Recovery of Phytosterols from Sunflower Oil Deodorizer Distillates

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ABSTRACT: Phytosterols are usually recovered by crystallization from the deodorizer distillate (DD) of vegetable oils. In this work, the impact of the principal process variables (*viz.*, solvents and cosolvents, cooling rate, crystallization temperature, and ripening time) on the quality and yield of the recovered phytosterols was studied by using a sunflower oil DD "enriched" (i.e., preconcentrated) *via* transesterification with ethanol (EDD) as a feedstock and commercial hexane as solvent (S), with S/EDD mass ratios of 3 to 5. Water (0 to 4.5 wt%) and ethanol (0 to 10 wt%) were used as cosolvents, with crystallization temperatures between 0 and –20°C and crystallization times from 4 to 96 h. The cooling rate was either –20°C/h or "brisk chilling" from 40 to –5°C. The nature and composition of the EDD solvent and cosolvent composite arose as the most important process variable, strongly influencing both the percentage of sterol yield and the purity of the crystals, as well as their filterability and washability. Water-saturated hexane sufficed to give good crystallization, yet the beneficial effect of adding water as the single cosolvent was enhanced by adding small and precise amounts of ethanol. A recovery of sterols as high as 84% (with 36% purity) was achieved by using a single-stage batch crystallization of the S/EDD mixture (S/EDD = mass ratio 4).

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KEY WORDS: Deodorizer distillates, phytosterol recovery, sunflowerseed oil.

During deodorization of edible vegetable oils, substances that usually give them a bad taste and/or foul odor are removed *via* steam-stripping distillation, along with tocopherols (vitamin E), phytosterols, FFA, TG, DG, and hydrocarbons. A valuable by-product, generally known as deodorizer distillate (DD), is then generated (1,2). The widespread recognition of vitamin E in human health, as well as the demand for phytosterols for manufacturing semisynthetic hormones or for their direct consumption as such (3,4), has led to numerous methods for recovering tocopherols and sterols from DD. Because these methods are customarily protected by patents, technical details about them are scarce (5,6).

The removal of undesirable volatile compounds from these DD is a relatively simple task, which can be accomplished *via* molecular distillation at reduced pressure and fairly low temperature (≈1 Torr and 120°C, respectively), with the optional aid of steam or nitrogen injection. FFA also can be distilled from DD ($P < 1$ Torr; $T \leq 220$°C), or can be first transformed into more volatile compounds such as methyl or ethyl esters. The advantage of this latter option is not only a reduction by almost 20°C in the evaporation temperature, but also the possibility of reducing or entirely eliminating DG and TG from the mixture through their previous transesterification with aliphatic low-carbon number alcohols (7,8).

Some other processes have addressed the separation problem by saponifying the fatty matter, followed by solvent extraction of tocopherols and sterols from the semisolid mass in an organic phase (9,10). Still other methods, based on successive liquid–liquid extractions with solvent mixtures of increasing polarity, have been developed. They allow the fractionation of the DD into three streams: tocopherols and sterols, compounds with higher polarity than the former (e.g., FFA and odorous compounds), and less polar compounds (hydrocarbons, TG, etc.) (11). Finally, enzymatic methods have been put forward in the last decade to purify and/or preconcentrate DD to recover tocopherols and phytosterols (12,13).

Whichever process is used to remove undesirable substances from DD, thus obtaining a deodorizer distillate enriched in tocopherols and sterols, the separation between both classes of compounds is usually performed *via* fractional crystallization. The information available in the open literature on the impact of the principal process variables on this particular crystallization process is practically nonexistent. In this work we present a detailed study focused on the enriched DD (EDD) of sunflower oil, a premium vegetable oil widely consumed in Argentina.

