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Technological Interventions in the Physicochemical Analysis of Food Products: Validation of Nutraceutical Content

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ABSTRACT: Physicochemical characteristics are important in various sectors, including pharmaceuticals, biotechnology, and food industry. It has an impact on many phases of food processing (acceptance, operation, packing, and transportation), as well as the food's final safety and quality. Food nutraceuticals are taken as a reference to understand the currently used procedures for assessing their molecules like proteins, vitamins, carbohydrates, fatty acids, triacylglycerols etc. by using modern techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), flame ionization detector (FID), supercritical fluid chromatography (SFC-UV) etc. Modern technical advancements in analytical equipment and devices used to assess the physicochemical analysis of the material, as well as their benefits over conventional approaches connected with physicochemical analysis and testing facilities across the world. This article discusses different types of physicochemical methods used for the analysis of nutraceutical food content.

Keywords: Nutraceuticals, analytical techniques, HPLC, GC-MS

INTRODUCTION

To ensure food and nutrition security of more than 1.3 billion people in India is a daunting task. To feed such population, requires to increased production of fish, fruits, grains, meat, milk, oil seeds, poultry, pulses, vegetables etc. Analysis of food is equally important to deal with the increased production as both animal and plant products are exposed to spoilage through biochemical changes, decay, and fermentation by microorganisms, and destruction by pests (Surwase, 2014). Consumer concern about food composition and the safety of the food they consume has fueled the development and implementation of analytical methods in food science. Food experts throughout the world are confronting difficult issues that will necessitate the use of the finest available science and technology in order to respond appropriately to rising consumer demand. Many products involve a variety of processed ingredients that are frequently delivered from different regions of the world and shared similar storage and manufacturing facilities. As a result, ensuring the safety, quality, and traceability of food items has never been more difficult or important than today (Garcia-Canas, 2012). Fruits and vegetables are in great demand because they are high in critical dietary micronutrients and dietary fiber. Furthermore, they have become a significant source of phytochemicals that may help with a variety of lifestyle-related disorders such as cancer, cardiovascular disease, diabetes, and others (Perumpuli *et al*., 2018).

In 1989, M.D. Stephen DeFelice has developed the term "nutraceutical" perhaps a combination of words nutrition and pharmaceuticals. The terms "medically and nutritionally functional foods" are synonymous to nutraceuticals (Télessy, 2018). These biologically active components play significant role in combating several diseases like cardiovascular, Alzheimer's, arthritis, osteomalacia, obesity, neurological, and cancer (Fig. 1) (Sachdeva *et al*., 2020). Nutraceuticals are can be derived from plant, animals, microorganisms (Chauhan *et al*, 2013) or it can be classified into traditional and non-traditional foods as in developed food product, enriched or fortified food (Galanakis, 2019). Bioactive compounds present in huge amount in nutraceuticals. They offer a lot of health benefits beyond the basic nutritional value these bioactive compounds are proteins, specialty carbohydrates, fatty acids, vitamins etc. However, the acquisition of active constituents from natural sources needs detailed blueprint so as to have uninterrupted supply irrespective of climatic conditions. This needs standard operation protocol that involves analytical techniques for identification and quantification of the bioactive compounds. The aim of the present work is review the current analytical tools being used identification and quantitation nutraceutical food.

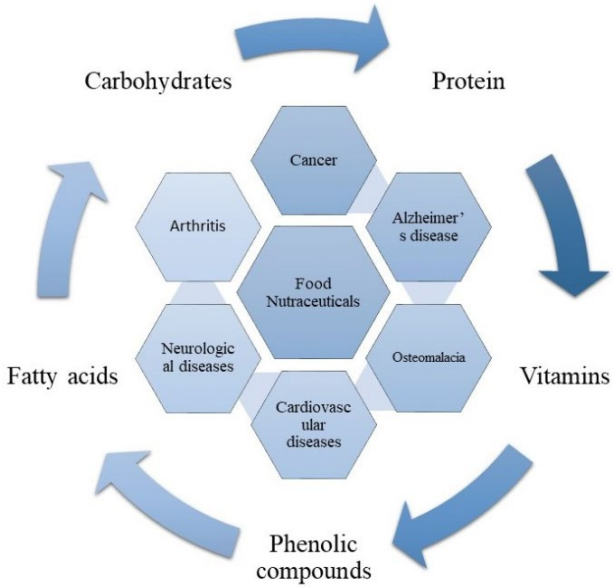

Fig. 1. Application of food nutraceuticals.

