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IMPROVED EXTRACTION OF LIPIDS FROM STRAWBERRY

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Abstract—A high degree of variability in strawberry lipid content and fatty acid composition, previously observed after two extractions with chloroform–methanol–water following the Bligh and Dyer method, was reduced by extracting three times with larger volumes. The previously observed variation was caused by incomplete lipid extraction while the internal standard, methylheptadecanoate, was quantitatively extracted. The improved procedure caused by itself little variation in the lipid determination. The remaining variability was predominantly due to the heterogeneity of the strawberry population. Ripe fruit contained slightly more lipid than unripe fruit, and these lipids were richer in oleic acid and less rich in linoleic acid. The improved extraction procedure was however more cumbersome than three extractions with chloroform–methanol followed by evaporation to dryness and purification according to Bligh and Dyer in a small volume of chloroform–methanol–water. The latter procedure yielded a slightly larger amount of lipids and was more suitable to the analysis of a large number of small samples. In conclusion, equivalent extraction of internal standard and sample lipids should not be taken for granted.

INTRODUCTION

During experiments where membrane lipid breakdown was investigated during ripening of strawberries, excessive variation was observed in the amounts of total lipids extracted, and the recovery of total fatty acids was low. The well established method of Bligh and Dyer [1] was used with an internal standard of methylheptadecanoate. In these experiments 4.8 g of strawberry homogenate were routinely extracted with 21.4 ml of chloroform–methanol–water (5:10:4, including tissue water), then with 9.5 ml of the same mixture, and purified as described in ref. [1]. The method was therefore re-investigated with the aim of improving recovery of lipids and reproducibility.

Several methods have been used for lipid extraction, among which methods based on chloroform and methanol which are excellent for polar lipids, mainly present in cell membrane [1, 2] and methods using apolar solvents, e.g. petrol, hexane or diethyl ether, which extract mainly the free lipids [3]: triglycerides, free fatty acids, etc. [4]. In recent years other solvents, less hazardous to health than chloroform, have been proposed, e.g. hexane–isopropanol [5]. Lipid extraction methods have been compared a number of times, e.g. by Sahasrabudhe and Smallbone for meat [3] and by de la Roche *et al.* for plant material [6], and these authors have considered chloroform–methanol mixtures to be the most satisfactory.

The present paper describes experiments attempting to evaluate the influence of the number of consecutive extractions, the volume of extraction solvent, and the length of time of stirring on lipid recovery. An improved procedure is described for the extraction of lipids from strawberries. It is efficient, rapid, reproducible and suitable for routine extraction of large numbers of small samples.

RESULTS

A first experiment aimed to establish the efficiency of the Bligh and Dyer [1] method for the extraction of strawberry lipids, and the number of consecutive extractions required to extract more than 90% of the lipids. The results (Table 1) show a recovery of 73.2% of the lipids after two extractions, 92.2% after three extractions, and 96.3% after four extractions with chloroform–methanol–water. Hexane–isopropanol extracted the remainder of the lipids, as subsequent refluxing with hexane failed to yield any fatty acids. However two extractions with chloroform–methanol–water were enough to completely extract the methylheptadecanoate used routinely as internal standard. The separation of neutral lipids by TLC showed that the transmethylation was quantitative. Although the consecutive fractions had relatively similar fatty acid compositions, they became gradually less rich in palmitic acid and richer in linoleic acid.

The volume of extraction solvent had great influence on the amount of lipid extracted but little effect on its fatty acid composition (Table 2). Three consecutive extractions with a relatively large volume of solvent (21.4 ml) were required for good recovery. The length of time during which the samples were stirred during lipid extraction had little influence on the amounts extracted or the fatty acid composition (Table 3).

The source of variation observed with the improved version of the lipid extraction procedure was investigated and the results are shown in Table 4. The variability due

Table 1. Fatty acid composition (area %) and content (mg/100 g fresh weight) of successive extractions of strawberry lipids

Fatty acids*	Extraction solvents				
	CMW†	CMW	CMW	CMW	HIP‡
16:0	9.9±0.5	6.0±0.6	5.4±0.6	6.2±1.5	5.4±3.8
18:1	23.3±0.9	22.7±1.5	22.7±0.8	20.6±3.2	22.8±1.9
18:2	36.5±1.2	38.7±2.1	40.3±1.9	42.2±3.2	44.6±2.8
18:3	30.3±1.1	32.6±1.9	31.6±0.6	31.1±1.9	28.3±2.2
Total	48.3±2.6	28.2±4.3	19.9±1.8	4.3±0.3	3.9±0.7
Yield (%)	46.2	27.0	19.0	4.1	3.7
Me-17:0 added (μg)	180	30	0	0	0

*Length of carbon-chain: double bonds. Stearic acid was not detected in this experiment.