EXPERIMENTAL PROCEDURES

Materials. Sunflower oil deodorizer distillate (DD) was provided by Aceitera General Deheza S.A. (Gral. Deheza, Argentina). The FFA and TG of the DD were transformed into FA ethyl esters using 37 wt% HCl as homogeneous catalyst (6:1 molar ratio of ethanol/TG; 2 wt% HCl on the basis of TG; 78–80°C and 12 h). After the reaction equilibrium was reached, the whole mass was evaporated under vacuum. Two overhead fractions were removed: HCl, ethanol, and water in a first cut ($T \leq 80$°C; 25 mm Hg), followed by odorous compounds and FA ethyl esters in the second fraction ($T \leq 200$°C; 1 mm Hg).

The product left in the bottom, a DD enriched in tocopherols and sterols, was semisolid at ambient temperature. Its main

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TABLE 1
Relevant Components of the Crude (DD) and Enriched (EDD) Sunflower Deodorizer Distillate

Class of compounds	DD	EDD
Saponification number (AOCS Cd 3b-76)[a]	116	93.2
Acid value (AOCS Cd 3a-63)[a]	89.1	<0.01
TG (as glyceryl trioleate)	16.3%	49.1%
FFA (as oleic acid)	45%	<0.01%
C18:1 + C18:2	—	25%
Tocopherols		
(GLC)[b]	6.0%	9.9%
(HPLC)[c]		10 %
Sterols		
(GLC)[b]	5.1%	9.3%
(HPLC)[c]		8.7%
Squalene		
(GLC)[b]	—	3.5%
Balance	28%	28%

[a]Reference 14.
[b]Relative SD correlation coefficient (RSD_r) = 5%.
[c]RSD_r = 4%.

characteristics, together with the most relevant compositional data of this sunflower oil DD, are detailed in Table 1.

Commercial hexane was a petrochemical cut customarily used in solvent extraction in Argentina. Ethanol 95/96° was USP grade. Double-distilled, deionized water was used as reagent, washing agent, and crystallization cosolvent. Standards of palmitic, stearic, oleic, and linoleic acids and their ethyl esters; α-, β-, γ-, and δ-tocopherol; β-sitosterol; stigmasterol; and squalene were from Sigma Chemical Co. (St. Louis, MO).

Analytical methods. The DD and EDD were characterized using AOCS recommended practices Cd 3b-76 and Cd 3a-63 (14). The contents of FFA, FA ethyl esters, sterols, and tocopherols were quantified by GLC using a Chrompack WCOT TAP 25 m × 0.25 mm column (Varian Inc., Palo Alto, CA). Analytical conditions were as follows: splitless injection; 300°C injector port; 370°C FID detector; H_2 (90 psig) carrier gas; temperature program of 1 min at 80°C, followed by heating to 150°C at 15°C/min and then to 350°C at 20°C/min.

HPLC was used to quantify tocopherols following AOCS Method Ce 8-89 by using a Silica A/10 250 × 4.6 mm column (PerkinElmer Analytical Instruments, Shelton, CT). Analytical conditions were as follows: UV/vis detector (292 nm); mobile phase: hexane/isopropanol 97.5:2.5 vol/vol, 1 mL/min flow rate; 30°C oven temperature; 10 μL injection volume. Quantitative determination of sterols was also performed by HPLC with a Spherisorb 5 μm 250 × 4.6 mm column (Waters Corp., Milford, MA). Analytical conditions were as follows: UV/vis detector (206 nm); mobile phase: methanol/water 99:1 vol/vol, 1 mL/min; 30°C oven temperature; 10 μL injection volume (15).

Selection of crystallization conditions. The precipitation of any given substance from a solution by crystallization is a direct consequence of its supersaturation, which can be achieved by solvent evaporation or by changing temperature if the solubility of the desired substance is temperature-dependent. A third mechanism used to cause crystallization is to add a cosolvent, which together with the primary solvent generates a mixture in which the substance is no longer soluble (a procedure known as "drawing-out" or "salting-out"). Likewise, the formation of adducts, hydrates, and so on, can lead to the precipitation of the compound if the new product is less soluble than the original (16).

