Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-023-05007-w

REVIEW ARTICLE

A narrative review on extraction techniques of phytosterols and their applications in food industry

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Received: 12 July 2023 / Revised: 6 October 2023 / Accepted: 11 October 2023

Abstract
Phytosterols are natural plant–based bioactive compound shaving various roles in human health that are widely used in the food, nutrition, pharmaceutical, and cosmetics industries. Phytosterol extraction and isolation techniques are difficult and time consuming. The growing demand for phytosterols encourages the development of easy-to-use technologies for extracting and isolating them from various plant sources. Free phytosterols extracted from plants are extensively used in fortified meals and nutritional supplements. Bioactivities of phytosterols have sparked interest in obtaining them from vegetable oils or industrial wastes. The growing demand for these bioactive compounds as a food supplement may spur additional advancements in extraction, isolation, and analytical processes that are more efficient, quick, and environmentally friendly. This review summarizes both conventional and non-conventional extraction techniques of phytosterols and their use in the food industry. Supercritical fluid extraction is the emerging technique to extract phytosterols due to its efficiency; however, further experiments are needed to create optimal working conditions and instruments.

Keywords Phytosterols · Extraction · Pharmaceutical · Human health · Functional food · Nutritional supplements

1 Introduction

Plant sterols or phytosterols are naturally occurring bioactive compounds that belong to the triterpene family. Every plant species has its own phytosterol composition, with over 250 phytosterols identified so far. All plant cell membranes include phytosterols, which are specifically present in abundance in legumes, nuts, cereals, vegetables, fruits, vegetable oils, and fats. Phytosterols can be found in a variety of plant sources, including oil seeds and herbaceous plants, as well as the waste from industrial softwood and hardwood processing. Phytostanols make up 10 to 15% of these phytosterols [1]. Phytosterols are classified into plant sterols and stanols, which are bioactive compounds found in foods of plant origin. Campesterol, sitosterol, and stigmasterol are commonly present in the diet, while the most common type of sterols are β-sitosterol [2]. Phytosterols are particularly found in oil seeds such as maize, soybean, and rapeseed oil [3].

Consumer interest in healthy and helpful meals has recently increased [4, 5]. Bioactive compounds can be added to foods and beverages to help achieve this goal [6–8]. Phytosterols have attracted a lot of interest among bioactive compounds because of their health benefits in terms of decreasing blood cholesterol and the ratio of high-density lipoprotein (HDL) to low-density lipoprotein (LDL) bound cholesterol in serum by reducing the intestinal cholesterol absorption. Phytosterol-enriched diets may stop the tumor growth and minimize the risk of cancer by 20% [9]. Phytosterols have many pharmacological benefits, acting as antioxidant, anti-inflammatory, antidiabetic, chemopreventive, and antiatherosclerotic agents [10, 11].

Vegetable oils [12] and resin oils are currently the two most prevalent raw materials for phytosterols extraction, which is the most common and commercially used approach.

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Published online: 26 October 2023

Glycophyte oilseeds such as sunflower, soybean, and rapeseed have been used to make crude vegetable oils [13]. The extraction and analysis of phytosterols is a complicated process that has yet to be thoroughly established. Phytosterols have an important role in the food, medicine, and cosmetics industries. Free phytosterols isolated from plant sources are commonly used in fortified meals and dietary supplements; most of the organic solvents used for the extraction of phytosterols from plant matrices are hazardous to human health and the environment [14–16]. Phytosterols are sparingly soluble in fats and oil, insoluble in water, and have high melting point. This limits their application in food in free form, and their bioavailability can be enhanced by esterification [17]. The type of the matrix, its physical condition (solid or liquid), and its chemical form all influence phytosterol extraction from food (free, esterified, or glycosylated) [18]. The main dietary sources of phytosterols are vegetable oils, cereals, and legumes. As a result, many phytosterol-containing functional foods (i.e., phytosterols-enriched diets) and supplements have emerged [19, 20].

2 Extraction of phytosterols by various techniques

Various extraction techniques are used to separate plant metabolites that are soluble in a solvent. The composition of the matrix and the shape of phytosterols affect phytosterol isolation procedures [21]. Figure 1 depicts the procedure for extracting, analyzing, and quantifying phytosterols.

2.1 Conventional methods of phytosterol extraction

Different conventional techniques such as soxhlet extraction, cold press, maceration, screw pressing, and some other techniques involving multistage extraction processes are used for extraction of phytosterols. Among these, the most commonly used conventional techniques of phytosterols extraction include soxhlet and cold press extraction methods [22]. Figure 2 shows the conventional techniques for extraction of phytosterols from different plant matrices.

2.1.1 Soxhlet extraction

Jafarian et al. (2020) used a modified soxhlet method for phytosterol extraction from deodorizer distillate of rapeseed oil (RODD). Silica gel was added to a mixture of ethanol and RODD sample. In the extraction chamber of the soxhlet apparatus, silica gel absorbed the polar part of the distillate which was extracted by ethyl ether. An increase in extraction temperature from 40 to 60 °C results in an increase in phytosterols concentration from 5% (wt) to 10% (wt). However, a further increase in temperature to 80 °C decreased the phytosterol concentration to 4% (wt), which is due to thermal degradation of phytosterols at higher temperatures.

