Galactolipids as Potential Health Promoting Compounds in Vegetable Foods

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Abstract: Galactolipids are a class of compounds widely found in the plant kingdom, including edible plants, and are an important part of the cell membranes. Galactolipids in plants consists mainly of monogalactosyldiacylglycerols and digalactosyldiacylglycerols containing one or two saturated and/or unsaturated fatty acids linked to the glycerol moiety. Several galactolipids have been shown to possess *in vitro* and/or *in vivo* anti-tumor promoting activity and anti-inflammatory activity. Recently, it has been demonstrated that the galactolipid, 1,2-di-*O*-*α*-linolenoyl-3-*O*-*β*-D-galactopyranosyl-*sn*-glycerol (**1**), may be important for the anti-inflammatory activity of dog rose (*Rosa canina*), a medicinal plant with documented effect on anti-inflammatory diseases such as arthritis. This galactolipid also occurs in relative high concentrations in certain legumes (e.g., common bean, pea), leaf vegetables (e.g., kale, leek, parsley, perilla and spinach), stem vegetables (e.g., asparagus, broccoli, brussels sprouts), and fruit vegetables (e.g., chilli, bell pepper, pumpkin). Furthermore, compound **1** has been isolated from spinach and several medicinal plants by bioassay-guided fractionation as a galactolipid with possible cancer preventive effects. In this review, the bioactivity of galactolipids is discussed and their potential role in human diet as important nutraceuticals. Moreover, recent patents on the bioactivity of specific galactolipids and inventions making use of this knowledge are presented and discussed.

Keywords: Galactolipids, anti-inflammatory, anti-cancer, bioactivity, bioavailability, extraction, vegetables.

INTRODUCTION

Epidemiological investigations have provided evidence that a diet high in vegetables and fruits is associated with a reduced risk for the development of cancer, cardiovascular diseases, diabetes and other diseases [1-5]. Compounds associated with the health promoting effects of vegetables are glucosinolates and other organosulphur compounds and their degradation products, carotenoids, phytosterols, polyphenols (e.g., flavonoids and phenolic acids), vitamins and dietary fibers [2,5]. These different classes of natural products may only partly explain the health effects of vegetables, and consequently focus has in recent years been on other types of potential health promoting compounds. For example, polyacetylenes of the falcarinol-type in carrots and related root vegetables have been shown to possess various biological activities, including anti-cancer and anti-inflammatory effects and are now considered as important bioactive compounds that may contribute significantly to the health effects of certain vegetables [5-8]. Another class of compounds that may contribute significantly to the health effects of vegetables is glycolipids and in particular galactolipids. Galactolipids have shown some interesting bioactivities *in vitro* and *in vivo* and hence may be considered as an important class of nutraceuticals.

Glycolipids and phospholipids represent the major building blocks for biological membranes. In plants, phosphoglycerolipids are the predominating lipid class in the extraplastidial membranes, whereas the chloroplasts are characterized by the occurrence of high proportions of galactolipids, and thus these lipids are especially important in the photosynthetic membranes of higher plants as well as in algae and certain bacteria [9,10]. Galactolipids in plants consists mainly of monogalactosyldiacylglycerols (MGDGs) and digalactosyldiacylglycerols (DGDGs) of which MGDGs and DGDGs constitute about 50% and 30% of total lipids in thylakoids, respectively [9,10]. Plant galactolipids are characterized by one or two saturated and/or unsaturated fatty acids with chain lengths typically varying from C_{16} to C_{20} linked to the glycerol moiety as shown in Fig. (**1**). Numerous studies have shown that galactolipids derived from plants, cyanobacteria and green algae exhibit various biological properties *in vitro* and/or *in vivo*, including anti-tumor activity [11-19], anti-inflammatory activity [12, 20-23] and antiviral activity [24].

This review focuses on the bioactivity and bioavailability of naturally occurring galactolipids as well as their distribution in vegetables and certain fruits. The effect of storage, processing and various stress conditions on the content of galactolipids is also described. Based on these findings the role of galactolipids as potential health promoting compounds in vegetable foods are discussed. The review cites recent patents relevant to the subject of galactolipids bioactivity as well as extraction and concentration of galactolipids from plant material for use in functional foods, dietary supplements, and medicinal preparations for the prevention of cancer and anti-inflammatory diseases [25-27].

