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Reducing the sample size of household nutrient monitoring based on the Fortification Assessment Coverage Tool (FACT) Surveys in four African countries

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Bachelor of Medicine
Fudan University
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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Reducing the sample size of household nutrient monitoring based on the Fortification Assessment Coverage Tool (FACT) Surveys in four African countries

By Yan Meng

Objective: Fortification quality is determined by analyzing the nutrient levels in household food samples and comparing them to international and national standards. This study has two main objectives: 1) Compare the fortification quality calculated from composite laboratory samples versus individual household samples to evaluate the feasibility of using composite samples as a replacement for individual samples, and 2) Identify the minimum number of samples required to provide an equivalent fortification quality estimation using a simulation study.

Method: Data analyses were based on 6665 household food (salt, maize flour, wheat flour and oil) samples collected from Nigeria, South Africa, Tanzania and Uganda using the Fortification Assessment Coverage Tool (FACT) survey instrument. For Objective 1, we utilized Fisher's Exact Test to compare the fortification quality of individual household food samples versus composite laboratory samples. For Objective 2, we adopted the point of stability (POS) framework via bootstrap resampling. The critical POS determined by a pre-specified confidence level and a tolerable estimation error (i.e., width) represents the proportion of the reduced sample size over the entire sample size.

Results: The fortification quality estimated from composite laboratory samples were substantially different from that obtained from individual household samples. For example, in Nigeria, 100% of the composite laboratory salt samples were found to be overfortified; while 42.73% of the individual household salt samples were overfortified. In analyses of reduced sample sizes, we found various reduced sample sizes with different values of critical POS (confidence levels: 80%, 90% and 95%; widths: ±1.25% and ± 2.5%). Importantly, 45%, 70%, 50%, and 50% of household salt samples from Nigeria, South Africa, Tanzania, and Uganda respectively were needed to estimate the iodine fortification quality in salt.

Conclusion: This study found the fortification levels among composite laboratory samples were not comparable to the levels among individual household samples, suggesting the testing of individual household samples remains essential. A specific reduced sample size that is comparable with the entire individual household data set to estimate the fortification quality under the corresponding confidence levels and widths can be calculated by multiplying the POS_{crit} by the entire sample size.

Keyword: food fortification, sample size reduction, point of stability, bootstrap

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Introduction

The burden of micronutrient malnutrition (MNM) across Africa remains persistent, especially among women of reproductive age, pregnant mothers, and children under five years of age (Barrett & Bevis, 2015). Micronutrients are vitamins and minerals that the body only requires in small amounts, yet are indispensable to development, disease prevention, and wellbeing (Sijbesma & Sheeran, 2011). The human body is incapable of synthesizing most of the micronutrients and must absorb them from the diet (Sijbesma & Sheeran, 2011). MNM exists in people lacking dietary diversity and causes plenty of adverse outcomes on human health (WHO & FAO, 2006). To reduce MNM prevalence, food fortification is one of the top four strategies identified by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (WHO & FAO, 2006). Food fortification is the addition of micronutrients to foods while they are processed (Dwyer et al., 2015). An effective food fortification program provides significant economic benefits measured, for example, cost per disability adjusted life-year (DALY) saved (Horton, 2006). Food fortification proves to be an effective strategy to reduce MNM in practice. For instance, a study including 29612 individuals in the Czech Republic confirmed that salt iodization was a determining factor in the success of the elimination of Iodine Deficiency (Zamrazil et al., 2004). Another study including 30329 participants in the United States also suggested that mandatory folic acid fortification is responsible for near-eliminating the prevalence of folate-deficiency anemia (Odewole et al., 2013). As such, fortification programs are considered as a high priority as a preventive healthcare intervention because of the significant health and economic benefits (Horton, 2006).

It is important to monitor every aspect of implementation to ensure the success of a food fortification program even in the presence of national legislation that mandates its implementation (van den Wijngaart et al., 2013). A monitoring and evaluation system for food fortification programs consists of two parts, regulatory monitoring by government and household/individual monitoring and evaluation (WHO & FAO, 2006). For the latter, we focused on coverage and specifically the proportion of households with quality-fortified foods (i.e., foods fortified according to the international and national standards).

Government agencies and other organizations can devote considerable resources to monitoring fortification programs. The household samples need to be collected by well-trained filed work teams in order to meet the data requirement of household monitoring (WHO & FAO, 2006). Then the household samples should be transported to an accredited laboratory securely where qualitative and/or quantitative testing can take place to determine the if the household food samples meet the international and national standards or not (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018). Normally, the annual household monitoring is supported by external donors (i.e., governments) (WHO & FAO, 2006). Plenty of laboratory tests are a barrier to household monitoring, particularly in African countries with limited government budgets. According to *the World Factbook* for the year 2016 and 2017, among all 228 countries, the government budgets of Nigeria, South Africa, Tanzania, and Uganda ranked as the 79th, the 32nd, the 97th, and the 115th, respectively (CIA, 2017).

The goal of the current study was to investigate the feasibility of using a reduced number of household samples to produce a reasonable estimate of fortification quality. This quality metric is represented by the proportion of food samples classified into different fortification categories based on national or international standards. Before expanding the goal, there are two definitions to clarify. The first one is the individual sample, which is the nutrient level of a single food item from each surveyed household. The second one is composite samples which are formed by physically mixing individual samples following a specific pattern (Patil, 2006). In the current research, the composites are created by the laboratory staff, which are termed as laboratory-composite samples.

There are two main objectives: 1) Compare the fortification quality calculated from composite laboratory samples versus individual household samples to evaluate the feasibility of using composite samples to replace individual samples, and 2) Identify the minimum number of household samples required to provide an equivalent fortification quality estimation using simulation studies. The ultimate goal is to provide recommendations for reduced sample sizes required to efficiently monitor the food fortification program quality.

Methodology

All food specimens were collected from cross-sectional, two-stage cluster household FACT surveys in four African countries. The current study was performed as the secondary data analyses on the basis of the data from the four FACT surveys.

Study data and food specimen collection

The study data consisted of nutrient values for fortified food specimens collected from household surveys using the Fortification Assessment Coverage Tool (FACT) survey instrument in four African countries (Nigeria (FFI et al., 2018), South Africa (CDC et al., 2017a), Tanzania (CDC et al., 2016), and Uganda (CDC et al., 2017b)). The FACT data of Nigeria were representative for two states: Kano and Lagos. The FACT data of South Africa were representative for two provinces: Gauteng and Eastern Cape. The FACT data of Tanzania and Uganda were nationally representative.

In Nigeria, fieldwork took place over a 20-day period beginning May 25, 2015; the investigators collected 1376 salt samples, 35 maize flour samples, 125 wheat flour samples, and 503 oil samples. In South Africa, fieldwork was completed between May 20, 2015, and June 26, 2015; the investigators collected 545 salt samples, 522 maize flour samples, and 43 wheat flour samples. In Tanzania, fieldwork took place from September 23, 2015, to October 22, 2015; the investigators collected 817 salt samples, 275 maize flour samples, 174 wheat flour samples, and 686 oil samples. In Uganda, fieldwork took place from June 8 to June 18, 2015; the investigators collected 818 salt samples, 238 maize flour samples, 47 wheat flour samples, and 277 oil samples.

Table 1. The number of food samples, the fieldwork period, and the number of enumeration area, food brand, and food producer sorted by country

Country	Food type	Number of food samples (n)	Fieldwork start date	Fieldwork end date	Enumeration area (n)	Food brand (n)	Food producer (n)
Nigeria (FFI et al., 2018)	salt	1376	25-May-15	13-Jun-15	60	18	12
	maize flour	35			13	3	3
	wheat flour	125			41	9	6
	oil	503			60	25	11
South Africa (CDC et al., 2017a)	salt	545	20-May-15	26-Jun-15	64	27	6
	maize flour	522			62	38	10
	wheat flour	43			21	9	6
Tanzania (CDC et al., 2016)	salt	817	23-Sep-15	22-Oct-15	70	6	6
	maize flour	275			49	6	5
	wheat flour	174			55	9	5
	oil	686			70	11	5
Uganda (CDC et al., 2017b)	salt	818	8-Jun-15	18-Jun-15	69	7	6
	maize flour	238			55	6	5
	wheat flour	47			31	6	6
	oil	277			66	16	8

The household samples salt, oil and flour of four countries were sent to BioAnalyt for the measurement of iodine, vitamin A and iron levels. The iCheck technology was used in the tests. The flour samples were also sent to an external laboratory (SGS INSTITUT FRESENIUS GmbH) for the measurement of the iron content because a reliable measurement of this iron type cannot be obtained by iCheck technology. The external laboratory did the tests on the flour samples according to DIN EN 15510 mod. ICP/OES method (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018).

