PROTOCOL FOR SAMPLING AND METHODS OF ANALYSIS FOR

MALAYSIAN FOOD COMPOSITION DATABASE

National Technical Working Group of
Malaysian Food Composition Database

2011

ACKNOWLEDGEMENT

On behalf of the National Technical Committee of Malaysian Food Composition Database (FCD), we would like to congratulate the Institute for Public Health (IKU) and Institute for Medical Research (IMR) for their commendable initiatives to establish the protocol for the sampling and methodology for updating Malaysian FCD. We would also like to thank and congratulate all the participating institutions that have contributed significantly and successfully for the completion of this document.

We are pleased that the National Technical Committee of Malaysian FCD has successfully initiated the first phase of the Malaysian FCD updated with the establishment of the standard protocol or methodology for creating Malaysian FCD. This protocol is an essential guideline for participating institutions to ensure a standard methodology is used for all food analyses.

It is hoped that this document is useful to all the stakeholders especially the participating agencies in ensuring a harmonized application of the methods. This new revision of FCD will benefit those in the field of food and nutrition from various institutions and industries. This strong collaboration should always be maintained for our present and future benefits.

We hope the publication of a harmonized sampling and method of FCD will achieve its objective in providing quality nutrient analysis of food for various uses in food and nutrition activities. Therefore, we would like to extend our greatest thanks and appreciations to:

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- Members of the Technical Working Group (TWG) of Malaysian FCD
- All the individuals who have directly and indirectly contributed toward the completion of this document.

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PART I: SAMPLING OF FOODS

1.1 INTRODUCTION

The main sampling method for new Food Composition Database (FCD) is the stratified sampling whereby units are taken from defined strata (subparts) of the parent population. Within each stratum, the samples are taken randomly. Strata may be regional, seasonal, retail sales point or others as defined by knowledge of the food being studied (Greenfield & Southgate, 2003).

Foods collected are divided into raw, processed and prepared (food as consumed) foods (Figure 1).

1.2 DEFINITIONS

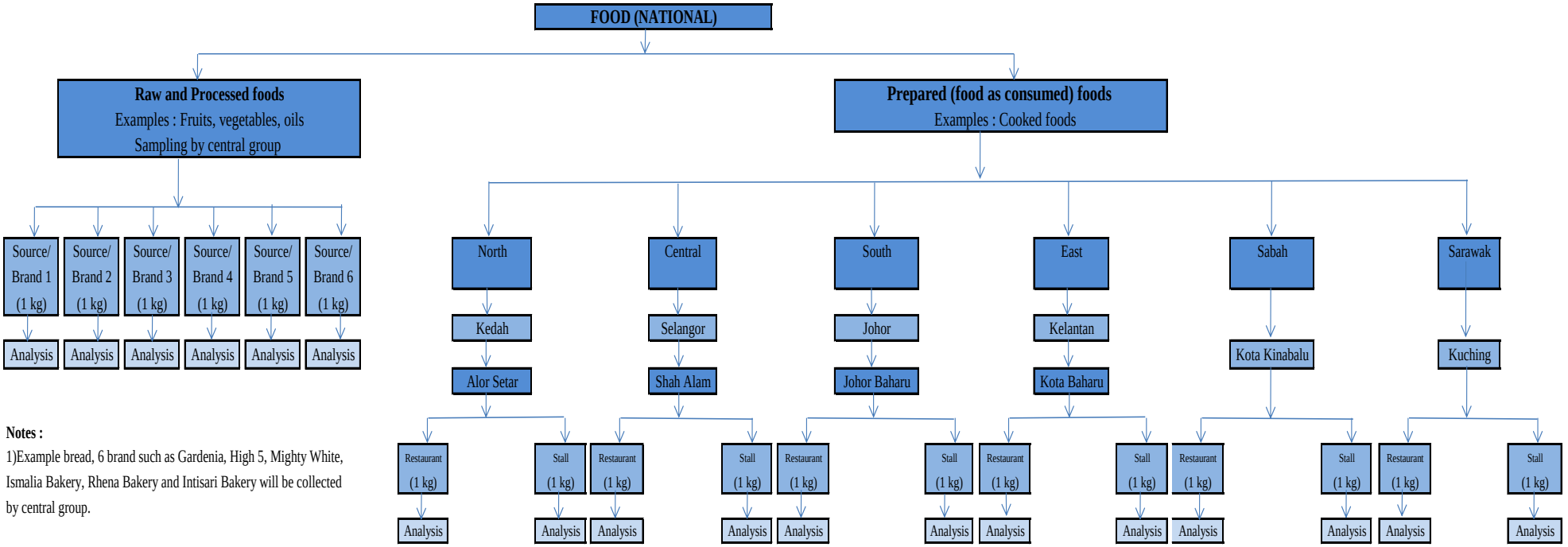
Raw food is a material in its natural unprocessed form; or, has not had the final stages of processing; or, food that has not been cooked (Wordiq, 2011).

Processed food is food that has been altered from their natural state for safety reasons and convenience. The methods used for processing foods include canning, freezing, refrigeration, dehydration and aseptic processing (Nutrition About, 2011).

Prepared food is food sold in a heated state or food that is heated by the seller; food where two or more food ingredients are mixed or combined by the seller for sale as a single item; or food sold with eating utensils provided by the seller such as plates, knives, forks, spoons, glasses, cups, napkins, or straws (Minnesota Revenue, 2011).

Restaurant is any place or premises, either permanently or temporarily sells food for consumption on the premises whether prepared or not prepared in situ (UHLG, 2006).

Stall includes any locked desk, food court, desk, storage, long benches, baskets, chairs, wood, wall display boxes, show boards of any kind or any other related to it, whether mobile or not mobile; and, whether wheeled or not, on which or in which food, beverages or goods of any kind are sold or exposed for sale at any private or public place (UHLG, 2006).



Notes :

1)Example bread, 6 brand such as Gardenia, High 5, Mighty White, Ismalia Bakery, Rhena Bakery and Intisari Bakery will be collected by central group.

2)Example sardine, 6 brand such as Ayam Brand, King Cup, Marina, Yeo's, Tc Boy and Nikko will be collected by central group.

Figure 1. Sampling of foods

1.3 CLASSIFICATION

Foods collected are classified into raw, processed and prepared (food as consumed) foods. Raw and processed foods include cereals and grain products (rice, white/wholemeal/flavoured breads, biscuits, flours, and processed cereal products); starchy roots, tubers and products (chips and flours); legumes and legume products (flours and sauces); nuts, seeds and products; fruits and fruits products; vegetables (starchy roots, tubers, bulbs, leaves, stems and shoots, flowers); and vegetables products; sugars and syrups; meat (beef, chicken, duck, lamb and mutton, pork) and meat products (burgers, patties, hotdogs/sausages, bologna etc); eggs (whole eggs, pasteurized eggs, frozen eggs, dried eggs, high selenium/omega-3 eggs); fish (freshwater and saltwater fish); shellfish (crustacean- crab, lobster, shrimp; mollusk- bivalve (clam, mussel, oyster, scallop), univalve; (abalone, conch, snail), cephalopod (octopus, squid); and fisheries products (crackers, snacks, sauces); milk & milk products (packed liquid/powdered milks, yogurts, cheeses, butter, creams, ghee), beverages (coffee/tea/cocoa mixtures other flavoured packed/ bottled beverages); and oils and fats.

As for prepared (food as consumed) foods, they include traditional Malaysian kuih (rice and rice flour-based, wheat flour-based, legume-based, glutinous rice-based, porridge and *pengat* and miscellaneous) cooked dishes and meals (cereal based, meat and egg dishes, fish and sea food dishes, vegetable dishes and miscellaneous) and franchised fast foods (chicken, burger, pizza, spaghetti, sandwiches, satay and miscellaneous).

1.4 SAMPLING PROCEDURES

For raw and processed foods, a total number of six samplings are required as primary samples (the collection of units initially taken from the total population of food) (Figure 2). For food products with familiar brand names, a total number of six brands are to be included as the primary samples. Each brand is to be analyzed individually, with analysis of each nutrient to be carried out in duplicate.

For prepared (food as consumed) foods, collection of samples shall follow the specifically-defined stratum (regional, seasonal, retail sale point, landing areas etc) of the individual food (Figure 3). For prepared or consumed foods such as *nasi goreng*, six regions are to be considered. The six regions are North (Perlis, Kedah and Penang), Central (Kuala Lumpur, Perak, Putrajaya and Selangor), South (Johor, Melaka and Negeri Sembilan), East (Pahang, Terengganu and Kelantan), Sabah/Labuan and Sarawak. In each region, one state will be chosen based on its comparatively highest population, with its capital city to be the focus of sample collection. Two primary samples will be collected from the capital city`s selected restaurant and stall which will be analyzed individually for each nutrient. The total samples of prepared foods is therefore twelve.

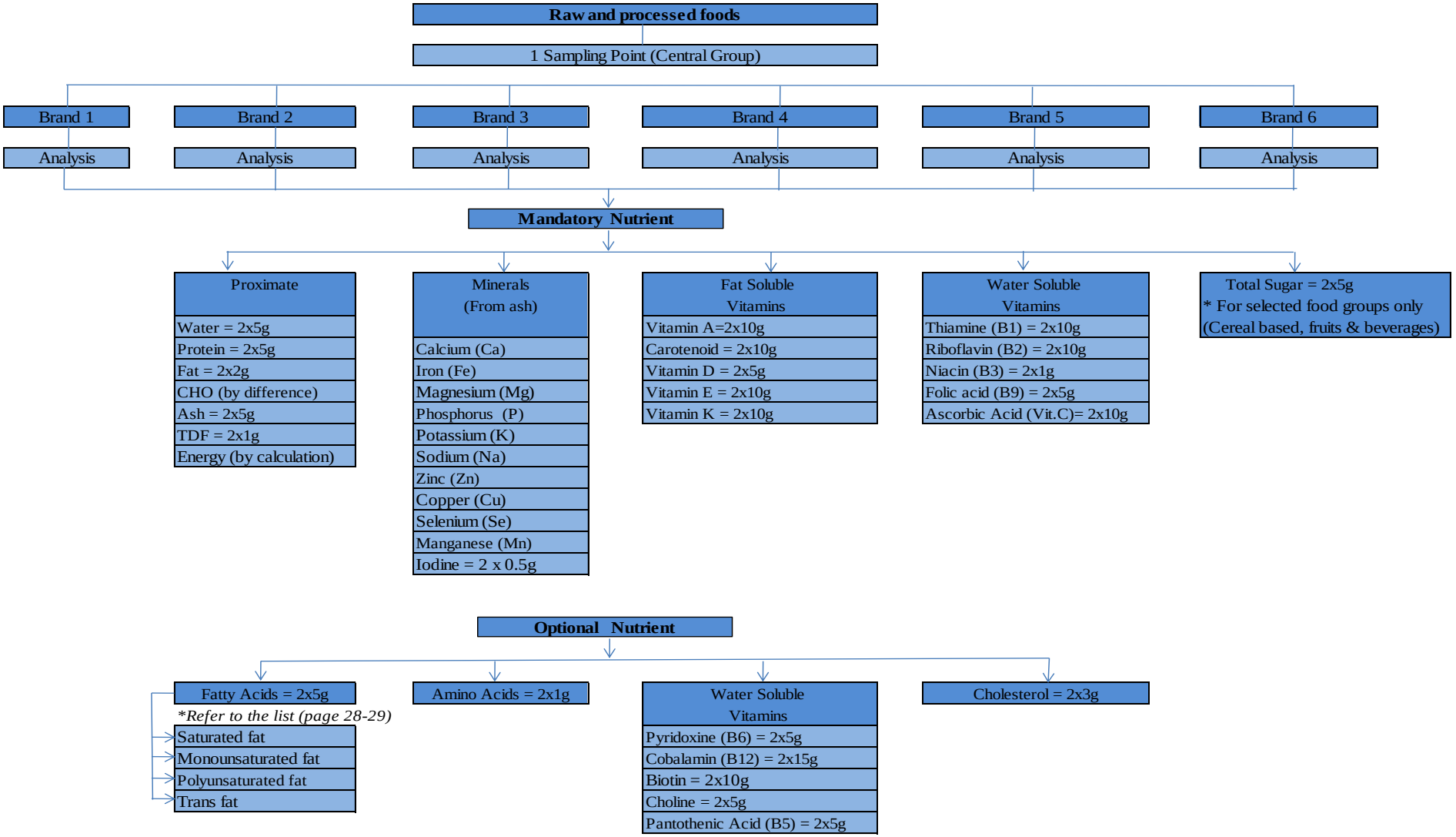


Figure 2. Sampling of raw and processed foods

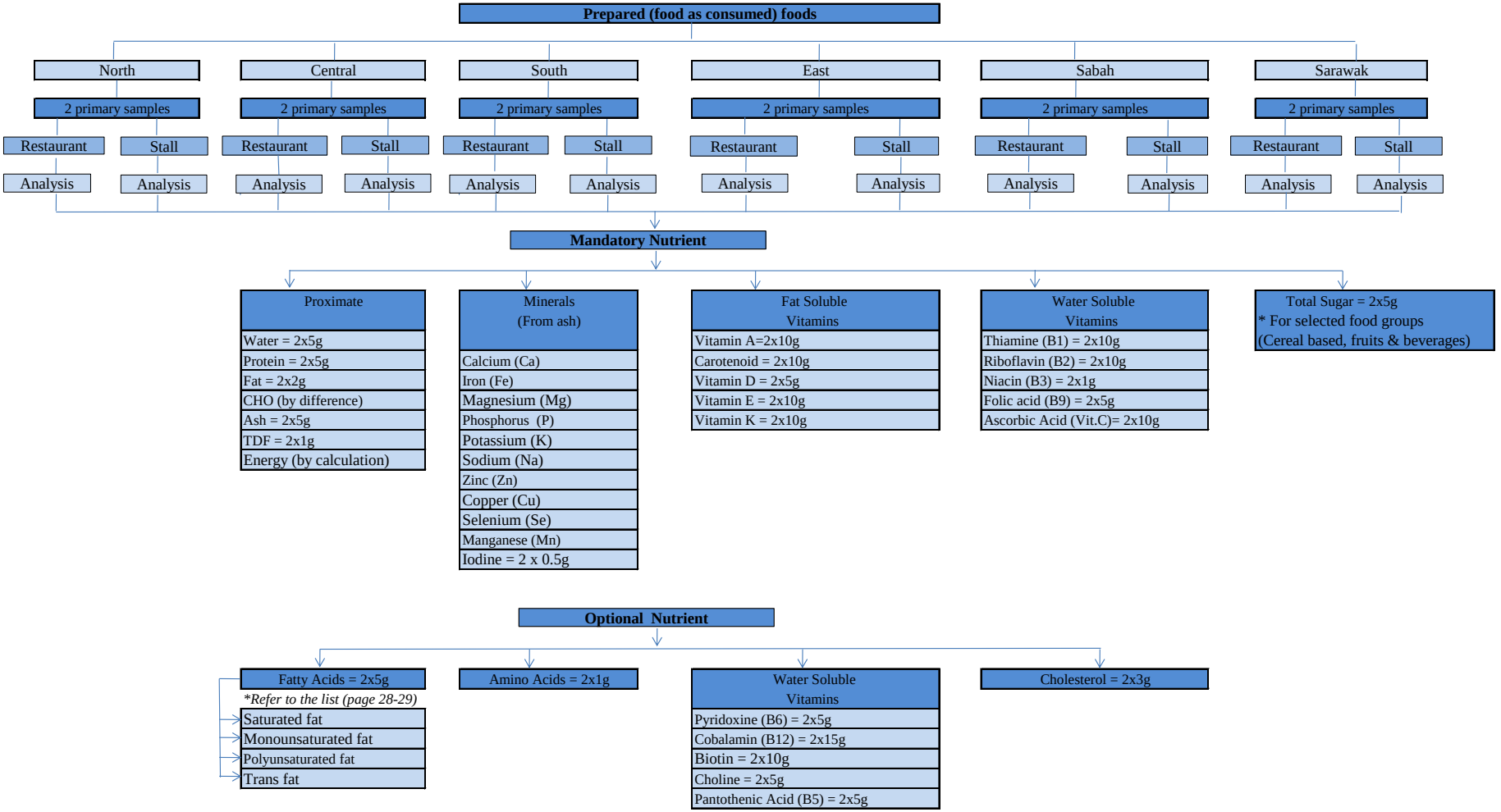


Figure 3. Sampling of prepared (food as consumed) foods

1.5 PORTIONS OF FOODS

Most databases use analytical values obtained from the analysis of edible material. During the selection of food for the database, it is therefore necessary to identify the edible material to be analysed. This will often be influenced profoundly by the cultural norms of the population for whom the database is being prepared. The inedible portion, or refuse, should also be measured and recorded in the database, since many users, particularly those in food service management, calculate nutrient content on the basis of foods as purchased. Table 1 provides examples of edible and inedible portions of some foods.

Table 1: Examples of inedible and edible portions of foods

Food	Inedible	Edible
Cereals and grain products • Barley	Nil	All
Starchy roots, tubers and products • Potato	Peel	Flesh (peel)
Legumes and legume products • Chickpea	Nil	All
Nuts, seeds and products • Almond	Nil	All
Vegetables and vegetable products • Cabbage	External yellow or wilted leaves, thick stalk	Remaining leaves and stalk
Fruits and fruit products • Banana • Orange • Passion fruit • Pineapple	Peel Peel, albedo, central pith Peel (seeds) Peel, tuft, base, core	Flesh Segments, residual albedo Flesh, (seeds) Flesh
Sugars and syrups • Honey	Nil	All
Meat and meat products • Meat • Liver • Chicken	Bone, gristle, (fat) Blood vessels, connective tissue Bones, (skin from back), leg, subcutaneous fat connective tissue	Muscle, (fat), connective tissue Remaining tissue Muscle, skin from chest and some fat pads
Eggs • Duck egg	Shell	Yolk and white egg
Fish, shellfish and products • Fish o fresh o canned in brine or (oil) o dried, small	Bone, viscera, (head), fins, (skin) Oil Bones, brine, (oil), (nil) Nil	Muscle, roe, (head), (skin) Flesh/bones All
Milk and milk products • Butter • Cheese	Nil (Rind)	All (Rind), inner part

Food	Inedible	Edible
Oils and fats • Margarine	Nil	All
Beverages • Sugar cane	Woody layers, pith	Juice
Prepared foods • *Nasi ayam*	Bones, (skin from back)	Rice, salad, cucumber, and muscle, skin from chest and some fat pads from chicken

Source: Greenfield & Southgate (2003)
Note: The inedible portions usually include spoiled material. The decision whether the part is edible or not depends on cultural norms and individual preference. The portions in parentheses may or may not be discarded.

References:

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PART II: PROTOCOL FOR COLLECTION AND HANDLING OF RAW, PROCESSED AND PREPARED (FOOD AS CONSUMED) SAMPLES

2.1 INTRODUCTION

Collection, handling and preparation of foods in laboratory are vital aspects of the analytical protocols. Care should be taken in the processes to separate edible and inedible portions (refuse, waste); and, in recording descriptions and weights of all parts.

2.2 OBJECTIVE

To ensure the process of collection, handling, storage and disposal of samples received for nutrient analysis are conducted according to systematic procedures.

2.3 SCOPE

2.3.1 Scope

The scope of the guideline covers the samples of nutrient analysis for the Malaysian Food Composition Database**.**

2.4 SAMPLE CODING SYSTEM

Category sample	Zone	State	Town	Food group	Food list	Sample number

Figure 4: Sample code number

i.e Category sample / Zone / State / Town / Food group / Food list / Sample number

2.4.1 Category sample:

	Code
Raw	1
Processed	2
Prepared	3

2.4.2 Zone Code

	Code
North	1
Central	2
South	3
East	4
Sabah	5
Sarawak	6

2.4.3 State Code

State	Code	State	Code	State	Code
Perlis	R	Negeri Sembilan	N	Terengganu	T
Kedah	K	Melaka	M	Kelantan	D
Penang	P	Johor	J	Sabah	S
Perak	A	Putrajaya	Putrajaya	Labuan	L
Selangor	B	Pahang	C	Sarawak	Q
Kuala Lumpur	W				

2.4.4 Town Code

Town	Code	Town	Code	Town	Code
Kangar	KR	Seremban	SM	Kuala Terengganu	KT
Alor Setar	AR	Bandaraya Melaka	BM	Kota Bharu	KB
Geogetown	GT	Johor Bahru	JB	Kota Kinabalu	KK
Ipoh	IP	Kuantan	KN	Labuan	LA
Shah Alam	SA	Putrajaya	PT	Kuching	KC
Kuala Lumpur	KL				

2.4.5 Food Groups

2.4.5.1 SECTION 1: RAW AND PROCESSED FOODS

Food groups	Code
Cereals and grain products	1.01
Starchy roots, tubers and products	1.02
Legumes and legume products	1.03
Nuts, seeds and products	1.04
Vegetables and vegetable products	1.05
Fruits and fruit products	1.06
Sugars and syrups	1.07
Meat and meat products	1.08
Eggs	1.09
Fish, shellfish and products	1.10
Milk and milk products	1.11
Oils and fats	1.12
Beverages	1.13
Miscellaneous	1.14

2.4.5.2 SECTION 2: PREPARED FOODS

Food group		Code
2.1 Traditional Malaysian Kuih	Rice and rice flour-based	2.1.1
	Wheat flour-based	2.1.2
	Legume-based	2.1.3
	Glutinous rice-based	2.1.4
	Tuber-based	2.1.5
	Bubur and pengat	2.1.6
	Miscellaneous	2.1.7
2.2 Cooked dishes and meals	Cereal-based	2.2.1
	Meat dishes	2.2.2
	Fish and sea-food dishes	2.2.3
	Vegetable dishes	2.2.4
	Miscellaneous	2.2.5
2.3 Franchised “fast food”	Chicken	2.3.1
	Burger	2.3.2
	Pizza	2.3.3
	Spaghetti	2.3.4
	Sandwiches	2.3.5
	Satay	2.3.6
	Miscellaneous	2.3.7

2.4.6 Food List:

Example: Atta for making capatti *(Tepung atta untuk membuat capatti)* – R101001

Sample number:

Raw and processed foods	Code
Brand 1	1
Brand 2	2
Brand 3	3
Brand 4	4
Brand 5	5
Brand 6	6

Prepared foods	Code
Restaurant	1
Stall	2

Example:

Atta for making capatti *(Tepung atta untuk membuat capatti)*

Raw food from Brand 1, collected from Central Zone (Selangor).

Category sample	Zone	State	Town	Food group	Food list	Sample number
1	**2**	**B**	**SA**	**1.01**	**R101001**	**1**

Figure 5: Sample code number for Atta for making capatti *(Tepung atta untuk membuat capatti)*

Sample code number:

1/2/B/SA/1.01/R101001/1

2.5 GENERAL PRECAUTIONARY MEASURES

Table 2: Effects of sample storage and preparation on nutrient content and precautions required to minimize them

Effects	Potential changes	Nutrients affected	Precaution
Drying out	Loss of water	All nutrients	Design of protocol. Keep samples in sealed or covered containers. Record food weight at initial and during preparation.
Absorption	Gain of water	All nutrients, especially in low-moisture and hygroscopic foods	Design of protocol. Keep samples in sealed containers
Microbial activity	Degradation/autolysis Synthesis	Losses of carbohydrates, proteins Gains in thiamin, vitamin B6, niacin and vitamin B12	Storage at low temperature. Pasteurization or addition of inhibitors may be necessary
Oxidation	Destruction of unsaturated fatty acids	Alterations in profile of fats	Store at –30 °C in sealed containers under nitrogen. Addition of antioxidants orbacteriostatic agents
	Loss of vitamins	Losses of vitamin C, riboflavin and folates	
Acid	Hydrolysis	Losses of sucrose and higher oligosaccharides	Store at low temperature. Neutralize acid
Alkaline	Destruction	Loss of thiamin	Avoid alkaline conditions and SO2
Light	Photodegradation	Loss of riboflavin	Protect from light
Contamination during sampling	From cooking vessels, soil, dust, etc.	Increase in inorganic nutrients	Design protocol to minimize contamination, gently rinse with distilled water
Contamination (from metallic blades, milling equipment, glassware, etc)	Increase in inorganic nutrients	Increase in major trace elements	Select apparatus with care. Clean all utensils thoroughly before use and store in plastic bags
Separation	Separation of fats. Fractionation of particles.	Changes in composition overall, alteration in fibre content	Avoid over vigorous mixing and thaw/freeze cycles
Enzymatic and metabolic activity	Change in organic nutrients	Losses of sugars, vitamin C, folate deconjugation	Store at low temperatures. Protect folates with ascorbate

Source: Greenfield & Southgate (2003)

2.6 RECORDING OF RAW SAMPLES

Zone:
State:
Capital City:
Retail Point: Supermarket/Pasar Tani/Wet Market/Stall

A – IDENTIFICATION OF FOOD (PRIMARY SAMPLE)

Common name of food :	
Sample code number :	
Date of receipt in laboratory :	
Date and time of collection	
Name of collector	
Food identification	**Examples of record**
Alternatives names	In language of country of origin
Scientific names	Genus, species, variety
Plant based food	☐ Entire plant ☐ Root/tuber ☐ Stem ☐ Leaves ☐ Flowers ☐ Seeds ☐ Fruits ☐ Shoot ☐ Others: specify
Animal based food	☐ Entire animal ☐ Leg ☐ head ☐ Internal organ ☐ Chest ☐ Drumstick ☐ Wings ☐ Others: specify
Sampling point	☐ Supermarket ☐ Pasar Tani ☐ Wet Market ☐ Stall
Address(es) of sampling point(s)	
Purchase price	If relevant
Graphical record (image)	Visual record with scales
Physical dimensions	

Transport conditions	Details including mode and conditions of transport and storage
Food type	☐ Cereals and grain products ☐ Starchy roots, tubers and products ☐ Legumes and legume products ☐ Nuts, seeds and products ☐ Vegetables and vegetable products ☐ Fruits and fruit products ☐ Sugars and syrups ☐ Meat and meat products ☐ Eggs ☐ Fish, shellfish and products ☐ Milk and milk products ☐ Oils and fats ☐ Beverages ☐ Miscellaneous
State of Maturity	☐ Immature ☐ Ripe ☐ Others: Specify
Physical state	☐ Liquid ☐ Solid ☐ Whole ☐ Divide ☐ Particle Size
Preparation method for consumption	Cooking method
Container or wrapping	☐ Can ☐ Glass ☐ Other: specify ☐ Paper ☐ Foil ☐ Plastic ☐ Polystyrene
Batch number for branded or pre-packed food	For branded foods
Weight of food collected	
Number of items	
Weights of individual items	
Weight of common measures or portion	
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

Modified from: Greenfield & Southgate (2003)

2.7 RECORDING OF PROCESSED FOOD SAMPLES

Zone:
State:
Capital City:
Retail point: Supermarket/Pasar Tani/Wet Market/Stall

A – IDENTIFICATION OF FOOD (PRIMARY SAMPLE)

Common name of food :	
Sample code number :	
Date of receipt in laboratory :	
Date and time of collection	
Name of collector	
Food identification	**Examples of record**
Alternatives names	In language of country of origin
Scientific names	Genus, species, variety
Plant based food	☐ Entire plant ☐ Leaves ☐ Fruits ☐ Root/tuber ☐ Flowers ☐ Shoot ☐ Stem ☐ Seeds ☐ Others: specify
Animal based food	☐ Entire animal ☐ Internal organ ☐ Wings ☐ Leg ☐ Chest ☐ Others: specify ☐ head ☐ Drumstick
Sampling point	☐ Supermarket ☐ Pasar Tani ☐ Wet Market ☐ Stall
Place of manufactured	If known (village, district, province, map reference, country)
Purchase price	If relevant
Graphical record (image)	Visual record with scales
Physical dimensions	
Transport conditions	Details including mode and conditions of transport and storage

Food type	☐ Cereals and grain products ☐ Starchy roots, tubers and products ☐ Legumes and legume products ☐ Nuts, seeds and products ☐ Vegetables and vegetable products ☐ Fruits and fruit products ☐ Sugars and syrups ☐ Meat and meat products ☐ Eggs ☐ Fish, shellfish and products ☐ Milk and milk products ☐ Oils and fats ☐ Beverages ☐ Miscellaneous
State of Maturity	☐ Immature ☐ Ripe ☐ Others: Specify
Physical state	☐ Liquid ☐ Solid ☐ Whole ☐ Divide ☐ Particle Size
Preparation method for consumption	Cooking method
Container or wrapping	☐ Can ☐ Glass ☐ Other: specify ☐ Paper ☐ Foil ☐ Plastic ☐ Polystyrene
Batch number for branded or pre-packed food	For branded foods
Weight of food collected	
Number of items	
Weights of individual items	
Weight of common measures or portion	
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

Modified from: Greenfield & Southgate (2003)

2.8 RECORDING OF PREPARED (FOOD AS CONSUMED) FOOD SAMPLES

Zone:
State:
Capital City:
Retail point: Restaurant/Stall

A – IDENTIFICATION OF FOOD (PRIMARY SAMPLE)

Common name of food :	
Sample code number :	
Date of receipt in laboratory :	
Date and time of collection	
Name of collector	
Food identification	**Examples of record**
Alternatives names	In language of country of origin
Scientific names	Genus, species, variety
Plant based food	☐ Entire plant ☐ Root/tuber ☐ Stem ☐ leaves ☐ flowers ☐ Seeds ☐ Fruits ☐ Shoot ☐ Others: specify
Animal based food	☐ Entire animal ☐ Leg ☐ Head ☐ Internal organ ☐ Chest ☐ Drumstick ☐ Wings ☐ Others: specify
State of maturity	☐ Immature ☐ Ripe ☐ Others
Grade	Where appropriate
Place of origin	If known (village, district, province, map reference)
Sampling point	☐ Restaurant ☐ Stall
Address(es) of sampling point(s)	
Purchase price	If relevant
Graphical record	Visual record with scales

Transport conditions	Details including mode and conditions of transport and storage
Food type	**Traditional Malaysian Kuih** ☐ Rice and rice flour based ☐ Wheat flour based ☐ Legume based ☐ Glutinous rice based ☐ Tuber based ☐ Bubur and pengat ☐ Miscellaneous **Cooked Dishes and Meals** ☐ Cereal based ☐ Meat and egg dishes ☐ Fish and sea-food dishes ☐ Vegetable dishes ☐ Miscellaneous **Franchised fast foods** ☐ Chicken ☐ Burger ☐ Pizza ☐ Spaghetti ☐ Sandwiches ☐ Satay ☐ Miscellaneous
Physical dimensions	
Physical state	☐ Liquid ☐ Solid ☐ Whole ☐ Divide ☐ Particle Size
Process and preservation method	☐ Canned ☐ Smoked ☐ Sun-dried
Preparation method for consumption	Cooking method
Extend of preparation	☐ Raw ☐ Uncooked ☐ Partially Cooked ☐ Fully Cooked ☐ Thawed ☐ Reheated
Container or wrapping:	☐ Can ☐ Paper ☐ Plastic ☐ Glass ☐ Foil ☐ Polystyrene ☐ Other: specify
Contact surface	☐ Glass ☐ Type of plastic ☐ Foil
Label or list of ingredients/Recipe	Retain label, estimated by inspection

Weight of food collected	
Number of items	
Weights of individual items	
Weight of common measures or portion	
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

Modified from: Greenfield & Southgate (2003)

B – SAMPLE HANDLING IN THE FIELD

1) Frozen Foods

Description	Examples of record
Quantity	1 kg minimum
Packaging	Sealed plastic bag for sample and wrapping with dark plastic.
Logistic	
• Timing	Within 24 hours should reach the lab.
Condition for storage during transportation	Cooled box with icepack, cooler or ice cube (0-4°C).
Transport conditions	Mode and conditions of transport
Note	• Sample from several packages for small food package must from the same production batch number.
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

2) Highly Perishable and Refrigerated Foods

Description	Examples of record
Quantity	1 kg minimum
Packaging	Sealed plastic bag for sample and wrapping with dark plastic.
Logistic	
• Timing	Within 24 hours should reach the lab.
Condition for storage during transportation	Cooled box with icepack, cooler or ice cube (0-4°C).
Transport conditions	Mode and conditions of transport
Note	• Sample from several packages for small food package must from the same production batch number. • Fresh eggs, outer cartoons are wrapped in several layers of bubble wrap and thick layers of newspaper are placed between each cartoon
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

3) Shelf-Stable Foods

Description	Examples of record
Quantity	1 kg minimum
Packaging	Sealed plastic bag for sample and wrapping with dark plastic.
Logistic	
• Timing	Within 24 hours should reach the lab. If delay, kept in room temperature.
Condition for storage during transportation	Cardboard boxes
Transport conditions	Mode and conditions of transport
Note	• Sample from several packages for small food package must from the same production batch number. • Items packed in glass bottles are wrapped in bubble wrap or newspaper to offer cushioning during transport. • Marked fragile for item like eggs.
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

4) Prepared (food as consumed) and Fast Foods

Description	Examples of record
Quantity	1 kg minimum
Packaging	Sealed plastic bag for sample and wrapping with dark plastic.
Logistic	
• Timing	Within 24 hours should reach the lab. If delay kept in the freeze.
Condition for storage during transportation	Cooled box with dry ice (<10°C).
Transport conditions	Mode and conditions of transport
Note	• Sample from several packages for small food package must be from the same production batch number. • Sealed plastic bags are not sealed until ten to fifteen minutes from time of purchase in order to allow foods to cool slightly. • Beverages such as sodas and coffee are transferred

Description	Examples of record
	to specially cleaned or prepared plastic bottles with leak-proof screw-type lids.
Other details	Any details that the recorder considers relevant (e.g. after fresh samples were collected they were vacuum sealed)

C – RECORD OF SAMPLE HANDLING IN LABORATORY

Common name of food	
Sample code number	
Lab identification number:	
Date of receipt in laboratory	
Handling stage	**Examples of record**
Total sample weight received (gm)	
Weight and nature of inedible matter (gm)	Prior to further preparation (e.g. head and feet of poultry, outer wilted leaves)
Weight and nature of edible matter (gm)	Prior to further preparation
Method of mixing and reduction	☐ Grinding ☐ Homogenizing in blender ☐ others
Type of storage	Temperature of storage, etc
Completed the result sheet (Refer to form in section 2.5)	
Name and signature of person completing record	
Date of record	
Other details:	Any details that the collector think may be relevant

D - SAMPLE PREPARATION IN LABORATORY

The food items received from the sampling points may require some simple preparations (e.g. wash and peel or use directly), while some will require detailed instruction for cooking (e.g. chicken). All food items are prepared as individual food.

1. Receiving of Samples

All received samples are to be sent to the sample preparation area for collection by respective laboratory analyst. Analysts are responsible to handle the samples as per request.

2. Identifying Sample

A sample is to be divided into 40 analyses. Each analyst must identify the sample type to determine the procedure for sample preparation prior to analysis.

3. Preparation of Sample

Sample preparation is to be done according to its sample type. The preparation of analytical samples for particular food types are as in Table 3.

The portion of laboratory samples to be analysed should be identified according to the type of analysis. For those samples which are requested for different types of analyses, the portions should be kept separately for each analysis.

Each sample portion should be placed in a clean, chemically-inert container and sealed to avoid any contamination and ensuring the integrity of the sample. Each sample should be correctly identified by a record and labeled accordingly by the respective analyst before storage.

Table 3: Preparation of analytical samples for particular food types

Type of Sample	Method of Sample Preparation	Storage conditions
Dry food/solid food (separate) except for milk powder	Homogenize with grinder / miller. Recommended particle size after homogenization is < 1 mm. For vitamin C analysis, keep the analytical portion into cold metaphosphoric acid.	Chilled at 4˚C or Room Temperature
Liquid samples	Shake the samples in the original bottle/ can and pour the samples into dedicated label bottle. e.g. syrup, juices, fresh milk, canned drink	Chilled at 4˚C
Liquid samples with solids	Use food processor or blender to homogenize the whole samples. e.g. mushroom soup, canned fruit or vegetables.	Chilled at 4˚C
Semi-solid samples	Use miller or mixer to homogenize the whole samples. e.g. marmalade, jam, etc	Chilled at 4˚C
Solid with liquid sample	Homogenize the solid part first then add the liquid part and use spatula to mix and finally homogenize all with mixer. e.g. instant noodles with seasoning and paste	Chilled at 4˚C
Milk Powder	Homogenize and put the samples into dedicated label bottle	Room Temperature

E - FLOW CHART OF SAMPLE PREPARATION IN LABORATORY

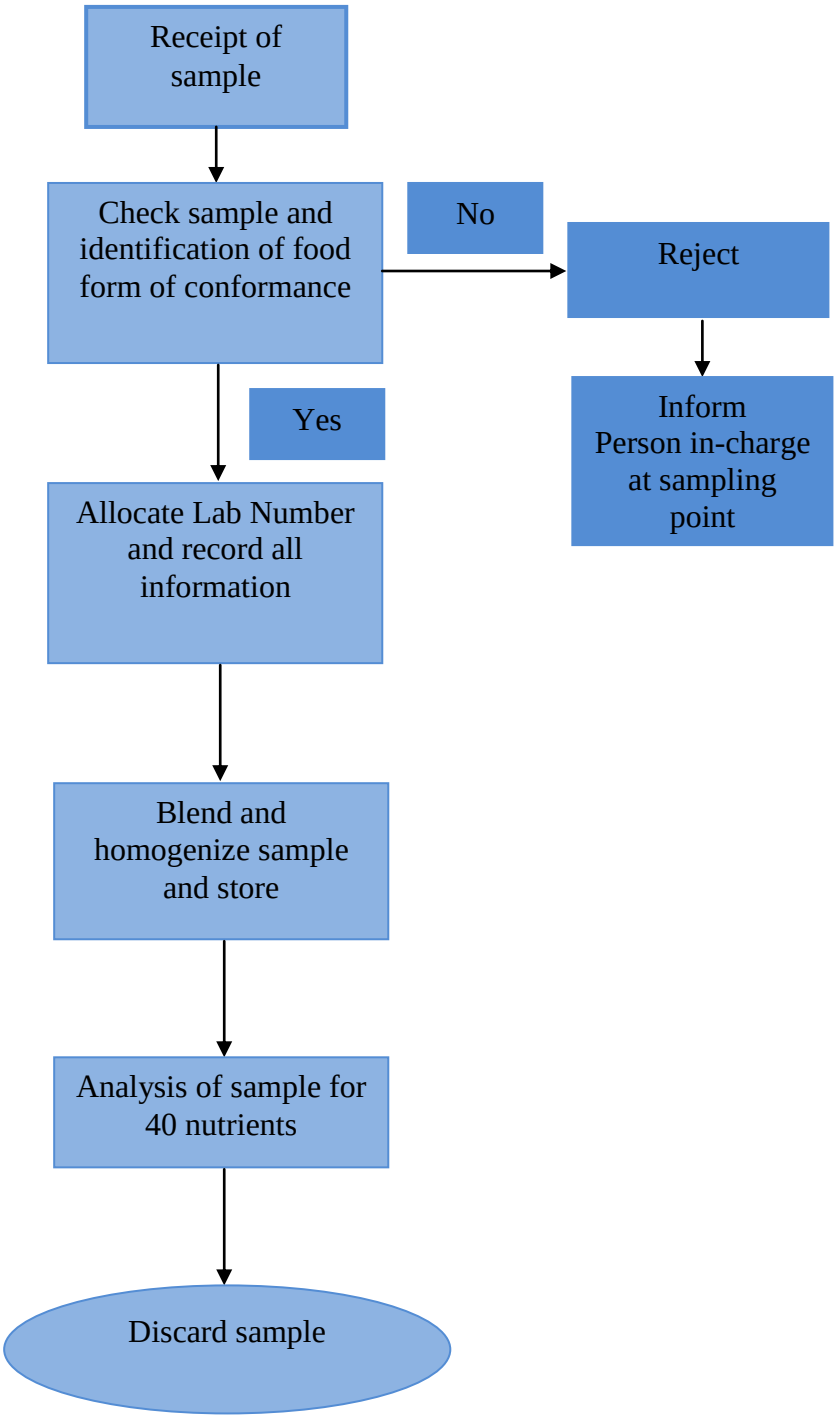

Figure 6: Flow chart of sample preparation in laboratory

F - EQUIPMENT REQUIREMENTS FOR HANDLING AND PREPARATION OF LABORATORY AND ANALYTICAL SAMPLES

A. General:

1. Trays (for carrying foods)
2. Bowls (0.5 litre to 4 litre capacity)
3. Spatulas
4. Chopping boards (polythene, wood)
5. Kitchen knives, knife-sharpener
6. Can-opener
7. Spoons (various sizes)
8. Plastic sieves, colanders
9. Electric heat-sealer (for freezer bags)
10. Large sheets of strong plastic (to cover benches, mix particulate foods)
11. Kitchen cutlery and tableware

B. Homogenizers:

Common domestic equipment:

1. Domestic food processor (can be equipped with titanium or other special blades
2. Food blender

2.11 RECORDING OF LABORATORY RESULTS

A - MANDATORY NUTRIENTS

Common name of food:
Sample code number:
Lab identification number:

No.	Nutrient	Unit	Results / 100 g sample	Methods of analysis	Known value results (QC)	Coefficient Variation (CV)
1	Energy	Kcal				
2	Water	g				
3	Protein	g				
4	Fat	g				
5	Available Carbohydrate, by difference	g				
6	Total Dietary Fibre, TDF	g				
7	Ash	g				
8	Calcium, Ca	mg				
9	Iron, Fe	mg				
10	Magnesium, Mg	mg				
11	Phosphorus, P	mg				
12	Potassium, K	mg				
13	Sodium, Na	mg				
14	Zinc, Zn	mg				
15	Copper, Cu	mg				
16	Selenium, Se	µg				
17	Manganese, Mn	µg				
18	Iodine	µg				
19	Ascorbic Acid (Vitamin C)	mg				
20	Thiamin (B1)	mg				
21	Riboflavin (B2)	mg				
22	Niacin (B3)	mg				
23	Folic Acid (B9)	µg				
24	Vitamin A (Retinol)	µg				
25	Carotenoid	µg				
	β-carotene	µg				
	Lycopene	µg				
	Lutein	µg				
26	Vitamin D	µg				
27	Vitamin E	mg				

No.	Nutrient	Unit	Results / 100 g sample	Methods of analysis	Known value results (QC)	Coefficient Variation (CV)
28	Vitamin K	µg				
29	Total sugar (Mandatory for cereal based foods, fruit and beverages)	g				
	Sucrose	g				
	Glucose	g				
	Fructose	g				
	Lactose	g				
	Maltose	g				

B - OPTIONAL NUTRIENTS

Common name of food:
Sample code number:
Lab identification number:

No.	Nutrient	Unit	Results / 100 g sample	Methods of analysis	Known value results (QC)	Coefficient Variation (CV)
1	Pantothenic Acid (B5)	mg				
2	Pyridoxine (B6)	mg				
3	Cobalamin (B12)	µg				
4	Choline	mg				
5	Biotin (B7)	mg				
6	Fatty acid, total saturated fat	g				
	4:0	g				
	6:0	g				
	8:0	g				
	10:0	g				
	12:0	g				
	14:0	g				
	16:0	g				
	18:0	g				
7	Fatty acids, total monounsaturated fat	g				
	16:1	g				
	18:1	g				
	20:1	g				
	22:1	g				

No.	Nutrient	Unit	Results / 100 g sample	Methods of analysis	Known value results (QC)	Coefficient Variation (CV)
8	Fatty acids, total polyunsaturated fat	g				
	18:2	g				
	18:3	g				
	18:4	g				
	20:4	g				
	20:5	g				
9	Trans fatty acids	g				
10	Cholesterol	mg				
11	Amino Acid:	g				
	Tryptophan	g				
	Threonine	g				
	Isoleucine	g				
	Leucine	g				
	Lysine	g				
	Methionine	g				
	Phenylalanine	g				
	Valine	g				

References:

H. Greenfield and D.A.T. Southgate. 2003. Food Composition Data: Production, Management and Use. 2nd Edition. Food and Agriculture Organization of United Nations. Rome.

Standard Operational Procedure, Sub-Sampling of Samples, National Public Health Laboratory, Ministry of Health (2009).

Standard Operating Procedure, Malaysia Total Diet Study, Food Safety & Quality Division, Ministry of Health (2010).

Trainer D, Pehrsson PR, Haytowitz DB, Holden JM, Philips KM, Rasor AS & Conley NA (2010). Development of sample handling procedures for foods under USDA's National Food and nutrient Analysis Program. *Journal of Food Composition and Analysis*.doi:10.1016/j.jfca.2010.03.020

PART III: LABORATORY METHODS OF ANALYSIS

3.1 Introduction

The composite laboratory method of analysis is used for updating the new Malaysian Food Composition Database to ensure standardization and reliability of the data.

Reliable data on nutrient composition of foods should only be obtained by accurate analytical methods in the hands of trained analysts. The choice of the appropriate method under quality assurance scheme is a crucial element in ensuring the quality of the values in a food composition database. For many nutrients, several alternative analytical methods should be made available for obtaining comparable results suitable for a given analysis and differences in food matrices.

The composition of laboratory methods is divided into proximate, minerals, fat soluble vitamins, water soluble vitamins, amino acids, fatty acids and cholesterol analyses. Several alternative methods may be selected by considering the matrix of the samples and lab capacity of the institution involved in a project.

3.2 Summary of method

3.2.1 Proximate

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Moisture content	Air oven (convection)	AOAC, 1984 Modified method by UKM (*Universiti Kebangsaan Malaysia*)	Most samples
		Vacuum oven	Doc. No. J04-002 Modified method by NPHL (National Public Health Laboratory)	Food samples containing high protein, sugar and fat contents
		Infra-red	Nielsen S. S., 1994 Modified method by UKM	Cereal and flour based products

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
2.	Protein	Kjeldahl	Doc. No. J04-004 Modified method by NPHL	Refer to Table 5
3.	Fat	Soxhlet	Doc. No. J04-009 Modified method by NPHL	Wet and dry samples
4.	Available carbohydrate	By difference (calculation)	Doc. No. J04-013 Modified method by NPHL	All samples
5.	Total Ash	Dry ashing	Doc. No. J04-003 Modified method by NPHL	All samples
6.	Total dietary fibre (TDF)	Enzymatic gravimetric method	AOAC Method 991.43, 1991	Total, soluble and insoluble fiber content
7.	Total sugar	High Performance Liquid Chromatography (HPLC) with Refractvie Index (RI) detector	Wills *et al.*, 1980 Modified method by UKM	Most samples. Sucrose, fructose, maltose, glucose and lactose
8.	Energy	Calculation	Doc. No. J04-013 Modified method by NPHL	All samples

3.2.2 Minerals

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Digestion based on food matrix	Dry Ashing	Tee *et al.*, 1997	Most samples
		Wet Digestion	Sim *et al.*, 2006	Most samples

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
		Microwave Digestion	Miller R.O, 1998 Modified method by UMS *(Universiti Malaysia Sabah)*	All samples
2.	Calcium (Ca) Ferum (Fe) Sodium (Na) Potassium (K) Magnesium (Mg) Copper(Cu) Zinc (Zn)	Atomic Absorption Spectrometer – Flame	Modified method by IMR (Institute for Medical Research)	All samples
		Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	Hua Zou & Jiang Hui Liu, 1997 Chamberlain, I *et al.*, 2000 Baker, S.A. *et al.*, 1999 Modified method by IMR	All samples
3.	Selenium (Se)	ICP-MS	Hua Zou & Jiang Hui Liu, 1997, Chamberlain, I *et al.*, 2000, Baker, S.A. *et al.*, 1999 Modified method by IMR	All samples
4.	Iodine (I)	ICP-MS	Khalid B. & Fabien B., 2006-2009	All samples
5.	Phosphorus (P)	Spectrophotometry	Tee *et al.*, 1997	All samples
6.	Manganese (Mn)	ICP-MS	Hua Zou & Jiang Hui Liu, 1997, Chamberlain, I *et al.*, 2000, Baker, S.A. *et al.*, 1999 Modified method by IMR	All samples

3.2.3 Fat Soluble Vitamins

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Vitamin A (Retinol)	HPLC Method	Tee *et al.*, 1997	All samples
	Carotenoids	HPLC Method	Tee *et al.*, 1997	All samples
2.	Vitamin D	HPLC Method	AOAC 995.05, 2000	Infant formulas and Enteral Product
		HPLC Method	Jasinghe, V.J. & Perera, C.O., 2005	All samples
3.	Vitamin E	HPLC Method	Fairus S *et al.*, 2006, Cunha S.C *et al.*, 2006, Nesaretnam K *et al.*, 2007, Kawakami Y *et al.*, 2007, Nielsen M.M & Hansen A., 2008 Modified method by MPOB (Malaysian Palm Oil Board)	All samples
		HPLC Method	A.O.C.S, 1990	All samples
4.	Vitamin K	HPLC Method	AOAC 999.15, 2000	Milk and infant formulas

3.2.4 Water Soluble Vitamins

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Thiamin (B1)	HPLC Method	Kumar, S. & Aalbersberg, B., 2006 Modified method by UMS	All samples
2.	Riboflavin (B2)	HPLC Method	Kumar, S. & Aalbersberg, B., 2006 Modified method by UMS	All samples
3.	Niacin (B3)	HPLC Method	Juraja *et el.*, 2003 Modified method by UMS	All samples
4.	Pantothenic acids (B5)	HPLC Method	Pakin *et el.*, 2004 Modified method by UMS	All samples
5.	Pyridoxine (B6)	HPLC Method	Kall, M.A., 2003 Modified method by UMS	All samples
6.	Biotin (B7)	Microbiological Method	Murakami *et al.*, 2008 Modified method by UMS	All samples
7.	Folic Acid (B9)	HPLC Method	Johansson *et al.*, 2008 Modified method by UMS	All samples
8.	Cobalamin (B12)	Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS) Method	Luo *et al.*, 2006 Modified method by UMS	All samples

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
9.	Choline	Enzymatic Colorimetric Method	AOAC 999.14	Infant formula and milk
		UPLC-MS Method	Koc *et al.*, 2004 & Zeisel *et al.*, 2003 Modified method by UMS	All samples
10.	Ascorbic Acid (Vitamin C)	HPLC Method	Phillips *et al.*, 1992 Modified method by UMS	All samples

3.2.5 Amino Acids

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Amino Acids	HPLC Method	Waters AccQ Tag Amino Acid Analysis	All samples

3.2.6 Fatty Acids

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Fatty Acids	Gas Chromatography (GC) Method in oil	Modified method by UMT *(Universiti Malaysia Terengganu)*	Oil
		Gas Chromatography (GC) Method in food	Morrison, W.R. & Smith, L.M., 1964, Hitchcock, C & Hammod, E.W., 1980 Modified method by MPOB	All samples

3.2.7 Cholesterol

No.	Type of nutrient	Method of analysis	Method Reference	Nature of sample
1.	Cholesterol	Direct Saponification-Gas Chromatographic Method	AOAC 994.10	All samples
		HPLC Method	Lopez-Carventas, J.et al., 2006, Sanchez-Machado, D.I.*et al.*, 2002 Modified method by UPM *(Universiti Putra Malaysia)*	All samples

3.3 Details on method of analysis

The details on method of nutrient analysis should include the principles of the analysis; the chemical and reagents; apparatus and instruments to be used; procedures and calculations and reference on the method.

3.3.1 PROXIMATE

3.3.1.1 DETERMINATION OF MOISTURE CONTENT

METHOD A: AIR OVEN

Principles

In the oven drying method, the amount of moisture in foods is the difference between the weight before and after drying. It is a simple and standard method for many kinds of food analyses. The process of drying is caused by the difference of the relative humidity between food and the atmosphere, of which the higher the temperature, the faster the drying. Food products which are unstable and decompose at high temperatures need to be dried at lower temperatures, such as 40 – 70°C under vacuum.

