effect on folate metabolism. Pernicious anemia is caused by the lack of vitamin B12. Vitamin B12 deficiency is rarely, if ever, caused by a lack of dietary B12; rather it is attributable to various disorders of absorption and transport. Liver is the outstanding dietary source of the vitamin, followed by kidney and heart. Muscle meats, fish, eggs, cheese and milk are other important foods. The cobalamins present in food are generally resistant to thermal processing and cooking in a non-alkaline medium. Humans appear to be entirely dependent on a dietary intake of vitamin B12. The mean percentage absorption of the extrinsic vitamin B12 label was as follows: lean mutton, 65 percent (Heyssel et al., 1966); chicken, 60 percent (Doscherholmen et al., 1978); fish, 39 percent (Doscherholmen et al., 1981); eggs, 24 to 36 percent (Doscherholmen et al., 1975); milk, 65 percent (Russell et al., 2001); and fortified bread, 55 percent (Russell et al., 2001). With the exception of eggs, in all of these foods, vitamin B12 was absorbed as efficiently as a comparable amount of crystalline cyanocobalamin administered orally in an aqueous solution. The

relatively poor absorption of vitamin B12 in eggs was attributed to the presence of distinct vitamin B12-binding proteins in egg white and egg yolk (Levine and Doscherholmen, 1983). There was no significant difference in the urinary excretion of vitamin B12 in humans receiving controlled diets supplemented with or without wheat bran (Lewis et al., 1986).

Vitamin C

Vitamin C is provided to humans through diet in the form of ascorbic acid. Its role is primarily that of reversible biological antioxidant. Ascorbic acid, in collaboration with α-tocopherol and β-carotene, plays an important role in the defense against cellular damage by oxidants. The principal natural compound with vitamin C activity is L-ascorbic acid. Fresh fruits (especially blackcurrants and citrus fruits) and green vegetables constitute rich sources of vitamin C. Liver (containing 10-40 mg/100 g), kidney and heart are good sources, but muscle meats and cereal grains do not contain the vitamin. In a human study involving 68 adult male nonsmokers, Mangels et al. (1993) found that there was no statistically-significant difference in bioavailability from tablets, orange juice and broccoli. In addition, the bioavailability of ascorbic acid in the tablet alone did not differ from that in the tablet plus iron, and there was no difference in bioavailability between orange segments and juice.

7b. Nutrient Interactions

In very general terms, nutrient interaction is a two-way or multiple-way effect or effects between nutrients. Many are one-directional with the presence of one nutrient affecting the absorption or metabolism of another nutrient. Also, the effect of these interactions is usually not additive (Caballero, n.d.). The interactions can be positive or negative and it affects the bioavailability and nutrient utilization by the body.

Vitamin A deficiency has been also related to protein malnutrition (Roels et al., 1963). Low serum vitamin A levels were found in in patients with severe protein malnutrition in Guatemala. When these patients were given a sufficient protein-rich diet without vitamin A, their serum vitamin A levels rose, provided they had sufficient liver reserves (Arroyave et al., 1961). Thus, dietary protein is needed for mobilization of vitamin A from liver into the bloodstream. A number of studies have shown that dietary fat is a major determinant of carotene absorption. In a Rwandan study (Roes et al., 1958) with boys having vitamin A deficiency (Bitot spots), the serving of 18g per day of olive oil to a carotene-sufficient but low-fat diet increased the absorption of vegetable carotenoids from 5 to 25 percent. The addition of 20 percent cooking oil per gram of dry matter carrot resulted in a 30 percent increase of bioaccessible β-carotene in pulped, raw carrot, but had no significant effect on raw carrot pieces (Hedren et al., 2002). This suggested an interaction of added fat with the integrity of the plant matrix. Long-chain PUFAs have a greater binding affinity for fatty acid binding proteins than do more saturated fatty acids. In other words, β-carotene ingested with a meal high in PUFA is absorbed more efficiently than when ingested with a meal containing more saturated fatty acids.

The potential risk of micronutrient interactions during food fortification programs have to be taken into account particularly when high doses of a single nutrient or when the supply on an individual micronutrient is inadequate (Sandstrom, 2001). The interactions

between iron, zinc and copper is significant. Iron deficient populations due to low availability of iron from diets escalate zinc deficiency as well. In addition to risks from low levels of iron on growth and development, low zinc status can compromise immune defense system. Additionally, zinc supplementation may affect copper-dependent iron metabolism. Some interactions also improve the availability of nutrients. For example, vitamin C enhances iron absorption but can affect selenium absorption positively or negatively depending upon chemical form and dietary conditions. Also, vitamin A and β-carotene enhance non-heme iron absorption.

Though small, there was an observed decrease in the bioavailability of folate, zinc and iron in milk and corn starch-based multi-micronutrient supplement (porridge) (Kamp et al., 2003). Although the food matrix did not contain phytates, the micronutrients had lower bioavailability. A 15 percent reduction in folate bioavailability was observed. Similarly, zinc and iron had lower bioavailabilities and the decrement was greater than that of folate. It has been reported that the bioavailability of zinc decreases from 70 percent to 20 percent when given in foods as compared to zinc taken from pure salts in aqueous form (Sandstorm et al., 1985). Similarly, 35 percent of iron absorption was observed from aqueous ferrous sulfate as compared to 5 percent when mixed with bovine milk (Stekel et al., 1983). Casein in whole milk also contributes to reduced iron and zinc bioavailability by binding these elements and reducing intestinal absorption (Lonnerdal, 2000).

Vitamin D is required for intestinal calcium and phosphorus absorption (Holmes and Kummerow, 1983). Peroxidation of lipids (PUFA) starts as soon as water is added to the cereal, as in the first stage of the drum drying process and is enhanced by presence of copper and iron.

A combination of calcium and vitamin D is more effective than both being consumed alone (Fairweather-Tait, 2002).

Table 2: Nutrient Interactions of Select Micronutrients

Nutrients	**Interacting nutrients and conditions**	**Effects**
Vitamins		
Vitamin A	Protein	Protein <10% and also higher levels between 20-40% inhibit Vitamin A absorption
	Fat, vitamin E, anti-oxidants	Improves vitamin A absorption
	Oxygen, iron, copper, zinc, UV rays, pH<5.0	Lowers vitamin A absorption

Nutrients	**Interacting nutrients and conditions**	**Effects**
Vitamin B2	Vitamin B1	Adequate amount of B1 would improve levels of vitamin B2
	Copper, zinc, manganese, iron	Chelates with riboflavin and interferes with riboflavin absorption in the intestines.
Vitamin B3 (Niacin)	Protein	Tryptophan deficiency can increase the risk of B3 deficiency.
	B1, B6, B12	Deficiencies of these vitamins will improve chances of vitamin B3 deficiency.
Folate	Vitamin B1, B2, B3	Adequate amounts of these need to be present for folate absorption.
Vitamin B12	Vitamin B6, vitamin E	Both these vitamins are needed for vitamin B12 absorption.
	Folic acid	Excess folic acid may mask vitamin B12 deficiency.
Vitamin B6	Dietary fiber	High fiber consumption lowers vitamin B6 levels as well as raises vitamin levels in fecal excretion.
Minerals		
Iron	Protein, organic acids	Helps in non-heme iron absorption.
	Zinc, calcium, phosphate,	Lowers iron absorption.
	Vitamin C	Increases iron absorption.
	Vitamin A	Vitamin A deficiency inhibits iron utilization and accelerates development of anemia.

Nutrients	Interacting nutrients and conditions	Effects
	Polyphenols, phytic acid	High levels of polyphenol and/or phytic acid contents may lower iron bioavailability.
Zinc	Protein	Helps zinc absorption.
	Folic acid, non-heme iron, tin, calcium, magnesium, fiber, phytates	Lowers zinc absorption.
Calcium	Protein	Dietary protein stimulates urinary calcium excretion but a moderate dietary protein increase doesn't affect calcium balance in healthy subjects.
	Fat, fibers, phytate, zinc, sodium	Lowers calcium absorption and levels in the body.
	Lactose	Shows improvement in calcium absorption in animal studies.
Copper	Protein	Improves copper absorption.
	Vitamin C, zinc, dietary fiber	Lowers copper absorption.