After the samples were transported to the labs, the laboratory staff formed 15 composite laboratory samples using individual salt samples from Nigeria, 5 composite laboratory samples using individual oil samples from South Africa, 10 composite laboratory samples using individual salt samples from South Africa, and 10 composite laboratory samples using individual maize flour samples from South Africa.

Ethical consideration

Institutional Review Boards (IRBs) in each country approved the conduct of this study (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018). The Emory IRB determined this project was IRB exempt because it does not meet the definition of research with "human subjects", and it was considered public health practice by CDC.

Data analyses

Data analyses were conducted using R version 3.5.2 (2018-12-20). Descriptive statistics are presented as mean, median, first quartile, third quartile, minimum, maximum, standard deviation.

To achieve objective 1, we assessed household coverage of four fortification categories based on both national and international regulations and standard using the composite laboratory samples and we compared the results to those of the individual household samples by Fisher's Exact Test. The null hypotheses of the Fisher's Exact Tests were that fortification quality calculated from composite laboratory samples and individual household samples are the same. The p-value less than 0.05 suggests that we reject the null hypotheses and conclude that the proportions calculated from individual household samples are different from those calculated from the composite laboratory samples.

To achieve objective 2, the *point of stability* (POS) framework was utilized. The framework was first proposed by Schönbrodt & Perugini in 2013 (Schönbrodt & Perugini, 2013). The POS framework was selected primarily because this framework provides an intuitive visualization that shows the process of sample size determination with pre-specified confidence levels.

Basic definitions of the POS framework:

In the process of identifying a reduced sample size for estimating the quantity of interest (i.e., the proportion of each fortification category: (a) unfortified, (b) inadequately fortified, (c) adequately fortified, and (d) overfortified, we need to specify an acceptable deviation from the true quantity that is usually estimated by using the maximum available sample size. The corridor of stability (COS) represents the area around the actual value, where all deviations were accepted as tolerable (Schönbrodt & Perugini, 2013). For example, if the quantity of interest is a percentage (the proportion of each fortification category) in this study, a COS of 5% means that any deviation within ± 2.5% (i.e., w = 2.5%) of the actual value is considered acceptable, where w denotes the half-width of the COS. With a pre-specified COS, POS is identified as the sample size where the value on the trajectory starts to stay within the COS (Schönbrodt & Perugini, 2013). In other words, POS indicates the largest sample size until it enters COS for the very first time and never leaves the COS again (Schönbrodt & Perugini, 2013). In the current study, we identified the POS for each fortification category (describe below) for each food type by country.

Visualization process and the critical POS (POS_{crit}) determination

Figure 1 is a visualization example created by the adequately fortified household salt samples of Tanzania. The thick blue line in **Fig. 1** is the so-called "actual trajectory" of the actual proportion of adequately fortified household salt samples in Tanzania, which displays the trend of the actual proportion as the sample size increases using the actual full data set. We retrieved 19 data sets containing between 10% and 100% of the Tanzania household salt samples (with increments of 5%). 19 proportion of being adequately fortified Tanzania household salt samples were calculated using these 19 data sets obtained from the actual data set. One proportion of adequately fortified household samples and the corresponding percentage of the entire sample size confirmed one point in the coordinate plane. Subsequently, 19 points were found via 19 subsets of the actual household salt samples of Tanzania. The actual trajectory of adequately fortified household samples are constructed by these 19 points. Specifically, in **Fig. 1**, the y-axis value of the blue line corresponding to the x-axis at 20% represents the proportion of adequately fortified household salt samples calculated from the first 20% of the entire Tanzania data set. Thus, the right-most point on the thick blue line represents the actual proportion of adequately fortified household salt samples in Tanzania.

The thin grey lines were constructed based on the same process as the actual trajectory.

However, the thin grey lines used the bootstrap samples instead of the actual household samples. The bootstrap method is a resampling technique to estimate statistics by sampling a data set repeatedly with replacement (Efron, 1992). It is commonly used in sample size determination, especially when the distribution of the variable of interest is unknown (Qumsiyeh, 2013). In the bootstrapping process, there are two vital parameters: the bootstrap sample size and the number of bootstrap replications. The maximum bootstrap sample size is the sample size of the original data set. A study showed that 1000 bootstrap replications were big enough to estimate accurate results (Efron & Tibshirani, 1994). As a result, we generated 1000 bootstrap replications with the bootstrap sample size equaling to the sample size of the original data set. 1000 bootstrapped trajectories were constructed based on 1000 bootstrap replications following the same procedures mentioned in the actual trajectory section. For example, there were 817 salt samples collected from Tanzania. Firstly, we generated 1000 bootstrap replications from the Tanzania household salt sample data set, each bootstrap replication contained 817 observations. Then 19 data sets were derived from one bootstrap sample. These data sets consisted of 82 (10%), 123 (15%), 164 (20%), 205 (25%), 246 (30%), 286 (35%), 327 (40%), 368 (45%), 409 (50%), 450 (55%), 491 (60%), 532 (65%), 572 (70%), 613 (75%), 654 (80%), 695 (85%), 736 (90%), 777 (95%), and 817 (100%) observations respectively. The proportions of the specific fortification category (unfortified, inadequately fortified, adequately fortified or overfortified) were calculated from these 19 data sets. As such, a total of 19 point estimates were obtained and used to construct one bootstrapped alternative trajectory from one bootstrap sample. This procedure was repeated 1000 times. 1000 bootstrapped alternative trajectories were obtained from 1000 bootstrap replications (thin grey lines in **Fig. 1**).

Each trajectory has its inherent POS since the POS is regarded as the largest sample size to start to stay within the COS with the pre-specified width (COS_w). In **Fig. 1**, the POS with the width of ± 2.5% of the actual trajectory are pointed out as an example of identifying the POS with a pre-specified width. The value of the x-axis where each grey line (bootstrapped trajectory) start to stay within the area circled by the dashed red lines is the POS with the width of ± 2.5%. Following the procedure, 1000 POS of 1000 bootstrapped trajectories are identified. Afterward, an empirical distribution of POS is obtained based on 1000 POS values. The percentiles of the empirical distribution allow us to identify the critical POS (POS_{crit}). For example, the 90th percentile of the POS is identified as POS_{crit} with 90% confidence level denoted as $POS_{crit(90\%)}$. The interpretation of $POS_{crit(90\%)}$ is that 90% of the bootstrap trajectories do not depart from the COS_w. The POS_{crit} represents the proportion of the reduced sample size over the entire sample size with the pre-specified width under the specified confidence level. The reduced sample size can be calculated by multiplying the entire sample size by the POS_{crit}. The proportion estimate calculated by the household food samples with the reduced sample size does not leave the COS_w (i.e., 5%) given a specific confidence level.