Oven method is used as a standard method of moisture determination for many types of foods. Eventhough weight changes in the oven drying method are assumed to be due to moisture loss, detected weight changes could also be due to oxidation of unsaturated fatty acids and other compounds. The infra-red method, although a simple and fast method, is not suitable for moisture content analysis of samples which harden or burn when heated. The reflux distillation method results in less thermal decomposition of some foods compared to oven drying at high temperatures. Adverse chemical reactions can be further reduced by using a solvent with a lower boiling point. However, care must be taken to ensure that the reading of the meniscus to determine the volume of water in the receiving tube is not any less accurate than using a weight measurement.

Apparatus/Instruments

1. Balance : Analytical, sensitivity ± 0.1mg
2. Desiccator: with some moisture absorbent – (silica gel, calcium chloride, concentrated sulphuric acid etc.)
3. Nickel dish with lid: filled with predried sand and a glass rod
4. Oven: set at 105°C
5. Tongs

Procedures

1. Perform the experiment in duplicates.
2. Dry the dish containing the sand, glass rod and its lid in the oven at 105°C overnight and transfer to the desiccator to cool (approx. 30 min).
3. Weigh the dish (containing the sand and glass rod) and lid.
4. Weigh about 5 g sample into the dish. Spread the meat with the glass rod. Place the glass rod in the dish.
5. Replace the lid and weigh the dish and its contents (W1).
6. Place the dish with its lid slipped to one side. Dry for 16 hr or overnight at 105°C.
7. After drying, use a pair of tongs to transfer the dish and lid to the desiccator to cool (approx. 45 min). Reweigh the dish, lid and its dried content.

8. Replace the dish with its lid partially covered in the oven for 1hr. Transfer to the desiccator to cool, and then weigh the dish and its content again. If the weight obtained at this step is less than that obtained at Step 7, it means the sample was not sufficiently dried. In this case, repeat this step until constant weight is obtained (W2).

Calculation

$$\text{Moisture (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where, W1 = weight (g) of sample before drying.
W2 = weight (g) of sample after drying.

Reference

Association of Official Analytical Chemists (1984). Official Methods of Analysis. 14th Edition (Williams, S. ed.), AOAC, Virginia.

METHOD B: VACCUM OVEN

Principle

Samples are dried to constant weight under a constant or reduced pressure at a prescribed temperature for a prescribed time. The moisture is the disparity of the weight measured before and after the drying. % Moisture = ($M_{initial}$ - M_{dried}) / $M_{initial}$ X 100.

Chemicals/Reagents

1. Silica sand, 3-5mm or Celite 545

Apparatus/Equipments

1. Vacuum Oven: thermostatically controlled and connected through a drying train to a vacuum pump capable of maintaining the pressure in the oven below 25mm of mercury. The oven should be provided with an air inlet connected to a silica gel drying bottle and a trap for releasing the vacuum.
2. Convection Oven
3. Dishes: glass and aluminum with covers
4. Desiccators: with silica gel as drying agent

Procedures

1. Decide drying condition based on type of sample whether it requires convection or vacuum oven; temperature and time for drying, and suitable amount to weigh.(Refer Table 4)
2. Dry sample (moisture less than 20%) should use Aluminum dish while wet sample should use glass dish with sea/silica sand / Celite 545 and glass rod.
3. Dry aluminum dish with cover or glass dish with silica sand / Celite 545 and glass rod to gain constant weight in convection oven at temperature 105^{o}C for more than 4 hours.
4. Transfer the dried aluminum dish or glass dish into desiccator with the lid partially opened for 20 minutes. Then, fully close the desiccator and cool it for at least 40 minutes.
5. Weigh out the dish using an analytical balance with four decimal places. Record the weight as W1 in worksheet.
6. Add suitable amount of homogenized sample and record the final weight W2.
7. Transfer the dish into an oven and dry the sample using prescribed temperature and time as in Table 4.
8. For sample in glass dish, mix sample thoroughly in silica sand / Celite 545 with glass rod. Place the sample on a steam water bath at temperature not more than prescribed in the drying condition (refer Table 4).
9. Sample with high sugar content need to be mixed by glass stick occasionally to avoid the sugar being crystallized and trapped with moisture. Then, dry the sample using prescribed condition (refer Table 4).
10. Operation of oven is referred to instrument operation manual, and for vacuum oven the reference is Equipment Operation Procedure for Vacuum Oven.
11. Take out the dried samples. Close the lid for Al dish.

12. Transfer the dish into desiccator with the lid partially opened for 20 minutes. Then, close the desiccator fully and cool it for at least 40 minutes.
13. Weigh out the final weight, W3.

Calculation

Moisture, g/100g = (W2 - W3)/(W2 - W1)×100

Where, W1 = Weight of dried dish
W2 = Weight of dried dish with sample
W3 = Weight of dried dish and dried sample

References

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.5.02,Method 927.05

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 31.1.02,Method 931.04

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 27.4.03,Method 945.15

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.7.04,Method 948.12

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 44.1.03,Method 924.45

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 44.4.04,Method 969.38

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 27.3.06,Method 935.29

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 39.1.02,Method 950.46

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.5.02,Method 927.05

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 32.1.02,Method 925.09

Training Material Provided by JICA's Expert (2004) extracted from Analytical Manual for Standard Tables of Food Composition in Japan, 5th Revised Edition (2000)

Table 4: Conditions of drying

Food Matrix	Sample Weight (g)	Temperature, (^{o} C)	Time (hour)	Type of Dish
Cereal	3-5	105	5	Al can
Grains flour	3-5	135	1	Al can
Bread	2-3	135	1	Al can
Bread with others, jam, cream etc	2-3	V 70	5	Glass dish*
Noodle	2-3	135	3	Glass dish*
Noodle with seasonings ingredients	2-3	105	5	Al can
Potatoes	2-3	100	5	Al can
Starch	2-3	135	1	Al can
Sugar powder	5	V 105	3	Glass dish*
Sugar liquid	1.5	V 100	3	Glass dish*
Honey	1-1.5	V 90	3	Glass dish*
Cake, Pie	2-3	V 70	5	Glass dish*
Cookies, Cracker, Biscuit	3-5	100	5	Al can
Candy	1.5	V 100	2	Glass dish*
Chocolate	2-3	V 70	5	Glass dish*
Snack	3-5	105	3	Al can
Nuts	2	130	2	Al can
Beans	5	130	3	Al can
Boiled beans	2	V 100	5	Glass dish*
Soybean	5	130	2	Al can
Defatted soybean	2	130	1	Al can
Fish and its processed products	5-10	105	5	Glass dish*
Meat and its processed products	2-3	135	2	Glass dish*
Egg	2-3	V 100	5	Glass dish*
Milk liquid	2-3	100	3	Glass dish*
Milk powder, Condensed milk	2	100	4	Al can
Cream, Ice cream	2-3	100	3	Glass dish*
Lactic beverages, yogurt	2	V 100	4	Glass dish*
Cheese	3	105	4	Glass dish*
Vegetables, Fruits its processed products	3-4	V 70	5	Glass dish*
Mushrooms, Seaweed	5	105	5	Al can
Coffee beans, Coffee powder	2-5	105	5	Al can
Salt	3	140	1.5	Al can
Seasoning	3-5	V 70	5	Al can
Soy sauce, Other sauces	5	V 70	5	Glass dish*

Food Matrix	Sample Weight (g)	Temperature, (^{o} C)	Time (hour)	Type of Dish
Processed food	If drying condition is different between ingredients, choose the lower temperature and/or vacuum condition. An example is canned fish in tomato paste, oil and seasoning shall use vegetable product condition or seasoning drying condition, NOT fish product drying condition.			

* : Use sea sand method
V: Vacuum condition

METHOD C: INFRA-RED

Principle

Infrared drying involves the penetration of heat into the food sample being dried. It is a rapid method of moisture content analysis as the heat penetration enables water to evaporate fairly quickly.

Apparatus/Instruments

1. Infra-red balance
2. Disposable aluminum pan liner
3. Desiccator with some moisture absorbent – (silica gel, calcium chloride, concentrated sulphuric acid etc.)

Procedures

1. Pre-dry the disposable aluminum pan liner at 100 °C overnight and then let it cool in a dessicator.
2. Balance the infra-red meter at zero level.
3. Accurately weigh 10 g flour and evenly spread it onto the pan liner. Do not compress the powder.
4. Place dish with sample on infra-red meter dish holder and switch on the heater in the moisture meter.
5. Note down the percentage of moisture loss every minute as shown on the display panel until no further changes in moisture content is detected.
6. Allow the moisture balance to cool before repeating this experiment.
7. Plot a graph showing “Moisture loss (g) against time (min)”.
8. Results can be read directly from the balance scale display panel.

Reference

Nielsen, S. S. 1994. Introduction to the Chemical Analysis of Foods. Boston: Jones and Bartlett Publishers, Inc.

3.3.1.2 DETERMINATION OF PROTEIN CONTENT

Principles

A food is digested with concentrated sulphuric acid to convert organic nitrogen to ammonium ions.

$N(food) \longrightarrow (NH_4)_2SO_4$

During neutralization, alkali is added and the liberated ammonia is distilled into an excess of boric acid solution.

$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H2O + Na2SO4$
$NH_3 + H3BO3$ (boric acid) $\longrightarrow NH_4^+ + H_2BO_3^-$ (borate ion)

The distillate is titrated with standardized hydrochloric acid to determine the ammonia absorption in the boric acid.

$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$

The amount of protein present is then calculated from the nitrogen concentration of the food.

Chemicals/Reagents

1. Catalyst tablet. (copper sulfate: potassium sulfate = 1:9)
2. Concentrated sulphuric acid
3. Hydrogen peroxide (chilled)
4. Hydrochloric acid 0.2N: Measure 200ml of 1N HCl in 1L volumetric flask and mark up to 1L distilled water.
5. Methyl Red Indicator: Weigh out 100mg of Methyl Red (MR) in 100ml volumetric flask. Dilute with 100ml methanol.
6. Bromocresol Green Indicator: Weigh out 100mg of Bromocresol Green (BCG) in 100ml volumetric flask. Dilute with 100ml methanol.
7. Sodium hydroxide (NaOH), 40%: Weigh out 400g of Sodium hydroxide (NaOH) and dissolve in 1L of distilled water.
8. 1% Boric Acid: Weigh out 10g of Boric Acid and dissolve in 1L of distilled water.

Apparatus/Equipments

1. Automated Digestion Unit: attached with scrubber.
2. Automated Protein Analyzer Machine.
3. Balance: Analytical sensitivity ± 0.1 mg
4. Digestion tube
5. Volumetric Flasks: 100 mL & 1L
6. Conical Flasks: 250 mL
7. Measuring Cylinder: 25 mL
8. Pipet: 5 mL

Procedures

A. Standardization of hydrochloric acid 0.2N

1. Weigh approx. 10g of anhydrous sodium carbonate (Na_2CO_3).Dry for 1h at 265°C or 2 h at 200°C. Transfer the sodium carbonate to a beaker with tight lid. Store in dessicator.
2. Weigh approx.0.4g anhydrous sodium carbonate (Na_2CO_3).Note the weight.
3. Transfer into receiver flask and add 40ml of distilled water. Add 6 drops from each indicator solutions.
4. Titrate to pink. Note amount in ml used. Boil this solution for a few minutes. The solution will turn green. Cool to room temperature under running water.
5. Titrate to pink. Note volume. Boil this solution for a few minutes. The solution will turn green. Cool to room temperature under running water.
6. Titrate to pink. Note volume. Boil this solution for a few minutes. The solution will turn green. Cool to room temperature under running water.
7. Titrate to pink. Note volume. Boil this solution for a few minutes. The solution will turn green. Cool to room temperature under running water.

B. Protein analysis

1. Weigh, to the nearest mg (dry 0.5-2g, wet 3-5g, liquid 3-5g) , of prepared sample on a piece of grease-proof paper for dry/semi solid sample, twist the paper and put into digestion tube. Liquid sample is to be pipetted directly into the digestion tube. Refer to Table 5 for guideline of sample size. Each sample is analysed in duplicate.
2. Place tube positions at No. 1 until 4 on rack for blank sample. Tube position No.5 until 8 are for sample check. Sample tube is started with position No.9 and above.
3. Add two Catalyst tablets into all the tubes.
4. Add 15 ml concentrated sulphuric acid to all sample tubes except for blank sample. Mix gently by swirling the liquid.
5. Add 5 ml of chilled hydrogen peroxide slowly into the tube to react with sample and acid. Continue adding another 5 ml hydrogen peroxide until mixture turn to blue or green or slightly brown. Do not add hydrogen peroxide into blank sample tube.
6. Place the rack into Digestion Unit and digest the sample at 420°C for 1½ hours. Refer to EOP – Automated Protein Analyzer Instrument Operation and Maintenance for the operation and Maintenance.
7. If traces of carbon still remain, cool the tube, add 3-5 ml concentrated sulfuric acid and continue digestion.
8. After the tube has cooled, transfer the rack into Automated Protein Analyzer machine.
9. Set the parameter such as the sample weight, conversion factor, blank and washing as well. Refer to Equipment Operation Procedure and Maintenance.
10. Run the machine and print the result.

Calculation

Nitrogen content, %***N*** = x /1000 X (***Vs*** – ***Vb***) /***S*** X 14 X 100
Where ***x*** =Normality of acid,
Vs and ***Vb*** **=** are titration volumes of the sample and blank (ml),
S =sample weight (g) and 14 is the molecular weight of nitrogen N.

% Protein = %***N*** X F
Where F is conversion factor referred to Table 6.

All the data and calculation are recorded into worksheet

References

Food Act 1983 (Act 281) & Regulations.18B (8)

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 32.3.13, Method 950.36

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 33.5.03, Method 930.29

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 37.1.35, Method 920.15

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 33.2.13, Method 991.22

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 31.5.02, Method 991.20

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 32.1.22, Method 922.06

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 32.1.14, Method 920.87

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 32.1.33, Method 920.87

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 40.1.06, Method 950.48

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 32.1.14, Method 922.06

Training Material Provided By JICA's Expert (2004) Extracted From Analytical Manual for Standard Tables of Food Composition in Japan, 5th Revised Edition, 2000.

Table 5: Estimate Sample Size for Kjeldahl Protein Analysis

Expected Protein Content (g/100g, %)	Food Sample (example)	Sample Size (g)
80-90	Soya bean extracted powder	0.3
60	Fish powder, chlorella	0.3
30-50	Bone powder	0.4 - 0.5
10-30	Mixture livestock feed	0.8
2-10	Others	1 - 2
1	Solid	2 – 4
	Liquid	5 – 10
Sugar, oils and fats		1 - 4

Table 6: Protein and Nitrogen Conversion Factor for Typical Food Commodity

Food Commodity	Type	N Factor
Cereals Products	Wheat, hard, medium or soft Wholemeal or flour or bulgur Flour, medium or low extraction Macaroni. Spaghetti, wheat pastes Bran Rice Rye, barley, oats	 5.83 5.70 5.70 6.31 5.95 5.83
Pulses, nuts and seeds	Groundnuts Soya bean, seeds, flour or products	5.46 6.25
Treenuts	Almond Brazil nut Coconuts, chestnuts, treenuts	5.18 5.71 5.30
Seeds	Sesame Safflower Sunflower	5.30 5.30 5.30
Milk and milk products	Regulation No.82- No 117*	6.38
Edible fats and edible oil	Regulation No.179- No.208*	6.25
Other foods	Other foods	6.25

***** Food Act 1983 (Act 281) & Regulations.18B (8)

3.3.1.3 DETERMINATION OF FAT CONTENT

METHOD A: DETERMINATION OF FAT CONTENT IN DRY FOODS

Principles

Soxhlet method is a semi continuous solvent extraction method. A ground sample is to be weighed in a porous thimble, and then placed in an extraction chamber suspended above a flask/container containing solvent. The flask is heated, the solvent is evaporated and converted into liquid at the condenser and passes through the sample, extract the lipids and channel them into the flask. After several hours of extraction, the flask containing the solvent is removed and evaporated. The Soxhlet method is also available in automated machine for a simpler and faster extraction technique such as FOSS Soxtec machine. Food is digested with concentrated sulphuric acid to convert organic nitrogen to ammonium ions.

The lipid content calculated as:
% lipid = M lipid / M sample X 100

Chemicals/Reagents

1. Petroleum ether
2. Diethyl ether

Apparatus/Equipments

1. Automated Fat Extraction System
2. Air oven
3. Balance: Analytical sensitivity ± 0.1mg
4. Desiccator
5. Ashless Filter paper
6. Aluminium cup/ glass cup
7. Thimble
8. Facial Cotton

Procedures

1. Prepare Aluminium cup/ glass cup by drying it in a hot air oven at 105ºC for one hour, cool in desiccator and weigh as W1.
2. Weigh dry sample 1.0~2.0 g, S on a folded filter paper.
3. Transfer the filter paper to the thimble and put on cotton as lid
4. Start the extraction procedures. See the Equipment Operation Procedure for operation of Automated Fat Extraction System machine.
5. After the program completes, take out the aluminum/glass cup from machine, dry at 105ºC for 1hr, cool it in a desiccator for 1hr, and weigh, W2

Calculation

Fat, g/100g = (W2 – W1)/S ×100
Where, W1 = Weight of dried aluminium/glass cup
W2 = Weight of dried aluminium/glass cup after extraction
S = Weight of sample

Reference

Training Material Provided by JICA's Expert (2004) extracted from Analytical Manual for Standard Tables of Food Composition in Japan, 5th Revised Edition (2000).

METHOD B: DETERMINATION OF FAT CONTENT IN WET FOODS

Principles

Soxhlet method is a semi continuous solvent extraction method. A ground sample is weighed in a porous thimble, placed in an extraction chamber suspended above a flask/container containing solvent. The flask is heated, the solvent is evaporated and converted into liquid at the condenser and passes through the sample, thus the lipids are extracted and channeled into into the flask. After several hours of extraction, the flask containing the solvent is removed and evaporated. The Soxhlet method is also available in automated machine for a simpler and less time extraction technique such as Automated Fat Extraction machine. The lipid content calculated as:
% lipid = M lipid / M sample X 100

Chemicals/Reagents

1. Petroleum ether
2. Diethyl ether
3. Na_2SO_4 anhydrous
4. Celite 545

Apparatus/Equipments

1. Automated Fat Extraction Machine (Soxhlet Method)
2. Waterbath
3. Air oven
4. Balance: Analytical sensitivity ± 0.1mg
5. Desiccator
6. Mortar and pestle
7. Ashless Filter paper
8. Aluminium cup/ glass cup
9. Thimble
10. Facial cotton
11. Glass beaker: 50 mL
12. Glass rod

Procedures

1. Prepare Aluminium/glass cup by drying it in a hot air oven at 105°C for one hour, cool it in desiccators and weigh as W1.
2. Weigh out sample 1.0~2.0g, S in a 50 ml beaker.
3. Add celite 2 – 3 spatula, glass rod and mix dry on the water bath over 2 hours.
4. Transfer the sample mixture qualitatively from beaker into a clean mortar, add 2 – 3 spatula of Na2SO4 and homogenize with pestle.
5. Transfer the sample mixture to the thimble.
6. Wipe the mortar and beaker with pieces of cotton dampened with diethyl ether and transfer the pieces to the thimble.
7. Set the thimble adaptor with thimble aluminum cups and thimbles, and place into the machine.

8. Start the extraction procedures. See the Equipment Operation Procedure for operation of Automated Fat Extraction machine.
9. After the procedures completed, take the aluminum cup out from machine, dry it at 105ºC for 1hour, cool in a desiccators for 1hour, and weigh, W2

Calculation

Fat, g/100g = (W2 – W1)/S ×100
Where, W1 = Weight of dried aluminium/glass cup
W2 = Weight of dried aluminium/glass cup after extraction
S = Weight of sample

References

Official Methods of Analysis of AOAC International, 17th Edition, Volume II, Section 39.1.08, Method 991.36

Training Material Provided by JICA's Expert (2004) extracted from Analytical Manual for Standard Tables of Food Composition in Japan, 5th Revised Edition (2000).

4.3.1.4 DETERMINATION OF AVAILABLE CARBOHYDRATE IN FOOD (BY DIFFERENCE)

Available carbohydrate content is calculated as follows:

Available Carbohydrate, g/100g = 100 – {[Ash]+ [Moisture] + [Fat] + [Protein]+ [Total Dietary Fibre]}

3.3.1.5 DETERMINATION OF ASH CONTENT

Principles

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperature between 500 ^{o}C to 600^{o}C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air into CO, H_2O & N_2. Most minerals are converted to oxides, sulfates, phosphates, chloride or silicates.

Apparatus/Instruments

1. Silica crucible: diameter 3.5cm, depth 4cm for dry sample or bigger for liquid sample
2. Desiccators: with silica gel desiccant
3. Muffle Furnace: thermostatically controlled at 550°C
4. Electric hotplate: with thermostatic controlled
5. Waterbath

Procedures

1. Place clean silica crucible into a muffle furnace at 550 ^{o}C for over 5 hours.
2. Cool for 1 hour in a desiccator and weigh out crucible as a constant weight, W1 using 4 decimal places analytical balance.
3. Weigh sample into a constant weight crucible and record as W2. Weight for dry sample is 3-5g while liquid sample is 5-10g.
4. Place crucible with liquid sample on a water bath or hot plate and heat until dry. Add a piece of ashless filter paper if liquid sample is viscous.
5. Pre-ash sample by heating the crucible on a hotplate. Cover crucible with a conical cap made from ashles filter paper for sample with high in carbon content to avoid sample from scattering.
6. Sample will turn black as charring is taking place.
7. Smoke will also appear and continue heating until smoking has finished.
8. Place the crucible into the muffle furnace and do ashing overnight at 550 ^{0} C.
9. Remove the crucible from muffle furnace to check the ash condition. Ashing is complete if its color is black carbon free, and weigh.
10. If traces of black carbon color are still seen, add a few drops of water after cool and stir with glass rod to break up the ash. Dry on a hotplate and then return to the muffle furnace and continue ashing. (duration depends on samples)
11. Cool for 1 hour and weigh the crucible. Record weight, W3.

Calculations

Ash, g/100g = (W3 - W1/ W2 - W1) ×100

Where, W1 = Weight of dried crucible
W2 = Weight of dried crucible with sample
W3 = Weight of dried crucible and dried sample

References

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 32.1.05,Method 923.03

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 31.1.04,Method 972.15

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.7.07,Method 977.11

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.5.01,Method 930.30

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.4.01,Method 945.48

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 37.1.18,Method 940.26

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 44.4.05,Method 920.18

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.1.10,Method 986.25

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 32.1.05,Method 923.03

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.2.10,Method 945.46

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 40.1.08,Method 950.49

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 35.1.40,Method 938.08

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 43.1.05,Method 941.12

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 44.1.05,Method 900.02

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 33.4.02,Method 920.115

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 30.1.05,Method 920.100

Official Methods of Analysis of AOAC International, 17th Edition, Volume II,Section 32.1.05,Method 923.03

Training Material Provided by JICA's Expert (2004) extracted from Analytical Manual for Standard Tables of Food Composition in Japan, 5th Revised Edition (2000).

3.3.1.6 DETERMINATION OF TOTAL, SOLUBLE AND INSOLUBLE DIETARY FIBER CONTENTS IN FOODS

Principles

The method of analysis is applicable to processed foods, grain, cereal products, fruits and vegetables. Duplicate samples of dried foods, fat-extracted if containing >10% fat, undergo sequential enzymatic digestion by heat stable ∝-amylase, protease, and amyloglycosidase to remove starch and protein. For total dietary fiber (TDF), enzyme digestate is treated with alcohol to precipitate soluble dietary fiber before filtering, and TDF residue is washed with alcohol and acetone, dried, and weighed. For insoluble and soluble dietary fiber (IDF and SDF), enzyme digestate is filtered, and residue (IDF) is washed with warm water, dried and weighed. For SDF, combined filtrate and washes are precipitated with alcohol, filtered, dried and weighed. TDF, IDF, and SDF residue values are corrected for protein, ash and blank.

Chemicals/Reagents

Use deionized water throughout.

1. Ethanol solutions - (1) 85%. Place 895 ml 95% ethanol into l L volumetric flask, dilute to volume with H2O. (2) 78%. Place 821 ml 95% ethanol into 1 L volumetric flask, dilute to volume with H_2O.
2. Heat-stable α-amylase solution - Cat.No. A 3306, Sigma Chemical Co., St. Louis, MO 63178, USA or Termamyl 300L Cat. No. 361-6282, Novo-Nordisk. Bagsvaerd, Denmark, or equivalent.
3. Protease - Cat. No. P 3910, Sigma Chemical Co. or equivalent. Prepare 50 mg/ml enzyme solution in MES/TRIS buffer fresh daily.
4. Amyloglucosidase solution- Cat. No. AMGA9913, Sigma Chemical Co., or equivalent. Store at 0-5°.
5. Diatomaceous earth - Acid washed (Celite 545 AW. No. C8656, Sigma Chemical Co. or equivalent).
6. Cleaning solution - Liquid surfactant-type laboratory cleaner, designed for critical cleaning (Micro International Products Corp. Trenton, NJ 08601 USA or equivalent). Prepare 2% solution in H2O.
7. MES - 2-(N-Morpholino) ethanesulfonic acid (No. M-8250, Sigma Chemical Co., or equivalent).
8. TRIS - Tris (hydroxymethyl) aminomethane (No. T-1503 Sigma Chemical Co., or equivalent).
9. MES/TRIS buffer solution. - 0.05M MES. 0.05M TRIS, pH 8.2 at 24°. Dissolve 19.52g MES 12.2g TRIS in 1.7 L H2O. Adjust pH to 8.2 with 6N NaOH, and dilute to 2L with H_2O. (Note: It is important to adjust pH to 8.2 at 24°. However, if buffer temperature is 20°, adjust pH to 8.3; if temperature is 28°, adjust pH to 8.1. For deviations between 20 and 28°, adjust by interpolation).
10. Hydrochloric acid solution - 0.561N. Add 93.5 ml 6N HCl to ca 700ml H2O in 1L volumetric flask. Dilute to 1L with H2O.

Apparatus/Instruments

1. Beakers: 400 or 600 ml tall form.
2. Filtering crucible: With fritted disk, coarse. ASTM 40-60 um pore size, Pyrex 60 mL (Corning No. 36060 Buchner, Corning, Inc., Science Products, Corning, NY 14831 USA, or equivalent). Prepare as follows. Ash overnight at 525° in muffle furnance. Let furnace temperature fall below 130° before removing crucibles. Soak crucible 1 h in 2% cleaning solution at room temperature. Rinse crucibles with H2O and then deionized H2O. For final rinse, use 15 ml acetone and then air-dry. Add approximately 0.5 - 1.0g Celite to dry crucibles, and dry at 130° to constant weight. Cool crucible approximately 1 hour in desiccator, and record weight, to nearest 0.1 mg, of crucible plus Celite.
3. Vacuum system: Vacuum pump or aspirator with regulating device. Heavy walled filtering flask, 1 L, with side arm. Rubber ring adaptors, for use with filtering flasks.
4. Shaking waterbaths: (1) Capable of maintaining 98 ± 2°, with automatic on-and off timer. (2) Constant temperature, adjustable to 60°.
5. Balance: Analytical, sensitivity ± 0.1 mg.
6. Muffle furnace: Capable of maintaining 525 ± 5°.
7. Oven: Capable of maintaining 105 and 130 ± 3°.
8. Desiccator: With SiO2 or equivalent desiccant. Biweekly, dry desiccant overnight at 130°.
9. pH meter: Temperature compensated, standardized with pH 4.0, 7.0 and 10.0 buffer solutions.
10. Pipetters: With disposable tips, 100-300 ul and 5ml capacity.
11. Dispensers: Capable of dispensing 15 ± 0.5 ml for 78% EtOH and acetone; 40 ± 0.5 ml for buffer.
12. Magnetic stirrers and stir bars.

Procedures

A. Sample Preparation

1. Determine total dietary fiber on dried sample.
2. Homogenize sample and dry overnight in 70°C oven, cool in desicator, and grind sample to fine mesh with mortar. If sample contains high fat content (>10%), defat with petroleum ether (3 times with 25 ml portions/g sample) before grinding.
3. Record loss of weight due to fat removal and make appropriate corrections to final % dietary fiber found in determination.
4. Store dry grind sample in capped jar in desiccator until analysis is carried out. For high sugar samples, desugar before determining dietary fiber by extracting 2-3 times with 85% EtOH or methanol 10 ml/g decauting, and then drying overnight at 40°.

B. Digestion:

1. Run 2 blanks assay with samples to measure any contribution from reagents to residue.

2. Weigh duplicate 1.000 ± 0.005g samples (M1 and M2), accurate to 0.1 mg into 400 ml (or 600 ml) tall-form beakers.
3. Add 40 ml MES/TRIS buffer solution, pH 8.2, to each. Stir on magnetic stirrer until sample is completely dispersed (to prevent lump formation, which would make test material inaccessible to enzymes).
4. Add 50ul heat-stable α-amylase solution, stirring at low speed.
5. Cover beakers with A1 foil and incubate in 95-100° H2O bath 15 min with continuous agitation. Start timing once bath temperature reaches 95° (total of 35 min is normally sufficient).
6. Remove all beakers from bath, and cool to 60. Remove foil. Scrape any ring from inside of beaker and disperse any gels in bottom of beaker with spatula. Rinse beaker walls and spatula with 10 ml H2O.
7. Add 100 ul protease solutions to each beaker. Cover with A1 foil, and incubate 30 min at 60 ± 1° with continuous agitation. Start timing when bath temperature reaches 60°.
8. Remove foil. Dispense 5 ml 0.561N Hcl into beakers while stirring.
9. Adjust pH to 4.0-4.7 at 60°, by adding 1N NaOH solution. (Note: It is important to check and adjust pH while solutions are 60° because pH will increase at lower temperatures). (Most cereal, grain, and vegetable products do not require pH adjustment. Once verified for each laboratory, pH checking procedure can be omitted. As a precaution, check pH of blank routinely; if outside desirable range, check samples too).
10. Add 300 ul amyloglucosidase solution while stirring. Cover with A1 foil, and incubale 30 min at 60° ± 1° with constant agitation. Start timing once bath reaches 60°.

I. Determination of Total Dietary Fiber

1. To each digested sample, add 225 ml (measured after heating) 95% EtOH at 60°. Ratio of EtOH to sample volume should be 4:1.
2. Remove sample from bath and cover beakers with large sheets of A1 foil. Let precipitate form for 1h at room temperature.
3. Wet and redistribute Celite bed in previously tared crucible B(b) using 15 ml 78% EtOH from wash bottle. Apply suction to crucible to draw Celite onto fritted glass as even mat.
4. Filter alcohol-treated enzyme digestate through crucible. (Note: If some samples form a gum, trapping the liquid, break film with spatula).
5. Using vacuum, wash residue 2 times each with 15 ml portions of 78% EtOH, 95% EtOH and acetone.
6. Dry crucible containing residue overnight in 105° oven. Cool crucible in desiccator for 1 h.
7. Weigh crucible, containing dietary fiber residue and Celite, to nearest 0.1 mg, and calculate residue weight by substracting weight of dry crucible with Celite B(b).
8. Use one duplicate from each sample to determine protein, by method 960.52 (see 16.4) using N x 6.25 as conversion factor.
9. For ash analysis, incinerate second duplicate 5 h at 525°. Cool in desiccator, and weigh to nearest 0.1 mg. Subtract weight of crucible and Celite, B(b), to determine ash weight.

II. Determination of Insoluble Dietary Fiber

1. Wet and redistribute Celite bed in previously tared crucible, B(b), using ca 3 ml H_2O. Apply suction to crucible to draw Celite into even mat.
2. Filter enzyme digestate, from E, through crucible into filtration flask.
3. Rinse beaker, and then wash residue 2 times with 10 ml 70° H_2O.
4. Combine filtrate and water washings, transfer to pretared 600 ml tall form beaker, and reserve for determination of soluble dietary fiber, H.
5. Using vacuum, wash residue 2 times each with 15 ml portions of 78% EtOH, and acetone, (Note: Delay in washing IDF redidues with 78% EtOH, 95% EtOH and acetone may cause inflated IDF values).
6. Use duplicates to determine protein and ash as in F.

III. Determination of Soluble Dietary Fiber

1. Proceed as for insoluble dietary fiber determination through instruction to combine the filtrate and water washings in pretared 600 ml tall-form beakers.
2. Weigh beakers with combined solution of filtrate and water washings, and estimate volumes.
3. Add 4 volumes of 95% EtOH preheated to 60°.
4. Use portion of 60° EtOH to rinse filtering flask from IDF determination.
5. Alternatively adjust weight of combined solution of filtrate and water washings to 80 g by addition of H2O and add 320 ml 60° 95% EtOH.
6. Let precipitate form at room temperature 1 h.

   Follow TDF determination, F, from "Wet and redistribute Celite Bed..."

Calculations

Blank (B mg) determination

$$B = \{ (BR1 + BR2)\} - P8 - A8$$

Where BR1 and BR2 = residue weights (mg) for duplicate blank determinations; and P8 and A8 = weights (mg) of protein and ash, respectively, determined on first and second blank residues.

Dietary fiber (DF, g/100g) determination:

$$\mathbf{DF = \{(R1 = R2)/\} - P - A - B\}/\{(M1 = M2)\ /2\} \times 100}$$

Where R2 and R2 - residue weights (mg) for duplicate samples; P and A = weights (mg) of protein ash respectively, determined on first and second residues; B = blank weight (mg); and M1 and M2 = weights (mg) for samples.
Total dietary fiber determination: Determine either by independent analysis, as in F, or by summing IDF and SDF, as in G and H.

References

J.AOAC Int.75, May/June (1992)
Methods of analysis for Nutrition Labelling (1993); Dietary Fibre, pg 210-213

3.3.1.7 DETERMINATION OF TOTAL SUGAR

Principles

Sugars are extracted with aqueous ethanol. After evaporation of the ethanol, the sugars left in aqueous solution are separated by high liquid chromatography (HPLC). The residue remaining after ethanol extraction is hydrolysed with amyloglucosidase to glucose quantified by HPLC. The final residue is considered to be dietary fibre after allowing for ash and protein.

Chemicals/Reagents

1. 85% (v/v) ethanol (AR Grade) in distilled water.
2. Acetone, AR Grade.
3. Acetate buffer, 2 M, pH 4.5. Mix 43 mL 2M sodium acetate with 57 mL 2 M acetic acid and dilute to 1 L.
4. Amyloglucosidase enzyme. Sigma Grade V, cat. No. A9268. From *Aspergillus oryzae*. Crude soln. Containing 0.2% benzoic acid as preservative. Activity: 1200-3000 units per mL.
5. Ethanol, absolute.
6. HPLC, mobile phase.
   Preparation of mobile phase (ACN75%H2O25%)
   1) Filter ± 250 ml deionised water using vacuum filter aqueous through nylon membrane filter (0.45 µm, Supor ® -450 Membrane).
   2) Filter ± 750 ml acetonitrile (ACN) using vacuum filter aqueous through nylon membrane filter (0.45 µm, PTFE).
   3) Mix the filtered ACN and deionised water into 1000 ml.
   4) Remove the mixture into blue cap bottle 1 L for analysis.
7. 0.5 N NaOH.
8. Sugar solution: 1 g/100 mL of each glucose, fructose, sucrose, maltose, lactose.
   Preparation of Standard and Mix Standard
   1) Mix 1 gram of each of sugar standard (glucose, sucrose, fructose, maltose and lactose) into 100 ml volumetric flasks.
   2) Add distilled water into volumetric flask until 100 ml and shake until sugar dissolved.
   3) Filter ± 2.0 ml solvent through nylon filter 0.45 µm into vial using syringe.

Apparatus/Instruments

1. Balance: Analytical sensitivity ± 0.1mg
2. Glass stirring rods
3. Measuring cylinder-25 mL
4. Whatman No.541 (15.0 cm) filter paper
5. Glass filter funnels
6. Round-bottom, short-neck flasks-250 mL
7. Air oven, set at 100°C
8. Test tubes (29 x 200 mm)
9. Boiling water bath

10. Steam bath, located in the fume cupboard
11. Incubator: at 37°C.
12. pH meter
13. Pipettes-50 mL
14. Volumetric flasks-50 mL and 10 mL
15. Ultrafiltration equipment: using 0.45 µm, 47 mm diameter membrane filters
16. Ultrasonic bath for degassing solvent
17. WISP sample vials
18. High performance liquid chromatography: Waters ALC/GPC 244 with differential refractive index detector, WISP Automatic injector and Data module integrator.
    Column: a) (for solutions with low salt, <150 mg/100 g food) Silica Rad-Pak, type 8S MHP 4µ used with RCM 8x10 cartridge holder;
    b) (for solutions with high salt) NH_2 Rad Pak used with RCM 8x10 cartridge holder.

Procedures

A. Extraction and analysis of sugars

1. Switch on steam bath and boiling water bath.
2. Weight approximately 5 g wet, homogenised food sample into a 100 mL beaker. To approximately 5 g of the same sample, add 100 mL 85% EtOH and measure the pH. Then, if necessary add sufficient 0.5N NaOH to increase the pH tp 7.0±0.5. Add this volume of NaOH to the 85% EtOH used for extraction of the accurately weighed food sample. Also weigh a Whatman No. 541 filter paper.
3. Add 25 mL boiling 85% ethanol to beaker containing food sample, place on steam bath, cover with watchglass and stir frequently while the solution boils for a few minutes.
4. Remove from the steam bath and immediately filter the solution through the preweighed Whatman No.541 filter paper into a 250 mL round-bottom, short-neck flask.
5. Repeat extraction with 25 mL boiling 85% ethanol another three times.
6. Evaporate off the ethanol on a rotary evaporator at 45°C leaving behind an aqueous solution of approximately 3 mL.
7. The aqueous solution is transferred using a syringe or pipette with distilled water to a 10 mL volumetric flask and made up to volume with distilled water.
8. The solution is passed through an ultrafilter until about 2 mL is collected.
9. Samples are placed in WISP sample vials and placed in the WISP injector when sufficient samples have been collected for an analytical run.
10. Inject about 20 µL into HPLC. A standard (1%) solution of sugars is also injected to check retention times and calibration of peak areas.

B. Starch

11. While the extract is being treated as above, analysis of the residue remaining after extraction with 85% ethanol is also carried out. Transfer all residue back into the 100 mL flask whilst washing with acetone. Discard washings.
12. Place the filter paper and residue in pre-weighed beaker, place the beaker and contents in an air oven at 100°C for 30 min. Weigh, replace in oven for a further 15 min, and remove and reweigh. Repeat to constant weight.
13. Remove from the oven, allow it to reach room temperature, zero the analytical balance and weigh the beaker and contents.
14. Immediately grind and mix the residue, weigh out 300-400 mg residue and transfer to a test tube.
15. Add 100 mL distilled water, seal test tube with aluminium foil then place it in a boiling water bath for 4 hours, shaking occasionally.
16. Remove from water bath, cool to room temperature and then add 0.3 mL acetate buffer, followed by 0.4 mL amyloglucosidase and a few drops of toluene. The mixture is then shaken and incubated at 37°C overnight.
17. The mixture is transferred to a volumetric flask (50 mL) with ethanol (30 mL) and the contents are mixed and made up to volume with ethanol.
18. The solution including the precipitate is filtered through a sintered glass filter, under vacuum.
19. Remove a 25 mL aliquot and evaporate off the ethanol in a rotary evaporator at 40°C, leaving behind an aqueous solution of approximately 3 mL.
20. The aqueous solution is transferred with distilled water to a 10 mL volumetric flask and made up to volume.
21. The solution is passed through an ultrafilter and then injected into the HPLC using the same HPLC system as for the sugars.

Calculations

Sugars:

1. Measure peak area of individual sugars in samples and standard solution.
2. Calculate weight of sugar (g/100 g fresh food)

$$= \frac{\text{Area sugar x 10 mL}}{\text{Vol injected (µL)}} \quad \frac{\text{x vol standard (µL) x conc (g/100 mL)}}{\text{Area standard x 100}} \quad \frac{\text{x100}}{\text{wt food (g)}}$$

Therefore, deducing from the above formula, weight of sugar (g/100g fresh food)

$$= \frac{\text{Area sugar x vol standard (µL) x conc standard (g/100 mL)}}{\text{Area std x vol sample (µL) x wt food (g)}} \times \frac{10}{1}$$

Starch:

1. Measure peak area of glucose and standard.
2. Calculate weight of starch as polymer (g/100 g food)

$$= \frac{\text{Area sugar x 10 mL}}{\text{Vol sample (µL)}} \times \frac{\text{Vol Std (µl) x conc (g/100 mL)}}{\text{Area standard x 100}}$$

$$\times \frac{\text{2 x total residue (mg)}}{\text{Sample residue (mg)}} \times \frac{100}{\text{wet wt food (g)}} \times \frac{1}{1.1}$$

Therefore, deducing from the above formula, weight of starch polymr (g/100g food)

$$= \frac{\text{Area sugar x vol std (µL) x conc standard (g/100 ml) x total residue (mg)}}{\text{Area std x vol sample (µL) x sample residue (mg) x wet wt food (g) x 55}} \times 1000$$

Reference

Wills, R.B.H., Balmer, N. and Greenfield, H. (1980). Composition of Australian foods. 2. Methods of analysis. Food Technologies. Australia. 32: 198-204

3.3.1.8 DETERMINATION OF ENERGY (CALCULATION)

Energy level in calculated as below:

Energy, kcal/100g = [Fat] X 9 + [Protein] X 4 + [Carbohydrate] X 4
Energy, kJ/100g = kcal X 4.184

3.3.2 MINERALS

3.3.2.1 DIGESTION METHOD FOR MINERAL DETERMINATION

METHOD A:DRY ASHING

Principle

The method consists of preparing an ash by using heat and hydrochloric acid to decompose the organic matter, and dissolving the inorganic residue in an appropriate volume of dilute hydrochloric acid. The organic matter is first destroyed by dry ashing at 550^{O}C overnight or until a whitish or greyish ash is obtained. The ash is dissolved in concentrated hydrochloric acid and filtered, diluted to volume and read directly on the atomic absorption spectrophotometer.

Chemicals/Reagents

1. Concentrated Hydrochloric Acid (HCl)
2. Deionized water

Apparatus/Instruments

1. Silica crucible: diameter 3.5cm, depth 4cm for dry sample or bigger for liquid sample.
2. Desiccators: with silica gel desiccant.
3. Muffle Furnace: thermostatically controlled at 550°C.
4. Electric hotplate: thermostatic controlled.
5. Waterbath
6. Volumetric flask-100 mL
7. Silica basin
8. Pipettes: 2 & 5 mL
9. Whatman No. 42 filter paper
10. Glass rod
11. Glass filter funnel

Note:Use borosilicate glassware and silica basins, precleaned with dilute nitric acid and rinsed in distilled water immediately before use.

Procedures

1. Carry out ashing procedure as described for the determination of ash (page 54). Add 5 ml concentrated HCl into the silica basin, breaking up ash with glass rod. Dry over waterbath.
2. Add 2 ml concentrated HCl to dissolve the ash, filter through whatman No. 42 filter paper into 100 ml volumetric flask.
3. Wash residue 3 times with hot water.
4. Dilute to volume.

Reference

Association of Official Analytical Chemists (1984). Official Methods of Analysis. 14th Edition (Williams, S. ed.), AOAC, Virginia.

METHOD B: WET DIGESTION

Principle

The method consists of decomposition of an organic material into an ash by treatment with nitric acid.

Chemicals/Reagents

1. Nitric acid (HNO_3), 65% Suprapur (Merck)
2. Hydrogen Peroxide (H_2O_2)

Apparatus/Instruments

1. Balance: Analytical sensitivity ±0.1 mg
2. Hotplate
3. Conical flask: 250 mL
4. Volumetric flask: 100 mL
5. Measuring cylinders: 10 mL
6. Pipette
7. Pasteur pipette
8. Watch glass

Procedures

1. Weigh accurately 2-3 g of food samples.
2. Add 10 ml of HNO_3, cover with watch glass and soak sample overnight.
3. Heat the sample on a hotplate.
4. Add few ml of HNO_3 if there is still brown fume and the level of solution is reduced. Cool the sample first before adding the HNO_3.
5. Heat until no brown fume given off.
6. Stop the heating when white fume is released.
7. Heat gently the sample again.
8. Allow the sample to cool down approximately 10-15 minutes.
9. Mix 2 ml of H_2O_2 and deionized water (H_2O_2:H_2O) in a beaker. Add (H_2O_2:H_2O) mixture dropwise into the sample to prevent reaction from becoming too vigorous. If upon addition brown fumes are given off, go back to step 4. If the H_2O_2:H_2O mixture has been used up, use concentrated H2O2 for the dropwise addition, but do not exceed 10 mL. Add till there are no further colour changes of the sample.
10. Cool the sample and filtrate.
11. Make up to final solution of 100ml with 2% HNO_3.

Reference

Sim SF, Devagi K, Sung CL (2006). Evaluation of the acid digestion method with different solvent combination for the determination of iron, zinc and led in canned sardines. Malaysian Journal of Chemistry.8 (1):010-015.

METHOD C: MICROWAVE DIGESTION

Principles

This digestion method is facilitated by the application of microwave power and elements volatilization is avoided by using closed digestion system. Microwave digestion method possess advantages such as less time consumption, easy handling, reduce consumption of high purity reagents and minimize contamination compared to conventional digestion method.

Chemicals/Reagents

1. Concentrated nitric acid (HNO_3), 65%
2. Hydrogen peroxide (H_2O_2), 30%
3. Ultra-pure water

Apparatus/Instruments

1. Microwave oven: Multiwave 3000, Anton Paar.
2. 8-position digestion rotor: 8SXQ80, Anton Paar.
3. Quartz digestion vessel: XQ80, Anton Paar.[1]
4. Grinder
5. Balance: Analytical sensitivity ±0.1 mg.
6. Pipette: 1 &10 mL
7. Volumetric flask: 100 mL
8. PTFE bottle

Procedures

1. Dry sample with oven at 105°C until a constant weight obtained.
2. Grind sample into powder form with conventional grinder.
3. Weigh accurately 0.1-0.5 g of food samples into digestion vessel.
4. Add 6 ml of HNO_3 and 1 ml of H_2O_2 into the vessel.[2]
5. Make sure sample in the vessel completely wetted by the reagent and seal every vessel using vessel seal.
6. Allow pre-digestion of sample for 30 minutes.
7. Prepare a blank by adding same amount of HNO_3 and H_2O_2 into a blank vessel.
8. After 30 minutes, place vessel with protective jacket and cap into the digestion rotor, make sure the venting screw of the vessel screw cap is tighten.
9. Start digestion in microwave oven by using the setting below:[3]

Power (Watts)	Ramp (min)	Hold (min)	Fan
600	5:00	5:00	1
1200	5:00	25:00	1
0		15:00	3

10. After digestion is completed, remove rotor from microwave oven and cool them in fume hood.
11. Vent the vessel by rotating the release venting screw one half revolution, until the vessel is completely depressurized.
12. Remove the vessel seal cap and rinse it with deionized water.
13. Transfer the digested sample quantitatively into a volumetric flask and make up to volume of 100 ml.
14. Store the diluted sample in PTFE bottle and keep it in a cool place prior to analysis.

[1]Rotor of 8XF100, with PTFE-TFM vessel also applicable for food analysis.

[2]Different sample might require different ratio of HNO_3 and H_2O_2. Some samples might need the aid of other reagent like hydrochloric acid for better digestion result.

[3]The digestion program use in microwave oven is sample dependent.

References

Michael Zischka. Microwave assisted digestion for food analysis. Anton Paar Application Note. B83lA18A.

Miller, R. O. 1998. Microwave digestion of plant tissue in a closed vessel. In Kalra Y. P. (ed.). Methods for plant analysis, pp 69-73. Boca Raton: CRC Press.

3.3.2.2 DETERMINATION OF MAGNESIUM, COPPER, ZINC, CALCIUM, IRON, SODIUM AND POTASSIUM IN FOOD

METHOD A: ATOMIC ABSORPTION METHOD

Principles

The atomic absorption spectrophotometric method can be used satisfactorily for the determination of several minerals. Sample preparation is similar to that used for preparing samples for determination by the colorimetric methods. The organic matter is first destroyed either by dry ashing, wet ashing or microwave digestion method. The ashing sample is diluted to volume and read directly on the atomic absorption spectrophotometer. The quantity of food material taken for analysis depends upon the amount available and expected mineral content. In general, representative sample of 5-10 g are weighed.

Chemicals/Reagents

1. Deionized water. Use for preparing standards and dilutions.
2. Magnesium standard solutions.
   a. Stock solution - 1000 ppm (magnesium nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard – 0.1, 0.2 and 0.3 ppm.

3. Copper standard solutions.
   a. Stock solution - 1000 ppm (copper (II) nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard – 0.5, 1.0 and 1.5 ppm.

4. Zinc standard solutions.
   a. Stock solution - 1000 ppm (zinc nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard - 0.25, 0.5, 0.75, 1.0 ppm.

5. Sodium standard solutions.
   a. Stock solution - 1000 ppm (sodium nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard - 0.1, 0.2, 0.3, 0.4 and 0.5 ppm.

6. Potassium standard solutions.
   a. Stock solution - 1000 ppm (potassium nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard – 0.3, 0.6 and 0.9 ppm.

7. Calcium standard solutions.
   a. Stock solution - 1000 ppm (calcium nitrate standard solution for atomic absorption spectrophotometry)
   b. Working standard - 1,2,3, 4, 5 and 6 ppm.

8. Iron standard solutions
    a. Stock solution - 1000 ppm (ferric nitrate standard solution for atomic absorption spectrophotometry)
    b. Working standard – 1, 2 and 3 ppm.

Apparatus/Instruments

1. Perkin Elmer atomic absorption spectrophotometer: model AAnalyst 400
2. Micropipette
3. Volumetric flasks: 100 mL

Procedures

A. Digestion of sample

1. Carry out ash, wet or microwave digestion procedure as described under digestion method for mineral determination (page 70 to 75). The digestion sample is diluted to volume and read directly on the atomic absorption spectrophotometer.

B. Determination

1. Set up instrument as given in the Table below. Prepare instrument and computer software for use as given in the operating manual.
2. Prepare calibration curves for each of the minerals to be determined, using the standard solutions prepared.
3. Obtain at least 3 readings for each sample solution prepared.

Operating Parameters for Atomic Absorption Spectrophotometer

Element	Flame type	Lamp Current (mA)	Wavelength (nm)	Slit Width (mm)	Working range (ppm)
Perkin Elmer atomic absorption spectrophotometer model AAnalyst 400					
Mg	Air-Acetylene	6	285.21	2.7/1.05	0.1-0.3
Cu	Air-Acetylene	15	324.75	2.7/0.8	0.5-1.5
Zn	Air-Acetylene	15	213.86	2.7/1.8	0.25-1.0
Na	Air-Acetylene	8	589.0	1.8/0.6	0.1-0.5
K	Air-Acetylene	12	766.49	2.7/0.45	0.3-0.9
Ca	Air-Acetylene	10	422.67	2.7/0.6	1-5
Fe	Air-Acetylene	30	248.33	1.8/1.35	1-3

Calculation

Obtain from the printed report concentration of the element in the sample, expressed in ppm. Convert results to mg per 100 g sample as follows:

$$\text{Ppm} \times \frac{1}{1000} \times 100$$

References

Association of Official Analytical Chemists (1984). Official Methods of Analysis. 14th Edition (Williams, S. ed.), AOAC, Virginia.

