Review

Optimizing biomedical and industrial products development based on flax

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Abstract

The principal goal of this review is to present the status of genetic modifications of flax (*Linum usitatissimum*) and its possible application in industry. Flax is a valuable source of oil and fibres, and development of genetic engineering allows the possibility of improving the quality of these products. There are different goals of flax modifications: improving the quality of flax fibres, improving oil properties, elevation of antioxidant level and creation of pathogen-resistant plants. These modifications made flax a more useful and precious source for a broad range of products applicable in industry. What makes flax valuable is the possibility of whole plant exploitation with almost no waste products.

Keywords: Transgenic flax, flax oil, flax fibers, phenylopropanoids, antioxidants, polyhydrxybutyrate, biocomposites, wound healing

Introduction

Flax (*Linum usitatissimum*) is an annual plant widely distributed in Mediterranean and temperate climate zone. It has a long history in cultivation as it has a great significance for industry; being a valuable source of oil and fibres. What makes this plant special for industry is the opportunity to exploit the whole plant with almost no waste products (Figure 4). Development of plant genetics and plant biotechnology has allowed scientists to obtain plants that are qualitatively better. There are different reasons for genetic modification of plants: improving nutritional qualities, taste, cultivate plants more easily and more economically (resistant to pathogens and diseases), and also obtaining plants that are able to produce pharmaceuticals or other valuable compounds. In this review, we present the possible application of different products based on genetically modified flax.

Flax as a Valuable Source of Oil and Fibres

Two main products are obtained from flax: oil, which is extracted from seeds, and fibre, which is produced from stem. Flax oil is one of the richest sources of α-linoleic acid (ALA), which confers about 44–57% of all fatty acids; it also contains 15–29% linoleic acid and 13–29% oleic acid [1]. As the human body cannot produce ALA, which belongs to ω-3 family, it is the essential fatty acid in our diet. ALA, as well as its metabolites, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are incorporated into cell membrane phospholipids. Increased level of ALA, EPA and DHA in cell membranes affects their fluidity and elasticity. High levels of ALA can enhance the elasticity of arteries in obese adults. ω-3 fatty acids can reduce the production of arachidonic acids and other eicosanoids, which are known mediators in inflammatory processes, promote inflammation and platelet aggregation [2]. Given the many favourable actions of ALA and other ω-3 fatty acids, there are many important applications of these compounds. Linseed oil plays an important role in food industry, health care and pharmaceutics. The flaxseed oil can lower total-, LDL-cholesterol (low density lipoproteins) and glucose level in blood [3], reduces inflammation and risk of coronary heart disease, stroke and cancer [4]. Populations with high consumption of ALA from different sources have lower risk of cancer diseases, stroke and myocardial infraction [5].

The dietary properties of linseed oil are also significant, as it has protection properties and it is used against respiratory tract diseases and gastrointestinal tract diseases. Linseed products are also recommended for treating skin diseases [6]. It is worth mentioning that linseed oil is rich in secoisolariciresinol diglucoside (SDG), which is a precursor of lignans. These compounds have many favourable actions on human health. They have anticancer properties as they inhibit cell proliferation and growth, especially in breast and prostate cancer [7]. SDG has also anti-viral, anti-bacterial and anti-fungal properties, is an antioxidant and it has been shown to enhance immune system functioning. Moreover linseeds contain some amount of other antioxidative compounds such as γ-tocoferol, carotenoids, phenolic acids and anthocyanins [1, 8]. These compounds are mainly in seeds and to some extent they may be present in the oil.