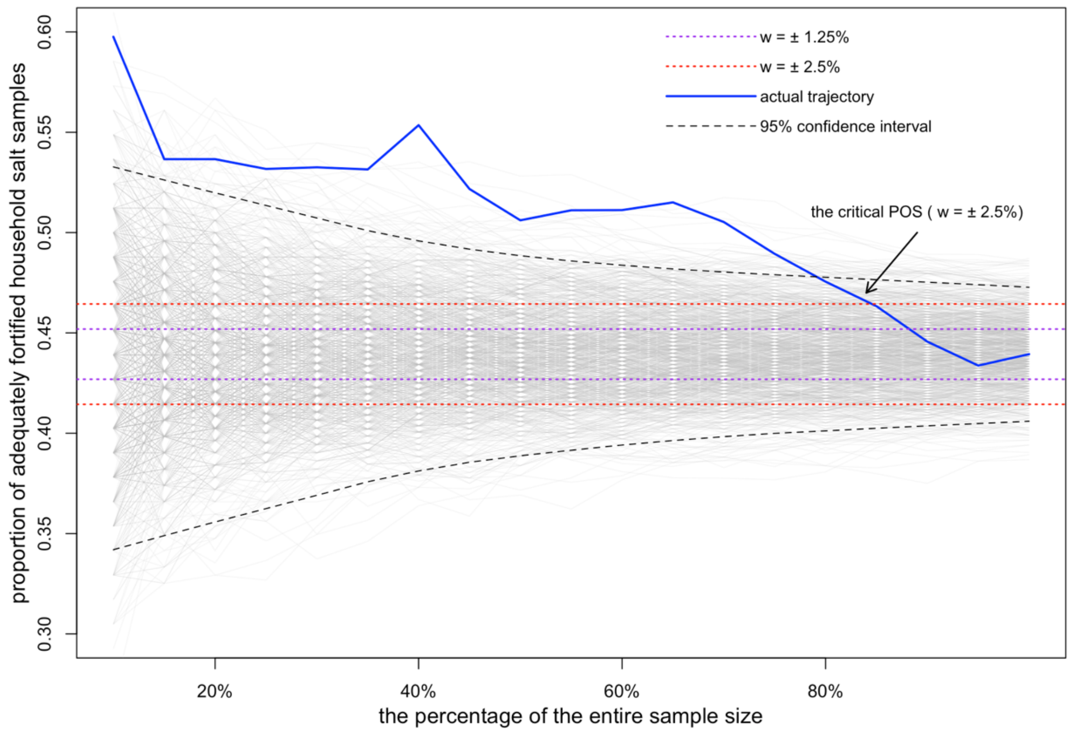

Figure 1. The point of stability graph of the adequately fortified household salt samples in Tanzania

1. The x-axis is the percentage of bootstrap sample size over the entire sample size.
2. The y-axis is the proportion of the specific fortification category (in this case, the proportion of adequately fortified household salt samples).
3. The thick blue line represents the actual trajectory of evaluation of the actual proportion of the adequately fortified household salt samples.
4. The thin grey lines are 1000 bootstrapped alternative trajectories.
5. The dashed black lines are the 95% confidence intervals of the proportion calculated by 1000 bootstrap replications.
6. The dashed purple lines show the COS when the width is ± 1.25%).
7. The dashed red lines show the COS when the width is ± 2.5%.

We conducted the simulation using both standard bootstrap and stratified bootstrap resampling method. The standard bootstrap method resamples the data from the entire data set. On the other hand, the stratified bootstrap method resamples the data within each stratum, which ensures that the bootstrap samples are selected from every stratum. We considered: (a) enumeration area, (b) food brand and (c) food producer as the “strata” and conducted separate stratified bootstrap resampling studies.

Analysis procedures

Objective 1. We compared the fortification quality calculated from composite laboratory samples versus individual household samples to evaluate the feasibility of using composite samples to replace individual samples in the following way:

In step 1, using existing individual household and composite laboratory samples from Nigeria and South Africa, we calculated the fortification quality by the proportions of (a) unfortified, (b) inadequately fortified, (c) adequately fortified, and (d) overfortified samples based on national or international fortification standards (see Appendix Table 1).

In step 2, we used Fisher's Exact Test to evaluate the difference in proportions calculated from Step 1 between the composite laboratory samples and individual household samples.

Objective 2. To identify the minimum number of samples required to provide a reasonable fortification quality estimation following the national and international food fortification standards for Nigeria, Tanzania, South Africa, and Uganda , the following steps were followed:

In step 1, we resampled the individual household samples by standard bootstrap method. We used the samples size of the original data set as the bootstrap sample size to draw 1000 bootstrap replications.

In step 2, we retrieved 19 subsets from every bootstrap replication. Then 19 proportions of the specific fortification category were calculated from each subset. These 19 values and the corresponded percentage of sample size confirming 19 points in the coordinate plane, which constructed one bootstrapped alternative trajectory. The procedure was repeated for each bootstrap replication. A total of 1000 bootstrapped alternative trajectories were plotted.

In step 3, the POS was identified for each bootstrap trajectory. We sought the trajectory from the biggest sample size until it left the COS_w (See the example in **Fig. 1**) for the very first time. This sample size was noted as the POS of this trajectory. We calculated three different percentiles of POS_{crit} (80%, 90%, and 95%). The reduced sample size for the specific fortification category was computed by multiplying the entire sample size by the POS_{crit}.

In step 4, we resampled individual household samples using stratified bootstrap method. 1000 bootstrap replications were generated by three strata: (a) enumeration area, (b) food brand, and (c) food producer. For each stratum, the procedures in step 2-3 were repeated based on the 1000 stratified bootstrap replications.

In step 5, we summarized all the POS_{crit} identified for each fortification category for each food type in the four countries and fitted a linear regression line of POS on the log-transformed proportion of all the four fortification categories to approximate the relationship between the observed fortification quality and the POS_{crit}. The approximate relationship presented an entire perspective on the trend of the POS_{crit} when the proportion of fortification quality changing.

Results

Summary statistics of household salt, maize flour, wheat flour, and oil samples by country

In Nigeria, the mean iodine level in household salt samples is 42.87 mg/kg (SD = 40.13) (**Table 2**). According to UNICEF/WHO criteria, 16.35% of the samples are unfortified; 20.49% are inadequately fortified; 20.42% are adequately fortified and 42.73% are overfortified (**Table 3**). In South Africa, the mean iodine level in household salt samples is 45.12 mg/kg (SD = 37.21) (**Table 2**). According to UNICEF/WHO criteria, 13.42% of the samples are unfortified; 2.94% are inadequately fortified; 24.45% are adequately fortified and 59.19% are overfortified (**Table 3**). In Tanzania, the mean iodine level in household salt samples is 28.82 mg/kg (SD = 15.95) (**Table 2**). According to UNICEF/WHO criteria, 21.79% of the samples are unfortified; 9.67% are inadequately fortified; 43.94% are adequately fortified and 24.60% are overfortified (**Table 3**). In Uganda, the mean iodine level in household salt samples is 36.33 mg/kg (SD = 10.13) (**Table 2**). According to UNICEF/WHO criteria, 0.49% of the samples are unfortified; 2.32% are inadequately fortified; 67.36% are adequately fortified and 29.83% are overfortified (**Table 3**).

Table 2. Summary statistics of the Iodine levels in household salt samples of 4 countries

	Iodine in salt(mg/kg)

Country	Number of food samples (n)	Mean	Median	First quartile	Third quartile	Minimum	Maximum	Standard deviation
Nigeria	1376	42.87	28.38	11.58	75.99	1.00	318.54	40.13
South Africa	545	53.14	44.54	25.93	53.97	1.00	4419.18	191.02
Tanzania	817	28.82	34.37	8.40	39.89	7.27	81.60	15.95
Uganda	818	36.33	36.87	32.25	41.26	7.00	132.50	10.13

Table 3. Iodine fortification quality in household salt samples of 4 countries*

Country	Unfortified	Inadequately fortified	Adequately fortified	Overfortified
Nigeria	16.35%	20.50%	20.42%	42.73%
South Africa	13.42%	2.94%	24.45%	59.19%
Tanzania	21.79%	9.67%	43.94%	24.60%
Uganda	0.49%	2.32%	67.36%	29.83%

* Fortification levels (mg/kg of iodine) for salt are classified according to the UNICEF/WHO criteria: unfortified (< 10 (7.6 in Tanzania and Uganda)), inadequately fortified (10 (7.6 in Tanzania and Uganda) to <15), adequately fortified (15 to <40) and overfortified (≥40) (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018).

Summary statistics of iron levels and the fortification quality in household maize flour samples are calculated by country. In South Africa, the mean iron level in household maize flour samples is 31.08 mg/kg (SD = 15.44) (**Table 4**). According to the national standards, 10.92% of the samples are unfortified; 55.36% are inadequately fortified; 21.46% are adequately fortified and 12.26% are overfortified (**Table 5**). In Tanzania, the mean iron level in household maize flour samples is 0.68 mg/kg (SD = 3.50) (**Table 4**). According to the national standards, 90.91% of the samples are unfortified; 5.82% are inadequately fortified; 2.55% are adequately fortified and 0.73% are overfortified (**Table 5**). In Uganda, the mean iron level in household maize flour samples is 16.48 mg/kg (SD = 5.41) (**Table 4**).

According to the national standards, 70.59% of the samples are unfortified; 25.63% are inadequately fortified; 3.36% are adequately fortified and 0.42% are overfortified (**Table 5**).

