Macronutrient analysis

Food component	Available method of analysis	Limitation	Application
Water (moisture)	Air oven*	Caramelization of sugars, degradation of unsaturated fat, loss of volatiles	This method is applicable to all foods at 60˚C. At 100˚C, it is applicable to all foods except those rich in fat and sugar
	Vacuum oven*	Loss of volatiles	
	Freeze-drying*	Slow. Care must be taken to avoid residual water in samples	Applicable to most foods
	Microwave oven	Charring	Applicable to medium- or high-moisture foods only
	Dean and Stark distillation	Safety of solvents used	Applicable to foods high in volatiles*
	Karl Fisher		Applicable to low-moisture, hygroscopic foods
	Physical methods (NMR, NIR)	High cost and needs calibration for each food group	NMR is applicable to most foods. NIR is only established for cereals and some other foods
	Chromatography (GLC, GSC)	High cost	GLC is applicable to meat and meat products only. GSC is applicable only to some meat products
Total fat	Continuous extraction (single solvent, also called Soxhlet)	Time consuming. Extracts cannot be used for fatty acid studies. Incomplete extraction from many foods (dry analytical samples). Non-comparable	Applicable to low-moisture foods and non-cereal foods

		value for cereals	
	Acid hydrolysis	Some hydrolysis of lipids. Extracts cannot be used for fatty acid studies	Applicable to all foods except dairy and high-sugar products
	Acid hydrolysis and capillary GLC	High cost. This method is NLEA-compliant	Applicable for most foods
	Mixed solvent extraction *	Complete extraction from most foods. Extract often needs clean-up	Applicable to most foods and extract can be used for fatty acid analysis
	Alkaline hydrolysis		Validated for dairy foods only
	NIR	High cost. Requires extensive calibration against other methods	Established only for cereals
Fatty acids	HPLC	High cost	
	GLC *	Moderate to high cost	Applicable to all foods. If used for *trans* fatty acids, capillary techniques are required
	Infrared absorption (for trans fatty acids)	High cost. Some interference	Applicable to all foods
Total nitrogen /protein	Kjeldahl (for total nitrogen) *	Minor interference from inorganic nitrogen. Toxic wastes	Applicable to all foods
	Dumas (for total nitrogen) *	Limitations are high cost, the inclusion of inorganic nitrogen and analytical portion size	Applicable to all foods
	Radiochemical methods (for total nitrogen)	Very high cost of instrumentation	Applicable to most foods
	Formol titration; Biuret; Folin's reagent (for protein)	Specificity	Applicable to dairy products only
	Alkaline distillation (for protein)	Specificity	Applicable to cereals only
	Dye-binding (for protein)	Specificity	Applicable only to specific foods, and some cereals

			and legumes
	NIR (for protein)	High cost. Number of calibration sample	Applicable to some foods
Amino acids (AA)	GLC (preceded by acid hydrolysis for most AA. Alkaline hydrolysis required for tryptophan. Special hydrolysis conditions required for sulphur AA and acid-sensitive AA.)	Moderate to high cost. Choice of derivative is critical. AA need to be derivatized prior to chromatography	Applicable to most foods
	HPLC* (Preceded by acid hydrolysis for most AA. Alkaline hydrolysis required for tryptophan. Special hydrolysis conditions required for sulphur AA and acid-sensitive AA. AA usually derivatized prior to chromatography)	High cost	Applicable to all foods
	Ion-exchange chromatography* (Preceded by acid hydrolysis for most AA. Alkaline hydrolysis required for tryptophan. Special hydrolysis conditions required for sulphur AA and acid-sensitive AA.)	High cost. Hydrolytic losses of more labile AAs and slow release of branched chain AAs	Applicable to all foods
	LC-MS	High cost	Applicable to all foods
	Colorimetry (Tryptophan and S containing AA, lysine)	Not sensitive enough	Applicable to all foods
	Microbiological assays	Tedious, time-consuming, non reproducibility	Applicable to all foods
Alcohol	Distillation*	Interference with volatiles	Applicable to all foods
	GLC*		Applicable to all foods
	Specific enzyme method*		Applicable to all foods
Sugars, total (mono and disaccharides)	Density	Accurate for sucrose	Applicable to sugar solutions
	Refractive index	Empirical calibration required	Applicable to sugar solution
	Polarimetry	Close attention to standardized methods is essential	Applicable to single sugars or simple mixture only

