Review

Phytosterols: Applications and recovery methods

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Abstract

Phytosterols, or plant sterols, are compounds that occur naturally and bear close structural resemblance to cholesterol, but have different side-chain configurations. Phytosterols are relevant in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick). Phytosterols can be obtained from vegetable oils or from industrial wastes, which gives an added value to the latter. Considerable efforts have been recently dedicated to the development of efficient processes for phytosterol isolation from natural sources. The present work aims to summarize information on the applications of phytosterols and to review recent approaches, mainly from the industry, for the large-scale recovery of phytosterols.
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1. Introduction

Phytosterols are triterpenes similar to cholesterol, both in structure, given the four-ring steroid nucleus, the 3β-hydroxyl group and often a 5,6-double bond, as in function, given their role in the stabilization of the phospholipid bilayers in cell membranes. However, cholesterol has a side-chain composed of eight carbon atoms, whereas more common phytosterols have a side-chain composed of 9 or 10 carbon atoms, out of a total of 28 or 29 carbon atoms. The alkyl side chain may also contain a double bond (Fig. 1). More than 100 types of phytosterols have been reported in plant species, but the more abundant are sitosterol, stigmasterol and campesterol (Moreau et al., 2002; Berger et al., 2004; Kritchevsky and Chen, 2005). Other rel-evant phytosterols that can be found in plants in minor amounts are brassicasterol, Δ^5-avenasterol, sitostanol and campestanol (Phillips et al., 2002). A detailed description of occurrence and nomenclature of phytosterols can be found in recent reviews (Moreau et al., 2002; Ostlund, 2002). Sterols in plants exist in the form of free alcohols, of fatty-acid esters, of steryl glycosides and of acylated steryl glycosides (Moreau et al., 2002; Phillips et al., 2002). In edible oils phytosterols are mainly present in free and ester-ified forms (Phillips et al., 2002). Phytosterols play major roles in several areas, namely in pharmaceuticals (produc-tion of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick). Academia has devoted consid-erable efforts into identifying the role of phytosterols in health, pharmaceutical and food and feed sectors, and a considerable number of publications resulted thereafter, particularly in the last decade. However research on ade-quate and efficient methodologies for phytosterol recovery from natural sources, mostly wastes from vegetable oils

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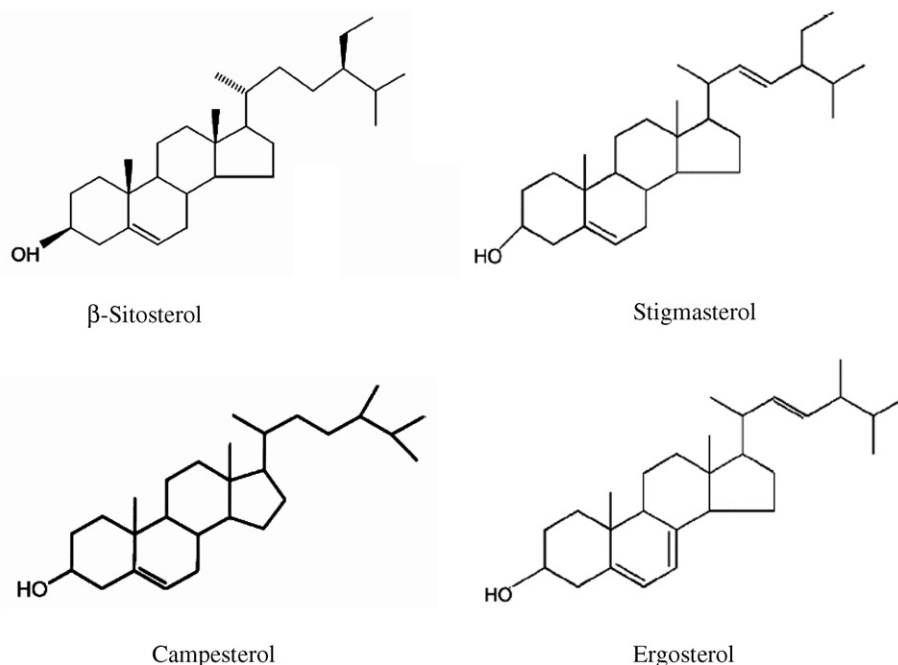*E-mail address:* pedro.fernandes@mail.ist.utl.pt (P. Fernandes).

Fig. 1. Structure of some representative phytosterols.

and cellulose processing, has been mostly performed by industry, academia publications on this matter being rather scarce. The present work aims to partially fill in such overlook. Again, in recent years, efforts from industry resulted in a significant number of reports, describing better and improved methods for phytosterol recovery and purification. Such surge is closely related to growing market for phytosterols, particularly given the widespread dissemination of functional foods. This review aims thus to provide a state of the art in phytosterols isolation and applications.

1.1. Main roles of phytosterols

High serum concentrations of total or low-density-lipoprotein (LDL)-cholesterol are major risk factors for coronary heart disease, a major cause for morbidity and mortality in developed countries (Tapiero et al., 2003; Plat and Mensink, 2005). It is understandable that efforts are made to minimize such risks, and reduction of serum cholesterol is a feasible approach, since a risk reduction of coronary heart disease of about 3% can be achieved through a 1% decrease in total cholesterol (Mel'nikov et al., 2004). The cholesterol-lowering effect of phytosterols was evidenced for the first time in 1951 (Peterson, 1951; Moghadasian and Frohlich, 1999). Shortly after, a phytosterol preparation from either tall oil or soybean oil, Cytellin™, was marketed by Eli Lilly (Tangedahl et al., 1979; Stewart et al., 1999). Afterward, experimental evidence has been piling up that consistently supports the benefits of plant sterols in reducing cholesterol levels in humans (Moghadasian and Frohlich, 1999; Tapiero et al., 2003; Berger et al., 2004; Kritchevsky and Chen, 2005). The mechanism played by phytosterols, which is more evident for 4-desmethyl sterols (mostly β-sitosterol, stigmasterol and campes-

terol), is based on the ability of plant sterol esters to reduce the intestinal absorption of diet and biliary cholesterol (Quilez et al., 2003; de Jong et al., 2004; Berger et al., 2004; Plat and Mensink, 2005). A thorough description of the mechanism of cholesterol lowering action of phytosterols is given in a recent review (Trautwein et al., 2003). This beneficial effect becomes measurable from the daily intake of an amount of phytosterol as small as 150 mg, which is within the current natural dietary intake of plant sterols, which roughly spans from 170 mg up to 440 mg. The former corresponds to the typical Western diet, the latter to a diet rich in vegetables (Ostlund, 2002; Quilez et al., 2003). Larger amounts are nevertheless required to have a significant cholesterol lowering effect. An intake of phytosterols of 1.5–3.0 g day^{-1} leads to a reduction of 8–15% in the blood levels of LDL-cholesterol (Quilez et al., 2003). Kritchevsky and Chen (2005) state that a 10% reduction in the blood levels of LDL-cholesterol can be obtained with an optimal intake of phytosterols of 2 g day^{-1}, beyond which only a marginal effect is observed, data also forwarded by Ostlund (2002). Tapiero et al. (2003) summarized the conclusions of several reports, to state that the intake of phytosterols could lower circulating cholesterol concentrations (total and LDL) by 5–15%. Apart from a carotenoid lowering effect resulting from the intake of plant sterols (Berger et al., 2004; Kritchevsky and Chen, 2005), that can be corrected by increasing the intake of food rich in carotenoids (Berger et al., 2004), no side effects have been related to the use of plant sterols to lower LDL-cholesterol. No significant alterations in high-density-lipoprotein (HDL)-cholesterol or triglycerides in general were reported, and the effectiveness of this approach has been positively tested in hypercholesterolaemic patients, as well as in individuals with normal cholesterol levels (Quilez