Adapted from Caballero (n.d.)

Table 2 demonstrates that micronutrient interactions within a food matrix plays an important role in the overall bioavailability and nutrient utilization. Proper understanding of the various synergistic and antagonist effects of this nutrient-nutrient interaction as well as the effects of other conditions such as light and pH should be factored in while designing the nutritional profile of foods.

7c. Micronutrients Used in Fortified Foods (different chemical forms)

Several strategies have been implemented to supplement micronutrients in undernourished populations. These include food fortification and supplementation, dietary diversification, education and food rationing. Among these different methods, food fortification may be the most cost-effective and can help maintain steady body stores if consumed regularly (Serdula, 2010). It was found that the bioavailability of ferric pyrophosphate in fortified rice was higher when cofortified with zinc sulfate than with zinc oxide (Hackl et al., 2017) using human stable isotope study. The authors attributed

this to the possibility of lowering the acid-driven dissolution of ferric pyrophosphate in the presence of zinc oxide (ZnO) as compared to zinc sulfate (ZnSO4). In light of this, overall solubility of ferric pyrophosphate decreases, thereby lowering the bioavailability in presence of ZnO.

The chemical forms, their ratios, interactions within the food matrix, storage and transportation stability of micronutrients (vitamins and minerals) are also a very important factor in their bioavailability. WHO/FAO (2006) has provided guidelines on various forms of these micronutrients and their suitability in different food matrices. Iron deficiency is the most widespread micronutrient deficiency globally. Therefore, it is of prime importance that the right form of the micronutrient be chosen for a particular food matrix. The most bioavailable forms of iron compounds have the highest solubility in the human proximal intestine. However, the chemical and physical properties cause these water-soluble iron compounds to react with the food matrix and causes unfavorable changes during storage. Therefore, less soluble forms of iron with lower bioavailability in higher quantities have to be used as a fortificant (Moretti et al., 2014). The chelating properties of ferric sodium EDTA (NaFeEDTA) and its water solubility make it a preferred form of iron fortificant in food matrices with high levels of phytate. NaFeEDTA has shown two-to-three-fold absorption than ferrous sulphate in high-phytate cereal diets (Davidsson et al., 2002) and similar bioavailability to ferrous sulphate in meals with low extraction flours (Bothwell and MacPhail, 2004). NaFeEDTA and electrolytic iron had relative bioavailability of 1.05 and 1.28 times higher respectively than that of ferrous sulphate when added to curry powder and was served to 40 households and 10 restaurants in Nepal (Karn et al.,2011). However, the cost of fortification with NaFeEDTA was 4.6 times higher than that of electrolytic iron and ferrous sulphate. Another class of iron fortificant is based on amino acid chelates like ferrous bis-glycinate and ferric tris-glycinate. Ferrous bis-glycinate was found to be superior in bioavailability as compared to ferrous sulphate but lower than NaFeEDTA in cooked maize flour (Layrisse et al., 2000). It also had organoleptic changes during storage (Bovell-Benjamin et al., 1999). Whereas, the tris-glycinate form caused limited organoleptic changes but the bioavailability has not been properly characterized.

Cercamondi et al. (2013) recommended that NaFeEDTA was not a good form of iron fortificant for an LNS or CFF (complementary food fortificant) as compared to iron II sulfate (FeSO4) due to lower iron absorption from NaFeEDTA. Reduced iron showed an inverse relation between particle size and iron bioavailability in a Caco-2 cell study (Arredondo et al., 2006). The effect of calcium salts on iron absorption was studied by Cook et al. (1991). It was reported that calcium carbonate at a dose of 600 mg did not inhibit absorption of ferrous sulfate (18mg) at an iron/calcium proportion of 1:33. However, using calcium citrate and calcium phosphate in the same ratio lowered the iron absorption by 44 percent and 62 percent respectively. Reddy and Cook (1997) observed that different iron/calcium proportions (above 1:40) and the types of salt sources of the minerals interfere with the bioavailability of iron.

Iron absorption from NaFeEDTA added to high extraction cereal flour-based foods or to a meal containing phytate is two- to threefold higher than for ferrous sulfate. Although it does not promote fat oxidation (i.e., rancidity) in stored wheat flour, NaFeEDTA may cause unacceptable color changes in some food vehicles (Hurrell,

1997b). In 1999, NaFeEDTA was approved by the Joint FAO/WHO Committee on Food Additives for use in supervised programs in areas with a high prevalence of iron deficiency, at a maximum intake of 0.2 mg Fe/kg body weight per day. NaFeEDTA may be a good option for the fortification of both wheat and corn flours with a high extraction rate which have a high amount of iron absorption inhibitors because they are less refined. NaFeEDTA is not yet available in the commercial market because the demand is low and because of this, the price is high. The price of NaFeEDTA is more expensive than ferrous sulfate (Hurrell, 2001) for an equivalent amount of iron but only slightly more expensive when bioavailability is factored into the cost (Bothwell and MacPhail, 2004).

The bioavailability of micronutrients like iron, vitamin A, zinc and folic acid in spices and condiments as vehicles for fortification was studied by Degerud et al. (2015). They tried to specify the form of nutrients to be used in dry or liquid matrices containing the spices and condiments. It was recommended that NaFeEDTA and ferrous sulfate with citric acid are good options for liquid matrices like fish and soy sauce. In dry matrices like curry powder and salt, iron sources like NaFeEDTA, encapsulated ferrous fumarate and micronized elemental iron are preferred. Vitamin A in dry forms–retinyl acetate or palmitate are bioavailable in dry matrices but no published studies are available for these fortificants in fluid vehicles. Food matrices do not affect folic acid bioavailability and it can be effectively used in spices and condiments. In a detailed review of micronutrient fortification of food and its impact on woman and child health by Das et al. (2013), they could not conclude on whether a specific compound was better than its other forms in impacting individual micronutrient status.

Table 3: Some Common Micronutrient Forms Used in Food Aid Products

Micronutrient	Chemical form	Used	Reference
Iron	Ferrous sulphate (dry)/Ferrous fumarate–can be encapsulated	Low extraction wheat or corn flour, cereal-based complementary foods, RUF	WHO and FAO, 2006; USAID 2015
	Ferric pyrophosphate	Fortified rice	USAID, 2016
	NaFeEDTA	SC+, CSB+, RUTF, RUF	WFP, 2014; USDA 2014; USDA, 2012; USAID, 2015
	Ferrous Fumarate	SC+, CSB+, HEB	WFP, 2014; USDA 2014; WFP,2016; USDA 2015
Vitamin A	Vitamin A Palmitate	HEB, SC, CSB+, flours, RUF, fortified rice	WFP,2016; USDA 2015; WFP, 2014; WHO and FAO,

Micronutrient	Chemical form	Used	Reference
			2006; USAID 2015; USAID, 2016
	Vitamin A palmitate or acetate (oily form)	Spreads	WHO and FAO, 2006;
Zinc	Zinc sulfate	SC+ CSB+, RUF, RUTF	WFP, 2014; USDA 2014; USAID 2015; USAID 2015; USDA, 2012
	Zinc oxide	Fortified rice	USAID, 2016
Vitamin B1	Thiamine hydrochloride	RUTF (paste)	USDA, 2012
	Thiamine mononitrate	RUTF (bars), CSB+, SC+, HEB	USDA, 2012; USDA, 2014; WFP 2014; WFP,2016; USDA 2015

Table 3 shows different forms of some micronutrients specified for different food matrices. The sodium EDTA form of iron is used in food products which contain phytates, whereas ferrous sulfate is used where the fortified foods are consumed quickly and do not have to be stored for long time. Ferrous sulfate is water-soluble and the most bioavailable form of iron but it can quickly cause rancidity (WHO and FAO, 2006). In foods that require longer storage time, ferrous fumarate is preferred, although it is poorly soluble in water but is soluble in dilute acids. Similarly, in RUTFs, thiamine hydrochloride is used as the source of vitamin B1 in pastes and thiamine mononitrate is used in bars. The hydrochloride form is more hygroscopic (Bailey, 1991) and is generally used where the food is not consumed as dry but in paste or liquid form. The mononitrate form is used in foods with dry matrices.