†21.4 ml $CHCl_3$–MeOH–H_2O (5:10:4, including tissue water) [1]

‡Hexane–isopropanol (3:2) [5].

Table 2. Effect of the volume of extraction solvent on the fatty acid composition (area %) and amounts (mg/100 g fresh weight) of lipids extracted from strawberries

Fatty acids	Extraction procedures*		
	(1)	(2)	(3)
16:0	9.0±0.3	8.2±0.3	7.8±0.2
18:0	3.4±0.3	3.1±0.2	3.0±0.2
18:1	18.5±0.6	18.7±0.6	19.2±0.7
18:2	43.5±0.6	44.9±1.1	44.4±1.6
18:3	25.7±0.5	25.2±0.5	25.6±0.7
Total	76.8±4.4	82.2±8.4	106.8±8.4

*Procedure (1): One extraction with 21.4 ml $CHCl_3$–MeOH–H_2O (5:10:4 including tissue water) followed by one extraction with 9.5 ml of the same mixture (procedure used in previous work). Procedure (2): one extraction with 21.4 ml followed by two extractions with 9.5 ml. Procedure (3): three extractions with 21.4 ml.

Table 3. Influence of length of time of stirring during extraction on fatty acid composition (area %) and amounts (mg/100 g fresh weight) of lipids extracted from strawberries

Fatty acids	Extraction procedures*		
	(1)	(2)	(3)
16:0	7.0±0.2	6.8±0.2	6.9±0.2
18:0	3.8±0.1	3.8±0.1	3.9±0.1
18:1	21.0±0.4	20.9±0.4	20.8±0.5
18:2	42.1±0.8	42.1±0.5	42.0±0.6
18:3	26.2±0.9	26.4±0.5	26.5±0.6
Total	151.2±7.0	154.7±3.1	152.9±2.9

*Lipids were extracted three times with 21.4 ml $CHCl_3$–MeOH–H_2O (5:10:4). Procedure (1): extraction 1, 2, 3: homogenized (Polytron) for 1 min., then vortexed for 2 min. Procedure (2): extraction 1: as above; extraction 2, 3: vortexed for 2 min. Procedure (3): extraction 1: as above; extraction 2,3: vortexed for 1 min.

to the extraction itself was evaluated on five aliquots from the same homogenate. It was low, as well for total amount of lipids extracted (3.4% of the mean), as for fatty acid composition. The degree of maturity accounted for relatively little variation: a little more lipid was extracted from the riper fruit, and those lipids contained more oleic acid and less linoleic acid than less ripe fruit. The greatest source of variation (11.9% of the mean) originated from the heterogeneity of the strawberry population, as measured on five different homogenates, with respect to amounts extracted and fatty acid composition.

The improved Bligh and Dyer lipid extraction procedure, with three extractions in 21.4 ml, was however relatively cumbersome because the increased volume did not allow centrifugation in one tube. It was compared with a simplified procedure where 4.8 g strawberry homogenate was extracted once with 21.4 ml chloroform–methanol–water (5:10:4, including the tissue water), and twice with 16.9 ml chloroform–methanol (1:2). The combined extracts were evaporated to dryness and purified according to ref. [1]. The results are shown in Table 5. This simplified procedure yielded slightly more lipid than the improved Bligh and Dyer extraction procedure.

DISCUSSION

The results shown in Table 1 strongly suggest that the high degree of variation previously observed with strawberry lipids was caused by incomplete extraction of the sample lipids after two extractions with chloroform–methanol–water, while the methylheptadecanoate was completely extracted. This observation underlines that the use of an internal standard is only valid if both sample lipids and standard are quantitatively extracted.

This difficulty was easily overcome by extracting three times (Table 1) with larger volumes (Table 2) of chloroform–methanol. This improved version of the

Table 4. Variability of strawberry lipids determinations: variation due to the extraction procedure (column 1), to the degree of maturity (columns 2–4) and to the heterogeneity of the material (column 5)

Fatty acids	Procedure (1)	Variability* due to degree of maturity: Unripe (2)	Intermediate (3)	Ripe (4)	Strawberry heterogeneity (5)
16:0	7.6±0.3	7.5±0.5	7.7±0.2	7.7±0.5	7.2±0.5
18:0	2.4±0.1	2.3±0.2	2.5±0.1	2.5±0.3	2.0±0.3
18:1	21.3±0.2	20.3±1.5	20.7±0.8	22.0±2.2	19.2±2.9
18:2	39.5±0.5	40.7±1.9	40.5±1.4	38.9±3.2	37.0±4.3
18:3	29.2±0.4	29.2±1.8	29.6±0.9	29.0±2.3	25.9±3.4
Total	158.3±2.3	149.6±5.4	158.8±6.9	162.6±5.2	122.2±14.5

*Data are expressed as area % (fatty acid composition) and mg/100 g fresh weight (total fatty acid content). They are the means of five samples of the same homogenate (column 1), of three samples in each of three different batches (columns 2–4), and of five different batches (column 5).