Flax is also a valuable source of fibre. There are three groups of plant fibres: the phloem or stem fibres (phloem stem fibres or xylem stem fibres) of dicotyledonous plants, the leaf fibres of monocotyledonous plants and the seed and fruit fibres [9]. Flax fibres belong to the first group, where the stem tissue is a source of fibres. Its stems are composed of 70% cellulose. They are hollow tubes that grow as bundles held together by complex carbohydrates such as pectins, gums and waxes. These bundles function as a plant support. Fibres are separated by retting, which is a process of separating fibres from non-fibre tissues in bast plants [10]. Retting is based on enzyme action, which degrades pectins. The main enzyme required for retting is polygalacturonase, which degrades polymer of the middle lamella into soluble galacturonic acid [11]. The proper degree of retting is required to obtain high-quality fibres [12]. There are some advantages that make flax fibre useful for different applications. It is flexible, lustrous and soft. It is stronger than cotton fibre but less elastic. The other benefits of flax fibre are that they are allergy-free, absorb humidity so they are good products for textile industry. Recently, other technologies have been developed to allow the use of flax fibre in different products such as car-door panels, plant pots and retaining mats.

Genetic Modification of Flax

Genetic modification of plants is a technique that has been practised for 26 years. The first species transformed by the *Agrobacterium* method were tobacco and petunia. Since then, genetic modification has been developed and improved. The aim is to make it more effective and safe, so it is necessary to monitor all introduced genes and their side effects, if any. The most common method of plant transformation involves the use of *Agrobacterium*, the soil bacteria that naturally infect the wound sites in dicotyledonous plants and cause formation of the crown gall tumours [13]. Tumour formation is the result of the gene transfer from *Agrobacterium* to infected plant cells, so the molecular basis of agrotransformation is transfer from bacterium and integration into the plant genome of a T-region of tumour-inducing (Ti) plasmid. Transport to the plant cell is a result of the activity of virulence genes (vir) located on Ti plasmid [14]. The final transformation efficiency depends on several factors such as species type of the vector, T-DNA components and finally on the *Agrobacterium* strain. Any plant can be potentially transformed by *Agrobacterium* if a proper transformation protocol is established. In 1988, Jordan and McHughen [15] published the successful and verified transformation in flax using *Agrobacterium tumefaciens*. However, this method has numerous limitations: low transformation efficiency, a high incidence of somaclonal variation resulting from *in vitro* culture of callus tissue, a high incidence of chimaeric regenerants [16], and a high incidence of escape shoots, i.e. shoots arising from non-transformed cells protected from selection by transformed cells [17]. To overcome these problems, protocols for flax transformation using *Agrobacterium* are still being developed and improved. According to Yildiz and Er [18], agrotransformation efficiency could be improved by peeling the hypocotyl explants. Shoots regenerated from hypocotyl segments become non-transgenic, since they can grow from non-transgenic cells protected by neighbour transformed cells. Peeling the layer of epidermis or preculturing the explants before inoculation with *Agrobacterium* results in increased level of transformation efficiency [18]. Another attempt to improve efficiency of flax agrotansformation was using a sonication-assisted, *Agrobacterium*-mediated, co-cultivation technique (SAAT). The results showed that treatment with ultrasound facilitates and increase uptake of plasmid DNA into the cells of flax hypocotyls and cotyledons [19]. Even though there are some difficulties in transforming flax via *Agrobacterium*, this is still the most commonly used technique for flax transformation [18, 20–22].

It is necessary to note that flax is a valuable industrial product and therefore some actions have been taken recently to generate new transgenic flax lines characterized with better properties of fibre and oil by use of genetic engineering methods (Figure 1). In this review, we present different flax modifications that aim to improve various properties of this plant and thus making it a very valuable source of oil and fibres, applicable in many industrial products.

Antioxidant Properties and Pathogen Resistance Improvements

Plants phenylopropanoids are large group of secondary metabolites, containing flavonoids, phenolic acids, phenols, lignans and tannins. Flavonoids are class of phenylopropanoids; its metabolic pathway begins with the chalcone synthesis catalysed by chalcone synthase (CHS), synthesizing of flavonons and flavonols by chalcone isomerase (CHI) leading to flavan production in a reaction catalysed by dihydroflavonol reductase (DFR) (Figure 2).