	Reductiometric	Non-reducing sugars, sucrose and invert sugar mixtures	Applicable to reducing sugars
	Colorimetric	Specificity	Applicable to single sugars and simple mixtures
	Specific enzyme method *	Reagents can be expensive	Applicable to glucose and complex mixtures
	GLC	Need for derivatives	Can be applied to complex mixture
	HPLC *	Moderate to high cost. Choice of columns, detectors are crucial	Can be applied to complex mixture
Polyols	Specific enzymatic method	Specificity of enzymes	Limited to a few polyols only
	HPLC*	Moderate to high cost. Lack of standardized procedures; choice of column	Can be applied to complex mixture
	Microbiology	Acyclic polyol only	All foods
Oligosaccharides	Specific enzymatic procedures	Moderate to high cost	Applied for selective hydrolysis and separation
	GLC	Moderate to high cost. Choice of column	Can be applied to complex mixture
	HPLC	Moderate to high cost	Complex mixtures
Starch	Polarimetry	Needs very careful calibration	Applicable only to some cereal foods
	Dilute acid hydrolysis using a general sugar method	Interference from any NSP present.	Applicable to highly refined foods that are low in NSP
	Dilute acid hydrolysis and glucose-specific method	Presence of β-glucans	Applicable only to foods low in β-glucans
	Enzymatic hydrolysis and glucose specific method*	Choice of enzymes and conditions	Applicable to all foods

Dietary fibres			
Total dietary fibre	AOAC method for dietary fibres (Prosky *et al.)* * - a enzymatic–***gravimetric*** method	Time-consuming	Applicable to all foods
Non-starch polysaccharides (NSP)	Enzymatic hydrolysis and removal of starch. Acid hydrolysis of NSP, GLC, HPLC separation of component monosaccharides. Colorimetric analysis of monosaccharide (Englyst et al.)	Moderate to high cost. Resistant starch must be treated before hydrolysis. GLC requires preparation of derivatives. Gives only total values. This method is not robust	Applicable to all foods
Resistant starch	Enzymatic hydrolysis of starch before and after treatment with alkali or DMSO	Choice of enzymes and conditions	Applicable to all foods

* recommended method

Inorganic material analysis

Applicable to all foods after defatting and drying, especially for food high in fat and/or water content

Food component	Available method of analysis	Limitations
Total Ash	Dry ashing	Not suitable for mineral analysis of volatile minerals because of their partial loss
	Wet ashing	Small sample throughput
Cations		
Na*, K*, Ca, Mg	Flame photometry	Interference
Na, K, Ca*, Mg*, Fe*, Cu*, Zn*, Mn*, Co*, Cr*	Atomic Absorption Spectrometry (AAS) with electrothermal furnace	Moderate to high cost. Interferences from anions; special suppression techniques
Se*	Hydride generation AAS	Moderate to high cost
	Fluorimetry	
all cations	Plasma emission spectrometry (= inductively coupled plasma spectroscopy ICP) ideally coupled with Mass spectrometry (MS)*	Very high cost. Matrix effects need to be controlled
K, Mg, Fe, Cu, Zn	Colorimetry	Extracting techniques. Difficult for K and Zn
Ca and Mg	Classical precipitation and titration	Size of analytical sample; skilled techniques
Anions		
Phosphorus	Colorimetry	
	ICP-MS	Very expensive
Chloride	Titrimetric	
	Ion-specific electrode (ISE)	Interference
	ICP-MS	Very expensive
	Automated conductimetry	High cost
Iodine	Microdistillation	Laboratory contamination
	ISE	
	ICP-MS	Very expensive
	Alkaline dry-ashing	
	GLC	High cost

Fluorine	Microdistillation	Time-consuming
	Ion-specific electrode (ISE)	
	Polarography	
Sulphur	Gravimetric	
	X-ray fluorescence	High cost
	ICP-MS	Very expensive
Nitrite	Colorimetry	
	Ion-specific electrode (ISE)	
Nitrate	HPLC	High cost

Vitamin analysis

Applicable to all foods

Food component	Available method of analysis	Limitations
Retinol	Colorimetry	Obsolete (Carr and Price, 1926). Low recoveries of retinoids
	HPLC *	Moderate to high cost.
Carotenoids	Open column chromatography	Identification of carotenoids. Lack of resolution of some geometrical isomers (lutein/zeaxanthin) and stereo-isomers (cis/trans).
	HPLC *	Moderate to high cost. Identification of carotenoids
Vitamin D	Bioassay	For low level only; animal facilities required
	Colorimetry	Lack of precision and sensitivity
	Gas-liquid chromatography (GLC)	New procedures under development
	HPLC*	High cost. Lipid interference; two stages, preparative followed by analytical separation needed for most foods
	Radio-immunoassay	High cost
Vitamin E	Colorimetry	Interfering compounds
	GLC	Derivation prior to chromatography required
	HPLC *	High cost. Extraction techniques
Vitamin K	Colorimetry	Lack of specificity
	Column chromatography, GC*	Moderate to high cost for GC
	HPLC*	High cost. Lipid interference
Vitamin C	Dye titration	Measure ascorbic acid only; pigments interfere; value lower as HPLC but comparable values for fresh fruits and vegetables
	Colorimetry	Measures inactive compounds also
	Fluorometry	Does not separate ascorbic and dehydroascorbic acid
	GLC	Derivitization prior to chromatography required
	HPLC *	High cost. Clean-up and separate detection of homologues add delays
Thiamin/ Riboflavin	Microbiological*	Time
	Fluorometry	

