



Sampling Protocol

for Rapid Assessment of *trans*-Fatty Acids in Foods

This document outlines the key procedural steps to design a study and collect samples to assess the levels of *trans*-fatty acids (TFA) in main food sources of industrially produced TFA (iTFA) intake in a specific population. The goal of the study is to raise awareness and support policy action for elimination of iTFA or to monitor compliance with existing regulations. It is not possible or necessary to analyze every available package or serving of a food. Limiting the geographical scope, food sector and food categories for sampling and testing foods will reduce the resources and time required, while also providing estimates of TFA levels in commonly available and consumed foods. A narrow and rapid assessment of foods is a low-cost way to raise awareness and spur policy action.

Use this document to guide study design and sample collection of foods for laboratory analysis of TFA. Reasons for conducting the assessment in foods (vs. in fats and oils) include the following:

- Key food categories have already been identified as probable sources of iTFA intake in the population;
- There is a large food import market in the country (packaged foods are made with fats and oils produced outside of the country); or
- There are political or policy reasons for testing foods, such as documentation that iTFA is present in the market, or to track baseline and follow-up levels before and after regulation.

Background

The World Health Organization (WHO) recommends that all individuals consume TFA less than 1% of total daily energy. To achieve this, countries should implement best practice policies—either a mandatory limit on iTFA to 2% of total fat in all fats, oils and foods, or ban on the use and sale of partially hydrogenated oils (PHO). PHO is the main source of iTFA intake in most countries. Lower levels of iTFA can also be formed during oil refinement processes using high temperatures. Preliminary research can be done in key geographies in a country to assess the quantity of TFA currently present in the food supply in order to support best practice policies.

Research Objective

Collect [food sector] samples from [food outlet types] in [geography] for TFA laboratory analysis.

Study Design

- 1 Geography Selection**
Select 1 geography (e.g. city) within the country for the assessment. Give preference to the most populous geography, geography with political influence, or geography with access to staff and a lab with capacity to carry out the assessment, and where products with iTFA are most likely to be available for purchase. If it is important for political or other reasons, 2 geographies may be selected for the assessment.
- 2 Food Sector Selection**
Select packaged and/or prepared foods. These sectors often comprise the major sources of iTFA consumption in a country. Restaurant foods may also contribute to iTFA consumption in countries. If food sectors other than packaged and prepared foods are thought to be major contributors of iTFA to the diet, then include those sectors. Note on prepared foods: If shipping logistics are complicated by the inclusion of perishable items and the need for dry ice, it may be advisable to not include perishable items in the assessment.

- 3 Food Outlet Types Selection**

Select supermarkets/markets, independent/chain bakeries, and/or informal food markets¹ as the food outlet types for the assessment. These are often popular outlet types for purchasing packaged and prepared foods in countries. If informal food market vendors are not popular food outlets, don't include in the assessment. If other outlet types are more popular in the country for the selected food sectors, select those for the assessment. The number of food outlet types should be based on the resources available. If the total number of samples in the assessment needs to be reduced based on available budget, remove less popular outlet types. For example, this initial assessment may only include prepared foods from bakeries. Table 1 provides examples of food outlet types by food sector.
- 4 Food Categories Selection**

Select 3 food categories for each outlet type included in the assessment. If other outlet types were selected for the assessment, select 3 categories for each. The categories should represent foods that are commonly consumed in the country and that are likely to be high in iTFA. For many countries, food categories that commonly contain TFA have been identified; review both published and grey literature to find existing TFA data. Nongovernmental organizations, academic researchers, industry and the government may also be sources of TFA data. Other sources of information on foods likely to contain TFA include: labels, food industry, other countries, and national nutrition or food composition databases. If more resources are available, additional food categories may be included; however, more complete assessments of food categories should be prioritized over partial assessments of many categories (i.e., prioritize testing more items within fewer food categories vs. testing fewer items across many categories). Table 1 provides examples of food categories by food sector and category that may contain iTFA.
- 5 Neighborhoods Selection**

For the selected geography (e.g., city), identify all neighborhoods, using available data (e.g., census data). Categorize the neighborhoods into 2 socio-economic status (SES) groups (e.g., high/middle, low-income) based on available data (e.g., rent prices, census data). Remove any neighborhoods that are not generally representative (e.g., sparsely populated, remote). Create numbered lists of all included neighborhoods for each of the 2 SES groups. Use a random number generator to select 2 neighborhoods to be visited for each SES group. If informal food markets are included in the assessment, limit the lists to only include neighborhoods where there are informal food markets. Table 2 is a template that can be used.
- 6 Specific Food Outlets Selection**

Within each of the 4 selected neighborhoods, select 1 popular bakery, 1 popular supermarket (or market or grocery store) and 1 popular informal food market where consumers frequently purchase foods within the previously selected food categories (in section 4). If different food sectors or outlet types were selected for the assessment, modify accordingly. Use key informant interviews (e.g., local guide, municipal council members, local health officials) or review media/social media to get information on most popular food outlets. If more than 1 outlet is popular (visited by a large number of consumers), create a list and choose 1 outlet randomly. For informal food markets, 1 market (comprising of several food vendors) is equivalent to 1 outlet.
- 7 Food Items and Brands Selection**

Select 3 popular food items from each selected food category for each outlet. Review sales data or interview owners or employees of the outlet to inform selection of the food items if there are more than 3 items in a category. For informal food markets, conduct a survey of a random sample of vendors in the selected markets to assess purchasing habits and identify most commonly purchased foods. (Annex 1)

This step of selecting food items can be done by the sample collector at the time of sample collection.