GALACTOLIPIDS IN VEGETABLES

Quantitative analysis of galactolipids in edible parts of vegetables have been performed by high-performance liquid

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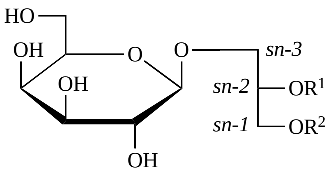
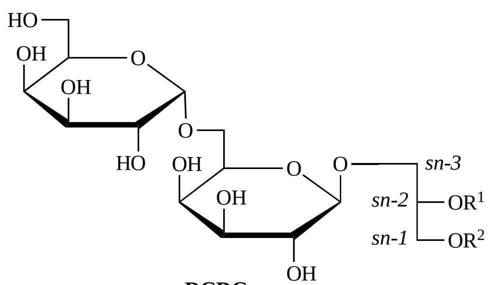


MGDG

Galactolipid No.	R[1]	R[2]
1	α-Linolenoyl (C18:3)	α-Linolenoyl (C18:3)
2	Linoleoyl (C18:2)	α-Linolenoyl (C18:3)
3	Oleoyl (C18:1)	α-Linolenoyl (C18:3)
4	Stearoyl (C18:0)	α-Linolenoyl (C18:3)
5	(7*Z*, 10*Z*, 13*Z*)-Hexadecatrienoyl (C16:3)	α-Linolenoyl (C18:3)
6	Palmitoyl (C16:0)	α-Linolenoyl (C18:3)
7	Palmitoyl (C16:0)	Linoleoyl (C18:2)



DGDG

Galactolipid No.	R[1]	R[2]
8	α-Linolenoyl (C18:3)	α-Linolenoyl (C18:3)
9	Linoleoyl (C18:2)	α-Linolenoyl (C18:3)
10	Oleoyl (C18:1)	α-Linolenoyl (C18:3)
11	Stearoyl (C18:0)	α-Linolenoyl (C18:3)
12	Palmitoyl (C16:0)	α-Linolenoyl (C18:3)
13	Stearoyl (C18:0)	Linoleoyl (C18:2)
14	Palmitoyl (C16:0)	Linoleoyl (C18:2)

Fig. (1). Chemical structures of the most common and abundant MGDGs and DGDGs (galactolipids) occurring in the edible parts of common vegetables. MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol.