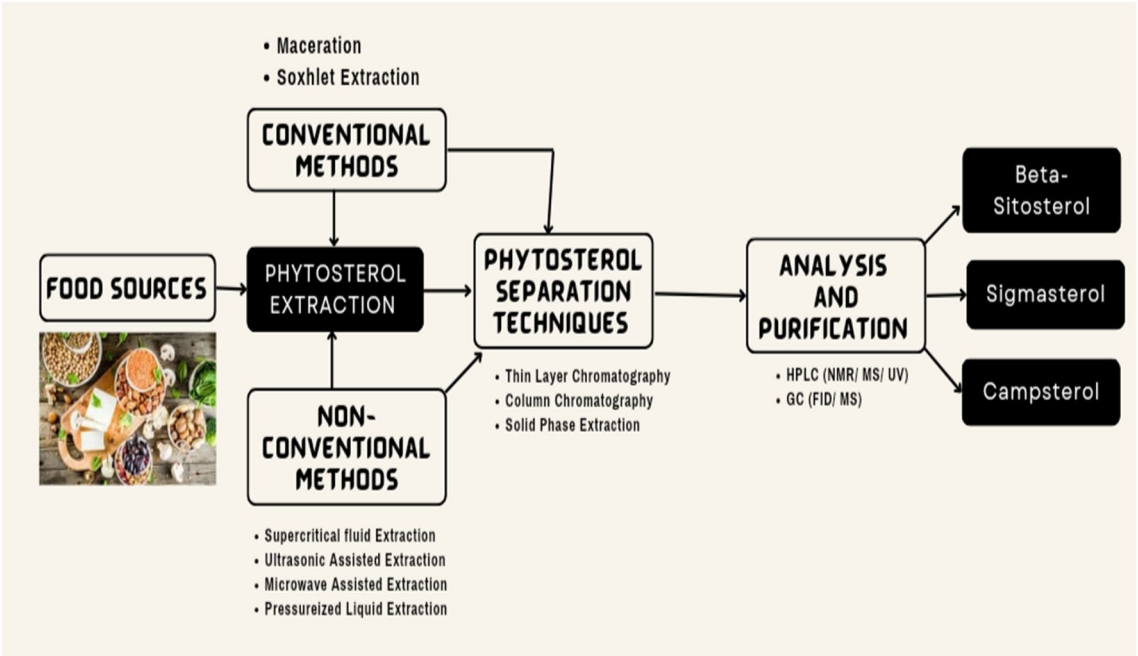

Fig. 1 Extraction, analysis, and quantification of phytosterols

Garcia-Gonzalez et al. (2021) concluded that soxhlet extraction (using hexane as solvent) results in higher phytosterols extraction as compared to the cold press technique. The phytosterols were extracted from sunflower seeds, and their composition was found to be similar for both the methods but solvent extraction resulted in higher phytosterols content (4849–9249 mg/kg) as compared to cold press method (2839–5284 mg/kg).

Poulose et al. [25] extracted phytosterols from *Gelidium spinosum* (a seaweed) through soxhlet extraction. GC–MS analysis revealed the presence of stigmasterol as the main phytosterol with a mass of 412.69 g/mol in seaweed. They further nano-emulsified the stigmasterol and incorporate it into biscuits. The product was acceptable with good sensory and quality characteristics. Moreover, Jaski et al. [26] extracted sunflower oil and olive leaf extract simultaneously to incorporate the active compounds (extracted from olive leaf extract) in sunflower oil. Soxhlet extraction (with hexane) and pressurized propane extraction were used. For soxhlet extraction, the extract was obtained with the sunflower grains in the bottom part and olive leaves in the upper part of the filter paper envelope. With both methods, it was possible to improve the bioactive compounds in sunflower oil. The main phytosterol extracted was β-sitosterol. With the conventional soxhlet method, β-sitosterol content was enhanced by 69% and with pressurized propane extraction; its content was improved by 91%. Zeng et al. [27] have observed a total phytosterol content of 327.76 mg/100 g oil in flaxseed oil extracted by solvent extraction technique using n-hexane. As different extracting solvents are used in soxhlet extraction, the yield and composition of extract vary. Extraction using soxhlet technique is time-consuming and requires significant amounts of organic solvents which are harmful to human health and the environment.

2.1.2 Cold press extraction

According to Bozdoğan et al. [28], cold-pressed oils are preferable to refined oils because of their naturally occurring distinctive flavor and modest bioactive components such as natural antioxidants. Many authors compared the cold press technique with different extraction techniques and found the cold press technique more beneficial. Naebi et al. [29] studied the effect of moisture content in seeds of *Lallemantia peltata* (Balangu) on oil quality. The highest oil yield was obtained at 7.5% moisture level. An increase in acid value, peroxide value, and tocopherol content was observed as the moisture content increased in raw seeds. However, phytosterol and fatty acid contents remained unchanged. Balangu oil contains three phytosterols, namely, stigmasterol, present in highest proportion (130.84 µg/g), campesterol (57.78 µg/g), and stigmasterol (10.6 µg/g).

Zeng et al. [27] found a phytosterol content of 288.81 mg/100 g oil in flaxseed oil extracted through a cold-pressing technique. β-sitosterol (111.11 mg/100 g oil) is the major phytosterol in flaxseed oil followed by campesterol (66.64 mg/100 g oil), Δ5-avenasterol (39.41 mg/100 g oil), cycloartenol (36.19 mg/100 g oil), stigmasterol (21.56 mg/100 g oil), and 2,4-methylene cycloartenol (13.89 mg/100 g oil). Mingyai et al. [30] observed total phytosterol content in rice bran oil. The comparison of different extraction methods shows that cold press extraction was more efficient in retaining more phytosterols (9.93 to 11.43 mg/g RBO) than solvent (hexane) (9.99 to 10.51 mg/g RBO) and supercritical extraction (8.78 to 11.21 mg/g RBO). Both the solvent extraction and supercritical extraction provided more yield but phytochemical retention was greater in the cold-press method. With the cold-press method, the proportion of β-sitosterol content was highest among other

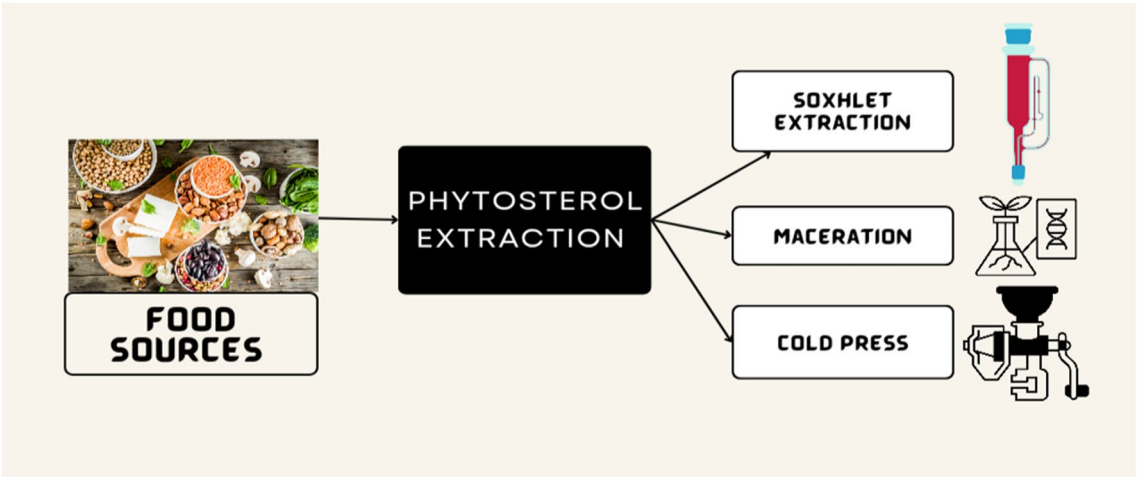

Fig. 2 Conventional methods of phytosterols extraction

types of phytosterols in rice bran oil and is in the range of 5.46 to 5.86 mg/g RBO, followed by campesterol (2.57 to 3.52 mg/g RBO) and stigmasterol (1.81 to 2.04 mg/g RBO).