Table 4. Summary statistics of the iron levels in household maize flour samples of 3 countries

Country	Number of food samples (n)	Iron in maize flour(mg/kg)						
		Mean	Median	First quartile	Third quartile	Minimum	Maximum	Standard deviation
South Africa	522	31.08	32.65	23.00	40.60	2.20	103.00	15.44
Tanzania	275	0.68	0.00	0.00	0.00	0.00	28.88	3.50
Uganda	238	16.46	14.00	14.00	16.22	14.00	51.36	5.41

Table 5. Iron fortification quality in household maize flour samples of 3 countries*

	Unfortified	Inadequately fortified	Adequately fortified	Overfortified
South Africa	10.92%	55.36%	21.46%	12.26%
Tanzania	90.91%	5.82%	2.55%	0.73%
Uganda	70.59%	25.63%	3.36%	0.42%

* Fortification levels (mg/kg of iron) for maize flour are classified as following national standards: unfortified (≤ 6.5 (South Africa), 0 (Tanzania) and <15 (Uganda)), inadequately fortified (>6.5 to <37.35 (South Africa), >0 to <5 (Tanzania) and 15 to <30 (Uganda)), adequately fortified (37.35 to 45.65 (South Africa), 5 to 25 (Tanzania) and 30 to <45 (Uganda)) and overfortified (>45.65 (South Africa), >25 (Tanzania) and ≥45 (Uganda)) (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018).

Summary statistics of iron levels and the fortification quality in wheat flour samples are calculated by country. In Nigeria, the mean iron level in household wheat flour samples is 39.73 mg/kg (SD = 27.53) (**Table 6**). According to the national standards, 1.60% of the samples are unfortified; 65.60% are inadequately fortified and 32.80% are adequately fortified (**Table 7**). In South Africa, the mean iron level in household wheat flour samples is

38.28 mg/kg (SD = 26.00) (**Table 6**). According to the national standards, 34.88% of the samples are unfortified; 27.91% are inadequately fortified; 18.60% are adequately fortified and 18.60% are overfortified (**Table 7**). In Tanzania, the mean iron level in household wheat flour samples is 22.01 mg/kg (SD = 16.12) (**Table 6**). According to the national standards, 11.49% of the samples are unfortified; 64.37% are inadequately fortified; 18.39% are adequately fortified and 5.75% are overfortified (**Table 7**). In Uganda, the mean iron level in household wheat flour samples is 52.86 mg/kg (SD = 5.41) (**Table 6**). According to the national standards, 23.40% of the samples are unfortified; 14.89% are inadequately fortified; 53.19% are adequately fortified and 8.51% are overfortified (**Table 7**).

Table 6. Summary statistics of the iron levels in household wheat flour samples of 4 countries

Country	Number of food samples (n)	Iron in wheat flour(mg/kg)						
		Mean	Median	First quartile	Third quartile	Minimum	Maximum	Standard deviation
Nigeria	125	39.73	28.70	24.70	50.60	15.10	240.00	27.53
South Africa	43	38.28	38.20	16.00	54.00	8.70	127.00	26.00
Tanzania	174	22.01	21.00	9.15	29.58	0.00	69.48	16.12
Uganda	47	52.86	53.39	37.00	65.88	14.00	96.33	20.92

Table 7. Iron fortification quality in household wheat flour samples of 4 countries*

Country	Unfortified	Inadequately fortified	Adequately fortified	Overfortified
Nigeria	1.60%	65.60%	32.80%	NA
South Africa	34.88%	27.91%	18.60%	18.60%
Tanzania	11.49%	64.37%	18.39%	5.75%
Uganda	23.40%	14.89%	53.19%	8.51%

* Fortification levels (mg/kg of iron) for wheat flour are classified according to national standards: unfortified (≤17 (Nigeria), ≤18 (South Africa), 0 (Tanzania) and <35 (Uganda)), inadequately fortified (>17 to <40.7 (Nigeria), >18 to <45.81 (South Africa), >0 to <30 (Tanzania) and 35 to <50 (Uganda)), adequately fortified

(≥40.7 (Nigeria), 45.81 to 55.59 (South Africa), 30 to 50 (Tanzania) and 50 to <80 (Uganda)) and overfortified (>55.59 (South Africa), >50 (Tanzania) and ≥80 (Uganda)) (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018).

Summary statistics of vitamin A levels and the fortification quality in household oil or maize flour samples are calculated by country. In Nigeria, the mean vitamin A level in household oil or maize flour samples is 9.62 mg/kg (SD = 12.51) or 0.46 mg/kg (SD = 1.06) (**Table 8**). According to the national regulations, 45.73% of the oil samples and 51.43% of the maize flour samples are unfortified; 15.11% of the oil samples and 48.57% of the maize flour samples are inadequately fortified. 39.17% of the oil samples are adequately fortified (**Table 9**). In Tanzania, the mean vitamin A level in household oil samples is 8.62 mg/kg (SD = 7.43) (**Table 8**). According to the national regulations, 37.76% of the samples are unfortified; 43.59% are inadequately fortified; 17.06% are adequately fortified and 1.60% are overfortified (**Table 9**). In Uganda, the mean vitamin A level in household oil samples is 19.72 mg/kg (SD = 10.86) (**Table 8**). According to the national regulations, 14.44% of the samples are unfortified; 27.80% are inadequately fortified and 57.76% are adequately fortified (**Table 9**).

Table 8. Summary statistics of the Vitamin A levels in household oil or maize flour samples of 3 countries

		Vitamin A in oil or maize flour (mg/kg)						
Country	Number of food samples (n)	Mean	Median	First quartile	Third quartile	Minimum	Maximum	Standard deviation
Nigeria (oil)	503	9.62	3.57	0.00	30.00	0.00	30.00	12.51
Tanzania	686	8.62	4.66	2.70	12.53	2.70	33.00	7.43
Uganda	277	19.72	22.27	11.86	27.14	1.00	35.00	10.86

Nigeria (maize flour)	35	0.46	0.00	0.00	0.39	0.00	4.49	1.06

Table 9. Vitamin A fortification quality in household oil or maize flour samples of 3 countries*

Country	Unfortified	Inadequately fortified	Adequately fortified	Overfortified
Nigeria (oil)	45.73%	15.11%	39.17%	NA
Tanzania	37.76%	43.59%	17.06%	1.60%
Uganda	14.44%	27.80%	57.76%	0.0%
Nigeria (maize flour)	51.43%	48.57%	0.00%	NA

* Fortification levels (mg/kg of vitamin A) for oil and maize flour are classified according to national standards: unfortified (≤3 (Nigeria oil), ≤1.11 (Nigeria maize flour), <3 (Tanzania) and <3 (Uganda)), inadequately fortified (>3 to <6 (Nigeria oil), >1.11 to <9 (Nigeria maize flour), ≥3 to <16 (Tanzania) and ≥3 to <20 (Uganda)), adequately fortified (≥6 (Nigeria oil), ≥ 9 (Nigeria maize flour), ≥16 and <28 (Tanzania) and ≥20 and <40 (Uganda)) and overfortified (>28 (Tanzania) and ≥40 (Uganda)) (CDC et al., 2016; CDC et al., 2017a; CDC et al., 2017b; FFI et al., 2018). NA represents that the specific category is not defined by the national regulations.

Comparison of the fortification quality calculated from composite laboratory samples versus individual household samples

Fig. 2 (a) shows that based on individual household samples, 16.35% of the salt samples are unfortified; 20.49% are inadequately fortified; 20.42% are adequately fortified and 42.73% are overfortified in Nigeria; while 100% of the composite laboratory salt samples are overfortified.

Fig. 2 (b) shows that based on individual household samples, 13.42% of the salt samples are unfortified; 2.94% are inadequately fortified; 24.45% are adequately fortified and 59.19% are overfortified in South Africa; while 50% of the composite laboratory salt samples are adequately fortified, and the remaining 50% of the composite laboratory salt samples are overfortified.

Fig. 2 (c) shows that based on individual household samples, 45.73% of the oil samples are unfortified; 15.11% are inadequately fortified and 39.17% are adequately fortified and 12.26% are overfortified in Nigeria. While 20% of the composite laboratory oil samples are unfortified; 60% of the composite laboratory oil samples are inadequately fortified, and 20% of the composite laboratory oil samples are adequately fortified.

Fig. 2 (d) shows that based on individual household samples, 10.92% of the household maize flour samples are unfortified; 55.36% are inadequately fortified; 21.46% are adequately fortified and 12.26% are overfortified in South Africa. While 90% of composite laboratory maize flour samples are inadequately fortified, and 10% composite laboratory maize flour samples are adequately fortified.