The main process used to separate phytosterols from DD is by cooling or chilling the solution after a suitable solvent has been added. The incorporation of cosolvents should have an impact as well, owing to either salting- or drawing-out or to adduct formation. In this work, we analyzed the effect of diluting a sunflower oil EDD with hexane as a primary solvent (S), because hexane is generally used in vegetable oil extraction plants and refineries. The influence of cosolvents (types, amount, and the combination of these), cooling rate, final crystallization temperature, and maturation time in terms of yield and/or purity of the recovered sterols was also studied. The experimental variables were selected based on practical or theoretical considerations given previously (17–19). A single-stage process was studied.

S/EDD mass ratio. In prior works, S/DD ratios from 30 to 1 were used. However, for any given (practical or economical) crystallization temperature, higher ratios led to lower recoveries. With soy oil, Sheabar and Neeman (6) found S/DD = 6 to be an optimal ratio. Likewise, Hunt *et al*. (20) reported using an S/DD ratio between 2 and 5, but the specific results of their work were not disclosed in their patent. From an industrial point of view, the lowest S/DD ratio is the most desirable because it minimizes both the mass to be cooled and the amount of solvent to be recycled. Even so, our preliminary tests showed that upon cooling, S/EDD mass ratios of 1 or 2 led to a semisolid mass of low fluidity. Therefore, we chose to begin with an S/EDD ratio = 3 and then to explore higher mass ratios until product properties and/or process performances were poor.

Cosolvents. Water and ethanol, both mentioned in the patent by Brown and Smith (11), were investigated as cosolvents. The choice of water was reasonable, as sterols tend to form hydrates, which reduces their solubility. Complementarily, ethanol acts by increasing the solubility of water in hexane, thus enhancing its role in hydration. Methanol is another choice, but its higher polarity (compared to ethanol) limits the amount of water than can be dissolved in hexane, as shown by hexane/methanol/water and hexane/ethanol/water phase-equilibrium diagrams (21).

Up to 4.5 wt% water (0.00, 1.50, 2.25, 3.00, and 4.50 wt% levels) and up to 10 wt% ethanol 96° (0.00, 1.25, 2.50, 5.00, 7.50, and 10.00 wt% levels) with respect to hexane were added. Invariably, two phases were formed. One of them was hexane-rich, with low mass fractions of water and ethanol, containing almost the whole mass of the system. The heavier phase, just a few drops, was polar and almost devoid of hexane.

Crystallization (ripening) temperature (T_C). A broad range of crystallization temperatures (from +5 to –20°C) has been tried in the literature. The lower the T_C, the lower the concentration of remaining sterols in the solution will be; thus, higher yields should be expected. Yet other solutes can also precipitate upon lowering the ripening temperature, and less pure crystals will be

TABLE 2
Mass Percentage of EDD Recovered, Sterol Purity, and Percentage of Sterol Recovery Using Different Cosolvents, Solvent (S)/EDD Ratios, Crystallization (ripening) Temperatures, and Cooling Rates