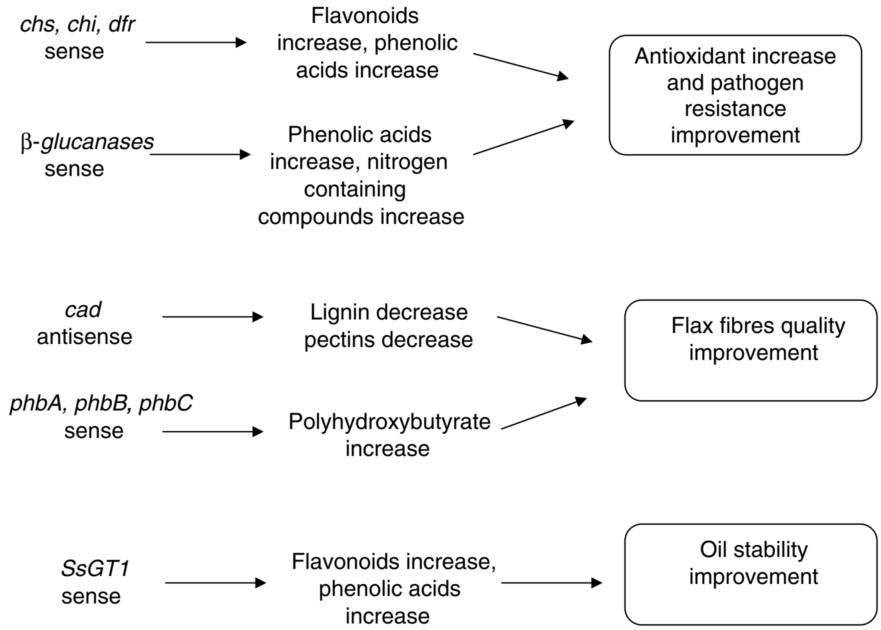

Figure 1 Targets for genetic modifications of flax

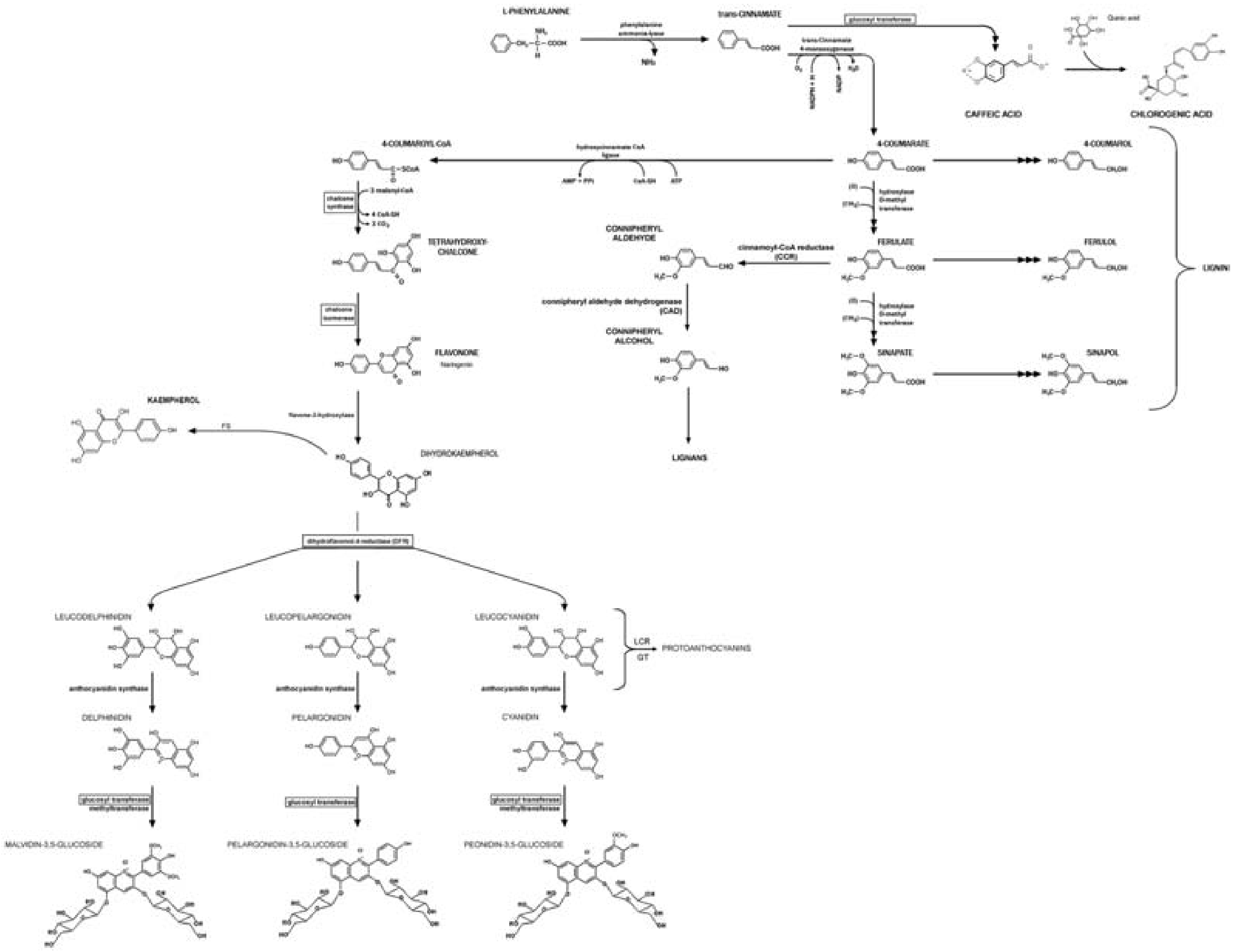

Figure 2 Scheme of the phenylopropanoid pathway

Flavan is precursor of anthocyanidins and pro-anthocyanidins. Flavonoids participate in plant growth and development; they are known antioxidants, photoreceptors and attractants [20]. They protect plants from pathogens [23], oxidative stress and ultraviolet light. Flavonols have anti-allergic, anti-viral and anti-inflammatory properties [20, 24]. All these features make flavonoids an interesting goal of genetic manipulation. Therefore, increasing the flavonoid level in plants should enhance their protection against biotic and abiotic stress. Transgenic flax with an increased level of flavonoids was generated. It was characterized by overexpression of three genes derived from *Petunia hybrida* coding for enzymes: CHS, CHI and DFR [25]. The aim of this manipulation was to create flax with enhanced antioxidant content and also that was more resistant to common flax diseases caused by *Fusarium*. Transformed plants exhibited an elevated flavonoids level in green parts and in seeds. There was a correlation between the flavonoid level and the mRNA level of the introduced genes. According to Lorenc-Kukuła *et al.* [25], transgenic flax had higher level of metabolites of flavonoids biosynthesis pathway, as higher activity of all three enzymes caused higher flow of metabolites. There was an increase of flavonols (kaemferol and quercitin), flavones (apigenin), flavonons and anthocyanins level. It was also proved that transgenic flax showed higher antioxidant properties. Antioxidative potential was elevated as much as 100 times compared with the control, non-transformed plants. Moreover, the calculated correlation coefficient between phenolic acid content and antioxidative potential was 0.82 for flaxseed and 0.7 for green part, what perfectly describes their strong relationship [25]. It is suggested that an increase in antioxidant properties may result in protection against infectious agents [26]. Indeed, the transgenic flax was more resistant to *Fusarium culmorum* and *Fusarium oxysporum*, the most common flax pathogen that cause *Fusarium* wilt. The higher resistance was correlated with elevated antioxidant properties, what proves the crucial role of flavonoids in the process of pathogenesis [27]. An important clue is that increased activity of three enzymes: CHS, CHI and DFR improved the infection reaction of flax. Additional confirmation of these conclusions comes from *in vitro* experiments, where *F. oxysporum* growth was strongly inhibited by the flavonoid extract isolated from transgenic flax [25].

