COMMON VEGETABLES AND FRUITS AS A SOURCE OF 1,2-DI-*O*-α-LINOLENOYL-3-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL, A POTENTIAL ANTI-INFLAMMATORY AND ANTITUMOR AGENT

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ABSTRACT

*1,2-Di-*O*-α-linolenoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (DLGG) has previously been found in a few medicinal plants and food plants, and has been shown to possess antitumor and anti-inflammatory activity. A range of common vegetables and fruits was investigated for this bioactive galactolipid in order to establish which vegetables and fruits are good sources of DLGG. Most green vegetables had a relatively high content of DLGG of over 200 mg/kg fresh weight (FW), while its concentration in most fruits and root vegetables was low, ranging from "not detectable" to less than 100 mg/kg FW. The highest content of DLGG was found in spinach, kale, sugar peas and green beans, with concentrations ranging from 546 to 396 mg/kg FW. The results indicate that the galactolipid DLGG may play a role in human diet as an important nutraceutical.*

PRACTICAL APPLICATIONS

As demonstrated in the present investigation, the potential bioactive galactolipid 1,2-di-*O*-α-linolenoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (DLGG) is common in the edible parts of many food plants. If the pharmacological effects of DLGG are established in preclinical and clinical trials, it may contribute significantly to the health-promoting effects of certain food plants.

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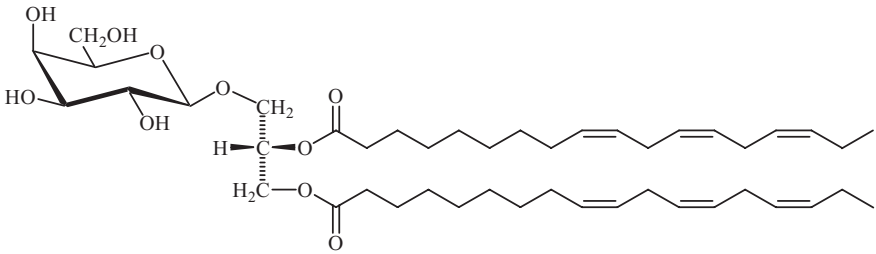
This information could be used to increase the content of DLGG in food plants and plant based products in order to improve their health promoting effects.

INTRODUCTION

Galactolipids are a class of compounds widely found in the plant kingdom, including edible plants, as they are an important part of the cell membranes. Galactolipids in plants consist mainly of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol containing one or two saturated and/or unsaturated fatty acids linked to the glycerol part (Dörmann and Benning 2002). Several galactolipids have been shown to possess *in vitro* and/or *in vivo* antitumor activity (Shirahashi *et al.* 1993; Nagatsu *et al.* 1994; Morimoto *et al.* 1995; Murakami *et al.* 1995, 2003; Wang *et al.* 2002; Kuriyama *et al.* 2005) and anti-inflammatory activity (Kikuchi *et al.* 1982; Murakami *et al.* 1995; Cateni *et al.* 2001; Larsen *et al.* 2003; Bruno *et al.* 2005). Particularly interesting is the MGDG galactolipid 1,2-di-*O*-α-linolenoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (DLGG, Fig. 1), which has been shown to possess various types of anti-inflammatory activity (Murakami *et al.* 1995; Cateni *et al.* 2001; Larsen *et al.* 2003). Further, it has been demonstrated that DLGG may be responsible for the antitumor-promoting activity of *Citrus hystrix*, a traditional herb in Thailand (Murakami *et al.* 1995) but also to some extent, the inhibitory effects of spinach (*Spinacia oleracea*) on human cancer cell proliferation (Wang *et al.* 2002; Murakami *et al.* 2003; Kuriyama *et al.* 2005).

The objective of this study was therefore to screen a large variety of common vegetables and fruits eaten in Europe and North America for DLGG,



DLGG

FIG. 1. CHEMICAL STRUCTURE OF THE BIOACTIVE GALACTOLIPID 1,2-DI-*O*-α-LINOLENOYL-3-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL (DLGG) PRESENT IN THE EDIBLE PARTS OF COMMON VEGETABLES AND FRUITS

in order to establish if this bioactive galactolipid may be a potential contributor to the health effects of vegetables and fruits.

MATERIALS AND METHODS

Plant Material

Samples of fresh (bell peppers, chili, broccoli, lettuce, cucumber, summer squash, celery, carrots, potato, tomato, onion, kiwi, plum, peach, nectarine, black currants, sweet cherries, mango, orange and apple) and frozen (peas, sugar peas, green beans, parsley, chive, kale, spinach and brussels sprouts) vegetables and fruits (Table 1) were obtained from a local grocery store. All fruits and vegetables were homogenized with an Ultra-Turrax homogenizer (model T25, IKA-Labortechnik, Staufen, Germany) at medium speed and stored at −25C before use.