chromatography (HPLC) using ultraviolet (UV) and evaporative light-scattering detection (ELSD). In a recent study by Larsen & Christensen [28], the content of 1,2-di-*O*-*α*-linolenoyl-3-*O*-*β*-D-galactopyranosyl-*sn*-glycerol (**1**), a compound with potential anti-inflammatory and anti-cancer effect [15, 19-21, 23, 28], was investigated in the edible parts of common vegetables and fruits by analytical reverse phase HPLC-UV. MGDG **1** composed over 200 mg/kg fresh weight (FW) among legumes, leaf vegetables, stem vegetables and fruit vegetables, while the concentration in most fruits and root vegetables were low ranging from 0 to 100 mg/kg FW Table **1**. These results are also in accordance with a study by Sugawara & Miyazawa [29], which analyzed similar vegetables for galactolipids (MGDGs and DGDGs) using HPLC-ELSD. The ratio between MGDGs and DGDGs was found to be of almost equal magnitude within most vegetables and fruits with MGDG and DGDG acylated with *α*-linolenic acid (C18:3) as the predominant class of galactolipids. The composition of MGDGs and DGDGs in vegetables may, however, be complex as demonstrated for bell pepper using HPLC-mass spectrometry (MS) analysis. HPLC-MS analysis of red bell pepper revealed the presence of a series of glycolipids including a complex mixture of MGDGs and DGDGs acylated with different types of fatty acids. The fatty acids of MGDG in red bell pepper were found to be C18:3/C18:3 (**1**), C18:2/C18:3 (**2**), C18:1/C18:3 (**3**), C18:0/C18:3 (**4**), C16:0/C18:3 (**6**), and C16:0/C18:2 (**7**), and those of DGDG were C18:3/C18:3 (**8**), C18:2/C18:3 (**9**), C18:1/C18:3 (**10**), C18:0/C18:3 (**11**), C16:0/C18:3 (**12**), C18:0/C18:2 (**13**) and C16:0/C18:2 (**14**) Fig. (**1**) [30]. Based on the HPLC-MS total ion chromatogram MGDG acylated with C18:3/C18:3 (**1**) was the predominant MGDG, whereas DGDGs acylated with C18:3/C18:3 (**8**), C18:0/C18:3 (**11**) and C16:0/C18:3 (**12**), respectively, were predominant with the former as being the most abundant DGDG. An even more complex fatty acid composition pattern has been observed for galactolipids isolated from fresh spinach leaves using chiral phase HPLC with the most abundant galactolipids being the MGDGs **1** and **5** and the DGDG **8** [31]. Galactolipids can theoretically have stereoisomers of two configurations, *α*-type (*α*-anomer) or *β*-type (*β*-anomer) between the sugar and the glyceride. MGDGs from dried spinach leaves and other dried vegetables contained the *α*-type, while the DGDGs had *α*-and *β*-types [17, 18] in contrast to the common *β*-types Fig. (**1**) isolated from fresh vegetables such as spinach leaves [15, 28, 30]. The differences in galactolipid content between dry and fresh spinach leaves and other dried vegetables may be explained by the investigation of dry versus fresh plant material or simply due to genotypic differences. DGDGs of the *α*-type have also been found in fresh olive fruits [32].

An investigation of bell pepper for MGDG **1** revealed that both the green and red varieties had a relatively high content of this galactolipid in amounts of 243 mg/kg FW and 167 mg/kg FW, respectively, while the content in the more mature yellow variety was only 37 mg/kg FW [28]. This indicates that the concentration of galactolipids may decrease with maturity in some vegetables. For spinach the content of MGDGs **1** and **5** has previously been shown to vary significantly with cultivars and culture conditions [15]. Large variations in the concentration of galactolipids is expected between individual vegetables and fruits as also shown in Table **1** and may depend upon the cultivar, storage condition, harvest time, maturity, processing etc. [15, 28, 33]. A clear trend in the galactolipid content of vegetables can be observed in Table **1**. It can be concluded that galactolipids are quite common in the edible parts of food plants and present in high concentrations in dark green vegetables such as leek, parsley, perilla, and spinach leaves. This is in good agreement with the fact that chloroplast membranes are good sources of galactolipids and consequently dark green vegetables have in most cases a higher content of galactolipids than light green vegetables or vegetables with no green color [9]. MGDG commonly exceeds DGDG in photosynthetic tissues, while DGDG is predominant in non-photosynthetic tissue [31, 34] in accordance with the quantitative results presented in Table **1**.