Fatty Acids: Fatty acids (FA) are long aliphatic chain of carboxylic acid which is either saturated (single bond) or unsaturated having one or more double bonds (Wynn, 2011). They are the building blocks or skeleton of lipids and also are the main components of fats and oils. Essential fatty acids (EFA), namely α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and linoleic acid (LA) cannot be synthesized by human body, hence needs to be supplemented with diet (Lands, 2015) as they represent 15-30% of the dry weight of the human brain (Hallahan & Garland, 2005). Biological activity of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in humans has been linked with lowering of cholesterol related oxidative stress (Karmally, 2005), prevention of colon and breast cancer (Tapiero *et al*., 2002; Giordano *et al*., 2020), prevention of coronary diseases due to its ability to reduce low-density lipoproteins cholesterol (Pérez-Martínez *et al*., 2017; Sokoła-Wysoczańska *et al*., 2018) and reported to trigger immune system in response to human inflammatory disorders (Ungaro *et al*., 2017). Also, the dietary preference and practice (such as vegetarian, vegan) enhances the risk of deficiency of EFAs (Davis & Kris-Etherton, 2003; Rosell *et al*., 2006). Nutraceuticals can play an important role in bridging the gap between requirement and preference. Estimation of FA using thin layer chromatography (TLC), Gas chromatography (GC) and High-performance liquid chromatography (HPLC) coupled with various detectors such as ultra violet (UV), flame ionization detector (FID), fluorescence detecto (FLD), mass spectrometry (MS) have been employed and reported by various research groups so far (Dołowy & Pyka, 2015). Chromatographic techniques reported in last five years for separation, identification and quantification of long chain saturated and unsaturated fatty acids from different food samples are summarized in Table 1.

Conventionally, FA profiling would involve use of single GC column or one dimensional GC (1D-GC) with non-polar capillary columns, but due to low resolution, polar column, namely ionic liquid, polyethylene glycol and cyanopropyl substituted polysiloxanes are preferred for better separation and elution of saturated and unsaturated FA (Zeng *et al*., 2013; Mota *et al*., 2021). However, the problem associated with co-elution of unsaturated and saturated FA still comes up. In newer developments, multidimensional gas chromatography (MDGC) is gaining importance due to better separation, identification and quantification with heightened peak capacity of individual analytes, wherein two GC column of different selectivity are connected and coupled with desired detectors (Dugo *et al*., 2001). When it comes to different column selectivity in MDGC, 1D-GC columns are non-polar, whereas short (<2 m long) polar microbore capillary columns are used in two dimensional (2D) phase of the GC x GC system, hence, the problem of co-elution of isovolatile components and peak overlapping in 1D will get resolved in 2D system (Tranchida *et al*., 2013). Likewise, branched fatty acid esters of hydroxy fatty acids (FAHFAs) are new class of endogenous bioactive lipids that are linked with improvement of insulin secretion and glucose tolerance (i.e. possible treatment of diabetes type 2) in mammalian cells (Yore *et al*., 2014). Liberati-Čizmek *et al*. (2019) have analysed different food sources includes fruits and vegetables to quantify FAHFAs using UPLC-MS.

Table 1: Analytical techniques used to quantitate fatty acids.