AAnalyst 400 Atomic Absorption Hardware Guide. 2004. Perkin Elmer Inc. 710 Brideport Aveneu Shelton, Connecticut 06483-4794. USA.

METHOD B: INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) METHOD

Principles

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry that is highly sensitive and capable of determination of a range of metals and several non-metals at concentrations below one part in 10^{12} (part per trillion). It is based on coupling of an inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method of separating and detecting the ions. ICP-MS is also capable of monitoring isotopic speciation for the ions of choice. As a droplet of nebulized sample enters the central channel of the ICP, it evaporates and any solids dissolved in the liquid vaporize and then break down into atoms. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely-bound electron to form a singly charged ion. The ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

Chemicals/Reagents

1. Nitric acid, 2% (HNO_3). Use for preparing standards and dilutions
2. Sodium standard solutions
    a. Stock solution - 1000 ppm (sodium nitrate standard solution)
    b. Working standard – 1ppb-1000ppb

3. Magnesium standard solutions
    a. Stock solution - 1000 ppm (magnesium nitrate standard solution)
    b. Working standard – 1ppb-1000ppb

4. Potassium standard solutions
    a. Stock solution - 1000 ppm (potassium nitrate standard solution)
    b. Working standard– 1ppb-1000ppb

5. Iron standard solutions
    a. Stock solution - 1000 ppm (ferric nitrate standard solution)
    b. Working standard - 1ppb-1000ppb

6. Copper standard solutions
    a. Stock solution - 1000 ppm (copper nitrate standard solution)
    b. Working standard - 1ppb-1000ppb

7. Zinc standard solutions
    a. Stock solution - 1000 ppm (Zinc nitrate standard solution)
    b. Working standard - 1ppb-1000ppb

8. Calcium standard solutions.
    a. Stock solution - 1000 ppm (calcium nitrate standard solution)
    b. Working standard – 1ppb-1000ppb

Apparatus/Instruments

1. ICP-MS, Elan 6100 Perkin Elmer
2. Volumetric flasks: 25 & 10 mL
3. Micropipette

Procedures

A. Digestion of sample

1. Carry out ash, wet or microwave digestion procedure as described under digestion method for mineral determination (page 70 to 75). The digestion sample is diluted to volume and read directly on the ICPMS

B. Determination

1. Set up instrument as given in the Table below. Prepare instrument and computer software for use as given in the operating manual.
2. Prepare calibration curve for each of the minerals to be determined, using the standard solutions prepared.
3. Obtain at least 3 readings for each sample solution prepared.

ICP-MS operating condition

ICP-MS, Elan 6100 Perkin Elmer	
Spray Chamber	Scott spray chamber
Nebulizer	Cross-flow
RF power	950 watts
Argon nebulizer gas flow ($Lmin^{-1}$)	0.93 (adjusted daily to maximum signal intensity)
Measures	
Scan mode	Peak hopping
Resolution (amu)	0.7
Replicate time (s)	1
Dwell time (s)	50
Sweeps	20
Replicates	3
Isotopes	^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{56}Fe, ^{63}Cu, ^{66}Zn,

Calculation

Obtain from the printed report concentration of the element in the sample, expressed in ppb. Convert results to mg per 100 g sample as follows:

$$\text{Ppb} \times \frac{1}{10}$$

References

Baker, S.A, Bradshaw, D.K. & Miller-I, N.J. (1999).Trace Elements Determinations in Food and Biological Samples using ICP-MS. Atomic Spectroscopy: 20(5).

Chamberlain, I., Adams, K. & Le, S. (2000). ICP-MS determination of Trace Elements in Fish. Atomic Spectroscopy: 21(4).

Hua Zou & Jiang Hui Liu. The simultaneous Determination of 15 Toxic elements in Foods by ICP-MS (1997). Atomic Spectroscopy: 18(4).

3.3.2.3 DETERMINATION OF SELENIUM AND MANGANESE USING ICP-MS

Principles

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry that is highly sensitive and capable of determination of a range of metals and several non-metals at concentrations below one part in 10^{12} (part per trillion). It is based on coupling of an inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method of separating and detecting the ions. ICP-MS is also capable of monitoring isotopic speciation for the ions of choice. As a droplet of nebulized sample enters the central channel of the ICP, it evaporates and any solids that were dissolved in the liquid vaporize and then break down into atoms. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely-bound electron to form a singly charged ion. The ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

Chemicals/Reagents

1. Nitric acid, 2% (HNO_3). Use for preparing standards and dilutions
2. Selenium standard solutions
   a. Stock solution - 1000 ppm (Selenium nitrate standard solution)
   b. Working standard - 1ppb-1000ppb

3. Manganese standard solutions
   a. Stock solution - 1000 ppm (Manganese nitrate standard solution)
   b. Working standard - 1ppb-1000ppb

Apparatus/Instruments

1. ICP-MS, Elan 6100 Perkin Elmer
2. Volumetric flsks-25 & 10 mL
3. Micropipette

Procedures

A. Digestion of sample

1. Carry out ash, wet or microwave digestion procedure as described under digestion method for minerals determination (page 70 to 73).

B. Determination

1. Set up instrument as given in the Table below. Prepare instrument and computer software for use as given in the operating manual.
2. Prepare calibration curves for each of the minerals to be determined, using the standard solutions prepared.
3. Obtain at least 3 readings for each sample solution prepared.

ICP-MS operating condition

ICP-MS, Elan 6100 Perkin Elmer	
Spray Chamber	Scott spray chamber
Nebulizer	Cross-flow
RF power	950 watts
Argon nebulizer gas flow ($Lmin^{-1}$)	0.93 (adjusted daily to maximum signal intensity)
Measures	
Scan mode	Peak hopping
Resolution (amu)	0.7
Replicate time (s)	1
Dwell time (s)	50
Sweeps	20
Replicates	3
Isotopes	^{34}Se ^{55}Mn

Calculation

Obtain from the printed report concentration of the element in the sample, expressed in ppb. Convert results to mg per 100 g sample as follows:

$$\text{Ppb} \times \frac{1}{10}$$

References

Baker, S.A, Bradshaw, D.K. & Miller-I, N.J. (1999).Trace Elements Determinations in Food and Biological Samples using ICP-MS. Atomic Spectroscopy: 20(5).

Chamberlain, I., Adams, K. & Le, S. (2000). ICP-MS determination of Trace Elements in Fish. Atomic Spectroscopy: 21(4).

Hua Zou & Jiang Hui Liu. The simultaneous Determination of 15 Toxic elements in Foods by ICP-MS (1997). Atomic Spectroscopy: 18(4).

3.3.2.4 DETERMINATION OF IODINE IN FOOD

Principles

Iodine is essential for the production of thyroid hormones. These hormones stimulate metabolism in the body, as well as mental growth and development. The recommended daily iodine intake is 0.1-0.2 mg, with the most common sources of iodine being fish, seafood, milk and food supplements. The determination of iodine in food has always been difficult due to the low concentrations (mg/kg), difficult sample preparation and the volatility of iodine.

Chemicals/Reagents

1. Iodide standard solutions
    a. Stock solution -1000ppm
    b. Working standard-5, 10, 15, 20 µg/L
2. Tetramethylammonium hydroxide, 25% (TMAH)
3. Tetramethylammonium hydroxide, 0.5% (TMAH)
4. Deionized water

Apparatus/Instruments

1. ELAN DRC™ II ICP-MS
2. Oven
3. Centrifuge
4. Balance: Analytical sensitivity ± 0.1 mg
5. PFA tube
6. Volumetric flasks: 10, 100 & 1000 mL
7. Measuring Cylinder: 10 & 50 mL
8. Whatman No. 541 filter paper
9. Micropipettes

Procedures

A. Sample Preparation

1. Prior to weighing, the samples are mixed or slightly crushed.
2. 0.25-0.5 g of samples is then added to PFA tubes, to be followed by 4.5 mL H20 (Milli-Q) and 1 mL TMAH (25%).
3. After capping, the tubes are placed in a drying oven at 90 °C for 3 hours.
4. After cooling, Milli-Q® water is added to a final volume of 10 mL. These solutions are then centrifuged at 3000 rpm for 15 minutes.
5. If any visible particulates remained after centrifuging, the samples were then filtered. The resulting solutions can then be analyzed directly or with an extra dilution if high matrix concentrations are present.

B. Determination

1. Set up instrument as given in the Table below. All measurements are to be done in standard mode.
2. Calibration standards ranging from 5 to 20 are to be used, prepared in 0.5% (v/v) TMAH.
3. After each standard and sample are analyzed, a 45 second rinse with of 0.5% TMAH is to be performed.

Instrumental Conditions:

ELAN DRC™ II ICP-MS	
Spray chamber	Cyclonic
Nebulizer	Meinhard®
Sample Uptake Rate	1 mL/min
RF Power	1100 W
Plasma Gas Flow (L/min)	15
Nebulizer Gas Flow (L/min)	0.93
Auxiliary Gas Flow (L/min)	1.2
Dwell Time (ms)	50
Sweeps per reading	25
Replicates	3
Delay Time (s)	50
Wash Time (s)	45

Reference

Khalid B & Fabien B, Scientific Institute of Public Health Brussels, Belgium in PerkinElmer Scienx Note. 2006-2009.

3.3.2.5 DETERMINATION OF PHOSPHORUS IN FOOD

Principles

The food sample is first ashed and the ash taken up with concentrated hydrochloric acid. Phosphate in the solution is then determined based on the Misson's reaction. Phosphorus present as orthophosphate reacts with a vanadate-molybdate reagent to produce a stable yellow-orange complex of vanadi-molybdiphosphoric acid, the optical density of which is measured at 420 nm.

Chemicals/Reagents

1. Vanadate-molybdate composite reagents
   Dissolve 20g ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in 400 ml warm water (50^oC) and cool. Dissolve 1.0 g ammonium metavanadate (NH_4VO_3) in 300 ml boiling distilled water, cool and add 140 ml concentrated nitric acid gradually with stirring. Then add the molybdate solution gradually to the acid vanadate solution with stirring and dilute to a litre with water.

2. Standard phosphate solution
   a. Stock solution. Dissolve 4.39 g potassium dihydrogen phosphate (KH_2PO_4) in water and dilute the solution to l litre. This solution contains l mg P per ml.
   b. Working solution. Dilute 5 ml of the stock solution to 100 ml with water. This solution contains 0.05 mg P per ml.

Apparatus/Instruments

1. Spectrophotometer
2. Waterbath
3. Balance: Analytical sensitivity ± 0.1 mg
4. Volumetric flasks: 100 & 1000 mL
5. Measuring cylinder: 25 mL
6. Glass rod

Procedures

Use ASH SOLUTION for this determination.

1. Preparation of ASH SOLUTION:
    a. Add 5 ml concentrated hydrochloric acid to the ash with rinsing of upper portions of the dish.
    b. Evaporate to dryness over water-bath.
    c. Add 2 ml of concentrated hydrochloric acid.
    d. Cover the dish with a watch glass and heat until the solution begins to boil.
    e. Add 20 ml distilled water, washing down the watch glass and filter through Whatman No. 42 paper into a 100 ml flask.
    f. Wash dish, residue and filter paper with 10 ml portions of boiling water, passing washings through filter into the 100 ml flask.
    g. Cool and make up to 100 ml. This solution is referred to as the ASH SOLUTION.
2. Transfer a suitable volume of this solution, containing 0.1 - 2 mg P (1-2 ml for most foods) to a 100 ml volumetric flask.
3. Prepare another flask for reagent blank.
4. Add 25 ml of the vanadomolybdate reagent to both flasks.
5. Dilute immediately to the mark with water.
6. Allow to stand for 10 minutes.
7. Read O.D. at 420 nm after zeroing the spectrophotometer using the reagent blank. The colour obtained is stable for a few hours.

Prepare a standard curve as follows:

1. Transfer a suitable volume of this solution, containing 0.1 - 2 mg P (1-2 ml for most foods) to a 100 ml volumetric flask.
2. Add 2.5, 5, 10, 20, 30, 40 and 50 ml of the working phosphate standard solution (containing 0.125-2.5 mg P) to a series of 100 ml volumetric flasks.
3. Add 25 ml of the vanadomolybdate reagent to each flask.
4. Dilute immediately and make up to the mark with water for all flasks.
5. Allow to stand for 10 minutes.
6. Read colour formed at 420 nm after zeroing the instrument with the reagent blank.

Calculation

Plot a standard curve of O.D versus mg P. Read off from this curve the concentration of P corresponding to the O.D. of the test solution. Phosphorus content of the sample being analysed can then be calculated as:
mg P in 100 g sample =

$$\text{mg P corresponding to test O.D. (from standard curve)} \times \frac{\text{total volume of ash solution}}{\text{volume of ash solution used}} \times \frac{100}{\text{weight, in g, of food sample}}$$

Reference

Egan, H., Kirk, R.S., and Sawyer, R. (1981). Pearson's Chemical Analysis of Foods. 8th Edition, Churchill Livingstone, London; pp. 29-30.

3.3.3 FAT SOLUBLE VITAMINS

3.3.3.1 DETERMINATION OF VITAMIN A AND CAROTENOIDS IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

High-pressure liquid chromatography (HPLC) has become a widely used procedure for accurate quantification of various carotenoids and retinoids in foods, mainly because of its ability to effect rapid separation, its non-destructiveness and, more importantly, the better resolution that is achieved.

The method developed by this laboratory is a non aqueous reverse-phase HPLC procedure, employing a ternary mixture of acetonitrile, methanol and ethyl acetate (88:10:2, v/v) as the mobile phase and a μBondapak C_{18} column. To enable simultaneous detection and quantitation of retinol and carotenoids, a UV-visible detector capable of dual wavelength detection is to be used. The detector is to be set to detect retinol at 325 nm and carotenoids at 450 nm.

The method is suitable for foods of animal origin and other foods in which preformed vitamin A and carotenoids are expected to be present, e.g. processed or composite foods. Various sample preparation procedures have to be carried out first, as described for the open column chromatography method.

Chemicals/Reagents

1. Acetonitrile, HPLC grade
2. Methanol, HPLC grade
3. Ethyl acetate, HPLC grade
4. Ethanol, analytical grade
5. Hexane, analytical grade
6. Benzene, analytical grade
7. Mixture of acetonitrile, methanol and ethyl acetate (88:10:2, v/v) as mobile phase

   Pipette 20 ml ethyl acetate into a one litre measuring cylinder. Add 100 ml of methanol into one litre with acetonitrile. Filter solvents through a 0.45 μm nylon membrane filter using the solvent filtration kit and degassed using an ultra-sonic bath.

8. Vitamin A standard solution

   a Retinol stock solution. Use 70% trans retinol (e.g. from Sigma Chemical Co.) as standard for vitamin A determination. Cut ampoule of 25 mg and dissolve

to 250 ml volume with absolute alcohol to give a suitable stock of 100 μg/ml and store in amber bottles in the freezer.

b Retinol working solution for spectrophotometric reading. Using micropipette, pipette 200 μl of stock solution of 100 μg/ml to make up to 10 ml with absolute alcohol to give a working solution of 2 μg/ml.

9. β-Carotene standard solution

   a Use synthetic crystalline trans-beta-carotene (e.g. from Sigma Chemical Co.) as standard for carotene determination. Weigh 10 mg of standard and make up to 100 ml with hexane to give a stock solution of 100 μg/ml and store in amber bottles in the freezer.

   b β-carotene working solution for spectrophotometric reading. Use micropipette, pipette 200 μl of stock solution of 100 μg/ml and make up to 10 ml volume with hexane to give a working solution of 2 μg/ml.

10. α-Carotene standard solution

   a Use α-carotene from carrots (e.g. Type V from Sigma Chemical Co.) as standard. Cut ampoule of 10 mg and make up to 100 ml volume with hexane to give a stock of 100 μg/ml and store in amber bottles in the freezer.

   b α-carotene working solution for spectrophotometric reading. Use micropipette, pipette 200 μl of stock solution of 100 μg/ml and make up to 10 ml volume with hexane to give a working solution of 2 μg/ml.

11. Lycopene standard solution

   a Use lycopene from tomato (e.g. from Sigma Chemical Co.). Cut ampoule of 5 mg and make up to 25 ml volume with benzene to give a stock of 200 μg/ml and store in amber bottles in the freezer.

   b Lycopene working solution for spectrophotometric reading. Using micropipette, pipette 100 μl of stock solution of 200 μg/ml and make up to 10 ml with benzene to give a working solution of 2 μg/ml.

12. Standard mixture solution for HPLC injection. Using micropipettes, pipette the following amount of retinol and carotenoid stock into a 10 ml volumetric flask, make up to volume and then filter with 0.45 μm regenerated cellulose membrane filter before injecting 50 μl into HPLC

   a 30 μl of 100 μg/ml of retinol stock to give a solution of 0.3 μg/ml.

   b 20 μl of 100 μg/ml of β-carotene stock to give a solution of 0.2 μg/ml.

   c 20 μl of 100 μg/ml of α-carotenel stock to give a solution of 0.2 μg/ml.

   d 5 μl of 200 μ/ml of lycopene stock to give a solution of 0.1 μg/ml.

Note: Preparation of all standards for retinol and carotenoids should be carried out in a room with subdued light and windows tinted with a light-protective film. All sample treatment and analytical procedures were also carried out in this room.

Apparatus/Instruments

1. Balance: Analytical sensitivity ± 0.1mg
2. Vortex mixer
3. Glass balls
4. Measuring cylinder: 50 mL
5. Round-bottom, short-neck flasks: 250 mL
6. Round-bottom, short-neck flasks: 250 mL
7. Steam bath: located in the fume cupboard
8. Pipettes: 5 mL
9. Micropipettes
10. Volumetric flasks: 10 mL
11. Separating funnel
12. Reflux apparatus
13. Electric heating mantle
14. Conical flask: 250 mL
15. Pasteur pipette
16. Ultrafiltration equipment: using 0.45 µm, 47 mm diameter membrane filters
17. Ultrasonic bath for degassing solvent
18. Syringe filter: 0.45 µm nylon
19. Syringe
20. Amber vials

HPLC conditions

1. 10-µm µBondapak C_{18} stainless steel column of 30 cm x 3.9 mm I.D. (e.g. from Waters).
2. Guard column holder housing a disposable pre-column insert packed with the same material as that of the analytical column.
3. A Gilson 234 autoinjector or 7125 Rheodyne injector with a 500 µl variable loop
4. A Gilson 305 piston pump to deliver the mobile phase.
5. A Gilson 805 manometric module to dampen the pulsations of the pump and to supply the current pressure value to the 305 piston pump.
6. A computer with a Gilson System Controller Software (i.e. the 715 controller software).
7. A HP LaserJet 5L printer to print the results and chromatograms.
8. Gilson UV 119 detector with dual wavelength detection (i.e. 325 nm for retinol detection and 450 nm for carotenoids).

Procedures

A. Alkaline hydrolysis

1. Weigh accurately 10 g of the foodstuff into a 250 ml boiling flask.
2. Add 40 ml of 95% alcohol and 40 ml of water to sample. Mix thoroughly.
3. Add slowly, with mixing, a volume of 100% potassium hydroxide solution equal to the weight of the sample.
4. Add a few boiling chips.
5. Attach flask to water-cooled refluxing apparatus. Heat on water-bath or electric heating mantle.
6. Adjust temperature to give a reflux rate of about 2 drops per second.
7. Agitate the flask frequently during refluxing to disperse any aggregates formed.
8. Reflux for 30 minutes from the time it starts boiling.
9. Cool the flask rapidly to room temperature.

B. Extraction

1. Extract the hydrolysate 3 times with 50-ml aliquots of hexane.
2. Combine the hexane extracts and wash with distilled water until washings are free of alkali (test with phenolphthalein).
3. Pass hexane extract through anhydrous sodium sulphate to dry.
4. Reduce volume of extract by heating on a water-bath at 60-65^{o}C under nitrogen.
5. Transfer to a 10 ml volumetric flask and make up to mark with hexane (V_1).

C. Instrument set up procedures

1. Warm up instrument for at least 1 hour before starting
2. Set the Gilson UV 119 detector to the dual wavelength mode (i.e. 325 nm at 0.01 aufs and 450 nm at 0.002 aufs)
3. Check that the solvent bottle is filled with the filtered and degassed mobile phase and immerse the inlet tubing filter into the solvent reservoir.
4. Bypass column by turning the knob above the injector anti-clockwise.
5. Attach the syringe to the luer fitting of the low pressure prime valve.
6. Draw liquid into the syringe with the low pressure prime valve in the SYRINGE-LOAD position.
7. Turn the vave to the SYRINGE-INJECT position, press the PRIME key on the front panel of the 305 piston pump; the pump will start running at its maximum speed (i.e. 10 ml/minute).
8. When no bubbles can be seen at the outlet tubing, press the STOP softkey to end the priming procedure.

9. Turn the valve to the RUN position, remove the syringe from the low pressure prime valve and remember to turn the knob above the injector clockwise so as to allow mobile phase to flow through the column.
10. Using the 715 software, open the mobile phase window and gradually increase the flow rate of the mobile phase to 2 ml/minute in 2 minutes.
11. Allow solvents to flow for at least 1 hour to equilibrate the column.
12. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards are the same for the 2 runs then the column is ready for sample injection.

D. Chromatography of carotenoids and retinol

1. Hexane in the extract is first evaporated off on a water-bath and the residue to immediately redissolve in a suitable volume of the mobile phase.
2. After filtering through a 0.45 μm nylon membrane filter (using a sample filtration kit), suitable volumes are injected into the chromatograph.
3. Identification and quantitation of carotenoids are carried out by comparing with reference retinol and carotenoids similarly chromatographed.
4. The appropriate extinction coefficients published in the literature (De Ritter, 1981) are used to calculate the exact concentration of each of the carotenoids.
5. Total peak areas from the chromatograms are used to determine the total carotenoid content, calculated using β-carotene standard curve.

Calculation

μg vitamin A alcohol (retinol) or μg carotene per 100g food =

$$\frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in } \mu g \times \frac{V1\ (ml)}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Tee, E.S. and Lim, C.L. (1992). Re-analysis of vitamin A values of selected Malaysian foods of animal origin by the AOAC and HPLC methods. Food Chemistry, 45:289-296.

3.3.3.2 DETERMINATION OF VITAMIN D IN FOOD

METHOD A: VITAMIN D IN INFANT FORMULAS (AOAC METHOD 995.05)

Principles

Test portions is saponified, extracted and evaporated to concentrate nutrient. Vitamin D is determined by reversed-phase LC equipped with UV detector at 265nm.

Reagents/Chemicals

(a) Solvents (HPLC grades)-N-hexane, dichloromethane, acetonitrile, isopropanol, methanol, ethyl Acetate,
(b) Ethanol-Absolute, Pharmaceutical grade
(c) Phenolphthalein solution-1%- Dissolve 1 g phenolphthalein in 100ml absolute ethanol
(d) Dichloromethane-isopropanol (IPA) solutions-
    (1) 99.8 + 0.2 (v/v) –transfer 2 ml isopropanol into 1L volumetric flask. Dilute to volume with dichloromethane and mix
    (2) 80 + 20 (v/v)-transfer 200 ml isopropanol inti 1L volumetric flasks, dilute to volume with dichloromethane and mix
(e) Acetic acid solution-10% -Transfer 10ml glacial acetic acid (AR grade) into 100 ml volumetric flask dilute to volume with H_2O and mix well.
(f) Ethanolic potassium hydroxide (KOH) solution-Dissolve 140g KOH pellets (AR grade) in 310 ml absolute ethanol and add 50mL H_2O. Prepare on day of use.
(g) Mobile phase-Gradient mixture of acetonitrile, methanol and ethyl acetate. See **Table 9995.05B** for concentration of mobile phase components.
(h) Vitamin D_2 standard solutions-
    (1) Stock standard solution (180 µg/ml)-Accurately weigh 45 mg vitamin D_2 and quantitatively transfer to 250 ml amber volumetric flask. Dilute to volume with absolute ethanol.
    (2) Working standard solution (2.88 µg/ml)-Transfer 4.0 ml stock standard solution into 250 ml amber volumetric flask and dilute to volume with absolute ethanol. Store in refrigerator ≤7 days
    (3) Internal standard solution (46 ng/ml). Use to quantitate vitamin D_3. Transfer 4.0 ml stock standard solution into 250 ml amber volumetric flask and dilute to volume with absolute ethanol. Store in refrigerator ≤7 days
(i) Vitamin D3 standard solutions-USP reference standard or traceable secondary standard. Prepare and store in refrigerator ≤7 days
    (1) Stock standard solution (180 µg/ml)-Accurately weigh 45 mg vitamin D_2 and quantitatively transfer to 250 ml amber volumetric flask. Dilute to volume with absolute ethanol.
    (2) Working standard solution (2.88 µg/ml)-Transfer 4.0 mL stock standard solution into 250 ml amber volumetric flask and dilute to volume with absolute ethanol. Store in refrigerator ≤7 days
    (3) Internal standard solution (46 ng/ml). Use to quantitate vitamin D_3.
(j) System suitability standard solution-Dissolve 125 mg certified vitamin D_2 standard and 125 mg certified vitamin D3 standard in 10 ml acetonitrile. Heat solution 45 min

at 90°C under reflux, and then cool. Transfer 1.0ml refluxed solution to 100ml amber volumetric flask and dilute to volume with acetonitrile.

(Note: Protect vitamin D_2 and D_3 standard solutions from high temperature, oxygen, and light to minimize degradation).

Apparatus/Instruments

1. Liquid chromatograph (LC)- with UV detector, meeting system suitability requirements. Operate at 27 ± 1°C higher temperature results in loss of resolution.2.
2. LC Colum-250 X 4.6 mm id, C18, 5µm particle size. Operating conditions:injection volume 250µl; column temperature, 27°C; wavelength, 265 nm; flow rates, see **Table 9995.05B** for flow rates; stop time, 35 min; retention times:vitamin D2, 19.5 min; vitamin D3, 23 min.

Table 995.05B Flow rates and concentrations of mobile phase components for determination of vitamin D

Time, min	Flow rate, ml/min	Acetonitrile %	Methanol %	Ethyl acetate %
0.0	0.7	91.0	9.0	0.0
28.0	0.7	91.0	9.0	0.0
28.5	2.5	0.0	0.0	100.00
31.0	2.5	0.0	0.0	100.00
31.5	2.5	91.0	9.0	0.0
33.0	2.5	91.0	9.0	0.0
34.0	0.7	91.0	9.0	0.0

3. Solid-phase extraction (SPE) column-Silica; 500 mg/2.8 ml
4. Vaccum manifold-For SPE column (c)
5. Evaporator-With N flow
6. Rotary Evaporator
7. Water bath shaker-Maintaining 60°C
8. Separatory funnel-250 ml
9. Amber volumetric flask-250 ml
10. Reflux apparatus

Procedures

A. Preparation of Standard Mixture and Test Portions

1) Standard mixture-Transfer 4.0ml vitamin D_2 internal standard solution, (h) and 4.0ml vitamin D_3 internal standard solution, (i) into Erlenmeyer flask and add 15 ml H_2O.
2) Test portion-prepare formula or enteral product according to label directions and use as test sample. Target vitamin D concentration will be 0.5IU/ml. Transfer 15.0ml test portion into 125ml Erlenmeyer flask using pipette or syringe (depending on viscosity). Add 4.0ml internal standard solution to flask. Use vitamin D_2 standard solution, (h)(3) to quantitate vitamin D_3 and vitamin D_3 standard solution, (i)(3) to quantitate vitamin D_2.

B. Saponification and Extraction

1. Add 15.0ml aliquots of ethanolic KOH solution, (f) to standards mixture, A1 and test portion from A2. Close flasks with stoppers and place them in water bath shaker for 30 min at 60ºC. Remove flask from shaker and let cool to room temperature.
2. Transfer contents of flask to 250ml separatory funnels. To empty flask add 15 ml H_2O, close with stopper and shake vigorously. Transfer rinse to corresponding funnel, rinse empty flask again with 60 ml hexane and transfer rinse to funnel. Close funnel with stopper and shake vigorously for 90s. Let layers separate ca 10 min. Drain and discard aqueous layer. Add 15 ml H_2O to hexane layer remaining in funnel, close funnel with stopper and shake vigorously. Let layers separate, drain and discard aqueous layer.
3. Add 1 drop phenolphthalein solution, (c) and 15.0ml H_2O to funnel. Add 10% acetic acid solution, (e) to funnel, dropwise with shaking, until washing is neutral to phenolphthalein (colourless). Drain and discard aqueous layer. Drain hexane layer through Na_2SO_4, supported by small cotton plug, into 100 ml round –bottom flask. Rinse funnel and Na_2sO_4 with few ml hexane, collecting hexane in same round-bottom flask.

C. Evaporation and solid-phase extraction

1. Place round bottom flask on rotary evaporator and evaporate hexane to dryness at 40°C. Immediately after evaporation, add 2.0ml dichloromethane-IPA solution, (l) to flask.
2. Wash SPE column with 4.0 ml dichloromethane-IPA solution, C(d)(2) and then with 5.0mllll dichloromethane-IPA solution, C(d)(l). Transfer solution from round-bottom flask to SPE column. Rinse empty flask with 1ml dichloromethane-IPA solution, C(d)(l) and transfer rinse to SPE column. Wash SPE column with 2.0ml dichloromethane-IPA solution, (l) and discard this fraction. Elute vitamins D_2 and D_3 with 7ml dichloromethane-IPA solution (l) into 16 x 100mm disposable culture tube. Place culture tube in water bath and evaporate dichloromethane-IPA solution at 40°C using N. When dry, immediately add 1 ml acetonitrile to tube and swirl to rinse down sides of tube. Transfer solution to LC vial using disposable dropper.

D. Chromatoghraphic Determination

1. Inject standards mixture, into LC column at beginning, middle and end of each run of test solutions.
2. To calculate content of vitamin D_2 and D_3, use internal standard procedure and peak heights.

E. Calculations

1. Perform following calculations:

(l) Calculate concentration of vitamin D_2 in standards mixture (CSD_2) as follows:

$$CS_{D2}(IU/ml)=\frac{W \times 4 \times 4 \times 4 \times 40000}{250 \times 250 \times 250} \times 1.05$$

Where W= weight of vitamin D_2 certified reference standard,mg, 4= dilution factor, 40 000=IU vitamin D/mg; 250= volumes of subsequent dilutions of vitamin D_2 standard solutions, 1.05=correction factor for pre-vitamin D.

(2) Calculate concentration of vitamin D_3 in standards mixture (CS_{D3}) as above using weight of vitamin D_3 certified reference standard.

(3) Calculate response ratio of vitamin D_2 in standards mixture (RS_{D2}) as follows:

$$RT_{D2}=\frac{PT_{D2}}{PT_{D3}}$$

Where PS_{D2}=peak height of vitamin $_{D2}$ in standard mixture and PS $_{D3}$=peak height of vitamin D_3 in standards mixture.

(4) Calculate response ratio of vitamin D_3 in standards mixture (RS_{D3}) as above, using peak heights of vitamins D_3 and D_2 respectively.

(5) Calculate response ratio of vitamin D_2 in test portion (RT_{D2}) as follows:

$$RT_{D2}=\frac{PT_{D2}}{PT_{D3}}$$

Where PT_{D3}=peak height of vitamin D_2 in test portion and PT_{D3}=peak height of vitamin D_3 in test sample.

(6) Calculate response ratio of vitamin D_3 in test portion (RT_{D3}) as above, using peak heights of vitamin D_3 and D_2 respectively.

(7) Calculate concentration of vitamin D_2 (CT_{D2}) or D_3 (CT_{D3}) in test portion as follows:

$$CT_{D2}\ (IU/ml) = \frac{RT_{D2} \times CS_{D2} \times U \times D}{RT_{D2} \quad V1}$$

$$CT_{D2}\ (IU/ml) = \frac{RT_{D3} \times CS_{D3} \times U \times D}{RT_{D3} \quad V1}$$

Where V1=volume of test portion, mL (usually 15); U= conversion factor to appropriate units (if necessary) and D =dilution factor for diluted powders or liquids.

F. System Suitability

For system suitability use standards mixture, D (i) during routine operation. Resolution factor between vitamin D_2 and vitamin D_3 should be 2.0. Calculate resolution factor as follows:

$$R = \frac{2(t2-t1)}{W1 + W2}$$

Where t1 and t2=elution times for vitamins D_2 and D_3 respectively and w1 and w2 = peak widths of vitamins D_2 and D_3. Peak widths are measured as the time interval between the points where the baseline intersects the 2 lines are produced by extending the relatively straight sides of the peak.

Separation between these peaks should be sufficient to allow additional peak to be resolved between vitamin D_2 and D_3 namely pre-vitamin D_3.

To verify that pre-vitamin D_3 is resolved, inject system suitability standard solution, C(j). The system suitability standard solution contains, in order of elution, pre-vitamin D2, vitamin D_2, pre-vitamin D_3 and Vitamin D_3.

Optimize chromatography by adjusting amount of methanol in mobile phase, decrease in methanol content increase retention times. Maintain column temperature at 27 ± 1°C; increased temperature decreases retention and vice versa. Six replicate injections of standard should have < 2% relative standard deviation (RSD). RSD values for standards throughout run should be <4%.

Reference

J.AOAC Int. 75, 566(1992);79, 73 (1996)

METHOD B: VITAMIN D IN FOOD USING HPLC WITH UV DETECTOR

Principles

The method for Vitamin D_2 and D_3 is a reverse phase HPLC procedure, employing mixture of acetonitrile and methanol (75:25, v/v) as the mobile phase at a flow rate of 2.3 ml/min and a C18 column. The UV detection of the evaluate is normally performed at 282 nm. The vitamins D_2 and D_3 and are determined by comparing the retention times of standards obtained, and quantification is done by using a calibration curve.

Chemicals/Reagents

1. Vitamin D_2 and D_3 standard
2. Cholecalciferol as the internal standard
3. Sodium ascorbate
4. Sodium hydroxide, NaOH
5. Ethanol (95% pure)
6. Potassium hydroxide (85% pure)
7. Ethanol
8. n-pentane
9. Potassium Hydroxide, KOH
10. Mixture of acetonitrile and methanol (HPLC grade, Merck Chemicals (75/25, v/v) as the mobile phase:
    Add 750 ml acetonitrile into a one litre measuring cylinder. Add 250 ml of methanol. Filter solvents through a 0.45 µm nylon membrane filter using the solvent filtration kit and degas using an ultrasonic bath.

Apparatus/Instruments

1. HPLC Condition:
   a) A Waters 600E HPLC system equipped with Waters 486 tunable absorbance UV detector (Waters, Milford, MA, USA).
   b) Eluted through a reverse phase C18 column (Maxsil 5 C18, 250 · 4.6 mm, Phenomenex, Torrance, CA, USA).
2. Balance: Analytical sensitivity ± 0.1 mg
3. Rotary evaporator
4. Round bottom flask: 250 mL
5. Measuring cylinder: 10 , 25 & 50 mL
6. Reflux apparatus
7. Separating funnel
8. Conical flask: 250 mL
9. Volumetric flasks: 5 ml
10. Pasteur pipette
11. Ultrafiltration equipment: using 0.45 µm, 47 mm diameter membrane filters
12. Ultrasonic bath: for degassing solvent
13. Syringe filter: 0.45 µm non-pyrogenic filter
14. Syringe
15. Vials

Procedures

A. Preparation of Sample

1. 0.5 g of samples are to be accurately weighed into 250 ml round bottom flasks and mixed with 4 ml of sodium ascorbate solution (17.5 g of sodium ascorbate in 100 ml of 1 M NaOH), 50 ml of ethanol (95% pure), 10 ml of 50% potassium hydroxide (85% pure) and 50 µg of cholecalciferol as the internal standard.
2. The mixture is to be saponified under reflux at 80ºC for 1h, then, it is to be immediately cooled to room temperature and transferred into a separating funnel.
3. The mixture is first to be extracted with 15 ml de-ionized water, followed by 15 ml ethanol and then with three-stages of n-pentane of volumes 50, 50 and 20 ml, respectively.
4. The pooled organic layers are washed three times with 50 ml of 3% KOH in 5% ethanol and then finally with de-ionized water until neutralized.
5. The organic layer is to be transferred into a round bottom flask, rotary evaporated to dryness at 40ºC, and immediately re-dissolved in 5 ml ethanol.
6. The samples are passed through a 0.45 µm non-pyrogenic filter.
7. A volume of 20 µl of filtered sample is injected into HPLC system.

Reference

Jasinghe, V.J. and Perera, C.O. 2005. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV irradiation. Food Chemistry, 92:541-546

3.3.3.3 DETERMINATION OF VITAMIN E IN FOOD

METHOD A: VITAMIN E IN FOOD USING HPLC WITH FLUORESCENE DETECTOR

Principles

Tocopherols and tocotrienols content in the food samples are detected and quantified using isocratic normal phase High Performance Liquid Chromatography (HPLC).

Chemicals/Reagents

1. Tocopherol standard solution, Davos, Singapore
2. Tocotrienol standard solution, Davos, Singapore
3. Internal standard, PMC (2,2,5,7,8-Pentamethyl-6 chromanol), Sigma-Aldrich, USA
4. Hexane, Merck, Germany
5. Isopropyl alcohol, J.T. Baker, USA
6. Ethanol, Merck, Germany
7. Dioxane, R&M, UK
8. NaCl, Merck, Germany

Apparatus/Instruments:

1. Balance: Analytical sensitivity ± 0.1 mg
2. Centrifuge
3. Nitrogen gas blower
4. Micropipettes
5. 12ml test tube: with screw cap
6. 7 ml Trident vial
7. IKA-VIBRAX-VXR shaker

Procedures

A. Extraction of food samples

1. Measure 1-5 g of homogenize food sample into a 12 ml test tube.
2. Add 1 ml 0.9% NaCl shake vigorously, and add 1 ml ethanol and 4 ml hexane.
3. Shake for 1 hour at 1000 rpm with IKA-VIBRAZ-VXR shaker.
4. Centrifuge at 2000 rpm for 15 minutes and transfer to 7 ml Trident vial.
5. Put 2 ml hexane into the 12 ml test tube.
6. Repeat the extraction.
7. Combine the supernatant and dry by blowing with nitrogen gas.
8. Reconstitute the sample with hexane.
9. Sample is ready to be injected into HPLC.

B. HPLC conditions

1. The determination of vitamin E tocopherol and tocotrienol are using HPLC (High Performance Liquid Chromatography).
2. The HPLC system used is Agilent 1100 series.

3. The HPLC is equipped with Agilent model FLD G1321 A fluorescence spectrophotometer and Agilent Chemstation for LC System Rev. A.06.0x.
4. The mobile phase consists of 970 hexane : 25 dioxane : 5 propyl alcohol (v/v).
5. The mobile phase is delivered and degassed with Agilent G1311A Quaternary Pump and JP73021896 Degasser at 1 ml / min flow rate.
6. The column used is Phenomenex® Luna 5 μ (250 x 4.6 mm I.D, 5 μM).
7. The injection system used is Agilent Auto Injector ALS G1313A and injection volume is 40 μl.
8. The fluorescence detector is set at excitation wavelength of 295 nm and emission wavelength of 325 nm.
9. The standard solution is prepared from 0.05 μg / ml to 10 μ g / ml consists of α, β, γ and δ tocopherol and tocotrienol including PMC as internal standard.
10. The calibration curve is based on the linearity of peak area response verses standard dilution / solution.
11. The sample is injected into HPLC and analyse with Agilent Chemstation.

References

Nielsen M.M and Hansen A. (2008). Rapid high-performance liquid chromatography determination of tocopherols and tocotrienols in cereals. Cereal Chem. 82 (2):248-251.

Cunha S.C., Amaral J.S., Fernandes J.O. and Oliveira B.P.P. (2006). Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems. J Agric Food Chem. 54(9):3351-3356.

Nesaretnam K, Gomez P.A., Selvaduray K.R. and Razak G.A. (2007). Tocotrienol levels in adipose tissue of benign and malignant breast lumps in patients in Malaysia. Asia Pac J Clin Nutr. 16(3).

Kawakami Y, Tsuzuki T, Nakagawa K, and Miyazama T. (2007). Biosci Biotechnol Biochem. 71(2): 464-471.

Fairus S, Nor R.M., Cheng H.M., and Sundram K. (2006). Postprandial metabolic fate of tocotrienol-rich vitamin E differs significantly from that of α-tocopherol. Am J Clin Nutr. 84: 835-42.

METHOD B: VITAMIN E IN VEGETABLE OILS AND FATS USING HPLC WITH FLUORESCENCE DETECTOR

Principles

The oils or fats (or unsaponifiable matter obtained from a processed product containing tocopherol esters) is dissolved in an organic solvent and subjected to direct high performance liquid chromatographic (HPLC) separation of the individual tocopherols and tocotrienols. Calibration factors are determined for each tocopherol from the chromatography of solutions of standard tocopherols; calibration factors for tocotrienols are taken to be equivalent to that of the corresponding tocopherols. The tocopherol and tocotrienol content of oil or fats is the quantity of tocopherols and tocotrienols, determined in the sample by the described procedure and expressed in micrograms per gram (µg/g).

Chemicals/Reagents

1. Alpha, beta, gamma and delta tocopherol standards
2. Methanol
3. Dichlromethane
4. Hexane
5. Propan-2-ol
6. Mixture of propan-2-ol and hexane (0.5/99.5, v/v) as mobile phase
   Pipette 5 ml propan-2-ol into a one litre measuring cylinder. Add 995 ml of hexane. Filter solvents through a 0.45 µm nylon membrane filter using the solvent filtration kit and degassed using an ultrasonic bath.

Apparatus/Instruments

1. HPLC system: consisting of a high pressure pump, sample injection device, fluorescence detector and recording integrator. A fluorescence detector should preferably be used with the excitation wavelength at 330 nm. An UV detector may be used if a fluorescence detector is not available. The wavelength of the UV detector should be set at 292 nm.
2. HPLC analytical column: (250 mm x 4 mm) packed with micro particulate silica (5µm)
3. HPLC Syringe (10 µm)
4. HPLC amber vial: size 3 ml
5. Rotary evaporator
6. Water bath
7. Round bottom flask: 250 mL
8. Buchner funnel
9. Whatman filter no.1

Note-All glassware should be of low actinic activity.

Procedures

A. Preparation of solutions of tocopherol standards

(a) *Alpha*-tocopherol standard stock solution:
Prepare a stock solution of alpha-tocopherol by accurately weighing about 10 mg of the standard into a 100 ml volumetric flask and making up to volume with hexane. Pipette 10 ml of this solution into an amber glass round bottomed flask and remove all hexane on rotary evaporator at a temperature not higher than 40ºC. Restore atmospheric pressure with nitrogen and remove the flask from evaporator as soon as the solvent has been removed. Pipette into the flask 10 ml of methanol and swirl to dissolve the 5tocopherol. Measure the absorbance of this solution at 292 nm and calculate the concentration (as µg/ml alpha tocopherol) by dividing the basorbance value by 0.0076.

(b) *Beta, gamma and delta*-tocopherol standard stock solutions:
Prepare similar stock solutions and aliquots for UV spectrometry of *beta, gamma and delta*-tocopherol standards as described for the *alpha*-tocopherol standard stock solution. Measure the absorbance of each of these solutions at the following wavelengths and use the corresponding divisor factors for for calculation of concentration-
296 nm *beta* –tocopherol= 0.0089
298 nm *gamma*-tocopherol= 0.0091
298 nm *delta*-tocopherol= 0.0087

(c) Mixed tocopherol standards working solution:
Mix appropriate volumes of the stock solutions of the tocopherol standards to obtain a mixed tocopherol standards working solution; and, dilute with hexane to give a solution containing between 1 and 5 µg per ml of each tocopherol.
Note: A more concentrated solution may have to be prepared if a UV detector is used. It is important that all standards are protected from light and stored refrigerated.

B. Optimization of working parameters

1. Condition the column, if necessary. Pump the propan-2-ol/hexane mobile phase through the column at a flow rate of 1 ml/min for at least 30 min. Inject about 20 µl of the mixed tocopherol standards working solution onto the column and if necessary adjust the propan-2-ol content of the mobile phase and the flow rate to achieve the following conditions-
   (a) Alpha-tocopherol retention time not less than 5 min.
   (b) Resolution factor (R) for the separation of beta and gamma-tocopherol not less than 1.0, an almost baseline separation.
2. Select the optimum setting detector and integrator sensitivity and cahart speed. Inject about 20 µl of the mixed tocopherol standards working solution. Repeat the injection and check that reproducible chromatograms are obtained.

C. Preparation of the test sample

1. The test sample should be prepared, in the case of liquid laboratory samples, by homogenization except that filtration should be avoided.

2. In the case of solid samples, transfer a representative portion (that is not less than 10% by weight of the laboratory sample) to a glass beaker and carefully homogenize by melting with gentle mixing in a water bath at a temperature not exceeding 40ºC.
3. The preparation of the test sample should carried out as far as it is practicable in subdued light and in any case out of direct sunlight.

D. Preparation of the test solution

1. Weigh accurately about 2 g of the prepared test sample into a 25 ml volumetric flask.
2. Add a quantity of hexane, swirling to dissolve the sample and make up to volume with the same solvent.
3. If a fluorescent detector is used it may be necessary to make further dilution of this solution prior to chromatography. It is important that the test solutions are protected from light prior to analysis and analyzed on the day of preparation.

E. HPLC determination of tocopherols in the test solution

1. Inject 20 µl of the mixed tocopherol standards working solution onto the column and record the areas of the tocopherol peaks. If integrator is not available record peak heights (measured in mm).
2. Inject 20 µl of the test solution onto the column and identify the tocopherols (and tocotrienols) present by reference to the chromatograms obtained from standards. Record the areas of the tocopherol peaks (or peak heights). Record the areas of any tocotrienol peaks if these are present and are to be quantified. Duplicate injections should be made.
3. Inject a further 20 µl of the mix tocopherol standards working solution and record the areas of the tocopherol peaks.

F. Number of determinations

1. Carry out two determinations (each consisting of duplicates injections of the prepared test solutions) in rapid succession, using a fresh test portion for each determination.

Calculations

1. The *alpha*-tocopherol content of the sample in µg/g is given by:

$$\frac{(C) \times (a) \times (D) \times 25}{(A) \times (m)}$$

Where:
C= concentration of the *alpha*-tocopherol standard (µg/ml)
A= mean of the peak areas obtained for the *alpha*-tocopherol standard
a= mean of the peak areas obtained for the *alpha*-tocopherol in test sample
m= mass of test sample taken
D= dilution factor

2. The *beta, gamma and delta*-tocopherol contents of the test sample are calculated in the same way using the data from chromatography of the corresponding tocopherol standard.
3. The tocotrienol content of a sample can be estimated using C and A values for the corresponding tocopherol.

Reference

A.O.C.S Official Method Ce 8-89. 1990.

3.3.3.4 DETERMINATION OF VITAMIN K IN MILK AND INFANT FORMULAS LIQUID CHROMATOGRAPHIC METHOD (AOAC METHOD 999.15)

Principles

Following enzymatic digestion of fat and precipation of fatty acids, vitamin K is extracted with hexane. Vitamin K is separated by HPLC with post-column reduction and quantitated by fluorescence detection with an external standard.

Phylloquinone geometric isomers quantitated either as a single unresolved peak with a C18 or may be estimated selectively with a C30 column (K trans< Kcis) under the reversed-phase employed.

(Note: Menaquinone-4 [MK-4] will also be present in milk and infant formulas incorporating milk fat, while 2'3'-dihydrophylloquinone may be present at appreciable levels in infant formulas containing hydrogenated soya or canola oils. If required, both may be estimated against appropriate standards. The order of elution is MK-4 <K1<2'3'dihydrophylloK1 [relative retention times ca 0.6, 1.0, and 1.2, respectively]

Chemicals/Reagents

Use AR grade reagents unless otherwise stated.

(a) Water-purified to >18 MΩ resistivity
(b) Nitrogen-Cylinder N (low 0 content)
(c) Lipase-From Candida rugosa, ca 1000 units.mg (Type VII, Sigma L-1754). Other sources of lipase can be used from Pseudomonas and Rhizopus species with the correct activity of enzyme.
(d) Monobasic potassium phosphate (KH_2PO_4)
(e) Potassium hydroxide solution -40% (w/v). Dissolve 40g KOH, (j) in water and dilute to 100ml.
(f) Phosphate buffer-0.8M. Dissolve 54.0 g KH_2PO_4 (d), in 350 ml water. Adjust pH to 7.9-8.0 with 40% KOH solution and and dilute to 500 ml.
(g) Ethanol
(h) Methanol-LC grade
(i) Reagent alcohol-Mix ethanol, (g), with methanol, (h), (95 + 5, v/v)
(j) Potassium hydroxide
(k) Zinc chloride
(l) Sodium acetate-Anhydrous
(m) Acetic acid-Glacial
(n) Potassium carbonate-Anhydrous
(o) Zinc powder-<60 µm, e.g., Merck 108774 and BDH 1029641. Use freshly opened bottle.
(p) Hexane-HPLC grade
(q) Dichlorometane-HPLC grade
(r) Isopropanol- HPLC grade

(s) Mobile phase- To dichlorometane, (q), methanol, (h), (100 + 900, v/v), add 5 mlmethanol containing $ZnCl_2$ (1.37g; k), anhydrous sodium acetate (0.41 g;1) and glacial acetic acid (0.30 g;m). Filter through 0.45 µm filter and degas.

(t) Zinc post-column redactor-Dry pack the 20 x 4 mm assembly B(c) with Zn powder, (0). Minimize voids by sequentially adding small amounts with frequent gentle tapping. With longer columns, use extra care. Re-prepare reductor column before each analytical schedule. Reduction efficiency of Zn powder should be monitored over time and replaced every 1-2 years as required.

(u) Vitamin K1 (phylloquinone)-USP grade. Conduct all operations must be conducted under low incandescent light conditions and in low-actinic volumetric flasks. (i) Stock solution-ca 1.0 mg/ml. Dissolve ca 100mg, weighted accurately, in 100.0 ml isopropanol (r) with warming at ca 30ºC and standing. Store under N, (b), at -10ºC for up to 6 months. (2) Intermediate I-ca 50 µg/ml. Dilute 5.0ml stock with methanol to 100.0mL and store under N at -10ºC for up to 1 month. (3) Intermediate II-ca 2.5 µg/ml. Dilute 5.0 ml Intermediate I with methanol to 100.0 ml. Prepare daily. (4) Working standards-ca 6.25, 12.50, 18.75, 25.00 and 31.25 ng/ml. Dilute 0.25, 0.50, 0.75, 1.00 and 1.25 ml, respectively, Intermediate II with methanol, (h), to 100.0ml. Prepare daily and calculate accurate concentration as follows: Accurate concentrations for working standards are calculated after estimation of phylloquinone purity from absorbance (248nm) of a solution prepared by evaporating 5.00ml. Intermediate I under N flow and re-dissolving in 25.0ml hexane (a1%=419)

$$\text{Theoretical A 248} = \frac{W}{100} \times \frac{5}{100} \times 5 \times 419 \times 100$$

Where W= weight of phylloquinone in stock standard (g)

Purity factor (PF)= measured A248/theoretical A248

$$\text{K1 in Intermediate II (µg/mL)} = PF \times \frac{W}{100} \times \frac{5}{100} \times 5 \times \frac{106}{100}$$

Accurate vitamin K1 concentration in the 5 working standards (ng/mL) is then calculated by multiplying PF by 2.5, 5.0, 7.5, 10.0 and 12.5 respectively.

Apparatus/Instruments

(a) HPLC system-manual or automated isocratic system: incorporating a fluorescence detector (λ 243_{ex} nm, λ 430_{em} nm) exhibiting a Raman spectra signal noise specification of >200:1(water). Since UV detection is insensitive to vitamin K at the working concentration levels, configure with single mode fluorescence detection only to minimize extra-column band broadening. For multi-detector LCs, configure with the fluorescence detector in the first position. Ensure the absence of leaks.