Another goal of genetic transformation aimed at improving antioxidant properties of flaxseeds was to increase the level of carotenoids. These compounds are mainly synthesized in plants and serve as light-harvesting antenna in photosynthesis. They transfer the energy and play a crucial role in the protection of the reaction centre from autooxidation. Humans appear to be incapable of synthesizing carotenoids, so they have to incorporate them from their diet. These antioxidant compounds are precursors of vitamin A, and they protect cells from the damaging effects of free radicals and enhance the functioning of immune system. Carotenoids provide health benefits by decreasing the risk of various diseases, particularly certain cancers and eye diseases [21]. In order to increase the amount of carotenoids in flaxseeds, the carotenoids biosynthetic pathways were altered through manipulation with phytoene synthase gene (*ctrB*) derived from bacterium *Pantoea ananatis*. *CtrB* was overexpressed under the constitutive promoter Cauliflower Mosaic Virus (CaMV) or the *Arabidopsis thaliana* fatty acid elongase 1 gene (*FAE1*) seed-specific promoter. The high performance liquid chromatography (HPLC) profile of transformed flax showed the presence of α-carotene, β-carotene and phytoene, which were not observed in control, non-transformed plants. The total carotenoids level in transgenic seeds was 7.8–18.6-fold higher in comparison with the control [28]. Moreover, the colour of transformed flax seed was changed by accumulation of carotenoids. Interestingly, the overexpression of carotenoids in transgenic seeds inhibited the flux of the xanthophyll biosynthetic pathway towards zeaxanthin. According to Fujisawa *et al.* [28], this inhibition might control the ABA level in flaxseeds to prevent the delay of their germination. This manipulation is the first known successful overproduction of carotenoids in plants without any effect on their growth. Flaxseeds containing the elevated level of carotenoids might be the valuable source of antioxidants and seeds oil with accumulated functional carotenoids has a great nutritional value for human.

Manipulation with genes from flavonoid metabolic pathway was not the only way to enhance flax protection against pathogen. The plant response to pathogen attack is a complex mechanism, which involves many different events: pathogenesis-related (PR) protein synthesis, phytoalexins and modification of cell wall [29, 30]. PR proteins includes β-1,3-glucanases and chitinases, the enzymes that hydrolyse the main components of fungal cell walls. It is suggested that β-glucanases and chitinases can influence plant pathogen response in two manners: by degrading pathogen cell wall or by releasing elicitors from fungal cell wall. The oligosaccharides elicitors are recognized by the host plant, what cause accumulation of phytoalexins [31] and activation of signalling cascades resulting in the up-regulation of different plant defence systems [32]. It was shown that PR proteins are related to pathogen plant resistance and plants overexpressing these proteins are characterized by higher resistance to fungi [33]. Flax plants were transformed with potato β-1,3-glucanase to improve their resistance to *Fusarium* infections. Seedlings of transgenic flax were tested for *F. oxysporum* and *F. culmorum* resistance. It was estimated that flax overexpressing β-1,3-glucanase was two- to three-fold more resistant than control plants [22]. Moreover, the transgenic plant extract inhibited mycelium growth to about 48%. These results definitely confirm an important role of β-1,3-glucanase enzyme in flax resistance to pathogens. Protective activity of β-1,3-glucanase lies in the direct interaction of enzyme with polysaccharides of fungal cell wall. Nevertheless it is necessary