System	Objective	Mobile phase	Column	Detector	Remarks	Reference
Liquid Chromatography						
UHPLC/HRMS	Cannabis FA profiling	Water: methanol/isopropanol (80:20 v/v) both containing 0.05% acetic acid and 5mM ammonium acetate with gradient flow rate of 0.3 mL/min	C18 column, 1.7 µm, 100 ×2.1 mm	HRMS with heated ESI having negative polarity operation mode	Extraction recovery of FA in the matrix ranged from 80-109%; Low RSD suggested reproducibility of the method	Piovesana *et al*. (2021)
UPLC-ESIMS/MS	Analysis of FA profile of triglycerides in vegetable oils	A: 95% aqueous 10 mM ammonium acetate/5% acetonitrile B: 100% acetonitrile Gradient flow rate of 0.5 mL/min	Bridged ethyl hybrid phenyl column, 1.7 µm, 100 × 2.1 mm	ESI-MS/MS	Vegetable oil and standard mixture were derivatized into FAME ester prior to injection, 93-108% FA profile accuracy and 0.32% imprecision with LOQ of derivatized sample 0.5 pg oncolumn	Nagumalli, Jacob, & Gamboa da Costa (2020)
UPLC-MS/MS	Analysis of FAHFA in fruits and vegetables	Methanol/ water in 93:7 v/v with isocratic flow rate of 0.7 mL/min	C18 column, 3.5 µm, 75 µm × 150 mm	MS based MRM assay with negative ESI mode	Extraction recovery for tested concentrations of FAHFA ranged from 93-106% with RSD <15%	Liberati-Čizmek *et al*. (2019)
HPLC-ESI-QToF	Analysis of FA and acylglycerols in olive oil and milk samples	A: Methanol/water in 85:15 B: iso-propanol Gradient flow rate of 0.3 mL/min	Poroshell C18 column (3×5 mm, 2.7 µm) with C18 guard column (4.6×12.5 mm, 5 µm)	MS/MS with both negative and positive ESI mode	Samples were derivatized with 2hydrazinoquinoline, LOD of derivatized sample: 0.2- 1.9 ng/g, short chromatographic run: 40 min	La Nasa *et al*., (2018)
UPLC/MS	Determination of unsaturated FA in Olive oil	Acetonitrile/water with gradient flow rate of 1.5 mL/min	C18 column, 1.9 µm, 50 × 2.1 mm	MS with negative ESI mode	Recovery of FA 89% with LOD: 0.09 to 0.24 µg/mL; LOQ: 0.29 to 0.71 µg/mL	Wabaidur *et al*. (2016)
Supercritical Fluid Chromatography						
SFC-UV; SFC-APCI-HRMS	Identification of lipid derivative in *Kniphofia uvaria*	CO_2 /methanol in 9:1 (v/v) with flow rate of 1.0 -1.5 mL/min	Six Kinetex C18 column (150 × 4.6 mm, 2.7 µm) and one Accucore C18 (150 × 4.6 mm, 2.6 µm) column were connected in series was used for better separation	UV; APCI-HRMS	53 compounds were identified and most of them were TAGs and diacylglycerols and FA This method requires 10% methanol, thereby reducing the consumption of toxic organic solvents in chromatographic studies	Duval *et al*. (2016)
SFC-ESI-MS	Determination of FFAs in edible oils	Organic modifiers (methanol, acetonitrile with formic acid) was used alongside CO_2 for better peak separation with constant flow rate of 1.6 mL/min	HSS C18 SB column (3.0 × 100 mm i.d., 1.8 µm)	MS with negative ESI mode	Satisfactory statistical indices: R^2 >0.994, RSD <13.5% (intraday) and <15% (interday)	Qu, Du, & Zhang (2015)
One dimension Gas Chromatography (1DGC)						
GC-FID	FA profiling of Naga King	Nitrogen	BPX70 capillary column (30 m × 0.32	FID	Sample derivatization: FAME	Ananthan *et al*., (2018)

	chilli		mm i.d.)			
GC-FID	Determination of FA in bovine colostrum	Helium	DB-FFAP Polar column (30 m × 0.53 mm i.d., 0.5 µm film thickness)	FID	Sample derivatization: FAME using acidic catalyst boron trifluoride LOD: 0.11-0.68 ppm; LOQ: 0.37-2.27 ppm	Yurchenko *et al*., (2016)
Two dimensional Gas Chromatography (2DGC)						
GC×GC- MS	FA profiling of Palm oil using 2D GC	Helium	1D: Polar Stabilwax® PEG column (30 m × 0.25 mm i.d., 0.25 µm film thickness); 2D: Non-polar Rxi®-5Sil MS column (0.79 m × 0.25 mm i.d., 0.25 µm film thickness)	ToF-MS	Palm oil resulted in twice FAME when separated using 2D GC unlike 1D GC-MS/FID	Kamatou & Viljoen (2017)
GC×GC-FID	FA profiling of fats and oil	Hydrogen	Combination of various ionic liquid columns 1D: (30 m × 0.25 mm i.d., 0.20 µm film thickness); 2D: (0.825 m × 0.1 mm i.d., 0.08 µm film thickness)		Inert IL columns were combined with conventional IL columns (varying polarity) in 2D GC setup with a solidstate temperature modulator resulted in rapid profiling of FAME with encouraging reproducibility	Pojjanapornpun *et al*. (2018)
GC×GC- MS	FA profiling of vegetable oils	Helium	1D: a polar 100% dimethyl polysiloxane (50 m × 0.2 mm i.d., 0.5 µm film thickness); 2D: moderately polar 50% phenyl-polysilphenylene siloxane (1.45 m × 0.25 mm i.d., 0.15 µm film thickness) FID	ToF-MS	Magnetic dispersive extraction was used to extract FFAs from the selected vegetable oils Recovery of FFA: 81107%; LOD: 5.6 to 25.8 ng/g	Zhu *et al*. (2019)