It is clear that the food matrix plays an important role in selecting the form of micronutrient to fortify a particular food.

7d. Processing and Its Role in Affecting Micronutrient Bioavailability

The losses seen in vitamins from foods are cumulative and every step from post-harvest storage to final cooking contributes to vitamin losses. The factors affecting vitamin losses during processing are oxidation (exposure to air), heat (time and temperature), catalytic effects of metals, pH, action of enzymes, moisture, etc. individually or in a combination of these factors.

Washing, Blanching, Cooking

Water soluble B vitamins are leached out during commercial washing, blanching and domestic cooking. Thiamin (vitamin B1) is heat sensitive in neutral and alkaline foods and is unstable in air. Riboflavin (vitamin B2) is decomposed by light. Niacin (vitamin B3) and vitamin B6 are relatively stable under a variety of processing conditions. Vitamin D is not greatly affected by processing and storage. Vitamins A and E are destroyed in conditions which oxidize unsaturated fat like light, heat, air and storage time, etc. Vitamin K is stable to heat but extremely sensitive to fluorescent light and sunlight. Vitamin C is extremely sensitive to chemical oxidation during cooking, processing and storage. Ascorbic acid is highly susceptible to chemical and enzymatic oxidation during the processing, storage and cooking of food. Ascorbic acid is very stable in canned or bottled foods after the oxygen in the headspace has been used up, provided the food is not subjected to high-temperature storage or exposed to light. Cold water washing or steeping does not normally leach out a significant amount of the vitamin in whole undamaged fruits and vegetables. Green peas retained 82 percent of ascorbic acid after treatment at 900 MPa and 43°C for five minutes (Quaglia et al., 1996). Wang et al. (1992) reported on the losses of added ascorbic acid during the pilot-scale processing and storage of potato flakes and during the reconstitution and holding of the mashed potatoes prior to serving. The amount of water used in domestic cooking and, to a lesser extent, the cooking time, affect vitamin C losses more than the source of energy or the type of cooking (Erdman and Klien, 1982).

Extrusion and Drum Drying

Industrial processes such as extrusion and drum drying used for manufacturing breakfast cereals from wheat flour saw vitamin E loss of 90 percent (Hakansson et al., 1987). This was attributed partly to lipid degradation. Nonenzymatic oxidation of vitamin E may also take place when the process temperature has passed the point at which the enzymes are heat-inactivated (about 60°C). Microwave treatment was another effective way of inactivating enzymes (lipoygenase and peroxidase) and improving vitamin E retention.

Milling

The milling of cereals removes most of the thiamin, so white flour, ready-to-eat breakfast cereals and, in certain countries, polished rice are enriched by the addition of the vitamin. A range of conditions, such as pH, temperature and moisture content, promote the loss of thiamin in foods. Thiamin is the most heat-labile of the B vitamins. Thiamin losses are enhanced when processed in alkaline environments like 50 percent destruction of thiamin from original thiamin content in flour when baking powder is used to make cakes (Benterud, 1977). They also observed that during the baking of bread with yeasts, the medium is slightly acidic and the thiamin loss is reduced to 15 to 25 percent mostly in the crust. In the processing of milk, the following losses of thiamin have been reported: pasteurization, 9 to 20 percent; sterilization, 30 to 35 percent; spray-drying, 10 percent; roller drying, 15 percent; and condensing (canning), 40 percent (Gubler, 1991).

The milling of cereals results in considerable loss (up to 60 percent) of vitamin B2, so white flour is enriched by the addition of the vitamin. Riboflavin is generally stable during heat processing and the normal cooking of foods if light is excluded. The alkaline conditions in which riboflavin is unstable are rarely encountered in foodstuffs. In mature

cereal grains, most of the niacin is present as bound nicotinic acid and is concentrated in the aleurone and germ layers. Milling to produce white flour removes most of the vitamin with the bran. Apart from leaching losses, niacin is stable during processing, storage and cooking of foods. The folic acid added to flour and cereal-grain products in accordance with the mandatory U.S. fortification policy shows good stability toward processing and storage. The baking of bread caused a 20 percent loss of added folic acid and a similar loss of native folate (Osseyi et al., 2001).

Aqueous Medium

In aqueous solution, folic acid is stable at 100°C for 10 h in a pH range 5.0–12.0 when protected from light but becomes increasingly unstable as the pH decreases below 5.0 (Paine-Wilson and Chen, 1979). Nguyen et al. (2003) found that folic acid was stable in high hydrostatic pressure (HHP) of 600 MPa and 60°C for 7h without degradation. Folate loss in UHT-processed milk amounted to 12.5 percent (Oamen et al., 1989); the loss in pasteurized milk is 10 percent (Renner, 1988). The presence of ascorbic acid in milk protects the folate from oxidation by the dissolved oxygen.

7f. Other Constituents Affecting Nutrient Bioavailability

Other than the food matrix, several other constituents acting within the body affect the nutrient bioavailability.

Gut Health

The microbes in the gut become established very soon after birth and its profile affects the nutrient extraction and immune function. Lack of food causes an imbalance in the gut flora which adds to the cause of undernutrition and its negative effects on the host's body. Microbiomes in the body of the undernourished may yield less capacity to harvest and utilize nutrients (Fluitman et al., 2017). The microbiota profile of undernourished children in a Bangladesh study did not correspond to their age and it showed them being "younger" as compared to the microbiota profile of healthy children (Subramanium et al., 2014). On feeding the undernourished with nutritious food, the microbiota matured but the change was temporary and returned to "young for age" status after the withdrawal of healthy food on regaining weight and nutritional status. The authors also pointed to the fact that the diversity in microbiome of malnourished was less as compared to healthy children and many species were less abundant than in healthy children of the same age.

Smith et al. (2013) conducted studies with 317 twin Malawian pairs up to the first three years of their life and found that differences in intestinal microbial mix could be a cause for kwashiorkor, caused primarily by protein deficiency. It was found that microbiota in Bangladeshi malnourished children contained less *Bacteroides* and contained more significantly pathogenic *Proteobacteria* (Monira et al., 2011).

A recent study by Cheung et. al. (2016) used LNS and CSB to assess its effect on gut microbiota of Malawian infants aged 6 to18 months. The 12-month study did not show any effect on the gut microbiome profile. Similar results were found by Aakko et al. (2017) by using comparable products: LNS (dairy or soy-based) and CSB. Interventions which can manipulate microbiota would be useful in promoting specific beneficial bacteria and suppressing others. In the context of undernutrition, the use of pro- and

prebiotics can promote nutrient uptake, mediate inflammation and have other beneficial effects (Pandey et al., 2015).

Infections

The absorption of nutrients by the body is also affected by illness and infections. Parasitic infections are common in countries facing undernutrition in Asia, Africa and Latin America. Children are more susceptible to these infections and therefore have lower levels of valuable trace elements (Black, 2003; Gibson 2005). Researchers have found the effect of these infections on the growth parameters, particularly height and weight (Egger et al., 1990; Filho et al., 2011; Amare et al., 2013). In a study conducted by Shalaby et al. (2017), it was found that serum micronutrients–zinc, iron and copper–and anthropometric indices were significantly lower in children (aged 2 to 15.5 years) with parasitic infection as compared to the control group (parasite-free, healthy children). Other studies have also shown lower serum levels of micronutrients in subjects having parasitic infection (Culha et al., 2007; Shady et. al., 2011). Studies have also shown improvement in serum levels of micronutrients after being treated for parasitic infections (Olivares et al., 2003; Quihui et al., 2010).

This indicated that nutrient bioavailability from food depends on both food and nonfood factors. The overall health of an individual is the net consequence of all affecting factors. To have a better understanding of the effect of food matrices on nutrient bioavailability, high-quality programmatic studies need to be designed and implemented. For example, studies which look into nonfood aspects as well in conjunction with food-related causes; studies which look into the overall effect of multiple food consumption by the recipients of food aid; as well as other studies designed to have a better understanding of all the causative factors which play a role in health outcome. It would help getting much-needed information on the cost and comparative cost-effectiveness of different integrated strategies for filling nutrient gaps and promoting healthy growth.

