



Low linolenic soybeans for biodiesel: Characteristics, performance and advantages

Eleonice Moreira Santos^{a,*}, Newton Deniz Piovesan^b, Everaldo Gonçalves de Barros^c,
Maurilio Alves Moreira^a

^a Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa, Viçosa, MG, Brazil

^b Instituto de Biotecnologia Aplicada a Agricultura, Universidade Federal de Viçosa, Viçosa, MG, Brazil

^c Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, MG, Brazil

HIGHLIGHTS

- ▶ Two soybean genotypes with low and normal linolenic acid content as sources of raw material for biodiesel production.
- ▶ Evaluation of the soybean characteristics, and main biodiesel properties.
- ▶ Biodiesel of the low linolenic soybean presented good properties.

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ABSTRACT

Soybean is one of the main raw materials used for biodiesel production. However, the polyunsaturated fatty acids present in soybean seeds are not desirable for this purpose due to their low oxidative stability. Therefore, it is expected that the use of soybean cultivars with low linolenic acid content for biodiesel production will improve its oxidative stability and the cold filter plugging point (CFPP). This work presents the main characteristics, the advantages and performance of low linolenic acid soybean (LL) as compared to a conventional soybean variety (CO) for biodiesel production. The results showed that LL oil and protein contents were similar to those of CO. Phosphatide concentration was higher in LL oil, while total tocopherol content was lower in relation to CO. With respect to LL biodiesel performance, oxidative stability was much higher than that produced from CO, and the CFPP did not change even with the improved fatty acid profile of LL.

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1. Introduction

Soybean (*Glycine max*) is the most important source of protein and oil worldwide [1]. It is widely cultivated in a number of countries, with the major producers being the US (33%), Brazil (27%), Argentina (21%) and China (7%) [2]. Soybeans are a valuable source of protein and oil. The protein is primarily used as feed, with some food applications, while the oil is more broadly incorporated into food, feed, and some industrial applications, especially biodiesel production [3]. As demand for soybean oil and protein increases, improvement of soybean quality and production through genetic breeding has become an important issue [4,5].

Among the different characteristics of soybean to be improved, the oil fraction has a special appeal, especially due to its extensive domestic and industrial use and, more recently, demand for it as a

raw material for biodiesel. Protein and oil contents in soybeans average approximately 40% and 20%, respectively [3]. Soybean oil is primarily composed of five fatty acids: palmitic acid (~13%), stearic acid (~4%), oleic acid (~18%), linoleic acid (~55%) and linolenic acid (~10%) [6]. Overall, 80–85% of esters are unsaturated, mainly characterized by high linoleic and linolenic acid contents which are responsible for low oil oxidative stability [7,8]. Thus, biodiesel produced from soybean oil has a high tendency to undergo oxidation, which makes it difficult to store and consequently affects overall quality [8–11]. In addition to oxidative stability, the composition and structure of fatty acids directly influence the properties of biodiesel, such as cetane number, cold flow, viscosity, lubricity and heat of combustion [7,12].

To address the problems related mainly to the low oxidative stability of soybean oil, genetic breeding programs have selected genotypes with altered palmitic, stearic, oleic, linoleic and linolenic acid contents in the seeds. These changes have been achieved through genetic modification of enzyme expression involved in fatty acid biosynthesis, either by induced mutagenesis or by direct DNA manipulation [1].

* Corresponding author. Address: Instituto de Biotecnologia Aplicada à Agropecuária – BIOAGRO, Universidade Federal de Viçosa, Av. PH Rolfs, s/n CEP 36570-000 Viçosa, MG, Brazil. Tel.: +55 31 3899 2977/2944; fax: +55 31 3899 2864.

E-mail addresses: emsquimica@hotmail.com (E.M. Santos), piovesan@ufv.br (N.D. Piovesan), ebarros@ufv.br (E.G.de. Barros), moreira@ufv.br (M.A. Moreira).

Reduced linolenic acid and increased oleic acid contents are the best characterized modifications. Low linolenic soybean (under 3%) was the first trait-modified crop introduced by Monsanto and its divisions. This was achieved through conventional breeding methods using marker assisted selection (Monsanto and Dupont). This content was further reduced to 1% (Asoyia). Oils from these cultivars were first used in the food industry due to gains in oxidative stability. These oils do not need hydrogenation and have an extended shelf life [5].

Several studies with low linolenic acid soybean varieties demonstrated that they present higher oxidative stability compared to conventional varieties [13,14].

In order to use soybean varieties with modified fatty acid profiles as raw material for biodiesel production, Graef et al. [15] and Tat et al. [16] evaluated a soybean line with high oleic and low palmitic acids. The results showed that the biodiesel produced presented better qualities than that from conventional soybeans.

In Brazil, soybean is responsible for over 80% of all biodiesel produced [17]. In Argentina it represents 100%, in the United States about 74% and in the European Union only 16% [18,19]. Therefore, soybean is a promising source for biodiesel production. However, the main problem associated with soybean biodiesel is its poor oxidative stability due to the high concentration of unsaturated fatty acids.