APCI: atmospheric pressure chemical ionization; ESI: electrospray ionization; FA: fatty acid; FAHFA: fatty acid esters of hydroxy fatty acids; FAME: fatty acid methyl ester; FFA: free fatty acid; FID: flame-ionization detection GC: gas chromatography; HPLC: high performance liquid chromatography; HRMS: high resolution mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MRM: multiple reaction monitoring; MS: mass spectrometry; QToF: quadrupole time of flight; RSD: relative standard deviation; SFC: supercritical fluid chromatography; TAGs: triacylglycerols; ToF: time of flight; UHPLC: ultra-high performance liquid chromatography; UPLC: ultra-performance liquid chromatography; UV: ultra violet.

Triacylglycerols: Edible oils are complex mixture of triacylglycerols (TAGs), mono- and di-acylglycerols, free fatty acids, phospholipids, tocopherols, tocotrienols, sterols, resins, pigments and other minor volatile compounds, and among these TAGs represent 95-98% of total vegetable oil composition (Indelicato *et al*., 2017). The minor volatile components that are supposedly responsible for stability, aroma, and bioactivity of the oil are comprised of 2-5% of total vegetable oil composition (Karuna & Prasad, 2015). These minor components are also used as target compound to ascertain adulteration and erosion (Alberdi-Cedeño *et al*., 2019).

The physico-chemical properties such as molar mass or total carbon number, degree of saturation, position and shape of double bonds in individual FA chains, and biological properties of vegetable oil depend on the composition of FAs and its position in the glycerol backbone (Himawan *et al*., 2006; Sato & Ueno, 2011). Identification of TAGs is comparatively complex as opposed to FA profiling because more than one TAGs exhibit similar physico-chemical properties that results in several isomers (Ruiz-Samblás *et al*., 2012). Mainly two techniques commonly used for identification of TAGs in vegetable oils are high temperature GC (HTGC) and HPLC (Ruiz-Samblás *et al*., 2012; Syed Idrus *et al*., 2017; Jialin Du *et al*., 2020). Identification of TAGs *Schisandrae chinesis fructus* oil by using LC-MS, FTICR-MS with APCI source. However, separation and identification of TAGs in Olive oil has been detect by Heart-cut MDGC-MS (Waktola *et al*., 2020).

Table 2: Analytical techniques used to quantitate triacylglycerides (TAGs).

System	Objective	Mobile phase	Column	Detector	Remarks	Reference
Heart-cut MDGC-MS	Separation and identification of TAGs in Olive oil	Helium	1D: non-polar SLB-5ms (15 m × 0.25 mm i.d., 0.25 µm film thickness) 2D: mid-polar Rtx-65 (9 m × 0.25 mm, 0.1 µm film thickness)	MS full scan mode with ESI	TAGs was resolved based on distinct mass fragmentations and compared with mass spectra of the standard TGAs; Retention time shift: <0.02% Area response: <8% RSD	Waktola *et al*., (2020)
HTGC-FID	Estimation of TAGs in rapeseed and olive oil	Hydrogen	RTX-65TG (30 m × 0.25 mm i.d., 0.1 µm film thickness)	FID	Oven temperature was raised up to 360 ºC	Qian *et al*., (2020)
LCMS/FTICR-MS	Identification of TAGs *Schisandrae chinesis fructus* oil	Acetonitrile: isopropanol (50:50 v/v) with 5mM ammonium formate and 1% (v/v) formic acid with flow rate of 1.5 mL/min	XB-C18 column (2.1 × 150 mm i.d., 1.8 µm)	LC-MS, FTICR-MS with APCI source	The FA profiling was done using GC- MS and based on the obtained profile, LC-MS followed by FTICR-MS was used to identify seven different TAGs in the oil	Jialin Du *et al*., (2020)
LCAPCI-MS	Separation of TAGs in edible oils	Toluene-isopropanaol- formic acid	Porous graphite carbon column (150 mm × 2.1 mm, 5.1 µm)	APCI-MS with positiveion acquisition mode	For TGA standards LOD: 0.13 to 1.38 µg/mL and LOQ: 0.43 to 4.36 µg/mL Repeatability (RSD): Retention time: 0.071% (intra-day); 0.091% (interday); Peak area: 1.3% (intra-day); 2.8% (interday)	Can *et al*., (2018)
HPLC-MS/MS	Determination of TGAs in fat and oils	Acetonitrile with 0.1% formic acid - isopropanol with 10 mM ammonium formate, varying flow rates (0.3 -0.6 mL/min) were tested	C18 core-shell column (2.1 × 100 mm^2 with avg. diameter of 2.6 µm and 1.6 µm inner core of solid silica circumvented by 0.5 µm porous silica shell	APCI MS/MS	TAGs were distinguished using positive-ion APCIMS by assigning protonated molecules $[M+H]^+$ for molar mass and $[M+H-RiCOOH]^+$ for individual FAs 0.4 mL/min was found out to be the efficient	Syed Idrus *et al*. (2017)