Reagents and Solvents

Acetonitrile (MeCN), tetrahydrofuran (THF), acetone, *n*-hexane and dichloromethane were of Rathburn high-performance liquid chromatography (HPLC) grade (99.9% HPLC grade) purchased from Sigma-Aldrich, Steinheim, Germany. The water used for HPLC analysis was generated by an Elgastat Maxima Analytica Water Purification System (Elga Ltd., High Wycombe, Bucks, U.K.). All eluents for HPLC were filtered through a 0.45-µm Cameo 25P syringe filter (nylon) (Bie and Berntsen, Rødovre, Denmark) and degassed with ultrasound for 20 min before use.

Isolation and Characterization

DLGG was isolated from freeze-dried spinach according to the method reported by Larsen *et al.* (2003) with a few modifications. Freeze-dried spinach (100 g) was homogenized in *n*-hexane (500 mL) and extracted overnight at room temperature followed by extraction with acetone (1,000 mL). The acetone extract was filtered and evaporated to dryness *in vacuo* (<40C) and the residue extracted with dichloromethane (200 mL). DLGG, used as standard, was isolated from the dichloromethane extract by column chromatography and preparative HPLC according to the procedure described by Larsen *et al.* (2003) and identified by optical rotation and by nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C NMR, and ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy recorded in *d*-$CDCl_3$ and d_3-CD_3OD with trimethylsilane [TMS] as internal standard) as described previously (Larsen *et al.* 2003).

TABLE 1.
CONTENT OF THE BIOACTIVE GALACTOLIPID 1,2-DI-*O*-α-LINOLENOYL-3-*O*-β-D-GALACTOPYRANOSYL-*sn*-GLYCEROL (DLGG) IN THE EDIBLE PARTS OF COMMON VEGETABLES AND FRUITS, AS DETERMINED BY ANALYTICAL REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Vegetables and fruits		DLGG in mg/kg fresh weight*
Spinach	*Spinacia oleracea*	546 ± 48
Peas (sugar peas)	*Pisum sativum* var. *sativum*	442 ± 20
Kale	*Brassica oleracea* var. *acephala*	415 ± 25
Green beans	*Phaseolus vulgaris*	396 ± 35
Red chili	*Capsicum annuum* var. annuum	321 ± 15
Lettuce (heart)	*Lactuca sativa*	320 ± 18
Broccoli	*B. oleracea* var. *italica*	316 ± 25
Rose hips†	*Rosa canina*	270 ± 25
Green bell pepper	*Capsicum annuum*	243 ± 28
Peas	*P. sativum* var. *sativum*	239 ± 16
Brussels sprouts	*B. oleracea* var. *gemmifera*	225 ± 11
Curly lettuce	*Lactuca sativa*	206 ± 14
Green chili	*C. annuum* var. *annuum*	178 ± 10
Red bell pepper	*C. annuum*	167 ± 9
Cucumber	*Cucumis sativus*	94 ± 11
Iceberg lettuce	*L. sativa*	88 ± 5
Summer squash	*Cucurbita pepo*	86 ± 20
Chive	*Allium schoenoprasum*	68 ± 9
Black currants	*Ribes nigrum*	57 ± 6
Kiwi	*Actinidia deliciosa*	45 ± 8
Potato	*Solanum tuberosum*	41 ± 10
Yellow bell pepper	*C. annuum*	37 ± 2
Tomato	*Lycopersicon esculentum*	32 ± 6
Feldt lettuce	*L. sativa*	32 ± 3
Nectarine	*Prunus persica* var. *nectarina*	17 ± 5
Apple	*Malus domestica*	13 ± 6
Peach	*P. persica*	11 ± 4
Mango	*Mangifera indica*	11 ± 3
Plum	*Prunus domestica*	10 ± 4
Carrot	*Daucus carota* var. *sativas*	ND
Celery	*Apium graveolens* var. *dulce*	ND
Onion	*Allium cepa*	ND
Orange	*Citrus sinensis*	ND
Parsley	*Petroselinum crispum*	ND
Sweet cherries	*Prunus avium*	ND

* Mean of three replicates ± SD.

† Medicinal plant with well-documented effect against arthritis (Kharazmi and Winther 1999; Warholm *et al.* 2003; Winther *et al.* 2005).

ND, not detected.