8. Improving Bioavailability of Food Aid Products

The more recent changes made in the formulation of food aid products have certainly improved the nutritional and health consequences in populations afflicted by undernutrition. These changes have been made primarily in the micronutrient premix added to the fortified foods. However, the macronutrient ingredients of choice have been corn and soy for FBFs, and peanuts and dairy proteins for lipid-based food systems. Although several variants of FBFs and RUTFs have been developed using local ingredients, those products have not been used on a large scale to tackle the undernutrition problems. FAQR's 2011 report has suggested many ways to improve the overall functionality of food aid products, including exploring nontraditional cereals and legumes and the use of dairy proteins.

8a. Macro Ingredients

In a review of complementary foods based on cereals, it was found that they were not nutritionally adequate (Treche and Mbome, 1999). Traditionally-prepared cereal-legume blends have better nutritional profiles than solely cereal-based complementary foods but they still are deficient in some essential nutrients including fat, iron, zinc and calcium (Gibson et al. 1998). Webb et al. (2011) recommended exploring different cereal

legume blends like sorghum-soy, rice-soy, potato-soy or rice-lentil blends as an alternate to CSB. Joseph (2016) studied the physicochemical characteristics of such fortified blended foods made from sorghum and cowpea or soy as a protein source. The study showed that these extruded products had better Bostwick flow properties (a measure of the flow/consistency of the porridge and an essential feature affecting porridge consumption by infants and children) than the presently-distributed variants of CSB. It is therefore prudent to explore different sources of macronutrients, especially those which are consumed in target countries or those which are sustainable. **Grains to consider include: sorghum, rice, millets and amaranth. Protein sources can be found from legumes such as cowpea, chickpea, Bengal gram, pea protein, wheat germ protein, etc. In addition, efforts should be made to at least look at the feasibility of proteins derived from single-cell microorganisms such as algae, fungi, yeasts, and bacteria.** The amino acid profile of these microorganisms is superior to that of soy and other plant sources (Billing and Spurrell, 2018). The technology of protein extraction is currently since the industry is at an emerging stage with small-scale production facilities.

Oil is a source for energy densification of fortified foods. It also serves as a superior carrier of oil-soluble vitamins. Vegetable oil fortified with vitamins A and D is the most commonly-supplied oil in food aid programs with soybean oil being the most common oil. Another important aspect of oil is the presence of essential fatty acids which are required for normal growth, immune function, cell function and anti-inflammatory response (Brenna et al., 2015). The essential fatty acids are ω-6 and ω-3 and the recommendation for the optimum ratio of ω-6: ω-3 is 5:1 to 10:1, which is similar to that of breast milk (Lutter and Rivera, 2003). The CSB has a high ω-6: ω-3 ratio, which is calculated around 14-15:1. This translates to lower ω-3 in the food. **Therefore, opportunity exists to choose oil with higher ω-3 content or with a lower ω-6: ω-3 ratio like canola oil or a blend of different oils to maintain a low ratio. The challenge in using such oils is the higher content of polyunsaturated fat which is prone to oxidation and rancidity. The use of antioxidants like rosemary derivatives, mixed tocopherols, etc. would potentially prevent oxidation of such oils.**

8b. Processing

Food aid programming generally consists of distributing most of the foods as staples (like corn, milled rice, dried beans, lentils, etc.) which can have antinutritional factors like phytic acid, tannins and trypsin inhibitors which would negatively affect the overall nutrition uptake from these foods in target populations. The current food aid basket is composed of almost 80 percent staples and 20 percent processed foods (Joseph et al., 2018). Several traditional/common household-level food processing techniques can be used to improve the bioavailability of micronutrients from plant-based diets. Thermal processing may improve the bioavailability of nutrients such as thiamin and iodine by destroying antinutritional factors like thiaminases and goitrogens. Phytate degradation depends on plant species, temperature and pH (Hotz and Gibson, 2007). **The pounding of grains at the household is effective in reducing phytate content in cereals which have phytate concentrated in the outer**

aleurone layer (like rice, sorghum wheat, etc.) or in the germ (maize). This would improve the bioavailability of minerals like iron, zinc and calcium but may lead to some loss of vitamins. Soaking of cereal and legume flours reduces phytate content and also passively diffuses water-soluble natrium (sodium), potassium, or magnesium phytate. Several studies have reported improvement in absorption of iron, zinc and calcium. **Fermentation produces phytase enzymes which break down phytate to lower inositol phosphates.** This is extremely helpful because inositol phosphates with <5 phosphate groups do no hinder zinc absorption (Lonnerdal et al., 1989.) and those with <3 phospahate groups do not bind non-heme iron (Sandberg et al., 1999, Hurrell, 2004). However, the effect of fermentation on phytates is reduced when the substrate has high tannin content (e.g. bulrush millet and red sorghum).

> ***Thermal processing may improve the bioavailability of nutrients such as thiamin and iodine by destroying antinutritional factors like thiaminases and goitrogens.***

Fermentation also improves protein quality and digestibility, vitamin B content, and microbiological safety and keeping quality. Germination/malting increases the activity of endogenous phytase found in cereals, legumes and oil seeds. α-Amylase activity is also increased during germination of cereals, especially sorghum and millet. The enzyme hydrolyzes amylase and amylopectin to dextrin and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson et al., 1998). **Use of such a combination of strategies like fermentation and/or germination along with the regular processing steps of milling, roasting, extrusion and cooking can almost completely remove phytate.** This is important because phytic acid is a potent inhibitor of iron absorption, even at low concentrations (Hurrell, 2004).

Consumption of plant-based diets at household levels have challenges relating to the bioavailability of nutrients (Gibson et al., 2006). The factors affecting them might be due to the chemical form of nutrient in the food and/or in the matrix, nutrients and other organic component interactions (phytate, dietary fiber, polyphenols, etc.), and preparation practices. **Food preparation and consumption strategies at household levels like soaking, germination, microbial fermentation, addition of ascorbic acid-rich fruits to diets to enhance non-heme iron absorption, heating to destroy heat labile antinutritional factors (goitrogens, thiaminases, etc.) can help increase the bioavailability of foods.** The major effect of these practices is on the bioavailability of minerals, primarily Fe, Zn and Ca (Gibson et al., 2006) rather than on macronutrients. Several studies (Kaur et al., 2015; Ghavidel and Prakash, 2007; Sinha and Kawatra, 2003) have reported improvement in starch digestibility due to processes like soaking and germination. These processes lower the antinutritional factors like phytic acid, polyphenols etc. and therefore improve the digestibility. No significant changes in in-vitro protein digestibility was found due to

the soaking of beans (Abd El-Hady and Habiba, 2003). However, Rasane et al. (2015) observed improved protein quality of oats due to germination.

> ***Use of such a combination of strategies like fermentation and/or germination along with the regular processing steps of milling, roasting, extrusion and cooking can almost completely remove phytate.***

Soaking rice flour for 1, 6, and 12 hours at 30°C to make rice-based complementary foods resulted in reduction in phytic acid levels by 60, 65, and 98 percent, respectively. Whereas the reduction of 10 and 17 percent of phytic acid was observed in mung bean flour (used as protein source with rice) soaked for one and six hours, respectively (Perlas and Gibson, 2002). Davidsson et al. (1995) conducted in vivo studies on 16 healthy adult volunteers and found that dephytinized soy infant formula had a 2.3-fold increase in manganese absorption (from 0.7 percent to 1.6 percent) as compared to the soy formula containing native phytic acid. However, manganese absorption was influenced by the addition of ascorbic acid in both types of soy formula.

The cost of treating anemia using a pharmacological dose of ferrous sulfate as supplementation costs $20,000/10,000 persons whereas treating anemia using iron cookware is estimated to cost $5000/10,000 persons (Adish et al., 1999). However, personal choice may limit the use of iron cookware. Domestic preparation of rice in iron cookware was observed to increase the bioavailability of iron by about 300 percent (from 0.249 to 0.747 mg/100g) and consumption of the prepared rice on a daily basis for 12 weeks reduced the iron anemia incidence from 31.2 to 5.3 percent among vegetarian teenagers (Quintaes et al., 2007).