BIOACTIVITY OF GALACTOLIPIDS FROM VEGETABLES

Several galactolipids isolated from medicinal plants, vegetables, algae, and bacteria by bioassay-guided chromatographic fractionation have indicated both anti-inflammatory and anti-cancer activity. MGDG **1** and **5**, isolated from fresh spinach leaves demonstrate inhibitory effects on tumor promoter-induced Epstein-Barr virus (EBV) activation [15]. Furthermore, it has been demonstrated that MGDG **1** and a mixture of 1-*O*-α-linolenoyl-2-*O*-palmitoyl-3-*O*-β-galactopyranosyl-*sn*-glycerol (**6**) and its counterpart 1-*O*-palmitoyl-2-*O*-α-linolenoyl-3-*O*-β-galactopyranosyl-*sn*-glycerol isolated from leaves of *Citrus hystrix*, a traditional herb in Thailand, are potent inhibitors of EBV activation of human B-lymphoblastoid cells (Raji) induced by teleocidin B-4 [12]. The former galactolipid has also been found to possess *in vivo* anti-tumor promoting activity as evaluated in a two stage carcinogenesis experiment on ICR mouse skin with dimethylbenz[α]anthracene and 12-*O*-tetradecanylphorbol 13-acetate (TPA). A dose of 160 nmol MGDG **1** significantly reduced tumor incidence by 39% ($P < 0.005$) and the number of tumors per mouse by 67% ($P < 0.001$) [12]. In the same study the anti-inflammatory activity of MGDG **1** and **6** was measured by TPA-induced edema formation on mouse ears. Both MGDG **1** and **6** exhibited higher anti-inflammatory activity than indomethacin, a well-known cyclooxygenase (COX) inhibitor. Hence, the inhibition of enzymes regulating the formation of prostaglandins and leukotrienes may partly be responsible for the anti-inflammatory effects of galactolipids and other glyceroglycolipids [12]. Galactolipid **1** has also been shown to be responsible for the anti-tumor promoting activity of jute (*Corchorus capsularis* and *C. olitotius*; Tiliaceae), a well-known medicinal vegetable in North Africa and Asia [16] as well as the anti-inflammatory activity of cypress spurge (*Euphorbia cyparissias*; Euphorbiaceae) [20]. In addition, MGDG **1** has also been isolated from the fruits of dog rose (rose hips, *Rosa canina*; Rosaceae) as an anti-inflammatory agent with inhibitory effects on chemotaxis of human peripheral blood neutrophils *in vitro*. The fruits of dog rose are used as an herbal medicine with well-documented effects on arthritis [23, 35–38], hence the anti-inflammatory effect of rose hips are likely due to this particular galactolipid [21, 23]. In this context it is interesting to note that the content of MGDG **1** in the fruits of dog rose is approximately 270 mg/kg FW [28], which is a lower concentration than found in many of the green vegetables listed in Table **1**. This could suggest that MGDG **1** is responsible for the significant anti-inflammatory activity of some vegetables. In a very recent study by Hou *et al.* [19], it was demonstrated that MGDG **1** isolated from *Crassocephalum rabens* (Asteraceae) a popular anti-inflammatory folk medicine and food supplement, has chemopreventive effects by suppressing cytoplasmic nuclear factor (NF)-κB and downstream inflammatory mediators, COX-2, inducible nitric oxide synthase (iNOS), nitrogen oxide (NO) and prostaglandin E_2 (PGE_2). Furthermore, MGDG **1** in this study also showed *in vivo* cancer prevention activity against B16 melanoma growth in mice following intraperitoneal administration, an effect comparable with that of cisplatin, a cancer therapeutic drug [19]. The α-anomer of MGDG **1** and other MGDGs of the α-type isolated from dry spinach have been shown to have potential anti-cancer effects by inhibition of proliferation of human cancer cells. The bioactivity was attributed to the inhibition of replicate DNA polymerases leading to apoptosis [17, 18]. On the other hand DGDG of the α-type does not seem to be cytotoxic [17, 18]. Finally, there have also been some reports of various anti-tumor-promoting activities of MGDGs and DGDGs from the green alga, *Chlorella vulgaris* [13] and DGDGs from the fresh-water cyanobacterium *Phormidium tenue* [11, 14] as well as anti-inflammatory activity of MGDGs from the marine sponge *Phyllospongia foliascens* [39].

α-Linolenic acid (C18:3n-3) accounts for more than 90% of the total fatty acids of MGDG in leaf and stem vegetables [29, 34]. Therefore galactolipids are also expected to be an important source of polyunsaturated n-3 essential fatty acids (ω-3-PUFA) such as α-linolenic acid. Consumption of the ω-3-PUFAs docosahexaenoic acid and eicosapentaenoic acid is associated with a reduction of platelet activation, lower (vitamin K-dependent) coagulation factors and/or a decrease of vascular tone and blood pressure, and hence may prevent the development of cardiovascular diseases [40–43]. Emerging evidence suggests that diets containing α-linolenic acid are associated with reductions in total deaths and sudden cardiac death [43, 44]. There is a continuing debate regarding whether α-linolenic acid has unique actions in relation to the cardiovascular system [44]. Finally, there is also some strong indications that dietary ω-3-PUFAs play an important role in preventing obesity and Type 2 diabetes [40, 45, 46]. The possible health effects of ω-3-PUFAs is probably associated with their ability to inhibit COX-1 and COX-2 and to contribute to the regulation of several transcription factors such as peroxisome proliferator-activated receptors (PPARs) and NF-κB that play an important role in health and disease [45].

