Indian J. Genet., 68(4): 456-458 (2008)

Short Communication

A simplified method for preparation of fatty acid methyl esters of *Brassica* oil

Sujata Vasudev[1], D.K. Yadava[1], Divya Malik[1], Rajendra Singh Tanwar[2] and K.V. Prabhu[1]

[1]Division of Genetics; [2]Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012

(Received: October 2008; Revised: November 2008; Accepted: November 2008)

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Vegetable oils are the most important source of fats in the diet. Fats are the essential component for the normal growth and development of any living being. For balanced body growth, the quality of oil in our diet is important. Oil quality in vegetable oils is determined for both nutritional and functional aspects. Edible oil quality depends on the content of different fatty acids [1]. Saturated fatty acids and erucic acid, high in some edible oils, are harmful as they aid deposition of cholesterol in the arteries [2].

The main challenge facing breeders dealing in oil quality improvement, is the development of superior quality oil varieties with high seed and oil yields. While selecting for better quality oil in terms of fatty acid profile which helps in determining nutritional quality and shelf life of oil, a large number of segregating materials is to be screened. This requires phenotyping for fatty acid profile of the seeds of large number of plants. It also requires a standardized laboratory technique that gives accurate results in lesser time.

For fatty acid analysis, Gas-liquid chromatography (GLC) is a rapid, cost effective method with high reproducibility [3]. However, before their analysis by GLC, fatty acids are required to be converted into their volatile forms, methyl esters. The currently followed method for preparing methyl esters in which oil is treated with sodium methoxide (in methanol) [4-6], is not a reliable one (7-10). This is because, when proper screw-capped vials are not used, methanol is lost due to leakage. Also, if proper temperature is not maintained, methyl ester formation is not complete. Such a sample when injected into GLC, would clog its column. In view of this, it was planned to develop a method in which chances of failure of methyl ester formation are minimized. On the basis of our experience and experimentation, a simplified method for formation of fatty acid methyl esters, has been developed, which is more precise and effective in determination of fatty acid profile of the both seeds and oil of materials coming from a breeding programme.

To carry out this study, five different genotypes of *Brassica juncea* seeds were taken (Table 1). Standard samples of methyl esters of fatty acids procured from Sigma Aldrich, were used. Other reagents used were H_2SO_4 (AR grade, 98% purity), Hexane (spectroscopic grade), Methanol (AR grade) and anhydrous sodium sulphate (AR grade).

Procedure for preparation of methyl esters

Five grams of each *Brassica* seed sample was taken in a pestle and mortar and ground well into a fine powder. The powder was transferred to a completely dry test tube (25 x 150 mm), to which 5 ml of methanol was added, followed by two drops of conc. H_2SO_4. These tubes containing oil:methanol:acid mixture were incubated in a water bath at 65°C for an hour. The tubes were then cooled to room temperature and 2 ml of hexane was added in each tube. The tubes were shaken well using a vortex mixer, and allowed to stand till the hexane layer containing methyl esters separated out. The hexane layer (1-1.5ml) was removed carefully using micropipette and transferred into a 2 ml screw capped vial. A pinch of anhydrous sodium sulphate was added

Corresponding author's e-mail: sujatavasudev@rediffmail.com

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

to each vial to remove moisture, if any. The vials were stored in a refrigerator if the samples were not analysed on the same day.

One µl of the hexane layer containing methyl esters was injected into pre-conditioned gas chromatograph (Perkin Elmer Claurus 500) fitted with megabore column (30 meter long and 0.53mm diameter) packed with OV-101, a polymer of methyl silicone. The detector used was Flame ionization detector (FID). The conditions maintained were: Column temperature: 150°C-270°C, Injector temperature: 250°C and Detector temperature: 250°C. GLC was programmed for the temperature increase at the rate of 10°C per minute, and finally it was maintained at 270°C. Each sample took 15 minutes (approx.) for its analysis.