		Cosolvent		Cooling	Crystallization			Solid	Sterols	
Test	S/EDD[a] (w/w)	Ethanol (wt%)	Water (wt%)	rate (°C/h)	T_C[b] (°C)	Onset (°C)	Crystal morphology[c]	phase (wt%)[d]	Purity (%)	Recovery[e] (%)
Effect of S/EDD mass ratio										
1	3	0	0	20	–5	3–5	P	2.5	81.5	21.9
2	3	0	1.5	20	–5	8–9	P	6.8	81.1	59.2
3	3	0	2.25	20	–5	8–9	F	7.3	79.9	62.6
4	3	0	3	20	–5	18–19	F	7.5	79.8	65.2
5	3	0	4.5	20	–5	18–19	F	7.6	77.6	63.4
6	3	1.25	0	20	–5	5–6	P	2.7	78.9	23.0
7	3	1.25	1.5	20	–5	7–9	P	14.4	48.7	75.4
8	3	1.25	2.25	20	–5	7–9	F	13.5	50.2	72.9
9	3	1.25	3	20	–5	15–18	F	18.6	40.7	81.3
10	3	1.25	4.5	20	–5	15–18	F	14.1	44.9	68.1
11	3	2.5	0	20	–5	5–6	P	2.9	82.4	25.7
12	3	2.5	1.5	20	–5	7–8	P + F	4.8	80.6	41.6
13	3	2.5	2.25	20	–5	14–15	F	5.0	81.9	44.0
14	3	2.5	3	20	–5	14–15	F	5.1	82.8	45.4
15	3	2.5	4.5	20	–5	14–15	F	8.2	69.5	61.3
16	3	5	0	20	–5	2–4	N	2.6[f]	75.8	21.2
17	3	5	1.5	20	–5	3–5	F	1.9	84.7	17.4
18	3	5	3	20	–5	0–1	F	3.8	80.0	32.7
19	3	7.5	0	20	–5	0–2	N	2.9[f]	73.4	23.0
20	3	7.5	1.5	20	–5	3–5	F	2.1	67.7	15.3
21	3	7.5	3	20	–5	3–6	P	NF[g]	—	—
22	3	10	0	20	–5	–2–1	N	NF	—	—
23	3	10	1.5	20	–5	–3–1	F	NF	—	—
24	3	10	3	20	–5	–4–2	P	1.3[f]	85.4	12.0
25	4	0	0	20	–5	3–4	P	6.2	67.8	45.2
26	4	0	1.5	20	–5	6–7	P + F	9.2	63.5	62.8
27	4	0	3	20	–5	15–16	F	10.7	59.9	68.9
28	4	0	4.5	20	–5	15–17	F	12.9	56.1	74.8
29	4	2.5	0	20	–5	0–3	P	15.9	36.4	83.9
30	4	2.5	1.5	20	–5	4–5	P + F	15.3	41.2	67.7
31	4	2.5	4.5	20	–5	4–5	F		46.6	64.7
32	4	5	1.5	20	–5	0–2	F		38.7	66.1
33	4	5	3	20	–5	–1–2	F	9.4	62.9	63.6
34	5	0	0	20	–5	0–1	P	5.7	67.4	41.3
35	5	0	1.5	20	–5	15–16	F	7.8	77.6	65.1
36	5	0	3	20	–5	15–16	F	7.7	68.4	57.4
37	5	2.5	3	20	–5	5–6	F	5.9	77.6	50.1
Effect of ripening temperature (combine with tests 1–18)										
38	3	0	0	20	0	3–5	P	4.1	81.0	35.7
39	3	0	1.5	20	0	8–9	P	7.1	85.2	65.1
40	3	0	3	20	0	18–19	F	6.9	75.4	56.2
41	3	2.5	1.5	20	0	7–8	P + F	5.7	82.9	50.8
42	3	2.5	3	20	0	13–14	F	6.2	78.3	52.2
43	3	2.5	4.5	20	0	13–14	F	7.6	63.5	51.9
44	3	5	1.5	20	0	5–6	F	1.4	—	—
45	3	5	3	20	0	0–1	F	3.6	71.6	27.7
46	3	0	0	20	–10	3–4	P	6.9	58.9	43.7
47	3	0	1.5	20	–10	8–9	P	7.4	68.4	54.4
48	3	0	3	20	–10	17–19	F	8.5	63.6	58.1
49	3	2.5	1.5	20	–10	7–8	P + F	NF	—	—
50	3	2.5	3	20	–10	13–14	F	NF	—	—
51	3	2.5	4.5	20	–10	13–14	F	6.0	61.7	39.8
52	3	5	1.5	20	–10	5–6	F	NF	—	—
53	3	5	3	20	–10	0–1	F	NF	—	—
Effect of cooling rate (combine with tests 1–14)										
54	3	0	1.5	∞ (“brisk”)	–5	NA		7.4	66.7	53.1
55	3	0	3	〃	–5	NA		28.3	24.1	73.3
56	3	0	4.5	〃	–5	NA		26.8	24.0	69.1
57	3	1.25	2.25	〃	–5	NA		20.8	31.9	71.4
58	3	1.25	4.5	〃	–5	NA		23.0	26.0	64.4
59	3	2.5	3	〃	–5	NA		13.8	48.5	71.9