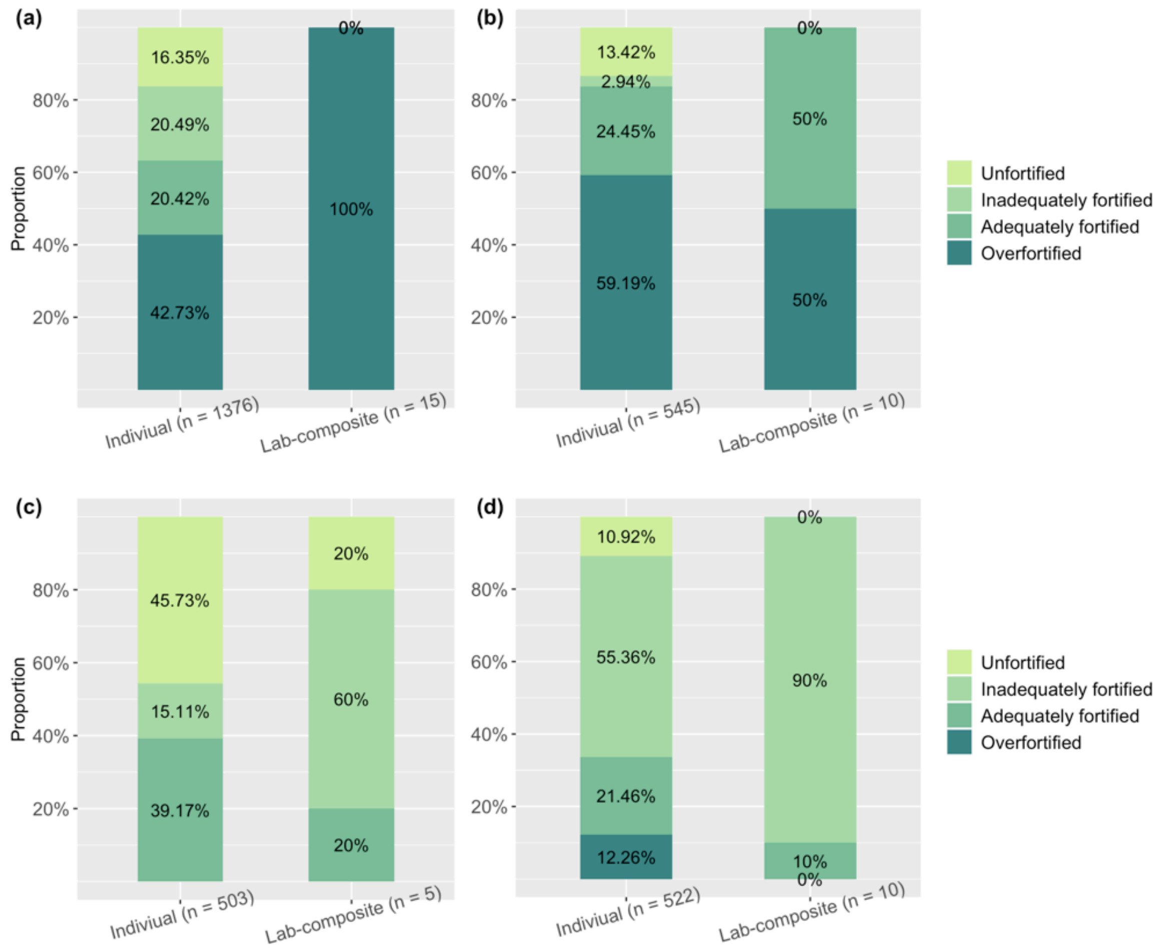

Figure 2. The fortification quality of composite laboratory salt samples versus (a) individual household salt samples of Nigeria (b) individual household salt samples of South Africa, (c) individual household oil samples of Nigeria and (d) individual household maize flour samples of South Africa

1. Fortification levels (mg/kg of iodine) for salt are classified according to the UNICEF/WHO criteria: unfortified (< 10), inadequately fortified (10 to <15), adequately fortified (15 to <40) and overfortified (≥40) (CDC et al., 2017a; FFI et al., 2018).
2. Fortification levels (mg/kg of iron) for maize flour are classified according to national standards: unfortified (≤ 6.5), inadequately fortified (>6.5 to <37.3), adequately fortified (37.35 to 45.65) and overfortified (>45.65) (CDC et al., 2017a).
3. Fortification levels (mg/kg of Vitamin A) for oil are classified according to national standards: unfortified (≤3), inadequately fortified (>3 to <6), adequately fortified (≥6) (FFI et al., 2018).

The p-values of all the four Fisher's Exact Test are less than 0.001, which suggests that the proportions calculated from individual household samples are different from those calculated from the composite laboratory samples.

Minimum required number of samples based on the theory of the POS_{crit}

Since household samples of four different food types (salt, maize flour, wheat flour, and oil) were collected from four African countries (Nigeria, South Africa, Tanzania, and Uganda)), plenty of repeated results are computed. The POS_{crit} values of household salt samples with different width and confidence level are displayed in **Tables 10 - 13** by country. The results of the rest of the foods can be found in Appendix Tables 2 - 8.

The lowest reduced sample size of household salt samples in Nigeria could be found under 80% confidence level with the width of ± 2.5% using stratified bootstrap method by food producer. The reduced sample size to obtain the estimate of the proportion of unfortified household salt samples within the range of the actual proportion ± 2.5% in Nigeria is 620 (POS_{crit} = 0.45) (**Table 10**). The reduced sample size to obtain one of inadequately fortified household salt samples is 688 (POS_{crit} = 0.50) (**Table 10**). The reduced sample size to obtain one of adequately fortified household salt samples is 826 (POS_{crit} = 0.60) (**Table 10**). The reduced sample size to obtain one of overfortified household salt samples is 826 (POS_{crit} = 0.60) (**Table 10**).

Table 10. The critical points of stability of household salt samples from Nigeria for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 1376) *

Stratum	Observed fortification quality	Critical point of stability (POScrit)					
		w = ± 1.25%			w = ± 2.5%		
		Confidence level			Confidence level		
		80%	90%	95%	80%	90%	95%

None	Unfortified	16.35%	1.00	1.00	1.00	0.45	0.60	0.80
	Inadequately fortified	20.49%	1.00	1.00	1.00	0.45	0.70	0.90
	Adequately fortified	20.42%	1.00	1.00	1.00	0.50	0.70	0.80
	Overfortified	42.73%	1.00	1.00	1.00	0.70	0.95	1.00
	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	16.35%	0.95	1.00	1.00	0.85	0.90	0.90
	Inadequately fortified	20.49%	0.95	1.00	1.00	0.90	0.90	0.90
	Adequately fortified	20.42%	0.95	1.00	1.00	0.85	0.90	0.90
	Overfortified	42.73%	0.95	1.00	1.00	0.95	0.95	0.95
	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	16.35%	0.95	1.00	1.00	0.80	0.85	0.90
	Inadequately fortified	20.49%	0.95	1.00	1.00	0.85	0.90	0.90
	Adequately fortified	20.42%	1.00	1.00	1.00	0.80	0.85	0.90
	Overfortified	42.73%	0.95	1.00	1.00	0.90	0.95	0.95
	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	16.35%	1.00	1.00	1.00	0.45	0.65	0.80
	Inadequately fortified	20.49%	1.00	1.00	1.00	0.50	0.65	0.85

	Adequately fortified	20.42%	1.00	1.00	1.00	0.50	0.70	0.88
	Overfortified	42.73%	1.00	1.00	1.00	0.60	0.85	1.00

*The POS_{crit} are listed by stratum as shown in the first column. The second column lists the observed proportion of each fortification category calculated from the individual household samples. The table shows the critical POS with two widths (±1.25% and ±2.5%) under 3 levels of confidence(80%, 90%, and 95%).

$$\text{Sample size}_{\text{reduced}} = \text{POS}_{\text{crit}} \times \text{Sample size}_{\text{entire}}$$

The reduced sample size can be calculated from this formula. In practice, the researchers can choose the fortification category that they are most interested in to select the POS_{crit}. For example, suppose that the researchers are interested in what percentage of households in Nigeria are have adequately fortified salt. The reduced sample size will be 0.5*1376 = 688 with a width of ± 2.5% under a confidence level of 80% using stratified bootstrap by food producer. It indicates that there is 80% of the possibility that the proportion of the adequately fortified household salt samples calculated from 688 samples is within the range of the actual proportion ± 2.5%. The researchers can choose the width and the level of confidence based on their priority. If the researches put much more emphasis on accuracy, then they should choose the POS with a thinner width and a higher level of confidence. If the researchers put much more emphasis on samples size reduction, then they should choose the POS with a wider width and a lower level of confidence.