APCI: atmospheric pressure chemical ionization; FID: flame-ionization detection; FTICR-MS: Fourier-transform ion cyclotron resonance mass spectrometry; GC: gas chromatography; HPLC: high performance liquid chromatography; LC: Liquid chromatography; LOD: limit of detection; MDGC: multidimensional gas chromatography; MS: mass spectrometry; RSD: relative standard deviation; TAGs: triacylglycerols.

Vitamins: Vitamins are micronutrients, an essential part of balanced diet in human nutrition, and classified based on their solubility i.e. water soluble (B-complex and C) and fat soluble (A, D, E and K) (Eggersdorfer *et al*., 2012). Human body can endogenously synthesize these essential organic compounds either completely or partially, and hence needs to be supplemented with diet as a whole or as precursor so as to stimulate its *in vivo* synthesis (Baj & Sieniawska, 2017). The health benefits of vitamins are known to humankind since antiquity, but its indispensable function in human health has garnered attention in recent past. Disease due to vitamin deficiency has led to consumption of vitamin as supplements, and at the same time toxicity upon overdose of the same has raised serious concern over its wise application and management (Kaur *et al*., 2015).

The basis of this study was to quantify the vitamers in different food samples so as to decipher its nutritional value because human body cannot synthesize Vit B6, but it can transform from one vitamers to other, and also these vitamers are widely utilized in various metabolic pathways inside the body. Similar studies were conducted by Tarvainen *et al*., (2019) to quantitate Vit K in fermented foods using Vit K vitamers, namely phylloquinone and menaquinone as internal standards using UHPLC-APCI-MS/MS. These analytical techniques for screening, quantification and confirmation of the target compounds in the extracted samples. Liquid chromatographic techniques coupled with MS detectors has become most preferred tool for analysis of vitamins in recent past. Moreover, vitamin D_3 has been detected in milk by HPLC-UV (two dimensional) detector at 265 nm (Sereshti, Toloutehrani, & Nodeh, 2020) while, determination of vitamin D_3 in milk sample detected by HPLC-PDA which is a three dimensional detector at 265 nm (Sazali *et al*, 2019). Likewise, studies involving analysis of vitamins in different food matrices are summarized in Table 3.

Table 3: Analytical techniques used to quantitate Vitamins.

System	Objective	Pre-treatment	Mobile phase	Column	Detector	Remarks	Reference
LCAPCIMS/MS	Analysis of Vit K_1 in fruits and vegetables	Accelerated solvent extraction followed by solid phase extraction	Water, methanol and 0.1% formic acid with gradient flow rate of 0.3 mL/min	Pentafluorophenyl core shell sillica column (100 × 2.1 mm, 2.6 µm particle size)	APCI-MS/MS	LOQ: 0.05 µg/100g; Recovery: 90 to 120%	Jäpelt & Jakobsen (2016)
UHPLC-APCIMS/MS	Quantification of Vit K in fermented food	Ultrasound assisted solvent extraction followed by solid phase extraction; Vit K vitamers	Water, methanol, formic acid and ethanol with gradient flow rate of 0.3 mL/min	Solid core C18 column (100 × 2.1 mm, 1.7 µm particle size)	APCI was used in positive ion mode MS/MS measurements were executed in timed MRM mode	LOD: 1.0 to 33.7 pg; LOQ: 3.2 to 112.2 pg; Recovery: 85.9 to 109.8%	Tarvainen, Fabritius, & Yang (2019)
HPLCUV	Quantification of Vit D_2 in milk and yogurt samples	DLLME	Acetonitrile and methanol with isocratic flow rate of 1.2 mL/min	C8 column (250 × 4.6 mm, 5 µm particle size)	UV detector at 265 nm	LOD: 0.9 ng/mL; LLOQ: 2 ng/mL; Recovery: 86.6 to 113.3% and RSD: 1.2 to 11.3%	Ghalebi *et al*., (2019)
HPLCPDA	Determination of Vit D_3 in milk sample	Salting-out assisted LLE	Methanol and water with isocratic flow rate of 0.8 mL/min	Hypersil ODS C18 column (250 × 4.6 mm, 5 µm particle size)	PDA detector at 265 nm	LOD:15 ng/g; LOQ: 25 ng/g; Recovery: 94.4 to 113.5%	Sazali *et al*. (2019)
LC-ESIMS/MS	Quantitation of B_6 vitamers in fruits and vegetables	Mild acid treatment, precipitation, centrifugation, solvent reduction under reduced pressure, reconstitution, membrane filtration (0.22 mm polyvinylidene	Water, methanol and 0.1% formic acid with gradient flow rate of 0.3 mL/min	Pentafluorophenyl propyl column (100 × 2.1 mm, 2.7 µm particle size)	MS with positive ESI mode MS/MS measurements were executed in timed MRM mode	LOD: 0.0028 to 0.02 mg/Kg; LOQ: 0.0085 to 0.059 mg/Kg; Recovery: 92 to 111%	Bachmann *et al*., (2020)