et al., 2003). Given the benefits of plant sterols, and since they are not present in sufficient amount in typical diets, phytosterols have to be administered as a supplement. This has to be performed in an adequate formulation, in order to assure the required availability for absorption into the mixed bile salt and acid micelles (Engel and Schubert, 2005). Given the hydrophobic nature of free phytosterols, they are poorly soluble in the digestive tract and their administration is far from simple, since they are almost insoluble in water and only slightly soluble in edible oils (Zawistowski, 2001; Engel and Schubert, 2005). The first main breakthrough in this area came with the discovery that the esterification of sterols with fatty acids, leading to steryl (or sterol) esters, can be easily formulated in food products and can therefore be useful in functional foods (Moreau et al., 2002). The use of these fat soluble sterol derivatives allowed a significant increase in the efficacy of these compounds, as compared with initial approaches for phytosterol administration, in crystal form. This required relatively large quantities to achieve a significant effect (Quilez et al., 2003; Kritchevsky and Chen, 2005). Ten to 20 grams per day of crystalline phytosterols were required to achieve the serum cholesterol reduction of roughly 10% achieved with 2–3 g per day of steryl esters (Engel and Schubert, 2005). Therefore, the incorporation of steryl esters into oily or fatty foods is currently the favored formulation (Ntanios et al., 2005; Engel and Schubert, 2005; Carr, 2005). There is however evidence showing that if free phytosterols are administered in a form that allows an efficient delivery into the micelles in the small intestine, they will be more effective in inhibiting cholesterol adsorption then their esterified counterparts (Mattson et al., 1982; Jones et al., 1999; Vanstone et al., 2002; Zawistowski, 2002; Engel and Schubert, 2005). Other approaches for the development of effective formulations that can incorporate free phytosterols into delivery vehicles, such as foods, nutraceuticals and pharmaceuticals, from which they can be readily absorbed into the bile salt and acid micelles in the intestine have been suggested, have thus been developed (Zawistowski, 2001; Engel and Schubert, 2005). Such approaches for phytosterol delivery include phytosterol-rich emulsions (Straub, 1993; Ostlund, 1999; Akashe and Miller, 2001; Auriou and Ferreres, 2002; Coote et al., 2002; Kutney et al., 2002; Moreau et al., 2002; Chen et al., 2004; Engel and Schubert, 2005; May and Slater, 2005; Zima et al., 2005), steryl esters-rich emulsions (Goulson et al., 2003), triglyceride-recrystallized non-esterified phytosterols (Perlman et al., 2003), formation of phytosterol microparticles (Christiansen et al., 2002). The latter can also be combined with the incorporation of these microparticles into an emulsion (Zawistowski, 2001). Other methodologies for phytosterol delivery include homogeneous dissolution, based in molten or melted phytosterols mixed with oils or fats (Zawistowski, 2002), liposome formulation (Spilburg, 2003) and formulations combining policosanols and/or policosanoic acids and sterol based ascorbic acid derivatives (Kutney and Wessman, 2005).

In addition to their cholesterol lowering properties, plant sterols possess anti-inflammatory (Gomez et al., 1999; Bouic, 2002; Lane, 2002; Niazi, 2003; Okoli and Akah, 2004) and anti-atherogenicity activity (Nashed et al., 2005) and may possess anti-cancer and anti-oxidative activities (Eugster et al., 1993; Choi et al., 2003; Berger et al., 2004; Platt et al., 2004).

An altogether different role for phytosterols is their use as raw materials for the production of therapeutic steroids. This is performed in processes involving biotransformations and/or biotransformations, eventually coupled with more conventional chemical transformations (Ahmad et al., 1992; Hogg, 1992; Mahato and Mazumder, 1995; Mahato and Garai, 1997; Schmid et al., 2001; Fernandes et al., 2003). The world market for building blocks for such steroid based drugs exceeded 1000 tonnes per year, as estimated recently (Schmid et al., 2001).

1.2. Sources of phytosterols

Phytosterols isolation in large scale is based in two major raw materials, vegetable oils and tall oil (Coss et al., 2000; Hayes et al., 2002; Quilez et al., 2003). Irrespectively of the source, they have been used in health, pharmaceutical and food applications (Lees et al., 1977; Kutney et al., 1998; Kritchevsky and Chen, 2005). Crude vegetable oils have to be processed in order to remove impurities such as proteinaceous material, phosphatides, odorous volatiles, waxes, free fatty acids, pigments, oxidation products, phosphatides, soaps, and trace metals (Copeland and Belcher, 2001). Two main schemes are currently used for oil refining: (a) a sequence involving degumming, neutralization, bleaching and deodorization, known as chemical refining; (b) a sequence composed of degumming, bleaching and steam-refining (deodorization or physical refining), usually known as physical refining (Copeland and Belcher, 2001; Chakrabarti et al., 2003). Chemical refining of vegetable oils involves removal of free fatty acids and gums in the neutralization step by using a hot alkali solution, to yield a neutral oil and soapstock, a by-product (Copeland and Belcher, 2001). The physical refining approach prevents formation of the latter by-product, but fails to remove non-hydratable phosphatides, common in oils such as soybean and sunflower seed (Copeland and Belcher, 2001). Also, physically refined oils lack flavor stability (Copeland and Belcher, 2001). In the overall the chemical refining approach is favored, although physical refining can be used for processing vegetable oils with low non-hydratable phosphatides (e.g. palm oil). Irrespective of the refining approach for crude vegetable oil processing, removal of phytosterols, mostly in the free form, takes place in the deodorization step of the refining process (Copeland and Belcher, 2001; Kamm et al., 2001; Quilez et al., 2003; Akimoto et al., 2004). This is the common industrial approach to obtain sterols from vegetable oils, since the low concentration of sterols in crude oils (Table 1) prevent their oriented recovery in an economical way

Table 1
Example of compositions of crude and refined vegetable oils (adapted from Guderjan et al., 2005; Hafidi et al., 2005)

Vegetable oil	Sterol content (mg/100 g_{oil})	
	Crude	Refined
Corn	850	730
Rapeseed	820	770
Sunflower	430	350
Soya	350	260

(Czuppon et al., 2003). Deodorization allows the removal of residual volatile components via high vacuum (1–8 mbar) and high temperature (180–270 °C) steam distillation, to yield a by-product, the deodorizer distillate (also called deodorizer sludge), which is rich in phytosterols. Fatty acids (C_{10}–C_{22}) are the major component of this by-product and tocopherols are also present (Coss et al., 2000; Kamm et al., 2001; Brench, 2002; Copeland and Belcher, 2003; Czuppon et al., 2003). Among vegetable oils, deodorizer distillates from corn oil, corn fiber oil, wheat germ oil and soybean oil are the most common (Coss et al., 2000; Hayes et al., 2002; Quilez et al., 2003). Typically a deodorizer distillate contains 30–85% free fatty acids, 1–8% tocopherols, 5–30% glycerides, 0–5% sterol esters and 2–15% free sterols (Czuppon et al., 2003). In soybean deodorizer distillates, phytosterols may account for up to 18% (w/w) (Copeland and Belcher, 2003), corresponding to a decrease in the free sterol content of the vegetable oil from 2.0 mg g_{oil}^{-1} to 1.6 mg g_{oil}^{-1} (Akimoto et al., 2004).

Another major source of phytosterols is tall oil (Quilez et al., 2003), a by-product of the wood pulp industry, primarily, but not exclusively, from soft woods of the *Pinus* genus. This is usually recovered in the sulfate or kraft paper process by acidulation of the soap skimming from black liquor. In the pulping process wood chips are cooked in a mixture of sodium sulfide and sodium hydroxide, in a steam heated digester, as to dissolve most of the lignin and only part of the hemicellulose, so that only cellulose is left to hold the fibers together. The alkaline mixture saponifies fats and converts fatty and resin acids into their sodium salts. The process yields pulp and a chemical mixture, the so-called black liquor. This pulping solution is concentrated until sodium salts, also called soaps, of fatty and resin acids, and unsaponifiables, which comprise fatty alcohols, fatty acid esters, free and esterified sterols, split and are skimmed off, since they float to the surface (Wong et al., 1999; Schultz and Sonnier, 2003; Sato et al., 2004). This skimmed material is known as tall oil soap. These salts are then acidulated with a mineral acid, usually sulfuric acid, to yield an aqueous phase and an oil phase. The latter phase, known as crude tall oil, contains unsaponifiables, free fatty acids and resin acids (Wong et al., 1999). Typically crude tall oil is composed of 35–50% fatty acids (C_{18}, such as linoleic acid, oleic acid and saturated stearic acid), 15–55% diterpenic resin (rosin) acids, and 5–35%

unsaponifiable matter (neutrals), such as hydrocarbon waxes, long chain alcohols, fatty acid esters and sterols and steryl esters. Total sterols may contain 3–7% by weight total sterols, mostly in esterified form (Huibers et al., 2000; Wu et al., 2004; Pearse, 2005; Rohr et al., 2005; http://www.uspto.gov/go/classification/uspc530/defs530.pdf, checked on 11 November 2005). The different compounds of crude tall oil are subsequently separated in a fractional distillation process to generate two phase. A light phase, the so-called depitched tall oil, containing mostly the more volatile fatty and rosin acids, and a heavy phase, the so-called pitch tall oil, containing the less volatile compounds, namely the major part of the unsaponifiables and a minor amount of fatty and rosin acids. The total sterol content in the pitch may range from 8% to 20% by weight (Wong et al., 1999; Huibers et al., 2000; Sato et al., 2004; Rohr et al., 2005). This separation, where more than 90% of fatty and rosin acids are removed, takes place under temperatures of 300 °C at least. Although effective, the high temperatures lead to the degradation of non-esterified sterols, e.g., more than half of the free β-sitosterol present in the crude tall oil is destroyed (Huibers et al., 2000). Phytosterols are isolated from tall oil soap, also called soap skimmings, from crude tall oil and from tall oil pitch (Wong et al., 1999; Hamunen, 2003; Schultz and Sonnier, 2003).