The lowest reduced sample size of household salt samples in South Africa could be found under 80% confidence level with the width of ± 2.5% using stratified bootstrap method by food producer. The reduced sample size to obtain the estimate of the proportion of unfortified household salt samples within the range of the actual proportion ± 2.5% in South Africa is 382 (POS_{crit} = 0.70) (**Table 11**). The reduced sample size to obtain one of inadequately fortified household salt samples is 55 (POS_{crit} = 0.10) (**Table 11**). The reduced sample size to obtain one of adequately fortified household salt samples is 518 (POS_{crit} = 0.95) (**Table 11**). The reduced sample size to obtain one of overfortified household salt samples is 518 (POS_{crit} = 0.95) (**Table 11**).

Table 11. The critical points of stability of household salt samples from South Africa for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 545)

Stratum	Observed fortification quality	Critical point of stability (POScrit)

			w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	13.42%	1.00	1.00	1.00	0.75	1.00	1.00
	Inadequate fortified	2.94%	0.80	1.00	1.00	0.25	0.35	0.45
	Adequate fortified	24.45%	1.00	1.00	1.00	0.95	1.00	1.00
	Overfortified	59.19%	1.00	1.00	1.00	1.00	1.00	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	13.42%	1.00	1.00	1.00	0.75	0.85	0.95
	Inadequate fortified	2.94%	0.80	0.95	1.00	0.30	0.45	0.55
	Adequate fortified	24.45%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	59.19%	1.00	1.00	1.00	0.95	1.00	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	13.42%	1.00	1.00	1.00	0.70	0.90	1.00
	Inadequate fortified	2.94%	0.80	0.95	1.00	0.10	0.20	0.20
	Adequate fortified	24.45%	1.00	1.00	1.00	0.95	1.00	1.00
	Overfortified	59.19%	1.00	1.00	1.00	0.95	1.00	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	13.42%	1.00	1.00	1.00	0.85	0.95	1.00
	Inadequate fortified	2.94%	0.75	1.00	1.00	0.25	0.45	0.45
	Adequate fortified	24.45%	1.00	1.00	1.00	0.95	1.00	1.00
	Overfortified	59.19%	1.00	1.00	1.00	1.00	1.00	1.00

The lowest reduced sample size of household salt samples in Tanzania could be found under 80% confidence level with the width of ± 2.5% using standard bootstrap method. The reduced sample size to obtain the estimate of the proportion of unfortified household salt samples within the range of the actual proportion ± 2.5% in Tanzania is 572 (POS_{crit} = 0.70) (**Table 12**). The reduced sample size to obtain one of inadequately fortified household salt samples is 409 (POS_{crit} = 0.50) (**Table 12**). The reduced sample size to obtain one of adequately fortified household salt samples is 736 (POS_{crit} = 0.90) (**Table 12**). The reduced sample size to obtain one of overfortified household salt samples is 613 (POS_{crit} = 0.75) (**Table 12**).

Table 12. The critical points of stability of household salt samples from Tanzania for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 817)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	21.79%	1.00	1.00	1.00	0.70	0.95	1.00
	Inadequately fortified	9.67%	1.00	1.00	1.00	0.50	0.68	0.79
	Adequately fortified	43.94%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	24.60%	1.00	1.00	1.00	0.75	0.95	1.00

	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	21.79%	1.00	1.00	1.00	0.85	0.85	0.85
	Inadequately fortified	9.67%	0.95	1.00	1.00	0.90	0.90	0.90

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
	Adequately fortified	43.94%	1.00	1.00	1.00	0.85	0.95	1.00
	Overfortified	24.60%	1.00	1.00	1.00	0.90	0.95	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	21.79%	1.00	1.00	1.00	0.90	0.95	0.95
	Inadequately fortified	9.67%	0.95	1.00	1.00	0.75	0.80	0.85
	Adequately fortified	43.94%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	24.60%	1.00	1.00	1.00	0.85	0.95	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	21.79%	1.00	1.00	1.00	0.90	0.90	0.95
	Inadequately fortified	9.67%	0.95	1.00	1.00	0.75	0.80	0.85
	Adequately fortified	43.94%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	24.60%	1.00	1.00	1.00	0.85	0.95	1.00

The lowest reduced sample size of household salt samples in Uganda could be found under 80% confidence level with the width of ± 2.5% using stratified bootstrap method by enumeration area. The reduced sample size to obtain the estimate of the proportion of unfortified household salt samples within the range of the actual proportion ± 2.5% in Uganda is 82 ($POS_{crit} = 0.10$) (**Table 13**). The reduced sample size to obtain one of inadequately fortified household salt samples is 409 ($POS_{crit} = 0.50$) (**Table 13**). The reduced sample size to obtain one of adequately fortified household salt samples is 696 (POS_{crit} =

0.85) (**Table 13**). The reduced sample size to obtain one of overfortified household salt samples is 655 (POS_{crit} = 0.80) (**Table 13**).

Table 13. The critical points of stability of household salt samples from Uganda for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 818)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	0.49%	0.25	0.25	0.35	0.16	0.20	0.20
	Inadequately fortified	2.32%	0.40	0.70	0.85	0.20	0.29	0.34
	Adequately fortified	67.36%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	29.83%	1.00	1.00	1.00	0.84	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	0.49%	0.20	0.20	0.25	0.10	0.15	0.15
	Inadequately fortified	2.32%	0.65	0.75	0.85	0.50	0.55	0.55
	Adequately fortified	67.36%	1.00	1.00	1.00	0.85	1.00	1.00
	Overfortified	29.83%	1.00	1.00	1.00	0.80	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	0.49%	NA	NA	NA	NA	NA	NA
	Inadequately fortified	2.32%	0.70	0.70	0.85	0.13	0.15	0.15
	Adequately fortified	67.36%	1.00	1.00	1.00	0.90	1.00	1.00

	Overfortified	29.83%	1.00	1.00	1.00	0.85	1.00	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	0.49%	NA	NA	NA	NA	NA	NA
	Inadequately fortified	2.32%	0.65	0.70	0.75	0.12	0.14	0.14
	Adequately fortified	67.36%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	29.83%	1.00	1.00	1.00	0.90	1.00	1.00

Fig. 3 shows the scatterplot of the observed proportions of all the four countries and the POS_{crit} under three confidence levels with the width of 2.5% by standard bootstrap method. The trendline equation under 80% confidence level is $y = 0.1798\ln(x) + 0.9629$ ($R^2 = 0.7452$). The trendline equation under 90% confidence level is $y = 0.1951\ln(x) + 1.1393$ ($R^2 = 0.8172$). The trendline equation under 95% confidence level is $y = 0.1901\ln(x) + 1.1873$ ($R^2 = 0.8875$).