(b) Column: Any C18 column (monometric or polymeric) containing 5 µm spherical silica with ≥10% carbon loading, proceeded by a guard column of similar packing.

(c) Post-column redactor: 20 x 4 mm stainless steel post-column assembly placed between analytical column and fluorescence detector (part NoWAT084550, Waters or equivalent). Alternative devices such as an empty (30-50 mm) LC column can be used.

(d) Spectrophotometer: UV/Vis spectrophotometer, digital readout to 0.001 A.
(e) Waterbath: 37 ± 1°C
(f) Centrifuge: Fixed or swing arm rotor
(g) Mechanical Shaker: orbital or wrist action
(h) Vortex mixer
(i) pH meter: Accurate to 0.01 pH with calibration buffers
(j) Test tubes: 200 x 24 mm with ground-glass stoppers
(k) Vials-Amber-tinted glass vial: (1.8-8.0ml) with Teflon-sealed caps.
(l) Volumetric flasks: low-actinic volumetric flasks, 100ml

Procedures

Perform all steps of assay under incandescent lighting in the absence of direct sunlight.

(a) Preparation of test solution

(1) Digestion
Weigh 1.0 g powder or 10.0 g liquid into test tube. Include both a reagent blank and a quality control test portion in each analytical schedule. Dissolve powder in ca 15 ml warm water (<40°C) with Vortex mixer (for liquids, add 5.0 ml warm water). Add 5.0ml phosphate buffer to each solution and mix. Add 1.0 g lipase powder and vortex mix. Stopper securely and shake until well dispersed (30-60 s). Incubate at 37 ± 2°C for 2 h. Shake each tube for 15 s at 20 min intervals. Cool to ambient temperature by standing in water. Add 10 ml reagent alcohol and mix. Add 1.0 g K_2CO_3 and mix.

(2) Extraction
(Note: Take precautions to prevent concentration of extracts through uncontrolled solvent evaporation). Add 30mL hexane, stopper and shake vigorously for ≥10 min using mechanical shaker. Let stand in the dark, preferably refrigerated, until layers separate. If this is slow (>10 min) or incomplete, centrifuge at ca 1000 rpm (ca 200g) for 10 min (decant a portion of supernatant into smaller centrifuge if necessary). Transfer 1 ml (fortified products) or 5 ml (nonfortified products) of upper hexane phase into vial. Evaporate under N flow to dryness.
(Note: larger extract volumes may require with the option of rotary evaporation dependent on the sensitivity of the fluorescence detector). These dried extracts may be held for 3-5 days in the dark at <0°C under N prior to LC analysis. Re-dissolve residue in 1mL methanol. Seal vial and Vortex mix. If using autosampler, use a low volume vial (or insert) to minimize evaporation into the headspace.

(b) HPLC determination

(1) Set-up
Prior to connection of post-column Zn redactor assembly, establish stable operating LC conditions over ca 30 min through equilibration of column with mobile phase (ca 1ml/min) and set fluorescence detector to λ_{ex} 243 nm and λ_{em} 430 nm (gain and sensitivity will be detector dependent). Install Zn redactor assembly between analytical column and detector and equilibrate system for a further 30-60 min, ensuring the absence of leaks (anhydrous LC eluent conditions

are required to minimize loss of reducing potential of Zn). Do not recycle mobile phase. Inject a standard (20-50 µL) and set flow rate in order to elute phylloquinone between 8-15 min; at typically 1.0-1.5 ml/min (retention may also be manipulated by modification of dichloromethane content in eluent between 8-12%). Allow total run time of ca 5 min beyond elution ok K1.

(2) Calibration and analyses-Inject methanol (standard blank) and confirm absence of chromatographic activity at retention time for phylloquinone. Inject lowest level working standard and ensure repeatable response, with signal: noise ≥10. Inject each working standard and ensure linear response. Sequentially inject reagent blank and test extracts and include a working standard after every 4-6 test solutions to monitor system stability. When analytical schedule is complete, flush system with methanol. Store analytical column in methanol when not in use. Dismantle and empty post-column assembly, soak in 5 M HCI to clean frits and rinse thoroughly with water and methanol. Dry thoroughly before repacking with Zn.

(c) Calculation

Construct a 5-level calibration using either peak area or height and calculate slope (s) by linear regression with forced zero. Calculate vitamin K1 (cis and trans) content in test samples as follows:

$$\text{Vitamin K1 (µg/100g)} = \frac{A'}{S} \times \frac{30}{V} \times \frac{100}{Wt} \times \frac{1}{1000}$$

Where A' = peak area (or height) of phylloquinone in test extract; Wt= weight of test portion (g);V= volume, 1ml (fortified) or 5 ml (nonfortified);S=slope of calibration graph.

Calibration and calculations may be achieved through data processing within the instrument or off-line.

Reference

J. AOAC Int. 83, 121 (2000)

3.3.4 WATER SOLUBLE VITAMINS

3.3.4.1 DETERMINATION OF THIAMINE (VITAMIN B1) AND RIBOFLAVIN (VITAMIN B2) IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Thiamine and riboflavin are water soluble vitamins stable in acid environment, therefore, thiamine is extracted simultaneously with riboflavin through acid hydrolysis followed by enzyme digestion. At the end stage of digestion thiamine and its phosphate ester are converted to thiochrome prior to HPLC analysis.

Chemicals & reagents

*Check included procedures

1. Acetonitrile, HPLC grade
2. Mobile phase preparation. Filter the mobile phase (Acetonitrile and deionized water) with 0.45 µm nylon membrane filter using solvent filtration apparatus daily prior to analysis.
3. 0.1 N hydrochloric acid (HCl). Pipette 8.7 ml of HCl in volumetric flask and make up to volume of 1 L.
4. 1 M sodium acetate. Dissolve 82 g of sodium acetate in deionized water and top up to volume of 1 L.
5. 1 M sodium hydroxide (NaOH). Dissolve 40 g of NaOH in deionized water and top up to volume of 1 L.
6. Thiamine standard solution
    a. Use thiamine hydrochloride (99% purity, e.g. from Sigma Chemical Co.) as standard for thiamine determination.
    b. Thiamine standard stock solution. Weight 10 mg of standard and make up to 100 ml with deionized water to give a concentration of 100 µg/ml. Prepare freshly prior to analysis.
    c. Thiamine standard working solution. Pipette 10 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 10 µg/ml.
7. Riboflavin standard solution
    a. Use riboflavin (≥ 98% purity, e.g. from Sigma Chemical Co.) as standard for riboflavin determination.
    b. Riboflavin standard stock solution. Weight 10 mg of standard and make up to 100 ml with deionized water to give a concentration of 100 µg/ml. Prepare freshly prior to analysis
    c. Riboflavin standard working solution. Pipette 10 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 10 µg/ml.

Apparatus/ Instruments

1. Balance: Analytical sensitivity ± 0.1 mg
2. Volumetric flask: 100 ml
3. Conical flask: 100 ml
4. Filter funnel
5. Homogenizer
6. Incubator: at 37°C
7. Water bath
8. Ultrafiltration system: using 0.45 µm, 47 mm diameter membrane filter
9. Vacuum pump
10. pH meter: accurate to 0.01 pH with calibration buffers
11. HPLC with DAD detector/ UV detector: (wavelength 268 nm)
12. Analytical column: reverse phase C_{18} column of 150 mm x 4.6 mm I.D and particle size 5 µm.
13. Guard column: 20 mm X 4.6 mm I.D
14. HPLC sample vial

HPLC conditions

1. Reverse phase C_{18} column of 150 mm x 4.6 mm I.D and particle size 5 µm (e.g. Atlantis. Waters).
2. Guard column holder with a guard column which has an I.D of 4.6 mm and length of 20 mm packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler, G1239.
4. Agilent 1200 series binary pump, G1312 to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser, G1379.
6. Agilent 1200 series diode arrays multiple wavelength detector (DAD), G1315 for thiamine and riboflavin detection at the wavelength of 268 nm.
7. Solvent cabinet to keep the mobile phases.
8. A computer with an Agilent ChemStation control software.
9. A HP LaserJet 5L printer to print the results and chromatograms

Procedures

A. Sample extraction

1. Weigh accurately 10 g (fresh sample) or 2 g (dried sample) of the foodstuff into a 100 ml extraction flask.
2. Add 60 ml of 0.1 N HCl into the flask and homogenize the extract for 10 min with a homogenizer.
3. Autoclave extracts at 120°C for 30 min, then cool the extract immediately on ice.
4. Adjust the pH of the acidic extract to pH 4.3 – 4.7 with 1 M of sodium acetate.
5. Add takadiastase (100 mg of takadiastase per g of sample) into the extract and incubate at 37°C for 18 hours.
6. After incubation, add 2 ml of 50% trichloroacetic acid (TCA) into the extract and heat in water bath for 10 min at 50°C.
7. Quantitatively transfer the extract to a 100 ml volumetric flask and top up to volume with deionized water (V1).
8. Filter the diluted extract with ashless filter paper.

B. Instruments set up procedure

1. Before turn on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase is used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.
4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump, close the purge valve, set the required mobile phase composition (acetonitrile: water, 70:30) and flow rate of 1 ml/min for thiamine and riboflavin analysis.
6. Turn on the pump again and allow the column to equilibrate with mobile phase which usually need 1 hour.
7. Make sure the UV and Visible lamps are turn on and set the DAD detector at wavelength of 268 nm.
8. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
9. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.

C. Chromatography of thiamine/ riboflavin

1. The cleaned extract (2 ml) is filtered through a 0.45 µm PVDF syringe filter into a sample vial for riboflavin determination.
2. For thiamine analysis, take 5 ml of extract and mix with 3 ml of 1% potassium ferricyanide, followed by 0.5 ml of phosphoric acid and 1.5 ml of water before filtering with 45 µm PVDF syringe filter.
3. Identify thiamine and riboflavin in sample by comparing their retention time with the retention time of standards.
4. Obtain a standard curve by using the peak area given by each standard solution (10 – 50 µg/ml)
5. Quantify both thiamine and riboflavin of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standards. The total amount of thiamine and riboflavin in sample was expressed in mg/100g of sample.

Calculation

mg thiamine/ riboflavin per 100g food =

$$\frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in µg} \times \frac{\text{V1 (ml)}}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Kumar, S. & Aalbersberg, B. 2006. Nutrient retention in foods after earth-oven cooking compared to other forms of domestic cooking, 2. Vitamin. *Journal of Food Composition and Analysis*. 19(4): 311-320.

3.3.4.2 DETERMINATION OF NIACIN (VITAMIN B3) IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Alkaline hydrolysis is used to extract niacin from sample as acid hydrolysis unable completely liberates bound niacin forms in food sample. Sample extract is then purified by using solid phase extraction with both C18 and SCX cation exchange column in series prior to HPLC analysis. PIC® A reagent is employed in mobile phase to obtain a better chromatography separation.

Chemicals & reagents

1. Methanol, HPLC grade
2. Methanol, analytical grade
3. Calcium hydroxide, e.g. from Sigma Chemical Co.
4. Oxalic acid, 10% (w/v).
5. Oxalic acid, 1%. (w/v)
6. Ammonium hydroxide, 2% (w/w).
7. PIC® A reagent, from Waters Co.
8. Mobile phase preparation. Prepare mobile phase consist of 5% methanol and 95% deionized water containing 0.005M PIC® A reagent. Filter mobile phase with 0.45 µm nylon membrane filter using solvent filtration apparatus daily prior to analysis.
9. Niacin standard solution
    a. Use niacin (99.5% purity, e.g. from Sigma Chemical Co.) as standard for niacin determination.
    b. Niacin standard stock solution. Weight 10 mg of standard and make up to 100 ml with deionized water to give a concentration of 100 µg/ml. Prepare freshly prior to analysis
    c. Niacin standard working solution. Pipette 1 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 1 µg/ml.

Apparatus/Instruments

1. Volumetric flask: 50 ml, 100 ml
2. Conical flask: 50 ml
3. Homogenizer
4. pH meter: accurate to 0.01 pH with calibration buffers
5. Centrifuge
6. Autoclave
7. Solid phase extraction (SPE) column: C18 (500 mg) and SCX (500 mg)
8. Vacuum manifold: for SPE column
9. Syringe filter: 0.45µm, PTFE
10. Ultrafiltration system: 0.45 µm, 47 mm diameter membrane filter
11. Vacuum pump
12. HPLC with DAD detector/ UV detector: wavelength 254 nm

13. Analytical column: reverse phase C_{18} column of 150 mm x 4.6 mm I.D and particle size 5 µm.
14. Guard column: 20 mm X 4.6 mm I.D
15. HPLC sample vial

HPLC conditions

1. Reverse phase C_{18} column: 150 mm x 4.6 mm I.D and particle size 5 µm (e.g. Atlantis. Waters).
2. Guard column holder: with a gurd column I.D of 4.6 mm and length of 20 mm, packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler G1239.
4. Agilent 1200 series binary pump G1312: to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser G1379.
6. Agilent 1200 series diode arrays multiple wavelength detector (DAD), G1315: for niacin detection at the wavelength of 254 nm.
7. Solvent cabinet: to keep the mobile phases.
8. A computer with an Agilent ChemStation control software.
9. A HP LaserJet 5L printer: to print the results and chromatograms.

Procedures

A. Sample extraction

1. Weigh accurately 1g of the foodstuff into a 50 ml extraction flask.
2. Add 0.75 g of calcium hydroxide and 20 ml of deionized water into the flask and homogenize the extract for 10 min with a homogenizer.
3. Autoclave extracts at 121°C for 2 hours, then cool the extract immediately on ice.
4. Dilute the cooled extract with deionized water and adjust the volume to 50 ml with volumetric flask.
5. Centrifuge the extract at 2500 rpm, 10°C for 20 min.
6. Take 15 ml of the supernatant and adjust the pH to pH 7 with 10% aqueous oxalic acid and make to volume to 25 ml.
7. Centrifuge the resultant supernatant at 2500 rpm, 10°C for 10 min to participate calcium oxalate.
8. Decant the supernatant for further purification.

B. Sample Purification

1. Connect C18 (500 mg) and SCX (500 mg) solid phase extraction (SPE) columns (e.g. from Sep-Pak) in a series, then connect them on a vacuum manifold.
2. Condition the SPE columns with 10 ml of methanol, follow by 10 ml of deionized water.
3. Load 10 ml supernatant onto the C18 column.
4. Wash the C18 column with 5 ml of water.
5. Discard C18 column, wash SCX column with 5 ml of methanol.
6. Elute niacin from SCX column with 5 ml of freshly prepare 2% solution concentrated ammonium hydroxide in methanol.
7. Evaporate eluate to dryness under a steam of nitrogen at room temperature.

8. Dissolve the residue with 1 ml (V1) of deionized water, then filter through 0.45µm PTFE syringe filter prior to HPLC analysis.

C. Instrument set up procedures

1. Before turning on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase are used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.
4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump and close the purge valve. Set the required mobile phase composition (isocratic) and flow rate of 1 ml/min for niacin analysis.
6. Turn on the pump again and allow the column to equilibrate with mobile phase which usually need 1 hour.
7. Make sure the UV and Visible lamps are turned on and set the DAD detector at wavelength of 254 nm.
8. To check whether column is equilibrated, inject the above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
9. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.

D. Chromatography of niacin

1. The cleaned extract (2 ml) is filtered through a 0.45 µm PVDF syringe filter into a sample vial.
2. Identify niacin in sample by comparing their retention time with the retention time of standards.
3. Obtain a standard curve by using the peak area given by each standard solution (10 – 50 µg/ml)
4. Quantify niacin of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standards. The total amount of niacin in sample is expressed in mg/100g of sample.

Calculation

mg niacin per 100g food =

$$\frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in µg} \times \frac{V1\ (\text{ml})}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Juraja, SM, Trenerry, VC, Millar, RG, Scheelings, P & Buick, DR. 2003. Asia Pacific food analysis network (APFAN) training exercise: the determination of niacin in cereals by alkaline extraction and high performance liquid chromatography. Journal of Food Composition and Analysis 16:93-106.

3.3.4.3 DETERMINATION OF PANTOTHENIC ACID (VITAMIN B5) IN FOODS (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Pantothenic acid (vitamin B5) exists in foodstuffs in its free form, as well as bound in coenzyme A (CoA) and acyl carier protein (ACP). Therefore, extraction method using enzymatic hydrolysis is required to release pantothenic acid from its bound forms. Microbiological assay is the most common approach for pantothenic acid determination. However it is time-consuming, low specificity and exhibit relatively low precision. Therefore, liquid chromatography (LC) methods is developed using post-column derevatization of pantothenic acid into a fluorescent compound- fluorescent 1-akkylthio-2-alkylisoindole. Fluorescent compound forms when pantothenic acid is hydrolyzed to β-alanin, under hot alkaline condition and then reacted with orthophthaldialdehyde in the presence of 3-mercaptopropionic acid. This method shows good recovery rate (96-101%) and posses low detection limit (0.65µg/g) thus makes it suitable for pantathonic acid determination in any foodstuff.

Chemicals & Reagents

1. Sodium bicarbonate, 200 mM
2. Sodium bicarbonate, 5 M
3. TRIS buffer, 200 mM, pH 8
4. HCl, 1M
5. HCl, 250 mM
6. Vitamin standard, calcium D-pantothenate, P2250 Sigma-Aldrich
7. Acetone-dried pigeon liver powder (contain pantetheinase), L8376 Sigma-Aldrich
    a. Purification: Dissolve 1 g of the powder in 10 ml of 20 mM sodium bicarbonate at 0°C. Then, centrifuge the solution at 3500 rpm for 10 min, collect the supernatant and transfer to a dialysis tubing (width 25 mm, diameter 16 mm; volume/length 2.0 ml/cm, Spectra/Por 4 membrane, MWCO 12,000 – 14,000). Place the filled dialysis tube in a 3 L volume of 20 mM sodium bicarbonate for 15 h at 4°C. Store purified pantetheinase solution in vial and frozen it until use.
8. Pepsin, P7125 Sigma-Aldrich
9. Alkaline phosphatase, P6772, Sigma-Aldrich
10. Methanol, HPLC grade – mobile phase
11. Phosphate buffer (33 mM, pH 2.5) – mobile pahse
12. Phophate buffer (300 mM, pH 3)
13. Reagent for post-column derivatization
    a. Prepare solution containing 200 mM sodium hydroxide, 1 mM orthophthaldialdehyde and 1.6 mM 3-mercaptopropionic acid
15. Mobile phase preparation.
    a. Filter mobile phase with 0.45 µm nylon membrane filter using solvent filtration apparatus daily prior to analysis.
16. Pantothenic acid standard solutions
17. Use calcium D-pantothenate (e.g. from Sigma Chemical Co) as standards for pantothenic acid determination
18. Standard stock solution. Weigh 10 mg of standards and make up to 100 ml with deionized water to give a concentration of 100 µg/ml. Prepare freshly prior to analysis

19. Standard working solution. Pipette 10 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 10 µg/ml.
20. Prepare a series of standard containing 0.05 – 2.00 µg/ml of pantothenic acid from working solution.

Apparatus/Instruments

1. Balance: Analytical sensitivity ± 0.1 mg
2. Extraction flask: 100 ml
3. Centrifuge
4. Pipettes: 1ml, 5 ml, 10 ml
5. Volumetric flask: 50 ml, 5 ml
6. Incubator
7. Centrifuge
8. Cellulose acetate filter: 0.45 µm
9. HPLC sample vials
10. SPE column: strong anion exchange, 400 mg
11. Vacuum manifold: for SPE
12. Post-column derivatization kit: containing T-connector, knitted PTFE coil reactor and peristaltic pump
13. HPLC with fluorescence detector and thermostatted column compartment
14. Analytical column: reverse phase (RP) C18 endcapped, octadecylsilyl, 250 mm x 5 mm I.D; particle size 5 µm.
15. Guard column: RP 18, 4 mm X 4 mm ID; 5 µm particle size

HPLC conditions

1. Reverse phase C_{18} endcapped column of 250 mm x 5 mm I.D; particle size 5 µm; octadecylsilyl (e.g. Lichrospher, Merck).
2. Guard column holder with a guard column that RP 18 (4 mm X 4 mm ID; 5 µm particle size; packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler, G1239.
4. Agilent 1200 series binary pump, G1312 to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser, G1379.
6. Agilent 1200 series fluorescence detector, G1321
7. Agilent 1200 series thermostatted column compartment, G1316
8. Solvent cabinet to keep the mobile phases.
9. A computer with an Agilent ChemStation control software.
10. A HP LaserJet 5L printer to print the results and chromatograms.
11. For post-column derivatization
    a. Set the derivatization kit between analytical column and fluorescence detector.
    b. Use peristaltic pump (e.g. Miniplus 3 peristaltic pump, Gilson) to pump in the derivatization solution. Peristaltic pump is set to flow rate of 1ml/min.
    c. Derivatization solution is added to column effluent through a T connector follow by a knitted PTFE coil reactor (0.5 mm ID X 40 m) which is housed in the thermostatted column compartment at 99°C.
    d. Another end of the PTFE reactor is connecting to the detector outlet for derivatized compound determination.

Procedures

A. Sample extraction

1. Weigh accurately 5g of the foodstuff into a 100 ml extraction flask.
2. Add 15 ml of 50 mM acetate buffer (pH 4.5) and 1 ml of pepsin solution (50 mg/ml [4500 U/ml]).
3. Homogenize the mixture and incubate at 50 °C for 3 h.
4. After 3 h, adjust the pH to 8 with 5 M of sodium hydroxide.
5. Add 10 ml of 200 mM Tris buffer (pH 8), 0.6 ml of an alkaline phosphatase solution (20 U/ml) and 2.5 ml of pantetheinase solution (80 mU/ml).
6. Incubate the mixture at 20°C for 18 h, then top up to 50 ml with deionized water.
7. Centrifuge the mixture at 11500 rpm for 10 min, and then filter through a 0.45 µm cellulose acetate filter.

B. Extract purification

1. Wash strong anion exchange cartridge (SPE catridge, 400 mg) successively with 5 ml of methanol and 5 ml of deionized water.
2. Load 5 ml of extract on a strong anion exchange cartridge, reject the first 2 ml.
3. Collect the following 3 ml of extract in 5 ml volumetric flask.
4. Add 0.25 ml of 250 mM HCl, then make up to 5 ml with 300 mM phosphate buffer (pH 3).
5. Prepare a strong cation exchange cartridge (SPE cartridge, 400 mg) by washing it successively with 5 ml of methanol and 5 ml of deionized water.
6. Load all solution (4) above onto the cartridge, reject the first 2 ml.
7. Collect the next 3 ml for HPLC determination.

C. Instrument set up procedures

1. Before turning on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase is used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.
4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump and close the purge valve. Set the required mobile phase composition of phosphate buffer (33 mM, pH 2.5) and methanol. Set flow rate of 1 ml/min for pantothenic acid analysis.
6. Gradient mode is to be used for analysis. The proportion of methanol in mobile phase is to increase linearly from 0 to 10% during 25 min. Maintain the composition of (90:10, v/v) for 8 min.
7. After 8 min, immediately adjust to initial composition (100% of phosphate buffer).
8. Turn on the pump again and allow the column to equilibrate with mobile phase initial composition, which usually need 1 hour.
9. Set fluorescence detector excitation at 345 nm and emission at 455 nm.

10. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
11. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.
12. Inject 50 µl of extract for HPLC analysis.

C. Chromatography of Pantothenic Acid

1. Identify pantothenic acid in sample by comparing their retention time with the retention time of standards.
2. Obtain a standard curve by using the peak area given by each standard solution (0.5 – 2 µg/ml)
3. Quantify pantothenic acid of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standards.
4. The total amount of pantothenic acid in sample is expressed in mg/100g of sample.

Reference

Pakin, C., Bergaentzlé, M., Hubscher, V., Aoudé-Werner, D., Hasselmann, C. 2004. Fluorimetric determination of pantothenic acid in food by liquid chromatography with post column derivatization. *Journal of Chromatography* A. 1035, 97-95.

3.3.4.4 DETERMINATION OF PYRIDOXINE (VITAMIN B6) IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Vitamin B6 in food is dephosphorylated by enzyme hydrolysis and detected using fluorescence as it provides a high sensitive and specific detection mode for the analysis of all vitamin B6 forms commonly encountered in foods. Pyridoxine hydrochloride (PN), pyridoxal hydrochloride (PL) and pyridoxamine dihydrochloride (PM) is detected and total vitamin B6 is calculated with the equation PN = PN + (1.01PL) + (0.79PM).

Chemicals/ Reagents

1. Acetonitrile, HPLC grade.
2. HCl, 0.01M
3. HCl, 0.1M
4. HCL, 1M
5. Sodium acetate, 2M
6. Potassium hydroxide, 3.5M
7. Acid phosphatase, 25 unit/ml, prepare freshly prior to analysis.
8. β-glucosidase, 45 unit/ml, prepare freshly prior to analysis.
9. Triethylamine, 99%, 4 mM
10. Potassium dihydrogen phosphate, 81 mM
11. Ortho-phosphoric acid, 85%, 19 mM
12. 1-octan-sulfonic acid, 2.2 mM
13. Sodium monohydrate, 99%
14. Mobile phase preparation.
    a. HPLC eluent-buffer. Prepare buffer (1L) consist of 2.2 mM 1-octan-sulfonic acid in 81 mM potassium dihydrogen phosphate and 19 mM 85% phosphoric acid and 4.0 mM triethylamine. Adjust pH to 2.75 with 3.5 M potassium hydroxide.
    b. Prepare mobile phase that contain 93% HPLC eluent-buffer and 7% acetonitrile.
    c. Filter mobile phase with 0.45 µm nylon membrane filter using solvent filtration apparatus daily prior to analysis.
15. Vitamin B6 standard solutions
    a. Use pyridoxine hydrochloride (PN), pyridoxal hydrochloride (PL) and pyridoxamine dihydrochloride (PM) (e.g. from Sigma Chemical Co) as standards for vitamin B6 determination.
    b. Standard stock solution. Weigh 10 mg of standards and make up to 100 ml with 0.1 M HCl to give a concentration of 100 µg/ml. Prepare freshly prior to analysis.
    c. Standard working solution. Pipette 1 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with 0.1 M HCl. The final concentration of working solution is 1 µg/ml.

Apparatus/Instruments

1. Ultrafiltration equipment: 0.45 µm, 47 mm diameter membrane filter
2. Volumetric flask: 100 ml, 1L
3. Balance: analytical sensitivity ± 0.1 mg
4. Conical flask: 50 ml
5. Beaker: 25 ml, 50 ml
6. Mechanical shaker
7. Autoclave
8. Centrifuge
9. Incubator: at 45°C
10. Filter paper, Whatman No.1
11. HPLC sample vials
12. Syringe filter: 0.45 µm PVDF
13. Analytical column: C18, particle size 3 µm, 150 mm x 4.6 mm I.D
14. HPLC with fluorescence detector

HPLC conditions

1. Reverse phase C_{18} column of 150 mm x 4.6 mm I.D and particle size 3 µm (e.g. Hypersil, Phenomenex).
2. Guard column holder with a gurd column with an I.D of 4.6 mm and length of 20 mm packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler, G1239.
4. Agilent 1200 series binary pump, G1312 to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser, G1379.
6. Agilent 1200 series fluorescence detector, G1321
7. Solvent cabinet to keep the mobile phases.
8. A computer with an Agilent ChemStation control software.
9. A HP LaserJet 5L printer to print the results and chromatograms.

Procedures

A. Sample extraction

1. Weigh accurately 5g of the foodstuff into a 100 ml extraction flask.
2. Add 50 ml of 0.1 m HCl; homogenize the extract for 10 min with a orbital shaker.
3. Autoclave extracts at 121°C for 30 min (animal origin sample); 5 min (vegetable origin sample).
4. Cool the extract to room temperature immediately on ice.
5. Adjust the cooled extract to pH 4.5 with 2 M sodium acetate.
6. Dilute the extract and make up to volume of 100 ml with deionized water.
7. Centrifuge the extract at 8500 g, 5°C for 10 min.
8. Decant the supernatant and filter with filter paper.
9. For animal origin sample, take 15 ml of the filtered supernatant and mix with 1 ml of acid phosphatase solution in 30 ml measurement flask.
10. For vegetable origin sample, take 15 ml of the filtered supernatant and then mix with 1 ml of acid phosphatase and 3 ml of β-glucosidase in 30 ml measurement flask.
11. Incubate the mixture at 45°C for 18 hours.

12. Cool the mixture to room temperature and add 5 ml of cool 1 M HCl. Then, fill up the flask with 0.01 M HCl until 30 ml (V1).
13. Filter the extract with 0.45 µm PVDF syringe filter into a HPLC vial. (For turbid sample, centrifuge it at 14,000 g for 10 min at 5°C prior to filtration.)

C. Instrument set up procedures

1. Before turning on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase is used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.
4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump and close the purge valve. Set the required mobile phase composition (isocratic) and flow rate of 1 ml/min for vitamin B6 analysis.
6. Turn on the pump again and allow the column to equilibrate with mobile phase which usually need 1 hour.
7. Set fluorescence detector excitation at 333 nm and emission at 375 nm.
8. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
9. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.
10. Inject 50 µl of extract for HPLC analysis.

D. Chromatography of Vitamin B6

1. Identify vitamins B6 in sample by comparing their retention time with the retention time of standards.
2. Obtain a standard curve by using the peak area given by each standard solution (1 – 50 µg/ml)
3. Quantify vitamins B6 of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standards.
4. Calculate vitamins B6 as pyridoxine hydrochloride (PN, HCl) with the calculation: PN, HCl = PN + (1.01PL) + (0.79PM)
5. The total amount of vitamin B6 in sample is expressed in mg/100g of sample.

Calculation

$$\text{mg PN/ PL/ PM in food} = \frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in } \mu g \times \frac{V1 \text{ (ml)}}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Kall, MA. 2003. Determination of total vitamin B_6 in food by isocratic HPLC: a comparison with microbiological analysis. Food Chemistry 82: 315-327.

3.3.4.5 DETERMINATION OF BIOTIN (VITAMIN B7) IN FOOD (MICROBIOLOGICAL METHOD)

Principles

Biotin occurs in relatively low concentration in most foods. Therefore, microbiological assay remains the most versatile method for determination of total biotin concentration in foods. It is a highly sensitive method that could detect biotin level up to 10^{-9}g/L. The assay based on the growth of *Lactobacillus plantarum* (biotin-dependent microorganism) according to the concentration of biotin in the medium.

Chemicals/Reagents

1. Methanol, analytical grade
2. Sulfuric acid, 2 N
3. Sulfuric acid , 6N
4. Sodium hydroxide (NaOH), 20%
5. Biotin assay medium
6. Folic acid standard solution
    a. Use biotin (from Sigma Chemical Co.) as standard for biotin determination.
    b. Folic acid standard stock solution. Weight 1 mg of standard and make up to 100 ml with 25% methanol to give a concentration of 10 µg/ml. Prepare freshly prior to analysis.
    c. Biotin standard working solution.
        1. Pipette 0.1 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water to give the final concentration of 10 ng/ml.
        2. Pipette 1 ml of diluted stock solution (ii) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 0.1 ng/ml.

Apparatus/ Instruments

1. Balance: analytical sensitivity ± 0.1 mg
2. Pipettes: 1 ml, 5 ml, 10 ml
3. Extraction flask: 50 ml
4. Homogenizer
5. Autoclave
6. pH meter: accurate to 0.01 pH with calibration buffers
7. Filter paper: ashless
8. Test tubes
9. Incubator

Procedures

A. Sample extraction

1. Weigh accurately 1g of hormogenized foodstuff into a 50 ml extraction flask.
2. Add 25 ml of sulfuric acid, 2N (for plant sample) or 6 N (for animal sample)
3. Homogenize the mixture with a homogenizer for 10 min
4. Autoclave the mixture at 121°C for 2 hours.
5. Cool the sample immediately; adjust to pH 7 with 20% NaOH.
6. Dilute the mixture to 100 ml with deionized water and filter through ashless filter paper.
7. Keep in refrigerator prior to analysis.

B. Microbiological assay

1. Prepare the biotin assay medium according to product manual (e.g. from Difco)
2. Inoculum preparation
   i) Add 5 ml of assay medium into a test tube, adjust the column to 10 ml with deionized water.
   ii) Autoclave the medium at 121°C for 5 min.
   iii) Supplement the autoclaved medium with 5 ng of biotin.
   iv) Subculture *Lactobacillus plantarum* from stock culture into the medium.
   v) Incubate culture for 16-24 hours at 35-37°C.
   vi) Centrifuge the culture at 3000 g, for 10 min.
   vii) Decant the supernatant liquid. Wash the cell 3 times with 10 ml of sterile 0.85% saline.
   viii) Resuspend the cell in 10 ml of sterile 0.85% saline.
   ix) Dilute the culture with the ratio of 1:100 with sterile 0.85% saline.
3. Prepare a series of standard test tube containing 0.0 (inoculated blank), 0.0 (uninoculated blank), 2, 4, 6, 8, 10 ml of the final biotin standard working solution.
4. Prepare a set of assay test tube containing 0.1 ml of sample solution.
5. Add deionized water into each test tube (standard and sample test tube) to make volume of 5 ml.
6. Add 5 ml of biotin assay solution into each tube.
7. Autoclave the mixture at 121°C for 10 min. Cool it rapidly.
8. Add 1 drop of diluted culture into each test tube (except uninoculated blank).
9. Incubate for 18-24 hours at 35-37°C until maximum turbidity reach.
10. Read the turbidity of sample and standard at 660 nm with UV/Vis spectrometer against inoculated blank.
11. Use uninoculated blank to set T at 100%
12. Prepare a standard curve of %T against concentration of biotin standard for sample quantification.

*** During all microbiological assay procedures, use clean glassware free from detergents and other chemical. Furthermore, heat glassware at 250°C for 1 hour to burn off any organic residues that might present. Conduct the whole assay under sterile and cool condition.*

Reference

Murakami, T., Yamano, T., Nakama, A., Mori, Y. 2008. Estimation of dietary intake of biotin and its measurement uncertainty using total diet sample in Osaka, Japan. Journal of AOAC international 91:6

3.3.4.6 DETERMINATION OF FOLIC ACID (VITAMIN B9) IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Folate in foods is extracted through tri-enzyme extractions, where protease, α-amylase and conjugase are employed. The tri-enzyme extractions enable the increase of total measurable folate from various food samples as each of the enzyme having different role in extraction. For example, α-amylase digestion increase folate extract from high starch and glycogen food. Folate content in food is identified by comparing ratio of fluorescence and UV peak height at different wavelength.

Chemicals/Reagents

1. Acetonitrile, HPLC grade
2. Protease, 2 mg/ml
3. α-Amylase, 20 mg/ml
4. Chicken pancreas conjugase, 5mg/ml
5. Hydrochloric acid (HCl), 0.1 M
6. Sodium hydroxide (NaOH), 0.1 M
7. Potassium phosphate buffer, 30 mM, pH 2.3
8. Elution buffer- Sodium acetate, 0.1 M (containing 10% sodium chloride, 1% ascorbic acid and 0.1% 2-mercaptoethanol)
9. Folic acid standard solution
   a. Use Folic acid (from Sigma Chemical Co.) as standard for folic acid determination.
   b. Folic acid standard stock solution. Weight 10 mg of standard and make up to 100 ml with deionized water to give a concentration of 100 µg/ml. Prepare freshly prior to analysis
   c. Folic acid standard working solution. Pipette 1 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 1 µg/ml.

Apparatus/Instruments

1. Balance: analytical sensitivity ± 0.1 mg
2. pH meter: accurate to 0.01 pH with calibration buffers
3. Extraction flask: 50 ml
4. Volumetric flask
5. Incubator: at 37°C
6. Pipettes: 1 ml, 5 ml, 10 ml
7. Shaking water bath
8. Vortex mixer
9. Centrifuge
10. SPE column: strong anion exchange (SAX, 500 mg)
11. Vacuum manifold
12. Vacuum pump
13. Syringe filter: 0.45µm PTFE
14. HPLC sample vials
15. HPLC with fluorescence detector and UV detector

16. Analytical column: RP C18, 150 mm x 4.6 mm I.D and particle size 3 µm
17. Guard column: 4.6 mm of I.D and length of 20 mm

HPLC conditions

1. Reverse phase C_{18} column of 150 mm x 4.6 mm I.D and particle size 3 µm (e.g. Atlantis. Waters).
2. Guard column holder with a guard column that have an I.D of 4.6 mm and length of 20 mm packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler, G1239.
4. Agilent 1200 series binary pump, G1312 to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser, G1379.
6. Agilent 1200 series fluorescence detector, G1321
7. Agilent 1200 series diode arrays multiple wavelength detector (DAD), G1315 for niacin detection at the wavelength of 254 nm.
8. Solvent cabinet to keep the mobile phases.
9. A computer with an Agilent ChemStation control software.
10. A HP LaserJet 5L printer to print the results and chromatograms.

Procedures

A. Sample extraction

1. Weigh accurately 5 g of hormogenized foodstuff into a 50 ml extraction flask.
2. Adjust pH to 4.5 with 0.1 M HCl.
3. Add 0.8 ml of protease, vortex and incubate at 37°C for 16 hours.
4. Place sample in 100°C shaking water bath for 10 min to deactivate protease. Cool the extract on ice to room temperature.
5. Add 0.8 ml of α-amylase, vortex and incubate at 37°C for 4 hours.
6. Place sample in 100°C shaking water bath for 10 min to deactivate α-amylase. Cool the extract on ice to room temperature.
7. Adjust pH to 7.2 with 0.1 m NaOH.
8. Add 1 ml of chicken pancreas conjugase, vortex and incubate at 37°C for 3 hours
9. Place sample in 100°C shaking water bath for 10 min to deactivate α-amylase. Cool the extract on ice to room temperature.
10. Centrifuge the extract at 3000 rpm, 10°C for 20 min.
11. Decant the supernatant and keep at -20°C for further purification.

B. Sample Purification

1. Clean the thawed sample with solid phase extraction (SPE) on strong anion exchange (SAX, 500 mg) column.
2. Connect the SPE SAX column on a vacuum manifold.
3. Condition the SAX columns with 5 ml of methanol follows by 5 ml of deionized water.
4. Load 2.5 ml (V1) supernatant onto SAX column.
5. Wash the column with 5 ml of water.
6. Elute folic acid from SAX column with 5 ml of freshly prepare elution buffer.
7. Discard the first 0.5 ml of eluate, then, collect the following 3.5 ml.
8. Filter the collected eluate through 0.45µm PTFE syringe filter prior to HPLC analysis.

C. Instrument set up procedures

1. Before turning on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase is used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.
4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump and close the purge valve. Set the required mobile phase composition (gradient) and flow rate of 0.4 ml/min for folic acid analysis.
6. Turn on the pump again and allow the column to equilibrate with mobile phase which usually need 1 hour.
7. Make sure the UV and Visible lamps are turn on and set the DAD detector at wavelength of 280, 290 and 300 nm.
8. Set the excitation and emission wavelength of fluorescence detector at 290 nm and 360 nm.
9. Perform gradient elution with acetonitrile (solvent A) and potassium phosphate buffer (solvent B). Start gradient with 6% acetonitrile and maintained isocratically for the first 5 min, thereafter the acetonitrile concentration is increased linearly to 25% within 20 min and total run time is 42 min.
10. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
11. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.

D. Chromatography of folic acid

1. Identify peak of folic acid by retention time and confirm the identity of folic acid by comparing ratio of fluorescence and UV peak height at different wavelength.
2. Obtain a standard curve by using the peak area given by each standard solution (10 – 50 µg/ml)
3. Quantify folic acid of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standards. The total amount of niacin in sample is expressed in mg/100g of sample.

Calculation

$$\text{mg folic acid per 100g food} = \frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in µg} \times \frac{\text{V1 (ml)}}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Johansson, M., Furuhagen, C., Frølich, W., Jägerstad, M. 2008. Folate content in frozen vegetarian ready meals and folate retention after different reheating methods. LWT 41: 528-536.

3.3.4.7 DETERMINATION OF COBALAMIN (VITAMIN B12) IN FOOD (ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY METHOD)

Principles

Vitamin B12 in food sample is extracted with enzyme digestion and analyzed by using the combination of UPLC-MS with positive ESI ionization.

Chemicals/Reagents

1. Acetonitrile, HPLC grade
2. Methanol, HPLC grade
3. Pepsin
4. α-Amylase
5. Sodium hydroxide (NaOH), 0.1 M
6. Sodium acetate buffer, 50 mM, pH 4
7. Sodium cyanide, 1%
8. Ammonium formate, 1M
9. Formic acid
10. Internal standard
    a. Use ginsenoside Re (from Sigma Chemical Co.) as internal standard.
    b. Prepare I.S. stock solution (40 µg/ml) by dissolving 4 mg of ginsenoside Re in deionized water and make up to volume of 100 ml.
    c. Prepare working solution (0.1 µg/ml) by pipetting 0.25 ml of stock solution (b) into 100 ml volumetric flask and make up to volume with deionized water.
11. Vitamin B12 standard solution
    a. Use vitamin B12 (from Sigma Chemical Co.) as standard for vitamin B12 determination.
    b. Vitamin B12 standard stock solution. Weight 1 mg of standard and make up to 100 ml with deionized water to give a concentration of 10 µg/ml. Prepare freshly prior to analysis
    c. Vitamin B12 standard working solution. Pipette 0.1 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with deionized water. The final concentration of working solution is 10 ng/ml.
    d. Prepare vitamin B12 standard in the concentration range of 10-150 ng/ml. Store working solution at 4°C before use.

Apparatus/Instruments

1. Balance: analytical sensitivity ± 0.1 mg
2. Homogenizer
3. Incubator: at 37°C
4. pH meter: accurate to 0.01 pH with calibration buffers
5. Volumetric flask: 50 ml
6. Centrifuge
7. Ultrafiltration system: with membrane filter, 0.45 µm
8. UPLC with PDA detector, column heater, MS system and Q-Tof
9. UPLC analytical column, RP C18: 50 mm x 2.1 mm I.D and particle size 1.8 µm.
10. Guard column: with I.D of 2.1 mm, length of 5 mm and 1.8 µm.

UPLC-ESI-MS conditions

1. UPLC Reverse phase C_{18} column of 50 mm x 2.1 mm I.D and particle size 1.8 µm (e.g. ACQUITY UPLC HSS T3, Waters).
2. Guard column with I.D of 2.1 mm, length of 5 mm and 1.8 µm packed with the same material as that of the analytical column (e.g. ACQUITY UPLC HSS T3 VanGuard Pre-column, Waters)
3. Waters ACQUITY UPLC® system
4. Waters ACQUITY UPLC® sample organizer.
5. Waters ACQUITY UPLC® binary pump to deliver the mobile phase.
6. Waters ACQUITY UPLC® column heater/ cooler.
7. Waters ACQUITY UPLC® PDA detector.
8. Waters SYNAPT™ mass spectrometry (MS) system.
9. Water Q-Tof Premier™
10. A computer with a MassLynx™ Imformatics control software.
11. A HP LaserJet 5L printer to print the results and chromatograms.

Procedures

A. Sample extraction

1. Weigh accurately 15 g of food sample into an extraction flask.
2. Add 40 ml of sodium acetate buffer, 1 ml of sodium cyanide, 0.25 g of α-amylase and 1 g of pepsin.
3. Homogenize the sample with homogenizer for 5 min.
4. Incubate the mixture with shaking incubator at 37°C for 3 hours.
5. Adjust pH to 4.8 with 0.1 M NaOH.
6. Heat the extract at 100°C for 35 min under agitation and nitrogen steam reflux.
7. Cool the extract immediately to room temperature.
8. Transfer the extract quantitatively in a 50 ml volumetric flask, add internal standard, top up to volume with deionized water.
9. Centrifuge the diluted extract at 8000 g, 4°C for 10 min.
10. Filter the supernatant through 0.45 µm membrane filter before injection.

B. Instrument for set up procedures

1. Before turning on the UPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase are used.
2. Turn on the MassLynx 4.1 control software and UPLC system, then, warm up instrument for at least 30 min before starting.
3. Turn on Start Up System, select purging for 5 minutes for valve A and B, as well as sample needle wash for 5 cycles.
4. Set the pump to allow the column to equilibrate with mobile phase which usually need 1 hour.
5. Make sure the UV and Visible lamps are turned on and set the PDA detector at wavelength of 200-500 nm.
6. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
7. Key in the sample identity and build the sequence in SAMPLE LIST for MassLynx.

C. Chromatography of niacin

1. UPLC Condition
   a. Set column temperature at 40°C and sample temperature at 4°C
   b. Set mobile phase flow rate at 0.6 ml/min
   c. Prepare mobile phase A in the combination of 990 ml of water: 10 ml of ammonium formate: 1 ml formic acid
   d. Prepare mobile phase B in the combination of 990 ml of methanol: 10 ml of ammonium formate: 1 ml formic acid
   e. Program the gradient elution as follow:

0.0 min	99% A
2.0 min	99% A
3.0 min	45% A
3.1 min	99% A
4.0 min	99% A

   f. Sample injection volume is 20 µl, full-loop injection
   g. Wash needle with 0.1% formic acid in water (weak wash); or 0.1% formic acid in methanol (strong wash)
   h. Total run time for each sample is 4 min.

2. MS condition
   a. Set 0.6 ml/min portion of column eleunt into MS
   b. Acquire ESI-MS spectra in positive ion mode
   c. Use nitrogen as both desolvation (350 L/hr) and cone (50 L/hr) gas.
   d. Set 350 °C for desolvation temperature.
   e. Set 105°C for source temperature.
   f. Capillary voltage is 4 kV; cone voltage is 180 V
   g. Use argon gas as collision gas at 3.5×10^{-3} mBar
   h. Use m/z 930.8 and 969.9 as quantifier ions for Vitamin B12 and ginsenoside Re respectively.
3. Obtain calibration curve by using the area counts given by each standard solution.
4. Express the total amount of vitamin B12 in sample in ng/g of sample.

Reference

Luo, X., Chen, B., Ding, L., Tang, F., Yao, S. 2006. HPLC-ESI-MS analysis of vitamin B12 in food products and in multivitamins-multimineral tablets. Analytica Chimica Acta 562: 185-189.

3.3.4.8 DETERMINATION OF CHOLINE IN FOOD

METHOD A: ENZYMATIC COLORIMETRIC METHOD

Method is applicable to the determination of choline in milk and infant formula containing 44-175 mg solids/100g. Method does not apply to powdered infant formula/milk containing more than 100 mg vitamin C/100g solids because of ascorbate suppression of color development.

Principles

The product is acid digested at 70ºC, to release most of the bound choline. Following pH adjustment, residual choline phospholipids are cleaved with phospholipase D and free choline is subjected to choline oxidase with liberation of H_2O_2. In the presence of peroxidise, phenol is oxidized and a quinoneimine chromophore is formed with 4-aminoantipyrine. Absorbance is measured at 505 nm and choline content calculated by interpolation from multilevel calibration. For this procedure, choline is defined and reported as the hydroxide.

See table for the results of the interlaboratory study supporting the acceptance of the method.

Chemicals/Reagents

If substituting enzymes come from another source than that specified, ensure that the declared activity (units/mg) are similar. Store enzymes and 4-aminoantipyrine at 4ºC or as instructed by the supplier.

(a) Water – Purified to > 18 MΩ resistivity.
(b) Hydrochloric acid – 1.0M. Measure 85 mL concentrated HCl inti 1L volumetric flask and dilute to the mark with water. Standardization is not required.
(c) Sodium hydroxide solution – f0% w/v. Dissolve 50g NaOH in ca 80 mL water in graduated cylinder with cooling, and dilute to 100 mL.
(d) Choline bitartrate – Sigma C-2654 or equivalent. Dry ca 0.7-1.0 g choline bitartrate at 102º to constant weight. Store desiccated at ambient temperature. (1) Stock choline standard – 2500 µg/mL as choline hydroxide. Add 523 mg dry choline bitartrate to 100 mL volumetric flask. Dissolve in water and dilute to the mark. Store refrigerated at 4 ± 2ºC. Do not use after 1 week. (2) Working choline standard – 250 µg/mL as choline hydroxide. Pipet 10 mL stock solution, (1), and dilute to 100 mL with water. Prepare fresh daily.
(e) Tris (hydroxymethyl) aminomethane (Trizma) buffer. – 0.05 M (pH 8.0). Add 6.057g tris (hydrixymethyl) aminomethane (Sigma T-1503 or equivalent) to 500 mL water in 1 L volumetric flask. Bring pH to 8.0 with 1M HCl, (b), and dilute to the mark with water. Store at 4ºC. Solution is stable for 1 month.
(f) Phospholipase D. – Sigma Type VI, P-8023, from Streptomyces chromofuscus, 150 units/mg. Unit definition: One unit will liberate 1.0 µmol choline from L-α-phosphatidyl choline (egg yolk) per hour at pH 5.0 at 30ºC. This form of phospholipase D is preferred over others due to its activity for sphingomyelins and lysophospholipids.
(g) Choline oxidase – Sigma, C-5896, from Alcaligenes species, 10 units/mg. Unit definition : One unit will form 1.0 µmol H_2O_2 with oxidation og 1.0µmol choline to betaine aldehyde per minute at pH 8.0 at 37ºC.

(h) Peroxidase – Sgma Type 1, P-8125, from horseradish, 80 unit/mg. Unit definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 s at pH 6.0 at 20ºC.
(i) 4-aminoantipyrine – sigma A4382 or equivalent.
(j) Phenol
(k) Chromogenic reagent – Into a 100 mL volumetric flask, sequentially weigh: 75-100 units phospholipase, (f); 100-120 units choline oxidase, (g); 250-280 unit peroxidise, (h); 15 mg 4-aminoantipyrine, (i); and 50 mg phenol, (j). Dissolve and dilute to volume with 0.05 M Trizma buffer, (e), (pH 8.0). Prepare fresh daily.

Apparatus/Instruments

(a) Spectrophotometer: Digital readout to 0.001 absorbance at 505 nm. Glass cuveltes (10 mm) of flow-through cell.
(b) Covered water baths: 37 ± 2ºC and 70 ± 4ºC.
(c) pH meter: Reading to 0.001 unit, with calibration buffers, 4.0 and 7.0.
(d) Pipets: Calibrated glass, or preferably auto-pipetors for 0.100 and 3.0 mL.
(e) Volumetric flasks: 10, 50 and 100 mL and 1 L.
(f) Digestion vessels: Conical flask, 100 and 150 mL, with stoppers. Alternatively, boiling tubes (dimensions ca 150 x 25 mm) with stoppers can be used, with suitable rack. Either vessel should permit insertion of a pH probe for post-digestion pH adjustment.
(g) Test tubes: 10 mL with glass stoppers, with rack. Alternatively disposable polypropylene or polystyrene plastic tubes fitted with screw-capped lids may be used.
(h) Filter paper: Medium speed, porosity 1.4-2.9 microns, Whatman No. 2, or equivalent.

Procedures

Note: Perform all steps of assay under incandescent lighting; avoid fluorescent lighting or direct sunlight.

(a) Hydrolysis

Weigh 5.00 g test portion into 100 mL conical flask (or boiling tube). Add 30 mL 1.0M HCl, C(b), stopper, and mix by shaking until well dispersed. Place flasks into water bath at 70ºC for 3 h, shaking occasionally. Loosen or remove stoppers occasionally to avoid excessive pressure buildup/ cool to ambient temperature, adjust pH to 3.4-4.0 with 50% NaOH, C(c), and quantitatively transfer discoloured solution to 50 mL volumetric flask. Dilute to the mark with water, filter through filter paper, and collect filtrate after discarding the first 5-10 mL. Ensure filtrate is free of particulates; otherwise refilter. Filtrate is stable and may be stored in the dark at 4ºC for up to 3 days. Return filtrate to ambient temperature before proceeding with assay. For each test solution, separately label two 10 mL test tubes as test solution (tube 1) and blank (tube 2). Into each tube, dispense a 0.100 mL aliquot of filtrate.