	HPLC*	High cost
Niacin	Microbiological*	Time
	Colorimetry	Hazardous reagent
	HPLC*	High cost.
Vitamin B6	Microbiological*	Time; response to different vitamers may not be equal; total values only
	HPLC*	High cost
	Radiometric-microbiological	High cost
Vitamin B12	Microbiological*	
	Radio-isotopic	High cost
Folates	Microbiological *	Response to different vitamers may not be equal; total values only
	HPLC	High cost. Not all vitamers measured properly
	LC-MS	Very high cost, but this method is able to quantify the different isomers of folates
Pantothenic acid	Microbiological*	
	HPLC	High cost
Biotin	Microbiological*	
	Isotope dilution	High cost
	Radiometric-microbiological	High cost
	Radio-immunoassay	High cost
	Protein-binding	High cost
	HPLC	High cost

* recommended method

Analysis of other components

Food component	Available method of analysis	Limitations
Hemagglutinins/Lectins	RBC agglutination	Not all blood samples of one animal species will react in an identical manner owing to the existence of several blood groups. Agglutination dilution test semi-quantitative
	Spectrophotometric methods	
	Radioactive labelling of lectin molecules	Requires specialized handling
Phytic acid	Anion exchange	Inability to resolve inositol phosphates adequately
	HPLC	High cost
	GLC	Detects derivatize volatile inositol phosphate forms only after separation by ion-exchange chromatography
	Capillary electrophoresis	Not applicable to all foods
	NMR-MS	High costs. Specialized application
Oxalates	Capillary electrophoresis	Not good for low oxalate content <1.8 mg/100 g. Meant for routine monitoring
	Ion chromatography	High running cost
	GLC	Some forms of oxalate are difficult to methylate; high instrument cost
	Enzymatic	Not applicable to all foods
	Colorimetry (AOAC)	Interference from other acids
	HPLC	High cost
Tannins (grouped into condensed tannins also called proanthocyanidins, hydrolysable and derived tannins)	UV-Spectrometry Vanillin HCL reagent	Parameters like extraction time, temperature, vanillin and HCL concentration need to be strictly controlled
	UV-Spectrometry Folin-Denis reagent	Non-specific as they can react with any phenol present in plant tissue
	UV-Spectrometry Prussian blue reagent	Non-specific as they can react with any phenol present in plant tissue, qualitative test

	HPLC	Modest success for smaller compounds of derived tannins
	Colorimetry	Limited to basic compounds of hydrolysable tannins
Saponins	Spectrophotometric method	Not suitable for determination of medicagenic acid for which titrimetric method for the quantitative determination of this aglycone content has to be employed
	Bioassays	
	HPLC	Identification of individual saponins
Trypsin inhibitor	Colorimetric	Does not differentiate between the different protease inhibitors
	ELISA method using monoclonal antibodies derived from mice	
Flavonoids	HPLC	Sample hydrolysis required for optimum resolution and quantisation of quercetin, kaempferol, myricetin, luteolin and apigenin. Separate extraction without hydrolysis required for analysis of anthocyanidins and flavan-3-ols
	LC-MS	Hydrolysis not required as long as masses of individual flavonoid conjugates differ by more than mass resolution of mass spectrometer
Isoflavones[1] and coumestrol	HPLC	Complex conjugates, and their numbers may be difficult to resolve with some reversed-phase columns and simple mobile-phase programs (isocratic)
	LC-MS	Hydrolysis not required as long as masses of individual conjugates differ by more than mass resolution of mass spectrometer
Lignans	HPLC	Isolariciresinol, pinoresinol, secoisolariciresinol and matairesinol
	GLC-MS	Only for matairesinol, secoisolariciresinol and shonanin in foods as trimethylsylyl derivatives

[1] Isoflavones are a subclass of flavonoids but because they have different and unique biological activities than other subclasses of flavonoids, they are analysed and compiled as a separate group