Chapter 4

Stripping of Vegetable Oils by Direct Mixing with Adsorbent Materials

Claire C. Berton-Carabin, Lucie Ribourg-Birault, and Thais Cristina Benatti Gallo

Abstract

It is often useful to conduct experiments with triacylglycerol oils, such as vegetable oils, that have been stripped from surface-active impurities and/or endogenous antioxidants. This chapter describes a rapid, simple, and solvent-free procedure to achieve such purification by the direct mixing of oil with an adsorbent powdered material (magnesium silicate or aluminum oxide), followed by gravitational separation. In particular, we give a step-by-step description of the method, as well as practical tips, and provide experimental data regarding the properties of a comprehensive set of oils obtained via this procedure.

Key words Vegetable oil, Stripping, Purification, Triacylglycerol oil, Adsorbent materials, Alumina, Silica, Surface-active impurities, Tocopherols

1 Introduction

Edible oils such as vegetable oils are mostly composed of triacylglycerols (TAGs), yet often still contain minor components that can have a noticeable effect on their stability and functional properties. Two major examples in that respect relate to (1) the presence of endogenous lipophilic antioxidants, such as tocopherols; and (2) the presence of surface-active impurities (such as free fatty acids, mono- and diacylglycerols, phospholipids, or lipid oxidation products) which, even in trace amounts, can largely alter the properties of adsorbed layers formed at the oil–water interface [1, 2].

It can therefore be relevant to strip vegetable oils from such components when conducting studies that pertain to the oxidative stability of the oil-based systems [3, 4], and/or to the characterization of structures and phenomena occurring at the oil–water interface [5]. For this, selected adsorbent materials have proven useful; they may be used either as chromatographic columns [6, 7], which implies first diluting the oil in an organic solvent, which shall then

Christelle Lopez et al. (eds.), *Multidimensional Characterization of Dietary Lipids*, Methods and Protocols in Food Science,
https://doi.org/10.1007/978-1-0716-3758-6_4,

be removed after elution, or by direct mixing with the oil followed by gravimetric separation [8, 9], which is a solvent-free procedure. The choice of the adsorbent material is of high importance depending on the targeted components to remove. For instance, magnesium silicate may be used to remove surface-active polar contaminants [10, 11] and alumina to remove not only such components but also lipophilic antioxidants such as tocopherols [9]. In addition, lipophilic pigments such as carotenoids and chlorophylls can be eliminated by activated charcoal [12]. Another protocol elaborated for the purification of mineral oils/nonpolar phases has also been proposed, which requires the consecutive treatment with iron (III) chloride for the reduction of peroxides, potassium hydroxide for the extraction of alcohols, silica gel for the adsorption of polar contaminants, and finally, sodium sulfate for the removal of water [13].

This chapter focuses on a solvent-free procedure for stripping vegetable oils, either using magnesium silicate (removal of surface-active impurities) or using aluminum oxide (removal of surface-active impurities + tocopherols and tocotrienols). We strongly recommend that the stripped oils obtained with this procedure are checked for interfacial tension against water (e.g., using an automated drop tensiometer; see for instance, ref. [11]) and, when antioxidant stripping is targeted, for residual tocopherol/tocotrienol content (see Chapter 10). A comprehensive series of examples of such properties for various reference lipids/oils and stripped oils is given later (Subheading 4 *See* **Note 1**, Table 1).

2 Material

2.1 Equipment

1. 50-mL conical centrifuge tubes (polypropylene).
2. Rotative tube agitator equipped with a 50-mL tube carousel.
3. Or glass beaker and magnetic bar/stirrer.
4. Centrifuge with swing rotor adapted for 50-mL conical tubes.
5. Plastic or glass transfer pipettes.

2.2 Chemicals

1. Magnesium silicate powder (also referred to as silica; CAS: 1343-88-0; example of commercial sample: Florisil®, 100–200 mesh, Supelco).
2. Or aluminum oxide powder (also referred to as alumina; CAS: 1344-28-1; an example of commercial sample: MP Alumina N—Super I, MP Biomedical).