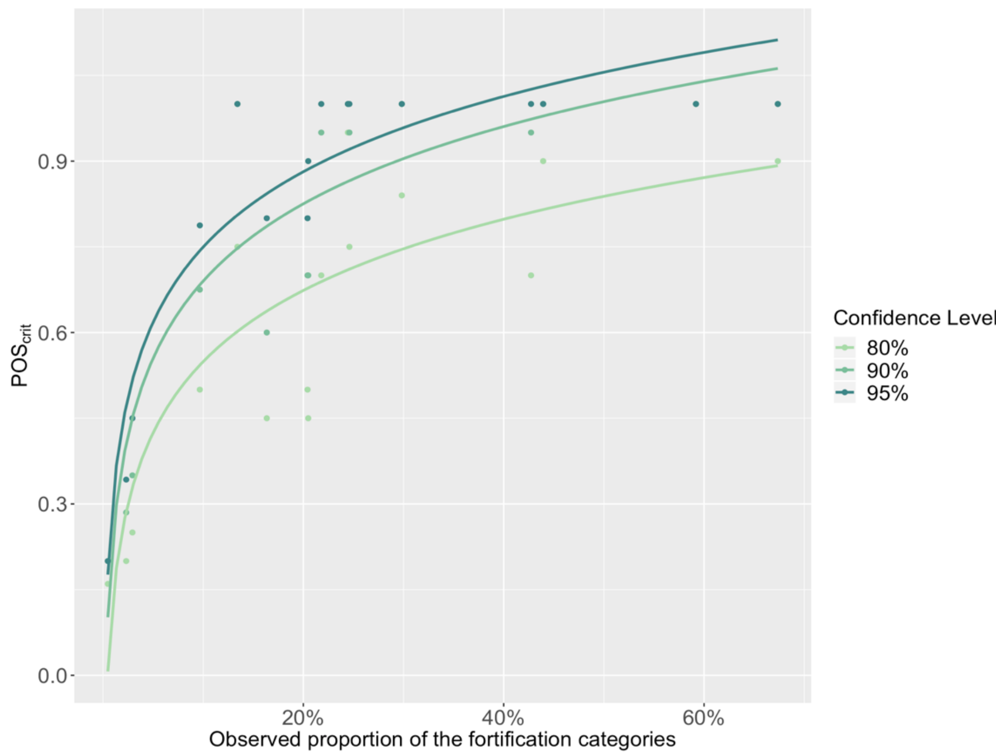

Figure 3. The scatterplot of the observed proportions of four fortification categories calculated by individual household salt samples of all the four countries and the corresponding critical POS under three confidence levels (80%, 90%, 95%) with width of 2.5% using standard bootstrap method and the trendlines of POS_{crit} on the log-transformed observed proportions by confidence level.

Discussion

The objectives of this project were to 1) Compare the fortification quality calculated from composite laboratory samples versus individual household samples to evaluate the feasibility of using composite samples to replace individual samples, and 2) Identify the minimum number of household samples required to provide an equivalent fortification quality estimation using simulation studies. To meet these objectives, we used laboratory-determined nutrient levels from household-sampled foods that should have been fortified in

Nigeria, South Africa, Tanzania, and Uganda. For the first objective, we found the fortification levels among composite laboratory samples were not comparable to the levels among individual household samples, suggesting the testing of individual household samples remains essential. For the second objective, we found a specific reduced sample size that is comparable with the entire individual household dataset to estimate the fortification quality under the corresponding confidence levels and widths can be calculated by multiplying the entire sample size by the POS_{crit}.

We found substantially different distributions of fortification quality of induvial samples versus the composite laboratory samples. The small number of composite laboratory samples does not yield a precise estimation. Only 15 composite laboratory salt samples from Nigeria and 10 composite laboratory salt samples from South Africa were available. However, there were 544 household salt samples from Nigeria and 1376 household salt samples from South Africa.

We show that reduced sample size can be determined by the critical POS. The smaller the POS is, the larger the reduction in the required sample size. The POS should be chosen after balancing the demands on accuracy and the sample size reduction. The width of COS defines an acceptable range of around the actual proportions of the fortification categories. A thinner width yields a more accurate the estimation of fortification quality and therefore a larger POS. The level of confidence shows the possibility that the proportion obtained from the reduced sample size is within the range of the actual proportion obtained from the original sample size. Therefore, when the confidence level increases, more samples are needed to make sure that enough bootstrap trajectories stay within the COS_w (See **Fig. 1**).

Furthermore, we find that the log-transformed observed proportion of the four fortification categories and the POS_{crit} appear to have a linear relationship. All the R-squares of the fitted models with a logarithmic function are greater than 0.7, indicating that a substantial amount of variability was explained by the models. The logarithmic trendline indicates that when the proportions of the fortification categories increase, the corresponding POS_{crit} grows but in a much slower pace once the observed proportion exceeds 20%. This may be due to the selection of the width relative to the true underlying proportion, which requires further investigation.

The strength of the research is that the reduced sample sizes are given with three levels of confidence (80%, 90%, and 95%) and two widths (±1.25% and ± 2.5%), which offers the multiple choices for the reduced sample size to be comparable with the entire household data set to estimate the fortification quality to the future researchers in practice. Additionally, the researchers can choose the smallest POS with standard or stratified sampling strategies. The stratified bootstrap method was considered to be more representative since the resulting bootstrap replications tended to be more balanced in terms of securing household samples from each stratum as opposed to random sampling. Standard bootstrap replicates might not contain samples from the certain stratum, especially when few household samples in the stratum. For instance, if one stratum only has 3 individual samples, it is possible that a majority of bootstrap replications fail to resample from this specific stratum. Thus, the samples from this certain stratum will be overlooked in the next steps.

However, the weakness of the study is that when the observed fortification quality is very close to zero (i.e., 0.49%), the fixed width (±1.25% or ± 2.5%) is no longer applicable. In our current study, it led to an incredibly small POS_{crit} or sometimes failed to form a POS_{crit}. The researches need to avoid using POS_{crit} if the observed percentage is so small that subtracting the pre-specified width causes a negative percentage. Besides, when the original sample size is too small (i.e., n<50), these POS converge to 100% regardless of COS width, levels of confidence, bootstrap strategy, and fortification category, indicating that the sample size reduction is not feasible using the POS framework. In these scenarios, more household samples will be required to obtain a reliable POS_{crit}.

In conclusion, the composite laboratory samples mixed in this specific way are inefficient to represent the fortification quality acquired from individual household samples. Future researchers could choose the reduced sample sizes based on Table 8 to Table 14 and Appendix Table 2 to Table 9. Specific reduced sample size can be computed by the entire sample size multiplying with the POS_{crit} . The width, level of confidence, and bootstrap method should be chosen according to the demands for the research. If the researchers seek a more accurate estimate of the fortification quality, they should use the POS_{crit} with a high confidence level and a thinner width. Future studies may focus on extrapolating the results to other countries.

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Appendices

Appendix Table 2 -9 are listed the POS_{crit} values for household maize flour, wheat flour, and oil samples from all the four countries. Since the original sample size of maize flour samples from Nigeria, wheat flour samples from South Africa, and wheat flour samples from Uganda are less than 50, the POS_{crit} values converge to 1.00 regardless of widths, confidence levels, bootstrap strategies, and fortification categories. The results of these three household samples are not listed in the Appendices.

Appendix Table 1. standards for food fortification by country and by food

		Nigeria	South Africa	Tanzania	Uganda
Iodine in salt (mg/kg)	Unfortified	<10	<10	<7.6	<7.6
	Inadequately fortified	≥10 and <15	≥10 and <15	≥7.6 and <15	≥7.6 and <15
	Adequately fortified	≥15 and <40	≥15 and <40	≥15 and <40	≥15 and <40
	Overfortified	≥40	≥40	≥40	≥40
Iron in wheat flour(mg/kg)	Unfortified	≤17	≤18	0	<35
	Inadequately fortified	>17 and <40.7	>18 and <45.81	>0 and <30	≥35 and <50
	Adequately fortified	≥40.7	≥45.81 and ≤55.99	≥30 and ≤50	≥50 and <80
	Overfortified	NA	>55.99	>50	≥80
Vitamin A in oil(mg/kg)	Unfortified	≤3	NA	<3	<3
	Inadequately fortified	>3 and <6	NA	≥3 and <16	≥3 and <20
	Adequately fortified	≥6	NA	≥16 and ≤28	≥20 and <40
	Overfortified	NA	NA	>28	≥40
Iron in maize flour(mg/kg)	Unfortified	NA	≤6.5	0	<15
	Inadequately fortified	NA	>6.5 and <37.35	>0 and <5	≥15 and <30
	Adequately fortified	NA	≤37.35 and ≤45.65	≥5 and ≤25	≥30 and <45

	Overfortified	NA	>45.65	>25	≥45

Appendix Table 2. The critical points of stability of household wheat flour samples from Nigeria for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 125)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	1.60%	1.00	1.00	1.00	0.75	0.95	0.95
	Inadequately fortified	65.60%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	32.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	1.60%	1.00	1.00	1.00	0.35	0.75	0.75
	Inadequately fortified	65.60%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	32.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	1.60%	0.55	0.80	1.00	0.95	0.95	1.00
	Inadequately fortified	65.60%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	32.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	1.60%	0.80	0.80	1.00	0.35	0.55	0.55
	Inadequately fortified	65.60%	1.00	1.00	1.00	1.00	1.00	1.00