Wastes produced during fermentation and processing of sugarcane juice in rum factories have been identified as another, although minor, source of phytosterols (Mitra and Kapadia, 1988; Nuissier et al., 2002). Cocoa hulls, a waste by-product of the roasting of cocoa beans with little value in chocolate manufacturing, have also been used to produce a phytosterol rich oil (Romanczyk and McClelland, 2001).

1.3. Recovery of phytosterols

Several methods have been suggested for phytosterol recovery from sterol containing materials. Sterols in raw materials are partially found in ester form. In such situations the starting material is processed as to hydrolyze the steryl esters into free sterols, which are unsaponifiable. This pretreatment involving hydrolysis of sterol esters may be either performed by contacting the sterol-ester-containing material with water and hydrolyzing the esters under pressure (1.5–50 MPa) at high temperature (200–260 °C); or by contacting the sterol-ester-containing material with a sodium or potassium hydroxide (saponification), at 90–120 °C, eventually with added pressure, and under stirring. The latter methodology advantageously combines hydrolysis and saponification (Rohr et al., 2005). Current processes used to separate and concentrate the unsaponifiable fraction from residues and by-products of the processing of animal and vegetable products and other raw materials, are either based in difference of solubility between unsaponifiable substances and the soap matrix in selected solvents or solvent mixtures; or in the difference of volatility between volatile unsaponifiables and non-volatile salts or

soaps, where separation is performed by high vacuum distillation/evaporation (Rohr et al., 2005). Representative examples of the different approaches are given.

1.4. From vegetable oils

Recovery of phytosterols from deodorizer sludge faces two major problems, the thermal degradation prone to take place if the distillate is exposed for extended periods to the temperatures at which phytosterols vaporize; and the close boiling points of phytosterols and tocopherols (Copeland and Belcher, 2003). Methodologies for phytosterol recovery from oils can be traced back to early 1940s (Yoder, 1943) and 1950s (Lange and Folzenlogen, 1951; Miller and Berry, 1954). Such pioneering processes were claimed to be applied to unsaponifiables of oily and fatty substrates (Yoder, 1943), or deodorizates of fats and oils (Lange and Folzenlogen, 1951; Berry and Miller, 1956), and aimed at obtaining a crystalline precipitate form the unsaponifiable fraction of the substrates. This was achieved by adding gaseous hydrogen bromide to a solution of the substrate in acetone (Yoder, 1943) or by adding perchloric acid to a solution of the substrate in chloroform (Lange and Folzenlogen, 1951). Berry and Miller (1956) focused on the residue from the distillation of a vegetable oil, which is saponified. The saponification mixture is cooled and eventually diluted with methanol, and a mixture of soaps and sterols were crystallized out, and were filtered off. The sterols could then be extracted with an organic solvent, such as ethylene dichloride, in which the remaining saponification products are insoluble, and precipitated by the addition of water. A sterol yield of 83%, based on the sterols present in the starting material, was claimed. Still, sterols precipitated in a gelatinous form, which rendered its separation difficult. This drawback was overcome if water and methanol or ethanol was added to the extract, and the resulting mixture allowed cooling down for steroid separation (Berry et al., 1958). These early processes were not satisfactory, for they required alkali in great excess to hydrolyze and saponify esters, high concentrations of steryl esters could result thereof (Brown and Smith, 1964), and the mineral acids eventually required are dangerous to work with and induce degradation of the distillate compounds (Copeland and Belcher, 2003). The approaches followed by Brown and Smith (1964) and Smith (1967) aimed at overcoming such drawbacks by introducing an esterification step that eased the separation of sterols (and tocopherols) from the remaining deodorizer distillate (Fig. 2a and b). Esterification of free fatty acids is performed in the processing of oils with high acid values, above 40 (Jeromin et al., 1997; Wollmann et al., 2005). Fizet (1996) refined the methodology by performing the esterification of the sterols with the fatty acids already present in the deodorizer sludge (Fig. 2c). The boiling point range of the formed sterol esters is much higher than that of the unreacted tocopherols, which makes the separation of the two groups of compounds simple using short-path distillation (Czuppon et al., 2003). Fizet (1996) introduced a transesterification step to convert steryl esters to the free form, to increase yield and purity of the final product. The processes developed later by Jeromin et al. (1997), Ho (2005) and Wollmann et al. (2005) also involved (trans)esterifications reactions (Fig. 3), and could be used in the processing of distillates with low sterol content, namely 5–30% (Wollmann et al., 2005) and high acid numbers (Jeromin et al., 1997; Wollmann et al., 2005). A second trans-esterification is carried out in order to improve purity. The process suggested by Jeromin et al. (1997) allowed a product with a sterol rich residue with a free sterol content of 40%. The extended process suggested by Ho (2005) and by Wollmann et al. (2005) led to a final product containing 90% or 94% sterols, respectively.

Czuppon et al. (2003) suggested removing firstly the free fatty acids from the deodorization distillate, as to reduce the quantity of the material at least by half, by either saponification or vacuum distillation. Copeland and Belcher (2003) specifically approached the latter, aimed at the separation of sterols, tocopherols and free fatty acids, with considerable detail. With the removal of fatty acids, the size of the equipment required for downstream processing concomitantly decreases. Their process can be performed for the processing of deodorizer distillates containing 2–15% sterols and 30–85% free fatty acids, and allows also the recovery of tocopherols (Fig. 4a). Phytosterol recovery methodologies can be simplified if the distillate to be processed has low acid values and high sterol content. Thus, deodorizer distillates, with acid number below 10 or with sterol content between 15% and 50%, were processed according to Hattori et al. (2001) and to Charlemagne et al. (2004), respectively (Fig. 4b). Fatty acids and their esters are saponified and removed in the saponification step, and sterols are crystallized out of the unsaponifiable fraction. The latter authors also claimed 100% sterol purity of the final crystals, albeit at the cost of a process yield of only 56%. Their approaches considerably decreased the amount of fatty acid esters that often contaminate the final phytosterol crystals (Hattori et al., 2001; Charlemagne et al., 2004). This methodology, when applicable, does not require additional equipment for vacuum distillation and trans-esterification, and sterols are not exposed to very harsh conditions (high temperature and vacuum), to which sterols, particularly in the free form, are sensitive and prone to decomposition (Charlemagne et al., 2004).