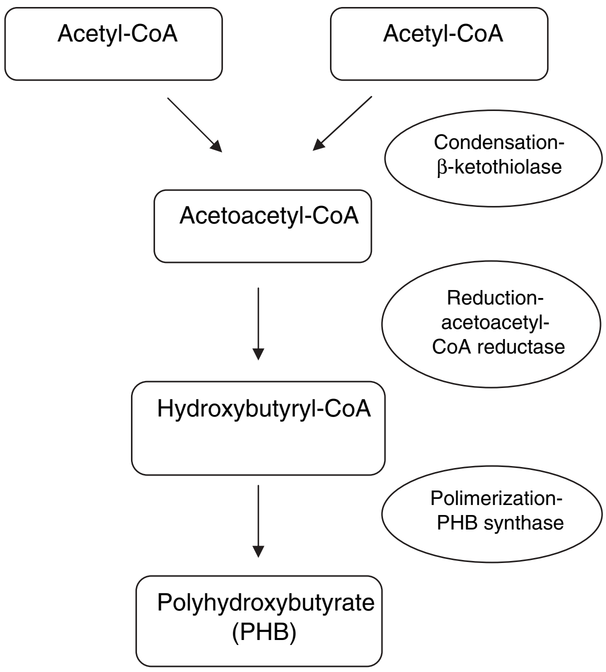

Figure 3 Polyhydroxybutyrate synthetic pathway

to consider putative metabolite modification of host plant that could act as pathogen inhibitors. It is then suggested that some of the secondary metabolites like phenolic acids and other antioxidants inhibit pathogen growth [34]. Plants overexpressing β-1,3-glucanase exhibited an increase in phenolic acid content. Indeed transgenic flax was characterized by higher phenolic acid level, but only in some transgenic lines [22]. Metabolic profile of transgenic flax showed increase in compound containing nitrogen like polyamides (spermidine and putrescine), some of the amino acids (arginine) and amides (glutamine and asparagines). All these compounds are accumulated in plants during stress [35]. Overexpression of β-1,3-glucanase resulted in the higher resistance to pathogen infection. It is proved that the enzyme directly affects the pathogen growth, which is suggested to be the primary reason for flax resistance to *Fusarium*.

Flax Fibre Quality Improvements

As flax fibre is a valuable material that has a wide range of possible applications, some experiments have been undertaken to improve the quality of flax fibres. Fibres from flax overproducing poly-β-hydroxybutyrate (PHB) exhibited better mechanical properties. PHB is non-toxic, insoluble in water and a thermoplastic material. It is polyester of hydroxyacids synthesized by bacteria. PHB accumulates as energy reserve material in many micro-organisms including *Alcaligenes, Azotobacter, Bacillus, Nocardia, Pseudomonas* and *Rhizobium*. Its properties are similar to polypropylene; it is stiff, brittle and resistant to UV light. The most important feature of PHB is its biodegradability, possibly through the action of micro-organisms that produce hydrolases and depolymerases [36]. So far, PHB has been mainly isolated from bacteria, which is rather an expensive technology. It has become clear that a promising option would be to use plants to produce PHB.

PHB is synthesized by bacteria in three-step reaction catalysed by β-ketothiolase (*phb*A), acetoacetyl-CoA reductase (*phb*B) and by PHB synthase (*phb*C) [37] (Figure 3). Recently some reports have appeared about PHB synthesis in transgenic plants, with promising effects of the PHB synthesis level but with significant growth inhibition [38]. The most interesting results have been shown for transgenic flax with overexpression of three genes crucial for PHB synthesis from *Ralstonia eutropha* with no negative effect on plant growth. For transformation, the 14-3-3 promoter was used as stem-specific for flax. Transformed plants were able to accumulate 70 times higher levels of PHB than non-transformed plants [39]. Mechanical properties of the stems of *in vitro* plants were examined and characterized by Young's modulus. The possible changes in stem mechanical properties through overexpression of PHB were evaluated by the stress–strain relationship of basal stems section. Young's module varied from 24.1 to 54.4 MPa in transformed flax and was twice much higher than in control plants. PHB overexpressing flax had higher average resistance of stem tissue to tensile loads and exhibited superior elastic properties. The approach of introducing PHB polymer to flax may generate the great source of commercially utilized bast fibres. Further studies of isolated flax fibres were carried out. The infrared (IR) spectra of the transgenic flax fibres showed some significant changes in fibre composition and chemical bound conformations. PHB located between flax fibres functioned as cross-linker between the cellulose chain. There was a greater structural disorder of the transgenic flax fibres than in control plants, which could be the result of formation of hemicelluloses with an amorphous structure and to the shortening of the cellulose chain lengths. Flax with introduced PHB exhibited stronger coupling between the fibres, which made them more stable [40].