(b) Preparation of standard curve

Pipet 2, 4, 6, and 8 mL aliquots of working standard, C(d)(2), (250 µg/mL) into four 10 mL volumetric flask and dilute to the mark with water. The fifth level calibrant is the undiluted working standard. Prepare a 5-level standard curve (50, 100, 150, 200 and 250 µg/mL0 by dispersing 0.100 mL of each standard into separately labelled 10 mL test tubes. A

reagent balnk tube (0 µg/mL) is also included by substituting 0.100 mL water in place of choline solution.

(c) Enzymatic determination

To each blank (tube 2), add 3.00 mL water. Add 3.00 mL chromogenic reagent, C(k), to each test solution (tube 1), the 5 standards, and the reagent blank.

Place test tubes in a covered water bath at 37 ± 2ºC for 15 min to develop a pink/red color. Remove from water bath and cool to ambient temperature for 15 min. Without delay, transfer contents of each tube into 10 mm optical cells, or use a flow-through cuvette.

Zero spectrophotometer at 505 nm against water and measure the absorbance of the standards (A_{std}), test solution (A'), blanks (A_{bl}), and reagent blank (A_{reag}).

Calculations

Create a standard calibration of net absorbance (A_{std}-A_{reag}) versus choline concentration (µg/mL) using linear least mean square regression analysis with forced origin.

Subtract the absorbance of the blanks and reagent blank from each test solution to eliminate nonspecific spectral background:

Net absorbance of test solution, $A = A' - A_{bl} - A_{reag}$

Determine the choline concentration in test solution, expressed as choline hydroxide:

$$\text{Choline hydroxide, mg/100g} = \frac{A}{S} \times \frac{V}{1000} \times \frac{100}{W}$$

where A = net absorbance of test solution; S = slope of standard curve; V = volume in mL of the hydrolysates (50 mL); W = weight in grams of test portion (5.00 g); 100 = conversion to 100 g basis; 1000 = conversion µg to mg. Report data to one decimal place.

Table 1 Interlaboratory study results for choline in milk and infant formula

	Sample[b]							
Parameter[a]	1	2	3	4	5	6	7	8
No. of labs	28	29	29	29	29	28	29	26
Average, mg/100 g	87.66	73.22	173.02	47.13	99.71	132.68	109.39	124.94
S_D, mg/100 g	3.03	2.03	5.95	1.52	2.23	3.07	2.01	3.16
RSD_D%	3.46	2.77	3.44	3.23	2.24	2.32	1.84	2.53
2.8s, mg/100 g	8.49	5.68	16.66	4.26	6.25	8.60	5.62	8.86
S_R, mg/100 g	4.55	4.19	8.04	2.99	5.26	5.85	4.66	6.54
RSD_R %	5.19	5.73	4.64	6.34	5.27	4.41	4.26	5.23
S_R, mg/100 g	12.75	11.74	22.50	8.37	14.72	16.39	13.06	18.31

[a] 1, 3, 4 = Milk/whey-based infant formula; 2 = milk-based follow on; 5 = soy-based infant formula; 6 = NIST SRM 1846, 7 and 8 = whole milks. Samples are variously vitaminized, oil filled, lecithinated, and supplemented with choline chloride.
[b] All values excluding invalid data, no outliers detected.

Reference

J. AOAC Int. 83, 131 (2000)

METHOD B: ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY METHOD

Principles

Choline, β-hydroxyethyltrimethyl-ammonium hydroxide, is a water soluble essential nutrient that required for synthesis of phospholipids in cell membranes, methyl metabolism, and normal cholinergic neurotransmission in human. Choline compounds in food samples are extracted and partitioned into organic and aqueous phases using methanol and chloroform and analyzed directly by liquid chromatography-electrospray ionization-isotope dilution mass spectrometry (LC-ESI-IDMS). Samples are analyzed for free choline (Cho) and choline-contributing compounds: glycerophosphoscholine (GPCho), phosphocholine (Pcho), phosphatidylcholine (Ptdcho) and sphingomyelin (SM). Total choline content in food sample is to be calculated as the sum of Cho, GPC, Pcho, Ptdcho and SM.

Chemicals & reagents

1. Methanol, HPLC grade
2. Chloroform, HPLC grade
3. Standards for calibration curve
    a. Choline chloride, C7017 Sigma
    b. Glycerophosphocholine, G5291 Sigma
    c. Phosphocholine, P0378 Sigma
    d. Phosphatidylcholine, P3556 Sigma
    e. Sphingeomyelin, S7004 SIgma
4. Isotopes
    a. Choline-[N,N,N-trimethyl-d_9]bromide
    b. Phosphocholine-[N,N,N-trimethyl-d_9] chloride
    c. 1,2-Dipalmitoyl-sn-3-glycero-phosphocholine-[N,N,N-trimethyl-d_9]
    d. Synthesis of glycerophosphocholine-[N,N,N,-trimethyl-d_9]
        i. Hydrolyze 10 µmol of 1,2-dipalmitoyl-sn-3-glycerophosphocholine-[N,N,N-trimethyl-d_9] in 1 ml of chloroform/methanol (1:4) mixture with 100 µL of 1.2 N NaOH in methanol/water (1:1) at 37°C for 10 min.
        ii. After incubation, the mixture is neutralized with 150 µL of 1N acetic acid. Then, add 2 ml of chloroform/methanol (9:1), 1 ml of isobutanol and 2 ml of water in the mixtue.
        iii. Re-extract the lower phase with 1 ml of methanol/water (1:2). Combine the aqueous phase, dry and purifies by HPLC.
    e. Synthesis of Sphingomyelin –[N-methyl-d_3]
        i. Dissolve 1 mmol of sphingomyelin and 6 mmol of 1,4-diazabicyclo(2,2,2)-octane in 50 ml of dimethyl formamide. Reflux the mixture under stream of nitrogen for 6 h.

ii. Cool the mixture and pour it in 20 ml ice-cold 1 N HCl and then extract with chloroform (50 ml). Collect chloroform extract and dry hot.

iii. Mix 200 mg of the dried extract with 30 µL of cyclohexylamine (0.35 mmol) and 90 µL of iodometahne-d_3 in 5 ml of methanol before it is tightly sealed with Teflon line screw cap tube and store at room temperature in dark for 18 h.

iv. After that, evaporate solvent under vacuum. Dissolve residue with chloroform and wash twice with 5% sodium thiosulfate, 2N HCl and water. Then dry it and purify with SPE cartridge (aminopropyl column, Bond Elut).

Apparatus/ Instruments

1. Pipettes: 10 ml, 1 ml
2. Micropipettes: 100 µl
3. Incubator
4. Screw cap tube, Teflon
5. SPE cartridge, aminopropyl column
6. Balance: analytical sensitivity ± 0.1 mg
7. Vortex mixer
8. Centrifuge
9. Fume hood
10. Volumetric flask: 10 ml
11. Ultrafiltration system with membrane filter, 0.45 µm
12. UPLC with PDA detector and column heater: equip with MS system and Q-Tof
13. Analytical column, silica column: 150 mm x 2.1 mm I.D and particle size 1.7 µm.

UPLC-ESI-MS conditions

1. LC Silica column of 150 mm x 2.1 mm I.D and particle size 1.7 µm (e.g. UPLC HILIC, Waters).
2. Guard column with I.D of 2.1 mm, length of 5 mm and 1.7 µm which is packed with the same material as that of the analytical column
3. Waters ACQUITY UPLC® system
4. Waters ACQUITY UPLC® sample organizer.
5. Waters ACQUITY UPLC® binary pump to deliver the mobile phase.
6. Waters ACQUITY UPLC® column heater/ cooler.
7. Waters ACQUITY UPLC® PDA detector.
8. Waters SYNAPT™ mass spectrometry (MS) system.
9. Water Q-Tof Premier™
10. A computer with an MassLynx™ Imformatics control software.
11. A HP LaserJet 5L printer to print the results and chromatograms.

Procedures

A. Sample preparation

1. Freeze sample at -80°C and pulverized it prior to analysis
2. Weigh accurately 100 mg of food sample then spike with deuterium labeled internal standard (isotopes) of all analytes.
3. Add 400 µL of methanol/chloroform (2:1, v/v), votex vigorously and left at -20°C overnight before centrifuge at 1500g for 5 min.
4. Transfer the supernatant to a new tube, re-extract the residues with 250 µL of methanol/ chloroform/ water (2:1:0.8, v/v)
5. Combine the supernatant from both extractions, then add 100 µL of chloroform follow by 100 µL of water to form 2 phases. Centrifuge it.
6. After centrifuge, separate aqueous phase and chloroform phase.
7. Dry aqueous phase and redissolve it in 20 µL of water. Then, add 800 µL of methanol, vortex, centrifuge and collect the supernatant for LCMS analysis (10 µL).
8. For chloroform phase, dry it, resuspend in chloroform and then purify it with SPE cartridge (aminopropyl column, Bond Elut) prior to LCMS analysis.

C. Instrument set up procedures

1. Before turning on the UPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase are used.
2. Turn on the MassLynx 4.1 control software and UPLC system, then, warm up instrument for at least 30 min before starting.
3. Urn on Start Up System, select purging for 5 minutes for valve A and B, as well as sample needle wash for 5 cycles.
4. Set the pump to allow the column to equilibrate with mobile phase which usually need 1 hour.
5. Make sure the UV and Visible lamps are turned on and set the PDA detector at wavelength of 200-500 nm.
6. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
7. Key in the sample identity and build the sequence in SAMPLE LIST for MassLynx.

D. Chromatography of choline

1. UPLC Condition
    a. Set column temperature at 40°C and sample temperature at 4°C
    b. Prepare mobile phase A in the combination of acetonitrile/water/ethanol/1 M ammonium acetate/ glacial acetic acid (800/127/68/3/2, v/v)
    c. Prepare mobile phase B in the combination of acetonitrile/water/ethanol/1 M ammonium acetate/ glacial acetic acid (500/500/85/27/18, v/v)
    d. Program the gradient elution as follow for aqueous phase:

0.0 min	0% B	0.4 ml/min
3.0 min	0%B	0.4 ml/min
10.0 min	40% B	0.4 ml/min
14.0 min	45% B	0.3 ml/min
18.0 min	60% B	0.3 ml/min
19.0 min	100%B	0.5 ml/min
27.0 min	100%B	0.5ml/min
29.0 min	0%B	0.4ml/min

    e. Program the gradient elution as follow for organic (chloroform) phase:

0.0 min	0% B	0.4 ml/min
3.0 min	0%B	0.4 ml/min
10.0 min	20% B	0.4 ml/min
14.0 min	60% B	0.3 ml/min
16.0 min	100% B	0.3 ml/min
17.0 min	100%B	0.5 ml/min
20.0 min	100%B	0.5ml/min
22.0 min	0%B	0.4ml/min

    f. Sample injection volume is 10 µl, full-loop injection
    g. Wash needle with 0.1% formic acid in water (weak wash); or 0.1% formic acid in methanol (strong wash)
    h. For aqueous phase, allow LC effluent from 5-26 min into MS; for organic phase, allow effluent from 4-14 min into MS.
2. MS condition
    a. Set 0.6 ml/min portion of column effluent into MS
    b. Acquire ESI-MS spectra in positive ion mode
    c. Use nitrogen as both desolvation (350 L/hr) and cone (50 L/hr) gas.
    d. Set 350 °C for desolvation temperature.
    e. Set 105°C for source temperature.

f. Capillary voltage is 5 kV; cone voltage is 180 V

g. Use argon gas as collision gas at 3.5 X 10^{-3} mBar

h. For aqueous phase. Use m/z 104 (Cho), m/z 113 (Cho-d_9), m/z 258 (GPCho), m/z 267 (GPCho-d_9), m/z 184 (PCho) and m/z 193 (PCho-d_9).

i. For organic phase. Use m/z 184 (PtdCho and SM); m/z 193 (PtdCho-d_9) and m/z 187 (SM-d_3).

3. Prepare calibration curve standard by mixing 20 nmol of deuterium-labeled internal standards with varying amount of analytes.
4. The calibration curves are constructed by relating the varying amounts of each analyte to their relative response factors (RRFs) as determined by the ratio of the peak area of the analyte to that of the corresponding deuterium-labeled internal standard.
5. Calculate total choline content (mg/100g food) in sample as the sum of Cho, GPCho, Pcho, PtdCho and SM. Report individual metabolites as mg choline moiety per 100g of food.

References

Koc, H., Mar, M-H., Ranasinghe, A., Swenberg, J.A. & Zeisel, S. H. 2002. Quantitation of choline and its metabolites in tissues and food by liquid chromatography/ electrospray ionization-isotope dilution mass spectrometry. Analytical Chemistry. 74: 4734-4740.

Zeisel, S.H., Mar, M-H, Howe, J-C, Hoilden, J.M. 2003. Concentrations of choline-containing compounds and betaine in common foods. Journal of Nutrition. 133: 1302-1307.

3.3.4.9 DETERMINATION OF ASCORBIC ACID (VITAMIN C) IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

High pressure liquid chromatography analytical methods are now replacing the traditional titrimetric and colorimetric methods for more accurate determination of total vitamin C. HPLC method is able to distinguish between L-ascorbic acid and isoascorbic acid and eliminate other deficiencies of traditional method. For example, colour interference by highly coloured extracts during the titration's end point, and overestimation of total vitamin C content due to reduction of dye by other substances like ferrous, tannin and cuprous.

Ascorbic acid in sample is extracted in acidic environment and completed rapidly in subdued light. Metaphosphoric acid is chosen as extractant because it inhibits L-ascorbic acid oxidase and metal catalysis, precipitates proteins and compatible to LC system. Besides, tris (2-carboxyethyl)-phosphine (TCEP) is included in extractant to convert dehydroascorbic into its reduced form and to stabilize ascorbic acid.

Reverse-phase HPLC procedure is employed in this method and 0.05% of formic acid is used as mobile phase with a Phenomenex C_{18} column. The diode array detector is set at 255 nm for L-ascorbic acid determination.

Chemicals/ Reagents

1. Extraction buffer preparation
    a. Prepare an extraction buffer containing 5 % of metaphosphoric acid (MPA), 1 mM of ethylenediaminetetraacetic acid (EDTA) and 5 mM of tris(2-carboxyethyl)phosphine (TCEP).
    b. Prepare 5 % MPA by dissolving 50 g of MPA with deionized water and make to volume of 1 L.
    c. Dissolve 150 mg of EDTA and 717 mg of TCEP with 5% of MPA, then top up to volume of 500 ml in volumetric flask. Refrigerate the extraction buffer until use.
2. Mobile phase preparation
    a. Prepare 0.05% of formic acid by dissolving 0.5 g of formic acid with deionized water and top up to volume of 1 L in volumetric flask. Filter the mobile phase with 0.45 µm nylon membrane filter using solvent filtration apparatus. Prepare mobile phase freshly prior to every analysis.
3. Vitamin C standard solution
    a. Use crystalline L-ascorbic acid (99% purity, e.g. from Sigma Chemical Co.) as standard for ascorbic acid determination.
    b. Ascorbic acid standard stock solution. Weigh 10 mg of standard and make up to 100 ml with 5% MPA to give a concentration of 100 µg/ml. Prepare freshly prior to analysis
    c. Ascorbic acid standard working solution. Pipette 10 ml of standard stock solution (b) in 100 ml volumetric flask and make up to volume with 5% MPA. The final concentration of working solution is 10 µg/ml.

Note: Preparation of all standards for ascorbic acid should be carried out in a room with subdued light. All sample treatment and analytical procedures were also carried out in this room.

Apparatus/Instruments

1. Analytical balance
2. Conical flask: 100 ml
3. Homogenizer
4. Centrifuge
5. Filtration system with 0.45 µm PVDF membrane filter
6. Volumetric flask: 50 ml
7. HPLC with UV detector.
8. Analytical column: RP C18, 150 mm x 4.6 mm I.D and particle size 5 µm
9. Guard column: length 20 mm, I.D 4.6 mm

HPLC conditions

1. Reverse phase C_{18} column: 150 mm x 4.6 mm I.D and particle size 5 µm (e.g. Atlantis. Waters).
2. Guard column holder with a guard column: an I.D of 4.6 mm and length of 20 mm packed with the same material as that of the analytical column.
3. Agilent 1200 series standard and preparative autosampler, G1239.
4. Agilent 1200 series binary pump, G1312 to deliver the mobile phase.
5. Agilent 1200 series micro vacuum degasser, G1379.
6. Agilent 1200 series diode arrays multiple wavelength detector (DAD), G1315 for ascorbic acid detection at the wavelength of 254 nm.
7. Solvent cabinet to keep the mobile phases.
8. A computer with an Agilent ChemStation control software.
9. A HP LaserJet 5L printer: to print the results and chromatograms.

Procedures

A. Sample extraction

1. Weigh accurately 10 g (fresh sample) or 2 g (dried sample) of the foodstuff into 100 ml conical flask.
2. Add 15 ml of cooled extraction buffer into the flask and extract for 10 min with a homogenizer.
3. Repeat the extraction procedure in (2) for 3-4 times and combine the extract.
4. Centrifuge the extract at 10°C, 5000 rpm for 30 min.
5. Decant the supernatant and filter with 0.45 µm PVDF membrane filter.
6. Quantitatively transfer the filtered extract to a 50 ml volumetric flask and top up to volume with extraction buffer (**V1**).

B. Instrument set up procedure

1. Before turning on the HPLC, check the column, reservoir and mobile phase to ensure that the correct column and mobile phase are used.
2. Turn on the Agilent Chemstation control software and HPLC system, then, warm up instrument for at least 30 min before starting.
3. Open the purge valve on the pump module by turning the knob counter clockwise several turns so that later priming solvent will directly deliver to waste container.

4. Set the pump 100% at flow rate of 5 ml/min to primp every channel in solvent delivery system with respective mobile phase for several minutes.
5. Turn off the pump, close the purge valve, set the required mobile phase composition (isocratic) and flow rate of 1 ml/min for ascorbic acid analysis.
6. Turn on the pump again and allow the column to equilibrate with mobile phase which usually need 1 hour.
7. Make sure the UV and Visible lamps are turn on and set the DAD detector at wavelength of 255 nm.
8. To check whether column is equilibrated, inject in above standard mixture for 2 runs and if the elution time of the standards is the same for the 2 runs then the column is ready for sample injection.
9. Key in the sample identity and build the sequence in SAMPLE SEQUENCE for autosampler.

C. Chromatography of ascorbic acid

1. The extract is filtered through a 0.45 µm PVDF syringe filter into a sample vial and 5.
2. Identify ascorbic acid in sample by comparing its retention time with the retention time of standard L-ascorbic acid.
3. Obtain a standard curve by using the peak area given by each standard solution (0.5 – 50 µg/ml)
4. Quantify ascorbic acid of sample by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standard ascorbic acid. The total amount of ascorbic acid in sample is expressed in mg/100g of sample.

Calculations

mg ascorbic acid per 100g food =

$$\frac{\text{Peak area of sample}}{\text{Peak area of std.}} \times \text{Amount of std. in µg} \times \frac{V1\ (\text{ml})}{\text{Inject volume in ml}} \times \frac{100}{\text{Wt. of food used}}$$

Reference

Phillips, K.M., Tarragó-Trani, M. T., Gebhardt, S. E., Exler, J., Patterson, K. Y., Haytowitz, D. B., Pehrsson, P. R., Holden, J. M. (1992). Stability of vitamin c in frozen raw fruit and vegetable homogenates. Journal of Food Composition and Analysis, 23:253-259.

3.3.5 AMINO ACIDS

3.3.5.1 DETERMINATION OF AMINO ACIDS IN FOOD (HIGH PRESSURE LIQUID CHROMATOGRAPHY METHOD)

Principles

Amino Acids content in the food samples is detected and quantified using High Performance Liquid Chromatography (HPLC) with fluorescence detector equipped with Waters AccQTag amino acid analysis column and buffer.

Chemicals/Reagents

1. Waters AccQ Tag Eluent A
2. Waters AccQ Tag Eluent B
3. Waters AccQ Fluor Reagents
4. Acetonitrile
5. Amino acid standard
6. Deionized water, DH_2O
7. 2 Amino butyric acid (AABA) as internal standard
8. Hydrochloric acid, HCI
9. Borate buffer
10. Methionine sulfonate ($MetO_2$)
11. Cysteic acid (Cya)
12. Formic acid (HCOOH)
13. Hydrogen Peroxide (H_2O_2)
14. Hydrogen bromide (HBr)
15. Sodium acetate
16. Methanol
17. Tryptophan Standard
18. Lithium hydroxide ($LiOH.H_2O$)

Apparatus/Instruments

1. Balance: analytical sensitivity ±0.1 mg
2. Vortex mixer
3. Nitrogen gas blower
4. Oven
5. Rotary evaporator
6. Refrigerator
7. pH meter
8. Test tubes: 10 mL
9. Graduated bottle: 250 mL
10. Volumetric flasks: 10, 50, 100, 500 & 2000 mL
11. Measuring cylinder: 10 & 200 mL
12. Pasteur pipette
13. Micropipette
14. Test tubes: black srew-capped
15. Test tubes with stopped
16. Filter funnel

17. Whatman no. 541 filter paper
18. Syringe filter: 0.45 µm cellulose acetate membrane
19. Syringe filter: 0.2 µm cellulose acetate membrane
20. Ultra filtration equipment: 0.45 µm ,cellulose acetate membrane filters
21. Syringe
22. Eppendorf tube: 1.5 mL
23. Low insert volume tubes
24. Vials

Procedures

(A) 6 N HCI Hydrolysate

Chromatographic Conditions:

1. Column: AccQ Tag Column (3.9 x 150 mm)
2. Mobile phase: AccQ Tag Eluent A, concentrate
   : AccQ Tag Eluent B or 60% acetonitrile
3. Derivatization : AccQ Fluor Reagents
4. Standards: Amino acid standard, hydrolysate (standard H, 'Pierce')
5. Flow rate: 1 ml/min
6. Column temperature: 36ºC
7. Detection: Fluorescence Detector
   Excitation λ=250 nm
   Emission λ =395 nm
   Gain =100
   Filter = 1.5 sec
8. Injection volume : 10 µl
   Program Gradient:

Time (min)	Flow (ml/min)	%A	%B	Curve
Initial	1.0	100.0	0.0	*
0.50	1.0	98.0	2.0	6
15.00	1.0	90.0	10.0	6
19.00	1.0	87.0	13.0	6
32.00	1.0	65.0	35.0	6
33.00	1.0	65.0	35.0	6
34.00	1.0	0.0	100.0	6
37.00	1.0	0.0	100.0	6
38.00	1.0	100.0	0.0	6
50.00	1.0	100.0	0.0	6

Shut-down and Storage of Column:

Time (min)	Flow (ml/min)	%A	%B	Curve
Initial	1.0	100.0	0.0	*
1.00	1.0	0.0	100.0	6
20.00	0.0	0.0	0.0	11

A. Preparation of Eluent A

1. Dilute 200 ml of AccQ Tag Eluent (concentrate) with 2000 ml of DH_2O.
2. Filter through 0.45 µm cellulose membrane filter.

B. Preparation of 2 Amino butyric acid (AABA) (internal standard) (50µmole/ml)

1. Dissolve 0.0516 g of AABA in 100ml of 0.1 N HCI.

C. Preparation of Amino Acids Standard

1. Pipette 5 ml of AABA (internal standard)(50µmole/ml) into 100ml volumetric flask.
2. Add 0.1 N HCI into the volumetric flask and make up to 100ml (1ml=2.5 µmole/ml AABA).
3. Pipette 1.5ml of 2.5µmole/ml AABA into test tube and mix with 1.5ml of Amino acid standard 'H' (1ml=2.5 µmole/ml of amino acids & 1.25 µmole/ml of Cys2) from 2 ampoules (1ml=1.25 µmole/ml of amino acids & 0.625 µmole/ml of Cys2).
4. Pipette 160 µl of 3 ml solution (1ml=1.25 µmole/ml of amino acids & 0.625 µmole/ml of Cys2) and add 840 µl water (1ml=0.2 µmole/ml of amino acids & 0.1 µmole/ml of Cys2).
5. Pipette 10 µl of 1000 µl solution (1ml=0.2 µmole/ml of amino acids & 0.1 µmole/ml of Cys2) (for derivatization)(1ml=0.2 nmole amino acids & 0.1nmole Cys2 and add 70 µl borate buffer and 20µl AccQ reagent.
6. Inject 10 µl (1ml=200pmole amino acids & 100pmole Cys2) into HPLC system.

D. Sample Preparation and Addition of Internal Standard

1. Weigh 0.1g-0.2g sample and put into black screw-capped test tube.
2. Add 10ml of 6N HCI, vortex the test tube and pass with N_2 gas.
3. Heat in oven at 110ºC for 24 hours.
4. Cool the sample in room temperature.
5. Pipette 400 µl of 50 µmole/ml of AABA (=20 µmole) into 100ml volumetric flask and add 10ml DH_2O.
6. Place filter funnel into the volumetric flask and pour in sample from test tube and make up to 100ml with DH_2O.
7. Filter sample through filter paper (No.541) into conical flask.
8. Filter an aliquot through syringe filter into tube.
9. Pipette 10 µl sample into 1.5 ml eppendorf tube and add 20 µl AccQ Fluor Reagent (2A), vortex immediately.
10. Add 70 µl borate buffer (AccQ Fluor 1), vortex immediately.
11. Put into HPLC bottle and inject into HPLC system.

E. Derivatization

(A) Prepare AccQ Fluor reagent (keep moisture away from the reagent at all times)

1. Tap bottle (2A) until the reagent powder settles down.
2. Add 1 ml acetonitrile (2B) to bottle 2A, cap quickly and vortex.
3. Heat at 55ºC for less than 10 min.
   (powder should dissolve completely, solution clear)

(B) Prepare the following mixtures:

Solution	Derivatization Blank	Standard	Unknown	Remarks
Borate Buffer (µL)	80	70	70	In 1.5 ml eppendorf tube
Std / Unk (µL)	-	10	10	Rinse pipette tip & vortex
AccQ reagent (µL)	20	20	20	Vortex at once after adding. Add & vortex one by one

1. Put 10 ml sample into 1.5 ml eppendorf tube.
2. Add in 20 µl AccQ, vortex immediately.
3. Add 70 µl borate buffer, vortex immediately.
4. Leave for 1 minute.
5. Transfer 10 µl into insert tube
6. Inject into HPLC.

Or

1. Put 10 ml sample into 1.5 ml eppendorf tube.
2. Add in 20 µl AccQ, vortex immediately.
3. Add 70 µl borate buffer, vortex immediately.
4. Leave for 1 minute.
5. Transfer 10 µl into insert tube
6. Inject into HPLC.

(C) Wait 1 min for derivatization to complete.

(D) Transfer to 150 µl low-volume insert with polyspring and place insert I a screw neck vial and cap with septa. Shake sample bottle.

The weight of amino acids injected

No.	Amino acid	Weight injected (ng)	No.	Amino acid	Weight injected (ng)
1	Asp	26.62	11	AABA	20.62 or 1
2	Ser	21.02	12	Cys 2	24.03
3	Glu	29.43	13	Tyr	36.24
4	Gly	15.01	14	Val	23.43
5	His	31.03	15	Met	29.84
6	NH3	1	16	Lys	29.24
7	Arg	34.48	17	Ile	26.23
8	Thr	23.82	18	Leu	26.23
9	Ala	17.82	19	Phe	33.04
10	Pro	23.03			

(B) Perfomic Acid hydrolysate

Chromatographic Conditions:

1. Column: AccQ Tag Column (3.9 x 150 mm)
2. Mobile phase: AccQ Tag Eluent A, concentrate
   : AccQ Tag Eluent B or 60% acetonitrile
3. Derivatization : AccQ Fluor Reagents
4. Standards: Amino acid standard, hydrolysate (standard H, 'Pierce')
   Methionine sulfonate ($MetO_2$)
   Cysteic acid (Cya)
5. Flow rate: 1 ml/min
6. Column temperature: 31ºC
7. Detection: Fluorescence Detector
   Excitation λ=250 nm
   Emission λ =395 nm
   Gain =100
   Filter = 1.5 sec
8. Injection volume : 10 µl

Procedures

A. Preparation of $MetO_2$(25 µmole/ml)

1. Dissolve 0.4530 g $MetO_2$ in 100ml of 0.1N HCI.
2. Filter through 0.45 µm cellulose membrane filter.

B. Preparation of cysteic acid (Cya) (25µmole/ml)

1. Dissolve 0.4679 g Cya.H_2O or 0.4229 Cya in 100mL of 0.1 N HCI.

C. Preparation of Standards

1. Pipette 5 ml of AABA (internal standard)(50µmole/ml), 10 ml Cya (25µmole/ml) and 10 ml$MetO_2$ (25µmole/ml), and amino acids std 'H' into 100ml volumetric flask.
2. Add 0.1 N HCI into the volumetric flask and make up to 100ml (1ml=2.5 µmole of AA, AABA, $MetO_2$, Cya).
3. Pipette 1.5ml of 2.5µmole of AA, AABA, $MetO_2$, Cya into test tube and mix with 1.5ml of Amino acid standard 'H' from 2 ampoules (1ml=1.25 µmole/ml of amino acids & 0.625 µmole/ml of Cys2). Store at -20ºC.
4. Pipette 160 µl of 3 ml solution (1ml=1.25 µmole/ml of amino acids & 0.625 µmole/ml of Cys2) and add 840 µl water (1ml=0.2 µmole/ml of amino acids & 0.1 µmole/ml of Cys2). Store at -20ºC.
5. Pipette 10 µl of 1000 µl solution (1ml=0.2 µmole/ml of amino acids & 0.1 µmole/ml of Cys2) (for derivatization)(1ml=0.2 nmole of each amino acids and add 70 µl borate buffer and 20µl ACCQ reagent.
6. Inject 10 µl (1ml=200pmole amino acids & 100pmole Cys2) into HPLC system.

D. Preparation of Performic Acid

No. of samples	Formic acid (HCOOH) (ml)	Hydrogen Peroxide (H_2O_2) (ml)
5	13.5	1.5
10	22.5	2.5
20	45.0	5.0
40	90.0	10.0

E. Sample Preparation

1. Weigh 0.1g sample into a stoppered tube.
2. Insert tubes into ice cubes.
3. Add 2 ml fresh, chilled performic acid.
4. Transfer to refrigerator for 16 hours.
5. Add 0.4 ml chilled hydrogen bromide (HBr), stand for 30 min.
6. Dry using rotary evaporator at 80ºC.
7. Proceed to 6 N hydrolysis.

The weight of Amino Acids injected:

No	Amino acid	Weight injected (ng)
1	Cya	24.03
2	$MetO_2$	29.842
3	AABA	20.624

(C) Analysis of Tryptophan (Alkaline Hydrolysis)

Chromatographic Conditions:

1. Column: Nova Pak C18, 5µm (25-30cm).
2. Mobile phase: 0.0085 M Sodium acetate at pH 4.0: Methanol (86.7 : 13.3)
3. Flow rate: 1 to 1.5 ml/min
4. Column temperature: ambient
5. Detection: Fluorescence Detector
   Excitation λ=285 nm
   Emission λ =345 nm
   Gain =10
   Filter = 1.5 sec
6. Injection volume : 10 µl for standard
   20 µl for sample

Procedures

A. Preparation of Tryptophan Standard

1. Weigh 0.05 g Trp into a 50 mL volumetric flask.
2. Add 0.1 N HCI into the volumetric flask and make up to 500ml (1ml=1000 µg Trp). Ultra sonicate to dissolve the standard.
3. Pipette 50µl of (1ml=1000 µg Trp) solution into 10ml volumetric flask and make up with mobile phase (0.0085 M Sodium acetate at pH 4.0: Methanol (86.7 : 13.3).
4. Inject 10 µl (=200pmole amino acids & 100pmole Cys2) into HPLC system.

B. Preparation of 4.3 N Lithium hydroxide ($LiOH.H_2O$)

1. Weigh 36.0856 g $LiOH.H_2O$ into a graduated 250 ml bottle.
2. Just before use, add water until 200 ml.
3. This should be enough for 10 samples.

C. Sample Preparation for Tryptophan

1. Weigh about 0.2 g sample into a stoppered tube.
2. Add 15ml fresh 4.3 N $LiOH.H_2O$.
3. Flush with nitrogen gas or vacuum sealed.
4. Heat at 120ºC in oven for 16 hours.
5. Transfer hydrolysate to a beaker, add water and 9ml of 6 N HCI. Make sure total volume is less than 100 ml. Adjust pH to 4.5 using dilute HCI.
6. Dilute to 100 ml with water in a volumetric flask.
7. Filter through filter paper and finally filter a small aliquot of filtrate through a syringe filter (0.2 µm cellulose acetate membrane).
8. Inject 20 µl.

Reference

Waters AccQ Tag Amino Acid Analysis

3.3.6 FATTY ACIDS

3.3.6.1 DETERMINATION OF FATTY ACIDS COMPOSITION

METHOD A: FATTY ACID COMPOSITION IN OIL SAMPLE USING GCMS

Principles

Gas chromatography (GC) is a technique for carrying out the separation and measurement of mixtures of materials that can be volatilized. These materials may be gases, liquids or solids with appreciable vapor pressures at temperatures up to a few hundred degrees. The sample should be vaporized and carried to the leading end of the column in negligible time. The detector, commonly flame ionization, monitors the composition of the carrier-gas stream as it leaves the flame ionization, monitors the composition of the carrier-gas stream as it leaves the column. Many compounds of interest do not posses sufficient volatility to be passed through a GC column. Examples of these are sugars, amino acids, metal and large fatty acids. However, it is often possible to react these compounds with reagents to produce new compounds or derivatives which may be analyzed by GC. One of the most common derivatization procedures is replacement of strongly hydrogen bonding of acids or sugars with methyl groups. The decrease in hydrogen bonding of methyl esters then allows sufficient volatility for analysis.

Chemicals/Reagents

1. Sodium methoxide solution (0.2 ml; 2 M $NaOCH_3$)
2. Hexane
3. Standard fatty acid methyl ester (FAME) mixture

Apparatus/Instruments

1. Shimadzu GC 2010
2. Dropper
3. 5 ml vials
4. Vortex

Procedures

GC Setup

GC Model	:	Shimadzu GC 2010
Column Type	:	Capillary Column (BPX-70;30 m length x 0.25 mm Internal diameter x 0.2 µm film thickness)
Mobile phase	:	Nitrogen
Carrier gas flow-rate	:	Split 10
Detector	:	Flame Ionisation Detector (FID)
Detector Temperature	:	250 ºC
Oven Temperature	:	
Programme	:	Holding for 1 min at 100ºC Heating from 100ºC to 180ºC (8ºC/min) Holding for 2 min at 180ºC Heating from 180ºC to 230ºC (8ºC/min) Holding at 230ºC for 1 min
Injection Temperature	:	230ºC

Preparation of FAME:

1. Weigh 0.05 g of oil in a 5-ml vial and dissolve it with 1 ml hexane
2. Add 0.2 ml of sodium methoxide solution
3. Stopper the vial and vigorously shake using vortex for 1 minute
4. Allow the mixture to separate into two layers (centrifuge if necessary)
5. Carefully pipette the upper layer (supernatant) (containing FAME) into another 2 ml vial
6. Prepare FAME from three independent oil samples

Retention Time for Standard FAME Mixture

1. Inject 1 µl of the standard FAME mixture and acquire the chromatogram.
2. Inject 1 µl of the upper layer (supernatant) into the GC column and acquire the chromatogram.
3. Identify the retem\ntion time for each fatty acid in both chromatograms.

Calculations

Quantification of Fatty Acid Concentration

From the standard chromatogram, for each fatty acid, record its area %, Response Factor (RF) and Corrected Response Factor (CRF) using the following formulations.

$$\text{Area \%} = \frac{\text{Area of each fatty acid}}{\text{Total area of all fatty acids}} \times 100$$

$$\text{RF} = \frac{\text{Amount (Wt \%) of each fatty acid}}{\text{Area \% of each fatty acid}}$$

$$\text{RF} = \frac{\text{RF of each fatty acid}}{\text{RF of each fatty acid}}$$

Using the CRF obtained, calculate the concentration of each fatty acid in test oil and finally report the concentration in Normalized Weight % by using the following formula.

$$\text{Amount of each fatty acid In test oil (WT \%)} = \text{(Area x CRF) of each fatty acid}$$

$$\text{Normalized Weight (N.WT) \%} = \frac{\text{Wt \% of each fatty acid}}{\text{Total WT \% of all fatty acids}} \times 100$$

References

Yusof, HM, Miles, EA and Calder PC. 2008. Influence of very long-chain n-3 fatty acids on plasma markers of inflammation in middle-aged men. Prostaglandins, Leukotrienes and Essential Fatty Acids 78: 219–228.

G.C. Burdge, P. Wright, A.E. Jones, S.A. Wootton, A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction, Br. J. Nutr. 84 (2000) 781–787.

METHOD B: FATTY ACID COMPOSITION IN FOOD SAMPLES USING GCMS

Principles

This method is applicable to solids. The usual sample quantity is about 10 g. Extraction is optimized if samples are previously dried and ground. The solvents used are petroleum ether 60 – 80 ºC or less preferably 40 – 60 ºC. Hexane may also be used. Complete extraction can usually be achieved in 2 hours.

Materials and Reagents:

1. H_2SO_4 – 2 % w/v in methanol
2. NaCl – 5% in distilled water
3. $KHCO_3$ – 2% w/v in distilled water
4. Toluene – AR
5. Hexane – AR or better
6. Chloroform
7. External standard – RM3, RM5, trans mix, PUFA – Sigma-Aldrich
8. Internal standard – C17:0
9. F.A.M.E. RM-3 mixture, Oil Reference Standard, AOCS No. 3 cat No. O7256-1AMP (Supelco)
10. Polyunsaturated Fatty Acid (PUFA) Mix No.1 (Animal Source), Cat No. 47033 (Supelco)
11. Supelco 37 component FAME mix, Cat No. 47885U Supelco
12. Cis/ Trans FAME Column Performance mix, Cat No. CB-000711(Supelco)

Apparatus/Instruments

1. Gas Chromatography Perkin Elmer Autosystem
2. Balance: analytical sensitivity ± 0.1 mg
3. IKA-VIBRAZ-VXR shaker
4. Nitrogen gas blower
5. Micropipette
6. Test-tube with condenser attachment: 20 mL
7. Heating block
8. Repeater pipette or graduated pipette and pipette-aid
9. Pasteur pipette
10. Test tube: 12 mL (to be used in centrifuge)
11. Vial
12. Vortex mixer
13. Centrifuge

Procedures

A. Extraction of food samples

1. Measure 1-5 g of homogenize food sample into a 12 ml test tube.
2. Add 1 ml 0.9% NaCl shake vigorously, and add 1 ml ethanol and 4 ml hexane.
3. Shake for 1 hour at 1000 rpm with IKA-VIBRAZ-VXR shaker.
4. Centrifuge at 2000 rpm for 15 minutes and transfer to 7 ml Trident vial.

5. Put 2 ml hexane into the 12 ml test tube.
6. Repeat the extraction.
7. Combine the supernatant and dry by blowing with nitrogen gas.

B. Methylation methods for FAME

1. Remove 50 µL lipid extract into a test-tube for refluxing.
2. Add 1 mL toluene.
3. Add 2 mL acidified methanol.
4. Attach condenser, place in heating block and reflux at 80ºC for hours preferably overnight at 55ºC.
5. Stop reaction by adding 5 mL NaCl.
6. Transfer contents into a 12 mL test tube.
7. Add 3 mL hexane.
8. Mix and centrifuge for at least 5 minutes at 2500 rpm.
9. Remove hexane layer into a test tube.
10. Repeat extraction steps with 2 mL hexane.
11. To combine hexane extracts, add $KHCO_3$ to maximum capacity. The pH may be tested with a litmus paper – it should not be < 6.5.
12. Mix and centrifuge.
13. Remove hexane upper layer into a vial and evaporate off hexane vacuum or under a nitrogen blanket.
14. Reconstitute with 70 – 100 µL chloroform.
15. The sample is now ready for gas chromatography analysis.

C. Condition of GCMS

1. Instrumen: Perkin Elmer Autosystem
2. Column: Restek-Rtx-2330, 105 m x 25 mm x 0.2 µm
3. Oven (or column) temperature: T1, 160 ºC, T2 200 ºC, T3 240 ºC,
4. Rate: R1 2.0 c/min, R2 3.0 c/min
5. Temperature holding time: Time 1, 0 minute, Time 2, 17 minutes
6. Injection temperature: 250 ºC
7. Detection temperature: 260 ºC
8. Carrier gas: 40 psi
9. Range: 1
10. Attenuation: 16
11. The above for time and rate will give a run time of 50 minutes. Values for the relevant option in the software programme can be keyed in so that the output (the chromatogram and report) can be obtained from a printout at the end of each run.
12. Identification of fatty acids is mainly based on known FAME standards supplied either as a mixture or singly. FAME will have a constant relative retention time for a set of conditions. If a standard is unavailable, then information supplied together with that column may be referred to.

References

Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. *Lipid Res*. 5(6):600-8.

Hitchcock, C & Hammod, E.W. (1980). The determination of lipids in foods. In: King, R.D. (ed.). Developments in food analysis techniques. Vol 2, London: Applied Science Pub. Ltd.

3.3.7 CHOLESTEROL

3.3.7.1 DETERMINATION OF CHOLESTROL IN FOODS

METHOD A: DIRECT SAPONIFICATION-GAS CHROMATOGRAPHIC

Principles

Lipid in test portion is saponified at high temperature with ethanolic KOH solution. Unsaponifiable fraction containing cholesterol and other sterols is extracted with toluene. Sterols are derivatized to trimethylsilyl (TMS) ethers and then quantified by gas chromatography.

Chemicals/Reagents

a) Dimethylformamide (DMF) – Distilled in glass
b) Hexamethyldisilane (HMDS)
c) 5α- Cholestane internal standard solution – 0.1 mg/mL in n-heptane. Standard 5α-cholestane available from Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178, is suitable.

Table 1f Interlaboratory study results for determination of cholesterol in foods by direct saponification methods

Sample	Mean rec., mg/100g	$S_{D,}$ mg/100g	$S_{R,}$ mg/100g	RSD %	RSD_R %	R^a, mg/100g	R^b, mg/100g
Butter cookies	28.1	0.75	0.83	2.69	2.95	2.11	2.32
Vegetable bacon baby food	3.71	0.36	0.47	9.60	12.6	1.00	1.31
Vegetable chicken baby food	4.69	0.32	1.00	6.82	21.3	0.89	2.79
Skinless wieners	76.7	2.79	5.98	3.63	7.80	7.80	16.8
NIST egg powder (SRM No. 1845)[c]	1965	60.0	82.1	3.05	4.18	168	230
Commercial powdered eggs	1195	65.7	66.6	5.50	5.57	184	188
Cheeze Whiz	66.9	1.61	3.78	2.40	5.64	4.50	10.6

[a] $r = 2.8 \times S_r$
[b] $R = 2.8 \times S_R$
[c] NIST certified value at 1900 ± 20 mg/100g

d) Cholestrol standard
1) Stock solution – 2.0 mg/mL dimethylfornamide (DMF)
2) Working solution – Dilute stock solution with DMF to obtain 6 solutions at concentration 0.0025-0.2 mg/mL (i.e., 0.0025, 0.005, 0.01, 0.05, 0.1 and o.2 mg/mL) . Eastman Kodak Co., is suitable.

e) Potassium hydroxide solutions
1) 50% KOH (w/w) – Dissolve 500 g KOH in 500g H_2O
2) 1M KOH – Dissolve 56g KOH in ca 800 mL H_2O with cooling and dilute to mark in 1 L volumetric flask.
3) 0.5M KOH – Dilute one part 1M KOH solution with one part H_2O.

f) Trimethylchlorosilane (TMCS) – No. 88531, Pierce Chemical Co., or equivalent.
g) Toluene – Distilled in glass
h) Sodium sulphate – Anhydrous
i) Glass wool

Apparatus/Instruments

a) Centrifuges tubes – 15 mL, Pyrex No.13.
Silanize tubes as follows: Fill tubes with 10% hydrofluoric acid and let stand 10 min. Rinse tubes thoroughly with H_2O, and then with anhydrous methanol. Dry tubes under stream of nitrogen. Fill tubes with 10% hexamethyldisilane (HMDS) in toluene and let stand 1 h. Rinse tubes thoroughly with toluene, and then with anhydrous methanol. Dry tubes in 100°C oven before use. Alternatively, commercial silinizing reagent may be used. Before each reuse, clean tubes with H_2O, ethanol, hexane and acetone, and dry in 100 °C oven. Tubes can be reused without resilylation if strong alkali wash is avoided. Resilanize tubes at least every 6 months.

b) Gas chromatography (GC)
With hydrogen flame ionization detector, capillary column, split-mode, 25 m x 0.32 mm x 0.17 µm film thickness, cross-linked 5% phenyl-methyl silicone or methyl silicone gum 9e.g. Hewlett Packard No. HP-5, Ultra 2, or HP-1), split inlet liner filled with 10% SP 2100 on 80-100 mesh Supelco packing, and 2 ramp oven temperature programming (Hewlett Packard Model 5890A, is suitable). Operating conditions: temperatures-injector 250 °C, detector 300 °C, column 190°C, hold 2 min; increase 20°/min to 230 °C, hold 3 min; increase 40°/min to 255 °C, hold 25 min. Flow rates: helium-column ca mL/min, split vent ca 30 mL/min, purge vent ca 3 mL/min, auxiliary make-up gas ca 20 mL/min, hydrogen-ca 35 mL/min; air-ca 280 mL/min.

c) Rotary evaporator
With glass condenser flask between concentration flask and metal shaft.

d) Magnetic stirrer-hot plate
With variable speed and heat controls

e) Micropipets - Capable of delivering 100 and 200 µL; metal body

f) Test tube mixer

g) Balance – Analytical, capable of weighing to 0.0001 g
h) Glassware – Erlenmeyer flask, 125 and 250 Ml; volumetric flask and pipets; graduated cylinders; separatory funnels, 500 mL.

Procedures

A. Saponification

Accurately weigh (usually 2-3g) test portion (W_1) to nearest 0.001g into 250mL Erlenmeyer flask. Amount of test portion should contain ≤ 1g fat or ≤ 5g H_2O (i.e., weigh 1g pure oils, 1.5g salad dressings, and ≤ 5g substances with high moisture content). Place magnetic stir bar into flask. Add to flask 40mL 95% ethanol and 8mL 50% KOH solution. (Note: Portion of ethanol maybe retained ad used as rinse after KOH addition. This will help prevent ground-glass joints of flask and condenser from freezing together.)

Place flask on magnetic stirrer-hot plate, attach condenser, turn on stirrer-hot plate, and reflux 70 ± 10 min. To ensure complete saponification, occasionally check test portion and disperse any clumps with glass rod or by adding KOH solution to test portion while stirring.

Turn off heat and add 60mL 95% ethanol through top of condenser while stirring solution. (Caution: Add carefully to avoid spurting of alcohol from top condenser, close with stopper and cool solution to room temperature. Test solution is stable 24h.

B. Extraction

Add 100 toluene (V_1) to saponified test portion while stirring. Stopper flask and stir ≥ 30s. Pour solution into 500 mL separatory funnel without rinsing. Add 110 mL 1M KOH solution, and shake funnel vigorously 10 s. Let layers separate and discard aqueous (lower) layer (will be turbid). Add 40 mL 0.5M KOH solution, to separatory funnel, invert funnel, and gently swirl contents 10 s. Discrad aqueous (lower) layer. Wash toluene layer with 40 mL H_2O by gently rotating separatory funnel. Allow layers to separate and discard aqueous phase. Repeat H_2O wash at least 3x, shaking more vigorously each time. If emulsification occurs, add small amount 95% ethanol, swirl contents of funnel, let layers separate, and continue with H_2O washes. After final wash, toluene layer should be crystal clear.

Pour toluene layer from top of separatory funnel through glass funnel, containing plug of glass wool and ca 20g Na_2SO_4 into 125 mL Erlenmeyer flask containing ca 2g Na_2SO_4. Stopper flask and swirl contents. Let mixture stand ≥ 15 min. Test solution may be held ≤ 24h if tightly sealed.

Pipet 25 mL extract (V_2) into 125 g flat-bottom boiling flask and evaporate contents to dryness on ratory evaporator at 40 ± 3ºC. Addca 3 mL acetone and evaporate contents to dryness again. Dissolve residue in 3.0 mL DMF (V_3). Final concentration of cholesterol in DMF should be within range of working standard solutions. If, after quantitation by gas chromatography, test portion concentration falls outside standard curve, change amount of toluene extract evaporated or volume of DMF used to dissolve the residue, or both, so final concentration of cholesterol in DMF falss within range of standards. If test portion contains little or no cholesterol, 75 mL toluene extract dried and redissolved in 2 mL DMF is adequate to detect 1 mg cholesterol/100g in 1 g test portion.

C.Derivatization

Pipet 1.0 mL aliquots of working standard solutions, and test solution into separate 15 mL centrifuge tubes. Add to each tube 0.2 mL HMDS and 0.1 mL TMCS. Stopper tubes and shake vigorously on test tube mixer or by hand for 30 s. Let solution stand undisturbed 15 min. Add to each tube 1.0 mL 5α-cholestane internal standard solution, and 10 mL H_2O. Stopper tubes, shake vigorously 30 s, and centrifuge ca 2 min.

Transfer sufficient portion of heptanes (upper) layer to injection vial. Make sure no aqueous layer is transferred. Derivatized standards and test solutions must be analyzed within 24h.

GC Analysis

Inject 1 µL or other appropriate volume into gas chromatograph. Determine area of 5α-cholestane and cholesterol peaks using height-width measurement or digital integrator (Note: 5α-cholestane and cholesterol should elute in 11-13 and 16-18 min, respectively. If these retention times are not met, adjust carrier flow and temperature).

Divide cholesterol peak area by internal standard peak to obtain standard response ratio. Plot response ratio of 4 high standards (0.01-0.20 mg/mL) against cholesterol concentrations. Standard response ratio plot should bracket test solution response ratio. If necessary, plot low standard curve (0.0025-0.05 mg/mL) for low level test solutions. Dilute high-level test solution to fall within standard range.

Calculation

Calculate g of test portion/mL derivatized as follows:

$$\text{g Test portion/mL derivatized} = (W_1/V_1) \times (V_2/V_3)$$

Where W1 = weight of test portion, g:
V_1 = volume of toluene used in extraction, 100 mL:
V_2 = aliquot of extract taken to dryness, 25 mL:
V_3 = volume of DMF used to dissolve residue, 3 mL.

Calculate cholesterol content in test portion as follows:

$$\text{mg Cholestrol/100g test portion} = \frac{\text{mg/mL cholesterol in test portion from standard curve} \times 100}{\text{g test portion/mL derivatized}}$$

Reference

J. AOAC Int. 78. 75 (1995)

METHOD B: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH PDA DETECTOR

Principles

The HPLC method is for the simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp waste hydrolysate lipid fraction. The method includes microscale saponification and extraction with *n*-hexane. Liposoluble vitamins and cholesterol are quantified by HPLC with UV detection (HPLC-UV), on a 25 cm×0.46 cm SS Exil ODS 5µm column, mobile phase 68:28:4 (v/v/v) methanol:acetonitrile:water; flow rate 1.4 ml/min; column temperature 36°C. The detection is operated using two channels of a diode-array spectrophotometer, 325 nm for retinol and 208 nm for α-tocopherol and cholesterol.