In conclusion, MGDG **1** as well as other galactolipids in vegetables seem to play a role in human diet as important nutraceuticals contributing significantly to the correlation between a high consumption of vegetables and fruits and reduced risk of cancer, cardiovascular diseases and other diseases [1–4]. However, to clarify the nutritional role of galactolipids as well as their potential health promoting effects it is necessary to determine the bioavailability and metabolism of galactolipids in humans. It has been shown that MGDGs and DGDGs are hydrolyzed *in vitro* by pancreas homogenates from sheep, rats and guinea pigs, and by human duodenal contents and pancreatic juice to free

Table 1. Content of Galactolipids (Monogalactosyldiacylglycerols (MGDGs) and Digalactosyldiacylglycerols (DGDGs)) in the Edible Parts of Vegetables and Fruits as Determined by Analytical HPLC-UV and/or HPLC-ELSD

Vegetables and Fruits		MGDG (mg/kg)[a]	DGDG (mg/kg)[a]
Legumes			
Azuki bean	*Vigna angularis*	28[b] [29][c]	431[b] [29]
Black soybean	*Glycine max*	66 [29]	309 [29]
Common bean (kidney bean)	*Phaseolus vulgaris*	396 [28]; 230 [29]	211 [29]
Pea	*Pisum sativum* var. *sativum*	22[b] [29]; 239-442 [28]	698[b] [29]
Soybean	*Glycine max*	ND[d] [29]	50[b] [29]
Leaf vegetables			
Cabbage	*Brassica oleracea*	105 [29]	83 [29]
Chinese cabbage	*Brassica rapa*	39 [29]	26 [29]
Chive	*Allium schoenoprasum*	68 [28]	—[e]
Kale	*Brassica oleracea* var. *acephala*	415 [28]	—
Leek	*Allium ampeloprasum* var. *porrum*	778 [29]	588 [29]
Lettuce	*Lactuca sativa*	135 [29]; 32-320 [28]	148 [29]
Parsley	*Petroselinum crispum*	1838 [29]	1157 [29]
Perilla	*Perilla frutescens* var. *japonica*	2851 [29]	2396 [29]
Spinach	*Spinacia oleracea*	546 [28]; 850 [29]; 3300-38800[b] [15]	563 [29]
Sweet potato (leaves)	*Ipomoea batatas*	53940[b] [71]	22640[b] [71]
Stem vegetables			
Asparagus	*Asparagus officinalis*	262 [29]	225 [29]
Broccoli	*Brassica oleracea* var. *italica*	316 [28][f]; 377 [29][f]	242 [29][f]
Brussels sprouts	*Brassica oleracea* var. *gemmifera*	225 [28]	-
Cauliflower	*Brassica oleracea* var. *botrytis*	108 [29]	141 [29]
Celery	*Apium graveolens*	ND [28]; 26 [29]	29 [29]
Green onion	*Allium fistulosum*	20 [29]	92 [29]
Onion	*Alium cepa*	ND [28]; 3 [29]	22 [29]
Root vegetables			
Aroid	*Xanthosoma sagittifolium*	228 [29]	264 [29]
Burdock	*Arcticum lappa*	18 [29]	18 [29]
Carrot	*Daucus carota* ssp. *sativus*	ND [28]; 40 [29]	66 [29]
Chinese yam	*Dioscorea opposite*	46 [29]	71 [29]
Garlic	*Allium sativum*	22 [29]	32 [29]
Japanese radish	*Raphanus sativus longipinnatus*	6 [29]	5 [29]
Lotus root	*Nelumbo nucifera*	68 [29]	75 [29]
Potato	*Solanum tuberosum*	19 [29]; 41 [28]	47 [29]
Sweet potato	*Ipomoea batatas*	97 [29]	226 [29]
Turnip	*Brassica napus*	20 [29]	15 [29]

(Table 1) Contd....