Though, in the computerized automated gas chromatograph, area under each peak is calculated automatically, it can be calculated by measuring the peak height and width at half height (Triangulation method) and multiplying them. After computing total peak area for the sample, percent area under each peak is calculated that would give percentage of respective fatty acid.

Five different genotypes of *Brassica juncea viz.*, Kranti, NPJ-93, LES-1-27, Varuna and Pusa Jaikisan were taken for the study and their fatty acid composition determined using the above method. Sample of each genotype was taken in triplicate, starting from extraction of oil from the seeds. Data for three replicates along with the mean are presented in Table 1.

Fatty acid profile of the genotypes reveals very good separation (Fig. 1), with good resolution. Seven major fatty acids present in Brassica oil revealed distinct peaks. The results show that the mean difference of three replicates, for all the fatty acids, in all the genotypes, was non-significant. Thus, the method was found to be repeatable and reliable. The only precaution to be taken is to have all the glassware completely moisture free. Water is the enemy of methyl ester formation of fatty acids. Although we have used *Brassica* seeds in our experiment, the method can be applied to any other oil-seed crops after minor modification. Not only it can be used for seeds, extracted oil sample can also be used for methyl ester formation. However, for screening purposes using seeds is a better option as oil extraction will become an additional step.

Thus, we conclude that the above method is efficient and reproducible for screening of large number of oilseeds for fatty acid analysis in breeding programmes.

Table 1. Fatty acid composition of different *Brassica* genotypes

S.No.	Genotype	Kranti				NPJ-93				LES-1-27				Varuna				Pusa Jaikisan			
	Fatty Acids	R1	RII	RIII	Mean	R1	RII	RIII	Mean	R1	RII	RIII	Mean	R1	RII	RIII	Mean	R1	RII	RIII	Mean
1	Palmitic	2.1	2.0	2.2	2.1	1.9	2.5	2.4	2.3	4.7	5.0	5.1	4.9	2.0	1.8	2.2	2.0	2.1	2.4	2.5	2.3
2	Stearic	1.0	0.7	0.75	0.8	0.8	1.3	1.2	1.1	3.4	3.3	3.6	3.4	1.3	1.1	1.3	1.2	0.57	0.66	0.69	0.6
3	Oleic	11.3	11.4	10.9	11.2	11.8	11.2	11.3	11.4	42.0	42.1	43.1	42.4	10.7	10.5	10.7	10.6	11.6	11.7	12.1	11.8
4	Linoleic	14.2	13.7	13.8	13.9	12.8	13.2	12.7	12.9	32.8	32.5	31.9	32.4	17.1	16.4	17.2	16.9	15.7	15.6	16.2	15.8
5	Linolenic	14.4	15.1	14.6	14.7	15.4	14.9	15.3	15.2	12.8	13.1	13.8	13.2	10.4	10.5	10.9	10.6	10.2	10.3	10.9	10.5
6	Eicosenoic	6.5	6.8	6.3	6.5	6.6	7.2	6.9	6.9	3.3	3.5	2.9	3.2	5.7	5.8	6.2	5.9	6.7	6.6	6.9	6.7
7	Erucic	53.0	52.0	48.0	51.0	51.1	51.9	53.2	52.1	0.48	0.46	0.38	0.44	51.3	52.8	52.9	52.3	52	52.3	52.8	52.4
CD 5% Reps.		—	—	—	NS	—	—	—	NS	—	—	—	NS	—	—	—	NS	—	—	—	NS
Fatty acids		—	—	—	1.76	—	—	—	0.85	—	—	—	0.86	—	—	—	0.67	—	—	—	0.27