		fluoride); Vit B6 vitamers were used as IS					
HPLCUV	Quantitation of Vit D_3 in bovine milk	Dispersive microsolid phase extraction using magnetic 3D graphene aerogel	Acetonitrile and methanol with isocratic flow rate of 1.0 mL/min	C18 column (250 × 4.6 mm, 5 µm particle size)	UV detector at 264 nm	LOD: 3.01 µg/L; intraday and inter-day RSD: 5.28% and 8.17%	Sereshti *et al*., (2020)
UPLC-MS/MS	Quantitation of multi-class nutirents in food matrices	Solid phase extraction (significant factors affecting the extraction were optimized using DOE tools)	Water, methanol, 20 mM ammonium formate, 0.1% formic acid with gradient flow at 0.30.4 mL/min	BEH C18 column (100 × 2.1 mm, 1.7 µm particle size)	MS with both positive and negative ESI mode MS/MS measurements were executed in timed MRM mode	Linearity range: 251000 ng/g; LOD: 1.29-29.17 ng/g; Recovery: 72.53 - 10.4.24%	Arvapally *et al*., (2021)
HPLC-ICPMS/MS	Quantification of B_{12} in human milk	Immuno-affinity column purification	Water, methanol, 5 mM EDTA disodium salt hydrate, 10 ppb Germanium with isocratic flow at 0.3 mL/min	Silica based RP C18 column (15 × 2.1 mm, 3 µm)	ICP-MS	Variability: 10-17%, Recovery: 80-120%	Dubascoux *et al*., (2021)
Gas chromatography							
GC-MS	Quantitation of four isomers (α-, β-, γ- and δ-) of tocopherol and tocotrienols tocopherols and tocotrienols	Ultrasonic assisted solvent extraction	Helium	DB-5MS apolar sillica capillary column (5% phenyl-95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness)	MS operated in electronic mode and analysis was performed in SIM mode	LOD: 0.3 - 2.5 ng/L; LOQ: 1.0 - 8.3 ng/L, Recovery: 83.7 - 117.2%; RSD: 1.9 - 7.5%	Zhang *et al*. (2016)
GCQTOF-MS	Quantitation of phytosterol and tocopherol in mango	Hot saponification to isolate unsaponifiable fraction	Helium	HP-5MS apolar column (5% phenyl-95% methylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness) RTX-200 MS mid-polar column (crossbond trifluoropropylmethyl polysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness)	QTOF-MS	LOD: 0.5781.304 µg/mL; LOQ: 1.925- 4.342 µg/mL; Recovery: 96.1 - 100.4%	López-Cobo *et al*., (2017)
GC-FID	Quantitation of phytosterols and tocopherol in vegetable oils	Solid phase extraction	Helium	DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness)	FID	LOD: 0.03 - 0.05 mg/100 g, LLOQ: 0.15 - 0.1 mg/100 g; Recovery: 83.4 to 97.7%; RSD: <10.1%	Xu *et al*, (2020)

APCI: atmospheric pressure chemical ionization; FID: flame-ionization detection; DLLME: Dispersive liquid–liquid microextraction; GC: gas chromatography; HPLC: high performance liquid chromatography; ICPMS: Inductively Coupled Plasma Mass Spectrometer; LC: Liquid chromatography; LLOQ: lower limit of quantification; LOD: limit of detection; LOQ: limit of quantification; MDGC: multidimensional gas chromatography; MS: mass spectrometry; QToF: quadrupole time of flight; RSD: relative standard deviation; UHPLC: ultra-high performance liquid chromatography; UPLC: ultra-performance liquid chromatography; UV: ultra violet.

