

Short Paper

GC determination of fatty acids in green beans grown in Galicia (N.W. Spain)

By J. López-Hernández, M. J. González-Castro, J. Simal-Lozano,
M. E. Vázquez-Blanco and M. L. Vázquez-Oderiz

Departamento de Química Analítica, Nutrición y Bromatología. Área de Nutrición y Bromatología.
Facultad de Farmacia. Universidad de Santiago de Compostela.
15706 Santiago de Compostela. La Coruña. España

RESUMEN

Determinación de ácidos grasos en judías verdes producidas en Galicia (N.O. España) mediante cromatografía gaseosa.

Se determinaron, mediante cromatografía gaseosa, las concentraciones de ácidos láurico, mirístico, palmítico, palmitoleico, esteárico, oleico, linoleico, linolénico y araquídico en judías verdes producidas en Galicia (N.O. España). El ácido palmítico fue el más abundante (113 mg/100 g. de liofilizado; 35,2% del contenido total de ácidos grasos), seguido por los ácidos linolénico (23,1%) y linoleico (21,6%).

PALABRAS-CLAVE: Ácido graso – Cromatografía gaseosa – Judía verde.

SUMMARY

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The concentrations of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic acids in green beans (*Phaseolus vulgaris* L.) grown in Galicia (N.W. Spain) were determined by capillary gas chromatography (GC). Palmitic acid was the most abundant fatty acid component (113 mg/100 g. of lyophilizate; 35.2% of total fatty acid content), followed by linolenic acid (23.1%) and linoleic acid (21.6%).

KEY-WORDS: Fatty acid – Gas chromatography – Green beans.

1. INTRODUCTION

The qualitative and quantitative analysis of constituent fatty acids is important in the characterization of an oil or fat; in effect, numerous properties of the oil are linked to the nature of fatty acids: viscosity, melting point, dietetic properties, stability when heated, oxidability, etc. (Farines et al., 1985).

Although the lipid content of vegetables is quite low, its study is nonetheless of interest due to the tendency

of this component to grow rancid, reducing the nutritional value of the food and producing unpleasant odours and flavours in the process (Badui Dergal, 1981).

On the other hand, the essential unsaturated fatty acids (linoleic and linolenic acids) are precursors of prostaglandins via arachidonic acid (Lehninger, 1980) and are necessary for the formation of biological membranes (Belitz and Grosch, 1988). They can also help to reduce blood cholesterol levels (Anderson et al., 1987).

Because of the importance of fatty acids in diet, many methods have been developed for their determination in foods. These include volumetric (AACC, 1984), gravimetric (AOAC, 1990) and colorimetric methods (Murray and Moss, 1990); FTIR spectroscopy (Lanser et al., 1991); and chromatography, particularly GC (Bakir et al., 1993; Park and Washington, 1993) and HPLC (Chaytor, 1987; Senatore, 1990), which are currently the most popular methods.

In this study, we used GC to determine the fatty acid composition of whole green beans (*Phaseolus vulgaris*, L. v Helda), for which data are scarce in the literature and they are widely cultivated in the autonomous region of Galicia (N.W. Spain).

2. EXPERIMENTAL

Sampling and sample preparation

Green beans (*Phaseolus vulgaris*, L. v Helda) were sown in March 1992 on a commercial plantation in Galicia (N.W. Spain) and harvested during July and August of the same year. Beans (26 samples) were selected for harvesting by the commercial criterion of having reached an appropriate podlength/beanlength ratio. Harvested beans were immediately homogenized, lyophilized and stored in air-tight flask pending analysis.

Reagents and lipids standards

Lauric, myristic, palmitic, palmitoleic, margaric, stearic, oleic, linoleic, linolenic and arachidic acids standars were from Sigma Chemical (Madrid, Spain). Reagent grade methanol and H₂SO₄ were from Scharlau (Barcelona, Spain). Hexane (LiChrosolv) was from Merck (La Coruña, Spain). Methyl esters of margaric acid and the other nine fatty acids were prepared as described below for the bean extracts.

Total fat and lipid extraction

Total fat content was determined by the AOAC method (1990).

The lipid component of each lyophilized sample (2 g) was extracted with refluxing hexane for 8 h in a Soxhlet extractor (Ajewole and Adeyeye, 1993; Spitzer et al., 1991). Each extract was concentrated under a nitrogen stream until all traces of solvent were removed.

Preparation of fatty acid methyl esters (FAME)

The extracts were esterified by refluxing in 1% methanolic H₂SO₄ for 30 min (Wolff, 1968). The methyl esters were extracted into 50 mL of hexane and 5 mL of this extract were evaporated to dryness under a nitrogen stream. The residues were redissolved in 1 mL of hexane and filtered through a 0.22 mm membrane (Millipore), after which the sample was spiked with a known quantity of an internal standard (margaric acid methyl ester).

Gas chromatographic analysis

The standards and each mixed FAME sample (1 mL) were analysed by GC in a Perkin-Elmer 8700 gas chromatograph equipped with a flame ionization detector (FID) and an acidified polyethyleneglycol capillary column SGL-1000 (25 m x 0.25 mm i.d. x 0.25 mm film thickness; Sugelabor S.A., Madrid, Spain). Nitrogen was used as a carrier gas flowing at 0.81 ml/min. The injector and detector temperatures were held at 230°C; the column temperature, initially 100°C, was increased to 160°C at a rate of 18°C/min, and then to 230°C at a rate of 1.5°C/min.

Quantification of FAMEs and evaluation of results

The component FAMEs were identified by comparison of their retention times with those of the standards. Quantitation was performed using the margaric acid methylester as internal standard. Statistical analysis of results was performed using the Statgraphics package (version 2.6).

3. RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of mixed fatty acid methyl esters obtained by esterifying hexane-soluble extracts of lyophilized green beans.

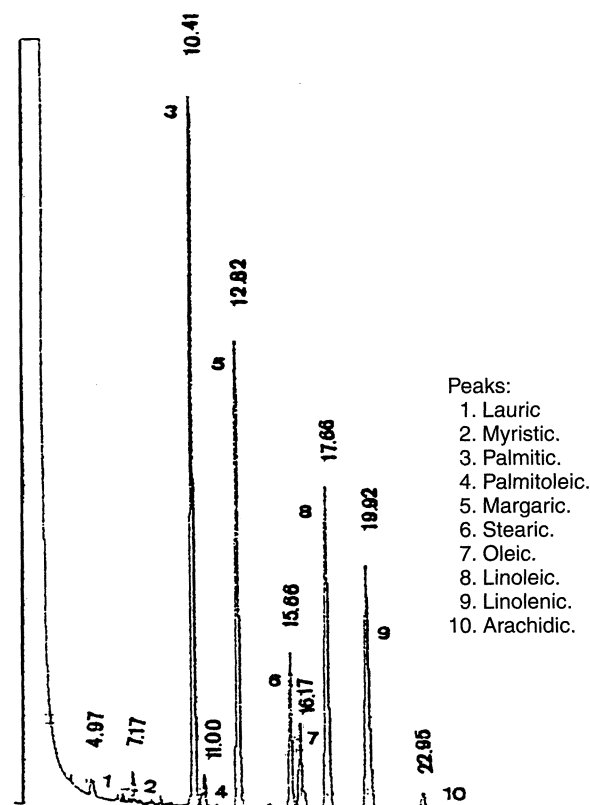


Figure 1
A typical chromatogram of mixed fatty acid methyl esters obtained by esterifying hexane-soluble extracts of lyophilized green beans

The precision of the method was calculated as the coefficient of variation of the amount of each FAME in 10 portions obtained independently from a single sample of lyophilized green bean. The precision of the measurement was calculated as the coefficient of variation of 10 GC determinations of the amount of each FAME in a single solution. The percent of recovery of method was calculated using 10 portions of the same lyophilized sample and spiking with varies quantities of each FFA. The limit of detection of each FAME was calculated following ACS (1980) guidelines. The results are given in table I.

The method was calibrated using a serie of standards of FAME (amounts varying from 1 to 100 ppm were derivatized and analysed in triplicate). Linear regression of the area of each FAME (y) on the concentration of the standard (x) yielded the equations given in table II.

Table I
 Repeatability, recovery and limit of detection of calculated fatty acids (n=10)

Fatty acid	Precision of chromatographic measurement RSDr (%)	Precisión of analytical method RSDr (%)	Recovery (%)	Limit of detection (mg/mL)
C12:0	2.51	4.23	83.3	1.53
C14:0	1.73	2.35	87.5	1.50
C16:0	0.76	1.30	94.6	2.06
C16:1	0.90	4.11	98.4	1.74
C18:0	0.58	1.60	92.1	3.40
C18:1	0.46	2.07	92.8	2.30
C18:2	0.39	0.93	98.6	5.78
C18:3	0.46	2.10	98.8	5.68
C20:0	1.79	3.61	97.8	6.02

Table II
 Parameters of calibration lines ($y=a+bx$) obtained from data for four analyte standards in the range 1-100 ppm (w/v)

Fatty acid	Intercept (a)	Slope (b)	Correlation Coefficient
C12:0	-12.88	11.95	0.99654
C14:0	-6.99	14.12	0.99871
C16:0	-79.4	14.37	0.99998
C16:1	-5.00	12.57	0.99742
C18:0	-1.65	11.23	0.99997
C18:1	3.12	10.27	0.99904
C18:2	-39.09	11.03	0.99992
C18:3	-21.46	9.41	0.99999
C20:0	-9.74	9.73	0.99999

The mean total fat content was low (0.97%). The main fatty acid component was palmitic acid, with a mean concentration for all 26 samples of 113 mg/100 g (35.2%). The other major fatty acid components were the essential polyunsaturated fatty acids linolenic acid (23.1%) and linoleic acid (21.6%). The results are given in Table III.

The variables determining the organoleptic and nutritive characteristics of a vegetable include its species and variety, the time of year it is sown, climate, soil type and agricultural practices (Desrosier, 1983). Data for the fatty acid content of legumes are scarce in the literature, and what data there are refer to the dried seed rather than the whole green bean (Apatá and Ologhobo, 1994). Seeds of *Phaseolus vulgaris* L. grown in California have been shown to contain the same three major fatty acids which were indicated by our results but in different relative proportions, in particular linolenic acid was most abundant (Hilditch and Williams, 1964).

Table III
 Fat and fatty acid content in green beans

Fat Constituents	mg/100 g dry weight	% FA/TFA
	$\bar{x} \pm SD$	$\bar{x} \pm SD$
C12:0	5.32 \pm 2.18	1.65 \pm 0.35
C14:0	3.65 \pm 1.14	1.07 \pm 0.29
C16:0	113 \pm 19.5	35.2 \pm 4.72
C16:1	4.72 \pm 1.80	1.47 \pm 0.64
C18:0	26.5 \pm 3.21	8.24 \pm 0.86
C18:1	20.7 \pm 5.48	6.44 \pm 1.59
C18:2	69.4 \pm 13.5	21.6 \pm 3.29
C18:3	74.1 \pm 18.3	23.1 \pm 4.17
C20:0	3.98 \pm 1.04	1.24 \pm 0.25
Total Fat		
0.97 \pm 0.11		
g/100 g dry weight		

\bar{x} : Mean; SD: Standard Deviation
 FA: Fatty acid; TFA: Total Fatty Acid

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