Whole grains are advantageous due to the presence of bioactive compounds. **Extrusion cooking can improve the bioavailability of these function compounds to an extent by forming complexes with protein which is broken down in the human body for antioxidant properties.** Incorporation of fruits and vegetables byproducts would enhance the bioactive levels in extrudates (Brenan et al., 2011). A plant-based, milk-free RUTF using sorghum, maize and soybean made via extrusion had a phytic acid: iron molar ratio of 0.8 and phytic acid: zinc molar ratio of 2.5 (Owino et al., 2014). In addition to the potential of varying the composition of RUTFs from the traditional peanut and milk-based formula, extrusion processing may provide an alternate method to reduce phytic acid content and thus contribute toward higher bioavailability of nutrients.

> ***Extrusion cooking can improve the bioavailability of these function compounds to an extent by forming complexes with protein which is broken down in the human body for antioxidant properties.***

An experiment conducted by Dust et al. (2004) on extruding different substrates such as barley grits, cornmeal, oat bran, soybean flour, soybean hulls and wheat bran showed that chemical composition of substrates affect the digestibility and resistant starch formation. The study revealed that only oat flour could have increasing amount of resistant starch

production on increasing the extrusion processing severity. Resistant starch can act as a proxy for traditional dietary fibers and can positively influence colonic health.

Nutritional value of legume seeds was examined after exposing them to different cooking methods–microwaving, boiling and autoclaving. It was found that microwave cooking was the most efficient in retaining minerals. The in vitro protein digestibility and protein-efficiency ratios of legumes under all regimes of cooking was improved. There was significant reduction in antinutritional factors–trypsin inhibitor, tannins and phytic acid—after cooking, as compared to raw foods. However, there were no significant differences in antinutritional factors due to cooking methods (Hefnawy, 2011).

Table 4: Effects of non-competetive interactions between organic substances on nutrient bioavailability in plant foods

Dietary component	Food sources	Major influences	Nutritional consequences
Inhibiting factors			
Phytate with magnesium, calcium, or potassium phytate	Unrefined cereals, legumes, nuts, oil seeds	Binds certain cations to form insoluble complexes in gut	Zinc, iron, calcium, and magnesium are poorly absorbed
Soybean protein	Some varieties of soybean, unfermented tofu, textured vegetable protein	Variety and processing methods impact the effect	Inhibits iron and zinc absorption in some varieties. Some contain iron as phytoferrin, which may be highly bioavailable.
Polyphenols	Red sorghum, legumes (red kidney beans, black beans, black gram), spinach, betel leaves, oregano, tea, coffee, cocoa, red wine	Form insoluble complexes with iron. Some polyphenols bind thiamine, some salivary and digestive enzymes. Enhances excretion of endogenous protein	Inhibits non-heme iron absorption. Reduces macronutrient digestibility, thiamine absorption
Oxalic acid	Amaranth, spinach, rhubarb, yam, taro, sweet potato, seasame seeds, black tea	Oxalates can form insoluble complexes with calcium and possibly iron	Reduces absorption of calcium and possibly iron. Increases urinary calcium
Dietary fiber	Unrefined cereals, legumes, nuts, oil	Lignin and pectin bind bile acid. Pectins, psyllium and gums retain	Reduces fat and fat-soluble vitamin absorption. Slows gastric emptying and digestion

	seeds, fruits and vegetables	water and form viscous matrix in GIT. Fermented in large intestine	and absorption of nutrients. SCFA produced increases calcium solubility
Enhancing factors			
Organic acids (citric, lactic, acetic etc.)	Fermented milk products, vegetables, sauerkraut, soya sauce, fermented cereals	May form soluble ligands with some trace minerals in GIT	Enhances absorption of zinc and possibly iron
Ascorbic acid	Citrus fruits and juices, guava, mango, papaya, kiwi, strawberry, tomato, asparagus, spinach etc.	Reduces ferric form of iron to ferrous form. May also increase stability of folate during processing and digestion	Enhances non-heme iron absorption. Counteracts effect of phytate. May increase folate bioavailability. May enhance absorption of other trace minerals like selenium and chromium.
Protein	Plants and animal sources	Amount and type influences soluble ligand formation with zinc, iron, and copper	Enhances absorption of zinc, iron and copper. Increases urinary calcium excretion
Fat	Oil seeds, nuts	Solubilize fat soluble vitamins and carotenoids	Enhances absorption of fat-soluble vitamins and provitamin A carotenoids

Source: Gibson et al., 2006

Awareness about the effects of consuming different foods on nutrient absorption as shown in Table 4 may help in better planning of diets where there is no scarcity of food. These strategies may not be sufficient in themselves to increase the bioavailability of micronutrients especially Ca, Fe and Zn, as has been reported in studies conducted in rural Mexico. In the Mexican study, even after providing 25 mg ascorbic acid twice a day at six days/week for eight months to Fe-deficient women did not show improvement in biochemical Fe status, even though there was a two-fold increment in Fe absorption as compared to those receiving the placebo (Garcia et al., 2003). Another study conducted by Mamiro et al. (2004) in Tanzania on six-month-olds who were fed processed complementary foods for months after soaking and germination (as compared to the same food which was unprocessed) did not show any significant difference in Fe status measured by hemoglobin and zinc protoporphyrin, and in growth probably because the preprocessing steps could only remove 34 percent phytate.

Therefore, an integrated approach of incorporating some of the above methods, the addition of small amounts of animal source foods, external addition of enzymes like phytase along with micronutrients may help increase the bioavailability of micronutrients (Gibson et al., 2006). Iron bioavailability was found to be highest in polished rice, followed by unpolished and bran portion respectively (Prom-u-thai et al., 2006). Garcia-Estepa et al. (1999) reported that enzymatic methods (fermentation and germination) followed by milling was most effective in reducing the phytate content of grains. These methods are employed singly or in combinations to improve the bioavailability of nutrients, primarily zinc from local foods. For example, soaking oats followed by sprouting the oats reduces phytate content and doubles the amount of absorbed zinc in comparison with untreated oats.

8c. Use of Technology

Mehansho et al. (2003) reported developing a fortification technology for using a single vehicle to deliver multiple micronutrients. They developed GrowthPlus® fortification technology for Procter and Gamble (Mehansho, 2006). This unique technology delivers better-absorbed and utilized iron, vitamin A and iodine (plus other vitamins and minerals) without compromising taste, color, and product and vitamin stability. This was accomplished by understanding and analyzing the factors which influence the reactivity, stability and bioavailability of the micronutrients in a food/beverage-based delivery system. The delivery system had a lock/unlock feature in which the nutrients remained unavailable/locked during consumption and transit inside the body. They tested it with iron (ferrous-bis-glycinate and FeS04). The micronutrient mix was added to water, chocolate and baby cereal and the off-color development associated with chemical changes in iron did not occur. After a market survey, they found that fruit flavored drinks are liked by the target population. Therefore, they added GrowthPlus® technology into fruit-based drinks under the name NutriStar®.

Thus, the fortified powder fruit drink consisted primarily of sweeteners, thickeners, clouds, acidulent, natural fruit flavors and GrowthPlus® (iron containing multiple-micronutrients). The overall acceptance of the fortified powder drink with the GrowthPlus® was compared with a placebo (a product with the same appearance and taste but without multiple-micronutrient fortification). The subjects were asked questions about the overall product acceptance, flavor and color during the five-day use test. The findings showed the multiple-micronutrients (including iron, iodine, vitamin A) delivered via GrowthPlus® fortification technology had no significant effect on the flavor, color and overall acceptance of the finally consumed fruit drink. The fortified powder fruit drink was packaged in a sachet and stored in temperature-controlled rooms for up to one year. As shown in **Table 3**, all nutrients (added as GrowthPlus®) including vitamin C, iodine, folic acid and B12 were stable after one year of storage at ambient temperature. The percent of recovery ranged from 91.5 percent for vitamin A to 113.9 percent for vitamin B6. It is important to note also all values after one year of storage are above the targeted value. The stability of the major micronutrients (namely, vitamin A, vitamin C, riboflavin and iodine) which are known to be sensitive to degradation in aqueous delivery systems was further evaluated after the fortified powder fruit drink was reconstituted. The results obtained after 1 hour and 24 hours storage at ambient temperature are shown in **Table 4.** As expected, there was no

change in the iron level. Riboflavin, vitamin C and vitamin A showed little or no degradation. However, the level of iodine in the reconstituted beverage was decreased by 21 percent and 16 percent after 1 hour and 24 hours, respectively. The stability observed in both the powder and beverage forms is due to the ability of the GrowthPlus® technology to keep the multiple micronutrients in a stable and/or non-reactive form.