Carbohydrates: Carbohydrates are one of the important nutrients which play a crucial role in human body. Maximum amount of calorie should be consumed from carbohydrate sources to maintain balanced diet. It has several health benefits in terms of chronic diseases like cancer, obesity, cardiovascular disease, diabetes, and GI disorders. Starchy products are known for its dynamic source of energy and contribute a significant role by maintaining digestive health of stomach (Lattimer & Haub, 2010). Non-starchy carbohydrates such as inulin, fructose, and other oligosaccharides improve the release of health stimulating bacteria like *Bifidobacterium* spp., which can overwhelm the activity of harmful microorganisms in the gut and also act as immunomodulators. The utilization of starch and non-starch products has been reported as functional food ingredients which can assist in combating various diseases (Sachdeva *et al*., 2020). It was reported that sulfated galactans can be used as anticoagulant in functional and nutraceutical foods. Microalgal biomass has been continuously fed to animal for their nutritional requirement (Ruocco *et al*., 2016). Marine waste as a potential source of polysaccharides thus, can be used for the extraction of carbohydrate molecules for the formulation of nutraceutical food. According to Vo *et al*., (2015) marine polysaccharides like alginate, porphyran, chitin, and their derivatives have been reported as possible substances which help in preventing numerous allergic reactions by enhancing the immune system, Th1 cells, inhibiting IgE production, and suppressing the activity of foreign agents. Therefore, incorporation of carbohydrates as a source of nutraceuticals can help in various physiological functions and also reduces the chance of metabolic diseases.

Carbohydrates present in nutraceutical food can determined by using various methods such HPLC, Gas chromatography, Nuclear magnetic resonance spectroscopy, GC coupled with mass spectroscopy and high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PED) (Malavaki *et al*., 2008). HPAEC-PED is an old yet important analytical method used for the detection of carbohydrate and further the separation can be facilitated to categorise the components on the basis of their shape, size, and molecular weight. This method has ability to separate carbohydrates into different class such as alditols, monosaccharide, oligosaccharides, and polysaccharides. Anion-exchange chromatography is suggested for the separation of highly acidic or weak acidic components at high pH which can partially transfer hydroxyl group into anionic membrane for the separation of analytes (Corradini *et al*., 2013). The sensitivity of pulsed electrochemical detectors is associated with gold electrodes which work efficiently under highly alkaline conditions (pH >12) for the detection of monosaccharide and oligosaccharides.

Proteins: Biological macro- and micro-molecules are responsible for bioactivity of any food substances, and the list includes proteins, peptides and amino acids as well. Bovine milk is a common example of bioactive proteins, peptides, immunoglobulin, oligosaccharides and lipids that provides several health benefits (Minj & Anand, 2020). Similarly, protein extracted from seaweeds *Undaria pinnatifida* exerting biological functions like antioxidant and anti-hypertension are suitable candidate for healthy non-animal source of protein (Nadeeshani *et al*., 2021). Proteins generally gets denatured under proteolytic conditions and generates short 2-20 amino acids peptides, that have biological activity due to its affinity, efficiency and precision towards tissues (Möller *et al*., 2008). The extraction, isolation and characterization of protein from plants source is quite tedious due to interference of secondary metabolites (Patil *et al*., 2020). Hence, advance analytical methods are being used for extraction and characterization of proteins, peptides and amino acids. Currently various methods have been used for the determination of proteins, amino acid and peptides which includes HPLC-ICPMS, GC-MS and High performance Anion Exchange Chromatography coupled with Pulsed Electrochemical Detection (HAEC-PED) (Maestri *et al*, 2019; Tie *et al*, 2018; Arias-Borrego *et al*., 2020). However, there are not much significant advancement happened in last five years with analytical tools for such purpose.

Some other biological molecules: Sphingolipids belong to complex family of phospholipids where a fatty acid is covalently attached to amino group of the long chain sphingosine containing a variable-polar head group (Sang & Zhu, 2014). One of the prominent sphingolipids of interest in food is gangliosides, which is basically a sialoglycolsphingolipid i.e. sphingolipids with one or more moieties of sialic acid (Fahy *et al*., 2005). Indian indigenous bovine colostrum and mature milk (120-140 days) reportedly are rich in sialic acid (Sharma *et al*., 2019), and its determination using spectrophotometry, HPLC and HPLC-MS has been critically reviewed by Lacomba *et al*. (2010). Yao *et al*. (2016) has quantified sialic acid in red meat using UPLC-FLD-MS with neuraminic acid as internal standard. The developed robust method used C18 reversed phase column and combination of water, acetonitrile and methanol as mobile phase having flow rate of 1 mL/min. The excitation and emission wavelength of fluroscene detector was 373/448 nm. Similarly, a cost efffective and versatile method was developed by Levonis *et al*., (2019) involved HPLC coupled with UV-Vis at 215 nm having C18 column with 50 mM tri-isopropanol amine/water as mobile phase at 0.4 mL/min flow rate. Sialic acids, namely *N*-acetylneuraminic acid and *N*- glycolylneuraminic acid used in this method had recovery of spiked samples ranged from 86.2 - 100% and 81.6 - 101%, respectively.