Albiez et al. (2004) also presented an approach to isolate sterols form deodorizer distillates without requiring toxicologically and ecologically unsafe solvents or metal-containing catalysts, that are furthermore expensive and require regeneration, and are a potential source of contamination of the final residue (Copeland and Belcher, 2003). In the method described by Albiez et al. (2004), a deodorizer sludge, containing 8.2% sterols and 19.5% steryl esters, is mixed with deionized water and heated to 220 °C for 3 h under pressure. The product of hydrolysis is separated
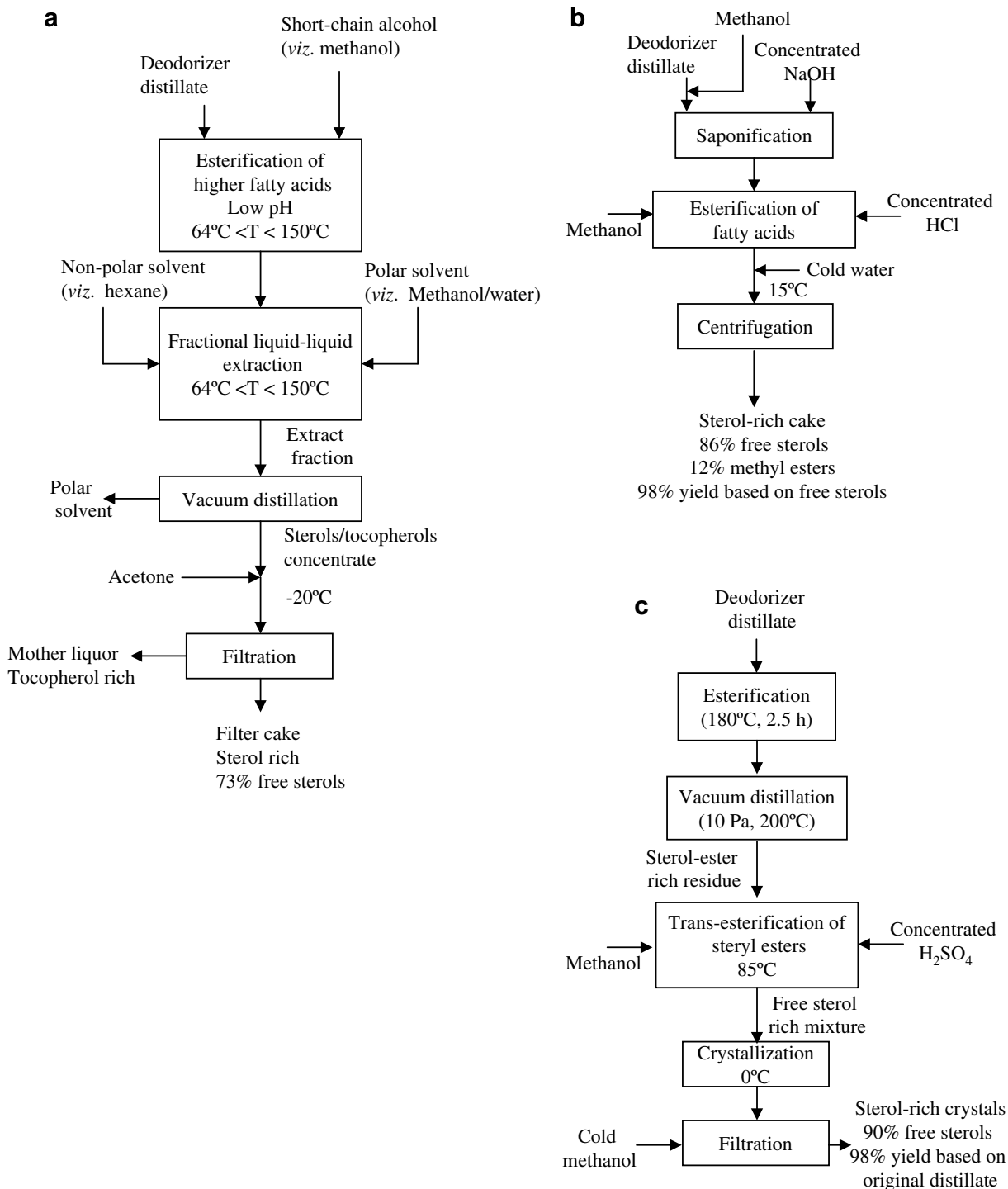
a

Deodorizer
distillate

Short-chain alcohol
(*viz.* methanol)

Esterification of
higher fatty acids
Low pH
64ºC <T < 150ºC

Non-polar solvent
(*viz.* hexane)

Polar solvent
(*viz.* Methanol/water)

Fractional liquid-liquid
extraction
64ºC <T < 150ºC

Extract
fraction

Polar
solvent

Vacuum distillation

Sterols/tocopherols
concentrate

Acetone   -20ºC

Mother liquor
Tocopherol rich

Filtration

Filter cake
Sterol rich
73% free sterols

b

Methanol

Deodorizer
distillate

Concentrated
NaOH

Saponification

Methanol

Esterification of
fatty acids

Concentrated
HCl

Cold water
15ºC

Centrifugation

Sterol-rich cake
86% free sterols
12% methyl esters
98% yield based on free sterols

c

Deodorizer
distillate

Esterification
(180ºC, 2.5 h)

Vacuum distillation
(10 Pa, 200ºC)

Sterol-ester
rich residue

Methanol

Trans-esterification of
steryl esters
85ºC

Concentrated
H_2SO_4

Free sterol
rich mixture

Crystallization
0ºC

Cold
methanol

Filtration

Sterol-rich crystals
90% free sterols
98% yield based on
original distillate

Fig. 2. Recovery of sterols from deodorizer distillates according to (a) Brown and Smith (1964), (b) Smith (1967) and (c) Fizet (1996).

from the water, rich in glycerol, and the fatty acids are removed by vacuum distillation, performed at 280 °C and 200 Pa. The sterol-rich residue was hydrolyzed at 220 °C under pressure for 6 h, which allowed a significant conversion of steryl esters into sterols. The fatty acids were removed by vacuum distillation and a final residue containing 50% sterols and 4% steryl esters was obtained.

Methods requiring a saponification and/or (trans)-esterification step increase process complexity and later process-

ing is necessary in order to convert steryl esters or soaps into free sterols. It should be pointed out, however, that the more complex processes, namely those comprising (trans)esterification reactions, also aim to efficiently recover tocopherols and fatty acids. Therefore, the sterol recovering step is one among several steps within complex processes, which aim to recover all valuable products from the deodorizer distillate, and thus favor the economic feasibility of the approach. In order to minimize thermal degradation, efforts
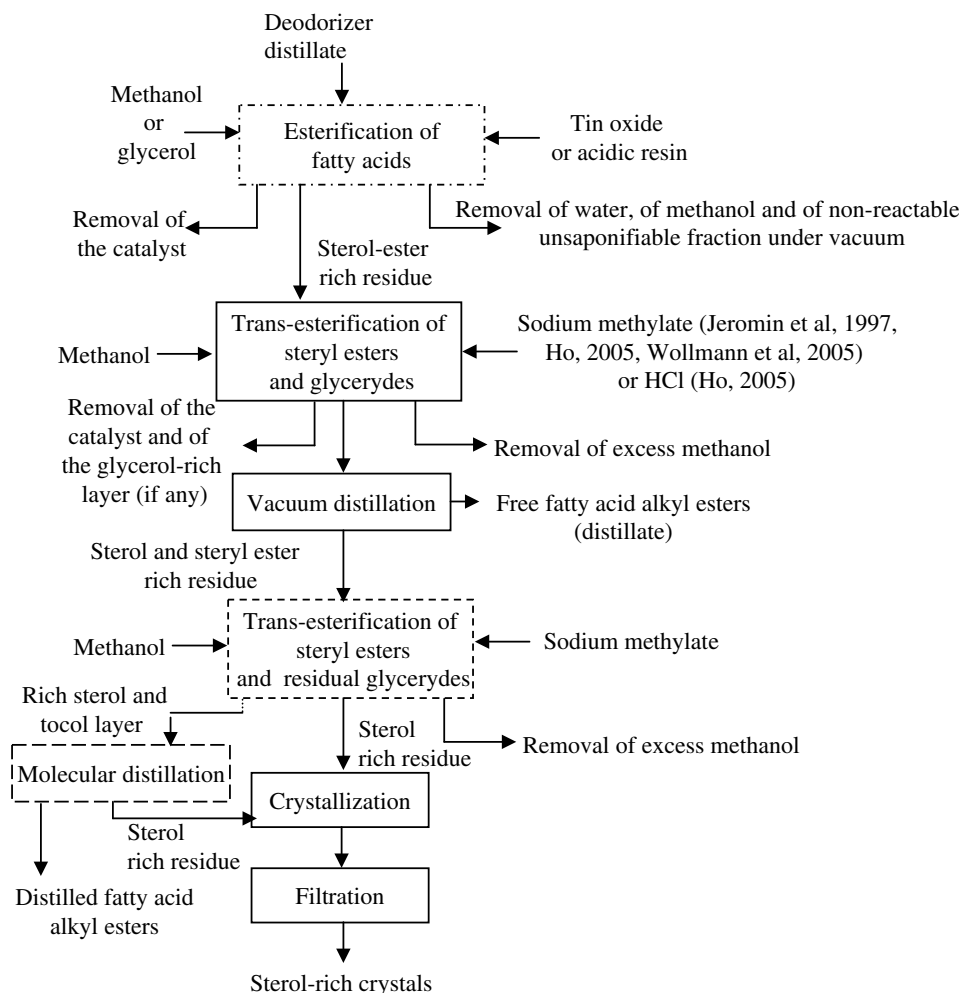
Fig. 3. Improved processes for recovery of valuable products, among them phytosterols, from deodorized distillates (adapted from Jeromin et al., 1997; Ho, 2005 and Wollmann et al., 2005). Esterification of fatty acids (dot and dash box) is not carried out in the process described by Ho (2005), the second transesterification (dash box) is not performed in the process described by Jeromin et al. (1997). Mollecular distillation (large dash box) is only performed in the process described by Ho (2005).

are consistently made to reduce the time period of sterol exposure to high temperatures and vacuum.