Vegetables and Fruits		MGDG (mg/kg)[a]	DGDG (mg/kg)[a]
Fruit vegetables			
Bell pepper (green)	*Capsicum annuum*	243 [28]	-
Bell pepper (red)	*Capsicum annuum*	167 [28]	-
Bell pepper (yellow)	*Capsicum annuum*	37 [28]	-
Chili (green)	*Capsicum annuum* var. *annuum*	178 [28]	-
Chili (red)	*Capsicum annuum* var. *annuum*	321 [28]	-
Cucumber	*Cucumis sativus*	94 [28]; 138 [29]	117 [29]
Eggplant	*Solanum melongena*	57 [29]	71 [29]
Olive	*Olea europea*	ND [32]	280 [32]
Pimento (cherry pepper)	*Capsicum annuum*	147 [29]	145 [29]
Pumpkin	*Cucurbita maxima*	617 [29]	686 [29]
Summer squash	*Cucurbita pepo*	86 [28]	-
Tomato	*Lycopersicon esculentum*	32 [28]; 21 [29]	23 [29]
Watermelon	*Citrullus lanatus*	12 [29]	33 [29]
Fruits			
Apple	*Malus domestica*	13 [28]; 7 [29]	12 [29]
Banana	*Musa manzano*	8 [29]	38 [29]
Black currants	*Ribes nigrum*	57 [28]	-
Grape	*Vitis vinifera*	10 [29]	9 [29]
Grapefruit	*Citrus* × *paradisi*	12 [29]	24 [29]
Japanese persimmon	*Diospyros kaki*	13 [29]	35 [29]
Kiwifruit	*Actinidia deliciosa*	45 [28]; 65 [29]	79 [29]
Lemon	*Citrus* × *limon*	6 [29]	7 [29]
Nectarine	*Prunus persica* var. *nectarina*	17 [28]	-
Mango	*Mangifera indica*	11 [28]	-
Orange	*Citrus sinensis*	ND [28]	-
Peach	*Prunus persica*	11 [28]	-
Pear	*Pyrus communis*	17 [29]	85 [29]
Plum	*Prunus domestica*	10 [28]	-
Strawberry	*Fragaria* × *ananassa*	11 [29]	10 [29]
Sweet cherries	*Prunus avium*	ND [28]	-

[a]Fresh weight basis unless otherwise noted; [b]Dry weight basis; [c]References in brackets; [d]ND = not detected; [e]Not investigated. [f] The content refers only to the edible part of broccoli i.e. the inflorescence.

fatty acids, intermediate products of MGDGs and DGDGs and water soluble galactose-containing compounds [47, 48]. In humans pancreatic lipase-related protein 2 (HPLRP2), a protein present in the exocrine pancreatic secretion and sharing around 70% amino acid identities with classical pancreatic lipase, displays a very high galactolipase activity towards MGDG and DGDG and it is the main enzyme involved in the digestion of galactolipids in the gastro-intestinal tract [49-52]. So far only a few studies have investigated the fate of galactolipids *in vivo*. Studies in rats suggest that galactolipids are not absorbed intact or as reacylated monoacyl compounds into blood but instead are degraded/hydrolyzed in the intestinal tract [53, 54]. The question remains whether the capacity to digest and absorb galactolipids is different among species such as humans, mice, guinea pigs and rats. The fate of galactolipids in humans is still an open question that clearly needs to be answered in order to establish whether this interesting group of compounds can be regarded as important health promoting compounds in vegetable foods and medicinal plants.

EFFECT OF STORAGE, PROCESSING, ENVIRONMENTAL AND NUTRIENT DEFICIENCY STRESS ON THE CONTENT OF GALACTOLIPIDS IN VEGETABLES

Lipid-acyl hydrolases (LAHs) play significant roles in lipid degradation in plant tissue [33]. Therefore, phospholipases and galactolipase seem to play a critical role in the quality deterioration of vegetables during storage both in relation to the production of off-flavours and the degradation of potential bioactive galactolipids [33]. LAHs also seem to play an important role for plants to cope with various types of stress conditions.