Another one is phytosterols (triterpene compounds) are either available freely or found in esterified forms; for example, oryzanol is an ester of ferulic acid and sterol that has been reported to have wide range of biological activities that includes antioxidant, lipid lowering effects (decrease in plasma cholesterol and low density lipoproteins), anti-diabetic, reducing coronary heart diseases etc. (Berger *et al*., 2004; MacKay & Jones, 2011; Lai *et al*., 2019).

Hence, physterols are converted to FAs steryl esters or phytosteryl esters to enrich fat-based foods due to its better solubility in fats and oils (Choi *et al*., 2017). GC-MS with selected ion monitoring (SIM) method was developed and validated by Tan *et al*. (2019) for identification and quatification of phytosteryl esters (PE), namely capesteryl oleate, stigmasteryl oleate and β-sitosteryl oleate with statisfactory precision (5.47% RSD) and recovery (89.85 to 97.65%) for each steryl esters. This method involved no spaonification and extraction or derivatization of PE from the selected oils (corn germ oil, rice bran oil and wheat germ oil), and also the target compounds were separated in a duration of 10 min.

Phenolic compounds or polyphenols are the group of small molecules generally distributed in plant tissues as secondary metabolites that are synthesized through shikimic acid, malonic acid, mevalonic acid and methylerythritol pathways (Rosa *et al*., 2019). Polyphenols are based on their chemical structure (number of phenolic rings, and functional derivative attached) are classified as flavonoids and non-flavonoids (phenolic acid, coumarins, lignans, stilbenes, isoflavonoids, phenolic polymers, such as tannins, proanthocyanidins) (Barba *et al*., 2014). Investigation on naturally occurring phenolic compounds has been on rise due to its aroma, colour, astringency and biological activity, such as anti-oxidative, anti-microbial, anti-cancer, anti-diabetic, anti-allergic, anti-thrombotic, immuno-regulatory to name a few (Liu, 2013; Roleira *et al*., 2018; Rashmi & Negi, 2020). Recent studies have begun to emphasize phenylpropanoids, and phenolic acids' potential as efficient antioxidants (Pazoki, 2015). In-depth study on phenolic compounds in various vegetables, fruits, and beverage crops are present in scientific literature (Shan *et al*., 2005; Mattila *et al*., 2006; Karasawa & Mohan, 2018).

From an analytical view point, no significant changes in chromatographic analysis of phenolic compounds from food samples were observed in last five years. However, the alternative approaches following green analytical chemistry in sample preparation has emerged. This includes, sorbent based micro-extraction (miniaturized SPE, dispersive micro SPE, miniaturized matrix solid-phase dispersion, micro-QuEChERS, micro-extraction by packed sorbents, SPME, Stir bar sorptive extraction), silica-based sorbent material (mesostructured silica, magnetic silica based material) and consolidating silica-based sorbent material in micro-extraction (Casado *et al*., 2020). Isolated polyphenols using micro-extraction techniques are quantified by chromatographic techniques, namely liquid chromatography (HPLC, UHPLC) or gas chromatography (GC) coupled with desired detectors (Q-TOF-MS/MS, PDA, UV, Q-MS).

CONCLUSION AND FUTURE SCOPE

The present work has reviewed the analytical techniques currently used to identify and quantify the biological active components in food matrices. The strategies of metabolic profiling mentioned in this scientific report are non-targeted metabolomics. The advent of targeted metabolomics in association with existing analytical techniques and statistical tools (principal component analysis, chemometrics etc.) is indispensable. With emergence of green consumerism, regulatory bodies have also started to consider the regulated use of bioactive components; hence, targeted metabolomics will play a vital role in days to come. The targeted metabolomics will result in acquisition of in depth information on nutraceuticals. This will help identify newer nutraceuticals and also its clinical trials so as to have better knowledge about its fate inside human body.

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