The bioavailability of the iron from the fortified powder fruit drink was determined. The treatments included the following: (a) fortified powder beverage alone; and (b) fortified powder beverage with rice. When the reconstituted fortified powder fruit drink was consumed alone, 23.4 percent of the iron was absorbed. This is comparable to the iron absorbed from meat and about five times the iron of milk fortified with ferrous sulfate. However, when consumed with rice, the percentage of iron absorbed was reduced by about half (23.4 percent versus 10.7 percent). Although there was a significant reduction of iron absorption by rice, the bioavailability value was still comparable to that of iron from fish, which is accepted as a good source of bioavailable iron.

Vinodkumar and Rajgopalan (2007) developed a multi-micronutrient fortified salt which had chelated ferrous sulfate and microencapsulated vitamins A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate and iodine. The efficacy test of salt on school children 5 to 15 years of age (N=119) in Chennai, India showed that the product was stable for 6 months storage and 20 minutes of cooking. They observed significant improvement in hemoglobin, red cell count, urinary iodine and serum vitamin A in experimental group as compared to control (N=126).

Gallier et al. (2015) characterized lipids in infant milk formula versus a newly-developed lipid architecture to human milk. It showed the newly-developed lipid architecture to be closer to lipids in human milk and would have better metabolic response (based on rat studies) than the existing lipids in infant milk formulas. They pointed out that the current lipids in infant milk formulas had lipids of smaller size than those found in human milk and was coated by proteins compared to bigger fat globules in human milk which are enveloped by phospholipids. The newly-developed lipid had a phospholipid coating which was more similar to human milk.

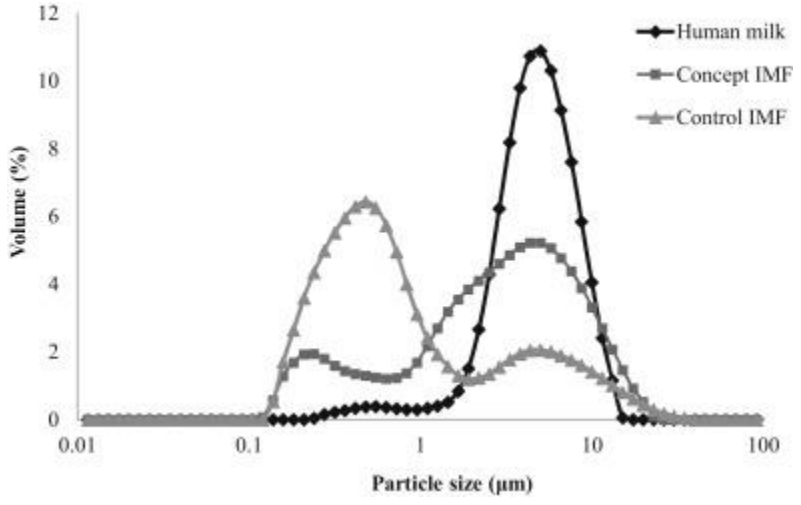

Figure 4: Droplet size distribution of lipid forms

Source: Gallier et al., 2015

8d. Controlled/Targeted Release

In reality, the benefits of food are lost because many bioactive components are not water soluble. For example, fat-soluble substances like vitamins are not readily processed and taken-up by the small intestine as water soluble components are, but are required to undergo a pretreatment phase involving micelle encapsulation to penetrate cells to release their contents. This delay results in the absorption of only 25 percent after fat digestion while the remaining is processed as waste in their undigested form (Sonkaria et al., 2012). The challenge has been to develop methods to solubilize these essential components to be effectively absorbed. With a view to increasing the bioavailability of nutrients susceptible to degradation and solubility concerns, efforts have been made to design novel formulations to incorporate food products which are not readily dissolvable in solution. Encapsulation, micro encapsulation or nanoencapsulation of bioactive ingredients like vitamins is a novel method to maintain the nutritional value, stability and controlled release of bioactive products over time. The encapsulation provides a barrier around the bioactive ingredient and prevents it from any unfavorable interactions with other ingredients. It further allows for the controlled release of the entrapped bioactive ingredient like vitamins at the point of absorption in the body.

The utilization of food-grade nanoparticles to encapsulate, protect and control the release of micronutrients provides a new direction in improving the bioavailability of micronutrients from food matrices (Joye et al., 2014). The capsule size (diameter) for microencapsulation varies between 5 to 300 microns (Gibbs et al. 1999) whereas the delivery systems with nanoencapsultions have a diameter less than one micron. The encapsulations may be fabricated from surfactants, lipids, proteins, and/or carbohydrates. The small size of the particles in these systems has a number of advantages over conventional delivery systems: higher stability to aggregation and gravitational separation; higher optical clarity; and, improved bioavailability. The higher surface-to-volume ratio offered by these enacapsulations enable speedier reactions than the traditional ingredient-delivery mechanisms (Neethirajan & Jayas, 2011). Edible nanoparticles can be fabricated from a variety of food-grade ingredients, including surfactants, lipids, proteins, carbohydrates and minerals (McClements, 2014).

9. Testing Methods for Analyzing In Vivo and In Vitro Bioavailability

Although in vivo methods provide direct bioavailability data, ethical restrictions and complex protocols limit this type of study when humans are used in biological research (Parada & Aguilera, 2007). Primarily, there are four methods to conduct in vitro assessment of bioaccesibility and/or bioavailability: solubility, dialyzability, gastrointestinal model (TIM) for bioaccessibility and Caco-2 model for bioavailability (Etcheverry et al., 2012). Van Campen and Glahn (1999) have reviewed different approaches to test micronutrient bioavailability. In vitro digestibility models are of two types: static and dynamic. When the final digestive products remain inside the reaction vessel, it is known

as static or biochemical model. Other physical movements like shear, mixing, etc. are not a part of this model.

The international consensus advises the use of pH 3 for in vitro gastric pH in adults. For infant simulated digestion, pH in the stomach should be higher than that of adults (Nguyen et al., 2015). The main advantages are low cost, simplicity and easy cleaning. It often becomes confusing to conduct in-vitro tests for which vitamins or minerals in multi micronutrients mix. The main disadvantages of the static models are that they cannot imitate the dynamic digestion process taking place in the human gastrointestinal tract which are the gastric emptying, peristaltic movements, pH change in the stomach, and enzyme and fluid secretion during digestion. These difficulties are overcome in a dynamic model. Two popular dynamic models are the TIM1 and TIM2. TIM1 has a gastrointestinal tract with stomach and three components for the small and large intestine. TIM1 takes into account most of the key parameters such as human temperature, pH change in the gastric system, gastric and pancreatic automatic secretion, gastric emptying, gastric and intestinal transit times, peristalsis movements and nutrient absorption in the intestine by a dialysis system (Guerra et al., 2012). In addition to these parameters, TIM2 can imitate the microbiota (Yoo and Chen, 2006). However, TIM1 is very expensive for a commercial product, plus complicated for cleaning and handling (Menard et al., 2014).

Following the hierarchy of indexing vitamins and minerals as per USP 24-NF19 (The United States Pharmacopeia) standards would help in overcoming the dilemma of choosing which micronutrients should be tested to give an insight for the solubility of all other vitamins (Srinivasan, 2001). Among vitamins, the first index vitamin is riboflavin (vitamin B2) in dissolution since it is the least soluble in water. If it's not in the micronutrient mix, then the second vitamin index is pyridoxine (vitamin B6), followed by niacin or niacinamide, thiamine (vitamin B1) and ascorbic acid (vitamin C), in that order. Similarly, for minerals, the indexing for the dissolution test starts with iron, followed by calcium, zinc and then magnesium. Therefore, the need for validated in vitro methods is urgent in order to evaluate and compare the effect of the microstructure with the amount and the rate of nutrients released in the gastrointestinal tract (GIT).