NB: Magnesium silicate powder (silica) should be used when the stripping procedure aims at removing surface-active impurities, but not endogenous tocopherols. Conversely, stripping with aluminum oxide (alumina) should be selected when both surface-active impurities and endogenous tocopherols/tocotrienols shall

Table 1
Interfacial tension values (oil–water interface) and tocopherol contents reported for various lipid phases, with a focus on vegetable oils

Oil or nonpolar phase	Stripping method	Interfacial tension value (mN/m)[a]	Total tocopherol content (mg/kg oil)[a]	Source/reference
Tetradecane	Stripped by consecutive treatment with iron (III) chloride, potassium hydroxide, silica gel, and sodium sulfate	51.5	NA	[13]
Hexane		50.0	NA	
1-Octanol		8.7	NA	
Medium chain triglycerides		21.0	–	
Commercial vegetable oils[b]	Nonstripped	19.5–23.5	–	[14]
Stripped commercial vegetable oils[b]	Stripped by passage through a packed column of magnesium silicate	30.0–31.5	–	
Commercial soybean oil with 0.1 wt.% lecithin	Nonstripped	~5	–	[15]
Commercial corn oil	Nonstripped	–	538.9 ± 12.8	[12] (in this article, data are also given for other (stripped) oils, namely camelina oil, chia oil, Sophia seed oil, olive oil and DHA single cell oil)
Stripped commercial corn oil	Simplified stripping method by direct mixing with hexane, activated silicic acid, and charcoal under N_2, followed by solvent evaporation	–	ND	
Stripped commercial rapeseed oil mixed with brominated oil[c] (65 wt.% – 35 wt.%)	Rapeseed oil: preliminary stripped with procedure in Subheading 3.1 with aluminum oxide, at 4 °C Brominated oil: nonstripped	25.4	–	Courtesy Sten ten Klooster, Wageningen University & Research (The Netherlands), 2021
Stripped commercial rapeseed oil mixed with brominated oil[c] (65 wt.% – 35 wt.%)	Rapeseed oil: preliminary stripped with procedure in Subheading 3.1 with aluminum oxide, at 4 °C Brominated oil: preliminary mixed with hexane (4:1, v/v), then stripped with procedure in Subheading 3.1 with aluminum oxide, at 4 °C. Hexane was then evaporated at 45 °C for 3 h under N_2 flow.	34.8	ND	
Commercial rapeseed oil[d]	Nonstripped	13.0	501.2	[16]
Stripped commercial rapeseed oil[d]	Procedure described in Subheading 3.1 with magnesium silicate, at 4 °C	28.7	513.4	
Stripped commercial rapeseed oil[d]	Procedure described in Subheading 3.1 with aluminum oxide, at 4 °C	28.7	1.8	

(continued)

Table 1
(continued)

Oil or nonpolar phase	Stripping method	Interfacial tension value (mN/m)[a]	Total tocopherol content (mg/kg oil)[a]	Source/reference
Stripped commercial rapeseed oil	Procedure described in Subheading 3.1 with a 50/50 mixture (w/w) of magnesium silicate/aluminum oxide, at 4 °C	–	30.7	
Stripped commercial rapeseed oil	Procedure described in Subheading 3.1 with magnesium silicate, at room temperature	27.8	–	Courtesy Jack Yang, Wageningen University & Research (The Netherlands), 2021
Stripped commercial rapeseed oil	Procedure described in Subheading 3.2 with aluminum oxide, at 20 °C	30.5	ND	Courtesy Jilu Feng, Wageningen University & Research (The Netherlands), 2021
Stripped commercial sunflower oil	Procedure described in Subheading 3.2 with aluminum oxide, at 20 °C	28.0 (against phosphate buffer 10 mM, pH 7.0)	–	[2]
Stripped commercial sunflower oil	Procedure described in Subheading 3.1 with aluminum oxide, at 4 °C	30.0 (against acetate buffer 50 mM, pH 4.5)	ND	[17]
Commercial rapeseed oil	Nonstripped oil	22.3	840.1 ± 5.2	Data from Thais Cristina Benatti Gallo and Lucie Birault, INRAE (France), 2021 (unpublished)
Stripped commercial rapeseed oil	Procedure described in Subheading 3.1 with magnesium silicate, at 4 °C	27.9	903.3 ± 7.8	
Stripped commercial rapeseed oil	Procedure described in Subheading 3.1 with aluminum oxide (*alumina bottle opened for >1 year*), at 4 °C	28.4	176.0 ± 3.0	
Stripped commercial rapeseed oil	Procedure described in Subheading 3.1 with aluminum oxide (*new alumina bottle*), at 4 °C	28.8	2.1 ± 0.0	

NA nonapplicable, *ND* nondetectable (below detection threshold)
[a]Interfacial tension was measured at room temperature against ultrapure water, unless otherwise stated. Tocopherols were measured by high-performance liquid chromatography (HPLC) as described in Chapter X
[b]Various oils tested (soybean, corn, canola, and olive oils)
[c]Commercial brominated vegetable oil, CAS number: 8016-94-2
[d]In these samples, the amount of hydroperoxides was also measured, and was found to be 1.1 mmol/kg oil for the nonstripped oil and 0.3 mmol/kg oil for the stripped oils (both with silica and with alumina)

be removed. After opening, it should be noted that the quality of adsorbent materials may decrease after a given storage period, which can decrease the stripping efficiency (see Subheading 4, *see* **Note 2**). Recommendations regarding handling of those materials are given in Subheading 4 (*see* **Note 3**).