A more innovative approach for the removal of fatty acids from oils is based in the use of supercritical CO_2 (sCO_2) extraction, which avoids the use of toxic and environmentally harmful organic solvents, which furthermore have to be removed from the final product. Crude rice bran oil was processed according to this technique in a continuous counter current fractionation mode technique, to allow the reduction of free fatty acids from 5% in the crude oil to 2.5% in the raffinate, while the amount of free sterols decreased slightly (0.7–0.5%, respectively) and the content in steryl esters increased from 2.6% to 2.9%, respectively (Dunford et al., 2003). Direct recovery of phytosterols from crude oils by sCO_2 extraction may also be a technically feasible approach (Bernardo-Gil et al., 2001). This was confirmed by the work performed by Mendes et al. (2005), who were able to isolate key components from a deodorizer distillate by sCO_2 extraction in a semi-batch process. Stigmasterol was isolated at 40 °C and 25 MPa.

Alternative approaches to purely physico-chemical methods for phytosterol recovery from deodorizer sludges have been reported. These are based in the specificity of lipases to esterify sterols into the corresponding steryl esters, among the wide variety of potential substrates present in the deodorizer distillate (Shimada et al., 2000; Nagao et al., 2004; Watanabe et al., 2004). A process for phytosterol isolation from deodorizer sludges, based in a two-step enzymatic process for esterification of fatty acids and sterols was recently reported (Watanabe et al., 2004; Nagao et al., 2005). The deodorizer distillate was pretreated, by vacuum distillation (240 °C and 2.6 Pa) for the separation of high boiling point substances, namely steryl esters, diacylglycerols, and triacylglycerols, to yield a distillate containing 52.8% free fatty acids, 8.4% sterols, 1.7% steryl esters and 15.8% tocopherols. Water was added to the complex mixture (5:95 w/w), together with a lipase preparation, and the whole incubated at 40 °C under 2.6 kPa for 24 h, with dehydration, to achieve 95% sterol esterification, which is required for an efficient purification from tocophe-
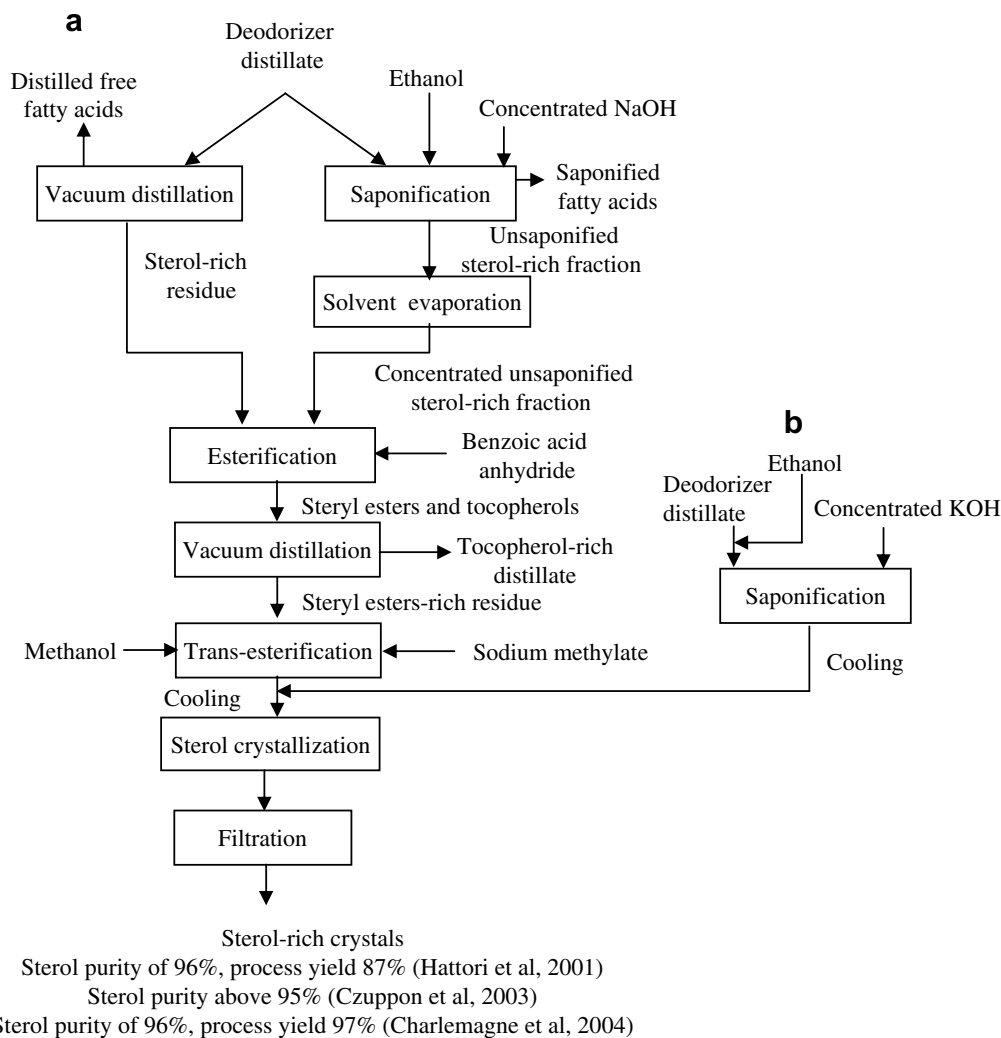
Fig. 4. Processes for the recovery of phytosterols, involving saponification step, from deodorizer distillates with (a) low sterol content and high acid value (adapted from Czuppon et al., 2003), and (b) high sterol content and low acid value based in saponification and crystallization steps (adapted from Hattori et al., 2001 and Charlemagne et al., 2004). Process yield based in the sterol content of the original distillate.

rols (Nagao et al., 2005). The reaction product contained 48.2% free fatty acids, 0.4% sterols, 15.2% steryl esters and 20.4% tocopherols. To this was added 20% water, 7 M amounts of methanol and a mixture of lipase, and the whole incubated at 30 °C without dehydration, for 8 h, to achieve 95% methyl esterification, without loss of tocopherols or hydrolysis of the steryl esters synthesized in the first-step reaction.

1.5. Recovery from tall oil

The amount of unsaponifiables in crude tall oil obtained from crude soaps, rich in phytosterols, may range from 5–30% by weight, depending on the kind of wood (Holmbom and Avela, 1976). Recovery of phytosterols from tall oil skimming soaps was put into commercial practice in the early 1980s by Oy Kaukas AB, in Finland (Schultz and Sonnier, 2003). Forbes Medi-Tech in Canada, and Cognis, in Germany, are also actively involved in phytosterol recovery from natural sources, namely tall oil. A consider-

able number of patents on this matter have been granted recently, which highlights its relevance. Recovery of phytosterols is made from tall oil soap or pitch, each raw material presenting advantages and drawbacks. Thermal decomposition of phytosterols may occur during the distillation process that ultimately yields pitch tall oil. This has nonetheless a higher sterol concentration than the soap (up to 11% mass ratio) and the volume is roughly six times smaller in comparison with the soap (Coss et al., 2000).

Processes for the recovery of sterols from tall oil pitch were described in the 1950s by Christenson (1951), Albr-echt and Herrlinger (1955) and Steiner and Fritz (1958). Major impurities in this sterol source are high molecular weight acids, that have commercial relevance, such as oleic acid or stearic acid, and high molecular weight alcohols, which are difficult to separate from sterols (Julian, 1974). Christenson (1951) removed unesterified fatty acids by extraction with a two-phase solvent system composed a hydrocarbon and a polar solvent containing water. The

sterol rich phase was washed free of water soluble contaminants with water, concentrated by evaporation of the solvent. The crude unsaponifiable residue was dissolved in methanol and crystallized to yield 2.1% of sterol based on tall oil soap concentrate, with purity of 82%.

In the process developed by Albrecht and Herrlinger (1955) free rosin and fatty acids were neutralized separated and discarded, afterwards the steryl esters were saponified and the sterols were forced to precipitate out of the solution by cooling.

In a process suggested by Steiner and Fritz (1958) either crude or pitch tall oil were solvent extracted in a propane tower. The overhead fraction, rich in sterols, was saponified and the sterols recovered by centrifugation, washed with methanol and dried.

Recovery of sterols from the unsaponifiable matter of tall oil soap using a counter current method was described by Christenson and Gloyer (1950). The method was based in the extraction of unsaponifiables from a water–alcohol–soap mixture using petroleum fractions, which were subsequently subjected to further treatment, such as evaporation. The sterols were then crystallized out of a suitable solvent. A similar approach was suggested later by Holmbom and Avela (1976), although using a water–acetone–soap mixture. Julian (1974) also used a mixture of a hydrocarbon–alcohol–water to remove fatty acids from tall oil pitch. Steryl esters were then saponified, and the resulting sterols were forced to precipitate out and were washed and dried. This approach compared advantageously with previous methods since it required neither excessive caustic use nor high pressure apparatus.