It is expected that the use of low linolenic acid soybean as raw material for biodiesel production will overcome the drawbacks of conventional soybean biodiesel. In this work we evaluated two soybean genotypes with low and normal linolenic acid contents with respect to their biochemical profiles and as sources of raw material for biodiesel production.

2. Material and methods

In this study two soybean genotypes were used: cultivar Monarca (CO), a conventional soybean cultivar with normal linolenic acid content (~8%), and A29 (LL), a soybean line developed by Dr. Walter Fehr from Iowa State University, with low linolenic acid content (~1%).

2.1. Oil extraction for biodiesel production

About 40 kg of grains from each soybean cultivar were pressed in a mini continuous press (model Ecirtec MPE-40, São Paulo, Brazil) for oil extraction. The oil was degummed prior to the transesterification reaction.

2.2. Soybean characterization

The soybean was characterized by: oil content, determined by direct extraction in a Soxhlet apparatus, according to method 920.39C of the AOAC [20]; total protein, determined according to method 991.20 of the AOAC [21]; free fatty acid (FFA) determined by AOCS Official Method Ca 5a-40 [22]; peroxide value (PV) determined by AOCS Official Method Cd 8-53 [23]; tocopherols determined by AOCS Official Method Ce 8-89 [24]. Due to insufficient separation under HPLC conditions, β -tocopherol was integrated with γ -tocopherol. Fatty acid methyl ester (FAME) composition of each oil was determined according to the method adapted from Bubeck et al. [25] in a gas chromatograph model GC-17A Shimadzu, equipped with FID detector using a DB-Wax column – J & W Scientific (30 m \times 0.25 mm), with the following conditions: injector temperature 245 °C, detector temperature 280 °C. The initial column temperature was 200 °C and its temperature was programmed to increase at a rate of 3 °C/min until a final temperature of 225 °C. The carrier gas used was nitrogen with a flow rate of

1.3 mL/min and the results were expressed as percentage of relative area of each FAME.

2.3. Biodiesel production

Biodiesel from soybean oil was produced by the alkaline transesterification reaction. The reaction was performed at 60 °C in a 5 L reactor with mechanical agitation. The molar ratio of methanol to oil was 6:1 along with 1.5% of catalyst – 30% sodium methylate in methanol. The total reaction time was 80 min under constant agitation. Subsequently, the residual methanol was removed and the glycerin separated by decantation. The biodiesel was dry washed using an ion exchange acid resin – Amberlite BD10DRY Dow-Rohm Haas.

2.4. Biodiesel characterization

The following properties of soybean oil biodiesel were determined: cold filter plugging point (D 6371-99); oxidative stability (EN 14112); % conversion of the oil in biodiesel, performed using biodiesel analyzer – InfraSpec VFA-IR Spectrometer of Wilks Enterprise, Inc.

3. Results and discussion

3.1. Soybean characteristics

3.1.1. Fatty acid composition

The fatty acid profiles of the soybean cultivars Monarca (CO) and A29 (LL) are quite distinct (Table 1), especially with respect to oleic (18:1) and linolenic (18:3) acid contents. The 18:1 content is greater in LL and corresponds to $31.14 \pm 2.23\%$ of the total fatty acid content, while the 18:3 content is lower in this cultivar and represents only $1.14 \pm 0.01\%$. The fatty acid profile of LL soybean oil is similar to that present in the soybean oil of the cultivar developed by Asoyia – Ultra low linolenic (C16:0–11%, C18:0–5%, C18:1–25%, C18:2–58% and C18:3–1%), designed primarily to meet the needs of the food industry [5].

Oil with increased C18:1 associated with decreased C18:3 contents has various industrial applications due to the high oxidative stability presented by this combination [26]. Partially hydrogenated vegetable oils have been used for these purposes. However, chemical hydrogenation results not only in simple reduction of unsaturated fatty acids, but in double bond migration and isomerization of cis double bonds into the thermodynamically more stable trans configuration [27].

Several studies have reported chemical modification of biodiesel fatty acid methyl esters (FAME) by hydrogenation [28–30]. The total saturated fatty acid percentage increases together with the formation of trans fatty acid methyl esters. The hydrogenated FAME shows higher oxidative stability and higher cetane number but poor cold flow properties, kinematic viscosity and

Table 1
Fatty acid profile from CO and LL soybeans.

Fatty acids (%)	LL	CO
C16:0	9.88 \pm 0.34	11.38 \pm 0.01
C18:0	5.06 \pm 0.19	4.07 \pm 0.06
C18:1	31.14 \pm 2.23	17.57 \pm 0.06
C18:2	52.77 \pm 1.67	58.95 \pm 0.004
C18:3	1.14 \pm 0.01	8.01 \pm 0.13
Σ Saturated (C16:0 + C18:0)	14.94	15.45
Σ Monounsaturated (C18:1)	31.13	17.57
Σ Polyunsaturated (C18:2 + C18:3)	53.91	66.96

CO:Y – carbon number: unsaturated number; \pm standard deviation.

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