3 Methods

3.1 Recommended Procedure: Agitation Using a Rotative Agitator

1. Fill in 50-mL conical centrifuge tubes with approximately 1 volume (15 mL) of adsorbent powder (silica or alumina) and 2 volumes (30 mL) of oil.
2. Mix cautiously the content of each tube with a spatula to disperse the adsorbent powder in the oil, and put immediately on rotative agitation at 15 rpm (be careful not to let the tubes stand still for too long before starting the agitation, as the adsorbent powder tends to sediment within minutes).
3. Incubate the tubes under rotative agitation at 15 rpm in the dark for at least a few hours (recommended: overnight, i.e., 10–12 h minimum). For oils that do not crystallize in refrigerated conditions (e.g., rapeseed oil), it is recommended to conduct the agitation at 4 °C. For oils with a higher melting point, a higher incubation temperature may be chosen (it is important that lipids are completely melted upon the stripping procedure).
4. At the end of the agitation period, pick the tubes and centrifuge them (centrifuge equipped with a swing rotor, 2000 × *g*, 20 min, 20 °C).
5. Collect the supernatant (oil layer) with a transfer pipette (stop collecting when reaching about 0.5 cm above the bottom adsorbent layer), and transfer it to a new 50-mL conical centrifuge tube.
6. Perform a second centrifugation in the same conditions as previously.
7. Collect the stripped oil with a transfer pipette (stop collecting when reaching about 1 cm above the bottom of the tube, i.e., the conical part. Generally, residual adsorbent particles are hardly visible for the second centrifugation). It is recommended to store the stripped oil in clean, glass tubes, under a nitrogen blanket and away from light, ideally at −20 °C or below, until further use.

3.2 Alternative Procedure: Agitation Using a Magnetic Stirrer

When a rotative agitator is not available, the aforementioned procedure can be modified to perform the mixing between oil and the adsorbent material directly in a beaker. The following steps should then be taken:

1. In a glass beaker of appropriate volume, put 1 volume of the adsorbent powder (silica or alumina) and 2 volumes of oil.
2. Mix cautiously with a spatula to disperse the adsorbent powder in the oil, and put immediately on magnetic stirring using a large enough magnetic bar. The stirring intensity should be high enough to keep the mixture well dispersed and avoid the formation of an oil layer depleted from the adsorbent material on top. It should be noted that the mixture is quite viscous, which may (1) cause the magnetic bar to slow down/stop at some point and/or (2) induce an increase in the temperature of the magnetic stirrer, which then heats the mixture. This may, in practice, not be resolved by putting the stirrer + mixture system in a refrigerated room because this would typically increase the viscosity of the mixture, resulting in stopping the magnetic bar from spinning.
3. Incubate the beaker under magnetic stirring at appropriate speed (see comment in the previous point), protecting it from light for at least a few hours (recommended: overnight, i.e., 10–12 h minimum) at room temperature.
4. At the end of the agitation period, rapidly transfer the content of the beaker into 50-mL conical centrifuge tubes.
5. Follow then **steps 4**–**7** as described in Subheading 3.1.

4 Notes

1. *Examples of typical properties of various reference lipids/oils and of stripped oils*: Table 1 summarizes typical interfacial tension values and tocopherol contents for different lipid phases, with an emphasis on vegetable oils.
2. *Useful remarks*: as can be seen from the last two lines of Table 1, the quality of adsorbent materials can decrease after a given storage period. In fact, it can be noticed that alumina loses its adsorbing properties toward tocopherols (only ~80% of tocopherols removed for an alumina bottle opened for >1 year, versus almost full removal for a freshly opened bottle). This can be solved by regenerating alumina, which can be done by heating (see conditions advised by the manufacturer). Nevertheless, this points out the importance of checking the quality of the stripped oil with regard to the important features targeted (interfacial tension and/or the removal of tocopherols or of other lipophilic components) for every batch.
3. *Safety*: Although the adsorbent materials used for the present procedure are not classified for specific hazards, please note that because of the very fine size of the powder particles and their use in a dry state, they may easily be suspended in the air. We

thus recommend that **steps 1** and **2** are conducted under a fume hood.

