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Kinetics of the incorporation of the main phenolic compounds into the lignan macromolecule during flaxseed development

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ABSTRACT

The main flax lignan, secoisolariciresinol diglucoside, is stored in a macromolecule containing other ester-bound phenolic compounds. In this study, NMR and HPLC-UV analyses were performed on flaxseeds harvested at different developmental stages to identify and quantify the main phenolic compounds produced during seed development. Extraction was carried out with or without alkaline hydrolysis to determine if these molecules accumulate in the lignan macromolecule and/or in a free form. Monolignol glucosides accumulate in a free form up to 9.85 mg/g dry matter at the early developmental stages. Hydroxycinnamic acid glucosides and flavonoid accumulate (up to 3.18 and 4.07 mg/g dry matter, respectively) in the later developmental stages and are ester-bound in the lignan macromolecule. Secosiolariciresinol diglucoside accumulates (up to 28.65 mg/g dry matter) in the later developmental stages in both forms, mainly ester-bound in the lignan macromolecule and slightly in a free form.

1. Introduction

In recent decades, interest in flax has increased. This plant is grown in temperate countries for its fiber or its seed, which have applications in the composite material and textile sector or in the food sector, respectively. Flaxseed, which is extensively used in the oil industry, is also the main source of lignans, a class of phenolic compounds presenting benefit for human health.

The flaxseed is formed in a capsule containing up to ten seeds of variable weight and size, according to the genotype. The color of the seed varies from yellow to dark brown. At maturity, the capsule opens and releases its seeds into the near environment. Flaxseed development is typical of dicots, with the plantlet showing two cotyledons (Gutierrez et al., 2006; Venglat et al., 2011). From a structural point of view, the seed is composed of an embryo surrounded by a very small albumen, itself surrounded by an integument. From a developmental point of view, the integument is a maternal tissue that comes from the differentiation of two internal integuments arising from the epidermis of the mature ovule. The differentiation of the albumen occurs when the embryo reaches the globular stage and continues until the torpedo stage. It involves a gradual decrease in the size and number of cells in this tissue. During embryogenesis, the embryo stiffens and becomes yellowish then greenish. The seed coat changes to a dark brown color, once the seed contains no water anymore. This corresponds to the seed desiccation stage that maintains the seed in dormancy until germination. From embryogenesis to reserve accumulation and final desiccation, flaxseed development takes around 50 days, depending on genotypes.

The main lignan in flaxseed is secoisolariciresinol diglucoside (SDG) (Fig. 1, structure 7), a dibenzylbutane-type lignan formed by the coupling of two coniferyl alcohol moieties (Fig. 1, structure 2′) through an 8–8′ linkage. It is noteworthy that the coniferyl alcohol can also be incorporated into lignin polymer. The major fraction of SDG is incorporated into the lignan macromolecule via a hydroxymethylglutaric acid (HMG) ester linkage.

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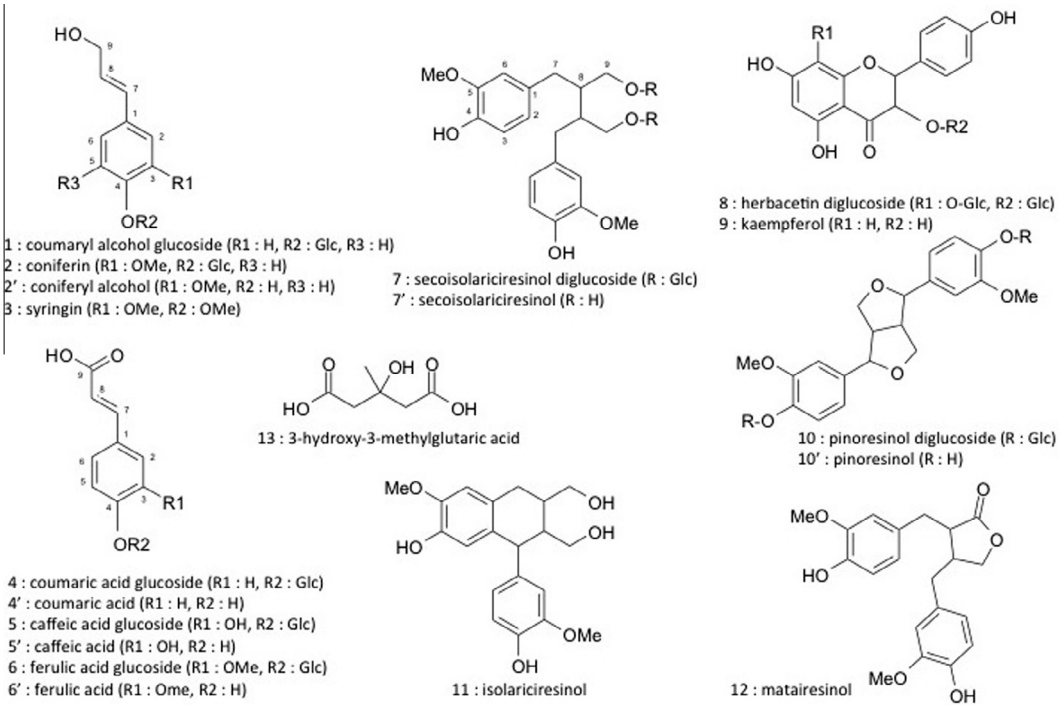

Fig. 1. Structures of the main molecules identified in flaxseeds.