In unblanched spinach leaves and peas, it has been observed that galactolipids and/or phospholipids decompose during frozen storage [55, 56], implying the involvement of LAHs in the degradation of these lipids in unblanched vegetables. In order to avoid degradation of galactolipids and other lipids in plant tissue, vegetables are subjected to a blanching process prior to frozen storage. The optimal blanching process for inactivation of LAHs in spinach and carrots have been studied and it appears that highly active and thermostable galactolipase and phospholipases can be used as indicator enzymes for the determination of quality deterioration of vegetables [33].

Membrane lipids may contribute to reorganization of cell components, conferring the ability to cope with various stress conditions. Therefore, environmental stress such as drought and metallic stress as well as nutrient deficiency stresses are believed to have significant impact on the metabolism and content of galactolipids and other lipids in plant tissue. Under optimal growing conditions, the amounts of galactolipids in extraplastidial membranes in plants are low. However, during phosphate deprivation, the biosynthesis of sulfoquinovosyldiacylglycerols and DGDGs and the expression of sulfolipid and galactolipid genes are upregulated [57-59]. Under phosphate-limiting conditions, DGDGs appear to replace phospholipids in plastidial and extraplastidial membranes in higher plants [59, 60]. Nitrogen (N) is one of the most important macronutrients and is often limiting for plant growth. N deficiency in *Arabidopsis* led to an increase in chloroplast DGDG and a decrease in chloroplast MGDG, which correlated with an elevated expression of DGDG synthase genes *DGD1* and *DGD2* [61]. The amounts of triacylglycerol and free fatty acids increased during N deprivation. Furthermore, phytyl esters containing C12:0 and C14:0 fatty acids accumulated in chloroplasts, in particular in the thylakoids and plastoglobules. Therefore N deficiency in higher plants results in a co-ordinated breakdown of galactolipids and the acyl groups released in this process are to a large extent incorporated in specific fatty acid phytyl esters in the chloroplasts [61].

Drought-induced modifications in plant membrane lipid composition have been reported in numerous species [62-64]. Water deficit induces changes in leaf galactolipids contents that are dependent on plant sensitivity or tolerance to drought [62, 64]. In sensitive plants, a reduction of galactolipid content occurs in response to mild water deficit, whereas drought-tolerant plants are significantly affected in response to severe water deficit only. The role of LAHs, such as galactolipases in these changes has been shown [65, 66]. However, similar to phosphate starvation, lack of water seem to enhance DGDG biosynthesis as shown in cowpea (*Vigna unguiculata*) leaves, which indicates that drought-induced DGDG accumulation in extraplastidial membranes contributes to plant tolerance to arid environments [62]. Galactolipids, in particular DGDG, has also been shown to play an important role in thermotolerance of higher plants [67].

Heavy metals are recognized as environmental pollutants and are released from both industrial and agricultural sources. For example, the intensive use of high-phosphate fertilizers in agriculture leads to an increased accumulation of metal ions, especially cadmium (Cd), in the soil [68]. The effect of Cd on lipid and fatty acid biosynthesis has been studied in young tomato leaves [69]. Exposure of tomato plants to high Cd concentrations resulted in significant decrease in the content of galactolipids and other lipid classes and a concentration-related decrease in the unsaturated fatty acid content. Thus, the biosynthesis of tri-unsaturated fatty acids (C16:3 and C18:3) was reduced while the biosynthesis of palmitic (C16:1), palmitoleic (C16:1), stearic (C18:0) and linoleic (C18:2) acids were enhanced resulting in a lower degree of fatty acid unsaturation in, for example, galactolipids. These results clearly show that Cd stress in higher plants may lead to an inhibition of polar lipid biosynthesis and reduced fatty acid desaturation process [69].

RECENT INVENTIONS ON GALACTOLIPIDS

A patent search revealed three recent patents or patent applications on galactolipids related to the development of functional foods and plant medicine with anti-cancer effect or cancer preventative effects as well as an effect against anti-inflammatory diseases such as arthritis.