[a]S (hexane)/EDD mass ratio. For abbreviation see Table 1.
[b]T_C, ripening temperature. In all cases, a ripening time (θ_C) of 24 h was used.
[c]P, planar (thin platelets, l ≤ 200 μm); N, needlelike (aciculate, d ≤ 50 μm, l ≤ 500 μm); F, flocs.
[d]Weight percent of the initial mass of EDD.
[e]Shown as SR% in the text.
[f]Hard to filter.

then obtained. In addition, refrigeration costs become appreciably higher the lower the T_C is made. On these grounds, we chose –5°C as a first, mild condition and later proceeded to testing at 0 and −10°C those mixtures of solvents and cosolvents (i.e., hexane and ethanol/water) with the EDD that had been promising in the first set of experiments. A constant mass ratio, S/EDD = 3, was maintained throughout these trials.

Cooling rate. We tested two different cooling procedures, both applicable in a real process plant: "slow cooling" (–20°C/h), which is typically used in batch processes, and "brisk chilling" down to the ripening temperature.

Crystallization (ripening) time (θ_C). A crystallization time of 24 h was taken as our base condition. Next, a new series of tests with θ_C = 4, 6, 8, 12, 24, and 96 h was used for those combinations of solvents and cosolvents that had given better percentages of solid (and sterol) recoveries and that, at the same time, had shown good or acceptable filterability.

Experimental procedures. Five grams of EDD, preheated to 60°C, was poured into screw-capped 70-mL culture test tubes (25 × 200 mm); hexane, ethanol, and/or water was added as needed. Each condition was tested in triplicate. The tubes were placed into a cryothermostatized bath at 40°C and stirred until the mixture was completely homogenized, after which the cooling program was started, at –20°C/h, down to the final ripening temperature (T_C). When brisk chilling was simulated, the tubes containing the homogenized mixture were transferred to a second bath kept at –5°C. In each case, the tubes were agitated every minute until T_C was reached and every 15–20 min during the following 2 h, after which the tubes were kept in the bath for another 20 h, except in those tests where the impact of crystallization time was studied.

Crystal recovery was performed by vacuum filtration by using Büchner funnels and 47 mm diameter, 0.2 µm pore diameter cellulose acetate membranes supported on filter paper (Whatman S 42, 2.7 µm nominal pore diameter). The content of the test tubes was discharged onto the filtering membrane and the retained crystals were washed with 10 mL of hexane, at –5°C. For some of the experimental conditions, the permeability of the filtering cake was low (or nil) owing to the presence of a gelatinous mass, which in some cases made filtration impossible. Those conditions are labeled NF (not filterable) in Tables 2 and 3, where the full set of experimental conditions and results is detailed. The filtering membrane and the crystal cake were dried *in vacuo* (60°C, 25 Torr, 6 h) and then weighed. Both the solid and the liquid (filtrate) phases were analyzed by GLC and/or HPLC.

The mass of crystals obtained after each crystallization (expressed as the weight percentage of EDD introduced into the test tubes), the percentage of sterols in these crystals (sterol purity), and the percentage of sterol recovery (SR%, the mass ratio of the sterols recovered in the solid phase divided by the amount originally present in the EDD, times 100) are indicated in Tables 2 and 3, together with the experimental conditions of each run and the temperatures at which the onset of crystallization was perceived. The values shown in the tables are the means of three replicates; the CV were always less than 10.5%.

TABLE 3
Mass Percentage of EDD Recovered, Sterol Purity, and Percentage of Sterol Recovery Using Different Crystallization (ripening) Times[a]

Test	$(\theta_C)^b$ (h)	Solid phase (wt%)	Sterols Purity (%)	Sterols Recovery (%)
60	4	9.4	66.8	67.5
61	6	12.2	55.4	72.7
62	8	9.0	62.9	60.9
63	12	10.2	63.5	69.6
64	24	9.5	60.2	61.5
65	96	10.0	60.5	65.1

[a]S/EDD mass ratio = 3; cosolvents: ethanol = 0 wt%, water = 1.5 wt%; cooling rate = –20°C/h. In all cases, crystallization temperatures of –5°C were used. Onset of crystallization was 8–9°C and crystals were planar in morphology (see footnotes *c–e* in Table 2).
[b]θ_C, ripening time.