These processes concentrate the valuable substances in tall oil by using solvent extraction processes, taking advantage of the difference of solubility between unsaponifiable substances and the soap matrix. Prior to extraction the phytosterols are converted into the free form by saponification with alkali metal hydroxides, whereas the free acids and the acid part of the esters form the soap. Still, it is often difficult to further purify the valuable substances, like sterols, to desired high purity degrees from the extracts without fractionation by distillation. These systems for phytosterol recovery from tall oil soaps are also hampered by difficult solvent regeneration in large-scale systems and solvent losses make them economically uninteresting. The solvents currently used are often environmentally hazardous (Hamunen, 2002). The unsaponifiable fractions are soluble in water-immiscible solvents, namely in hydrocarbons and halogenated hydrocarbons. Still, the separation of sterols from such solvents by concentration of the solution and fractional crystallization is hardly effective, for the fraction rich in sterol thus obtained is very often much too contaminated by other components present in the solution. In order to obtain a highly pure final product repeated recrystallizations from one or several different solvents, among them acetone, ethanol, isopropanol, are required. This often leads to expensive process design as well as rather low yields

(Johansson et al., 1977). These authors suggested an approach for sterol separation from tall oil soap unsaponifiables, which could be obtained according to Holmbom and Avela (1976), based in the dissolution of the unsaponifiables in a suitable water-immiscible solvent, such as hexane, or in a solvent mixture composed of hexane, acetone and methanol; the extraction of the sterols from this solution into a methanol–acetone–hexane mixture, containing a small amount of water as to affect phase separation; concentration of this methanol–acetone phase by evaporation; cooling the solution at 4 °C overnight to induce crystallization of the sterols; and separation of the crystalline sterol by filtration. Solvents recovered can be used in adequate phases of the process. The process could be performed in counter-current extraction continuous mode or as a multistage batch operation. Process yield of 75% (with reference to the total amount of β-sitosterol present in the original neutral substance) for sterol purity from 80% to roughly 100% was reported. Kutney et al. (1998) described a process for the isolation and purification from pulping soap. This soap was mixed with acetone and water and extracted with hexane. The extraction product was concentrated by evaporation and the residue treated with methanol, to yield a mixture with a phytosterol purity of 82%. These methodologies based in solvent extraction are nevertheless getting technically harder to develop, given the increasing environmental restrictions regarding the use of solvents (Diaz et al., 1999, 2001). Furthermore they often fail to provide highly pure crystals (Schersl, 2001). A method for the efficient separation of unsaponifiables from black-liquor soaps or from crude tall oil, based on distillation, was presented by Diaz et al. (1999, 2001), as to overcome the drawbacks of solvent extraction. Their approach is in some order a follow-up on the improvements in the distillation process of crude tall oil to yield pitch tall oil in order to enhance the recovery of both esterified and free fatty acids from tall oil pitch, and concomitantly facilitating the recovery of sterols that had been published earlier (Hughes, 1985). Diaz et al. (1999, 2001) based their methodology in repeated distillations over a previously dried raw material, until a current of sterols with the desired purity is obtained. A sterol concentrate with a level of purity of up to 96% can thus be obtained. Despite requiring about five successive distillations, hence bound to be costly, their process introduced the possibility of isolating all the neutral or unsaponificable compounds through distillation. Huibers et al. (2000) also based their approach for recovering phytosterols from crude tall oil in distillation. Given the higher boiling point of steryl esters, the process yield could be increased if thermal esterification was promoted prior to distillation of the crude tall oil (Fig. 5). The authors furthermore disclose details of specially designed pitch collecting apparatuses where fractionation may be carried out, as well as detailed information on the procedures for determination of the sterol content and sterol identification in the crude tall oil and in the residue.
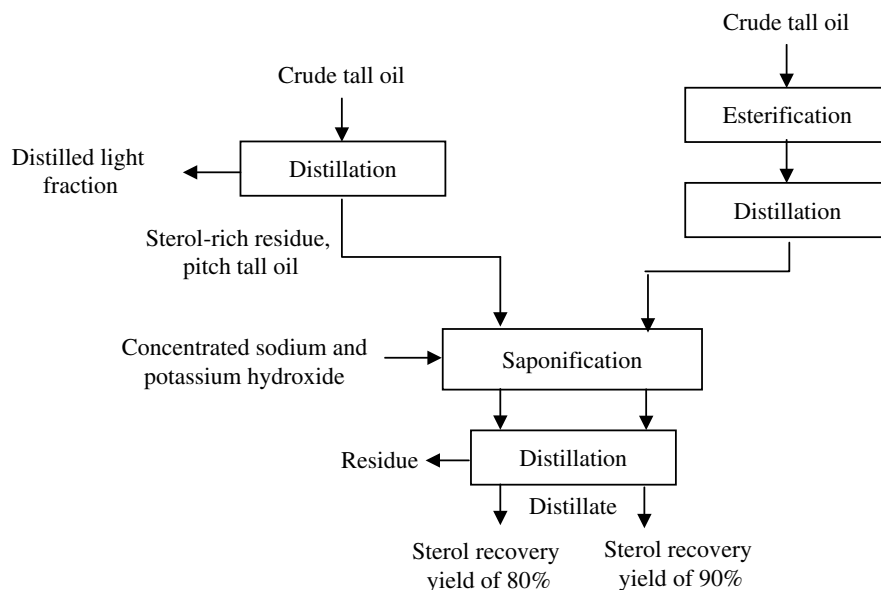
Fig. 5. Process for the recovery of phytosterols from crude tall oil according to Huibers et al. (2000). Esterification step prior to distillation improves final product yield.

It has been recognized that in order to enhance the removal of impurities that are bound to interfere with sterol crystallization from the extracted neutrals, a combination of various processes may be required (Hamunen and Ukkonen, 2000). The processes described in Fig. 6 were based in such concept. In the approach suggested by Coss et al. (2000) the recovery of phytosterols from tall oil soap or pitch is based in the preferential formation of a phytosterol/salt metal complex, which is hydrolyzed afterwards (Fig. 6a).

Wong et al. (1999) suggested pitch modification prior to evaporative fractionation in order to increase the content of free phytosterols and ease free phytosterol recovery (Fig. 6b). Schersl (2001) described a process quite similar to the one from Wong et al. (1999) but using an extraction step of the unsaponifiables following saponification. The process by Hamunen (2003) can also effectively recover and purify sterols from tall oil pitch or soap, involving a transesterification step (Fig. 6b). Schultz and Sonnier (2003) suggested a preliminary distillation of tall oil pitch in order to remove excess rosin acids and fatty acids. This distillation also makes the ensuing saponification step more efficient in terms of conversion of steryl esters to free sterols, and reduces the amount of alkali metal base required for saponification and the amount of acid required for the subsequent neutralization (Fig. 6b).

Hamunen and Ukkonen (2000) and Hamunen (2002) combined the extraction of unsaponifiables with a mixture of aliphatic and cycloaliphatic hydrocarbons with evaporative fractionation, in processes that use pitch or soap as raw materials (Fig. 6c).

Processes combining expensive extraction steps with evaporation fractionation, may prove effective for sterol recovery, yet they require considerable investments and operation costs of the separation processes tend to become very expensive (Hamunen and Ukkonen, 2000).

1.6. Other recovery processes of phytosterols

Recently Rohr (2003) and Rohr et al. (2005) presented a general methodology for the separation and recovery of valuable products, including unsaponifiable materials, from any given matrix of raw materials that is mainly composed of saponifiable components and unsaponifiable components. This includes all potential sources of phytosterols. The method comprises the conversion of sodium or potassium soaps obtained from the saponification of a raw material into metallic soaps, using a metal soap-forming compound. The mixture comprising metal soaps and unsaponifiable compounds is separated by distillation, to obtain a distillate comprising the unsaponifiable compounds and a residue comprising the metal soaps.

With their approach the authors aimed to overcome the drawbacks of current processes. In those based on solvent extraction often a reasonable separation between the unsaponifiable components and the fatty acid and the rosin acid soaps, requires the use of more than one solvent, usually in large proportion as compared with the amount of material extracted. In the overall, this increases the complexity of the recycling and increases the costs of the process. On the other hand, although the large difference of the boiling points of unsaponifiables and sodium (or potassium) organic acid soaps allows efficient separation, given the temperatures involved, the latter soaps are prone to melt, resulting in extremely viscous liquids. which makes handling difficult. Furthermore, the prolonged exposure at the high temperatures involved may compromise the output yield, given the thermal sensitivity of the valuable unsaponifiable products (Rohr et al., 2005). The overall outline of this approach is given in Fig. 7.