Chemicals/Reagents

1. Methanol, HPLC-grade
2. Hexane, HPLC-grade
3. Acetonitrile, HPLC-grade
4. Pyrocatechol, Analytical-grade
5. Cholesterol, Analytical-grade (Aldrich, St. Louis, USA)
6. Potassium hydroxide, KOH (J.T. Baker, Xalostoc,M´exico)
7. Retinol and α-tocopherol (Fluka, Steinheim, Switzerland)
8. Ultrapure water prepared using NANOpure DiamondUVsystem (Barnstead International Dubuque, Iowa, USA).
9. KOH solution prepared in methanol (0.5 M).
10. The pyrocatechol solution (1 g in 5ml of methanol) prepared fresh daily and stored at approximately 4°C away from light.

Apparatus/Instruments

1. The HPLC-UV system (GBC, Dandenong, Australia): equipped with an auto injector LC 1650, an on-line solvent degasser LC1460, a system controller WinChrom, a pump LC1150, a column oven LC1150, a 20 µl injection loop (Rheodyne, Cotati, CA, USA), and a photodiode-array detector LC5100.
2. Balance: analytical sensitivity ± 0.1 mg
3. Vortex mixer
4. Centrifuge
5. Nitrogen gas blower
6. Test tube: screw capped
7. Micropipettes
8. Membrane-filtered (pore size 0.50µm; Whatman).
9. Ultra filtration equipment

Chromatographic conditions

Chromatographic analysis is to be performed using an analytical scale (25 cm×0.4 cm I.D.) SS Exil ODS column with a particle size 5 µm (SGE, Dandenong, Australia). HPLC conditions are to be as follows: mobile phase 68:28:4 (v/v/v) methanol:acetonitrile:water; a flow rate of 1.4 ml/min; column temperature 36°C. The detection is operated using two channels of a diode-array spectrophotometer, 325 nm for retinol, and 208 nm for α-

tocopherol and cholesterol. Retinol, α-tocopherol, and cholesterol are identified by retention and spectral data.

Standards and quantification

1. Stock standard solutions of retinol (0.5 mg/ml), a-tocopherol (0.5 mg/ml), and cholesterol (2.5 mg/ml) are prepared in 100% methanol and stored at −10°C away from light.
2. The purity of the reference standards is ≥97.0% for retinol, ≥98.0% for α-tocopherol, and ≥95.0% for cholesterol.
3. Working solutions are prepared from these solutions and diluted with methanol prior to analysis.
4. For the determination of retinol, α-tocopherol, and cholesterol in lipid fraction, the stock solution is, in all the cases, be analyzed together with the samples.
5. Analyte concentrations in samples are estimated on the basis of peak areas. The study team analyzed the samples in triplicate. Retinol, α-tocopherol, and cholesterol contents are cited as means±standard deviation.

Procedures

A. <u>Saponification & Extraction</u>

Retinol, α-tocopherol, and cholesterol are extracted from the lipid fraction by the method of Sanchez-Machado *et al.* (2002) with minor modification by Lopez-Cervantes *et al*. (2006).

1. A subsample of 0.40 g (±0.001 g) is weighed out in a screw-top assay tube.
2. Two hundred microlitres of pyrocatechol solution is used as an antioxidant.
3. Five milliliters of KOH solution (0.5M in methanol) are added and immediately vortexed for 20 s.
4. The tubes are to be placed in a water bath at 80°C for 15 min, removed every 5 min, and vortexed again for 15 s.
5. After cooling in iced water, 1 ml of distilled water and 5ml of hexane is added, and the mixture is rapidly vortexed for 1 min, then centrifuged for 2 min at 425×g.
6. Three milliliters of the upper phase are transferred to another test tube and dried under nitrogen.
7. The residue is redissolved in 3ml of the HPLC mobile phase (68:28:4 (v/v/v) methanol:acetonitrile:water), then membrane-filtered (pore size 0.50µm; Whatman, Clifton, New Jersey, USA).
8. Aliquot of 20µl is injected into the HPLC column (extracts are maintained at -10°C away from light).

References

Lopez-Carventas, J., D.I. Sanchez-Machado, D.I., and Rıos-Vazquez, N.J. (2006). High-performance liquid chromatography method for the simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp waste hydrolysate. *Journal of Chromatography A*. 1105:135–139.

Sanchez-Machado, D.I., Lopez-Hernandez, J., and Paseiro-Losada, P. (2002) High-Performance liquid chromatographic determination of α-tocopherol in macroalgae. *Journal of Chromatography A*. 976: 277-284.

3.4 Method Performance Characteristics

Every participating laboratory involves in the analysis of nutrients will be tested for competence before engaging with the actual analysis. It is a pre-requisite to indicate their laboratories performances in order to minimize inter-laboratories variations; hence producing precise and accurate results. Comparisons of results can be made with other participating laboratories to ensure the results are centred on a mean value, not on the extremes of the distribution. The statistics of a normal distribution mean that about 95% of data will lies between a z-score of ± 2.

The analysis of external quality check sample will be conducted twice a year for certain critical parameters especially the mandatory nutrients (mainly proximates). Distributions of blind check samples will be coordinated by Chemistry Department consisting of Proficiency Test (PT) samples and Standard Reference Material (SRMs) provided by Chemistry Department as Quality Control (QC) Coordinator. Chemistry Department will assess the results of participating laboratories and submit the assessment report. Nevertheless, every individual laboratory should perform their Internal Quality Control to minimize deviations and eliminate outliers.

A- MANDATORY NUTRIENTS

No.	Nutrient	Method of Analysis	Reference	Method Performance
1	Energy	Energy by calculation JKM F 1205	Guide to Nutrition Labelling and Claims by Food safety and quality Division MOH 2006	-
2	Water	Determination of Moisture In Air Oven (JKM F 0932) or Vacuum Oven(JKM F 0933)	1) AOAC, 15th Ed. 2)FAO Food and Nutrition Paper, Manual of food Quality Control, No 7	QC based on replicate testing Repeatability: CV : 2.4 – 2.6 %
3	Protein	Protein by Kjedahl Digestion Method JKM F 0908 or Total Protein by Dumas Method JKM F 0931	1) Pearson's Chemical Analysis of Foods,8th Ed. 2) JAOAC 73, 849	QC based Reference Material Repeatability: CV : 2.5 % (JKM F0908) CV : 1.6 % (JKM F0931) Mean Recovery : 97%
4	Fat	Total Fat in Food by Automatic Soxhlet Extraction JKM F 0925	Pearson, D (1970) The chemical Analysis of Food, pp 14.	QC based on replicate testing Repeatability: CV : 1.69% Measurement Uncertainty: 0.04x Conc. of Fat

No.	Nutrient	Method of Analysis	Reference	Method Performance
5	Available Carbohydrate	Available Carbohydrate by calculation JKM F 1204	Guide toNutrition Labelling and Claims by Food safety and quality Division MOH 2006	-
6	Total Dietry fiber TDF	Total dietary fiber in foods by Enzymatic-gravimetric Method – (phosphate buffer) JKM F 1206	Ref : AOAC 985.29 (1995)	QC based on replicates / Reference Material Repeatability: CV: 14.9% Measurement Uncertainty: 0.35 x % DF
7	Ash	Determination of Total Ash and Acid Insoluble Ash JKM F 0902	Pearson's Chemical Analysis of Food (8th Edition)	QC based on replicate Repeatability: CV = 6.5% (total ash) CV = 12% (ash insoluble in acid)
8	Calcium, Ca	Determination of Minerals (Calcium, Sodium, Magnesium & Iron) in Food Products by ICP-OES after Dry Ashing JKM F1209	Journal of AOAC International Vol. 87, No.1, 2004. Comparison of Partial Digestion Procedures of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn in Milk by Inductively Coupled Plasma-Optical Emission Spectrometry	QC based on spiked sample / reference material Repeatability: CV : 13 % Mean Recovery : 109% Measurement Uncertainty: 0.15 x Conc. of Ca
9	Iron, Fe	Determination of Minerals (Calcium, Sodium, Magnesium & Iron) in Food Products by ICP-OES after Dry Ashing JKM F1209	1) Journal of AOAC International Vol. 87, No.1, 2004. Comparison of Partial Digestion Procedures of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn in Milk by Inductively Coupled Plasma-Optical Emission Spectrometry	QC based on spiked sample / reference material Repeatability: CV : 15 % Mean Recovery : 102% Measurement Uncertainty: 0.28 x Conc. of Fe

No.	Nutrient	Method of Analysis	Reference	Method Performance
10	Magnesium, Mg	Determination of Minerals (Calcium, Sodium, Magnesium & Iron) in Food Products by ICP-OES after Dry Ashing JKM F1209	1) Journal of AOAC International Vol. 87, No.1, 2004. Comparison of Partial Digestion Procedures of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn in Milk by Inductively Coupled Plasma-Optical Emission Spectrometry	QC based on spiked sample / reference material Repeatability: CV : 10% Mean Recovery : 117% Measurement Uncertainty: 0.15 x Conc of Mg
11	Phosphorus, P	-	-	-
12	Potassium, K	-	-	-
13	Sodium, Na	Determination of Minerals (Calcium, Sodium, Magnesium & Iron) in Food Products by ICP-OES after Dry Ashing JKM F 1209	1) Journal of AOAC International Vol. 87, No.1, 2004. Comparison of Partial Digestion Procedures of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn in Milk by Inductively Coupled Plasma-Optical Emission Spectrometry	QC based on spiked sample / reference material Repeatability: CV : 9 % Mean Recovery : 108% Measurement Uncertainty: 0.11 x Conc. of Na
14	Zinc, Zn	Determination of Trace Metals (Pb, Cd, Zn & Cu) in Food by Atomic Absorption Spectrophotometry after Dry Ashing JKM F 0501	JAOAC July/August 1993, Volume 76 No 4. Determination of Metals in Foodstuffs by Atomic Absorption Spectrophotometry after Dry Ashing	QC based on spiked sample / reference material Repeatability: CV : 5 % Mean Recovery : 97%
15	Copper, Cu	Determination of Trace Metals (Pb, Cd, Zn & Cu) in Food by Atomic Absorption Spectrophotometry after Dry Ashing JKM F 0501	JAOAC July/August 1993, Volume 76 No 4. Determination of Metals in Foodstuffs by Atomic Absorption Spectrophotometry after Dry Ashing	QC based on spiked sample / reference material Repeatability: CV : 4 % Mean Recovery : 98%
16	Selenium, Se	-	-	-

No.	Nutrient	Method of Analysis	Reference	Method Performance
17	Manganese,Mn	-	-	-
18	Iodine	-	-	-
19	Ascorbic Acid (Vitamin C)	Vitamin C in Fruit Juices and Related Products by Liquid Chromatography JKM F 1202	JAOAC Vol.86, No.2,2003	QC based on spiked sample Repeatability: CV : 5.9 % Mean Recovery : 97.7% Measurement Uncertainty: 0.12 x Conc of Vit C
20	Thiamine (B1)	Vitamin B Rapid Determination of B1,B2.B3,B6 and B9 in Food by Liquid Chromatography JKM F 1201	1) Pearson's Chemical Analysis of Food, 8th Ed.,pg 73-74 2) JAOAC Vol 85, No 4, 2002	QC based on spiked sample/reference material Repeatability: CV : 13.69 % Mean Recovery : 77.6% Measurement Uncertainty: 0.3 x Conc. of B1
21	Riboflavin (B2)	Vitamin B Rapid Determination of B1,B2.B3,B6 and B9 in Food by Liquid Chromatography JKM F 1201	1) Pearson's Chemical Analysis of Food, 8th Ed.,pg 73-74 2) JAOAC Vol 85, No 4, 2002	QC based on spiked sample /reference material Repeatability: CV : 16.98 % Mean Recovery : 61.3% Measurement Uncertainty: 0.36 x Conc of B2
22	Niacin (B3)	Vitamin B Rapid Determination of B1,B2.B3,B6 and B9 in Food by Liquid Chromatography JKM F 1201	1) Pearson's Chemical Analysis of Food, 8th Ed.,pg 73-74 2) JAOAC Vol 85, No 4, 2002	QC based on spiked sample /reference material Repeatability: CV : 22.29 % Mean Recovery : 68.5% Measurement Uncertainty: 0.5 x Conc. of B3

No.	Nutrient	Method of Analysis	Reference	Method Performance
23	Folic Acid (B9)	Vitamin B Rapid Determination of B1,B2.B3,B6 and B9 in Food by Liquid Chromatography JKM F 1201	1) Pearson's Chemical Analysis of Food, 8th Ed.,pg 73-74 2) JAOAC Vol 85, No 4, 2002	QC based on spiked sample /reference material Repeatability: CV : 11.09 % Mean Recovery : 45.8% Measurement Uncertainty: 0.27 x Conc. of B9
24	Vitamin A (Retinol)	Vitamin A (Retinol) and Vitamin E (alpha-Tocopherol) in Food by HPLC JKM F 1203	In-house method based AOAC Vol 85, No 2, 2002, pg 424-434	QC based on spiked sample /reference material Repeatability: CV : 3.8 % Mean Recovery : 115.2% Measurement Uncertainty: 0.13 x Conc. of Vit A
25	Carotenoid α-carotene β-carotene Lycopene Lutein	-	-	-
26	Vitamin D	-	-	-
27	Vitamin E	Vitamin A (Retinol) and Vitamin E (alpha-Tocopherol) in Food by HPLC JKM F 1203	In-house method based AOAC Vol 85, No 2, 2002, pg 424-434	QC based on spiked sample /reference material Repeatability: CV : 7.9 % Mean Recovery : 98.4% Measurement Uncertainty: 0.25 x Conc. of Vit E
28	Vitamin K	-	-	-
29	Total sugar Sucrose Glucose Fructose Lactose	Determination of Total Sugars by HPLC ELSD JKM F 0923	In-house method	QC based on Reference Material Repeatability: CV : 5.0 %

No.	Nutrient	Method of Analysis	Reference	Method Performance
	Maltose			Measurement Uncertainty: 0.10 x Conc of Total Sugars

B – OPTIONAL NUTRIENTS

No.	Nutrient	Method of Analysis	Reference	Method Performance
1	Pantothenic Acid (B5)	-	-	-
2	Pyridoxine (B6)	Vitamin B Rapid Determination of B1,B2.B3,B6 and B9 in Food by Liquid Chromatography JKM F 1201	1. Pearson's Chemical Analysis of Food, 8th Ed.,pg 73-74 2. JAOAC Vol 85, No 4, 2002	QC based on spiked sample /reference material Repeatability: CV : 9.66 % Mean Recovery : 92.0% Measurement Uncertainty: 0.21 x Conc. of B6
3	Cobalamin (B12)	-	-	-
4	Choline	-	-	-
5	Biotin (B7)	-	-	-
6	Fatty acid , total saturated fat 4:0 6:0 8:0 10:0 12:0 14:0 16:0 18:0	Analysis of saturated, monounsaturated, polyunsaturated and trans fatty acid in food by GC JKM F 1210	AOAC Official Method of Analysis, Method 996.06	QC based on replicates Repeatability: CV : 5.5 % Mean Recovery : 91.0% Measurement Uncertainty: 0.34 x Conc. of sat. fat

No.	Nutrient	Method of Analysis	Reference	Method Performance
7	Fatty acid , total monounsaturated fat	Analysis of saturated, monounsaturated, polyunsaturated and trans fatty acid in food by GC JKM F 1210	AOAC Official Method of Analysis, Method 996.06	QC based on replicates Repeatability: CV : 5.0 % Mean Recovery : 92.2%
	16:1			
	18:1			
	20:1			
	22:1			
8	Fatty acid , total polyunsaturated fat	Analysis of saturated, monounsaturated, polyunsaturated and trans fatty acid in food by GC JKM F 1210	AOAC Official Method of Analysis, Method 996.06	QC based on replicate Repeatability: CV : 6.3 % Mean Recovery : 65.6% Measurement Uncertainty: 0.16 x Conc of polyunsat. Fat
	18:2			
	18:3			
	18:4	-	-	-
	20:4	Analysis of saturated, monounsaturated, polyunsaturated and trans fatty acid in food by GC JKM F 1210	AOAC Official Method of Analysis, Method 996.06	QC based on replicate Repeatability: CV : 6.3 % Mean Recovery : 65.6% Measurement Uncertainty: 0.16 x Conc of polyunsat. Fat
	20:5	-	-	-
9	Trans fatty acids	Analysis of saturated, monounsaturated, polyunsaturated and trans fatty acid in food by GC JKM F 1210	AOAC Official Method of Analysis, Method 996.06	QC based on replicate Repeatability: CV : 6.3 % Mean Recovery : 99.5% Measurement Uncertainty: 0.16 x Conc of trans fatty acid
10	Cholesterol	Determination of Cholesterol in Food by Direct Saponification	AOAC Official Method of Analysis (2000). Method	QC based on spiked sample

No.	Nutrient	Method of Analysis	Reference	Method Performance
		JKM F 1208	994.10	Repeatability: CV : 10.15 % Mean Recovery : 102.2% Measurement Uncertainty: 0.22 x Conc of cholesterol
11	Amino Acid:	-	-	-
	Tryptophan			
	Threonine			
	Isoleucine			
	Leucine			
	Lysine			
	Methionine			
	Phenylalanine			
	Valine			

PART IV: FOOD LIST

SECTION 1 – RAW AND PROCESSED FOODS

1.01 Cereals and cereal products

Old code	Revised code	Food	Classification of food
101037	R101001	Atta for making capatti *(Tepung atta untuk membuat capati)*	Processed
N101001	R101002	Barley flour *(Tepung barli)*	Processed
101001	R101003	Barley, pearl *(Beras Belanda); Hordeum vulgare*	Processed
N101034	R101004	Corn flakes *(Kepingan jagung)*	Processed
101003	R101005	Corn flour, maize flour *(Tepung jagung)*	Processed
101004	R101006	Corn snack, cheese flavoured *(Snek jagung berperisa keju)*	Processed
101005	R101007	Corn snack, chicken flavoured *(Snek jagung berperisa ayam)*	Processed
101007	R101008	Corn stick, chocolate flavoured *(Snek jagung berperisa coklat)*	Processed
101006	R101009	Corn/rice snack, chicken flavoured *(Snek jagung/beras, berperisa ayam)*	Processed
101009	R101010	Custard powder *(Tepung kastad)*	Processed
N101035	R101011	Egg noodles	Processed
N101036	R101012	Flour, rye *(Tepung rye)*	Processed
N101037	R101013	Flour,wheat, self-raising *(Tepung naik sendiri)*	Processed
101058	R101014	Macaroni *(Makaroni)*	Processed
101002	R101015	Maize *(Jagung); Zea Mays*	Raw
101025	R101016	Milk cereal (rice) for infants *(Bijirin bersusu untuk bayi)*	Processed
101059	R101017	Milk cereal (wheat) for infants *(Bijirin bersusu untuk bayi)*	Processed
N101038	R101018	Milk chocolate bar with rice cereal *(Coklat dengan bijirin)*	Processed
101010	R101019	Millet *(Sekoi); Eleusine coracana*	Processed
N101039	R101020	Muesli	Processed
N101040	R101021	Nestum cereal, dry *(Bijirin nestum, kering)*	Processed
101031	R101022	Noodle Laksa, thick, dry *(Mee laksa, kering)*	Processed
101030	R101023	Noodle Laksa, thick, wet *(Mee laksa, basah)*	Processed
101063	R101024	Noodle snack, chicken flavoured *(Snek mee berperisa ayam)*	Processed
101060	R101025	Noodle, dry *(Mee kering)*	Processed
101061	R101026	Noodle, dry, instant *(Mee kering, segera)*	Processed
101026	R101027	Noodle, rice *(Kuih-teow)*	Processed
101027	R101028	Noodle, rice *(Lo-see-fun)*	Processed
101028	R101029	Noodle, rice *(Mee-hoon)*	Processed
101029	R101030	Noodle, rice *(Mee-sua)*	Processed
101062	R101031	Noodle, wet *(Mee basah)*	Processed
N101043	R101032	Oatmeal cereal, dry *(Bijirin oat, kering)*	Processed
101011	R101033	Oats, processed, tinned *(Oat dalam tin); Avena sativa*	Processed
101012	R101034	Oats, rolled *(Oat, digelek)*	Processed
101008	R101035	Pop corn, durian flavoured *("Pop corn" berperisa durian)*	Processed

Old code	Revised code	Food	Classification of food
N101044	R101036	Premix flour	Processed
101018	R101037	Rice bran, coarse *(Dedak kasar)*	Processed
101019	R101038	Rice bran, fine *(Dedak halus)*	Processed
101020	R101039	Rice flour *(Tepung beras)*	Processed
101024	R101040	Rice flour, glutinous *(Tepung pulut)*	Processed
101021	R101041	Rice porridge, fish, instant *(Bubur ikan, segera)*	Processed
N101045	R101042	Rice, boil in beg rice *(Ketupat)*	Processed
101013	R101043	Rice, broken *(Beras hancur)*	Processed
101014	R101044	Rice, cooked *(Nasi)*	Prepared
101022	R101045	Rice, glutinous, black *(Pulut hitam)*	Processed
101023	R101046	Rice, glutinous, white *(Pulut putih)*	Processed
101015	R101047	Rice, husked, unpolished *(Beras, tidak dikilang)*	Processed
101016	R101048	Rice, parboiled *(Beras, "parboiled")*	Processed
101017	R101049	Rice, polished *(Beras kilang)*	Processed
N101046	R101050	Rice, siam *(Beras siam)*	Processed
N101047	R101051	Rice, Basmati *(Beras basmati)*	Processed
N101048	R101052	Rice, fragrant (Beras Wangi)	Processed
N101049	R101053	Spaghetti, dry *(Spageti, kering)*	Processed
101033	R101054	Wheat flour *(Tepung gandum)*	Processed
101034	R101055	Wheat flour, high protein *(Tepung gandum, protein tinggi)*	Processed
101035	R101056	Wheat flour, wholemeal *(Tepung gandum, mil penuh)*	Processed
101036	R101057	Wheat germ *(Germa gandum)*	Processed
101032	R101058	Wheat, whole grain *(Gandum, biji)*	Processed
101038	R101059	Biscuit, alphabet *(Biskut bentuk abjad/Biskut ABC)*	Processed
N101002	R101060	Biscuit, chocolate chip *(Biskut coklat cip)*	Processed
101039	R101061	Biscuit, coconut *(Biskut kelapa)*	Processed
N101003	R101062	Biscuit, corn *(Biskut jagung)*	Processed
N101004	R101063	Biscuit, cracker with sugar *(Biskut kraker bergula)*	Processed
N101005	R101064	Biscuit, crackers, vegetable flavor *(Biskut kraker perisa sayuran)*	Processed
101040	R101065	Biscuit, cream crackers *(Biskut krim kraker)*	Processed
N101006	R101066	Biscuit, cream filled *(Biskut berkrim)*	Processed
101041	R101067	Biscuit, finger cream *(Biskut jejari berkrim)*	Processed
101042	R101068	Biscuit, green gram *(Biskut kacang hijau)*	Processed
101043	R101069	Biscuit, lemon puff *(Biskut "lemon puff")*	Processed
101044	R101070	Biscuit, marie *(Biskut marie/Biskut manis)*	Processed
N101007	R101071	Biscuit, milk *(Biskut susu)*	Processed
N101008	R101072	Biscuit, oatmeal *(Biskut oat)*	Processed
101045	R101073	Biscuit, peanut and coconut *(Biskut kacang dan kelapa)*	Processed
N101009	R101074	Biscuit, raisin *(Biskut kismis)*	Processed
101047	R101075	Biscuit, savoury *(Biskut "savoury")*	Processed
N101010	R101076	Biscuit, shortbread	Processed
101046	R101077	Biscuit, soda/plain *(Biskut soda/tawar)*	Processed
101048	R101078	Biscuit, sultana *(Biskut sultana)*	Processed
N101011	R101079	Biscuit, wholemeal crackers *(Biskut kraker mil penuh)*	Processed
101049	R101080	Bread, coconut *(Roti kelapa)*	Processed
N101012	R101081	Bread, croissants	Processed
N101013	R101082	Bread, garlic *(Roti bawang putih)*	Processed
N101014	R101083	Bread, high fiber *(Roti tinggi serat)*	Processed

Old code	Revised code	Food	Classification of food
N101015	R101084	Bread, naan, frozen *(Roti naan, beku)*	Processed
N101016	R101085	Bread, pita *(Roti pita)*	Processed
N101017	R101086	Bread, raisin *(Roti kismis)*	Processed
101050	R101087	Bread, ryemeal *(Roti mil rai)*	Processed
N101018	R101088	Bread, wheat *(Roti gandum)*	Processed
101051	R101089	Bread, white *(Roti putih)*	Processed
N101019	R101090	Bread, wholegrain fibremeal	Processed
101052	R101091	Bread, wholemeal *(Roti mil penuh)*	Processed
N101020	R101092	Bun, anchovies *(Ban ikan bilis)*	Processed
N101021	R101093	Bun, blueberry *(Ban bluberi)*	Processed
N101022	R101094	Bun, cheese *(Ban keju)*	Processed
N101023	R101095	Bun, chocolate *(Ban coklat)*	Processed
N101024	R101096	Bun, coconut *(Ban kelapa)*	Processed
N101025	R101097	Bun, corn *(Ban jagung)*	Processed
N101026	R101098	Bun, cream *(Ban berkrim)*	Processed
N101027	R101099	Bun, kaya *(Ban kaya)*	Processed
N101028	R101100	Bun, potato *(Ban kentang)*	Processed
N101029	R101101	Bun, raisin *(Ban kismis)*	Processed
N101030	R101102	Bun, red beans *(Ban kacang merah)*	Processed
N101031	R101103	Bun, sardine *(Ban sardin)*	Processed
N101032	R101104	Bun, strawberry *(Ban strawberi)*	Processed
N101033	R101105	Cookies, butter *(Biskut mentega)*	Processed
101053	R101106	Cookies, cashewnut *(Biskut Gajus)*	Processed
101054	R101107	Cookies, cornflakes *(Biskut konflek)*	Processed
101055	R101108	Cookies, oats *(Biskut oat)*	Processed
101056	R101109	Cookies, peanut *(Biskut kacang)*	Processed
101057	R101110	Cookies, sesame seed *(Biskut bijan)*	Processed
N101040	R101111	Muffin, banana *(Mufin pisang)*	Processed
N101041	R101112	Muffin, chocolate *(Mufin coklat)*	Processed
N101042	R101113	Muffin, vanilla *(Mufin vanilla)*	Processed
N101050	R101114	Wafer, chocolate, full coated *(Wafer salut coklat)*	Processed

1.02 Starchy roots, tubers and products

Old code	Revised code	Food	Classification of food
102001	R102001	Breadfruit *(Sukun)*	Raw
N102001	R102002	Breadfruits chips *(Kerepek sukun)*	Processed
102002	R102003	Coleus tubers *(Ubi kembili)*	Raw
N102002	R102004	Flour, sago *(Tepung sagu)*	Processed
102003	R102005	Potato *(Ubi kentang)*	Raw
102004	R102006	Potato chips *(Keropok ubi kentang)*	Processed
N102003	R102007	Potato chips, spicy *(Keropok ubi kentang berperisa rempah pedas)*	Processed
N102004	R102008	Potato, pink *(Ubi kentang merah jambu)*	Raw
102006	R102009	Sago noodles *(Tang-hoon)*	Processed
102005	R102010	Sago, pearl *(Sagu, biji-bijian)*	Processed
102008	R102011	Sweet potato, dried *(Ubi keledek, kering)*	Raw
N102005	R102012	Sweet potato, purple, chips *(Kerepek ubi keledek)*	Processed
102007	R102013	Sweet Potato, white *(Ubi keledek putih)*	Raw
102011	R102014	Tannia *(Keladi telur)*	Raw
N102006	R102015	Tapioca chips, barbeque *(Kerepek ubi kayu perisa barbeque)*	Processed
N102007	R102016	Tapioca chips, black pepper *(Kerepek ubi kayu perisa lada hitam)*	Processed
N102008	R102017	Tapioca chips, plain, salted *(Kerepek ubi kayu, bergaram)*	Processed
N102009	R102018	Tapioca chips, plain, unsalted *(Kerepek ubi kayu, tanpa garam)*	Processed
N102010	R102019	Tapioca chips, spicy *(Kerepek ubi kayu pedas)*	Processed
102010	R102020	Tapioca flour *(Tepung ubi kayu)*	Processed
102009	R102021	Tapioca, fresh tuber *(Ubi kayu)*	Raw
102012	R102022	Taro (*Ubi keladi Cina)*	Raw

1.03 Legumes & legume products

Old code	Revised code	Food	Classification of food
103030	R103001	Appalam	Processed
103031	R103002	Baked bean, canned *(Kacang panggang dalam tin)*	Processed
N103001	R103003	Black eye bean *(Kacang mata hitam)*	Processed
N103002	R103004	Carob flour *(Tepung kacang kuda)*	Processed
103001	R103005	Chickpea / Common gram *(Kacang kuda); Cicer arietinum*	Processed
103002	R103006	Dhal, Australian (yellow) *(Dhal Australia, kuning)*	Processed
103003	R103007	Dhal, Mysore (orange) *(Dal, Mysor)*	Processed
103004	R103008	Dhal, red *(Dal merah)*	Processed
103005	R103009	Dhal, yellow *(Dal kuning)*	Processed
103006	R103010	Egyption kidney bean/Hyacinth bean *(Kacang sepat)*	Processed
103007	R103011	Gram, black *(Kacang hitam); Phaseolus mungo*	Processed
103008	R103012	Gram, green/Mung bean *(Kacang hijau); Phaseolus aureus*	Processed
103009	R103013	Gram, red *(Kacang merah); Phaseolus angularis*	Processed
103010	R103014	Lentil *(Lentil); Lens esculenta*	Processed
103011	R103015	Lima beans *(Kacang Cina); Phaseolus lunatus*	Processed
103015	R103016	Soya bean cake, fermented *(Tempeh)*	Processed
103019	R103017	Soya bean curd *(Tau-hoo)*	Processed
103023	R103018	Soya bean curd *(Tau-hoo-pok)*	Processed
103016	R103019	Soya bean curd *(Tau-kua)*	Processed
103017	R103020	Soya bean curd, fried *(Tau-kua goreng)*	Prepared
103020	R103021	Soya bean curd, sheet/film *(Fucok)*	Processed
103022	R103022	Soya bean curd, sheet/strip *(Tim-cok)*	Processed
103018	R103023	Soya bean curd, spiced *(Ngor-hiong tau-kua)*	Processed
103021	R103024	Soya bean curd, strands *(Fucok)*	Processed
103024	R103025	Soya bean curd, unsweetened *(Tau-hoo-fah, tanpa gula)*	Processed
103025	R103026	Soya bean milk, packet *(Susu kacang soya, kotak)*	Processed
103026	R103027	Soya bean milk, unsweented *(Susu kacang soya, tanpa gula)*	Processed
103014	R103028	Soya bean paste, fermented *(Tau-ceo)*	Processed
103012	R103029	Soya bean, black *(Kacang soya hitam)*	Processed
103013	R103030	Soya bean, white *(Kacang soya putih)*	Processed
103027	R103031	Soya been noodles *(Mee kacang soya)*	Processed
N103003	R103032	Soya flour*(Tepung kacang soya)*	Processed
103028	R103033	Soya sauce, "thick" (viscous) *(Kicap pekat)*	Processed
103029	R103034	Soya sauce, "thin" (dilute) *(Kicap cair)*	Processed
N103004	R103035	Soya sauces, sweet *(Kicap manis)*	Processed

1.04 Nuts, seeds and products

Old code	Revised code	Food	Classification of food
104001	R104001	Almond *(Buah badam); Prunus amygdalus*	Processed
104002	R104002	Arecanut shavings *(Buah pinang); Areca catechu*	Processed
104003	R104003	Brazil nut *(Kacang Brazil); Bertholletia excelsa*	Processed
N104001	R104004	Broad bean *(Kacang parang)*	Processed
104004	R104005	Candlenut *(Buah keras); Aleurites moluccana*	Processed
104005	R104006	Cashew nut *(Biji gajus); Anacardiumoccidentale*	Processed
104006	R104007	Chestnut, Chinese *(Buah berangan/Kao-lak); Castanea spp.*	Processed
104007	R104008	Coconut *(Kelapa tua), old; Cocos nucifera*	Processed
104008	R104009	Coconut cream *(Krim kelapa)*	Processed
104009	R104010	Coconut flesh, old *(Isi kelapa tua)*	Raw
104010	R104011	Coconut flesh, young *(Isi kelapa muda)*	Raw
104011	R104012	Coconut milk *(Santan kelapa)*	Processed
104012	R104013	Coconut milk, powder, insatnt *(Serbuk santan kelapa)*	Processed
104013	R104014	Coconut water *(Air kelapa)*	Raw
N104002	R104015	Coconut, shreded *(Kelapa kisar)*	Processed
N104003	R104016	Flaxseed, golden *(Benih lenan)*	Processed
104014	R104017	Gingelly/Sesame seed *(Biji bijian); Sesamum indicum*	Processed
N104004	R104018	Ginkgo Nuts	Processed
N104005	R104019	Hazel nut *(Kacang hazel)*	Processed
104016	R104020	Jackfruit (cempedak), seed *(Biji cempedak); Artocarpus integer*	Raw
104015	R104021	Jackfruit (nangka), seed *(Biji nangka); Artocarpus heterophyllus*	Raw
104017	R104022	Lotus seed *(Biji teratai); Nelumbo nucifera*	Processed
N104006	R104023	Macadamia nuts *(Kacang macadamia)*	Processed
104018	R104024	Peanut / Groundnut (without shell) *(Kacang tanah tanpa kulit); Arachis hypogea*	Processed
104019	R104025	Peanut butter *(Mentega kacang)*	Processed
N104007	R104026	Peanut, crush *(Kacang tumbuk)*	Processed
N104008	R104027	Peanut/Groundnut, flour coated *(Kacang tanah bersalut tepung)*	Processed
N104009	R104028	Pecans, nuts *(Kacang pecan)*	Processed
N104010	R104029	Pistachio nut *(Kacang pistacio)*	Processed
N104011	R104030	Pumpkin seed *(Biji labu)*	Processed
N104012	R104031	Sunflower seed	Processed
104020	R104032	Walnut, dried *(Walnut kering); Juglans regia*	Processed
104021	R104033	Watermelon seed, dried, black *(Kua-ci); Citrullus vulgaris*	Processed

1.05 Vegetables and vegetable products

Old code	Revised code	Food	Classification of food
N105001	R105001	Agal-agal – Sabah	Raw
N105002	R105002	Alfalfa	Raw
105001	R105003	Asam gelugor, pucuk *(Asam gelugor, pucuk); Garcinia atroviridis*	Raw
105003	R105004	Asparagus, canned *(Asparagus dalam tin)*	Processed
105002	R105005	Asparagus, fresh *(Asparagus); Asparagus officinalis*	Raw
N105003	R105006	Baby corn *(Jagung sayur)*	Raw
105004	R105007	Bamboo shoot *(Rebung); Dendrocalamusand Bambusa spp.*	Raw
105005	R105008	Bamboo shoot, braised, canned *(Rebung direbus, dalam tin)*	Processed
105006	R105009	Bamboo shoot, pickled *(Rebung, dijeruk)*	Processed
105007	R105010	Bean, four-angled *(Kacang botor); Psophocarpus tetragonolobus*	Raw
105008	R105011	Bean, French *(Kacang buncis); Phaseolus vulgaris*	Raw
105009	R105012	Bean, string *(Kacang panjang); Vigna sinensis*	Raw
105010	R105013	Beetroot *(Akar bit); Beta vulgaris*	Raw
105011	R105014	Betel leaf *(Sireh); Piper betel*	Raw
105012	R105015	Broccoli *(Brokoli); Brassica oleracea*	Raw
105014	R105016	Cabage, celery, straight cylindrical *(Wong-nga-paak); Brassica pekinensis var cylindrica*	Raw
N105004	R105017	Cabage, red *(Kobis merah)*	Raw
105013	R105018	Cabbage, celery, wrapped head *(Wong-nga-paak); Brassica perkinensis var. cephalata*	Raw
105015	R105019	Cabbage, Chinese *(Pak-coy); Brassica chinensis*	Raw
105016	R105020	Cabbage, Chinese, salted *(Hum-coy)*	Raw
105017	R105021	Cabbage, common *(Kobis); Brassica oleracea*	Raw
N105005	R105022	Capsicum, green *(Lada bengala, hijau)*	Raw
N105006	R105023	Capsicum, red *(Lada bengala, merah)*	Raw
N105007	R105024	Capsicum, yellow *(Lada bengala, kuning)*	Raw
105018	R105025	Carrot *(Lobak merah); Daucas carota*	Raw
105019	R105026	Cashew leaves *(Pucuk janggus/gajus); Anacardium occidentale*	Raw
105020	R105027	Cauliflower *(Bunga kobis); Brassica oleracea*	Raw
105021	R105028	(Cekur manis); Sauropus androgynus	Raw
105022	R105029	Celery *(Daun seladeri); Apium graveolens*	Raw
105023	R105030	(Cemperai); Champereia griffithii	Raw
105027	R105031	Chilli sauce, bottle *(Sos cili dalam botol)*	Processed
105024	R105032	Chilli, green *(Lada hijau); Capsicum annuum*	Raw
105025	R105033	Chilli, red *(Lada merah); Capsicum annuum*	Raw
105026	R105034	Chilli, small *(Cili padi)*	Raw
105028	R105035	Chives, Chinese *(Kucai); Allium odorum*	Raw
105029	R105036	Chrysanthemum / Crowndaisy *(Tong-ho); Chrysanthemum coronarium*	Raw
105030	R105037	Coriander leaves *(Daun ketumbar/Yuen-sai)*	Raw
105031	R105038	Cucumber *(Timun); Cucumis sativus*	Raw

Old code	Revised code	Food	Classification of food
105032	R105039	Cucumber, hairy *(Timun bulu); Cucumis spp.*	Raw
N105008	R105040	Cucumber, japanese *(Timun Jepun)*	Raw
105033	R105041	Drumstick, fresh pods *(Kelor / Remunggai); Moringa oleifera/M.pterygosperma*	Raw
105034	R105042	Drumstick, leaves *(Kelor / Remunggai)*	Raw
105035	R105043	Egg plant / Brinjal *(Terung); Solanum melongena*	Raw
105036	R105044	Fern shoots *(Pucuk paku); Diplazium esculentum*	Raw
N105009	R105045	Fungus	Raw
105037	R105046	Garlic, bulbs *(Bawang putih); Allium sattivum*	Raw
105038	R105047	Garlic, plants *(Pucuk bawang putih)*	Raw
105040	R105048	Gourd, bottle / Calabash *(Labu jantung); Lagenaria vulgaris/L. leucantha*	Raw
105041	R105049	Gourd, snake *(Ketola ular); Tricosanthes anguina*	Raw
105042	R105050	Gourd, wax / Winter melon *(Kundur / Tong-kuah); Benincasa hispida*	Raw
105039	R105051	Ground, bitter / Balsam pear *(Peria); Momordica charantia*	Raw
105043	R105052	Indian Pennywort *(Pegaga); Hydrocotyle asiatica*	Raw
N105010	R105053	*Jampang - Sarawak*	Raw
N105011	R105054	*Jering*	Raw
105044	R105055	Jew's ears / Juda ear's (dried) *(Mok-yi); Auricularia polytricha*	Processed
105045	R105056	(*Kadok*); Piper sarmentosum	Raw
105046	R105057	Kale, Chinese *(Kai-lan-coy); Brassica alboglabra*	Raw
105047	R105058	(*Kantan*); Phaeomeria speciosa	Raw
105048	R105059	(*Kesom*); Polygonum minus	Raw
105049	R105060	Lady's fingers /Okra *(Kacang bendi); Hibiscus esculentus*	Raw
N105012	R105061	*Latok - Sabah*	Raw
105050	R105062	Leek *(Tai-suen); Allium porrum*	Raw
105051	R105063	Lettuce *(Daun salad/Sang-coy); Lactuca sativa*	Raw
N105013	R105064	Lettuce, red coral	Raw
105052	R105065	Loofah, angled *(Ketola segi); Luffa acutangula*	Raw
105053	R105066	Loofah, smooth *(Ketola air); Luffa cylindrica*	Raw
105054	R105067	Lotus root *(Akar teratai); Nelumbo nucifera*	Raw
105055	R105068	(*Maman*); Gynandropsis gynandra	Raw
N105014	R105069	Melon, sharkfin	Raw
105056	R105070	(*Mengkudu*); Morinda citrifolia	Raw
105057	R105071	Mint *(Daun pudina); Mentha arvensis*	Raw
N105015	R105072	Mixed coral	Raw
105058	R105073	Mung bean / Green bean sprouts *(Tau-geh); Phaseolus aureus*	Raw
N105016	R105074	Mushroom, button *(Cendawan butang)*	Raw
105059	R105075	Mushroom, Chinese, dried *(Cendawan Cina, kering); Agaricus bretscheideri*	Processed
N105017	R105076	Mushroom, enoki *(Cendawan enoki)*	Raw
105060	R105077	Mushroom, grey oyster, fresh *(Cendawan tiram, segar)*	Raw
N105018	R105078	Mushroom, king oyster *(Cendawan raja)*	Raw

Old code	Revised code	Food	Classification of food
N105019	R105079	Mushroom, shitake *(Cendawan shitake)*	Raw
105064	R105080	Mustard leaf & stem, highland, pickled *(Hum kai-coy)*	Raw
105063	R105081	Mustard leaf & stem, lowland, pickled *(Hum kai-coy)*	Raw
105061	R105082	Mustard leaves, Chinese *(Sawi/Coy-sam); Brassica juncea*	Raw
105062	R105083	Mustard leaves, Indian *(Kai-coy); Brassica juncea*	Raw
N105020	R105084	Mustard leaves, Jepanese (Sawi jepun)	Raw
105065	R105085	Onion, large *(Bawang besar); Allium cepa*	Raw
105067	R105086	Onion, small, pickled *(Jeruk bawang merah)*	Processed
105066	R105087	Onion, small/Shallot *(Bawang merah); Allium fistulosum*	Processed
105068	R105088	Papaya shoots *(Pucuk betik); Carica papaya*	Raw
105069	R105089	Paprika / Bell peppers *(Lada hijau besar); Capsicum annuum*	Raw
105070	R105090	Parsley *(Pasli); Petroselinum crispum*	Raw
105071	R105091	Peas, garden, fresh *(Kacang pea, segar); Pisum sativum*	Raw
105072	R105092	Peas, gren, canned *(Kacang pea hijau dalam tin)*	Processed
105073	R105093	Peas, processed, canned *(Kacang pea, diproses, dalam tin)*	Processed
105074	R105094	Peas, salted, fried *(Kacang pea, goreng bergaram)*	Cooked
105075	R105095	(*Pegaga gajah*); Hydrocotyle javanica	Raw
105076	R105096	(*Petai*); Parkia speciosa	Raw
105077	R105097	Plantain, flower *(Jantung pisang); Musa sapientium*	Raw
105078	R105098	Pumpkin *(Labu merah); Cucurbita maxima*	Raw
N105021	R105099	Pumpkin leaves, raw	Raw
105079	R105100	Purslane *(Gelong pasir); Portulaca oleracea*	Raw
105080	R105101	Radish, Chinese *(Lobak); Raphanus sativus*	Raw
105081	R105102	Radish, Chinese, pickeled *(Lobak dijeruk)*	Processed
105082	R105103	Rhubarb / Pie plant, petioles *(Tai-wong); Rheum rhaponticum*	Raw
N105022	R105104	Rose mary	Raw
N105023	R105105	*Sabong - Sarawak*	Raw
N105024	R105106	Sage	Raw
105083	R105107	Salang *(Salang); Claoxylon longifolium*	Raw
N105025	R105108	*San sawi dayak (Sayur ensabi) - Sarawak*	Raw
N105026	R105109	*Sayur buah betik - Sabah*	Raw
N105027	R105110	Sea moss	Raw
105084	R105111	Seaweed, dried *(Hai-tai)*	Processed
N105028	R105112	Seaweed, fresh	Raw
105085	R105113	(*Selom*); Oenanthe javanica	Raw
105086	R105114	Sesbania *(Daun Turi/Geti); Sesbania grandiflora*	Raw
105087	R105115	Soya bean sprout *(Tau-geh kacang soya); Glycine max*	Raw
105090	R105116	Spinach *(Bayam duri); Amaranthus viridis*	Raw
105091	R105117	Spinach *(Bayam pasir)*	Raw
105089	R105118	Spinach *(Bayam putih); Amaranthus viridis*	Raw
105088	R105119	Spinach *(Por-coy); Spinacia oleracea*	Raw
105092	R105120	Spinach, Ceylon *(Remayong); Basella rubra*	Raw
105093	R105121	Spinach, red *(Bayam merah); Amaranthus spinosus*	Raw

Old code	Revised code	Food	Classification of food
105094	R105122	Spring onion *(Daun Bawang); Allium fistulosum*	Raw
105095	R105123	Swamp cabbage / Water convulvolus *(Kangkung); Ipomoea aquatica/I. reptans*	Raw
N105029	R105124	Sweet peas (Kacang manis)	Raw
105096	R105125	Sweet potato shoots *(Pucuk ubi keledek); Ipomoea batatas*	Raw
105097	R105126	Tapioca shoots *(Pucuk ubi kayu); Manihot utilissima*	Raw
N105030	R105127	Taro shoots	Raw
N105031	R105128	Terung masam - Sarawak	Raw
105098	R105129	Tomato *(Tomato); Lycopersicum esculentum*	Raw
105099	R105130	Tomato juice, canned *(Jus tomato dalam tin)*	Processed
105100	R105131	Tomato ketchup *(Sos tomato/Ketcup tomato)*	Processed
105101	R105132	Tomato soup, canned *(Sup tomato dalam tin)*	Processed
N105032	R105133	Tomato, cherry (Tomato ceri)	Raw
105102	R105134	Tree tomato; C*yphomandra betacea*	Raw
105103	R105135	Turnip *(Lobak/Coy-tau); Brassica rapa*	Raw
N105033	R105136	Tutan - sabah	Raw
105104	R105137	Ubi kembili, leaves *(Ubi kembili, daun); Coleus tuberosus*	Raw
105105	R105138	Ulam raja *(Ulam raja); Cosmos caudatus*	Raw
N105034	R105139	Umbut apong - Sarawak	Raw
N105035	R105140	Umbut lalis - Sarawak	Raw
N105036	R105141	Umbut nenas - Sarawak	Raw
105106	R105142	Waterchesnut *(Ma-tai); Scirpus tuberosus/Eleocharis dulcis*	Raw
105107	R105143	Watercress *(Semanggi/Sai-yong-coy); Nashturtium officinale*	Raw
N105037	R105144	Wintermelon	Raw
105108	R105145	Wolfberry leaves/Chinese box thorn *(Kau-kei); Lycium chinense*	Raw
105109	R105146	Yam bean *(Sengkuang); Pachyrrhizus erosus / P. bulbosus*	Raw
N105038	R105147	Yam bean, chinese (Sengkuang cina)	Raw
105110	R105148	Yam stalks *(Batang keladi)*	Raw
N105039	R105149	Zucchini, raw	Raw

1.06 Fruit & fruit products

Old Code	Revised Code	Food	Classification of food
106002	R106001	Aplle, red *(Epal merah); Pyrus malus*	Raw
N106001	R106002	Apple, fuji *(Epal fuji)*	Raw
106001	R106003	Apple, green *(Epal hijau); Pyrus malus*	Raw
N106002	R106004	Apple, royal gala *(Epal royal gala)*	Raw
N106003	R106005	Asam kulit limau	Raw
106003	R106006	Avocado pear *(Apokat); Persea americana*	Raw
N106004	R106007	Bambangan - sabah	Raw
106005	R106008	Banana *(Pisang abu)*	Raw
N106005	R106009	Banana *(Pisang awak)*	Raw
106006	R106010	Banana *(Pisang berangan)*	Raw
106008	R106011	Banana *(Pisang embun)*	Raw
N106006	R106012	Banana *(Pisang kapas)*	Raw
106007	R106013	Banana *(Pisang kari)*	Raw
106009	R106014	Banana *(Pisang kelat)*	Raw
106010	R106015	Banana *(Pisang mas)*	Raw
106011	R106016	Banana *(Pisang nangka)*	Raw
N106007	R106017	Banana *(Pisang nipah)*	Raw
106012	R106018	Banana *(Pisang rajah udang/merah)*	Raw
106013	R106019	Banana *(Pisang rastali)*	Raw
N106008	R106020	Banana *(Pisang susu)*	Raw
N106009	R106021	Banana *(Pisang talang)*	Raw
N106010	R106022	Banana *(Pisang tanduk raja)*	Raw
106014	R106023	Banana *(Pisang tanduk)*	Raw
106004	R106024	Banana, common varieties *(Pisang); Musa sapientium/ M. paradisiaca*	Raw
106015	R106025	Banana, smoked *(Pisang salai)*	Raw
106016	R106026	Belimbi *(Belimbing masam / buluh); Averrhoa bilimbi*	Processed
N106011	R106027	Belunu - sabah	Raw
106017	R106028	(Binjai); Mangifera caesia	Raw
N106012	R106029	Blackberry *(Beri hitam)*	Raw
106018	R106030	Carambola / Star-fruit *(Belimbing manis/besi); Averrhoa carambola*	Raw
106019	R106031	Cashew apple *(Jambu gajus); Anacardium occidentale*	Raw
N106013	R106032	Cherry *(Ceri)*	Raw
106020	R106033	Custard apple *(Buah nona); Annona squamosa*	Raw
106021	R106034	Date, dried *(Buah khurma, kering); Phoenix dactylifera*	Raw
N106014	R106035	Dragon fruit, red *(Buah naga, isi merah)*	Raw
N106015	R106036	Dragon fruit, white *(Buah naga, isi putih)*	Raw
106022	R106037	(*Duku*); Lansium domesticum	Raw
106023	R106038	Durian (Durian); Durio zibethinus	Raw
106025	R106039	Durian cake (Lempuk)	Processed

Old Code	Revised Code	Food	Classification of food
106024	R106040	Durian, fermented *(Tempoyak)*	Processed
N106016	R106041	Fig *(Buah tin)*	Processed
106026	R106042	Fruit cocktail in syrup, canned *(Koktel buah dalam tin)*	Processed
106027	R106043	Fruit, mixed, spicy pickled *(Jeruk buah campuran)*	Processed
106028	R106044	Grape *(Buah anggur); Vitis vinifera*	Raw
106029	R106045	Grape fruit *(Limau gedang); Citrus paradisi*	Raw
106030	R106046	Guava *(Jambu batu); Psidium guajava*	Raw
N106017	R106047	Guava, dried *(Jambu batu kering)*	Processed
106031	R106048	Guava, with "asam buoy" *(Jambu batu dengan asam buoy)*	Processed
106032	R106049	Hog plum / Ambarella *(Kedondong); Spondias cytherea*	Raw
106033	R106050	Hog plum, pickled *(Jeruk kedondong)*	Processed
N106018	R106051	Honeydew *(Tembikai susu)*	Raw
106034	R106052	Jackfruit *(Cempedak); Artocarpus integer*	Raw
106035	R106053	Jackfruit *(Nangka); Artocarpus heterophullus*	Raw
N106019	R106054	Java plum / Jambolan	Raw
106036	R106055	(Jering); Pithecellobium lobatum/P.jiringa	Raw
N106020	R106056	*Jeruk bambangan* - sabah	Raw
N106021	R106057	Jeruk cermai	Processed
N106022	R106058	Jeruk kelubi	Processed
106037	R106059	Kiwi fruit *(Buah kiwi)*	Raw
N106023	R106060	Kiwi fruit, gold *(Kiwi emas)*	Raw
106038	R106061	(Kundang); Bouea macrophylla	Raw
106039	R106062	Kundang, candied *(Halwa buah kundang)*	Processed
106040	R106063	Kundang, preserved *(Jeruk buah kundang)*	Processed
106041	R106064	Kundur, candied *(Halwa buah kundur); Benincasa cerifera*	Processed
106042	R106065	(Langsat); Lansium domesticum	Raw
106043	R106066	Lemon *(Limau susu/Limau mata kerbau)*	Raw
106045	R106067	Lime, common *(Limau kapas)*	Raw
106044	R106068	Lime, common *(Limau nipis/Limau asam); Citrus aurantifolia*	Raw
106046	R106069	Lime, musk *(Limau kesturi); Citrus microcarpa*	Raw
106047	R106070	Lime, wild *(Limau purut); Citrus hystrix*	Raw
N106024	R106071	Longan, canned in syrup *(Mata kucing, dengan sirap dalam tin)*	Processed
106048	R106072	Lychee *(Lai-ci); Litchi chinensis*	Raw
N106025	R106073	Lychee, canned in syrup *(Laici, dengan sirap dalam tin)*	Processed
106052	R106074	Manggo *(Bacang siku); Mangifera foetida*	Raw
106051	R106075	Mango *(Bacang gelok); Mangifera foetida*	Raw
106050	R106076	Mango *(Kwini); Mangifera odorata*	Raw
106049	R106077	Mango *(Mangga); Mangifera indica*	Raw
106053	R106078	Mango, candied *(Halwa bacang)*	Processed