Mizushina *et al.* [25] obtained a glycolipid enriched fraction, purified by a convenient extraction and fractionation method from dry spinach leaves. The dry spinach leaves were first extracted with water at 60ºC followed by extraction of the residue with ethanol at 60ºC resulting in an oily extract that was finally fractionated by column chromatography resulting in a fraction primarily consisting of glycolipids. The enriched glycolipid fraction contained three types of glycolipids, i.e., MGDGs, DGDGs and sulphoquinovosyldiacylglycerol at a high ratio. This glycolipid-containing composition has useful physiological activities such as a DNA synthase inhibitory activity, inhibition of cancer cell growth and anti-tumor activity. When treated with a lipase, the glycolipid fraction shows further enhanced physiological activities and becomes useful in functional foods. This patent primarily concerns MGDG and DGDG of the α-type. According to an older Japanese patent filed by Osamu and Itsu [70], some parts of sweet potato, especially the leaves, stalks and rhizomes, can be employed as functional foods or drinks presenting anti-carcinogenic activities. The galactolipid fraction of the plant material, which probably consists of MGDGs and DGDGs of the *β*-type [71], was shown to suppress proliferation and promote differentiation of cancer cells and was hence claimed to be responsible for the carcinostatic properties described in the patent [70].

Another invention by Larsen *et al.* [26], relates to the use of glycosides of mono- or diacylglycerol such as MGDG **1** isolated from the fruits of dog rose by bioassay-guided fractionation for the treatment of inflammatory conditions e.g., treatment of inflammation by alleviating chemotaxis and oxidative burst response by leukocytes. The use of this invention is primary focused on developing plant medicine for the treatment of inflammatory diseases such as arthritis and osteoarthritis but not topical inflammation. Two patents by Winget [72, 73] describe the use of MGDGs esterified with at least one eicosapentaenoic acid moiety isolated from marine algae for treatment of topical inflammation.

The invention by Bagger *et al.* [27] provides a method for preparing a product containing MGDGs and/or DGDGs from the fruits of dog rose or any other plant material. The invention is focused on developing a method for the preparation of products, with relative high concentrations of galactolipids, in particular MGDG **1**. This invention therefore builds on the recent invention on the anti-inflammatory activity of MGDG **1** [26] isolated from rose hip extracts, and the invention on the anti-inflammatory activity of a rose hip concentrate having a high content of vitamin C being able to alleviate the symptoms associated with inflammation [74]. The invention by Bagger *et al.* [27] is comprised of: (i) milling the freeze-dried plant material, (ii) extraction of the milled plant material with a first aqueous extraction solution obtaining a first liquid phase and a first solid phase, and (iii) separating the liquid phase from the solid phase to obtain a product containing MGDGs and/or DGDGs. This invention leads to a product enriched in galactolipids and in particular MGDG **1** to be used in functional foods, dietary supplements and medicinal preparations for the treatment of inflammatory conditions.

In conclusion, current inventions use the potential health promoting properties of galactolipids to produce products that can be used in the treatment or prevention of inflammatory conditions and cancer.

CURRENT & FUTURE DEVELOPMENTS

The compounds responsible for the health promoting properties of vegetables and fruits are still to debate, although several groups of natural products have been identified as important contributors to the health effects of plant based foods. Galactolipids have not received much attention in relation to the health effects of vegetable foods. The anti-cancer and anti-inflammatory activity of galactolipids, as demonstrated in animal studies and *in vitro* studies clearly suggests that galactolipids can be used in the treatment and prevention of inflammatory conditions and cancer and that they may be considered as an important group of nutraceuticals. Future investigations, however, need to be directed towards the fate of galactolipids in humans i.e. their metabolism and absorption due to the fact that animal studies and *in vitro* studies suggests that galactolipids are not bioavailable as intact galactolipids but are degraded or hydrolyzed by galactolipases, in particular HPLRP2, before they reach the blood circulation. If galactolipids are found to have low bioavailability, galactolipid enriched medical preparations given as intravenous therapy may still be considered as important pharmaceuticals from vegetable foods.

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