Industrial Crops and Products 48 (2013) 124–132

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

The effect of genotype and environment on biodiesel quality prepared from Indian mustard (*Brassica juncea*) grown in Australia

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ARTICLE INFO

Article history:
Received 4 February 2013
Received in revised form 27 March 2013
Accepted 2 April 2013

Keywords:
Biodiesel
Genotype
Environment
Mustard
Fatty acid

ABSTRACT

Two experiments were conducted in north-western New South Wales, Australia to determine the effect of genotype (G), growing site (S) and year (Y) on the suitability of Indian mustard (*Brassica juncea*) as a biodiesel feedstock. The first experiment analyzed the effect of growing environment on six mustard genotypes while the second experiment analyzed the effect of sowing on the same genotypes across two seasons. The results demonstrate that late sowing forced maturity of the seed and decreased the yield whilst early sowing resulted in economically viable seed yields (>1.3 t/ha). The oil content of the seed ranged from 34 to 39.8% and the main fatty acids present in the oil were oleic (C18:1) and linoleic acid (C18:2) in both experiments. The main factor that impacted on the fatty acid profile in a single season was the seed genotype while in the second experiment the growing year and interactions between year and the other parameters had a major impact on the fatty acid profile. The main fatty acids affected by the growing year were oleic, linoleic and erucic (C22:1). Oleic and linoleic acids were inversely correlated with erucic acid content which tended to be higher in cooler growing conditions. Two of the genotypes were processed into biodiesel and assessed for quality and the fuel met most requirements except for oxidation stability and kinematic viscosity. The relatively high concentration of polyunsaturated fatty acids was deemed to be responsible for the poor oxidation stability and higher amounts of erucic acid and glycerol would contribute to poor kinematic viscosity values. The mustard genotypes analyzed may prove to be both a viable break crop as well as providing a good feedstock for the establishment of a biodiesel industry in this area.

1. Introduction

Biodiesel is a fuel prepared by transesterification of the long-chain fatty acids contained in 'non-fossilized' oil feedstocks such as vegetables, seeds, animal fats, algae and used cooking oils. The substitution of traditional petroleum-based fuels with renewables such as biodiesel is seen as a long-term strategy that will provide both a secure and diverse range of energy sources while reducing greenhouse gas emissions. In addition to production from renewable feedstocks, biodiesel possesses other 'green' credentials such as low sulfur content, biodegradability, reduced toxicity, and generally lower emissions compared with current petroleum-based fuels (Jham et al., 2009; Moser, 2009). Biodiesel does have some disadvantages when compared to traditional fuels including poor low temperature operability and oxidative stability (Moser, 2009) which are mainly attributed to the fatty acids contained in the oil feedstock. In some cases the use of fuel additives may attenuate some of these problems however the use of appropriate feedstocks that possess better chemical properties may prove to be a sustainable alternative.

Even though Australia can currently supply most of its transportation fuels, increased demand will see further pressure to develop alternative fuel sources. Renewable energy sources accounted for just over 5% of energy consumption in Australia in 2008/09 and biofuels accounted for only 8% of the renewable energy consumed (Schultz and Petchey, 2010). To improve the sustainability of energy resources the Australian federal government set an annual production target of 350 million L of biofuels. Production capacity for biodiesel reached 100 million L in 2010/11 and used mainly tallow, canola and recycled cooking oils as the feedstock (ACCC, 2011). Despite the advantages of biodiesel, the high cost of the oil is a constraint to developing a biodiesel industry; around 85–88% of production expenses are attributed to purchasing the feedstocks (Retka-Schill, 2008). It has been reported that high feedstock prices has led to the closure or production stoppages of some established Australian biodiesel plants (ACCC, 2010);alternatives to

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0926-6690/$ – see front matter
http://dx.doi.org/10.1016/j.indcrop.2013.04.016

expensive commodity feedstocks are critical to the continued survival and expansion of the biodiesel industry. However, sourcing oil for biodiesel from crop land may compete with food and fiber production, potentially driving up the price of essential commodities. Therefore, if a biodiesel industry is to be sustainable a feedstock must be identified that; provides a source of energy equivalent in quality to fossil-based fuels; can be produced in large quantities; and does not directly compete with food and fiber crops for available arable land.

Indian mustard (*Brassica juncea* L.) is an annual herbaceous oilseed-bearing plant belonging to the Brassicaceae family. Conventional breeding methods have focused on improving seed yield and fatty acid profile and mustard is now grown widely in South Asia for cooking oil, and on a small scale in Australia for condiment mustard production (Oram et al., 2005). However, it may prove to be a reliable crop for biodiesel production like other *Brassica* species that have already gained widespread acceptance as feedstocks for biodiesel production such as *B. napus* (canola), *B. alba* (white mustard), and *B. carinata* (Ethiopian mustard) (Jham et al., 2009; Moser, 2009).