Zhang et al. [31] observed 11,800 mg/kg of total phytosterol content in oil extracted from flax seeds with cold press. Out of six phytosterols identified, β-sitosteorol (5350 mg/kg) was present in the highest quantity followed by cycloartenol (3410 mg/kg), Δ5-Avenasterol (690 mg/kg), 2,4-methylenecycloartenol (440 mg/kg), and stigmasterol (145 mg/kg). In a study conducted by Cheng et al. [32], it was observed that flaxseed oil extracted by cold press provide 547 mg/100 g oil of total phytosterols, while a phytosterols content of 515.24 mg/100 g oil was obtained by applying microwave pre-treatment at a frequency of 2450 MHz for 7 min prior to extraction by screw oil expeller. On the other hand, Aguirre et al. [33] observed 37–38% higher content of total sterols in sunflower oil extracted with solvent extraction as compared to pressed oils. In this study, oil extraction was done with the pressed method followed by subsequent extraction with the soxhlet extraction method using hexane at 60 °C for 4 h. The pressing method provided 4757 to 9956 mg/kg total sterols in sunflower oil, while solvent extraction provides 6520 to 13,703 mg/kg total sterols. Sterol composition analysis revealed β-sitosterol to be the major sterol present in sunflower oil from 67.7 to 68.9%. Other sterols like campesterol, stigmasterol, Δ5-Avenasterol, Δ7-Stigmastenol, and Δ7-Avenasterol were also observed and their concentration varies for both methods. Sangpradab et al. [34] found that subjecting rice bran to ohmic heating (at 70 V/cm, 90 °C for 3 min) before mechanical extraction (in a screw type expeller) improved the total phytosterol content in extracted rice bran oil from 3568.02 to 4415.27 mg/kg.β-sitosterol, campesterol, and stigmasterol content in rice bran oil was 2420, 795, and 355 mg/kg, respectively, which was enhanced to 2980, 955, and 480 mg/kg, respectively, in ohmic assisted-mechanically extracted rice bran oil.

Ozcan et al. [35] extracted *M. peregrina* and *M. oleifera* oils through soxhlet and cold press extraction. According to this study, cold press method was recommended because it provides more quantity of sterols as compared to soxhlet extraction. The most abundant sterols in moringa oils were stigmasterol, campesterol, β-sitosterol, and δ5-avenasterols with β-sitosterol present in highest quantity in both types of oils. Stigmasterol contents of *M. peregrina* and *M. oleifera* oils obtained with soxhlet extraction were found as 18.54% and 15.41%, whereas cold press extraction systems were found as 19.62% and 17.84%, respectively. Similarly, β-sitosterol contents of *M. peregrina* and *M. oleifera* oils extracted with cold press were found as 46.81% and 48.56%; β-sitosterol contents of *M. peregrina* and *M. oleifera* oils obtained by soxhlet extraction system were determined as 45.84% and 47.56, respectively. Other phytosterols present in minor quantities in both oils include 24-methylene cholesterol, stigmastanol, campestanol, ergostadienol, clerosterol, δ7-avenasterol, δ7-campestanol, 28-isoavenasterol, and δ7,14-stigmastanol. Cold press technique is more environment-friendly since no hazardous compounds are used. However, the yield of extract is not high and composition of extract vary.

2.1.3 Other conventional methods of extraction

Jaski et al. [26] applied a multistage process comprising of solid–liquid extraction, saponification, liquid–liquid extraction, crystallization, and vacuum filtration steps to obtain phytosterols. Phytosterols were extracted from a solid residue obtained during the extraction of vitamin E from palm fatty acid distillate (PFAD).The total sterols (84%) were recovered with stigmasterol forming the major part (59–64%) followed by β-sitosterol (21–22%) and campesterol (13–20%). Fithriani et al. [36] used maceration technique to extract phytosterols from *Nannochloropsis oculata* (a microalgae) with varying the concentrations of ethanol solution (containing 7.5% KOH in ethanol) and extraction time. With 70% ethanol, the level of phytosterols extracted was 0.4%, which was increased to 1.63% with 90% ethanol. Moreover, an extraction time of 1 h yielded maximum phytosterol content. Marques et al. [37] determined the phytosterol content in baru seed oil extracted by screw press and hydraulic press methods. β-sitosterol was found to be the major compound in oil followed by stigmasterol for both extraction techniques. Conventional techniques are the oldest methods used for extraction. However, these techniques have some limitations as these are time-consuming and are not efficient enough. Table 1 explain about the extraction methods of phytosterols using conventional techniques.

2.2 Non-conventional ways of phytosterol extraction

Non-conventional techniques include super critical fluid extraction, enzymatic extraction, solid phase extraction, direct hydrolysis extraction, microwave-assisted hydrodistillation, silica gel adsorption, and ultrasound-assisted extraction. Among these non-conventional techniques, SC-CO_2 extraction method has been commonly used for phytosterols extraction [22]. Figure 3 presents various non-conventional methods for phytosterols extraction from different plant matrices.

2.2.1 Super critical fluid extraction

By changing the extraction parameters, the quantity of phytochemicals extracted varies. Many authors explained optimum conditions for extraction of phytosterols through

Table 1 Extraction methods of phytosterols using conventional techniques

Extraction methods	Sources	Solvent	Phytosterols detected	References
Soxhlet extraction	Rapeseed oil deodorizer distillate	Ethanol and silica gel	Total phytosterols (10%)	[23]
	Sunflower seeds	Hexane	Total phytosterols (4849–9249 mg/kg)	[24]
	M. oleifera oil	Petroleum ether	β-sitosterol (47.56%), stigmasterol (18.54%), campesterol (17.43%), δ5-avenasterols (10.38%)	[35]
	M. peregrina oil		β-sitosterol (45.84%), stigmasterol (15.41%), campesterol (13.21%), δ5-avenasterols (8.83%)	
	Gelidium spinosum	n-Hexane	Stigmasterol	[25]
	Olive leaves	n-Hexane	Phytosterols	[26]
	Flaxseed oil	n-hexane	Total phytosterols (327.76 mg/100 g oil)	[27]
Cold press extraction	*M. oleifera* oil	-	β-sitosterol (48.56%), stigmasterol (19.62%), campesterol (18.21%), δ5-avenasterols (12.74%)	[35]
	M. peregrina oil		β-sitosterol (46.81%), stigmasterol (17.84%), campesterol (15.57%), δ5-avenasterols (9.71%)	
	Blackcurrant, raspberry, chokeberry, and strawberry seed oils	-	Rassicasterol, campesterol, campestanol, stigmasterol, and β-sitosterol	[38]
	Lallemantia peltata (Balangu) seeds	-	β-sitosterol (130.84 µg/g), stigmasterol (10.6 µg/g), campesterol (57.78 µg/g)	[29]
	Flaxseed oil	-	Total phytosterols (288.81 mg/100 g oil), β-sitosterol (111.11 mg/100 g oil), campesterol (66.64 mg/100 g oil), Δ5-avenasterol (39.41 mg/100 g oil), cycloartenol (36.19 mg/100 g oil), stigmasterol (21.56 mg/100 g oil), 2,4-methylene cycloartenol (13.89 mg/100 g oil)	[27]
	Rice bran oils	-	Campesterol (2.57–3.52 mg/g RBO), stigmasterol (1.81–2.04 mg/g RBO), β-sitosterol (5.46–5.86 mg/g RBO), total phytosterols (9.93–11.43 mg/g RBO)	[30]
	Flaxseeds oil	-	β-sitosteorol (5350 mg/kg), cycloartenol (3410 mg/kg), stigmasterol (145 mg/kg), Δ5-avenasterol (690 mg/kg), 2,4-methylenecycloartenol (440 mg/kg), total phytosterols (11,800 mg/kg)	[31]
	Flaxseed oil	-	Total phytosterols (547 mg/100 g oil)	[32]
	Sunflower oils	-	Total sterols (4757 and 9956 mg/kg), β-sitosterol (from 67.7 to 68.9%)	[33]
	Rice bran oil	-	Total phytosterols (3568.02 mg/kg), campesterol (795 mg/kg), β-sitosterol (2420 mg/kg), stigmasterol (355 mg/kg)	[34]
	Sunflower seeds	-	Total phytosterols (2839–5284 mg/kg)	[24]