Where judged appropriate, Tukey's Studentized range test (22) was used to determine significance of differences among means.

RESULTS AND DISCUSSION

Effect of cosolvents. In the absence of water, easily sedimenting, free, needlelike, or planar crystals were observed, but flocs, or "cumuli," began to appear when water was added as cosolvent. These aggregates grew in both size and quantity when higher amounts of water were added, and they eventually clouded (and uniformly filled) the entire volume. The morphology of the crystals—planar (P), needlelike (N), or the presence of flocs (F)—is also given in Tables 2 and 3.

The addition of ethanol as the single cosolvent slightly increased the amount of crystallized solids and the SR% as compared to pure hexane (16 and 17.3%, respectively); this is apparent by direct inspection of tests 1, 6, 11, 16, 19, and 22 in Table 2. Nonetheless, upon increasing the amount of alcohol, a gelatinous precipitate was formed that was almost impossible to filter.

Conversely, the addition of water as the only cosolvent (tests 1–5) noticeably augmented the yield (by more that 200%) with respect to pure hexane. Furthermore, in every case the cake of crystals retained in the membrane was easily filterable, and a clean, off-white mass was left. The SR% surpassed 65%, with sterol purities between 77.6 and 81.1% regardless of the percentage of water. From a statistical point of view, however, the increase in sterol purity upon addition of water was not significant ($P < 0.05$), whereas both the amount of crystallized solids and the SR% were significantly higher than in the absence of water.

The amount of crystallized mass did not increase in the different S/DDE mixtures prepared by adding both cosolvents to the hexane, except in those cases where 1.25% of alcohol was used. In these cases, whatever the amount of water added, a maximum percentage of precipitate was obtained (about 14% of the initial mass of EDD), which was almost double the percentages obtained with 0 and 2.5% ethanol (*cf.* tests 2–5, 7–10,

and 12–15 in Table 2). Conversely, the purity of the crystals did not improve (40.7–48.5%); rather, this was far below the values found using only hexane (81.5%) or with the addition of either ethanol or water as the single cosolvent (75–82.5%).

Figure 1 compares the mass percentage of crystals obtained from the EDD, the percentage of sterols recovered, and their purity as a function of the contents of alcohol and/or water in hexane. No significant ($P < 0.05$) differences in SR% or sterol purity were found among the precipitates obtained upon addition to the hexane of 1.25% ethanol together with various amounts of water. One can assume, then, that the presence of water is essential for obtaining a good yield of fairly pure sterol crystals, with a morphology appropriate enough to allow their easy filtering/washing. Yet our results revealed a certain insensitivity regarding the percentage of water incorporated into the mixture. Because drops of water were consistently observed to coalesce at the bottom of the tubes, we judged that it was sufficient to use water-saturated hexane to achieve good crystallization.

Last, for the samples with nil to moderate percentages of ethanol added to the mixture, the temperature indicating the onset of crystallization consistently increased as higher amounts of water were incorporated as cosolvent, whereas for 10 wt% ethanol, the onset temperature did not change appreciably, irrespective of the amount of water added. Additionally, the crystals formed in the absence of alcohol were planar (thin platelets) and changed to needlelike—and then to flocs—upon increasing the percentage of alcohol added as cosolvent.

These combined results led us to eliminate from the experimental grid the ethanol/water cosolvent combinations that had produced barely filterable crystallisates (namely, ethanol percentages higher than 5 wt%) or those where reproducibility was poor, owing to their excessive sensitivity to small variations in cosolvent composition (around 1.25 wt% ethanol).

