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Short communication

Gas chromatographic determination of the fatty-acid content of heat-treated green beans ☆

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Abstract

A gas chromatographic method that employs flame ionization detection, a DB-Wax capillary column with helium as the carrier gas, and a split–splitless (1:15) injector was used to determine the effects of different heat treatments on the fatty-acid content of whole green beans (*Phaseolus vulgaris*, L.). A one-step-extraction–methylation method was used to obtain fatty acid methyl esters from raw and steamed, boiled, pressure-cooked, and microwave-cooked green beans. The fatty-acid profile changed slightly, but heat treatment produced an increase in the fatty-acid content.

Keywords: Vegetables; Food analysis; Fatty acids

1. Introduction

The lipid content of vegetables is quite low but its study is of interest due to the tendency of this component to grow rancid, reducing the nutritional value of the food and producing unpleasant odors and flavors in the process [1]. In addition, this small amount of lipids is of considerable dietary importance since it includes two polyunsaturated fatty acids, linoleic and linolenic acid, which are not synthesized by humans and which are important for health [2]. The elaboration and cooking of foods inevitably alters their composition and can thus affect organoleptic and nutritive properties [3]. Some vegetables can be eaten raw, but there are many, like green beans, that need to be cooked before being acceptable to the consumer. In this work, we examined the effect of four heat treatments (steaming, boiling, pressure-cooking and microwave heating) on the fatty acid (FA) content of green beans (*Phaseolus vulgaris* L.). Many methods have been developed for the determination of fatty acids in foods. These include Fourier transform (FT) IR spectroscopy [4], HPLC [5] and GC [6–8], with GC-based procedures currently being the most popular. In this work, FAs were extracted and methylated in a single step, and a GC method was developed to determine them.

2. Experimental

2.1. Samples

Green beans were purchased in a local supermar-

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ket. Five representative 40 g batches of diced beans were obtained. One batch was for raw analysis and each of the others was immediately subjected to one of the following heat treatments.

1. Boiling in a covered pot in 1 L of water for 30 min.
2. Steaming in a covered pot for 40 min.
3. Pressure cooking in 200 mL of water for 5 min after the first issue of vapor.
4. Microwave cooking in 150 mL of water for 5 min.

All of the treatments used the minimum level of tap water and the minimum cooking time to reach a similar level of tenderness that would give adequate palatability and taste; the least tender product was obtained by microwave cooking. After heat treatment, raw and cooked batches were homogenized and freeze-dried.

2.2. One-step methylation–extraction method

After drying, the batches were carefully ground with a glass pestle and divided into three, 0.7 g samples (5×3 samples). The samples were then processed as described by Sukhija and Palmquist [9]. In brief, about 0.7 g of each sample was weighed in a 160×16 mm Pyrex tube fitted with a PTFE-lined screw cap. Then, 2 ml of toluene (Carlo Erba, Milan, Italy) and 3 ml of freshly prepared 5% methanolic HCl (methanol, HPLC grade, and HCl; Scharlau, Barcelona, Spain) were added. After carefully mixing the contents and flushing the headspace of the vial with nitrogen, the tubes were heated for 2 h in a water bath at 70°C. To the cooled tubes, 5 ml of 6% K_2CO_3 (Scharlau), 1 ml of toluene and 1 ml of nonadecanoic acid methyl ester (Sigma, St. Louis, MO, USA) (I.S. toluene solution) were added and the mixture was thoroughly vortex-mixed. Phase separation was accomplished by centrifugation for 5 min at 1100 rpm in an Ettich EBA 12 centrifuge. The organic layer was dried with anhydrous Na_2SO_4 (Scharlau) and 1 μl was injected.