	Adequately fortified	32.80%	1.00	1.00	1.00	1.00	1.00	1.00

Appendix Table 3. The critical points of stability of household oil samples from Nigeria for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 503)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Individual	Unfortified	45.73%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	15.11%	1.00	1.00	1.00	0.85	1.00	1.00
	Adequately fortified	39.17%	1.00	1.00	1.00	1.00	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	45.73%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	15.11%	1.00	1.00	1.00	0.85	0.95	1.00
	Adequately fortified	39.17%	1.00	1.00	1.00	0.95	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	45.73%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	15.11%	1.00	1.00	1.00	0.75	0.95	1.00
	Adequately fortified	39.17%	1.00	1.00	1.00	1.00	1.00	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	45.73%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	15.11%	1.00	1.00	1.00	0.80	1.00	1.00

Adequately fortified	39.17%	1.00	1.00	1.00	1.00	1.00	1.00

Appendix Table 4. The critical points of stability of household maize flour samples from South Africa for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 522)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	10.92%	1.00	1.00	1.00	0.65	0.90	1.00
	Inadequately fortified	55.36%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	21.46%	1.00	1.00	1.00	0.95	1.00	1.00
	Overfortified	12.26%	1.00	1.00	1.00	0.75	0.95	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	10.92%	1.00	1.00	1.00	0.95	0.95	0.95
	Inadequately fortified	55.36%	1.00	1.00	1.00	0.95	1.00	1.00
	Adequately fortified	21.46%	1.00	1.00	1.00	0.85	1.00	1.00
	Overfortified	12.26%	1.00	1.00	1.00	0.80	0.85	1.00
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	10.92%	1.00	1.00	1.00	0.85	0.90	0.90
	Inadequately fortified	55.36%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	21.46%	1.00	1.00	1.00	0.90	1.00	1.00
	Overfortified	12.26%	1.00	1.00	1.00	0.80	0.85	0.95

	Observed fortification quality		w = ± 1.25% Confidence level 80%	90%	95%	w = ± 2.5% Confidence level 80%	90%	95%
Producer	Unfortified	10.92%	1.00	1.00	1.00	0.85	0.90	0.95
	Inadequately fortified	55.36%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	21.46%	1.00	1.00	1.00	0.95	1.00	1.00
	Overfortified	12.26%	1.00	1.00	1.00	0.75	0.95	1.00

Appendix Table 5. The critical points of stability of household maize flour samples from Tanzania for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 275)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	90.91%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	5.82%	1.00	1.00	1.00	0.70	1.00	1.00
	Adequately fortified	2.55%	0.95	1.00	1.00	0.40	0.50	0.70
	Overfortified	0.73%	0.70	0.90	0.94	0.20	0.30	0.30
	Observed fortification quality		w = ± 1.25% Confidence level 80%	90%	95%	w = ± 2.5% Confidence level 80%	90%	95%
Enumeration Area	Unfortified	90.91%	1.00	1.00	1.00	0.90	1.00	1.00
	Inadequately fortified	5.82%	1.00	1.00	1.00	0.70	0.95	1.00
	Adequately fortified	2.55%	0.85	1.00	1.00	0.35	0.40	0.65
	Overfortified	0.73%	1.00	1.00	1.00	0.75	0.75	0.75
	Observed fortification quality		w = ± 1.25% Confidence level 80%	90%	95%	w = ± 2.5% Confidence level 80%	90%	95%
Brand	Unfortified	90.91%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	5.82%	1.00	1.00	1.00	0.70	1.00	1.00

	Adequately fortified	2.55%	0.95	1.00	1.00	0.40	0.55	0.75
	Overfortified	0.73%	0.35	0.50	0.70	0.20	0.30	0.30

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	90.91%	1.00	1.00	1.00	0.90	1.00	1.00
	Inadequately fortified	5.82%	1.00	1.00	1.00	0.80	1.00	1.00
	Adequately fortified	2.55%	0.95	1.00	1.00	0.40	0.55	0.70
	Overfortified	0.73%	0.35	0.50	0.50	0.20	0.30	0.30

Appendix Table 6. The critical points of stability of household wheat flour samples from Tanzania for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 174)

Stratum	Overserved fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	11.49%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	64.37%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	18.39%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	5.75%	1.00	1.00	1.00	0.95	1.00	1.00
	Overserved fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	11.49%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	64.37%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	18.39%	1.00	1.00	1.00	1.00	1.00	1.00

	Overfortified	5.75%	1.00	1.00	1.00	0.75	0.91	1.00

	Overserved fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	11.49%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	64.37%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	18.39%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	5.75%	1.00	1.00	1.00	0.95	1.00	1.00

	Overserved fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	11.49%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	64.37%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	18.39%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	5.75%	1.00	1.00	1.00	0.90	1.00	1.00

Appendix Table 7. The critical points of stability of household oil samples from Tanzania for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 686)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	37.76%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	43.59%	1.00	1.00	1.00	0.95	1.00	1.00
	Adequately fortified	17.06%	1.00	1.00	1.00	0.75	0.95	1.00

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
	Overfortified	1.60%	0.40	0.50	0.65	0.15	0.20	0.27

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	37.76%	1.00	1.00	1.00	0.95	0.95	1.00
	Inadequately fortified	43.59%	1.00	1.00	1.00	0.95	1.00	1.00
	Adequately fortified	17.06%	1.00	1.00	1.00	0.85	0.95	0.95
	Overfortified	1.60%	0.70	0.75	0.80	0.25	0.30	0.60

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	37.76%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	43.59%	1.00	1.00	1.00	0.95	1.00	1.00
	Adequately fortified	17.06%	1.00	1.00	1.00	0.85	0.95	1.00
	Overfortified	1.60%	0.65	0.65	0.70	0.13	0.15	0.18

	Observed fortification quality		w = ± 1.25% Confidence level			w = ± 2.5% Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	37.76%	1.00	1.00	1.00	0.95	1.00	1.00
	Inadequately fortified	43.59%	1.00	1.00	1.00	0.95	1.00	1.00
	Adequately fortified	17.06%	1.00	1.00	1.00	0.70	0.90	1.00
	Overfortified	1.60%	0.65	0.75	0.85	0.45	0.55	0.60

Appendix Table 8. The critical points of stability of household maize flour samples from Uganda for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 238)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	70.59%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	25.63%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	3.36%	1.00	1.00	1.00	0.55	0.75	1.00
	Overfortified	0.42%	0.75	0.75	1.00	0.25	0.25	0.30
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	70.59%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	25.63%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	3.36%	1.00	1.00	1.00	0.75	0.75	0.85
	Overfortified	0.42%	0.50	0.50	0.75	0.25	0.25	0.40
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	70.59%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	25.63%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	3.36%	1.00	1.00	1.00	0.60	0.75	1.00
	Overfortified	0.42%	0.75	0.75	1.00	0.55	0.55	0.70
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	70.59%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	25.63%	1.00	1.00	1.00	1.00	1.00	1.00

	Adequately fortified	3.36%	1.00	1.00	1.00	0.55	0.65	0.95
	Overfortified	0.42%	0.75	0.75	1.00	0.40	0.55	0.55

Appendix Table 9. The critical points of stability of household oil samples from Uganda for the different widths (±1.25%, ± 2.5%) of the corridor of stability under different levels of confidence (80%, 90%, 95%) with standard bootstrap, and stratified bootstrap by enumeration area, food brand, and food producer (n = 277)

Stratum	Observed fortification quality		Critical point of stability (POScrit)					
			w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
None	Unfortified	14.44%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	27.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	57.76%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	0.00%	NA	NA	NA	NA	NA	NA
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Enumeration Area	Unfortified	70.59%	1.00	1.00	1.00	0.90	1.00	1.00
	Inadequately fortified	25.63%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	3.36%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	0.42%	NA	NA	NA	NA	NA	NA
	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Brand	Unfortified	14.44%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	27.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	57.76%	1.00	1.00	1.00	1.00	1.00	1.00

	Overfortified	0.00%	NA	NA	NA	NA	NA	NA

	Observed fortification quality		w = ± 1.25%			w = ± 2.5%		
			Confidence level			Confidence level		
			80%	90%	95%	80%	90%	95%
Producer	Unfortified	14.44%	1.00	1.00	1.00	1.00	1.00	1.00
	Inadequately fortified	27.80%	1.00	1.00	1.00	1.00	1.00	1.00
	Adequately fortified	57.76%	1.00	1.00	1.00	1.00	1.00	1.00
	Overfortified	0.00%	NA	NA	NA	NA	NA	NA