Effect of the S/EDD mass ratio. Tests 25–33 and 34–37 determined the percentages of crystallized solids, sterol purity, and SR%, with respect of the amount originally present in the EDD, for the S/EDD mass ratios = 4 and 5, respectively. Taking into account only the hexane/water mixtures (tests 1–5, 25–28, and 34–36), one can observe that by using S/EDD = 4 the mass of crystallisate was consistently higher (63 to 148% greater) than the amount of solids obtained with an S/EDD mass ratio of 3. A higher S/EDD mass ratio also increased the SR%. This increment ranged from 18 to 106% with respect to the SR% reached using S/EDD = 3. Unfortunately, some other (unidentified) compounds also precipitated for this S/EDD mass ratio, thus reducing the purity of the sterols obtained. Conversely—as was foreseeable—the S/EDD mass ratio of 5 led to lower sterol recoveries, although crystal purity improved consistently.

Influence of cooling rate. Several solvent–cosolvent/EDD combinations were tested to analyze the impact of this process variable. When the test tubes, previously tempered at 40°C, were immersed (under vigorous agitation) into a thermostatic bath kept at –5°C, the S/EDD mixture changed from fluid and clear to semisolid and opaque with fine grains in about 1 min. The tubes were then allowed to rest for 24 h (still inside the –5°C bath), after which their contents were filtered and washed

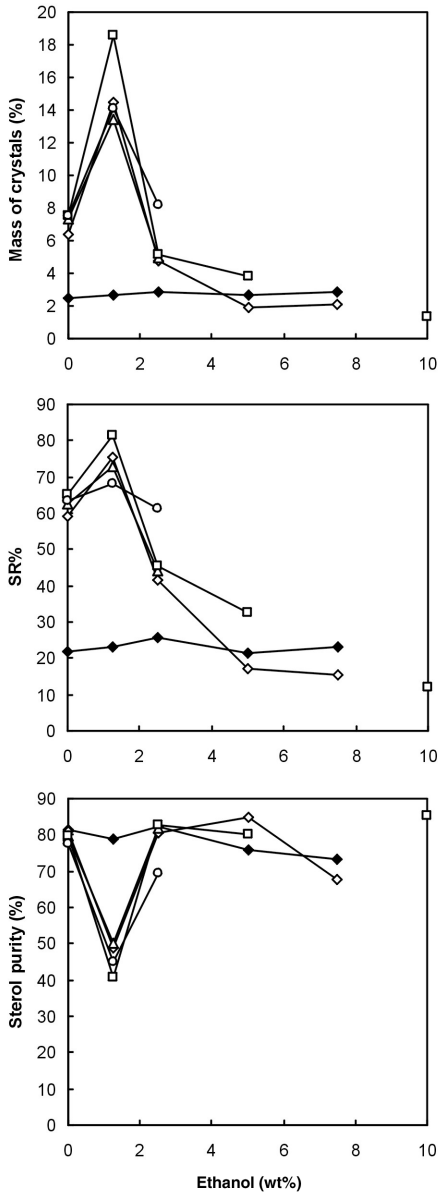

FIG. 1. Amount of crystallized solids [as wt% of enriched deodorizer distillate (EDD)], percentage recovery of sterols (SR%), and percentage of sterols in the crystallisate (sterol purity) obtained using different combinations of cosolvents [mass ratio of hexane to EDD, S/EDD = 3; crystallization temperature (T_C) = –5°C; crystallization (ripening) time (θ_C) = 24 h]. The values presented are means of three replicates; the CV were consistently less than 10.5%. Symbols (wt% water): ◆ = 0; ◇ = 1.5; △ = 2.25; □ = 3.0; ○ = 4.5.

(see tests 53–58 in Table 2, which are equivalent in S/EDD composition to tests 2, 4, 5, 8, 10, and 14, respectively). In general, the cakes were voluminous and had low permeability as a consequence of the small size of the crystals, which hindered displacement of the mother liquor. This explains the high percentage of solids retained in the filtering membrane and their low purity as well, although the SR% was similar to that obtained under equivalent working conditions (namely, solvent types and S/EDD mass ratios) in the slow cooling procedure (–20°C/h).