Table 1 (continued)

Extraction methods	Sources	Solvent	Phytosterols detected	References
Other methods				
Maceration	*Nannochloropsis oculata*	Ethanol	β-sitosterol and stigmasterol	[36]
Screw press and hydraulic press	Dipteryxalata Vogel,		Campesterol, stigmasterol, and β-sitosterol	[37]

supercritical fluid extraction. CO_2 is most commonly used for extraction purpose as it is non-toxic, non-flammable, and does not contaminate the extracts [39]. β-sitosterol content of guava seed oil extracted by SC-CO_2 extraction was (1048.9 ± 48.4 mg/100 g oil) and campesterol was (23.9 ± 1.4 mg/100 g oil), respectively, with β-sitosterol being the dominant phytosterol in guava seed oil. However, stigmasterol or stigmastanol were not present in guava seed oil. In their study, the extraction was done at a temperature of 52 °C, pressure of 35.7 MPa with a flow of 30 g CO_2/min for a period of 150 min, while according to Alvarez-Henao et al. [40], the use of a co-solvent like ethanol can be effective at low pressure along with supercritical CO_2, but has no effect at high pressure. In their study, the phytosterol extraction was done from sugarcane bagasse. Ethanol was used as a co-solvent, which was effective at low pressure but has no effect on phytosterol content at high pressure. The optimum extraction parameters including temperature of 40 °C, 6 mL/min, 400 bar, and 0% ethanol provide maximum phytosterols content. At optimum conditions, the campesterol, stigmasterol, and β-sitosterol contents were 0.063, 0.084, and 0.153 mg/g d.m., respectively, and total phytosterol content was 0.300 mg/g d.m. Asl et al. [41] applied the supercritical extraction to separate phytosterols from rapeseed oil deodorizer distillate (RODD). In this study, the use of a co-solvent (ethanol 5%, wt) with CO_2 showed results in an increase in purity, extraction efficiency, and sterol content in the product. A sterol recovery of 76% and purity

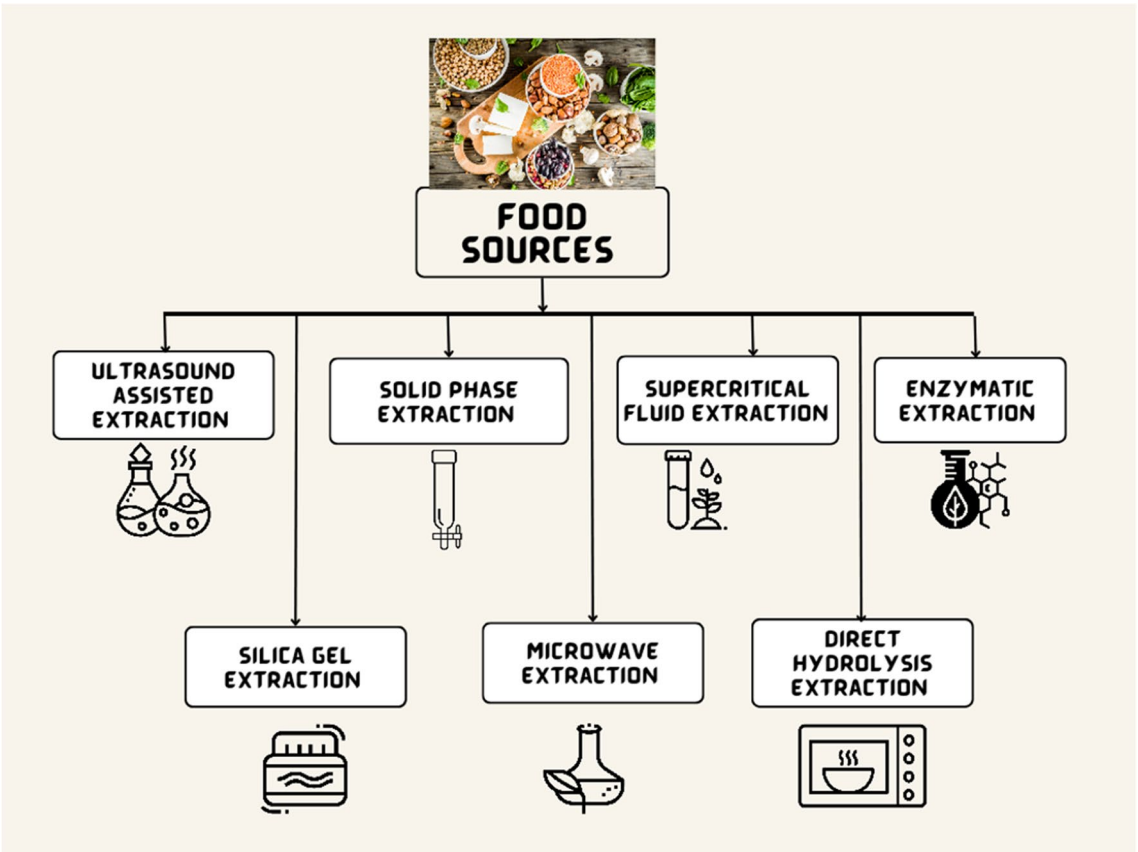

Fig. 3 Non-conventional methods of phytosterols extraction

of 60% were observed from sterols in saponified RODD at 35 MPa, 313 K, and a solvent to feed mass ratio (S/F) of 5. β-sitosterol was present abundantly (50%) followed by campesterol and brassicasterol which accounts for 36.25% and 23.91%, respectively.