2.3. Fatty acid methyl ester (FAME) analysis

A Fisons GC-8000 series (Manchester, UK), fitted with a split–splitless injector and a Fisons EL-980 flame ionization detection (FID) system, was used to separate and quantify individual FAMEs. Individual FAMEs were separated using a J&W (Folsom, CA, USA) DB-Wax capillary column (60 m×0.32 mm I.D. fused-silica column coated with a 0.25 μm polyethylene glycol film). Peak areas were processed using Chrom-Card for Windows (Carlo Erba) software (version 1.18). Samples (1 μl) were injected with a split ratio of 1:15 at a column temperature of 110°C and an injector temperature of 250°C. The carrier gas used was helium at a flow-rate of 1.37 ml/min, an average linear velocity of 22.76 cm/sg and a head pressure of 15 p.s.i. (1 p.s.i.=6894.76 Pa). Five minutes after injection, the temperature was raised at 5°C/min to 220°C and held for 10 min (39 min total). The detector temperature was 260°C. The FAMEs were identified by comparison of their retention times with standards (all from Sigma) and by comparison of their mass spectra with the Wiley (New York, USA) mass spectra library (version 1.4). For mass spectra analysis, a Fisons MD-800 (Manchester, UK) mass detector and Finnigan GC/MD software (version 1.4) were used.

3. Results

The precision of the method was calculated as the coefficient of variation of the amount of each FAME in eight portions obtained independently from a single sample of lyophilized green beans. The precision of the measurement was calculated as the coefficient of variation of nine GC determinations of the amount of each FAME in a single solution. The recovery of the method was calculated using ten portions of the same lyophilized sample and spiking with various quantities of each free fatty acid. The limit of detection of each FAME was calculated following ACS guidelines [10]. The results were similar for each component, so these values are expressed by their means. For the precision of chromatographic measurement, we found a RSD of 0.957%; the precision of the analytical method gave a RSD of 3.40%; the recovery was 101.5%, and the limit of detection was 1.82 μg/ml.

The fatty-acid content was increased by all of the heat treatments (see Table 1). Pressure-cooked green beans showed an increase in fatty-acid content of about 15% compared to raw beans. Steamed, boiled

Table 1
Fatty-acid composition of raw and heat-treated green beans (mg/100 g raw beans±SD; $n=3$)

Fatty acid	Content (mg/100 g)				
	Raw	Pressure-cooking	Steaming	Boiling	Microwaving
$C_{16:0}$	18.01±1.82	17.93±1.09	20.99±1.78	21.70±2.61	23.79±2.84
$C_{18:0}$	4.06±0.49	3.52±0.19	4.36±0.34	4.98±0.54	4.93±0.90
$C_{18:1}$	3.19±0.45	1.98±0.39	3.12±0.32	4.41±0.77	4.25±0.98
$C_{18:2}$	17.65±2.12	20.30±1.68	25.82±2.23	27.68±2.96	27.85±3.86
$C_{18:3}$	24.21±2.74	33.40±2.27	41.28±3.13	41.50±3.86	44.30±5.68
Total	67.12	77.13	95.57	100.27	105.11

Table 2
Fatty-acid profile (%) of raw and heat-treated green beans

Fatty acid	Profile (%)				
	Raw	Pressure-cooking	Steaming	Boiling	Microwaving
$C_{16:0}$	26.8	23.2	22.0	21.6	22.6
$C_{18:0}$	6.1	4.6	4.6	5.0	4.7
$C_{18:1}$	4.7	2.6	3.3	4.4	4.0
$C_{18:2}$	26.3	26.3	27.0	27.6	26.5
$C_{18:3}$	36.1	43.3	43.2	41.4	42.1

and microwave-cooked beans showed increases of between 42 and 57% in the fatty-acid content. In spite of those changes in the fatty-acid content, the profile of the fatty acids only changed slightly after heat treatment (see Table 2). This agreed with the results of Minka et al. [8], who observed that heat treatment does not induce changes in levels of fatty acids in cooked black beans (*Phaseolus vulgaris* L.) and of Attia et al. [11], who found that cooking and parching caused only slight changes in lipid classes and fatty-acid composition of chickpea (*Cicer arietinum* L.).

4. Discussion

Heat treatments inhibit lipase and cause a marked drop in lipoxygenase [11]. This could explain why only raw green beans showed a minor content of linolenic acid, because this fatty acid is a very good lipoxygenase substrate and could easily be degraded. The thermal inactivation of lipoxygenase could produce an increase of its substrates, linoleic and linolenic acids, but cannot explain the similar increase in each FA. Neither can it explain the similar FA profile obtained following all of the treatments. This regular increase in these components may be due to a sample concentration of FAs in the sample because of the losses of water-soluble components such as free sugars, starch, pectins, pigments or organic acids during the heat treatments. Treatments that produce minor losses of some of these components, like pressure cooking [3,12], showed a minor increase in FAs.

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