Extraction for HPLC Analysis

Frozen homogenized samples (5 g) for quantitative analysis were further homogenized for 60 s with 20-mL acetone. After 18 h at 5C, the samples were centrifuged for 10 min at 20,845 × g. The supernatants were filtered through a 0.45-µm Cameo 25P syringe filter (Bie and Berntsen) directly into a 2-mL brown vial and subjected to HPLC analysis. The concentration of DLGG in common fruits and vegetables as determined by reverse-phase (RP) HPLC is shown in Table 1. Other extraction procedures were investigated using different extraction times, number of extractions and solvents. Optimal extraction conditions were obtained using a single extraction with acetone overnight as described. Other solvents, such as THF, work equally well and may be more useful when extracting dried plant materials.

HPLC Analysis and Quantification

Analytical RP-HPLC was carried out on a Dionex HPLC system (Dionex Denmark A/S, Hvidovre, Denmark) equipped with a photodiode array detector (PDA 100) operating between 200 and 700 nm. The photodiode array detector was employed at 203 nm for detection of DLGG. Separations were performed on a LiChrospher 100 RP-18 column (5 µm; 244 × 4 mm i.d., Merck, Darmstadt, Germany) protected with a LiChrosorb RP-18 guard cartridge (5 µm; 15 × 4 mm i.d.; Merck). The column temperature was maintained at 45C and the mobile phases consisted of solvent A (100% MeCN), solvent B (20% MeCN–80% H_2O) and solvent C (100% THF). Chromatographic separations were performed by the following solvent gradient: 0–10 min (100% B), 25 min (50% B, 50% A), 55 min (0% B, 100% A), 64 min (100% A), 74 min (80% A, 20% C), 85 min (80% A, 20% C), 95 min (100% A, 0% C), 105 min (0% A, 100% B) and 115 min (100% B). The flow rate was 1 mL/min and the injection volume 20 µL. Retention time for DLGG was 54 min. The concentration of DLGG in extracts was determined using DLGG as external standard. The HPLC method was validated with regard to linearity, accuracy and precision. The calibration curve of DLGG was linear in the concentration range 0.001–1 mg/mL with a correlation coefficient of $r^2 = 0.9996$. Mean recovery rate (~ accuracy) for DLGG was determined by spiking a known amount of authentic standard (from stock solutions) of DLGG to a rose hip sample with a known content of DLGG. Mean recovery rate of DLGG was 96.5 ± 2.6%. The precision of the HPLC method was determined by four injections of a rose hip sample in 1 day (intraday variability) and by analyzing the same rose hip sample on four different days (interday variability) with a relative SD <5% for DLGG. Limit of detection ($S/N = 3$) and limit of quantification ($S/N = 10$) for DLGG were determined to be 0.1 and 0.3 µg/mL, respectively.

RESULTS AND DISCUSSION

The content of DLGG in the edible parts of common vegetables and fruits was determined by analytical RP-HPLC, and the results are shown in Table 1. Most green vegetables had a relatively high content of DLGG of over 200 mg/kg fresh weight (FW), while the concentration in most fruits and root vegetables was low, ranging from "not detectable" to less than 100 mg/kg FW (Table 1). An interesting result was obtained with bell pepper as both the green and red variety had a relatively high content of DLGG of 243 and 167 mg/kg FW, respectively, while the content in the more mature yellow variety was only 37 mg/kg FW. This indicates that the concentration of DLGG may decrease with maturity in some vegetables. For spinach, the content of DLGG and other galactolipids has previously been shown to vary significantly with cultivars (Wang *et al.* 2002). Although large variations in the concentration of DLGG may be found in individual vegetables and fruits as the content may depend on cultivars, storage conditions, harvest time, maturity, processing etc., a general trend is observed (Table 1). It is also interesting to note that vegetables such as spinach, sugar peas, kale, green beans, heart lettuce and broccoli had a higher content of DLGG compared to the fruits of dog rose (rose hips, *Rosa canina*) (Table 1), which is used as an herbal medicine with a well-documented effect on arthritis (Kharazmi and Winther 1999; Warholm *et al.* 2003; Winther *et al.* 2005). This anti-inflammatory effect of rose hips is likely to be due to this particular galactolipid as demonstrated recently (Larsen *et al.* 2003), and hence it is not unlikely to assume that vegetables having a relative high content of DLGG (Table 1) may contribute with an anti-inflammatory effect.

From the results shown in Table 1, it can be concluded that DLGG is quite common in the edible parts of food plants, although largely confined to green vegetables. This is in good agreement with the fact that chloroplast membranes are good sources of galactolipids (Dörmann and Benning 2002). Consequently, DLGG may play a role in human diet as an important nutraceutical that contributes significantly to the correlation between a high consumption of vegetables and fruits, and reduced risk of cancer and other diseases as demonstrated in various epidemiological studies (Steinmetz and Potter 1991, 1996; Block *et al.* 1992). Further research is warranted to establish the bioavailability and the metabolism of DLGG *in vivo* as well as its effect in preclinical and clinical studies.

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