Similarly, Nyam et al. [42] concluded that the use of ethanol as entrainer along with SC-CO_2 can improve the solubility and extraction yield of seed oil as compared to using only SC-CO_2 extraction. They recovered the phytosterols from roselle (*Hibiscus sabdariffa* L.) seeds. The total phytosterol content of 7262.80 mg/kg was observed at a low temperature of 40 °C, high pressure of 400 bar, and supercritical fluid flow rate of 20 mL/min in the presence of 2 mL/min ethanol as entrainer. Ekinci et al. [43] determined phytosterols content in melon seeds (*Cucumis melo*) oil extracted by supercritical carbon dioxide (SC-CO_2) extraction. The GC–MS analysis shows stigmasterol and β-sitosterol contents in the range of 121 mg/kg seed and 304 mg/kg seed, respectively, under optimum extraction conditions (temperature of 33 °C, 200 bar pressure, 11 g CO_2/min flow rate, 0.4 mm mean particle size of the seeds, and 3 h time period).

Hrabovski et al. [44] examined phytosterols content in *Cucurbita pepo* convar. citrullina (pumpkin) seed oil extracted by SC-CO_2 at 400 bar and 40 °C. Derivatizing agents were used for determining the phytosterols content and were found effective. The total phytosterol content in pumpkin seed oil was in the range from 190 to 320 mg/100 g obtained by either SC-CO_2 or solvent extraction. Δ7-sterols were present in highest quantities ranging 91.0 to 94.2% of total phytosterols. The total phytosterol content extracted by SC-CO_2 extraction was 294 mg/100 g oil, which was 20% and 30% higher than that obtained by solvents like petroleum ether and hexane, respectively. However, SC-CO_2 extraction technique provides less oil yield (36.17%) as compared to that obtained by hexane (43.37%) and petroleum ether (44.65%). Mass spectrometric results also revealed the presence of campesterol, desmosterol, stigmasterol, spinasterol, avenasterol, etc., present in *citrullina* seed oil.

Santos et al. [45] extracted *Morus alba* leaves by SC-CO_2 extraction. β-sitosterol content varies between 99.19 and 155.74 mg/100 g leaves depending upon the temperature and pressure during extraction. It was observed that with an increase in temperature and pressure, the β-sitosterol content extracted increases. The maximum quantity of β-sitosterol (155.74 mg/100 g leaves) was obtained at a temperature of 60 °C and pressure of 200 bar. Mingyai et al. [30] observed a total phytosterol content from 8.78 to 11.21 mg/g oil by SC-CO_2extraction in rice bran oil. Cheng et al. [32] also observed total phytosterol content of 352 mg/100 g oil in flaxseed oil extracted by SC-CO_2 extraction. In this study, extraction was done at a time, pressure, temperature, and CO_2 flow rate of 1.5 h, 30 MPa, 42 °C, and 18 L/h, respectively. Supercritical fluid extraction is widely used in food analysis as well as in pharmaceutical and polymer applications.

2.2.2 Enzymatic extraction

Zeng et al. [27] observed a total phytosterol content of 347.56 mg/100 g oil in flaxseed oil extracted through aqueous enzymatic extraction. The enzyme alkaline protease (1.5%, w/w) was used for extraction purpose. β-sitosterol (115.39 mg/100 g oil) was abundant phytosterol in flaxseed oil followed by cy-cloartenol, campesterol, Δ5-avenasterol, stigmasterol, and 2,4-methylene cycloartenol, which are present in the proportion of 85.45, 65.80, 40.92, 20.96, and 19.05 mg/100 g oil, respectively. It was also inferred from the results that enzymatic extraction was found to be more efficient and provide more quantity of phytosterols as compared to other methods like solvent extraction (327.76 mg/100 g oil), cold press (288.81 mg/100 g oil), and hot press method (270.10 mg/100 g oil).

Silva et al. [46] extracted sunflower seed oil through enzymatic aqueous extraction (EAE). The optimum conditions for extraction determined in their study were a temperature of 60 °C, 1:5 seed:water ratio (g/g), and 1% (v/v) enzyme concentration. Oil yield was increased up to 20.34% by using buffered medium. EAE provided higher quantity of total phytosterols (from 95.86 to 135.59 mg/100 g) under different concentrations of buffered medium. β-sitosterol, stigmasterol, and campesterol were also present in sunflower oil with β-sitosterol being abundant.

Feng et al. [47] separated the phytosterol glycosides and free phytosterols from rice bran. Ultrasonic-assisted extraction at 585 J/mL, 240 W was used for 1 h to extract phytosterols from rice bran. The extraction solvent used was chloroform–methanol solution in 2:1 (v/v) ratio. Among free phytosterols, β-sitosterol (305 μg/g rice bran) was the most abundantly present followed by cycloartenol, 24-methylenecycloartenol, stigmasterol, campesterol, and ergosterol in the range of 80.5 μg/g, 87.1 μg/g, 106 μg/g, 126 μg/g, and 129 μg/g rice bran, respectively. Similarly, β-sitosterol glycoside (133 ± 7 μg/g rice bran) was the most abundant phytosterol glycoside. Other phytosterol glycosides include campesterol glucoside (16.0 ± 1.3 μg/g rice bran) and stigmasterol glycoside (99.0 μg/g rice bran). Enzymatic extraction is an alternative to conventional solvent extraction methods for extracting biologically active compounds from plants.

2.2.3 Direct hydrolysis extraction

Feng et al. [48] used direct citric acid hydrolysis extraction method to extract phytosterols from hickory husk. A pH of 2.0, liquid to solid ratios of 17.12 °C and 55.81 °C temperature were concluded as the optimum extraction parameters.

Phytosterol quantity extracted was 912.452 µg/g DW. The main phytosterols detected were stigmasterol, β-sitosterol, ergosterol, and campesterol present in hickory husk. The yield of β-sitosterol was 755.03 ± 26.59 µg/g DW in case of direct citric acid hydrolysis extraction as compared to 626.20 ± 42.73 µg/g DW obtained through solvent extraction. Direct hydrolysis extraction is an efficient technique that has shorter extraction time and cost-effective.

2.2.4 Microwave-assisted hydrodistillation

In a study conducted by Noormazlinah et al. [49], microwave-assisted hydrodistillation was used to extract phytosterols from legume pods. Their findings show that by changing the ethanol concentration, the phytosterol content varies. The β-sitosterol quantity in *L. leucocephala*, *A. pauciflorum*, and *P. speciosa* was 0.2191, 0.0313, and 0.1101 mg/mL, respectively. The best conditions for the β-sitosterol extraction from legume pod were irradiation power of 600 W, solvent concentration (ethanol) of 75%, and irradiation temperature of 75 °C for a irradiation period of 6 min. Hu et al. [50] extracted tiger nut oil by microwave-assisted extraction and compared it to solvent extraction. Firstly, they determined the optimized parameters of MAE by response surface methodology, which were petroleum ether and acetone (2:1, v/v) mixture, 420 W power, temperature of 75 °C, 7.0 mL/g liquid to solid ratio, and a irradiation period of 55 min. The phytosterol content was high (658.68 mg/100 g) in oil extracted with MAE under optimized conditions as compared to solvent extraction, which provided 578.71 mg/100 g oil. Similarly, β-sitosterol, stigmasterol, and campesterol contents were 518.26, 70.42, and 25.78 mg/100 g, respectively, for MAE extracted oil. On the other hand, solvent extraction provided 432.19, 55.86, and 23.70 mg/100 g β-sitosterol, stigmasterol, and campesterol contents, respectively, which was lower than those extracted by MAE. Microwave-assisted extraction is a quick extraction technique that uses less organic solvents, and it is cost-effective to extract bioactive compounds from plant.