Many studies have been performed to prove the biological activities of secoisolariciresinol (both the aglycone and diglucosylated forms) and its efficacy in the treatment or prevention of several diseases. *In vitro* and *in vivo* studies have revealed the complex action of this molecule by both non-hormonal and hormonal effects (Rickard-Bon & Thompson, 2003). The non-hormonal action is due to the polyphenolic structure of the molecule giving it an antioxidant capacity (Ganorkar & Jain, 2013). The hormonal action is due to the estrogenic or anti-estrogenic properties of the molecule. SDG has shown benefits in the prevention of cancers and in the treatment of cardiovascular, inflammatory and metabolic diseases (Adolphe, Whiting, Juurlink, Thorpe, & Alcorn, 2010).

Due to the interest for SDG in human health, several studies have been performed to characterize its biosynthesis in flaxseed. It is well established that SDG accumulates in the seed coat of flaxseed (Ford, Huang, Wang, Davin, & Lewis, 2001; Hano et al., 2006). The tissular location has been identified through experiments describing the Pinoresinol Lariciresinol Reductase (PLR) transcript detection in the hull by RT-PCR and by promoter-reporter transgenesis (Hano et al., 2006). Polyclonal antibodies against secoisolariciresinol have been developed to immunolocalize lignans in flaxseed, which are mainly found in the secondary wall of the sclerite cell layer of the outer integument of the seed (Attoumbre et al., 2010). Most of the studies on SDG have been performed on mature seeds and only a few papers have reported on the spatio-temporal synthesis of lignan and its precursors in developing flaxseeds (Attoumbre et al., 2010; Dalisay et al., 2015; Fang et al., 2013; Ford et al., 2001; Hano et al., 2006).

Lignan macromolecule composition has been the focus of many studies (Ford et al., 2001; Johnsson, Kamal-Eldin, Lundgren, & Aman, 2000; Kamal-Eldin et al., 2001; Kosinska, Penkacik, Wiczkowski, & Amarowicz, 2011; Li, Yuan, Xu, Wang, & Liu, 2008; Rickard et al., 1996; Strandas, Kamal-Eldin, Andersson, & Ñüman, 2008; Struijs, Vincken, Doeswijk, Voragen, & Gruppen, 2009; Struijs, Vincken, Verhoef, Voragen, & Gruppen, 2008; Struijs et al., 2007; Yuan, Li, Xu, Wang, & Liu, 2008). The linkage of SDG in the macromolecule has been elucidated (Rickard et al., 1996) but the exact composition of the lignan macromolecules is still not clearly determined. The composition of polyphenols in the lignan macromolecule may vary but tends to encompass SDG, coumaric acid glucoside (CAG) (Fig. 1, structure 4), ferulic acid glucoside (FAG) (Fig. 1, structure 6) and herbacetin diglucoside (HDG) (Fig. 1, structure 8) (Johnsson et al., 2000; Strandas et al., 2008) with a monomeric unit composed of SDG-HMG (Kamal-Eldin et al., 2001). SDG and HDG are linked to HMG (Fig. 1, structure 13) (Struijs et al., 2007) while CAG and/or FAG are linked to the glucose of SDG. The core structure consists of three SDG units and may vary between one and seven SDG units. One out of seven SDG units can be replaced by an HDG unit. Usually, CAG and FAG acids tend to be located in the terminal position of polymer chains (Li et al., 2008; Struijs et al., 2008, 2009; Yuan et al., 2008). After hydrolysis of the lignan macromolecule, the release of caffeic acid (Fig. 1, structure 5′) and kaempferol (Fig. 1, structure 9) has been reported (Kosinska et al., 2011; Li et al., 2008; Struijs et al., 2009; Yuan et al., 2008). Up to date, most of the studies of the lignan macromolecule composition were mainly performed on plant material obtained from mature flaxseed. The only study of the lignan macromolecule formation during seed development was carried out by Ford et al. (2001) through plant feeding experiments with labeled phenylalanine to follow its conversion into phenolics. These authors identified some metabolites that are present in the free form, including coniferin (Fig. 1, structure 2), SDG, and SDG-HMG analogues, whereas some metabolites such as coumaric acid (Fig. 1, structure 4′), CAG, ferulic acid (Fig. 1, structure 6′), FAG, coniferyl alcohol, pinoresinol (Fig. 1, structure 10′), isolariciresinol (Fig. 1, structure 11), secoisolariciresinol (Fig. 1, structure 7′) and matairesinol (Fig. 1, structure 12) are released after alkaline treatment. However no quantification was undertaken.

The notion of a macromolecule is important when thinking about the extraction of phenolic compounds of interest. Many experiments have been designed to optimize the extraction of SDG from flaxseed to improve its valorization in industry. Several extraction techniques have been evaluated to improve lignan yield from flaxseed. Herchi et al. (2014) published a review on the