Table 5. Comparison between the improved Bligh and Dyer lipid extraction procedure* and a simplified procedure† [data are expressed as area % (fatty acid composition) and mg/100 g fresh weight (total fatty acid content)]

Fatty acids	Extraction procedures: Improved Bligh and Dyer procedure	Simplified procedure
16:0	6.9±0.3	7.6±0.4
18:0	2.4±0.2	2.5±0.2
18:1	14.8±0.4	14.9±0.5
18:2	47.1±1.1	46.5±0.7
18:3	28.8±0.8	28.6±0.7
Total	112.6±1.3	122.4±3.5

*Three extractions with 21.4 ml $CHCl_3$–MeOH–H_2O (5:10:4, including tissue water), then addition of one volume each of chloroform and water.

†extraction with 21.4 ml $CHCl_3$–MeOH–H_2O (5:10:4, including tissue water) followed by two extractions with 16.9 ml $CHCl_3$–MeOH (1:2), evaporation to dryness and purification by the Bligh and Dyer 2-phase system.

Bligh and Dyer method caused by itself little variation in the lipid determination (Table 4). The remaining variability was predominantly due to the heterogeneity of the strawberry population. Ripe fruit contained slightly more lipid than unripe fruit, and these lipids were richer in oleic acid and less rich in linoleic acid.

Three consecutive extractions with larger volumes of chloroform–methanol-water according to Bligh and Dyer were however more cumbersome than three extractions with chloroform–methanol followed by evaporation of the lipids to dryness and purification according to Bligh and Dyer in a small volume of chloroform–methanol–water. The latter procedure yielded a slightly larger amount of lipids and was more suitable for routine analysis of large numbers of small samples. The main conclusion to be drawn from this work is that the equivalent extraction of an internal standard and the sample lipids should not be taken for granted.

EXPERIMENTAL

Plant material. Strawberries were purchased locally and selected for uniformity of colour and size. When fruits of different degrees of maturity were compared, they were selected on the basis of colour: unripe (1/3 red), intermediate, ripe.

Lipid extraction. In the initial experiment (Table 1) five strawberries (approximately 60 g) were steamed for 3 min in a pressure cooker to inactivate lipid degrading enzymes and homogenized (Polytron, Brinkmann Instrum., Rexdale, Ont.). Aliquots of 4.8 g of the homogenate, to which suitable amounts of internal standard (methylheptadecanoate) were added, were extracted ×4 with 21.4 ml $CHCl_3$–MeOH–H_2O (1:2:0.8) [1] once with 18 ml hexane–*i* PrOH (3:2) [5] and once by refluxing with 60 ml hexane for 18 hr at 60°. For each liquid extraction, the material was homogenized for 1 min with a Polytron and vortexed for 2 min Modifications to this initial procedure in subsequent experiments are described in the text. All extractions were made in triplicate unless stated otherwise. For each experiment a different batch of strawberries was used.

Fatty acid analysis. After each extraction the lipids were transmethylated with NaOMe (0.5 M in MeOH) [7]. The efficiency of the transmethylation was tested by TLC separation of the neutral lipids in hexane–Et_2O–HOAc (80:20:1). The FAMEs were separated by capillary GLC (model 5890A, Hewlett Packard, Mississauga, Ont.; 30 m SP2330, Supelco, Oakville, Ont.). The temperature program included holding at 70° for 0.2 min, increasing to 180° at 30° per min, holding for 4 min, increasing to 200 at 30° per min and holding for 3 min. Injector and detector (FID) temperatures were held at 225 and 250° respectively. Peaks were identified by comparison with a reference mixture (Supelco) and their areas were determined with a model 3390A (Hewlett Packard) integrator. Three injections were made for each triplicate extract. Unless otherwise stated, results are means of 3 extractions +s.d.

REFERENCES

1. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
2. Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497.
3. Sahasrabudhe, M. R. and Smallbone, B. W. (1983) *J. Am. Oil Chem. Soc.* **60**, 801.
4. Anon (1980) *Official Methods of Analysis of the Association of official Analytical Chemists*, 13th Edn, AOAC, Washington, D.C.
5. Hara, A. and Radin, N. S. (1978) *Anal. Biochem.* **90**, 420.
6. de la Roche, I. A., Andrews, C. J. and Kates, M. (1973) *Plant Physiol.* **51**, 468.
7. Slack, C. R. and Roughan, P. G. (1975) *Biochem. J.* **152**, 217.