Acknowledgments

Anja Schröder, Jack Yang, Sten ten Klooster, and Jilu Feng (Wageningen University & Research, the Netherlands) are gratefully acknowledged for kindly providing experimental values measured on stripped oils.

References

1. Abousalham A, Fotiadu F, Buono G, Verger R (2000) Surface properties of unsaturated non-oxidized and oxidized free fatty acids spread as monomolecular films at an argon/water interface. Chem Phys Lipids 104:93–99
2. Berton-Carabin CC, Schröder A, Rovalino Cordova A et al (2016) Protein and lipid oxidation affect the viscoelasticity of whey protein layers at the oil-water interface. Eur J Lipid Sci Technol 118:1630–1643. https://doi.org/10.1002/ejlt.201600066
3. Chaiyasit W, Elias RJ, McClements DJ, Decker EA (2007) Role of physical structures in bulk oils on lipid oxidation. Crit Rev Food Sci Nutr 47:299–317. https://doi.org/10.1080/10408390600754248
4. Hoppenreijs LJG, Berton-Carabin CC, Dubbelboer A, Hennebelle M (2021) Evaluation of oxygen partial pressure, temperature and stripping of antioxidants for accelerated shelf-life testing of oil blends using 1H NMR. Food Res Int 147:110555. https://doi.org/10.1016/j.foodres.2021.110555
5. Bergfreund J, Bertsch P, Fischer P (2021) Effect of the hydrophobic phase on interfacial phenomena of surfactants, proteins, and particles at fluid interfaces. Curr Opin Colloid Interface Sci 101509. https://doi.org/10.1016/j.cocis.2021.101509
6. Boon CS, Xu Z, Yue X et al (2008) Factors affecting lycopene oxidation in oil-in-water emulsions. J Agric Food Chem 56:1408–1414. https://doi.org/10.1021/jf072929+
7. Villiere A, Viau M, Bronnec I et al (2005) Oxidative stability of bovine serum albumin- and sodium caseinate-stabilized emulsions depends on metal availability. J Agric Food Chem 53:1514–1520. https://doi.org/10.1021/jf0486951
8. Maldonado-Valderrama J, Woodward NC, Patrick Gunning A et al (2008) Interfacial characterization of β-lactoglobulin networks: Displacement by bile salts. Langmuir 24:6759–6767. https://doi.org/10.1021/la800551u
9. Berton C, Genot C, Ropers M-H (2011) Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. J Colloid Interface Sci 354:739–748
10. Mishra K, Bergfreund J, Bertsch P et al (2020) Crystallization-induced network formation of tri- and monopalmitin at the middle-chain triglyceride oil/air interface. Langmuir 36:7566–7572. https://doi.org/10.1021/acs.langmuir.0c01195
11. Hinderink E, Sagis L, Schroën K, Berton-Carabin CC (2020) Behavior of plant-dairy protein blends at air-water and oil-water interfaces. Colloids Surfaces B Biointerfaces 192:111015. https://doi.org/10.1016/j.colsurfb.2020.111015
12. Abad A, Shahidi F (2020) A robust stripping method for the removal of minor components from edible oils. Food Prod Process Nutr 2:1–9. https://doi.org/10.1186/s43014-019-0015-2
13. Bergfreund J, Bertsch P, Kuster S, Fischer P (2018) Effect of oil hydrophobicity on the adsorption and rheology of β -lactoglobulin at oil–water interfaces. Langmuir 34:4929–4936. https://doi.org/10.1021/acs.langmuir.8b00458
14. Gaonkar AG (1989) Interfacial tensions of vegetable oil/water systems: Effect of oil purification. J Am Oil Chem Soc 66:1090–1092
15. Johansson D, Bergenståhl B (1995) Lecithins in oil-continuous emulsions. Fat crystal wetting and interfacial tension. J Am Oil Chem Soc 72:205–211. https://doi.org/10.1007/BF02638901
16. Berton C (2011) Construction raisonnée d'interfaces pour protéger les lipides émulsionnés contre l'oxydation. Université de Nantes
17. Schröder A, Laguerre M, Tenon M et al (2021) Natural particles can armor emulsions against lipid oxidation and coalescence. Food Chem 347:129003. https://doi.org/10.1016/j.foodchem.2021.129003