Cilla et al. (2011) used the Caco-2 cell model to study the effect of processing and food matrix on calcium and phosphorous bioavailability from milk-based fruit beverages. They found that bioaccessibility of Ca in high pressure processing (HPP) increased compared with thermal processing but bioavailability remained the same. This could be due to calcium being in a more soluble state in HPP but other factors might also be involved in its absorption. However, for phosphorous, the bioaccessibility and bioavailability were higher for HPP samples in Caco-2 cell analysis. Davidsson and Haksell (2011) highlighted the usefulness of stable isotope techniques in their review to assess the bioavailability of non-heme iron and provitamin A carotenoids from fortified foods. They also noted that β-carotene (and α-carotenoid) is poorly absorbed since it is a hydrocarbon carotenoid as compared to β-cryptoxanthin (xanthophylls).

The most common problem in measuring the mineral bioavailability from a diet is that different methodologies provide different results due to our limited knowledge of physiological factors affecting absorption. Because of this, comparison of these results

can become difficult. The technique of radiolabeling a single meal has provided very useful information in identifying the enhancers and inhibitors of iron absorption (Benito and Miller, 1998). Both in vivo and in vitro methods have been used to assess trace element bioavailability, however no one method is useful for all elements and model systems. Additionally, both stable and radioactive isotopes occurring either intrinsically in foods or added extrinsically to diets or foods have been used as tracers to assess the bioavailability of mineral elements. The use of tracers incorporated naturally (intrinsically) into edible portions of plant foods during plant growth will likely provide the most reliable estimates of trace elements' bioavailability consumed in mixed diets (House, 1999). Since extrinsic labeling is less expensive than intrinsic labelling, studies using extrinsic labelling are more popular.

Etcheverry et al. (2012) reviewed the in vitro bioaccessibility/bioavailability methods for different micronutrients and recommended methods which could be the best predictor of bioavailability for different micronutrients. They added that the recommended in vitro methods in their review may not absolutely predict the nutrient absorption in humans but could be used as a preliminary screen to identify the most promising food matrix, processing conditions, staple crop, growing conditions, etc. and their relative potential to impact nutrient bioavailability. The Caco-2 cell model is good for predicting calcium and zinc bioavailability in humans (Etcheverry et al., 2012). For carotenoids, the measure of bioaccessibility might be sufficient to estimate the bioavailability (O'Sullivan, 2010; Etcheverry et al., 2012). Because of this, the in vitro solubility method of measuring the soluble micellarized carotenoids instead of soluble carotenoids should give more reliable information. An in-vitro test for folate bioavailability was Seyoum and Selhub's method (1998) since it had significant correlation between folate bioavailability indices from foods like egg yolks, cow's liver, orange juice, etc. as reported in human studies (Tamura and Stokstad, 1973; Babu and Srikantia, 1976).

The dynamic TIM method only measures bioaccesibilituy of folate and not its absorption so it is not a preferred method. The in vitro digestion/Caco-2 uptake model is the recommended bioavailability method for iron because it is an analysis which can provide more information than bioaccessibility studies alone, such as the impact of food components on absorption rate and efficiency, and the possible competition among nutrients or between nutrients and food components for the same absorptive site (Etcheverry et al., 2012). In the review of in vitro methods to assess bioavailability of micronutrients, Etcheverry et al. (2012) stated that for magnesium, none of the in vitro methods have been validated against human studies and also none of the methods have been used extensively. They observed the same lack of validated studies with humans for in vitro testing of polyphenols, vitamin B6 (pyridoxine), vitamin D and vitamin E. For vitamin B12 (cobalamin), the recommended method was absorption studies (using either fecal excretion or body retention methods) in human subjects.

Caco-2 cell model was used to test the bioavailability of iron and zinc from multiple micronutrient beverage mixes with and without phytate (Pullakhandam et al., 2011). They discovered that iron absorption was higher in samples with phytate but those which contained ascorbic acid and zinc, similar uptake was found in samples with and without phytate. Hallberg and Hulthen (2000) used a radioiron tracer to measure the

absorption of iron while developing an algorithm to predict the bioavailability of iron from different meals.

Lynch et al. (2007) compared physical properties, screening procedures and human efficacy trials for predicting bioavailability of commercial elemental iron powders used for food fortification. They suggested that physical properties such as particle size and surface area affect bioavailability but that other factors also affect the bioavailability so these may not be very effective methods. Further, in vitro screening procedures–dialysis and Caco-2 cell uptake (using the method of Yeung et al., 2004)—were not found to be satisfactory in predicting bioavailability. They found a high degree of variability in replicates for dialyzable iron and the Caco-2 test seemed to have a higher effect of enhancers and inhibitors of iron absorption than they do on human iron absorption (Lynch, 2005). The dissolution test in 0.1mol/L HCl under standardized conditions was found to be the best predictor of bioavailability of elemental iron powders. Its results closely matched the RBV values by the AOAC rat hemoglobin repletion method and predicted the same results as those with human efficacy trials.

A comparative assessment of iron (FeSO4) bioavailability, in vitro (dialysis method) and in vivo serum iron measurement (n=22 and between 18 to 50 years with eutrophic = 7 and obese = 15) showed similar results. Higher bioavailability was seen in the aqueous form of iron. Lower bioavailability was observed in the presence of inhibitors (fiber) and higher bioavailability was observed with enhancers (ascorbic acid) (Bueno et al., 2013). There are several algorithms which have been developed to predict the bioavailability of iron, zinc, protein, folate, vitamin A and carotenoids but there is still no consensus among countries about which are the best algorithms to use. In some countries, fixed bioavailability factors are still used for certain nutrients even though their efficiency of absorption may vary with the dietary level of the nutrient or the life-stage group (Gibson, 2007).

Enzymatic digestion in vitro followed by the flame atomic absorption spectrometry method was used by Suliburska et al. (2011) to assess iron bioavailability in 29 fortified and non-fortified commercial cereal products. Johns et al. (2014) developed an in vitro method to determine soluble iron in fortified rice analogue/natural rice blends. This method was not a substitute of in vivo assays but an inexpensive, reliable and fast tool to identify issues worthy of in-vivo study. On the basis of experimental assessments of linearity (R2 >0.9995), precision (day-to-day RSD ≤20% for soluble iron concentrations ≥0.1 mg per 100 g of rice), accuracy (spike recovery average=97.5±0.4%) and FerroZine™ selectivity (no more than a minimal bias from copper expected), the in-vitro assay is regarded as capable of reliably discriminating the soluble iron concentration differences associated with rice flour/iron ingredient blends and with natural rice/fortified rice analogue blends.

Zinc bioavailability from fortified rice (Ultra Rice®) was studied using Wistar rats. The rats were fed fortified rice containing zinc oxide and zinc carbonate (50 percent or 100 percent of the recommended levels for this animal), and control diet which did not have zinc. Control diet showed higher weight gain, feed efficiency ratio, retention of Zn and Zn concentration in the femur. However, no differences were observed for dietary intake, length and thickness of the femur, erythrocyte and plasmatic Zn between groups.

Rice fortified with zinc oxide showed a lower bioavailability compared to zinc carbonate *(ZnCO3) (Lucia et al., 2014).