2.2.5 Ultrasound-assisted extraction

Stevanato and Silva [51] extracted radish seed oil by ultrasound-assisted extraction using ethanol and obtained a higher content of phytosterols (346.18 mg/100 g oil) as compared to 273.47 and 173.03 mg/100 g oil obtained by Soxhlet extraction method using ethanol and hexane, respectively. This can be due to changes in structure of cells due to the bubbles collapse that helps to increase the mass transfer of phytosterols and other compounds in the solvent. Raddish seed oil mainly contains stigmasterol, campesterol, and β-sitosterol at a concentration of 49.19, 81.14, and 215.86 mg/100 g oil, respectively, as obtained by ultrasound assisted extraction. Santos et al. [45] observed a β-sitosterol content of 55.55 to 103.49 mg/100 g M. *alba* leaves extracted by ultrasound-assisted extraction. Ultrasound-assisted extraction is a rapid extraction process that requires less energy and solvent consumption. It provides three times higher efficiency for the extraction of phytosterols. Table 2 explains about the extraction methods of phytosterols using non-conventional techniques.

3 Applications of phytosterols

Phytosterols find many applications such as they can be used for developing nutraceuticals and functional foods, have potential to lower cholesterol level, used as natural antioxidants, and as fat replacers. However, there are some barriers which limit their use like high temperature sensitivity, lipophilicity, limited solubility, and high melting points. They are also prone to oxidation and sparingly absorbed by the human body. This limits their use in food and other applications. Microencapsulation, liposomal formulations, and lipid nanoparticles can be used to overcome these challenges. In a study conducted by Tolve et al. [52], soyabean oil enriched with phytosterols (5% and 10%) was encapsulated using inulin, chitosan, and whey protein isolate as carrier agents. Microcapsules with a size less than 50 µm were produced with excellent encapsulation efficiency (85%), surface oil level (11%), and water activity ranging 0.2–0.4, thus meeting the industrial requirements. However, the microcapsules' oxidation stability was poor as evidenced by an increase in peroxide value to 101.7 meq O_2/kg of oil with an increase in phytosterol concentration even right after production. This can be due to the pro-oxidant activity of phytosterols and high temperature emulsification process.

Poudel et al. [53] developed liposomes incorporated with brassicasterol, campesterol, and β-sitosterol. These phytosterols were extracted from canola waste, and the liposomes prepared were added in a model orange juice as a food additive for increasing the oxidative stability and efficacy of phytosterols. The results indicated that phytosterols (entrapped within liposomes) remained stable to pasteurization temperature (72 °C) for 15 s and therefore, can be used in different foods. Also, the model orange juice was physically stable for one month of storage at 4 °C. In a study by Santos et al. [54], nano-structured lipid careers (NLCs) were developed for carrying phytosterols using hydrogenated crambe oil, canola oil, and high oleic sunflower oil as raw materials. Both canola and crambe oils showed sufficient thermal stability and ability to completely incorporate phytosterols. Thermal resistance and crystallinity of lipid matrices were also improved with the addition of phytosterols. It was observed that NLCs developed with 30% phytosterol level can effectively deliver phytosterols in different food products. These

Table 2 Extraction methods of phytosterols using non-conventional techniques

Extraction methods	Sources	Solvent used	Phytosterols detected	References
Super critical fluid extraction	Guava seeds oil	CO_2	β-sitosterol (1048 ± 48.4 mg/100 g oil), campesterol (23.9 ± 1.4 mg/100 g oil)	[39]
	Sugarcane bagasse	CO_2 and ethanol	β-sitosterol (0.153 mg/g d.m.), campesterol (0.063 mg/g d.m.), stigmasterol (0.084 mg/g d.m.), total phytosterols (0.300 mg/g d.m.)	[40]
	Flaxseed oil	CO_2	Total phytosterols (352 mg/100 g oil)	[32]
	Roselle (*Hibiscus sabdariffa* L.) seeds	CO_2, ethanol	Total phytosterols (7262.80 mg/kg)	[42]
	Rapeseed oil	CO_2, ethanol	B-sitosterol, brassicasterol, and campesterol	[23]
	Cucurbita pepo convar. citrullina (pumpkin) seed oil	CO_2	Total phytosterols (294 mg/100 g), Δ7-sterols (from 91.0 to 94.2%)	[44]
	Rapeseed oil Deodourizer Distillate	CO_2 and ethanol (5%)	Campesterol (36.25%), β-sitosterol (50%) and brassicasterol (23.91%)	[41]
	Morusalba leaves	CO_2	β-sitosterol (155.74 mg/100 g leaves)	[45]
	Rice bran oil	CO_2	Total phytosterols (from 8.78 to 11.21 mg/g oil)	[30]
	Flaxseed oil	CO_2	Total phytosterols (352 mg/100 g oil)	[32]
	Melon (*Cucumismelo*) seed oil	CO_2	Stigmasterol (121 mg/kg seed), β-sitosterol (304 mg/kg seed)	[43]
Enzymatic extraction	Flaxseed oil	Alkaline protease	β-sitosterol (115.39 mg/100 g oil), cycloartenol (85.45 mg/100 g oil), campesterol (65.80 mg/100 g oil), Δ5-avenasterol (40.92 mg/100 g oil), stigmasterol (20.96 mg/100 g oil), 2,4-methylene cycloartenol (19.05 mg/100 g oil), total phytosterols (347.56 mg/100 g oil)	[27]
Ultrasound-assisted extraction	Sunflower oil	Cellulase (Celluclast 1.5 L, Novozyme)	Total phytosterols (from 95.86 to 135.59 mg/100 g)	[46]
	Rice Bran	Chloroform–methanol (2:1, v/v)	β-sitosterol (305 µg/g rice bran), cycloartenol (80.5 µg/g), 24-methylenecycloartenol (87.1 µg/g), stigmasterol 106 µg/g), campesterol (126 µg/g), and ergosterol (129 µg/g)	[47]
	Morus alba leaves	n-hexane	From 55.55 to 103.49 mg/100 g leaves	[45]
Direct hydrolysis extraction	Hickory husk	Citric acid	β-sitosterol, stigmasterol, campesterol, ergosterol, and lupeol, total phytosterols (912.452 µg/g d.m.)	[48]
Microwave-assisted hydrodistillation	Different Legume Pods	Ethanol	β-sitosterol	[49]
Ultrasound-assisted extraction	Tiger nut (*Cyperusesculentus L.*)	Petroleum ether and acetone	β-sitosterol (518.26 mg/100 g) stigmasterol (70.42 mg/100 g) and campesterol (25.78 mg/100 g), total phytosterols (658.68 mg/100 g)	[50]
	Radish seed oil	Ethanol	Stigmasterol (49.19 mg/100 g), campesterol (81.14 mg/100 g), β-sitosterol (215.86 mg/100 g), total phytosterols (346.18 mg/100 g oil)	[51]