Old Code	Revised Code	Food	Classification of food
106054	R106079	Mango, candied *(Halwa mempelam)*	Processed
106055	R106080	Mango, pickled *(Jeruk bacang)*	Processed
N106026	R106081	Mango, pickled *(Jeruk mangga)*	Processed
106057	R106082	Mango, pickled *(Jeruk manis mempelam padi)*	Processed
106056	R106083	Mango, pickled *(Jeruk manis mempelam)*	Processed
106058	R106084	Mangosteen *(Manggis); Gardinia mangostana*	Processed
106059	R106085	Mata kucing *(Mata kucing); Nephelium malaiense*	Raw
N106027	R106086	Nectarine	Raw
106060	R106087	Nutmeg, fresh *(Buah pala); Myristica fragrans*	Raw
106061	R106088	Nutmeg, pickled *(Jeruk buah pala)*	Processed
106062	R106089	Olive *(Buah zaitun); Olea europaea*	Raw
106063	R106090	Orange *(Limau manis); Citrus nobilis*	Raw
106065	R106091	Orange, Mandarin / Tangerine *(Limau Cina); Citrus reticulata*	Raw
106064	R106092	Orange, neck *(Limau cembol)*	Raw
N106028	R106093	Orange, valencia *(Oren valencia)*	Raw
106066	R106094	Papaya *(Betik); Carica papaya*	Raw
106067	R106095	Papaya, candied *(Halwa betik)*	Processed
106068	R106096	Papaya, Exotica *(Betik Eksotika)*	Raw
N106029	R106097	Passion fruit	Raw
106069	R106098	Peach *(Buah pic); Prunus persica*	Raw
N106030	R106099	Peach, canned, heavy syrup	Processed
N106031	R106100	Pear, fragrant *(Pir manis)*	Raw
N106032	R106101	Pear, gong *(Pir gong)*	Raw
106070	R106102	Pear, green *(Buah pir hijau); Pyrus communis*	Raw
N106033	R106103	Pear, sanji *(Pir sanji)*	Raw
106071	R106104	Pear, yellow, Chinese *(Buah lai); Pyrus sinensis*	Raw
106072	R106105	Persimmon *(Pisang kaki); Diospyros kaki*	Raw
106073	R106106	Persimmon, dried *(Pisang kaki, kering)*	Processed
106074	R106107	Pineapple *(Nenas); Annas comosa*	Raw
106075	R106108	Pineapple canned in syrup *(Nenas, dengan sirap, dalam tin)*	Processed
106076	R106109	Pineapple syrup, canned *(Jus nenas dalam tin)*	Processed
106077	R106110	Pineapple, candied *(Halwa nenas)*	Processed
N106034	R106111	Pineapple, canned in heavy syrup, with syrup *(Nenas, dengan sirap dalam tin)*	Processed
106078	R106112	Plums *(Buah plum); Prunus spp.*	Raw
N106035	R106113	Pomegranate *(Delima)*	Raw
106080	R106114	Pomelo *(Limau Betawi/Limau Bali); Citrus grandis/ C. maxima*	Raw
106079	R106115	Prune *(Prun)*	Raw
N106036	R106116	Prune, ready to eat, semi dried *(Prun sedia dimakan,separuh kering)*	Processed
106081	R106117	Pulasan *(Pulasan); Nephelium mutabile*	Raw
106082	R106118	Raisin *(Kismis); Vitis vinifera*	Processed

Old Code	Revised Code	Food	Classification of food
106083	R106119	(Rambai); Baccaurea motleyana	Raw
106084	R106120	Rambutan *(Rambutan); Nephelium lappaceum*	Raw
N106037	R106121	Rockmelon	Raw
106085	R106122	Rokam, candied *(Halwa rokam); Flacourtia jangomas*	Processed
N106038	R106123	Roselle, dried	Processed
106086	R106124	(Salak); Zalacca edulis	Raw
106087	R106125	Sapodilla *(Ciku); Manilkara achras*	Raw
106088	R106126	Sentul, pickled *(Jeruk buah sentul); Sandoricum koetjape*	Processed
106089	R106127	Soursop *(Durian Belanda); Annona muricata*	Raw
106090	R106128	Strawberry *(Strawberi); Fragaria grandiflora*	Raw
N106039	R106129	Sun melon	Raw
106091	R106130	Tamarind, fresh pods *(Buah Asam Jawa); Tamarindus indica*	Raw
N106040	R106131	*(Tarap)* - Sabah	Raw
N106041	R106132	*(Tarap kinoring)* - Sabah	Raw
106092	R106133	Water Apple *(Jambu air); Eugenia aquea/Syzygium aqueum*	Raw
106093	R106134	Watermelon *(Tembikai); Citrullus vulgaris*	Raw
N106042	R106135	Watermelon, red *(Tembikai merah)*	Raw
N106043	R106136	Watermelon, yellow *(Tembikai kuning)*	Raw

1.07 Sugars and syrups

Old code	Revised code	Food	Classification of food
N107001	R107001	Aspartame *(Aspartam)*	Processed
N107002	R107002	Butterscotch candy *(Gula-gula butterscotch)*	Processed
107001	R107003	Candy, coconut *(Gula-gula kelapa)*	Processed
N107003	R107004	Caramel candy *(Gula-gula karamel)*	Processed
N107004	R107005	Cordial root beer *(Kordial rut bir)*	Processed
N107005	R107006	Cordial, soursop (Kordial durian belanda)	Processed
N107006	R107007	Cordial, grape *(Kordial anggur)*	Processed
N107007	R107008	Cordial, guava *(Kordial jambu batu)*	Processed
N107008	R107009	Cordial, kiwi *(Kordial kiwi)*	Processed
N107009	R107010	Cordial, lime *(Kordial limau nipis)*	Processed
N107010	R107011	Cordial, mango *(Kordial mangga)*	Processed
N107011	R107012	Cordial, orange *(Kordial oren)*	Processed
N107012	R107013	Cordial, pineapple*(Kordial nenas)*	Processed
N107013	R107014	Cordial, roselle *(Kordial rosel)*	Processed
N107014	R107015	Cordial, sarsi *(Kordial sarsi)*	Processed
N107015	R107016	Glucose liquid *(Cecair glukosa)*	Processed
107002	R107017	Honey *(Madu)*	Processed
N107016	R107018	Jam, apricot *(Jem aprikot)*	Processed
N107017	R107019	Jam, blueberry *(Jem blueberi)*	Processed
107003	R107020	Jam, egg *(Seri kaya)*	Processed
N107018	R107021	Jam, grape *(Jem anggur)*	Processed
107004	R107022	Jam, mango *(Jem bacang)*	Processed
107005	R107023	Jam, mango *(Jem mempelam)*	Processed
107006	R107024	Jam, papaya *(Jem betik)*	Processed
107007	R107025	Jam, pineapple *(Jem nenas)*	Processed
N107019	R107026	Jam, rambutan *(Jem rambutan)*	Processed
107008	R107027	Jam, rokam *(Jem buah rokam)*	Processed
N107020	R107028	Jam, roselle *(Jem rosel)*	Processed
N107021	R107029	Jam, strawberry *(Jem, strawberi)*	Processed
107009	R107030	Jam, water apple *(Jem jambu air)*	Processed
N107022	R107031	Jelly beans	Processed
107010	R107032	Jelly crystals, strawberry flavoured *(Jeli, berperisa strawberi)*	Processed
107011	R107033	Jelly from rokam fruit *(Jeli buah rokam)*	Processed
107012	R107034	Jelly, honey dew *(Jeli "honey dew")*	Processed
107013	R107035	Jelly, pineapple *(Jeli nenas)*	Processed
N107023	R107036	Jelly, roselle *(Jeli rosel)*	Processed
107014	R107037	Jelly, strawberry *(Jeli strawberi)*	Processed
107015	R107038	Jelly, watermelon *(Jeli Tembikai)*	Processed
N107024	R107039	Lollipop, chocolate flavor *(Gula-gula berperisa coklat)*	Processed
N107025	R107040	Lollipop, fruit flavor *(Gula-gula berperisa buah-buahan)*	Processed

Old code	Revised code	Food	Classification of food
107016	R107041	Marmalade *(Marmalade)*	Processed
N107026	R107042	Nougat candy	Processed
107017	R107043	Sugar, brown *(Gula merah)*	Processed
107018	R107044	Sugar, coconut palm in inflorescene *(Gula Melaka)*	Processed
107019	R107045	Sugar, granulated *(Gula pasir)*	Processed
N107027	R107046	Sugar, icing *(Gula ising)*	Processed
N107028	R107047	Sugar, maple	Processed
107020	R107048	Sugar, rock *(Gula batu)*	Processed
107021	R107049	Syrup, rose *(Sirap, ros)*	Processed
107022	R107050	Treacle, black *(Air gula, hitam, pekat)*	Processed

1.08 Meat and meat products

Old code	Revised code	Food	Classification of food
108031	R108001	Bacon, fatty *(Bakon, lemak)*	Processed
108032	R108002	Bacon, lean *(Bakon, tanpa lemak)*	Processed
N108001	R108003	Beef, marrow *(Sum-sum lembu)*	Raw
108003	R108004	Beef burger patty *("Burger" daging lembu)*	Processed
108004	R108005	Beef extract, proprietary brand *(Pati daging lembu)*	Processed
108005	R108006	Beef frankfurter *("Frankfurter" daging lembu)*	Processed
108006	R108007	Beef rendang, canned *(Rendang daging lembu, dalam tin)*	Processed
N108002	R108008	Beef, bacon *(Bakon, lembu)*	Processed
N108003	R108009	Beef, bone *(Tulang lembu)*	Raw
N108004	R108010	Beef, brain *(Otak lembu)*	Raw
108001	R108011	Beef, corned, canned *(Daging lembu diasinkan, dalam tin); Bos Taurus*	Processed
N108005	R108012	Beef, ham *(Ham, lembu)*	Processed
108002	R108013	Beef, lean *(Daging lembu tanpa lemak)*	Raw
N108006	R108014	Beef, sausage *(Sosej daging lembu)*	Processed
N108007	R108015	Beef, spleen *(Limpa lembu)*	Raw
N108008	R108016	Beef, tail *(Ekor lembu)*	Raw
N108009	R108017	Beef, tongue *(Lidah lembu)*	Raw
108033	R108018	Brain, pig *(Otak khinzir)*	Raw
N108010	R108019	Buffalo meat *(Daging kerbau)*	Raw
N108011	R108020	Buffalo, bone *(Tulang kerbau)*	Raw
N108012	R108021	Buffalo, brain *(Otak kerbau)*	Raw
N108013	R108022	Buffalo, heart *(Jantung kerbau)*	Raw
N108014	R108023	Buffalo, liver *(Hati kerbau)*	Raw
N108015	R108024	Buffalo, lungs *(Paru kerbau)*	Raw
N108016	R108025	Buffalo, spleen *(Limpa kerbau)*	Raw
N108017	R108026	Buffalo, tail *(Ekor kerbau)*	Raw
N108018	R108027	Buffalo, tongue *(Lidah kerbau)*	Raw
N108019	R108028	Camel, liver *(Hati unta)*	Raw
N108020	R108029	Camel, lungs *(Paru unta)*	Raw
N108021	R108030	Camel, meat *(Daging unta)*	Raw
N108022	R108031	Camel, spleen *(Limpa unta)*	Raw
108016	R108032	Chicken burger patty *("Burger" daging ayam)*	Processed
108017	R108033	Chicken curry, canned *(Kari ayam dalam tin)*	Processed
108018	R108034	Chicken frankfurter *("Fankfurter" daging ayam)*	Processed
N108023	R108035	Chicken, bacon *(Bakon, ayam)*	Processed
N108024	R108036	Chicken, bone *(Tulang ayam)*	Raw
108013	R108037	Chicken, breast meat *(Daging ayam, bahagian dada)*	Raw
N108025	R108038	Chicken, ham *(Ham, ayam)*	Raw
108012	R108039	Chicken, matured, dressed carcass *(Ayam dibersihkan)*	Raw
N108026	R108040	Chicken, sausage *(Sosej daging ayam)*	Processed

Old code	Revised code	Food	Classification of food
108014	R108041	Chicken, thigh *(Daging ayam, bahagian paha)*	Raw
108015	R108042	Chicken, wing *(Daging ayam, sayap)*	Raw
N108027	R108043	Duck, bone *(Tulang itik)*	Raw
108024	R108044	Duck, matured, dressed carcas *(Itik, dibersihkan)*	Raw
108025	R108045	Duck, roasted *(Itik panggang)*	Prepared
108019	R108046	Feet, chicken, deboned *(Kaki ayam, tanpa tulang)*	Raw
N108028	R108047	Frog, leg *(Daging katak bahagian kaki)*	Raw
N108029	R108048	Frog, meat *(Daging katak)*	Raw
108020	R108049	Gizzard, chicken *(Hempedal ayam)*	Raw
108026	R108050	Goat meat, lean *(Daging kambing tempatan)*	Raw
N108030	R108051	Goat, bone *(Tulang kambing)*	Raw
N108032	R108053	Goat, lungs *(Paru kambing)*	Raw
N108033	R108054	Goat, spleen *(Limpa kambing)*	Raw
108034	R108055	Ham *(Ham)*	Processed
108021	R108056	Chicken, heart *(Jantung ayam)*	Raw
108027	R108057	Goat, heart *(Jantung kambing)*	Raw
108007	R108058	Ox, heart *(Jantung lembu)*	Raw
108022	R108059	Chicken, intestine (Usus ayam)	Raw
108008	R108060	Kidney, ox (Buah pinggang lembu)	Raw
108023	R108061	Liver, chicken (Hati ayam)	Raw
108009	R108062	Ox, liver (Hati lembu)	Raw
108035	R108063	Pig, liver (Hati khinzir)	Raw
108010	R108064	Ox, lungs (Paru-paru lembu)	Raw
108011	R108065	Maw, imported (Perut lembu)	Processed
108029	R108066	Mutton curry, canned (kari daging kambing dalam tin)	Processed
108028	R108067	Mutton, lean (Daging kambing)	Raw
N108034	R108068	Pork, bacon (Bakon, daging khinzir)	Processed
N108035	R108069	Pork, ham (Ham, daging khinzir)	Raw
108036	R108070	Pork, lean (Daging khinzir tanpa lemak)	Raw
108037	R108071	Pork, medium fat (Daging khinzir separa lemak)	Raw
N108036	R108072	Pork, sausage (Sosej, daging khinzir)	Processed
N108037	R108073	Quail, meat (Daging burung puyuh)	Raw
108030	R108074	Rabbit, domesticared, whole carcas (Arnab)	Raw
108038	R108075	Sausage, Chinese (Lup cheong/Sosej Cina)	Processed

1.09 Eggs

Old code	Revised code	Food	Classification of food
109001	R109001	Century egg *(Pei-tan)*	Processed
109004	R109002	Duck egg, salted, whole *(Telur asin, sebiji)*	Processed
109005	R109003	Duck egg, salted, yolk *(Telur asin, kuning telur)*	Processed
N109001	R109004	Duck egg, white *(Telur itik, putih telur)*	Raw
109002	R109005	Duck egg, whole *(Telur itik, sebiji)*	Raw
109003	R109006	Duck egg, yolk *(Telur itik, kuning telur)*	Raw
N109002	R109007	Eggnog	Processed
N109003	R109008	Goose egg, whole, fresh, raw *(Telur angsa)*	Raw
N109004	R109009	Hen egg, lower cholestrol, with vitamin E (Telur ayam rendah kolestrol, dengan vitamin E)	Raw
N109005	R109010	Hen egg, omega 3 *(Telur ayam omega 3)*	Raw
109008	R109011	Hen egg, white *(Telur ayam, putih telur)*	Raw
109006	R109012	Hen egg, whole *(Telur ayam, sebiji)*	Raw
109007	R109013	Hen egg, yolk *(Telur ayam, kuning telur)*	Raw
109009	R109014	Quail egg, whole *(Telur puyuh, sebiji)*	Raw
N109006	R109015	Turki egg, whole, fresh, raw *(Telur ayam turki, sebiji)*	Raw
109012	R109016	Turtle egg, white *(Telur penyu, putih telur)*	Raw
109010	R109017	Turtle egg, whole *(Telur penyu, sebiji)*	Raw
109011	R109018	Turtle egg, yolk *(Telur penyu, kuning telur)*	Raw

1.10 Fish and Fisheries Products

Old code	Revised code	Food	Classification of food
N110001	R110001	Abalone, canned *(Abalon dalam tin)*	Processed
N110002	R110002	Abalone, dried *(Abalon, kering)*	Processed
N110003	R110003	Abalone, raw *(Abalon)*	Raw
110003	R110004	Anchovy, dried, whole *(Ikan bilis, kering, bahagian keseluruhan)*	Raw
110002	R110005	Anchovy, dried, without head & entrails (Ikan bilis, kering, tanpa kepala dan usus)	Raw
110001	R110006	Anchovy, fresh, whole *(Ikan bilis, segar, bahagian keseluruhan)*	Raw
N110004	R110007	Anchovy, canned *(Sambal ikan bilis dalam tin)*	Processed
N110005	R110008	Barb *(Kerai kunyit) ; Hyrsibarbus sp.*	Raw
N110006	R110009	Barb *(Lomah) ; Labiobarbus ocellatus*	Raw
N110007	R110010	Barb *(Pucuk pisang) ; Lobiobarbus fasciatus*	Raw
N110008	R110011	Barb *(Tengalan) ; Puntius bulu*	Raw
N110009	R110012	Barb, spanner *(Bagoh); Puntius lateristriga*	Raw
N110010	R110013	Barracuda *(Ikan alu-alu/kacang-kacang); Sphyreana spp.*	Raw
110005	R110014	Bladder, fish, fried *(Yi-piu, digoreng)*	Prepared
110004	R110015	Bladder, fish, unspecified, dried *(Yi-piu, kering)*	Processed
N110011	R110016	Bombay-duck *(Lumi-lumi); Harpodon nehereus*	Raw
110006	R110017	Tilapia, black (*Tilapia hitam*); *Oreochromis niloticus*	Raw
110007	R110018	Tilapia, red *(Tilapia merah); Oreochromis spp.*	Raw
N110012	R110019	Tilapia fillet (Filet ikan tilapia)	Processed
N110013	R110020	Bream, monacle *(Puyu laut); Scolopsis spp.*	Raw
110008	R110021	Bream, threadfin, Japanese *(Kerisi); Nemipterus japonicus*	Raw
N110014	R110022	Carp *(Lalang); Oxygaster anomalura*	Raw
N110015	R110023	Carp *(Sebarau); Hampala macrolepidota*	Raw
N110016	R110024	Carp *(Semilang batu); Epbalzeorthynhus kalopterus*	Raw
N110017	R110025	Carp *(Sia); Mystacoleucus marginatus*	Raw
110009	R110026	Carp *(Tebal sisik); Puntius binotatus*	Raw
N110018	R110027	Carp *(Temperas); Cyclochelichthys apogon*	Raw
N110019	R110028	Carp *(Tengas); Acrossocheilus hexagonolepis*	Raw
N110020	R110029	Carp *(Terbol); Osteochilus hasseltii*	Raw
110010	R110030	Carp , big head *(Kap kepala besar); Aristichthys nobilis*	Raw
N110021	R110031	Carp, black *(Kap hitam); Mylopheryngodon piceus*	Raw
110011	R110032	Carp, common *(Lee koh); Cyprinus carpio*	Raw
110012	R110033	Carp, grass *(Kap rumput); Ctenopharyngodon idellus*	Raw
110013	R110034	Carp, Javanese *(Lampam Jawa); Puntius gonionotus*	Raw
N110022	R110035	Carp, Malaysian *(Temoleh); Probarbus jullieni*	Raw
N110023	R110036	Carp, mud *(Kap lumpur); Cirrhina molitorella*	Raw
N110024	R110037	Carp, river *(Kelah); Tor tambroides*	Raw

Old code	Revised code	Food	Classification of food
N110025	R110038	Carp, river *(Lampam sungai); Puntius schwanenfeldi*	Raw
N110026	R110039	Carp, silver *(Kap perak); Hypophthalmichthys molitrix*	Raw
110014	R110040	Catfish *(Keli); Clarias batrachus*	Raw
N110027	R110041	Catfish, canned (keli dalam tin)	Processed
110018	R110042	Catfish eel *(Semilang); Plotosus canius*	Raw
N110028	R110043	Catfish fillet (Filet ikan patin)	Processed
110015	R110044	Catfish, Giant sea *(Jahan); Netuma thalassina*	Raw
110016	R110045	Catfish, Malaysia river *(Patin); Pangasius pangasius*	Raw
110017	R110046	Catfish, river *(Baung); Mystus nemurus*	Raw
110019	R110047	Clam *(Lala)*	Raw
N110029	R110048	Clam, carpet *(Retak seribu); Paphia undulata*	Raw
N110030	R110049	Cobia *(Aruan tasik); Rachycentron canadum*	Raw
110021	R110050	Cockles, boiled 5 mins. *(Kerang, direbus 5 minit)*	Raw
110020	R110051	Cockles, fresh *(Kerang); Arca granosa*	Raw
N110031	R110052	Cod raw *(Ikan kod)*	Raw
110022	R110053	Grouper, sixbar (Kerapu); Epinephelus sexfasciatus	Raw
110023	R110054	Crab, blue/Sea crab, boiled *(Ketam bunga, direbus)*	Prepared
N110032	R110055	crab, blue/Sea crab, fresh (Ketam bunga, segar)	Raw
110024	R110056	Crab, swimming/Live crab, boiled *(Ketam batu, direbus)*	Prepared
N110033	R110058	Crab, swimming/Live crab, fresh *(ketam batu, segar)*	Raw
N110034	R110059	Croaker, tiger-tooth *(Tengkerong); Otolithes sp.*	Raw
110027	R110060	Cuttlefish crackers *(Keropok sotong)*	Processed
110026	R110061	Cuttlefish, dried *(Sotong, kering)*	Processed
110025	R110062	Cuttlefish, fresh *(Sotong); Sepia officinalis*	Raw
110030	R110063	Dart / Ladyfish/ Moonfish *(Nyior-nyior); Trachinotus blochii*	Raw
N110035	R110064	Eel, swamp, *raw (Belut); Fluta alba*	Raw
N110036	R110065	Emperors *(Pelandok); Lethrinus spp.*	Raw
110031	R110066	Featherback *(Belida); Notopterus spp.*	Raw
110036	R110067	Fish "satay" snack *("Satay" ikan)*	Processed
110033	R110068	Fish ball *(Bebola ikan)*	Processed
N110037	R110070	Fish cake *(Kek ikan)*	Processed
N110038	R110071	Fish crackers *(Amplang)*	Processed
110110	R110072	Fish crackers, fried *(Keropok ikan, digoreng)*	Prepared
N110039	R110073	Fish crackers, fried *(Keropok lekor, digoreng)*	Prepared
110109	R110074	Fish crackers, raw *(Keropok ikan, mentah)*	Raw
N110040	R110076	Fish crackers, raw *(Keropok lekor, mentah)*	Processed
110034	R110077	Fish curry, canned *(Kari ikan dalam tin)*	Processed
N110041	R110078	Fish floss *(Serunding ikan)*	Processed
N110042	R110079	Fish nugget *(Nuget ikan)*	Processed
110035	R110080	Fish roe, fresh *(Telur ikan kerapu)*	Raw
N110043	R110081	Fish, salted, in brine *(Ikan pekasam)*	Processed
110037	R110082	Fish sauce (*Budu)*	Processed

Old code	Revised code	Food	Classification of food
N110044	R110083	Fish, smoked *(Ikan Salai)*	Processed
N110045	R110084	Fish snack *(Maruku ikan)*	Processed
N110046	R110085	Fish sausage *(Sosej ikan)*	Processed
110032	R110086	Fish, unspecified, dried, salted *(Ikan asin)*	Processed
N110047	R110087	Frigate tuna *(Tongkol selasih); Auxis thazard*	Raw
N110048	R110088	Fringescale sardine *(Tamban sisek); Sardinella spp.*	Raw
N110049	R110092	Goatfish *(Ikan biji nangka); Upeneus sp.*	Raw
110038	R110093	Goby *(Ketutu); Oxyeleotris marmoratus*	Raw
110039	R110094	Gouramy, giant *(Kalui); Osphronemus goramy*	Raw
110040	R110095	Gouramy, snakeskin *(Sepat siam); Trichogaster pectoralis*	Raw
N110050	R110096	Grouper *(Kerapu); Epinephelus sp.*	Raw
110041	R110097	Grouper, greasy *(Kertang); Epinephelus tauvina*	Raw
110042	R110098	Grunter, silver *(Gerut-gerut); Pomadasys hasta*	Raw
110043	R110099	Halibut, indian *(Togok); Psettodes erumei*	Raw
110044	R110100	Herring, round *(Tamban bulat); Dussumieria hasselti*	Raw
110045	R110102	Herring, wolf *(Ikan parang); Chirocentrus dorab*	Raw
N110051	R110103	Indian threadfish *(Ikan cermin); Alectis indicus*	Raw
N110052	R110104	Indo-pacific tarpon *(Bulan-bulan); Megalops cyprinoides*	Raw
110046	R110105	Carp, river (Jelawat); Leptobarbus hoevenii	Raw
N110053	R110106	Jellyfish (Obor-obor); Rhopilema spp.	Raw
110047	R110107	Jewfish, brown *(Gelama kling); Sciaena dussumieri*	Raw
110048	R110108	Jewfish, silver *(Gelama papan); Johnius (Preudosciaena) soldado*	Raw
N110054	R110109	Kahanga (Sejenis siput laut) – Sabah	Raw
110050	R110110	Giant freshwater prawn *(Udang galah); Macrobrachium rosenbergi*	Raw
N110055	R110111	Lobster, slipper *(Udang lobok); Thennus orientalis*	Raw
N110056	R110112	Lobster, spiny *(Udang karang); Panulirus polyphagus*	Raw
110051	R110114	Long tongue sole *(Lidah pasir); Cynoglossus lingua*	Raw
N110057	R110115	Longtail tuna *(Ikan tongkol hitam); Thunnus tonggol*	Raw
110052	R110116	Mackerel, indo-pacific king *(Tenggiri batang); Scomberomorus commersonii*	Raw
110053	R110117	Mackerel, Indian *(Kembong); Rastrelliger kanagurta*	Raw
110054	R110118	Mackerel, Spanish *(Tenggiri); Scomberomorus guttatus*	Raw
N110058	R110119	Majorras *(Kapas laut); Gerres spp.*	Raw
N110059	R110120	Moustached thryssa *(Ikan bulu ayam); Thryssa mustax*	Raw
110055	R110121	Mullet, bluetail *(Belanak); Valamugil seheli*	Raw
110111	R110122	Mussel *(Siput sudu / Kupang); Perna viridis*	Raw
N110060	R110123	Octopus *(Sotong kurita); Octopodidae*	Raw
N110061	R110125	Otoshimi	Processed
110057	R110126	Oyster, sauce *(Sos tiram)*	Processed
110056	R110127	Oyster, without shell *(Tiram); Ostrea spp.*	Raw

Old code	Revised code	Food	Classification of food
110058	R110128	Painted sweetlip *(Kaci); Spilotichthys pictus*	Raw
N110062	R110129	Parrotfish *(Ikan bayan); D Scarus spp.*	Raw
N110063	R110130	Perch *(Kepar); Pristolepis fasciatus*	Raw
110059	R110131	Perch, climbing *(Puyu/Betok); Anabas testudineus*	Raw
110060	R110132	Perch, Giant Sea/Seabass *(Siakap); Lates calcarifer*	Raw
N110064	R110133	Pike congerl (Malong); Muraenesox spp.	Raw
110062	R110134	Pomfret, black *(Bawal hitam); Parastromateus niger*	Raw
110063	R110135	Pomfret, Chinese *(Bawal tambak); Pampus chinensis*	Raw
110064	R110138	Pomfret, white *(Bawal putih); Pampus argenteus*	Raw
110065	R110139	Ponyfish, greater *(Kikek gedebang); Leiognathus equulus*	Raw
110068	R110140	Prawn crackers *(Keropok udang)*	Processed
110069	R110141	Prawn paste *(Hay-ko)*	Raw
N110065	R110142	Prawn, banana *(Udang puteh); Penaeus merguiensis*	Raw
110066	R110143	Prawn, pink *(Udang merah ros); Metapenaeus affinis*	Raw
N110066	R110144	Prawn, rainbow *(Udang kulit keras); Parapenaeopsis sculptilis*	Raw
N110067	R110145	Prawn, red *(Udang merah); Solenocera subnuda*	Raw
110067	R110146	Prawn, salted, dried *(Udang kering)*	Processed
N110068	R110147	Prawn, sand *(Udang pasir); Metapeneopsis stridulans*	Raw
N110069	R110149	Prawn, sharp-rostrum *(Udang minyak); P. coromandelica*	Raw
N110070	R110150	Prawn, small white *(Udang puteh kecil); Metapenaeus lysianassa*	Raw
N110071	R110151	Prawn, tiger *(Udang harimau); Penaeus monodon*	Raw
N110072	R110152	Prawn, yellow *(Udang kuning); Metapenaeus brevicornis*	Raw
N110073	R110153	Prawn, white (Udang kuning); *Penaeus vannamei*	Raw
110028	R110154	Prepared cuttlefish snack (brand K) *(Snek sotong)*	Processed
110029	R110156	Prepared cuttlefish snack (brand LB) *(Snek sotong)*	Processed
110070	R110157	Queenfish *(Talang); Scomberoides spp*	Raw
110071	R110158	Rabbitfish, streaked *(Dengkis); Siganus javus*	Raw
N110074	R110159	Rainbow sardine *(Tamban buloh); Dussumieris spp.*	Raw
110072	R110160	Ribbonfish *(Timah); Trichiurus haumela*	Raw
N110075	R110161	River Eel *(Tuna); Anquilla bicolour*	Raw
110073	R110162	Ruhu (Rohu); Labeo rohita	Raw
N110076	R110163	Sailfish *(Layaran); Istiophorus spp.*	Raw
N110077	R110164	Salmon *(Ikan salmon)*	Raw
110074	R110166	Sardine *(Pucuk tamban); Clupeoides spp.*	Raw
110075	R110168	Sardine, canned *(Sardin dalam tin)*	Processed
N110078	R110170	Scad, bigeye *(Selar mata besar); P Selar crumenopthalmus*	Raw
110076	R110171	Scad, hairtail *(Cincaru); Megalaspis cordyla*	Raw
110077	R110172	Scad, hairtail, dried *(Cincaru, kering)*	Processed
N110079	R110173	Scad, Ox-eye *(Lolong); Selar boops*	Raw

Old code	Revised code	Food	Classification of food
110112	R110174	Scad, round *(Selayang); Decapterus russelli*	Raw
110079	R110175	Scad, yellowtail *(Pelata); Atule mate*	Raw
N110080	R110176	Sclerapages *(Kelisa); Scleropages formosus*	Raw
N110081	R110177	Sea catfish *(Ikan duri); D Arius sp.*	Raw
N110082	R110178	Sea cucumber *(Trepang/gamat); Holothuriodea*	Raw
110081	R110179	Sea slug/Sea cucumber, cleaned *(Trepang, dibersih)*	Processed
N110083	R110180	Sea cucumber, dried (Gamat kering)	Processed
N110084	R110181	Seaweed, dried (Rumpai laut, kering)	Processed
N110085	R110182	Seaweed, dried (Rumpai laut, basah)	Raw
110082	R110184	Shad, gizzard *(Selangat); Anodontostoma chacunda*	Raw
110083	R110185	Shad, longtail *(Terubok); Tenualosa macrura*	Raw
110084	R110186	Shad, slender *(Beliak mata); Ilisha elongata*	Raw
110085	R110187	Shark, dog *(Yu pasir); Scoliodon sorrakowah*	Raw
110086	R110188	Shark's fin *(Yi-cee)*	Raw
110087	R110189	Sharptooth bass *(Kerisi Bali); Pristipomoides typus*	Raw
110090	R110190	Shrimp paste *(Belacan)*	Processed
110088	R110191	Shrimp, fermented *(Cincaluk)*	Processed
110091	R110192	Sicklefish, spotted *(Daun baharu); Drepane punctata*	Raw
N110086	R110193	Snakehead *(Bujuk); Ophicephalus lucius*	Raw
110092	R110194	Snakehead *(Haruan); Ophicephalus (Channa) striatus*	Raw
N110086	R110195	Snapper *(Remong/Kunyit-kunyit); Lutjanus lineolatus*	Raw
110093	R110196	Snapper, golden stripped *(Jenahak); Lutjanus johni*	Raw
110094	R110197	Snapper, red *(Merah); Lutjanus argentimaculatus*	Raw
110095	R110198	Snapper, Russell's *(Tanda); Lutjanuss russelli*	Raw
N110087	R110199	Spadefish *(Peluru); Ephippus orbis*	Raw
110096	R110200	Starry triggerfish *(Jebong); Abalistes stellaris*	Raw
110097	R110201	Sting ray *(Pari nyiru); Dasyatis zugei*	Raw
N110088	R110202	Surimi	Raw
N110089	R110203	Tayum *(Sejenis landak laut)* – Sabah	Raw
N110090	R110204	Tehek-tehek – Sabah	Raw
110098	R110205	Threadfin *(Kurau); Polynemus indicus*	Raw
110100	R110206	Threadfin *(Senangin); Eleutheronema tetradactylum*	Raw
110099	R110207	Threadfin, dried *(Kurau, kering)*	Processed
110101	R110208	Snakehead (Toman); Channa micropeltes	Raw
N110091	R110209	Trevally, bigeye *(Ikan kerepoh); Caranx sexfasciatus*	Raw
N110092	R110210	Trevally, black-banded *(Ikan aji-aji); Seriolina nigrofasciata*	Raw
N110093	R110211	Trevally, false *(Shrumbu/Lemah); Lactarius lactarius*	Raw
110102	R110212	Trevally, Malabar *(Rambai); Carangoides malabaricus*	Raw
110103	R110213	Scad, yellow-striped *(Selar kuning); Selaroides leptolepis*	Raw
110104	R110214	Scad, yellow-striped, dried *(Selar kuning, kering)*	Raw
N110094	R110215	Tuna spread with mayonis	Processed

Old code	Revised code	Food	Classification of food
N110095	R110216	Tuna spread with tomato and chilli	Processed
N110096	R110217	Tuna, big eye *(Tuna mata besar); Thunnus obesus*	Raw
110105	R110218	Tuna, little / Bonito *(Aya kurik); Euthynnus affinis*	Raw
N110097	R110219	Tuna, skipjack *(Ikan ayu/Kayu); Katsuwonus pelamis*	Raw
N110098	R110220	Tuna, yellowfin tuna *(Tuna sirip kuning); Thunnus albacares*	Raw
N110099	R110221	Umai-Sarawak	Prepared
N110100	R110222	Unagi *(Belut)*	Prepared
N110101	R110223	White spot *(Kepala timah); Aplocheilus panchax*	Raw
110107	R110224	Whiting, trumpeter *(Bulus-bulus / Puntung damar); Sillago maculate*	Raw
110106	R110225	Whitting, silver *(Bulus-bulus / Puntung damar); Sillago sihama*	Raw
110108	R110226	Yellowtail fusilier *(Delah); Caesio erythrogaster*	Raw

1.11 Milk & Milk Products

Old code	Revised code	Food	Classification of food
N111001	R111001	Butter *(Mentega kurang masin)*	Processed
111001	R111002	Butter *(Mentega)*	Processed
N111002	R111003	Cheese cottage, low fat *(Keju cottage, kurang lemak)*	Processed
N111003	R111004	Cheese spread *(Keju sapuan)*	Processed
N111004	R111005	Cheese, blue *(Keju, biru)*	Processed
N111005	R111006	Cheese, brick *(Keju, brick)*	Processed
N111006	R111007	Cheese, brie *(Keju, brie)*	Processed
N111007	R111008	Cheese, camembert *(Keju, camembert)*	Processed
N111008	R111009	Cheese, caraway *(Keju caraway)*	Processed
N111009	R111010	Cheese, cheddar, reduced fat *(Keju cheddar, kurang lemak)*	Processed
N111010	R111011	Cheese, chesdale slices, processed *(Kepingan keju chesdale)*	Processed
N111011	R111012	Cheese, cheshire *(Keju, cheshire)*	Processed
N111012	R111013	Cheese, colby *(Keju, colby)*	Processed
N111013	R111014	Cheese, cottage *(Keju, cottage)*	Processed
N111014	R111015	Cheese, mozarella *(Keju mozarella)*	Processed
N111015	R111016	Cheese, parmesan *(Keju parmesan)*	Processed
111002	R111017	Cheese, processed, cheddar *(Keju, dalam bungkusan plastik)*	Processed
111003	R111018	Cheese, processed, cheddar, canned *(Keju, dalam tin)*	Processed
N111016	R111019	Cheese, ricotta *(Keju, ricotta)*	Processed
N111017	R111020	Cream, whipped *(Krim putar)*	Processed
N111018	R111021	Cream cheese *(Krim keju)*	Processed
111004	R111022	Cream, 26% fat *(Krim, 26% lemak)*	Processed
N111019	R111023	Creamer, powder *(Krimer serbuk)*	Processed
111005	R111024	Ghee *(Minyak sapi)*	Processed
111006	R111025	Ice cream powder *(Serbuk ais krim)*	Processed
N111020	R111026	Milk , pasteurised, chocolate flavor *(Susu berpasteur perisa coklat)*	Processed
N111021	R111027	Milk, camel *(Susu unta)*	Raw
111007	R111028	Milk, cow, fresh *(Susu lembu segar)*	Raw
N111022	R111029	Milk, cultured *(Susu kultur)*	Processed
111008	R111030	Milk, evaporated *(Susu sejat)*	Processed
111009	R111031	Milk, filled *(Susu isian)*	Processed
111010	R111032	Milk, filled, sweetened condensed *(Susu isian pekat manis)*	Processed
N111023	R111033	Milk, goat *(Susu kambing)*	Processed
N111024	R111034	Milk, high calcium *(Susu kalsium tinggi)*	Processed
N111025	R111035	Milk, human *(Susu badan)*	Raw
N111026	R111036	Milk, low fat *(Susu rendah lemak)*	Processed
N111027	R111037	Milk, pasteurised, coffee flavor *(Susu berpasteur perisa kopi)*	Processed

Old code	Revised code	Food	Classification of food
N111028	R111038	Milk, pasteurised, natural *(Susu berpasteur perisa asli)*	Processed
N111029	R111039	Milk, pasteurised, strawberry flavor *(Susu berpasteur perisa strawberi)*	Processed
111011	R111040	Milk, powder, infant formula *(Susu tepung bayi)*	Processed
111012	R111041	Milk, powder, instant, full cream *(Susu tepung penuh krim)*	Processed
111013	R111042	Milk, powder, skim *(Susu tepung skim)*	Processed
111014	R111043	Milk, sterelised *(Susu steril)*	Processed
111015	R111044	Milk, sweetened, condensed *(Susu pekat manis)*	Processed
111016	R111045	Milk, UHT, chocolate flavoured *(Susu UHT, berperisa coklat)*	Processed
N111030	R111046	Milk, UHT, coffee flavoured *(Susu UHT berperisa kopi)*	Processed
111017	R111047	Milk, UHT, full cream, recombined *(Susu UHT, penuh krim, campuran)*	Processed
111018	R111048	Milk, UHT, low-fat, recombined *(Susu UHT, rendah lemak, campuran)*	Processed
N111031	R111049	Milk, UHT, strawberry flavoured *(Susu UHT berperisa strawberi)*	Processed
N111032	R111050	Milkshake, thick chocolate *(Susu kocak berperisa coklat)*	Processed
N111033	R111051	Milkshake, thick vanilla *(Susu kocak berperisa vanilla)*	Processed
111019	R111052	Yoghurt, apricot flavor *(Yogurt, berperisa aprikot)*	Processed
N111034	R111053	Yoghurt, drinking, pasteurised, different flavours *(Air yoghurt, berperisa)*	Processed
N111035	R111054	Yoghurt, drinking, pasteurised, natural flavours *(Air yoghurt, perisa asli)*	Processed
N111036	R111055	Yoghurt, high calcium *(Yogurt, tinggi kalsium)*	Processed
N111037	R111056	Yoghurt, low fat *(Yogurt, rendah lemak)*	Processed
N111038	R111057	Yoghurt, low fat, natural *(Yoghurt, rendah lemak, berperisa asli)*	Processed
N111039	R111058	Yoghurt, natural *(Yogurt, berperisa asli)*	Processed

1.12 Oils and fats

Old code	Revised code	Food	Classification of food
N112001	R112001	Fish oil, cod liver oil *(Minyak ikan kod)*	Processed
112001	R112002	Margarine *(Marjerin)*	Processed
N112002	R112003	Margarine spread, with salt *(Sapuan merjerin bergaram)*	Processed
N112003	R112004	Margarine spread, without salt *(Sapuan merjerin tanpa garam)*	Processed
N112004	R112005	Mayonnaise *(Mayonis)*	Processed
N112005	R112006	Oil, almond *(Minyak kacang almond)*	Processed
N112006	R112007	Oil, apricot kernel *(Minyak aprikot kernel)*	Processed
N112007	R112008	Oil, avocado	Processed
N112008	R112009	Oil, blend *(Minyak campuran)*	Processed
112002	R112010	Oil, blended (palm, peanut, sesame) *(Minyak campuran)*	Processed
N112009	R112011	Oil, canola *(Minyak canola)*	Processed
N112010	R112012	Oil, cocoa butter	Processed
N112011	R112013	Oil, coconut *(Minyak kelapa)*	Processed
112003	R112014	Oil, corn *(Minyak jagung)*	Processed
N112012	R112015	Oil, corn and canola *(Minyak jagung dan canola)*	Processed
112004	R112016	Oil, gingelly / Sesame *(Minyak bijan)*	Processed
112005	R112017	Oil, olive *(Minyak zaitun)*	Processed
112007	R112018	Oil, palm olein *(Minyak olein sawit)*	Processed
112006	R112019	Oil, palm, crude *(Minyak sawit mentah)*	Processed
112008	R112020	Oil, soya bean *(Minyak kacang soya)*	Processed
N112013	R112021	Oil, sunflower *(Minyak bunga matahari)*	Processed
N112014	R112022	Oil, vegetable *(Minyak sayuran)*	Processed
N112015	R112023	Sauce, thousand island	Processed
N112016	R112024	Soft margarine *(Marjerin lembut)*	Processed
112009	R112025	Vanaspati *(Vanaspati)*	Processed

1.13 Beverages

Old code	Revised code	Food	Classification of food
N113001	R113001	Air batu campur (ABC)	Processed
N113002	R113002	Apple, juice (Jus epal)	Processed
N113003	R113003	Asam boi	Processed
N113004	R113004	*Bahar* – Sabah	Processed
N113005	R113005	Barli	Processed
N113006	R113006	Carrot, juice *(Jus lobak merah)*	Processed
N113007	R113007	Cendol	Processed
N113008	R113008	Cereal drink with oat, 3 in 1 *(Minuman bijiran oat, 3 dalam 1)*	Processed
N113009	R113009	Chocolate, 3 in 1 *(Minuman coklat, 3 dalam 1)*	Processed
N113010	R113010	Cincau	Processed
N113011	R113011	Coconut *(Air kelapa)*	Raw
113002	R113012	Coffe powder, instant *(Serbuk kopi segera)*	Processed
113001	R113013	Coffee mixture, powder *(Serbuk kopi campuran)*	Processed
N113012	R113014	Coffee, 3 in 1 *(Minuman kopi, 3 dalam 1)*	Processed
N113013	R113015	Coffee, ginseng *(Kopi ginseng)*	Processed
N113014	R113016	Corn *(Air jagung)*	Processed
N113015	R113017	Cucumber, juice *(Jus timun)*	Processed
N113016	R113018	Dates (Kurma)	Processed
N113017	R113019	Dragon fruit, juice *(Jus buah naga)*	Processed
N113018	R113020	Energy drink *(Minuman tenaga)*	Processed
N113019	R113021	Grape *(Air anggur)*	Processed
N113020	R113022	Guava, juice *(Jus jambu batu)*	Processed
N113021	R113023	Honeydew, juice *(Jus tembikai susu)*	Processed
N113022	R113024	Isotonic drink *(Minuman isotonik)*	Processed
N113023	R113025	Kiwi *(Air kiwi)*	Processed
N113024	R113026	Laicikang	Processed
N113025	R113027	*Lihing* - Sabah	Processed
N113026	R113028	Lime *(Limau nipis)*	Processed
N113027	R113029	Longan *(Air mata kucing)*	Processed
N113028	R113030	Lychee, juice *(Jus laici)*	Processed
113004	R113031	Malted milk drink (packet) *(Minuman susu bermalt, bungkus)*	Processed
113003	R113032	Malted milk powder *(Tepung susu bermalt)*	Processed
N113029	R113033	Mango, juice *(Jus mangga)*	Processed
113005	R113034	Milk based diet supplement, powder *(Tambahan diet berasaskan susu)*	Processed
N113030	R113035	Mixed fruit, juice *(Jus buah-buahan campuran)*	Processed
N113031	R113036	Mocha drink, 3 in 1 *(Mocha 3 dalam 1)*	Processed
N113032	R113037	*Montoku* - Sabah	Processed
113006	R113038	Orange flavoured drink, powder *(Serbuk minuman berperisa oren)*	Processed

Old code	Revised code	Food	Classification of food
N113033	R113039	Orange, juice *(Jus oren)*	Processed
N113034	R113040	Pennywort, juice (Jus pegaga)	Processed
N113035	R113041	Pineapple, juice *(Jus nenas)*	Processed
N113036	R113042	Sarsi	Processed
N113037	R113043	Sirap	Processed
N113038	R113044	Sirap bandung	Processed
N113039	R113045	Soursop, juice *(Jus durian belanda)*	Processed
N113040	R113046	Soya *(Air soya)*	Processed
N113041	R113047	Star fruit, juice *(Jus belimbing)*	Processed
N113042	R113048	Strawberry, juice *(Jus strawberi)*	Processed
113007	R113049	Sugar cane, juice *(Air tebu)*	Processed
N113043	R113050	Tea, crysantimum *(Teh Bunga krisantimum)*	Processed
N113044	R113051	Tea, green *(Teh hijau)*	Processed
N113045	R113052	Tea, kundur *(Teh kundur)*	Processed
N113046	R113053	Tea, milk, foam *(Teh tarik)*	Processed
N113047	R113054	Tea, with sugar *(Air teh)*	Processed
N113048	R113055	Watermelon, juice *(Jus tembikai)*	Processed

1.14 Miscellaneous

Old code	Revised code	Food	Classification of food
114017	R114001	Anise seed, dried *(Jintan manis)*	Processed
114018	R114002	Asam gelugor, pieces *(Asam gelugor keping)*	Raw
N114002	R114003	Bird nest, dried *(Sarang burung, kering)*	Processed
N114003	R114004	Bird nest, soup *(Sarang burung, sup)*	Cooked
114019	R114005	Cardamon *(Buah pelaga)*	Raw
114020	R114006	Chilli, dried *(Lada kering)*	Processed
N114001	R114007	Chocolate *(Coklat bertih)*	Processed
114007	R114008	Chocolate biscuit *(Biskut coklat)*	Processed
114004	R114009	Chocolate sprinkle *(Percikan coklat)*	Processed
114006	R114010	Chocolate wafer *(Wafer coklat)*	Processed
114005	R114011	Chocolate wafer, rounded *(Wafer coklat berbentuk bulat)*	Processed
N114004	R114012	Chocolate, bread spread	Processed
N114005	R114013	Chocolate, cashew nut *(Coklat kacang gajus)*	Processed
N114006	R114014	Chocolate, dark *(Coklat hitam)*	Processed
N114007	R114015	Chocolate, hazelnut *(Coklat kacang badam)*	Processed
114002	R114016	Chocolate, milk *(Coklat susu)*	Processed
114003	R114017	Chocolate, raisin *(Coklat berkismis)*	Processed
N114008	R114018	Chocolate, roasted almond *(Coklat dengan kacang badam)*	Processed
N114009	R114019	Chocolate, white *(Coklat putih)*	Processed
N114010	R114020	Cincau	Processed
114021	R114021	Cinnamon *(Kayu manis)*	Raw
114023	R114022	Cioriander seeds *(Ketumbar)*	Raw
114022	R114023	Clove (Bunga cengkih)	Raw
114010	R114024	Cocoa, powder (Koko, serbuk)	Processed
N114011	R114025	Cohocolate, mixed nuts *(Coklat kacang campuran)*	Processed
114011	R114026	Creamer, non-dairy *(Krimer, bukan tenusu)*	Processed
114024	R114027	Cumin seeds, black *(Jintan hitam)*	Processed
114025	R114028	Cumin seeds, white *(Jintan putih)*	Processed
114026	R114029	Curry leaf *(Daun kari)*	Raw
114027	R114030	Curry powder *(Serbuk kari)*	Processed
114012	R114031	Essence of chicken, proprietary brand *(Pati ayam)*	Processed
114028	R114032	Fenugreek seeds *(Halba/Ventheum)*	Raw
114029	R114033	Galangal *(Lengkuas)*	Raw
N114012	R114034	Garlic spread *(Sapuan bawang putih)*	Processed
114030	R114035	Ginger root, fresh *(Halia)*	Raw
114031	R114036	Ginger, pickled *(Jeruk halia)*	Processed
N114013	R114037	Jelly *(Jeli)*	Processed
114032	R114038	Lemon grass *(Serai)*	Raw
114009	R114039	Milk chocolate beans *(Butiran coklat bersusu)*	Processed
114008	R114040	Milk chocolate with peanuts *(Coklat bersusu dan berkacang)*	Processed