Besides its potential as a fuel source, mustard has agronomic benefits and can be used as a break crop in the drier areas of Australia where canola is poorly adapted. Field trials indicate competitive seed yields, a requirement for fewer inputs such as fertilizer, and improved resistance to shattering when compared to canola (Hocking et al., 1997; Angadi et al., 2000; Oram et al., 2005). When grown as a break crop, mustard may improve the yield of crops such as wheat and barley grown in the following winter season. According to Angus et al. (1991), growing mustard as a break crop increased the yield of a subsequent wheat crop by 30% compared to wheat grown after wheat. Break-crop benefits may also extend to a second successive wheat crop resulting in an average increase of 13% in grain yield (Kirkegaard et al., 1997). The rotational benefits of *Brassica* crops are due to the glucosinolates exuded from root tissues which are hydrolysed to form isothiocyanates that act as biofumigants suppressing the growth of disease-causing microorganisms (Angus et al., 1994; Kirkegaard and Sarwar, 1998). In the north western areas of NSW, *B. juncea* has been shown to effectively reduce the incidence of crown root rot caused by *Fusarium pseudograminearum* (Kirkegaard et al., 2003). Thus, the benefits of growing *B. juncea* are twofold – the root exudates act to break a disease cycle thereby improving cereal crop growth, and the oil can be processed into biodiesel.

In the north west of NSW, the potential growing area available for *B. juncea* oil seed production is around 4 mha, however, the major impediment for establishment of this crop is a lack of suitable mustard varieties that provide both biodiesel quality and an economic return to growers. Hancock (2005) reports that yields would need to reach a minimum of 1.3 t/ha for mustard to be competitive as a feedstock for biodiesel production. However, the influence of genotype and environment on fatty acid profile in *B. juncea*, and hence biodiesel quality, is not well understood. The main biodiesel properties affected by fatty acid properties are the cetane number (CN), oxidative stability and cold flow properties. The CN is related to the combustion properties and longer, saturated fatty acids result in good cetane numbers (Ramos et al., 2009). Saturated fatty acids also have superior oxidative properties compared with polyunsaturated fatty acids of equivalent chain lengths; however saturated fatty acids result in biodiesel with poor cold flow properties (Moser, 2009). Therefore, good biodiesel quality requires a fine balance of fatty acids of appropriate chain length and saturation to provide sufficient combustion while maintaining oxidative stability and cold flow properties. It appears that a high concentration of oleic acid in the raw feedstock results in biodiesel that satisfies most of these parameters (Ramos et al., 2009). This project examines the effect of environment on the yield and fatty acid profile of six mustard genotypes grown in replicated trials across northwestern NSW, Australia.

2. Materials and methods

Fatty acids and biodiesel were prepared from the oilseed of two Indian mustard genotypes (Muscon BM11 and Hermola-805) grown in a preliminary study in 2008. The biodiesel was analyzed in a commercial laboratory and compared to the Australian standards as outlined in the Fuel Standard (Biodiesel) Determination 2003 which uses a combination of both ATSM and EN standards (http://www.comlaw.gov.au/Details/F2009C00146). These fatty acid profiles were also used as a standard for comparison of the samples prepared from the field trial samples.

As fatty acid profile in the seed is controlled by the genetics of the plant as well as the growing environment (Hou et al., 2006) two separate experiments were then conducted to analyze the effect of environmental conditions on a range of Indian mustard genotypes and whether variation in the growing environment (location and/or sowing date and year), and/or genotype significantly impacts on the fatty acid profile of the seed and subsequent biodiesel quality prepared from the oils. In the first experiment six *B. juncea* genotypes were grown in a single season (2009) across five different locations in unique randomized complete block designs with two replications. In the second experiment the same oil seed genotypes were sown at a single location at two different sowing dates in two different seasons (2009 and 2010) in a randomized complete block design with three replications.

2.1. Oilseed genotypes and growth locations

To determine the viability of a biodiesel industry six mustard genotypes were grown in experiments at five sites in north western NSW in 2009. The sites were Narrabri (early and late sown), Mungindi, Come-by-Chance and Nowley. Weather data, including rainfall and temperature, and crop growth parameters were recorded at Narrabri (Table 1; Fig. 1). The six mustard genotypes for fatty acid analysis were selected based on high yield and diverse genetic background. The second experiment was sown at Narrabri in 2010 at two planting dates to examine the effect of season on seed and oil yield, and fatty acid profile of the same six genotypes evaluated in the 2009 experiments. The six mustard genotypes were 65-3CSIRO*60–9/Pollen bulk; JM97.3*43B-2/Pollen bulk; JM97.3*5-7/Pollen bulk, Muscon BM11, Hermola-805 and Canadian #2. The canola check cultivar was not assessed for fatty acid profile as the grain yield was significantly lower than the selected mustard genotypes at all sites. Samples of grain were taken from each replicated plot from all genotypes at all locations. Oil content was determined using NIR using the canola calibration (Foss NIRSystems Inc.) and then extracted from the seed by cold pressing and stored at 4 °C until the preparation of fatty acid methyl esters (FAME) for gas chromatography (GC) analysis. Oil from two separate field samples was prepared for each genotype.

2.2. Preparation of FAMEs

An esterification reagent was prepared immediately before use by adding 2 g of NH_4Cl to methanol (60 mL) in a 150 mL Quickfit round bottom flask. Concentrated sulfuric acid (98%, 3 mL) was added, and the mixture refluxed for 15 min using a water condenser. The esterification reagent was then allowed to cool before use.

Both of the replicate samples of oil were used to prepare duplicate laboratory FAME samples for GC analysis. Oils (0.1–0.2 g)