NLCs can be used to enrich different products like spreads, margarine, and beverages with phytosterols. In other study conducted by Bagherpour et al. [55], β-sitosterol was used to improve the antioxidant and nutritional properties of butter. For this purpose, NLCs carrying the β-sitosterols were developed and added in butter. The NLCs remained stable for 3 months of storage. The antioxidant capacity of β-sitosterol in butter was improved and remained stable over time. An initial increase in the peroxide and acid values was observed which remained unchanged throughout the storage duration.

Phytosterols have the potential to replace fat in different food products. They have fat-like characteristics and do not provide energy to the human body. Goh et al. [56] applied phytosterol esters as fat replacers in skim milk. The fatty acids and aldehyde compounds increased, while an adequate lubrication and whiteness properties were observed in milk. With an increase in phytosterol concentration, the fat globule size and viscosity increased, while friction coefficient declined. Similarly, lightness and yellowness of milk also increased with increasing the phytosterol concentration and were comparable to commercial milk containing same fat content. The milk enriched with phytosterols was free from any off-flavors. It was concluded that phytosterols can be added at a concentration of 1.6 or 2% in milk without affecting the physical properties and sensory characteristics except a grainier texture was perceived.

Ningtyas et al. [57] studied the impact of phytosterols and β-glucan, individually and in combination, as fat replacers, on the textural properties of reduced-fat cream cheese. Phytosterol powder and esters extracted from soyabean were used. Although the β-glucan was more effective to improve the moisture content, viscosity, adhesiveness, and firmness of product as compared to phytosterols. Phytosterols (both in esterified and emulsified form) reduced the coefficient of friction and enhanced the lubrication property of the product more effectively as compared to β-glucan, resulting in a more spreadable cream cheese. Also, the formulation containing phytosterol emulsion showed larger size of fat globules among all the formulations. Moreover, an open structure of casein matrix in cream cheese was observed when phytosterols and β-glucan were used alone or in combination. It was also inferred from the results that phytosterols have a synergistic effect with β-glucan to improve the texture of reduced fat cream cheese as the cream cheese containing a combination of β-glucan and phytosterols (either emulsified or esterified) showed higher firmness. Similarly, Amaral et al. [58] suggested light cream cheese spread to be an excellent matrix for phytosterols addition. Goat milk cream cheese spread containing phytosterols was prepared. Total protein content increased, while a reduction in fat and total calories was observed. Also, the product met the microbiological standards during refrigeration storage of 90 days. However, the product showed a grainy texture due to non-solubilization of phytosterol powder. According to this study, 6% level was recommended to be the optimum level for phytosterols addition in food products. On the other hand, Nzekoue et al. [59] evaluate the suitability of whey cheese for phytosterols-enrichment. During cheese processing, phytosterols were stable to heat. Their bioactivity was well maintained without any decline in their level during a storage period of 4 weeks. Moreover, the phytosterols were homogenously distributed in the product and ricotta cheese was considered as a suitable food for adding phytosterols. Ahangari et al. [60] prepared probiotic Ayran (a fermented drink) enriched with phytosterols. The pH, acidity, and viscosity of the product were not affected by phytosterols, and according to sensory scores, phytosterols (0.5 mg/L) were found to be suitable for addition to probiotic Ayran. Seyhan et al. [61] developed reconstituted whey beverage containing probiotic bacteria and phytosterols and isoflavones as bioactive compounds. Phytosterols incorporated samples were more preferred by the sensory panel as compared to those containing isoflavones. Nagaraj et al. [62] fortified the milk with phytosterols. The study suggested that 10% phytosterols in the form of an o/w emulsion can be added in milk without affecting the sensory parameters. It was observed that phytosterol-enriched milk could be stored for 7 days without presenting any difference in physicochemical and sensory parameters.

In a study, the use of phytosterols to inhibit the formation of *trans*-fatty acids in food showed their excellent anti-isomerization potential. The effect of different phytosterols on trans-fatty acid production during heating in peanut oil was determined. Peanut oil, triollein, trilinollein, and trilinolenin (each incorporated with 1% phytosterols) were heated at 180 °C for 24 h. A decrease in the formation of *trans*linoleic acids, *trans*linolenic acids, *trans*oleic acid, and total fatty acids was observed, and this effect increased with heating time [63]. Ben Moussa et al. [64] prepared yoghurt enriched with phytosterols (1.6%) or a combination of phytosterol (1.6%) and lactulose (6%). A reduction in post acidification and syneresis was observed in all samples. Phytosterols did not affect starter counts during storage period. The yoghurt presented a lower oxidation and good rheological characteristics when phytosterols and lactulose were used together. According to a study conducted by Comunian et al. [65], the phytosterol mixture comprising of stigmasterol (0 – 2%), campesterol (0 – 5%), campesterol (0 – 15%), β-sitosterol (70 – 80%), and β-sitostanol (0 – 15%) was coencapsulated with echium oil along with sinapic acid (as an antioxidant and cross linking agent) and microcapsules formed were incorporated in yoghurt. The result indicated good sensorial characteristics of functional yoghurt and rheological as well as physicochemical stability was maintained.

Tolve et al. [66] developed dark chocolates containing microencapsulated phytosterols and cocoa. The product was

chemically stable with peroxide value not exceeding 2 meq O_2 /kg of fat even after a period of 3 months. The antioxidant activity of 92 μg trolox/g was observed, and the product got good acceptability scores by the sensory panel. Chen et al. [67] also used β-sitosterol and γ-oryzanol as natural antioxidant to prevent off-flavor and enhance oxidative stability of algae oil nano-emulsion and spray-dried powder. The oxidative stability was enhanced by β-sitosterol and γ-oryzanol, and less fishy off-flavor was developed as compared to control samples.