Table 5: Advantages and Limitations of Methods Used to Assess Iron and Zinc Bioavailability

Source: Dias et al., 2017

Methods	Advantages	Limitations
In vitro (Caco-2 cells)	✓ It is less costly and less time-and work-intensive ✓ It enables a substantial number of breeding lines to be compared in a single experiment ✓ It is a better indicators of bioavailability than the solubility method ✓ It simulates gastric and intestinal digestion of food	✓ It cannot simulate all physiological and metabolic responses of the human body ✓ There is variability in protocols ✓ There are changes in intestinal epithelial permeability because of modifications in transporters and metabolic enzyme expression in carcinoma cells ✓ There are no biomarkers of zinc uptake.
In vivo Animal models	✓ It enables the identification of genetic markers for iron bioavailability ✓ It enables the analysis of individual tissues to provide a whole-body assessment of absorption. ✓ There are faster physiological responses than in humans. ✓ The poultry model has a quick response to micronutrient deficiency. ✓ Piglets have similarities to humans with respect to gastrointestinal anatomy and physiology.	✓ It lacks communication with other organs. ✓ No animal model exactly simulate the physiological responses of humans ✓ Food intake, energy expenditure, body proportions, intestinal morphologies and enteric microbiota are different from those of humans ✓ Rats endogenously synthesize ascorbic acid and phytase ✓ The body fat contents of pigs differs from that of humans. ✓ Animals practice coprophagy
Human model	✓ It provides the most applicable results. ✓ It assess the true absorption of nutrients from foods. ✓ Stable isotopes allow discrimination between the dose of the micronutrient provided and endogenous forms of the micronutrient, allowing for a more accurate measurement of bioavailability.	✓ There is a risk of radiation exposure, and studies are costly and complex ✓ Stable isotopes are costly, and the procedures required are labor-intensive ✓ Studies are difficult to perform because of the social and ethical considerations that govern

Methods	Advantages	Limitations
	✓ It is possible to assess alterations in zinc and iron nutritional status, allowing a direct assessment of the effectiveness of biofortification.	invasive medical procedures

10. Biomarkers Used for Micronutrient Status

Biomarkers indicate changes or variations in cellular or biochemical components, structure or functions in biological systems or samples through observable properties of an organism (Bearer, 1998). It could be considered as a key measure to pinpoint the specific exposure from a dietary compound to a health outcome and can be used as early signals to know the health status of the organism. Biomarkers are scanned through laboratory work in clinics or at facilities which have experts to run the tests. These tests must be reliable in order to correctly predict the outcome, which is malnutrition. Jensen et al. (2009) defined adult malnutrition as "decline in lean body mass with potential for functional impairment." They further categorized malnutrition causes as: 1) inflammation caused by either acute or chronic diseases; or 2) non-inflammation-related chronic starvation and anorexia nervosa. Some of the biomarkers for assessing nutritional status are serum proteins–albumin and prealbumin, retinol-binding protein, transferrin, total cholesterol, indicators of inflammation–C-reactive protein and total lymphocyte count (Bharadwaj et al., 2016). They compared laboratory markers and nutritional assessment (nutrition-focused physical examination) for diagnosing malnutrition and found that physical examination was a better tool for diagnosing malnutrition. Even though the laboratory markers are popular for providing quantitative results, the literature points to these markers as unreliable. **Because of this, laboratory markers should be used to complement the findings from a physical examination.**

The WHO (2011) recommends the use of ferritin levels in the blood as the primary measure of population-level iron deficiency and complementing this with the assessment of hemoglobin levels. The biomarker recommended by WHO for vitamin A status (WHO, 2011) in populations is measuring retinol levels in the blood. These markers are usually measured by ELISA and is a very good method, provided there is enough sample and there is not a need to screen a large population. Brindle et al. (2014) have developed a multiplex immunoassay technique which has excellent potential as a cost-effective tool for use in large-scale deficiency assessment efforts. This method can measure five markers relevant to assessing inflammation, vitamin A and iron status. The new method was found to be highly correlated (Pearson correlation of 0.606 to 0.991, $p<0.0001$) to the conventional ELISA (enzyme-linked immunosorbent assay).

Recently, Brindle et al. (2017) have revised and expanded the version of multiplex micronutrient assay to human micronutrient assay (7-plex). It measures up to seven biomarkers with relevance to the assessment of the key micronutrients of iron, iodine, and vitamin A, and inflammation and malaria biomarkers: α-1-acid glycoprotein, C-reactive protein (both for adjusting for the influence of inflammation), ferritin (for iron status), retinol binding protein 4 (for vitamin A status), soluble transferrin receptor (for iron status), thyroglobulin (for iodine status) and histidine-rich protein (for malarial

infection). The new tool was used to simultaneously measure the iodine, iron, vitamin A, malarial antigenemia and inflammation status biomarkers on a population of pregnant Niger women. The correlation between assays for each biomarker measured from this cohort was found to be good, with the exception of thyroglobulin. The sensitivity ranged from 74 percent to 93 percent, and specificity from 81 percent to 98 percent.

Plasma amino acid patterns could be a used as a biomarker for diseases and physiological states. Ajinomoto Co., Inc., Japan have used plasma amino acids to screen for cancer and moderate malnutrition. Ajinomoto reported that until July 2012, 300 hospitals and clinics had adopted it as a blood test option (Combs Jr. et al., 2013). The authors also presented blood-based markers as another method for assessing nutrition status and its association with metabolic risk. In this method, the relationship of nutrients' blood measures (including fatty acids, sterols, amino acids, etc.) with metabolic outcomes is examined. This would provide a simpler, more accurate picture of the nutritional status.

11. Modeling

A simulated model to estimate micronutrient levels in FBFs developed by Fleige et al. (2009) recommended two types of fortified commodities: one targeted to infants and young children and the other targeted for older groups, including pregnant and lactating women. They further recommended that the micronutrients in each group should provide 75 percent of the Recommended Nutrient Intake (RNI) and 25 percent of energy needs for the age group with highest nutrient density requirement.

Hallberg and Hulthen (2000) developed an algorithm to predict the bioavailability of iron from different diets. With reasonable accuracy (r^2=0.987), the algorithm could predict the bioavailability of iron from 24 different complete meals. It could predict reasonably well the iron absorption (r2 =0.958) from whole meals served with labeled heme- and non-heme iron tracers over a period of five days.

12. Conclusions and Future Work

Different strategies have been used effectively to alleviate nutritional deficiencies around the world. Opportunities still exist to further improve the nutritional efficiency of food aid products through changes in formulation and processing. This review is limited by the heterogeneity of studies reviewed: varying concentrations of nutrients and study populations. The majority of studies are designed to look at the effect of food aid consumption on health outcomes, not the effect of food matrices on nutrient bioavailability. The effort to tackle malnutrition needs to be examined beyond the nutrient contents of a food aid product to include the overall food matrix. The review was useful in identifying some common issues which have impacted the nutrient uptake by the body. Addressing those issues in a concerted manner would help improve the nutrient bioavailability from food aid products and make it more "cost-effective" in terms of overall benefits.

Based on the above information gathered on different aspects of food matrices and nutrient bioavailability, some of the next steps to improve the bioavailability of nutrients from food aid products are listed below:

1. The efficacy and effectiveness of improving micronutrient bioavailability from food aid products either through fortification and/or dietary diversity should be implemented in realistic settings. Studies should be undertaken to evaluate the potential of the foods which are provided as well as the condition of the populations to assess real nutritional efficiency of the products. This would lead to more sustainable food aid products which could continue to benefit the consumers even after the controlled feeding programs are over.
2. There is a need for more validation studies on nutrient absorption from different food aid products in which the in vivo results are compared to in vitro results. This would allow for designing better food product formulations to meet the needs of the target population at lower cost in the long run.
3. Sensory studies of current and new products should be a part of the research design and health outcomes should be correlated to this aspect as well.
4. Developing an algorithm to predict nutrient bioavailability of at least important micronutrients from different diets should be considered to provide a quick and reliable data on the food formulation which can be modified (if needed) to meet the intended goal of nutrition.
5. Identify minimum quantities of different animal source foods which can support growth and development of children with moderate malnutrition.
6. Plan cost-effective ways to reduce antinutrients and fibers from plant-based foods like developing products which have fermentation or germination steps involved as a part of the overall food manufacturing process.
7. Explore alternate animal source foods like small fish, insects, rodents and offal to be incorporated into foods.
8. Promote the use of local foods and recipes which have been processed in simple ways to attain the maximum health benefits.
9. Remove antinutritional factors (especially phytates) which should be prioritized since it has been a major impediment in improving nutrient bioavailability of food aid products and making them cost-effective.
10. Include newer ingredients like essential fatty acids, primarily ω-3, prebiotics, flours, etc. with better quality and aiding in physiological functions of the body should be incorporated in the formulation.
11. Use food-grade nanoparticles to improve micronutrient delivery systems through encapsulation and controlled/targeted release of nutrients.
12. Plan on addressing the aflatoxin contamination in foods.

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