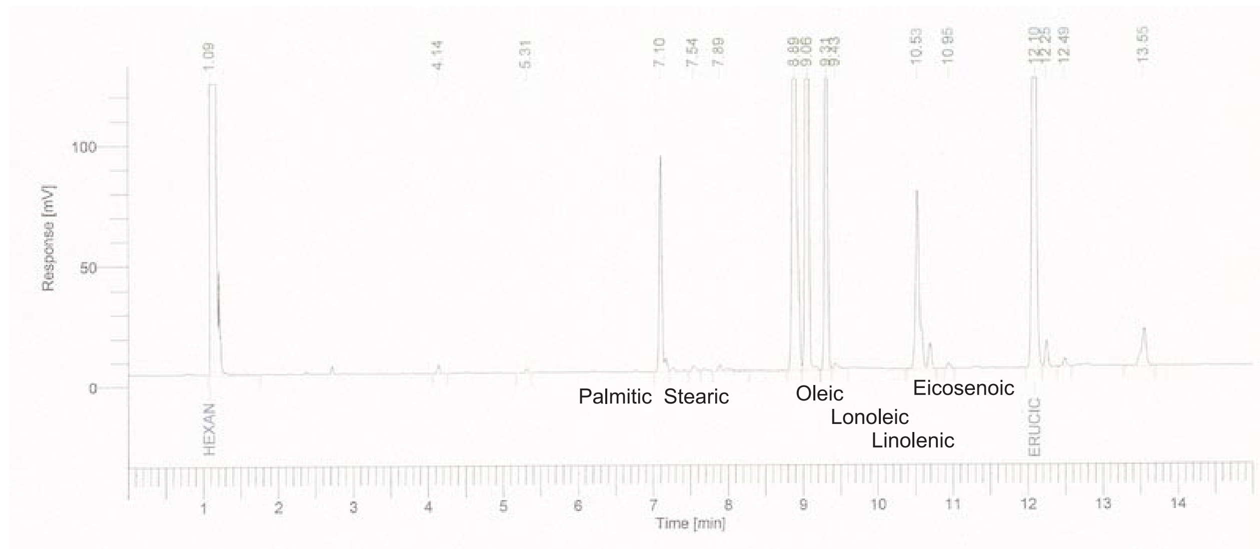

Fig. 1. Real-time gas chromatogram for fatty acid separation of *B. juncea*

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

References

1. **Peter B. E.M. and Scarth R.** 2002. Breeding for improved oil quality in Brassica oilseed species. In: Quality Improvement for Field Crops, A.S. Basra and L.S. Randhawa (ed.). Haworth press, Philadelphia, USA: 345-370.
2. **Hu F. B., Stampfer M. J. Manson J. E., Rimm E., Colditz G. A., Rosner B. A., Hennekens C. H. and Willett W. C.** 1997. Dietary fat intake and the risk of coronary heart disease in women. N Engl. J. Med., **337**: 1491-1499.
3. **Chapman G. W.** 2008. Gas chromatographic determination of free fatty acids in vegetable oils by a modified esterification procedure. J Amer. Oil Chemists' Society, 77-79.
4. **Metcalfe L. D. and Schmitz A. A.** 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem., **33**: 363-364.
5. **Morrison W. R. and Smith L. M.** 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron trifluoride-methanol. J. Lipid Res., **5**: 600-608.
6. **Christie W.W.** 1993. Extraction of lipids from samples. Lipid Technology, **5**: 18-19.
7. **Christie W. W.** 1993. Preparation of ester derivatives of fatty acids for chromatographic analysis. *In:* Advances in Lipid Methodology - Two, W.W. Christie (ed.), The Oily Press, Dundee, pp. 69-111.
8. **Tuckey R. C. and Stevenson P. M.** 1979. Methanolysis of cholesteryl esters: Conditions for quantitative preparation of methyl esters. Anal Biochem., **92**: 402-408.
9. **Ackman R. G.** 1998. Remarks on official methods employing boron trifluoride in the preparation of methyl esters of the fatty acids of fish oils. J. Am. Oil Chem. Soc., **75**: 541-545.
10. **Winfred K. F. and Mary S. S.** 1970. Production of an artifact during methanolysis of lipids by boron trifluoride-methanol, J. Lipid. Res., **1**: 276-277.