Phytosterols have a synergistic effect with other compounds in lowering cholesterol level on body. Hussain et al. [68] explored the cholesterol lowering effect of phytosterols. Encapsulated phytosterols esters were added in cheddar cheese. The aim was to obtain a synergistic cholesterol lowering effect of a fermented dairy product and phytosterols. The product got high sensory scores for texture but the taste was decreased as the phytosterol content increases. Also, the hardness of cheese increased with high percentage of phytosterol incorporation. The cheese was fed to rats for 6 weeks, and results indicate a decline in total cholesterol and triglycerides level by 19% and 11%, respectively, while HDL cholesterol increased by 8%. In case of liver lipids, a decrease in total cholesterol (by 9.3%) and triglycerides (7%) was observed. However, body weight did not change significantly. Ferguson et al. [69] suggested that phytosterol-enriched bread can be an efficient mean of delivering phytosterols with greater potential of lowering the blood cholesterol level in individuals. Bread enriched with 2.3 g phytosterols

Table 3 Applications of phytosterols in food industry

Application	Uses	Effects	Dosage	References
Soyabean oil enriched with phytosterols	Microcapsules	Excellent efficiency, oxidative stability	5% and 10% phytosterols	[52]
Phytosterols from canola waste	Orange juice containing liposomes	Food additive	-	[53]
Phytosterols from hydrogenated crambe oil, canola oil and high oleic sunflower oil	Nano-structured lipid careers	Food products	30% phytosterols	[54]
β-sitosterol	Nano-structured lipid careers	Enhanced antioxidant and nutritional properties of butter	-	[55]
Phytosterol esters	Phytosterol enriched milk	Fat replacers in skim milk	1.6% or 2% phytosterols	[56]
Phytosterol powder and ester	Phytosterols as fat replacers	Reduced fat cream cheese	-	[57]
Goat milk	Cream cheese spread enriched with phytosterols	Total protein content increased and fat and total calories reduced	6% phytosterols	[58]
Whey cheese enriched with phytosterols	Ricotta cheese	Excellent bioactivity	0.8 g/100 g	[59]
Probiotic Ayran enriched with phytosterols	Encapsulated probiotics	Protective role for probiotic bacteria	0.5 mg/L phytosterols	[60]
Whey powder enriched with phytosterols	Whey beverages	Nutraceuticals	1.0%	[61]
Fortified milk with phytosterols	Water soluble emulsion in milk	Functional food	10% phytosterols	[62]
Phytosterols from peanut oil	Inhibit the formation of trans-fatty acids	Excellent anti-isomerization potential	-	[63]
Yogurt enriched with phytosterols	Fortified yogurt	Lower oxidation and good rheological characteristics	1.6% phytosterols	[64]
Phytosterols mixture	Microcapsules incorporated in yogurt	Physicochemical stability	-	[65]
Dark chocolate	Microencapsulated phytosterols	Antioxidant activity	92 μg trolox/g	[66]
β-sitosterol and γ-oryzanol	Algae oil nano-emulsion and spray dried powder	Antioxidant activity	-	[67]
Cheddar cheese	Encapsulated phytosterols esters	Cholesterol lowering effect	5%	[68]
Phytosterol-enriched bread	Novel food product	Cholesterol lowering effect	2.3 g phytosterols	[69]
Phytosterols addition in poultry feed	-	Antioxidant characteristics improve growth performance and meat quality	40 mg/kg	[70]
Phytosterols supplementation in diet	-	Antioxidant characteristics improve growth performance and meat quality	50 g and 75 g β-sitosterol	[71]

or 228 mg curcumin or a combination of these was given to hypercholesterolemic patients for a period of four weeks. A reduction in total cholesterol, LDL cholesterol, LDL particle number and size, and cardiovascular disease risk was observed in individuals given phytosterol-curcumin bread. Results showed that phytosterol enriched bread with or without curcumin have the potential to lower blood cholesterol. Other applications of phytosterols include their use in poultry feed. In their research, Zhao et al. [70] conclude that addition of phytosterols (40 mg/kg feed) can be effective in improving overall growth, antioxidant characteristics, and meat quality of chickens. Similarly, Naji et al. [71] concluded that dietary phytosterols can enhance meat quality by rising antioxidant enzyme levels in broiler chicken without leaving any effect on texture of meat. Table 3 explains about the applications of phytosterols in the food industry.

4 Future trends and/or challenges

Phytosterols have been increasingly popular in recent decades as a result of their human health-promoting characteristics. The global market for phytosterols appropriate for use as food additives is expected to reach $USD 710 million in 2019, with a growth rate of roughly 9% expected in the next years [72]. Bioactive substances such as phytosterols are encapsulated using a novel nano-delivery technology. Despite the positive effects of nano-based formulations on human health and the environment, there are some worries about their effects. Nanoencapsulation is a new and well-established approach for preserving their natural features. It improves phytosterol solubility and bioavailability, as well as their stability and long-term release profile [3]. When phytostanols are given to meals, they reduce serum cholesterol levels by limiting sterol absorption from the digestive system. Treatment of moderate hypercholesterolemia with products enhanced with plant phytostanols is beneficial [73]. It is worth noting that phytosterol bioactivities are beneficial to human health, which makes them suitable for inclusion in functional foods. With the rising demand for phytosterols in pharmaceuticals, food, and cosmetics, sustainable and environment friendly manufacturing processes are used along with new sources other than plant-related sources [72]. Plant sterols were granted a cholesterol-lowering health claim by the European Food Safety Authority (EFSA) in 2008, although their mixes have yet to be used as olegelators in meals. If these technical hurdles can be overcome, phytosterol oleogels will have a dual effect on blood cholesterol levels as plant phytosterols are also claimed to have a reducing effect on blood cholesterol in their own right, with a concurrent effect on a significant cardiovascular disease risk factor [74].

5 Conclusions

Phytosterols are naturally occurring sterols found in plants, particularly in oils, nuts, legumes, and cereals. Consumer demand for healthier diets has increased the need for novel functional foods to be developed. A functional diet with bioactive substances can improve health or lower illness risk. The growing demand for bioactive compounds such as phytosterols has prompted the development of simple and effective methods for extracting and isolating them from a variety of plant sources. Organic solvents are used in large quantities in traditional extraction methods, which must be properly disposed of in order to safeguard the environment. Several parameters can be manipulated simultaneously in non-conventional extraction techniques. Reduced solvent consumption can be achieved by selecting the best suited method and adjusting the extraction conditions. Furthermore, some non-traditional procedures, such as SFE with CO_2, could be utilized without the use of potentially harmful organic solvents. Furthermore, with green products, the majority of non-conventional procedures delivered substantial quantities of phytosterols. SFC can also be used to prevent or limit the usage of organic solvents; however, further experiments are needed to create optimal working conditions and instruments. The increased demand for these bioactive compounds in food industry may lead to more efficient, fast, and environmentally friendly extraction, isolation, and analytical processes.

Author contribution Rimsha Younas: investigation-equal, writing—original draft. Amna Sahar: conceptualization, writing—review and editing, supervision. Aysha Sameen: writing—review and editing. Muhammad Issa Khan: writing—review and editing. Muhammad Azhar Ali: writing—review and editing. Muhammad Arbaz Tahir, Muhammad Mohsin, and Muhammad Usman: writing—review and editing. Rana Muhammad Aadil: writing—review and editing, supervision.

Funding This work was supported by the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

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