Based on steps **1** – **7**, create a sample collection framework/list that data collectors will use to visit outlets and collect food samples (see Table 3 as a template). The number of samples for each item will be determined by the sample collector and will depend on the weight of individual items (a minimum of 150g of each food sample is required for fatty acid analysis). The total number of samples will be updated accordingly.

1 For the purposes of this protocol, an “informal food market” is defined as a public area with a concentration of at least 10 “street food” vendors (carts, stalls) selling prepared, ready-to-eat foods.

Sample Collection, Storage and Shipping

Recruit and train local field researchers to collect, store and transport the food samples to the laboratory.

Sample collection, storage and shipping procedures:

1. For the first SES group, travel to the first neighborhood and go to the first food outlet.
2. Once inside the outlet, look for and purchase each food item on the sample collection list. If selection of items for sampling is being done at the time of sample collection, see instructions in step 7 of the study design.
 - a. Collect a minimum of 150g of each food item. This may require purchasing several packages or servings of an item. Place samples of each item in a Ziploc bag with a unique ID. If multiple samples of the same item are collected, they should go into 1 Ziploc bag with 1 unique ID.
 - b. While at the outlet, record the price of each item on a sticker and place the sticker on each item.
 - c. Place each item purchased from 1 vendor in 1 bag and label the bag with the city, vendor outlet type, SES, neighborhood, date, and total # of items purchased.
 - d. Place all the perishable samples in an icebox with icepacks. Perishable items are anything stored in a refrigerator. Non-perishable items do not need to be transported or stored in cold chain.

Note

If the outlet does not have all the food items in each category, purchase what is available then go to any nearby vendor outlet that meets the SES/vendor/outlet type criteria and purchase the remaining items. If the second outlet does not have the remaining items available, try a third outlet. If the remaining food items still cannot be found at a third outlet, purchase the next similar item and move on to the next outlet on the list.

Note

Informal food markets, although comprised of multiple vendors, will be treated as 1 outlet. After completing the questionnaire and steps outlined in Annex 1, data collectors should purchase the three identified popular food items for each food category documented in Table 3 (Sample collection framework). From the sample of vendors surveyed, data collectors can purchase from any of the vendors that provide the selected popular food items, and as many needed to ensure a sample is collected from for each popular food item for each food category.

3. Still in the first neighborhood of the first SES group, go to the second and third food outlets and follow the same steps.
4. Still in the first SES group, travel to the second neighborhood and go to the first, second and third food outlet and follow the same steps.
5. For the second SES group, follow the same steps as for the first SES group.
6. Once back in the office, label each product with a unique identifier, and log information about each product in an Excel database (Annex 2). The following details for each product should be documented in Excel:

<ol style="list-style-type: none"> a. Unique identifier b. Food category c. Fat/oil item name d. Brand name e. Manufacturer name and location 	<ol style="list-style-type: none"> f. Nutrition information, including SFA, TFA, total fat (<i>if available on nutrition label</i>) g. Ingredient list (<i>if PHO, shortening, hydrogenated oil, or similar, is listed as an ingredient</i>) h. Product weight (grams) 	<ol style="list-style-type: none"> i. Product quantity j. Product price k. Printed expiration date l. Purchase city m. SES group n. Vendor outlet name o. Date purchased
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7. Deliver or ship samples to the laboratory for analysis. Perishable samples should be shipped on dry ice. If shipping time is greater than 3 days, dry ice must be changed every 3 days. This is to avoid deterioration of the samples. Non-perishable samples should be shipped without the cold chain.

Note on Laboratory Selection

Laboratories must be able to perform gas chromatograph with flame ionization detector (GC-FID) with a 100-meter GC column. Full guidance on how to select a laboratory to conduct analysis of the food samples is available on the WHO REPLACE webpage (see Annex 2 of the [REPLACE Module 4](#)).

Note on Shipping Samples

Preference should be given to acceptable domestic laboratories to avoid international shipping challenges. If samples must be shipped internationally to a laboratory, customs requirements will depend on the receiving country and contents of the shipment. For example, some countries do not permit dry ice into their countries; some countries have lengthy customs review and approval processes for animal products or research subjects. The customs logistics process should be verified before the sample collection process begins. Customs agents may be able to aide in verifying the requirements and process. If feasible to transport items with a traveler, this may be recommended.

Note on Perishable Items

If shipping logistics are complicated by the inclusion of perishable items and the need for dry ice, it may be advisable to remove these items from the sample set.