The use of sCO$_2$ for extraction of phytosterols was approached by González-Vila et al. (2000), following

a

Tall oil soap or pitch

↓

| Extractiun | ← Water:hexane:acetone

↓ Unsaponifiable fraction

| Evaporation | → Hexane:acetone

↓ Concentrated sterol rich residue

| Complexation | ← Ketone or petroleu ether
              ← Calcium chloride

↓ Precipitated sterol/salt metal complex

| Hydrolysis | ← Hot water

↓ Slurry

| Filtration |

↓

Sterol rich cake
Sterol purity of 80%,
Process yield of 90%

c

Tall oil pitch   Concentrated NaOH

↓   ↓

| Saponification |

↓ Unsaponifiable fraction

Tall oil soap →

Heptane or Aliphatic and cycloaliphatic hydrocarbons → | Extraction | ← C_6 to C_8 hydrocarbons

↓ Organic phase

Inorganic salts Soap residues ← | Washing with hot water (optional) |

↓ Organic phase

Solvents ← | Evaporation |

↓ Dried neutrals   Concentrated sterol rich residue

Light fraction ◄ | Distillation | ◄------
                                       Methanol

↓

Hydrocarbon/ methanol/water → | Crystallization |

↓

Sterol crystals
Sterol purity of 97.5% and process yield 80% (Hamunen
and Ukkonen, 2000) or sterol purity of 96.5% and process
yield of 70% (Hamunen, 2002)

b

Tall oil pitch

↓

Rosin and fatty acids ◄ | Distillation | Crude tall oil
                                             Tall oil pitch

Concentrated NaOH or KOH → | Saponification | ┄┄┄┄┄┄► | Drying | ┄► Water

Phosphoric acid → | Neutralization | → Water   ↓

Modified, sterol-rich pitch   Sterol-rich mixture | Trans-esterification | ◄ Sodium methoxide in methanol

↓

| Evaporative fractionation | → Distilled fatty and rosin acids

↓ Sterol-rich residue

| Evaporative fractionation | → Residue

↓ Distilled sterols

Short chain alkanols → | Crystallization |

↓

Sterol crystals
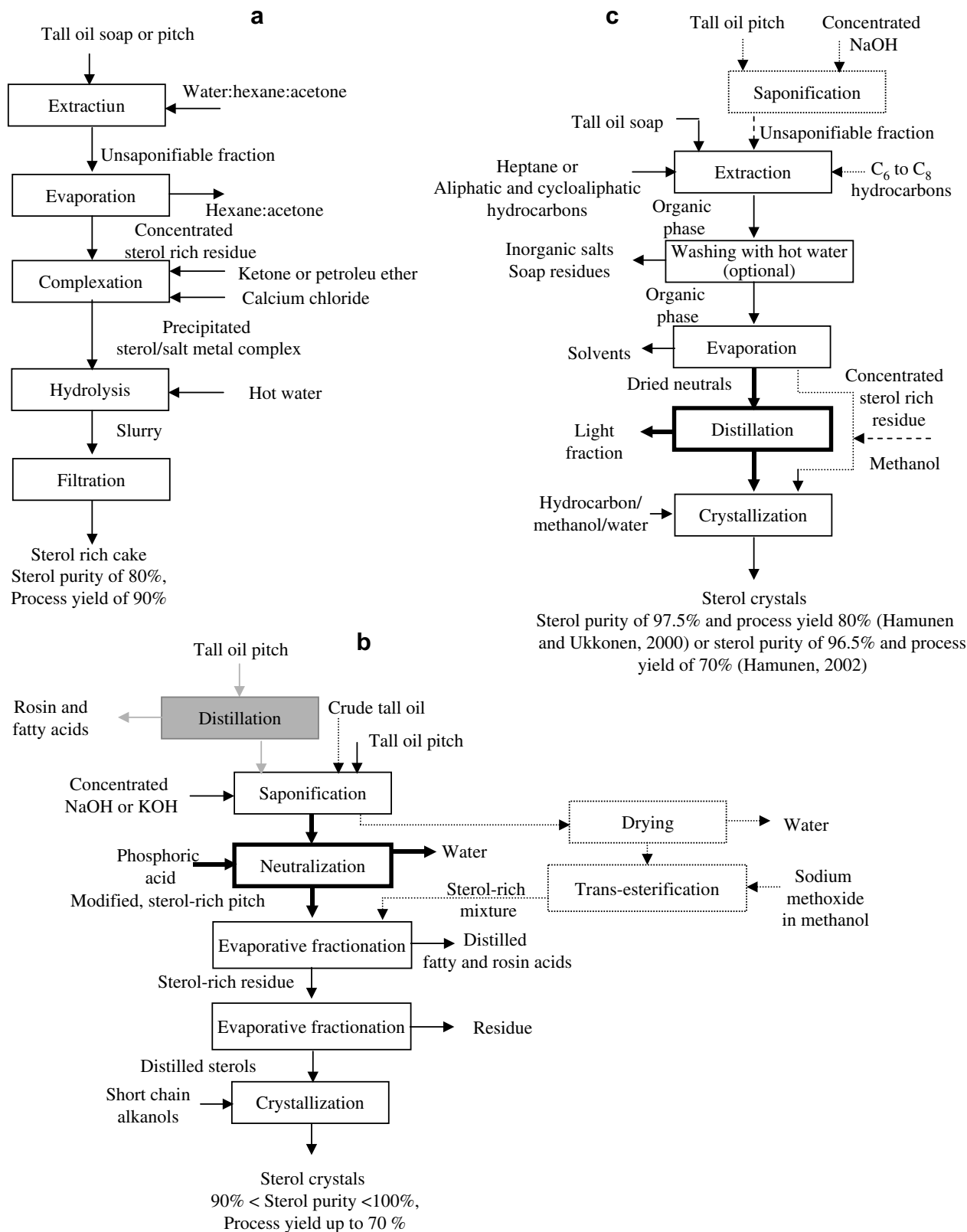90% < Sterol purity <100%,
Process yield up to 70 %

Fig. 6. Processes for the recovery of phytosterols from tall oil according to (a) Coss et al. (2000), (b) Wong et al. (1999), Hamunen (2003) and Schultz and Sonnier (2003), and (c) Hamunen and Ukkonen (2000) and Hamunen (2002). In (b) gray box and lines refer to processing steps according solely to Schultz and Sonnier (2003), bold boxes and lines refer to processing steps according to Wong et al. (1999) and Schultz and Sonnier (2003), whereas dotted boxes and lines refer to processing steps according solely to Hamunen (2003), all other being common to both approaches. In (c) bold boxes and lines refer to processing steps according solely to Hamunen and Ukkonen (2000) whereas dotted boxes and lines refer to processing steps according solely to Hamunen (2002), all other being common to both approaches.
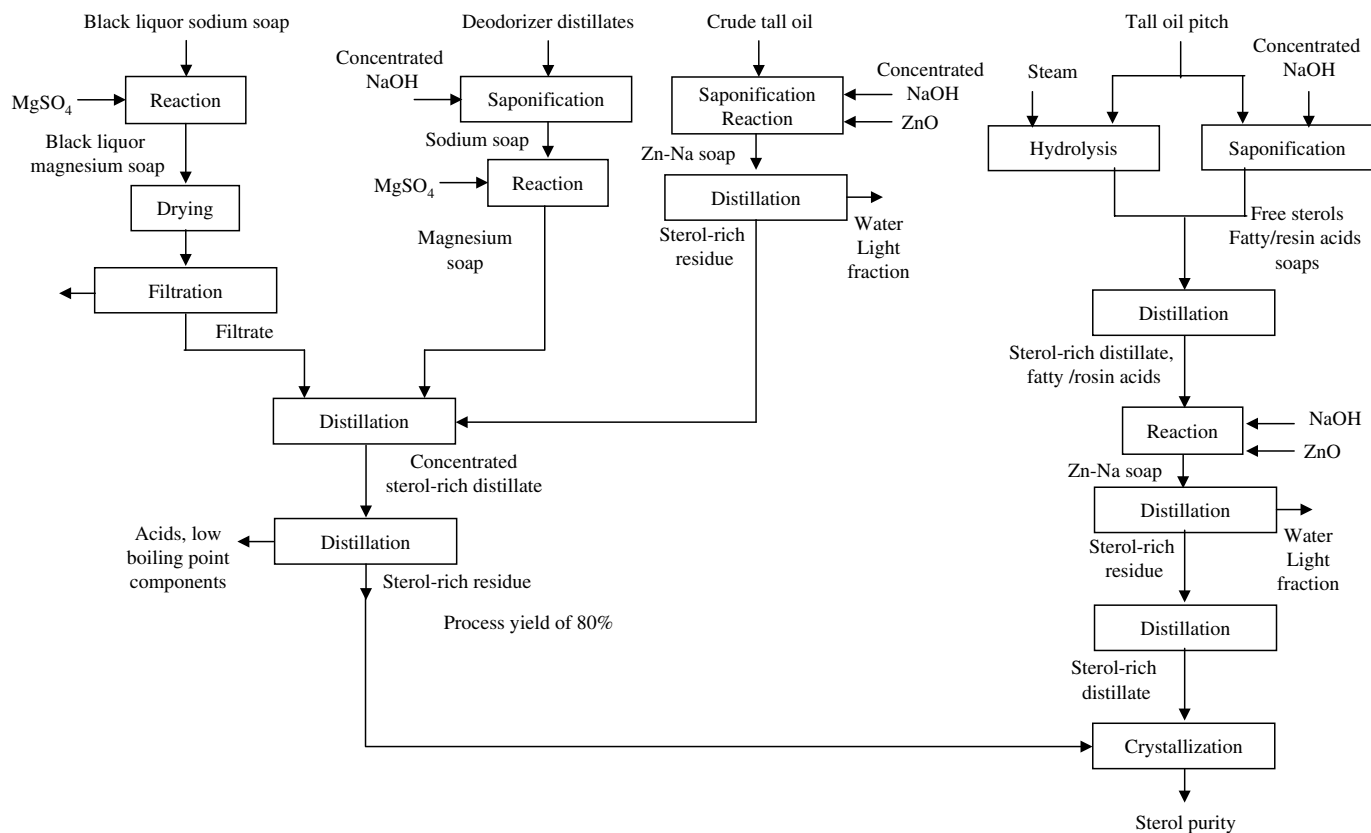
Fig. 7. Generic process for the recovery of sterols from several raw materials (adapted from Rohr et al., 2005).