Influence of ripening temperature (T_C). As already pointed out, some of the S/EDD mixtures–in general rich in water and with no or a low content of alcohol—began to crystallize above 10°C, and even close to 20°C. This observation opened questions about the impact of T_C on the yield (and economy) of the process. To further explore whether it was possible to decrease supersaturation by using a higher ripening temperature without sacrificing the percentage yield of sterols, two additional sets of tubes were prepared, using the hexane/water–ethanol solvent/cosolvent mixtures that had given "clean" cakes, good or excellent drainability, and ease of filtering in the previous experiments. The new mixtures (see tests 38–45 and 46–53) were cooled from 40°C to either 0 or –10°C (at –20°C/h), under agitation, and then left to stand for another 24 h at each of these final T_C. Last, the crystals were separated by filtration and handled as already described.

In general terms, the percentages of solids recovered by ripening at either 0 or –10°C did not differ substantially from those obtained at –5°C. Thus, one can assume that within the range of the experimental conditions tested, the crystallization/separation of sterols from the EDD was rather insensitive to the final tempering temperature. Nevertheless, when cooling to –10°C, it was possible to test only a limited range of cosolvent combinations because the precipitate became unfilterable. Also, for about the same SR% (*viz.,* 60–65 wt%, using 1.5 wt% water as the only cosolvent), better crystal purity was achieved by cooling to only 0°C instead of –5°C: 85.2 vs. 78.9% (see tests 39 and 2, respectively). These combined values of sterol purity and degree of recovery were the highest we achieved by using this single-stage laboratory process.

Despite the narrow range of process conditions studied, which were purposely chosen to improve crystal quality and SR% in the subset of samples cooled to –10°C, one of the samples (test 51) was quite hard to filter, whereas others (tests 49, 50, 52, and 53) could not be filtered at all. At this low ripening temperature, hard-to-wash, "dirty" cakes were obtained in most of the samples, except when ethanol was not added. These features, together with the low SR% and the poor purity of the crystals compared to those obtained at –5°C, were telltale signs of the coprecipitation of unwanted substances (which were not identified further). Thus, it seemed advisable not to chill the S/EDD any further.

Influence of ripening time (θ_C). To study the effect of this variable on process performance, another set of tubes was prepared using S/EDD = 3 and 1.5 wt% water as the single cosolvent and cooling from 40 to –5°C (at –20°C/h), with θ_C from 4 to 96 h (tests 60–65, Table 3). In all cases, yields and purities of the crystals were about the same. The filterability and washability of the cakes was also similar. Therefore, it follows that, within the span of experimental conditions used, there were no compounds in the EDD whose crystallization was time-dependent, and foremost, that 4 h of ripening was sufficient.

The nature and composition of the EDD solvent and cosolvent composite, from which mixture the sterols could be recovered by crystallization, arose as the most important process variable, strongly influencing both the SR% and the purity of the crystals, as well as their filterability and washability. This was found irrespective of the ripening temperature conditions used. The impact of adding water as a cosolvent and modifier of sterol solubility was remarkable. Water can lead to significant improvements in sterol yield and quality, presumably because the onset of crystallization can be changed greatly by adding different amounts of water. Furthermore, the beneficial effect of adding water as the single cosolvent can be enhanced by adding small and precise amounts of ethanol.

As for the impact of diluting the EDD with solvent(s), our results indicate that better recoveries of sterols can be achieved by using an S/EDD mass ratio = 4. However, the somewhat lower sterol yield obtained with a lower ratio (e.g., S/EDD = 3) should be evaluated economically (within the context of the additional mass of solvent that must be chilled and later recovered *via* evaporation) considering the purity of the crystals as well. Purity was higher when the dilution of EDD was lower. On the other hand, for any given T_C the use of a higher S/EDD mass ratio (5 or more) was not convenient, as recovery did not improve compared with lower dilutions. Again, simple economic considerations make the use of additional amounts of solvent objectionable owing to the detrimental impact of cooling and evaporation costs.

Brisk chilling led to low sterol yields and to contaminated (impure) cakes, which would require further purification stages. It is desirable to keep $T_C \geq -5$°C for a single-stage crystallization process, although it would be advisable to study the impact of higher ripening temperatures for the recovery of sterols in cascade since, for certain solvent combinations, the first crystals had already appeared at 18–19°C.

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