Old code	Revised code	Food	Classification of food
114034	R114041	Mustard powder, tinned *(Serbuk biji sawi dalam tin)*	Processed
114033	R114042	Mustard seed *(Biji sawi)*	Raw
N114014	R114043	Patty, chicken *(Pati ayam)*	Processed
N114015	R114044	Patty, fruits *(Pati buah)*	Processed
114035	R114045	Pepper, powder, white *(Serbuk lada putih)*	Processed
N114016	R114046	Pickled, fruits *(Jeruk buah-buahan)*	Processed
N114017	R114047	Plum sauce *(Sos plum)*	Processed
114036	R114048	Saffron *(Kuma)*	Raw
114037	R114049	Salt, table *(Garam)*	Processed
114001	R114050	Seaweed, agar *(Agar-agar)*	Processed
114038	R114051	Tamarind, paste *(Asam Jawa)*	Processed
114039	R114052	Tumeric root, dried *(Kunyit, kering)*	Processed
N114018	R114053	Vanilla wafer *(Wafer vanilla)*	Processed
N114019	R114054	Vinegar, palm (Cuka nipah)	Processed
114013	R114055	Vinegar, white *(Cuka, putih)*	Processed
N114020	R114056	Wafer, orange flavor *(Wafer berperisa oren)*	Processed
N114021	R114057	Wafer, strawberry flavor *(Wafer berperisa strawberi)*	Processed
N114022	R114058	Yeast *(Yis beku)*	Processed
114016	R114059	Yeast extract (Proprietary brand)	Processed
114014	R114060	Yeast, dried, brewers *(Yis)*	Processed
114015	R114061	Yis, granules, tinned *(Yis biji dalam tin)*	Processed

SECTION 2-PREPARED FOODS

2.1 Traditional Malaysian Kuih

2.1.1 Rice and flour based

Old code	Revised code	Food	Classification of food
211001	R211001	(Bidaran)	Prepared
N211001	R211002	(Bingka beras)	Prepared
N211002	R211003	(Bingka labu)	Prepared
211002	R211004	(Bingka tepung beras)	Prepared
211003	R211005	(Chee-pah)	Prepared
N211003	R211006	(Jerongkong) -Sarawak	Prepared
N211004	R211007	(Komis) – Sabah	Prepared
N211005	R211008	(Kuih akok)	Prepared
211006	R211009	(Kuih bakul)	Prepared
N211006	R211010	(Kuih basung)	Prepared
212017	R211011	(Kuih bawang)	Prepared
N211007	R211012	(Kuih beras)	Prepared
211009	R211013	(Kuih buah rotan)	Prepared
211010	R211014	(Kuih karas)	Prepared
211011	R211015	(Kuih kasui)	Prepared
N211008	R211016	(Kuih khasidah)	Prepared
211014	R211017	(Kuih lapis)	Prepared
211015	R211018	(Kuih lompang)	Prepared
N211009	R211019	(Kuih nyonya)	Prepared
211017	R211020	(Kuih peneram)	Prepared
N211010	R211021	(Kuih penjaram) -Sabah	Prepared
N211011	R211022	(Kuih som-som)	Prepared
N211012	R211023	(Kuih tako)	Prepared
211019	R211024	(Kuih talam seri kaya)	Prepared
211020	R211025	(Kuih tepung pelita)	Prepared
N211013	R211026	(Lompang)	Prepared
N211014	R211027	(Lompat tikam)	Prepared
211021	R211028	(Lor-mai-fan)	Prepared
N211015	R211029	(Nagasari)	Prepared
N211016	R211030	(Putu bambu)	Prepared
N211017	R211031	(Putu beras)	Prepared
N211018	R211032	(Putu mayang)	Prepared
N211019	R211033	(Putu nagor)	Prepared
211023	R211034	(Putu piring)	Prepared
211024	R211035	(Rempeyek)	Prepared
N211020	R211036	(Serabai)	Prepared
N211021	R211037	(Talam keladi)	Prepared
N211022	R211038	(Talam keledek manis)	Prepared

Old code	Revised code	Food	Classification of food
N211023	R211039	(Talam labu)	Prepared
N211024	R211040	(Talam pandan)	Prepared
N211025	R211041	(Talam suji)	Prepared
N211026	R211042	(Tepung torak)	Prepared
211027	R211043	(Tumpi)	Prepared
211028	R211044	(Wajik)	Prepared

2.1.2 Wheat flour based

Old code	Revised code	Food	Classification of food
212001	R212001	Baulu cermai	Prepared
N212001	R212002	Apam seri ayu	Prepared
N212002	R212003	Bahulu kemboja	Prepared
N212003	R212004	Bingka ubi kentang	Prepared
N212004	R212005	Cake, banana *(Kek pisang)*	Prepared
N212005	R212006	Cake, carot *(Kek lobak merah)*	Prepared
N212006	R212007	Cake, fruit *(Kek buah kukus)*	Prepared
212002	R212008	Cake, plain *(Kek biasa)*	Prepared
N212007	R212009	Cake, sponge *(Kek span)*	Prepared
212003	R212010	Cake, swiss roll *(Kek swiss roll)*	Prepared
212004	R212011	Cake, swiss roll, chocolate flavor *(Kek swiss roll perasa coklat)*	Prepared
N212008	R212012	Cakoi	Prepared
N212009	R212013	Cek mek molek	Prepared
N212010	R212014	Cekodok nestum	Prepared
213003	R212015	Cokodok pisang	Prepared
213004	R212016	Cucur badak	Prepared
N212011	R212017	Cucur bawang	Prepared
N212012	R212018	Cucur durian	Prepared
N212013	R212019	Cucur ikan bilis	Prepared
N212014	R212020	Cucur jagung	Prepared
N212015	R212021	Cucur kelapa	Prepared
N212016	R212022	Cucur kentang	Prepared
N212017	R212023	Cucur manis	Prepared
N212018	R212024	Cucur sayur	Prepared
212005	R212025	Cucur udang	Prepared
N212019	R212026	Cupcake *(Kek cawan)*	Prepared
N212020	R212027	Currypuff *(Karipap isi keledek)*	Prepared
212006	R212028	Currypuff *(Karipap)*	Prepared
N212021	R212029	Currypuff, beef *(Karipap daging)*	Prepared
N212022	R212030	Currypuff, chicken *(Karipap isi ayam)*	Prepared
212008	R212031	Currypuff, mini *(Karipap mini)*	Prepared
N212023	R212032	Currypuff, sardine *(Karipap sardin)*	Prepared
N212024	R212033	Currypuff, tuna *(Karipap tuna)*	Prepared
212007	R212034	Currypuff, twisted *(Karipap pusing)*	Prepared
N212025	R212035	Dim sum	Prepared
212025	R212036	Doughnut *(Donut)*	Prepared
N212026	R212037	Doughnut, chocolate coated *(Donut bersalut coklat)*	Prepared
N212027	R212038	Dumpling, beef *(Kuih pau daging)*	Prepared
212009	R212039	Dumpling, chicken *(Kuih pau ayam)*	Prepared
N212028	R212040	Dumpling, chocolate *(Kuih pau coklat)*	Prepared

Old code	Revised code	Food	Classification of food
N212029	R212041	Dumpling, coconut *(Kuih pau kelapa)*	Prepared
N212030	R212042	Dumpling, corn *(Kuih pau jagung)*	Prepared
N212031	R212043	Dumpling, kaya *(Kuih pau kaya)*	Prepared
212010	R212044	Dumpling, red gram *(Kuih pau kacang merah)*	Prepared
N212032	R212045	Dumpling, sardine *(Kuih pau sardin)*	Prepared
212011	R212046	Halwa	Prepared
N212033	R212047	Halwa maskat	Prepared
N212034	R212048	Kek batik	Prepared
212013	R212049	Kesari	Prepared
N212035	R212050	Kuih akak	Prepared
212014	R212051	Kuih apam	Prepared
212015	R212052	Kuih apam balik	Prepared
212016	R212053	Kuih apam gula hangus	Prepared
N212036	R212054	Kuih apam pisang	Prepared
N212037	R212055	Kuih bakar	Prepared
N212038	R212056	Kuih campiang	Prepared
N212039	R212057	Kuih cara berlauk	Prepared
N212040	R212058	Kuih cara manis	Prepared
N212041	R212059	Kuih jintan	Prepared
212018	R212060	Kuih kapit	Prepared
212019	R212061	Kuih keria	Prepared
N212042	R212062	*Kuih kering* – Sabah	Prepared
212020	R212063	Kuih ketayap	Prepared
212021	R212064	Kuih lidah kucing	Prepared
N212043	R212065	Kuih pau goreng/sambal	Prepared
N212044	R212066	Kuih sari bulan	Prepared
N212045	R212067	Kuih siput	Prepared
N212046	R212068	Kuih taik itik	Prepared
N212047	R212069	Lempeng kelapa	Prepared
N212048	R212070	Lempeng pisang	Prepared
N212049	R212071	Lepat labu	Prepared
213018	R212072	Lepat pisang	Prepared
N212050	R212073	Maruku	Prepared
N212051	R212074	Mooncake *(Kuih bulan)*	Prepared
N212052	R212075	Pancakes *(Pankek)*	Prepared
N212053	R212076	Pau goreng sambal	Prepared
212022	R212077	Pineaple tart *(Kuih tat nenas)*	Prepared
N212054	R212078	Roti jala	Prepared
212023	R212079	Sandwich, sardine *(Sandwic sardin)*	Prepared
N212055	R212080	Talam berlauk	Prepared
N212056	R212081	Talam cendol	Prepared
N212057	R212082	Tepung talam	Prepared
212024	R212083	Yau-car-kue	Prepared

2.1.3 Legume based

Old code	Revised code	Food	Classification of food
N213001	R213001	(Bepang kacang)	Prepared
N213002	R213002	(Kacang goreng bersalut)	Prepared
N213003	R213003	(Kacang tumbuk)	Prepared
N213004	R213004	(Koleh kacang)	Prepared
213012	R213005	(Kuih kasturi)	Prepared
N213005	R213006	(Kuih ku)	Prepared
213016	R213007	(Laddu)	Prepared
N213006	R213008	(Masalodeh)	Prepared
213025	R213009	(Putu kacang)	Prepared
213028	R213010	(Vadai, kacang dal kuning)	Prepared
213029	R213011	(Vadai, kacang hitam)	Prepared

2.1.4 Glutinous rice based

Old code	Revised code	Food	Classification of food
N214001	R214001	(Abuk-abuk sagu)	Prepared
N214002	R214002	(Badak berendam)	Prepared
N214003	R214003	(Bepang bijan)	Prepared
N214004	R214004	(Dangai)	Prepared
211005	R214005	(Dodol berdurian)	Prepared
211004	R214006	(Dodol)	Prepared
212012	R214007	(Ham-chi-peng with glutinous rice (Ham-chi-peng)	Prepared
N214005	R214008	(Jejari kering bersira)	Prepared
N214006	R214009	(Ketupat daun palas)	Prepared
211007	R214010	(Kuih bom)	Prepared
211008	R214011	(Kuih buah melaka)	Prepared
N214007	R214012	(Kuih cina) (Sabah)	Prepared
N214008	R214013	(Kuih denderam)	Prepared
N214009	R214014	(Kuih kelupis pulut kukus) - sabah	Prepared
N214010	R214015	(Kuih kipang) - sabah	Prepared
211013	R214016	(Kuih koci pulut hitam)	Prepared
211012	R214017	(Kuih koci pulut putih)	Prepared
211016	R214018	(Kuih lopes pulut)	Prepared
N214011	R214019	(Kuih pintal)	Prepared
N214012	R214020	(Kuih sri muka durian)	Prepared
211018	R214021	(Kuih sri muka)	Prepared
N214013	R214022	(Lepat liat)	Prepared
N214014	R214023	(Loh Mai kai)	Prepared
N214015	R214024	(Onde-onde)	Prepared
N214016	R214025	(Pulut berinti)	Prepared

Old code	Revised code	Food	Classification of food
N214017	R214026	(Pulut dakap)	Prepared
N214018	R214027	(Pulut kukus)	Prepared
N214019	R214028	(Pulut kuning)	Prepared
211022	R214029	(Pulut panggang/Pulut udang)	Prepared
N214020	R214030	(Pulut serunding)	Prepared
N214021	R214031	(Puteri mandi)	Prepared
211025	R214032	(Tapai pulut)	Prepared
211026	R214033	(Tepung bungkus)	Prepared
N214022	R214034	(Tepung gomak/Abuk-abuk)	Prepared

2.1.5 Tuber based

Old code	Revised code	Food	Classification of food
213001	R215001	(Bingka ubi kayu)	Prepared
213002	R215002	(Bingka ubi kayu, gula merah)	Prepared
N215001	R215003	(Cakar ayam)	Prepared
N215002	R215004	(Keledek goreng)	Prepared
213007	R215005	(Kerepek ubi kayu)	Prepared
213014	R215006	(Kuih talam ubi kayu)	Prepared
213019	R215007	(Lepat ubi)	Prepared
N215003	R215008	(Ubi kayu goreng)	Prepared
N215004	R215009	(Ubi kayu sambal)	Prepared
N215005	R215010	(Ubi rebus)	Prepared

2.1.6 Vegetables and fruits based

Old code	Revised code	Food	Classification of food
N216001	R216001	Cempedak goreng	Prepared
N216002	R216002	Kastad labu	Prepared
N216003	R216003	Kerepek pisang	Prepared
213011	R216004	Kuih kastad jagung	Prepared
N216004	R216005	*Kuih terap* - Sabah	Prepared
213017	R216006	Lengat pisang	Prepared
N216005	R216007	Mango puding *(Puding mangga)*	Prepared
N216006	R216008	*Pais jagung* - Sabah	Prepared
N216007	R216009	Pisang bersira	Prepared
213023	R216010	Pisang goreng	Prepared
N216008	R216011	*Pisang pepek* - Sabah	Prepared
213024	R216012	Puding jagung	Prepared
N216009	R216013	Samosa inti isi ayam dan sayur campur	Prepared
213005	R216014	Samosa inti keladi manis	Prepared
213006	R216015	Sukun goreng	Prepared
N216010	R216016	Bayam goreng	Prepared
N216011	R216017	Cendawan goreng	Prepared

2.1.7 Porridge dan *pengat*

Old code	Revised code	Food	Classification of food
N217001	R217001	Bubur asyura	Prepared
N217002	R217002	Bubur asyura manis	Prepared
N217003	R217003	Bubur candil	Prepared
N217004	R217004	Bubur gandum	Prepared
N217005	R217005	Bubur caca	Prepared
N217006	R217006	Bubur durian	Prepared
N217007	R217007	Bubur jagung	Prepared
N217008	R217008	Bubur kurma	Prepared
N217009	R217009	Bubur kanji	Prepared
N217010	R217010	Bubur nangka	Prepared
N217011	R217011	Bubur pisang hijau - Sabah	Prepared
224004	R217012	Pengat keledek, gula putih	Prepared
224003	R217013	Pengat keledek, gula merah	Prepared
N217012	R217014	Pengat labu	Prepared
224005	R217015	Pengat pisang nangka	Prepared
224006	R217016	Pengat pisang nipah	Prepared
224007	R217017	Pengat pisang tanduk	Prepared
N217013	R217018	Pengat ubi	Prepared
224001	R217019	Green gram porridge with coconut milk (Bubur kacang hijau)	Prepared
224002	R217020	Red gram porridge *(Bubur kacang merah)*	Prepared

2.1.8 Miscellaneous

Old code	Revised code	Food	Classification of food
N218001	R218001	Agar-agar coklat	Prepared
N218002	R218002	Agar-agar gula melaka	Prepared
N218003	R218003	Agar-agar merah	Prepared
N218004	R218004	Agar-agar santan	Prepared
N218005	R218005	Agar-agar seri kaya	Prepared
N218006	R218006	Bengkang roti	Prepared
N218007	R218007	Caramel puding *(Puding karamel)*	Prepared
N218008	R218008	*Celorot* - Sarawak	Prepared
N218009	R218009	Cheese cake *(Kek keju)*	Prepared
N218010	R218010	Cream roll, strawberry flavor	Prepared
N218011	R218011	Cream roll, vanilla flavor	Prepared
N218012	R218012	Egg tart *(Tat telur)*	Prepared
213005	R218013	Emping muda	Prepared
213006	R218014	Emping tua	Prepared
N218013	R218015	Jala mas	Prepared
N218014	R218016	Kek batik	Prepared

Old code	Revised code	Food	Classification of food
N218015	R218017	Kek gula hangus	Prepared
N218016	R218018	Kek lapis	Prepared
213008	R218019	Kuih bangkit	Prepared
213009	R218020	Kuih bangkit sagu	Prepared
N218017	R218021	*Kuih buras* - Sabah	Prepared
N218018	R218022	*Kuih cempiang* - Sabah	Prepared
N218019	R218023	*Kuih cincin* - Sarawak	Prepared
N218020	R218024	*Kuih gerigit* - sabah	Prepared
213010	R218025	Kuih kacang	Prepared
N218021	R218026	*Kuih panganan* - sabah	Prepared
N218022	R218027	Kuih ros	Prepared
213013	R218028	Kuih sagu	Prepared
213015	R218029	Kuih telur labah	Prepared
N218023	R218030	Maruku	Prepared
N218024	R218031	Mysore pak	Prepared
N218025	R218032	*Pais pulut hitam* - Sabah	Prepared
213021	R218033	Pakoda	Prepared
213022	R218034	Papadam	Prepared
N218026	R218035	Puding pandan	Prepared
N218027	R218036	Puding roti	Prepared
N218028	R218037	Puding susu	Prepared
213020	R218038	*Sagu goreng* - Sarawak	Prepared
N218029	R218039	Sagu gula melaka	Prepared
N218030	R218040	Sagun	Prepared
N218031	R218041	Sardine roll *(Sardin gulung)*	Prepared
N218032	R218042	Spring roll *(Popia basah)*	Prepared
213026	R218043	Spring roll *(Popia)*	Prepared
213027	R218044	Spring roll, fried *(Popia goreng)*	Prepared
N218032	R218045	*Tabaloi* - Sarawak	Prepared

2.2 Cooked Dishes and Meals

2.2.1 Cereal based

Old code	Revised code	Food	Classification of food
N221001	R221001	*Ambuyat* - Sabah	Prepared
N221002	R221002	*Ambuyat bersama lauk* - sabah	Prepared
N221003	R221003	Bakso	Prepared
N221004	R221004	Bread, naan, butter *(Roti naan butter)*	Prepared
N221005	R221005	Bread, naan, cheese *(Roti nan keju)*	Prepared
N221006	R221006	Bread, naan, plain *(Roti nan kosong)*	Prepared
N221007	R221007	Bread, sausage *(Roti sosej)*	Prepared
N221008	R221008	*Burasa* - sabah	Prepared
221001	R221009	Capati	Prepared
N221009	R221010	Char-kuih-teow	Prepared
N221010	R221011	Chee cheong fun - Sarawak	Prepared
237002	R221012	Chicken soto *(Soto ayam)*	Prepared
221003	R221013	Dosai	Prepared
237001	R221014	Fried rice with chicken pieces *(Nasi goreng dengan daging ayam)*	Prepared
N221011	R221015	Fried rice, chinese style *(Nasi goreng cina)*	Prepared
N221012	R221016	Fried rice, kampung style *(Nasi goreng kampung)*	Prepared
221002	R221017	Green gram gravy served with Capati *(Kuah kacang hijau untuk Capati)*	Prepared
221005	R221018	Idli	Prepared
N221013	R221019	Kebab ayam	Prepared
N221014	R221020	Kebab daging	Prepared
N221015	R221021	Ketupat daun palas	Prepared
N221016	R221022	*Konomi* - Sabah	Prepared
221006	R221023	Kuih-teow (rice noodle), Bandung style *(Kuih-teow Bandung)*	Prepared
221007	R221024	Kuih-teow (rice noodle), fried *(Kuih-teow goreng)*	Prepared
N221017	R221025	Kuih-teow (rice noodle), kolok *(Kuih-teow kolok)*	Prepared
N221018	R221026	Kuih-teow (rice noodle), Kungfu *(Kuih-teow kungfu)*	Prepared
N221019	R221027	Kuih-teow (rice noodle), Ladna *(Kuih-teow ladna)*	Prepared
N221020	R221028	Kuih-teow (rice noodle), Tomyam *(Kuih-teow tomyam)*	Prepared
N221021	R221029	Kuih-teow (rice noodle),Hailam *(Kuih-teow hailam)*	Prepared
N221022	R221030	Kuih-teow (rice noodle),Soup *(Kuih-teow sup)*	Prepared
N221023	R221031	Laksa asam	Prepared
N221024	R221032	Laksa goreng	Prepared
N221025	R221033	Laksa Johor	Prepared
N221026	R221034	Laksa Penang	Prepared
N221027	R221035	Laksa Sarawak	Prepared
N221028	R221036	Laksa Singapura	Prepared
N221029	R221037	Laksa Terengganu	Prepared
N221030	R221038	Laksam	Prepared
N221031	R221039	Lemang	Prepared

Old code	Revised code	Food	Classification of food
N221032	R221040	Lontong	Prepared
N221033	R221041	Lontong goreng	Prepared
N221034	R221042	Macaroni, baked *(Makaroni bakar)*	Prepared
N221035	R221043	Macaroni, fried *(Makaroni goreng)*	Prepared
221008	R221044	Mee (wheat noodle), Bandung style *(Mee Bandung)*	Prepared
N221036	R221045	Mee (wheat noodle), cantonese *(Mee kantonis)*	Prepared
221009	R221046	Mee (wheat noodle), curry *(Mee kari)*	Prepared
221010	R221047	Mee (wheat noodle), fried *(Mee goreng)*	Prepared
N221037	R221048	Mee (wheat noodle), Hailam *(Mee hailam)*	Prepared
N221038	R221049	Mee (wheat noodle), Jawa *(Mee jawa)*	Prepared
N221039	R221050	Mee (wheat noodle), kampua *(Mee kampua)*	Prepared
N221040	R221051	Mee (wheat noodle), Mamak style *(Mee goreng mamak)*	Prepared
N221041	R221052	Mee (wheat noodle), prawn *(Mee udang)*	Prepared
N221042	R221053	Mee (wheat noodle), siam *(Mee siam)*	Prepared
221011	R221054	Mee (wheat noodle), soup *(Mee sup)*	Prepared
N221043	R221055	Mee (wheat noodle), Tomyam *(Mee tomyam)*	Prepared
N221044	R221056	Mee (wheat noodle),Ladna *(Mee ladna)*	Prepared
N221045	R221057	Mee (wheat noodle),Rebus *(Mee rebus)*	Prepared
N221046	R221058	Mee kolok, fried *(Mee kolok goreng)* - Sarawak	Prepared
N221047	R221059	Mee kolok, soup *(Mee kolok sup)* - Sarawak	Prepared
N221048	R221060	Mee, sizzling *(Mee sizzling)*	Prepared
N221049	R221061	Mee wantan	Prepared
N221050	R221062	Mee-hoon (rice noodle) , fried *(Mee-hoon goreng putih)*	Prepared
221012	R221063	Mee-hoon (rice noodle), Bandung style *(Mee-hoon Bandung)*	Prepared
221013	R221064	Mee-hoon (rice noodle), fried *(Mee-hoon goreng)*	Prepared
N221051	R221065	Mee-hoon (rice noodle), Hailam *(Mee-hoon hailam)*	Prepared
N221052	R221066	Mee-hoon (rice noodle), Ladna *(Mee-hoon ladna)*	Prepared
N221053	R221067	Mee-hoon (rice noodle), Tomyam *(Mee-hoon tomyam)*	Prepared
N221054	R221068	Nasi ambang	Prepared
N221055	R221069	*Nasi bangus* - Sabah	Prepared
N221056	R221070	Nasi impit	Prepared
N221057	R221071	Noodle, claypot *(Mee claypot)*	Prepared
N221058	R221072	Porridge *(Bubur lambuk)*	Prepared
N221059	R221073	Porridge, chicken *(Bubur ayam)*	Prepared
N221060	R221074	Porridge, vegetarian *(Bubur sayuran)*	Prepared
N221061	R221075	*Putu* - Sabah	Prepared
221014	R221076	Putu bambu	Prepared
221015	R221077	Putu mayam	Prepared
221016	R221078	Rawadosai	Prepared
221020	R221079	Rice, "dagang" *(Nasi dagang)*	Prepared
221022	R221080	Rice, "oily" *(Nasi minyak)*	Prepared
N221062	R221081	Rice, arab *(Nasi arab)*	Prepared

Old code	Revised code	Food	Classification of food
N221063	R221082	Rice, beriani gam *(Nasi beriani gam)*	Prepared
221017	R221083	Rice, briyani (rice only) (Basmathi rice) *(Nasi briyani, nasi sahaja)*	Prepared
221018	R221084	Rice, chicken *(Nasi ayam)*	Prepared
N221064	R221085	Rice, claypot *(Nasi claypot)*	Prepared
221019	R221086	Rice, coconut milk *(Nasi lemak)*	Prepared
221021	R221087	Rice, fried *(Nasi goreng)*	Prepared
N221065	R221088	Rice, kerabu *(Nasi kerabu)*	Prepared
N221066	R221089	Rice, tomato *(Nasi tomato)*	Prepared
N221067	R221090	Rice, tumeric *(Nasi kunyit)*	Prepared
N221068	R221091	Rice, ulam *(Nasi ulam)*	Prepared
N221069	R221092	Rice, yellow *(Nasi kuning)* - Sabah	Prepared
N221070	R221093	Roti arab	Prepared
N221071	R221094	Roti bawang	Prepared
N221072	R221095	Roti boom	Prepared
221023	R221096	Roti canai	Prepared
N221073	R221097	Roti john	Prepared
N221074	R221098	Roti planta	Prepared
N221075	R221099	Roti sardin	Prepared
221024	R221100	Roti telur	Prepared
N221076	R221101	Roti tisu	Prepared
N221077	R221102	*Soto makasar* - Sabah	Prepared
N221078	R221103	Spaghetti, fried *(Spagheti goreng)*	Prepared
N221079	R221104	*Tumbuk* - Sabah	Prepared
221025	R221105	Yellow dhal gravy (serve with Roti canai / Roti telur) *(Kuah kacang dal kuning dihidang dengan Roti canai/Roti telur)*	Prepared

2.2.2 Meat and egg dishes

Old code	Revised code	Food	Classification of food
N222001	R222001	Beef beriani *(Daging beriani)*	Prepared
222003	R222002	Beef burger *("Burger" daging lembu)*	Prepared
222004	R222003	Beef curry *(Kari daging lembu)*	Prepared
222005	R222004	Beef rendang *(Rendang daging lembu)*	Prepared
222006	R222005	Beef satay *(Satay daging lembu)*	Prepared
N222002	R222006	Beef, black pepper *(Daging lembu masak lada hitam)*	Prepared
N222003	R222007	Beef, bone, soup *(Sup tulang)*	Prepared
N222004	R222008	Beef, floss *(Serunding daging)*	Prepared
222001	R222009	Beef, fried *(Daging lembu goreng)*	Prepared
N222005	R222010	Beef, ginger *(Daging masak halia)*	Prepared
N222006	R222011	Beef, in coconut milk gravy *(Daging lembu masak lemak)*	Prepared
N222007	R222012	Beef, in soya sauce *(Daging lembu masak kicap)*	Prepared
222002	R222013	Beef, in tomato sauce *(Daging lembu masak merah)*	Prepared
N222008	R222014	Beef, kurma *(Daging lembu masak kurma)*	Prepared
N222009	R222015	Beef, roasted *(Daging lembu bakar)*	Prepared
N222010	R222016	Beef, samur *(Daging masak samur)* - Sabah	Prepared
N222011	R222017	Beef, smoke *(Daging salai)*	Prepared
N222012	R222018	Beef, soup *(Sup daging lembu)*	Prepared
N222013	R222019	Beef, stewed *(Stew daging)*	Prepared
N222014	R222020	Beef, tail, soup *(Sup ekor lembu)*	Prepared
N222015	R222021	Char siew (Pork with fish sauce) *(Daging khinzir dengan sos ikan)*	Prepared
N222016	R222022	Chicken chop	Prepared
222013	R222023	Chicken curry *(Kari ayam)*	Prepared
222014	R222024	Chicken kurma *(Ayam kurma)*	Prepared
222015	R222025	Chicken liver rendang *(Rendang hati ayam)*	Prepared
N222017	R222026	Chicken liver, fried *(Hati ayam goreng)*	Prepared
N222018	R222027	Chicken liver, in coconut milk gravy *(Hati ayam masak lemak cili api)*	Prepared
N222019	R222028	Chicken rendang *(Ayam rendang)*	Prepared
222016	R222029	Chicken satay *(Satay ayam)*	Prepared
N222020	R222030	Chicken, cooked with honey *(Ayam madu)*	Prepared
N222021	R222031	Chicken, cooked with tomato *(Ayam masak tomato)*	Prepared
N222022	R222032	Chicken, flour coated *(Ayam goreng tepung)*	Prepared
222012	R222033	Chicken, fried *(Ayam goreng)*	Prepared
N222023	R222034	Chicken, fried in chilli *(Ayam goreng belada)*	Prepared
N222024	R222035	Chicken, fried, marinated *(Ayam goreng berempah)*	Prepared
N222025	R222036	Chicken, ginger *(Ayam masak halia)*	Prepared
N222026	R222037	Chicken, green curry *(Kari hijau ayam)*	Prepared
N222027	R222038	Chicken, grilled *(Ayam panggang)*	Prepared
N222028	R222039	Chicken, in coconut milk gravy *(Ayam masak lemak)*	Prepared
N222029	R222040	Chicken, in tomato sauce *(Ayam masak merah)*	Prepared

Old code	Revised code	Food	Classification of food
N222030	R222041	Chicken, pansuh *(Ayam pansuh)* - Sarawak	Prepared
N222031	R222042	Chicken, percik *(Ayam percik)*	Prepared
N222032	R222043	Chicken, roasted *(Ayam bakar)*	Prepared
N222033	R222044	Chicken, soup *(Sup ayam)*	Prepared
N222034	R222045	Chicken, soya sauce *(Ayam masak kicap)*	Prepared
N222035	R222046	Chicken, tandoori *(Ayam tandoori)*	Prepared
N222036	R222047	Chiken, masala *(Ayam masala)*	Prepared
N222037	R222048	Duck meat, fried *(Daging itik goreng)*	Prepared
N222038	R222049	Duck meat, soup *(Daging itik masak sup)*	Prepared
N222039	R222050	Egg, in coconut milk *(Masak lemak telur)*	Prepared
N222040	R222051	Egg, kurma *(Kurma telur)*	Prepared
N222041	R222052	Egg, packed *(Telur bungkus)*	Prepared
N222042	R222053	Japanese tofu *(Tauhu telur/tauhu jepun)*	Prepared
N222043	R222054	*Kinoring* - Sabah	Prepared
222007	R222055	Liver rendang *(Rendang hati lembu)*	Prepared
222008	R222056	Lungs, fried *(Paru lembu goreng)*	Prepared
N222044	R222057	Lungs, fried in chilli *(Paru goreng bercili)*	Prepared
222009	R222058	Maw in coconut milk gravy *(Perut lembu masak lemak)*	Prepared
222010	R222059	Maw satay *(Satay perut lembu)*	Prepared
N222045	R222060	Maw, kerabu *(Kerabu perut lembu)*	Prepared
N222046	R222061	Maw, soup *(Sup perut)*	Prepared
222017	R222062	Mutton curry *(Kari kambing)*	Prepared
N222047	R222063	Pork, fried *(Khinzir goreng)*	Prepared
N222048	R222064	Pork, soup *(Khinzir masak sup)*	Prepared
N222049	R222065	Pork, sweet sour *(Daging khinzir masak masam manis)*	Prepared
N222050	R222066	Quail, fried *(Daging burung goreng)*	Prepared
N222051	R222067	Quail, fried in chilli *(Daging burung masak cili)*	Prepared
N222052	R222068	Soup gearbox *(Sup gearbox)*	Prepared
222011	R222069	Spleen rendang *(Rendang limpa lembu)*	Prepared

2.2.3 Fish and sea-food dishes

Old code	Revised code	Food	Classification of food
223001	R223001	African bream, fried in chilli *(Ikan tilapia goreng berlada)*	Prepared
223002	R223002	African bream, in coconut milk *(Ikan tilapia masak lemak)*	Prepared
223003	R223003	Anchovy, dried, fried in chilli *(Ikan bilis sambal)*	Prepared
N223001	R223004	Anchovy, fried *(Ikan bilis goreng)*	Prepared
N223002	R223005	*Bat masak tumis* – Sabah	Prepared
223004	R223006	Black pomfret, fried *(Ikan bawal hitam goreng)*	Prepared
223005	R223007	Black pomfret, fried in chilli *(Ikan bawal hitam goreng berlada)*	Prepared
N223003	R223008	Black pomfret, sweet sour *(Ikan bawal masak masam manis)*	Prepared
N223004	R223009	*Bosou ikan* – Sabah	Prepared
N223005	R223010	Botok-botok	Prepared
N223006	R223011	*Buduh selisip* – Sabah	Prepared
N223007	R223012	Butter Prawn *(Udang mentega)*	Prepared
223006	R223013	Catfish eel, fried *(Ikan sembilang goreng)*	Prepared
N223008	R223014	Catfish in coconut milk *(Ikan keli masak lemak)*	Prepared
N223009	R223015	Catfish, fried in chilli *(Ikan keli goreng berlada)*	Prepared
N223010	R223016	Catfish, Malaysia river *(Ikan patin gulai tempoyak)*	Prepared
N223011	R223017	Clam, cooked with ginger *(Lala masak halia)*	Prepared
N223012	R223018	Clam, fried in chilli *(Lala masak cili)*	Prepared
N223013	R223019	Cockles, boiled *(Kerang rebus)*	Prepared
N223014	R223020	Cockles, roasted *(Kerang bakar)*	Prepared
N223015	R223021	Crab ball, deep fried *(Bebola ketam)*	Prepared
N223016	R223022	Crab finger *(Jejari ketam)*	Prepared
N223017	R223023	Crab, flour coated *(Ketam goreng tepung)*	Prepared
N223018	R223024	Crab, fried *(Ketam goreng)*	Prepared
N223019	R223025	Crab, fried in chilli *(Ketam goreng berlada)*	Prepared
N223020	R223026	Crab, fried in spices *(Ketam goreng berempah)*	Prepared
N223021	R223027	Crab, in coconut milk *(Ketam masak lemak cili api)*	Prepared
N223022	R223028	Cuttlefish *(Sotong sumbat)*	Prepared
N223023	R223029	Cuttlefish eggs, fried *(Telur sotong goreng)*	Prepared
N223024	R223030	Cuttlefish, dried, fried in chilli *(Sambal sotong kering)*	Prepared
N223025	R223031	Cuttlefish, fried, coated with flour *(Sotong goreng / sotong goreng tepung)*	Prepared
N223026	R223032	Cuttlefish, kerabu *(Kerabu sotong)*	Prepared
223007	R223033	Cuttlefish, small, fried in chilli *(Sotong kecil sambal)*	Prepared
N223027	R223034	Dollin, in coconut milk *(Dollin masak lemak)* – Sabah	Prepared
N223028	R223035	Eel, soup (Sup belut)	Prepared
N223029	R223036	Fish roe *(Telur ikan goreng)*	Prepared
N223030	R223037	Hairtail scad with chilli *(Ikan cincaru sumbat cili)*	Prepared
223008	R223038	Hairtail scad, cooked in vinegar *(Ikan cincaru masak cuka)*	Prepared
223009	R223039	Hairtail scad,fried in chilli *(Ikan cincaru goreng berlada)*	Prepared
N223031	R223040	Herring, wolf, cooked in tamarind *(Ikan parang asam pedas)*	Prepared

Old code	Revised code	Food	Classification of food
N223032	R223041	*Hinava ikan* – Sabah	Prepared
N223033	R223042	*Inaba* – Sabah	Prepared
N223034	R223043	Indian mackerel, *(Ikan kembong masak taucu dengan timun)**	Prepared
N223035	R223044	Indian mackerel, ampap *(Ikan kembong masak ampap)*	Prepared
N223036	R223045	Indian mackerel, cooked in tamarind *(Ikan kembong masak asam rebus)*	Prepared
223010	R223046	Indian mackerel, curry *(Ikan kembong kari)*	Prepared
223011	R223047	Indian mackerel, fried *(Ikan kembung goreng)*	Prepared
223013	R223048	Indian mackerel, fried in chilli *(Ikan kembong goreng berlada)*	Prepared
223012	R223049	Indian mackerel, in soya sauce *(Ikan kembong masak kicap)*	Prepared
N223037	R223050	Indian mackerel, pais *(Pais ikan kembung)*	Prepared
N223038	R223051	Indian mackerel, percik *(Ikan kembong percik)*	Prepared
N223039	R223052	Indian mackerel, roasted *(Ikan kembong bakar)*	Prepared
N223040	R223053	*Kima basah masak tumis* – Sabah	Prepared
N223041	R223054	*Kima masak lemak* – Sabah	Prepared
N223042	R223055	*Kinilau* – Sabah	Prepared
N223043	R223056	Perch sea, *(Ikan siakap tiga rasa)**	Prepared
N223044	R223057	Perch sea, fried in chilli *(Ikan siakap masak sambal)*	Prepared
N223045	R223058	Perch sea, percik *(Ikan siakap percik)*	Prepared
N223046	R223059	Perch sea, steamed *(Ikan siakap kukus)*	Prepared
N223047	R223060	*Pinarasakan* – sabah	Prepared
N223048	R223061	Prawn sweet sour *(Udang masak masam manis)*	Prepared
N223049	R223062	Prawn, *(Sambal petai udang)**	Prepared
N223050	R223063	Prawn, fried with tumeric *(Udang goreng kunyit)*	Prepared
N223051	R223064	Prawn, fried, flour coated *(Udang goreng tepung)*	Prepared
N223052	R223065	Prawn, in coconut milk *(Udang masak lemak cili api)*	Prepared
223014	R223066	Red snapper, cooked in tamarind *(Ikan merah masak asam)*	Prepared
N223053	R223067	Red snapper, curry *(Ikan merah masak kari)*	Prepared
223015	R223068	Red snapper, fried in chilli *(Ikan merah goreng berlada)*	Prepared
N223054	R223069	Red snapper, head, soup *(Sup kepala ikan)*	Prepared
223016	R223070	Red snapper, in coconut milk *(Ikan merah masak lemak)*	Prepared
N223055	R223071	Red snapper, soup *(Ikan merah masak sup)*	Prepared
N223056	R223072	*Sagol ikan buntal* – Sabah	Prepared
N223057	R223073	Sardine, fried in chilli *(Ikan sardin sambal)*	Prepared
223017	R223074	Shrimp, small, cooked in chili *(Udang kecil sambal)*	Prepared
N223058	R223075	*Sinagul ikan pari* – Sabah	Prepared
N223059	R223076	Snails in coconut milk *(Siput sedut masak lemak)*	Prepared
223018	R223077	Snakehead, salted, fried *(Ikan haruan kering goreng)*	Prepared
223019	R223078	Spanish mackerel, fried *(Ikan tenggiri goreng)*	Prepared
223020	R223079	Spanish mackerel, fried in chilli *(Ikan tenggiri goreng berlada)*	Prepared
223021	R223080	Stingray, cooked in tamarind *(Ikan pari masak asam pedas)*	Prepared

Old code	Revised code	Food	Classification of food
N223060	R223081	Threadfin bream, fried *(Ikan kerisi goreng)*	Prepared
223022	R223082	Threadfin bream, fried in chilli *(Ikan kerisi goreng berlada)*	Prepared
223023	R223083	Threadfin bream, in soya sauce *(Ikan kerisi masak kicap)*	Prepared
N223061	R223084	Threadfin, in coconut milk *(Ikan senangin masak gulai)*	Prepared
N223062	R223085	Threadfin, salted, in coconut milk *(Ikan kurau masin gulai lemak)*	Prepared
N223063	R223086	Trevally, yellow-banded, fried in chilli *(Ikan selar sambal tumis)*	Prepared
223024	R223087	Tuna, cooked in coconut milk *(Ikan tongkol masak lemak)*	Prepared
N223064	R223088	Tuna, fried *(Ikan tongkol goreng)*	Prepared
N223065	R223089	Tuna, in soya sauce *(Ikan tongkol masak kicap)*	Prepared
N223066	R223090	Tuna, singgang *(Ikan tongkol singgang)*	Prepared
N223067	R223091	*Umai* – Sarawak	Prepared
223025	R223092	Yellow-banded travelly, in tamarind *(Ikan selar kuning masak asam pedas)*	Prepared

2.2.4 Vegetable dishes

Old code	Revised code	Food	Classification of food
N224001	R224001	Acar buah	Prepared
N224002	R224002	Acar rampai	Prepared
N224003	R224003	Acar timun	Prepared
N224004	R224004	Asparagus, fried *(Asparagus goreng)*	Prepared
N224005	R224005	Banana, unripe *(Masak lemak kuning pisang muda)*	Prepared
N224006	R224006	Bean, four-angled *(Kacang botol goreng)*	Prepared
N224007	R224007	Bean, french *(Kacang buncis goreng)*	Prepared
N224008	R224008	Bean, string, fried *(Kacang panjang goreng)*	Prepared
N224009	R224009	Beansprouts in coconut milk *(Taugeh masak lemak putih)*	Prepared
N224010	R224010	Beansprouts, kerabu *(Kerabu taugeh)*	Prepared
N224011	R224011	Beansprouts, stir fried *(Taugeh goreng)*	Prepared
N224012	R224012	Brocolli, stir fried *(Brokoli goreng)*	Prepared
N224013	R224013	Cabbage in coconut milk *(Kobis masak lemak putih)*	Prepared
N224014	R224014	Cabbage, fried *(Kobis goreng)*	Prepared
N224015	R224015	Eggpant, fried in chilli *(Terung goreng belada)*	Prepared
N224016	R224016	Fern shoots in coconut milk *(Masak lemak pucuk paku)*	Prepared
N224017	R224017	Fern shoots, kerabu *(Kerabu pucuk paku)*	Prepared
N224018	R224018	Gado-gado	Prepared
N224019	R224019	Ground, bitter, fried *(Peria goreng)*	Prepared
N224020	R224020	Gulai betik	Prepared
N224021	R224021	Gulai rebung	Prepared
N224022	R224022	Gulai umbut kelapa	Prepared
N224023	R224023	Jelatah	Prepared
N224024	R224024	Kailan goreng	Prepared
N224025	R224025	Kailan masak sos tiram	Prepared
N224026	R224026	Kale with salted fish *(Kailan ikan masin)*	Prepared
N224027	R224027	Kerabu pegaga	Prepared
N224028	R224028	Kerabu pucuk beko	Prepared
N224029	R224029	Kerabu pucuk paku	Prepared
N224030	R224030	Kuah lodeh	Prepared
N224031	R224031	*Lada kuning masak daun bawang merah* - sabah	Prepared
N224032	R224032	*Lembiding masak sup* - Sabah	Prepared
N224033	R224033	Mango, kerabu *(Kerabu mangga)*	Prepared
N224034	R224034	Mix vegetables, stir fried *(Sayur campur goreng)*	Prepared
N224035	R224035	Mushroom, fried, coated with flour *(Cendawan goreng tepung)*	Prepared
N224036	R224036	Mushroom, soup *(Sup cendawan)*	Prepared
N224037	R224037	Paceri buah	Prepared
N224038	R224038	Paceri terung	Prepared
N224039	R224039	Petola air	Prepared
N224040	R224040	Pineapple in coconut milk gravy *(Pajeri nenas)*	Prepared

Old code	Revised code	Food	Classification of food
N224041	R224041	Plaintain, flower, kerabu *(Kerabu jantung pisang)*	Prepared
N224042	R224042	Sambal/sayur goreng (Nasi ambang)	Prepared
N224043	R224043	Sare, kerabu (Kerabu rumpai laut)	Prepared
N224044	R224044	*Sinalau sayur nangka* - Sabah	Prepared
N224045	R224045	Solok lada	Prepared
N224046	R224046	Spinach, *(Bayam masak air)**	Prepared
N224047	R224047	Sup sayur	Prepared
N224048	R224048	Swap cabbage, fried with belacan *(Kangkung goreng belacan)*	Prepared
N224049	R224049	Tapai ubi kayu	Prepared
N224050	R224050	Tauhu goreng	Prepared
N224051	R224051	Tauhu sumbat	Prepared
N224052	R224052	Tempe goreng	Prepared
N224053	R224053	Tempe goreng cili	Prepared
N224054	R224054	*Tuhau* - Sabah	Prepared

2.2.5 Miscellaneous

Old code	Revised code	Food	Classification of food
N225001	R225001	Air asam	Prepared
N225002	R225002	*Amplang ikan* - Sabah	Prepared
N225003	R225003	Bergedil ayam	Prepared
N225004	R225004	Bergedil daging	Prepared
N225005	R225005	Bergedil ikan	Prepared
N225006	R225006	Budu	Prepared
N225007	R225007	Budu goreng	Prepared
N225008	R225008	Cencalok	Prepared
N225009	R225009	Keropok leko	Prepared
N225010	R225010	*Linopot* - Sabah	Prepared
N225011	R225011	Otak-otak	Prepared
N225012	R225012	Pasembor	Prepared
N225013	R225013	Pecal	Prepared
N225014	R225014	Rasam	Prepared
224001	R225015	Rojak	Prepared
N225015	R225016	Rojak mamak	Prepared
N225016	R225017	Sambal belacan	Prepared
N225017	R225018	Sambal betik	Prepared
N225018	R225019	Sambal kelapa	Prepared
N225019	R225020	Sambal kicap	Prepared
N225020	R225021	Sata	Prepared
N225021	R225022	Somtam	Prepared
N225022	R225023	Ulat mulung - Sarawak	Prepared

2.3 Franchised fast food

2.3.1 Chicken

Old code	Revised code	Food	Classification of food
231004	R231001	Bun *(Roti)*	Prepared
231008	R231002	Chicken breast *(Daging ayam, bahagian dada)*	Prepared
231010	R231003	Chicken drumstick *(Daging ayam, bahagian paha)*	Prepared
N231001	R231004	Chicken meat ball *(Bebola ayam)*	Prepared
231002	R231005	Chicken meat, shaped and fried *(Daging ayam, digoreng)*	Prepared
N231002	R231006	Chicken nuggets *(Naget ayam)*	Prepared
231012	R231007	Chicken shoulder *(Daging ayam, bahagian bahu)*	Prepared
231011	R231008	Chicken thigh *(Daging ayam, bahagian "drumstick")*	Prepared
231009	R231009	Chicken wing *(Daging ayam, sayap)*	Prepared
231005	R231010	Coleslaw	Prepared
231007	R231011	French fries *(Kentang goreng)*	Prepared
231001	R231012	Fried chicken, various portions *(Ayam goreng, berbagai bahagian)*	Prepared
N231003	R231013	Grilled chicken *(Ayam panggang)*	Prepared
231003	R231014	Mashed potatoes *(Ubi kentang, lenyek)*	Prepared
231006	R231015	Salad	Prepared

2.3.2 Burger

Old code	Revised code	Food	Classification of food
232001	R232001	Beef burger *("Burger" daging lembu)*	Prepared
N232001	R232002	Burger fish, with tartar sauce and cheese *("Burger" ikan dengan sos tartar dan keju)*	Prepared
N232002	R232003	Burger, chicken *("Burger" ayam)*	Prepared
N232003	R232004	Burger, fish *("Burger" ikan)*	Prepared
N232004	R232005	Burger, fish, with tartar sauce *("Burger" ikan dengan sos tartar)*	Prepared
232002	R232006	Cheese burger *("Burger" keju)*	Prepared
232003	R232007	Cheese burger with extra beef pattie *("Burger" keju dgn daging lembu)*	Prepared
232005	R232008	Coney dog	Prepared
221004	R232009	Egg banjo *("Banjo" telur)*	Prepared
232004	R232010	Fried fish cake with bun *(Daging ikan goreng dengan roti)*	Prepared
232006	R232011	Hot dog	Prepared

2.3.3 Pizza

Old code	Revised code	Food	Classification of food
233006	R233001	Pizza with beef and onion *(Piza yang mengandungi daging lembu dan bawang)*	Prepared
233003	R233002	Pizza with beef, chicken, onion *(Piza yang mengandungi daging lembu,ayam, dll)*	Prepared
233011	R233003	Pizza with beef, salami, etc *(Piza yang mengandungi daging lembu, salami, dll)*	Prepared
N233001	R233004	Pizza with cheese only *(Pizza yang mengandungi keju sahaja)*	Prepared
233002	R233005	Pizza with chicken and pineapple *(Piza yang mengandungi ayam dan nenas)*	Prepared
233004	R233006	Pizza with chicken curry and peas *(Piza yang mengandungi kari ayam dan kacang)*	Prepared
233008	R233007	Pizza with chicken, mushroom, tomato *(Piza yang mengandungi daging ayam, cendawan dll)*	Prepared
233005	R233008	Pizza with curry beef and peas *(Piza yang mengandungi kari lembu dan kacang)*	Prepared
233007	R233009	Pizza with onion, tomatoes, etc *(Piza yang mengandungi bawang, tomato, dll)*	Prepared
233001	R233010	Pizza with pepperoni, beef, etc *(Piza yang mengandungi pepperoni dll)*	Prepared
233009	R233011	Pizza with shrimp and cucumber *(Piza yang mengandungi udang dan timun)*	Prepared
233010	R233012	Pizza with shrimp, squid and mushroom *(Piza yang mengandungi udang, sotong & cendawan)*	Prepared
N233002	R233013	Pizza with tomyam flavor *(Pizza tomyam)*	Prepared

2.3.4 Spaghetti

Old code	Revised code	Food	Classification of food
N234001	R234001	Spaghetti Carbonara	Prepared
234001	R234002	Spaghetti with cheese and meat sauce *(Spageti yang mengandungi keju dan sos daging)*	Prepared
234003	R234003	Spaghetti with chicken, mushroom, etc *(Spageti yang mengandungi ayam, cendawan, dll)*	Prepared
234002	R234004	Spaghetti with shrimp, mushroom, etc *(Spageti yang mengandungi udang, cendawan, dll)*	Prepared
234004	R234005	Spaghetti with vegetables, sauce, etc *(Spageti yang mengandungi sayur-sayuran, sos, dll)*	Prepared

2.3.5 Sandwiches

Old code	Revised code	Food	Classification of food
235004	R235001	Sandwich with chicken, etc *(Sandwic yang mengandungi daging ayam, dll)*	Prepared
235003	R235002	Sandwich with chicken, salad, etc *(Sandwic yang mengandungi ayam, salad, dll)*	Prepared
N235001	R235003	Sandwich with egg *(Sandwic telur)*	Prepared
235002	R235004	Sandwich with fish, salad, mayonnaise *(Sandwic yang mengandungi ikan, salad, dll)*	Prepared
235005	R235005	Sandwich with pepperoni, salad, etc *(Sandwic yang mengandungi pepperoni, salad, dll)*	Prepared
235001	R235006	Sandwich with tuna fish, etc *(Sandwic dgn ikan tuna, dll)*	Prepared

2.3.6 Satay

Old code	Revised code	Food	Classification of food
236002	R236001	Beef satay *(Satay daging lembu)*	Prepared
236001	R236002	Chicken satay *(Satay daging ayam)*	Prepared
236003	R236003	Mutton satay *(Satay daging kambing)*	Prepared
236004	R236004	Satay sauce *(Kuah satay)*	Prepared

2.3.7 Miscellaneous

Old code	Revised code	Food	Classification of food
N237001	R237001	Beef murtabak *(Murtabak daging)*	Prepared
N237002	R237002	Chicken murtabak *(Murtabak ayam)*	Prepared
N237003	R237003	Jacket potato *(Kentang jaket)*	Prepared
N237004	R237004	Lasagna	Prepared
237003	R237005	*Murtabak*	Prepared
237004	R237006	Murtabak sauce *(Sos murtabak)*	Prepared
N237005	R237008	Puff, chicken *(Paf ayam)*	Prepared
N237006	R237009	Puff, sardine *(Paf sardin)*	Prepared
N237007	R237010	Tortilla, beef *(Tortila daging)*	Prepared
N237008	R237011	Yong Tau Fu	Prepared

APPENDIX

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