promising results reported by Jansson et al. (1993). Supercritical extraction of sterols from eucalyptus wood chips matched a conventional Soxhlet extraction with acetone, and its effectiveness was enhanced when sCO$_2$ was used with methanol.

1.7. Phytosterol purification

Along with research focused in the overall process of recovery and purification of phytosterols from deodorizer sludge or tall oil pitch or other raw materials, several publications address individual steps within that complex process, aiming to enhance yield and/or simplify it. Moreira and Baltanás (2004) published recently a thorough evaluation on the influence of the relevant process variables on the yield and quality of phytosterols recovered from a deodorizer distillate by crystallization, after a transesterification step with ethanol. The semi-solid residue from this reaction, rich in phytosterols, was diluted in *n*-hexane. Best results, corresponding to a phytosterol recovery yield of 84%, for a purity of 36% was obtained when a 1:4 mass ratio of residue to hexane was used, with 2.5% ethanol as co-solvent for salting out the crystals, with a cooling rate of −20 °C h^{-1}, a crystallization temperature of −5 °C and a ripening time of 24 h. Detailed approaches to enhance yield of the crystallization step and concomitantly the purity of the obtained crystals have also been published (Sicre et al., 2004). It was also reported a processes for purifica-

tion of β-sitosterol from a sitosterol mixture of α and β-sitosterol by crystallization (Ukkonen and Simpura, 1981), to obtain a product with a α-sitosterol content of 0.5% for a process yield of 60%. Hamunen (1983) also described a process for β-sitosterol purification by crystallization from a sterol rich mixture isolated from the unsaponifiables of tall oil, the neutral substance extracted from the crude soap. The separation and purification of β-sitosterol and stigmasterol from phytosterol concentrates by solvent crystallization method was also addressed using *n*-pentanol and cyclohexanone as a solvent (Xu et al., 2005). A final product with a content of β-sitosterol over 85% was obtained after three stages of recrystallization in cyclohexanone, for a process yield of 39%. The latter could be increased to 54% albeit at the cost of a reduction in product purity to 75%. This pattern was more evident when the purification of stigmasterol from recrystallization in either *n*-pentanol or cyclohexanone was addressed, since a final product with stigmasterol content above 93% after five stages of fractional crystallization, with a process yield of 6–8%. The increase of the latter to roughly 50% led to a concomitant decrease in purity to about also 50%.

The isolation and recovery of β-sitosterol from a sterol mixture was described by Koskenniska (1981). The raw material is dissolved under reflux in a mixture of a polar organic solvent having the ability to form hydrogen bonds, water and either an aromatic hydrocarbon or a mixture of an aromatic and an aliphatic hydrocarbon. Cooling the

solution to 5–0 °C for 4 h, leads to the precipitation of a product with β-sitosterol content in excess of 90%.

Yoichiro and Takayuki (2000) described a process for purification of sterols from tall oil neutrals, with a sterol content of 45%. The neutral ingredients were contacted with methanol or isopropanol and water and heated to 90 °C or to 110 °C, respectively. After the precipitation of insolubles, which were drawn off, the mixture was cooled, so that crystals were formed. These were then washed and dried. The crystals of the tall oil treated with methanol had a purity of 96% for a recovery yield of 45%, whereas the crystals of the tall oil treated with isopropanol–water had a purity of 82% for a recovery yield of 87%.

Vincente et al. (2000), Robinson et al. (2000a) and Yan et al. (2000) also described a method for the isolation of the sterol component of the neutral fraction of saponified tall oil pitch. The goal is to control the growth of sterol crystals. Thus after saponification of the tall oil pitch and separation of the neutral fraction, the latter is dissolved in heptane and the mixture is blended with methanol, at a temperature above the temperature of crystallization of the sterol, usually above 50 °C, to which water at 70 °C or more is added, to result in two phases. The mixture is cooled to 25 °C, inducing the crystallization of sterols in the heptane phase. Sterol yield of 72% based in the amount of sterols in the neutrals and crystals with 98% purity were reported: An increase in the recovery yield to 80% led to a decrease in purity to 92% (Yan et al., 2000). In a variation of this process, the mixture of the two liquid phases is decanted and the water phase removed. The remaining upper organic phase is again washed with hot water, which is then removed. The remaining organic phase is allowed to cool down to produce sterol crystals. Sterol yield of 70% based in the amount of sterols in the neutrals and crystals with 98% purity were reported (Robinson et al., 2000b).

Purification of sterols from tall oil pitch feed mixture with a sterol content of 20–40%, along with undesirables by the selective adsorption of sterols onto activated-type carbon sieve, was described by Barder (1989). Undiserable products comprehended di- and triterpene alcohols, fatty alcohols, diterpene aldehydes, squalene and polymeric materials. Desorption was achieved by elution with chlorobenzene. A feed with 25% sterols (about 14% β-sitosterol) was concentrated to yield a product with a overall sterol purity of 78% (51% β-sitosterol) with 90% recovery (more then 99% β-sitosterol).

The successful separation of β-sitosterol from campesterol has been performed by distillation (Clark et al., 1977) and by selective adsorption, using zeolite NaY as molecular sieve (Berezin et al., 2001). In the former approach the ratio of sitosterol to campesterol increased 1.5-fold after processing, but process yield allowing for this was below 50%. In the latter, a sterol concentrate containing a mixture of sitosterol:campesterol:stigmasterol (59:34:7) was enriched in sitosterol. After treatment with zeolite a mixture of sitosterol:campesterol:stigmasterol (74:16:10) was obtained.

2. Conclusions

The beneficial role of phytosterols in health, pharmaceutical and food and feed sectors is being well established and research is also focused in understanding the phenomena underlying their positive action. Thorough research efforts have been recently made, mostly by the industrial sector, in order to develop cost effective approaches to efficiently recover phytosterols from wastes of the vegetable oil and cellulose branches, and to obtain pure crystals from the resulting sterol concentrate slurries, in high yield processes. Most of these approaches involve conventional separation processes, namely solvent extraction, distillation and evaporative fractionation, saponification and chemical (trans)-esterification, aiming to reach cost-effective processes, with reduced environmental impact. Enzyme induced esterification has been recently tried out and proved effective. This finding, coupled to the encouraging results obtained when direct extraction of sterols from plants using supercritical fluid technology was tentatively explored, suggest that methodologies for phytosterol recovery from natural sources are foreseen to increasingly incorporate a green chemistry based approach. More environmentally friendly methodologies are thus expected as an outcome of research.

Acknowledgements

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