Available online at www.sciencedirect.com
ScienceDirect
Industrial Crops and Products 26 (2007) 337–344
INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL
www.elsevier.com/locate/indcrop

Dynamics of fruit growth, accumulation of wax esters, simmondsins, proteins and carbohydrates in jojoba

A. Benzioni [a,*], M. Van Boven [b], S. Ramamoorthy [a], D. Mills [a]

[a] *The Institutes for Applied Research, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel*
[b] *Laboratory for Food Chemistry, Katholieke Universiteit Leuven, O&N2, Herestraat 49, B-3000 Leuven, Belgium*

Received 7 March 2007; received in revised form 12 April 2007; accepted 18 April 2007

Abstract

The aim of this study was to learn the developmental aspects and compositional changes during the development of jojoba [*Simmondsia chinensis* (Link) Schneider] fruit and seed. Three Israeli clones: Forti, Benzioni and Shiloh were used. By 50–60 days after pollination (DAP) the capsules had grown to attain their full dimensions. Only then did the embryos begin to rapidly gain weight and accumulate storage reserves. Seed growth followed a sigmoid pattern, reaching final weight by about 140 DAP. The appearance of wax bodies began at 55–65 DAP and wax deposition continued during the whole period of seed maturation, including late seed maturation. Protein concentration remained constant during the entire period of seed growth, increasing proportionately with seed weight. Sugar concentration was high at the beginning of the maturation period and declined throughout maturation. The sugar profile changed during maturation as the ratio of sucrose to reducing sugars increased several fold by 50–60 DAP, just as the embryos switched from the embryogenesis stage to maturation and began accumulating storage reserves.

Simmondsins were present at concentrations of 3–4% when sampling first began (47–55 DAP) but no ferulate was detected until 75–86 DAP. During maturation, simmondsins concentration rose gradually and by late maturation, when dehydration began, simmondsins concentration had increased considerably to concentrations of 9–15%.

Three developmental phases were identified: early embryogenesis lasting around 2 months from pollination, maturation with accumulation of storage reserves, and late maturation when simmondsins accumulate.

Keywords: Wax; Simmondsins; Fatty acids; Fatty alcohols; Wax bodies; Protein bodies; Seed development

1. Introduction

Jojoba is a crop grown for its seeds, which contain, along with proteins and sugars, 50–56% liquid wax – used mainly in the cosmetic industry – and a group of cyanoglucosides known as simmondsins. Simmondsins have an anorexic effect and can be extracted and used as a satiating factor (Flo et al., 1998).

When female and male flowers reach anthesis, usually in early spring, pollination and double fertilization of the embryo sac take place and seed production commences. The full development of the fruit and embryo may take 3–4 months, depending on genotype and climate (Gentry, 1958; Wardlaw and Dunstone, 1984). The capsule (pericarp) develops soon after pollination and within a few weeks forms a large cavity, which the fertilized ovule and embryo begin to fill. During this period the embryo goes through several morphogenetic stages, during which cell division and differentiation take place. The endosperm is reabsorbed during this time. Maturation begins about

* Corresponding author. Fax: +972 8 6472984.
E-mail address: benzioni@bgu.ac.il (A. Benzioni).

0926-6690/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2007.04.004

50–60 days after pollination (DAP) when the embryo cells expand and storage products begin to accumulate. The storage lipids – wax esters of monounsaturated long-chain fatty acids (18–24 carbons) and long-chain fatty alcohols (20–24 carbons) (Miwa, 1971) – are stored in wax bodies (Rost and Paterson, 1978). Proteins, consisting of 79% albumins and 21% globulins (Shrestha et al., 2002), are stored in protein globules (Rost and Paterson, 1978). The mature seed also contains a large amount of simmondsins (Verbiscar et al., 1980; Benzioni et al., 2005).

Data concerning the kinetics of fruit development, particularly the accumulation of storage products in jojoba, are very scarce, and no information is available to date on the kinetics of the accumulation of simmondsin derivatives (Ohlrogge et al., 1978; Xiang-Yu et al., 1981).

In this study, developmental aspects and compositional changes during the development of jojoba seeds are presented.

2. Materials and methods

2.1. Plant material

Three commercial jojoba genotypes ('Benzioni', 'Forti' and 'Shiloh'), planted in 1999 and growing at the Hazerim plantation (western Negev, Israel), were used. Forty to sixty intact branches each with 15–35 swollen but still closed flower buds were bagged in paper bags fitted with a transparent plastic window. The branches were pollinated as their flowers began to open (anthesis) with pollen collected and stored as described by Vaknin et al. (2003). Each branch was pollinated a second time 6 days later to ensure pollination of all the flowers reaching anthesis. Pollination was performed in February 2004. The dates were 15 and 21 for the genotype 'Forti', 21 and 27 for the genotype 'Benzioni' and 22 and 28 for the genotype 'Shiloh'. The intermediate date between the two pollinations was used for reckoning of DAP (18, 24 and 25 February, respectively). Pollen was injected into the bags with a syringe without opening them.

2.2. Growth measurement during fruit filling

On designated dates, five to nine branches bearing 60–200 developing fruit were harvested. Capsules and seeds were separated and each pooled randomly into five groups (replications) each consistent of 12–30 seeds for fresh weight determination. The pooled samples were then dried for 48 h at 70 °C, and their dry weight was determined. The tissue was finely ground, pooled for each clone and date, and stored for further analysis.

Fruit and seed lengths were recorded for five fruit and seeds in each of five replications (25 seeds per date, and clone) until 70 DAP, after which their dimensions remained constant.

2.3. Wax content

Two grams of the dried ground seed were placed in tubes containing 20 ml of hexane. The tubes were sealed hermetically and placed in an oven at 85 °C for 3 h. After cooling, the supernatant was collected. The pellet was re-extracted a second and third time, each time using 20 ml of hexane. The amount of wax in the three (pooled) supernatants was determined gravimetrically. The defatted meal remaining after wax extraction was pooled and used for determination of simmondsins, sugars and protein.

2.4. Wax composition

The extracted seeds oil was subjected to ethanolysis, as described by Tonnet et al. (1984) or placed in petroleum ether and used for direct determination of wax components. The compositions of the ethanolysis products and of the intact wax esters were determined by gas chromatography (CP9001–Chrompack,10 m × 0.25 mm i.d.; CP-Sil-5-CB OVI column; FID detector).

2.5. Protein determination

One hundred milligram samples of the defatted meal were sonicated in 4 ml of 1 M NaCl for 1 h and kept overnight. On the next day they were centrifuged at 10,000 × *g* for 10 min. After collecting the supernatant the procedure was repeated twice and the supernatants were pulled. The extracts were diluted 1:50 in water and the protein concentration was estimated by the Bradford (1976) method using bovine serum albumin to prepare a standard curve.

2.6. Sugar determination

The defatted meal was dried in an air stream and two 100 mg samples (per clone and date) were extracted with 10 ml of methanol:water (90:10, v/v) in 20 ml extraction tubes for 2 h in a Branson-1200 bath sonicator (Branson, CT). Reducing sugars were detected spectrophotometrically by the Summner (1921) method, sucrose by the anthrone reagent method, and fructose by the resorcinol reagent method.