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Table 1. Food Sampling Framework

① **Geography:** _____

<p>② Food Sector <i>check box for sector(s)</i></p>	<input type="checkbox"/> Packaged foods		<input type="checkbox"/> Prepared foods			
<p>③ Food Outlet Type <i>check box for outlet types within selected sectors</i></p>	<input type="checkbox"/> Supermarkets/ Markets	<input type="checkbox"/> Other: _____ _____ _____	<input type="checkbox"/> Bakeries (chain/ independent)	<input type="checkbox"/> Informal food market	<input type="checkbox"/> Restaurants (chain/fast food)	<input type="checkbox"/> Other: _____ _____ _____
<p>④ Food Category <i>check box for 3 categories for each selected outlet type</i></p>	<input type="checkbox"/> Baked goods <input type="checkbox"/> Cereals <input type="checkbox"/> Coffee creamer <input type="checkbox"/> Condiments <input type="checkbox"/> Confectionaries <input type="checkbox"/> Dairy <input type="checkbox"/> Frozen foods <input type="checkbox"/> Instant noodles <input type="checkbox"/> Pastries <input type="checkbox"/> Snacks <input type="checkbox"/> Other (commonly consumed and prepared using fats/oil): _____ _____	<input type="checkbox"/> _____	<input type="checkbox"/> Biscuits (savory) <input type="checkbox"/> Biscuits (sweet) <input type="checkbox"/> Cakes <input type="checkbox"/> Confectionaries <input type="checkbox"/> Doughnuts <input type="checkbox"/> Pastries (savory) <input type="checkbox"/> Pastries (sweet) <input type="checkbox"/> Other (commonly consumed and prepared using fats/oil): _____ _____ _____ _____	<input type="checkbox"/> Baked goods <input type="checkbox"/> Confectionaries <input type="checkbox"/> Doughnuts <input type="checkbox"/> Fried entrées <input type="checkbox"/> Fried snacks <input type="checkbox"/> Pastries <input type="checkbox"/> Other (commonly consumed and prepared using fats/oil): _____ _____ _____ _____	<input type="checkbox"/> Baked goods <input type="checkbox"/> Confectionaries <input type="checkbox"/> Doughnuts <input type="checkbox"/> Fried entrées <input type="checkbox"/> Fried snacks <input type="checkbox"/> Pastries <input type="checkbox"/> Other (commonly consumed and prepared using fats/oil): _____ _____ _____ _____	<input type="checkbox"/> _____

Table 2. Locations Sampling Framework

<p>⑤ Select Neighborhoods using random number</p>	<p>SES group</p>	<p>High Income (HI)</p>	<p>Low Income (LI)</p>
	<p>Random number</p>	<p>_____ , _____</p>	<p>_____ , _____</p>
<p>⑥ Select Specific Outlets using key informant interviews</p>	<p>Neighborhood 1</p>	<p>HI-N1 _____</p>	<p>LI-N1 _____</p>
	<p>Bakery Outlet 1</p>	<p>HI-N1-B1 _____</p>	<p>LI-N1-B1 _____</p>
	<p>Market Outlet 1</p>	<p>HI-N1-M1 _____</p>	<p>LI-N1-M1 _____</p>
	<p>Informal Food Market 1</p>	<p>HI-N1-IM1 _____</p>	<p>LI-N1-IM1 _____</p>
	<p>Neighborhood 2</p>	<p>HI-N2 _____</p>	<p>LI-N2 _____</p>
	<p>Bakery Outlet 1</p>	<p>HI-N2-B1 _____</p>	<p>LI-N2-B1 _____</p>
	<p>Market Outlet 1</p>	<p>HI-N2-M1 _____</p>	<p>LI-N2-M1 _____</p>
	<p>Informal Food Market 1</p>	<p>HI-N2-IM1 _____</p>	<p>LI-N2-IM1 _____</p>

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Table 3. Sample Collection Framework

*more than 1 sample of individual items may be necessary to meet the minimum weight requirement.

Sector Packaged Foods

Outlet Type Market/Supermarket

Outlet	Category	Item/brand	# of samples purchased*	# of samples for lab analysis	Total # of samples
HI-N1-M1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
HI-N2-M1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	

LI-N1-M1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
LI-N2-M1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
SUBTOTAL, MARKET/SUPERMARKET FOOD					36

Sector Prepared Foods

Outlet Type Bakery

Outlet	Category	Item/brand	# of samples purchased*	# of samples for lab analysis	Total # of samples
HI-N1-B1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
HI-N2-B1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	

LI-N1-B1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
LI-N2-B1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
SUBTOTAL, BAKERY FOODS					36

Sector Prepared Foods

Outlet Type Informal Food Market

Outlet	Category	Item/brand	# of samples purchased*	# of samples for lab analysis	Total # of samples
HI-N1-IM1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
HI-N2-IM1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	

LI-N1-IM1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
LI-N2-IM1	1 _____	1	1	1	9
		2	1	1	
		3	1	1	
	2 _____	1	1	1	
		2	1	1	
		3	1	1	
	3 _____	1	1	1	
		2	1	1	
		3	1	1	
SUBTOTAL, INFORMAL MARKET FOOD FOOD					36
TOTAL, MARKET, BAKERY AND INFORMAL MARKET FOODS					108