Another modification that could influence fibre quality is retting improvement. The efficiency of this process also influences fibre quality. Partial degradation of stem by bacterial and fungal enzymes enables to receive pure fibre proper for industrial use. The retting efficiency strongly depends on the method used, so different procedures have been examined: water retting in a tank under precisely controlled conditions, retting of standing flax following desiccation with glyphosate, and treatment with formic acid, propionic acid, formaldehyde, Ethylene diaminetetra-acetic acid (EDTA) or sulphur dioxide were used, in the hopes of improving the fibre bundle separation from the rest of the plant stem. Most of the methods were satisfactory but only on a laboratory scale, so the traditional dew retting method is mostly used for industrial purpose. Harvested stalks are left in the field and soil micro-organisms digest the cell matrix polysaccharides. This process strongly depends on weather conditions and thus cannot be properly controlled. Moreover, the chemical composition of flax stems influences the retting process; the more lignified fibres are, the longer the exposure to fungal and bacterial enzymes that is needed, long treatment causes cellulose decomposition and weakens the fibres. The possible solution to this problem would be reduction of lignin synthesis in flax. Lignins are complex polymers of three aromatic alcohols: coniferyl, sinapyl and *p*-coumaryl [41], and cinnamyl alcohol dehydrogenase (CAD) is an enzyme that catalyses the biosynthesis of lignin monomers (hydroxycinnamoyl-alcohols) from the corresponding aldehydes [42]. It is said to be a specific marker of lignification [43]. Flax fibres have 3–5% lignin, which provides mechanical resistance. Lignins are synthesized and deposited in response to pathogen attack and stress. They create the physical barrier from pathogen infection [44]. Nevertheless, lignin accumulation lowers fibres plasticity and has negative influence on retting efficiency and thus quality of flax fibre. To improve fibre quality the *cad* gene was silenced in flax. The transgenic flax with silenced *cad* gene was expected to have lower lignin content and indeed this was the case. CAD enzyme activity decreased by about 20–40% and was characterized by lower lignin level of up to 40% [45]. Silencing of the *cad* gene influenced the composition of cell wall. There was a remarkable decrease in the pectin level (rhamnose, arabinose, galactose and galacturonic acid) and hemicelluloses. As all these compounds are degraded by micro-organisms during retting, decreasing their level facilitated this process. What is more, the mechanical properties of transgenic flax fibres were improved because of the increased ratio of cellulose to lignin. The strength and stiffness of the stem were measured and transgenic flax with the silenced *cad* gene exhibited more favourable properties. These data may have potential commercial meaning in improving the retting process and also mechanical properties of flax fibres.

Oil Stability Improvements

Another goal of genetic manipulation in flax was directed towards improving flax oil properties. For this purpose, flax was transformed with *Solanum sogarandinum*-derived glycosyltransferase (UGT) protein, which we designated SsGT1 under seed-specific napin promoter. UGTs are large group of enzymes found in all living organisms [46]. The glycosyltransferase from *S. sogarandinum* was cloned and analysed [47]. Detailed analysis of the enzyme revealed its broad substrate specificity, with the highest activity against kaemferol and peonidin [48]. These flavonoids are known antioxidants and their glucosylation performed by overexpressed SsGT1 stabilizes these metabolites. Accumulation of glucosylated flavonoids in transgenic flax might improve the quality of flax oil. Overproduction of SsGT1 in transgenic flax resulted in proanthocyanin, phenolic acids and unsaturated fatty acids accumulation in the seeds. The consequence of this modification was also a significant increase in the lignan level. SDG, the major component of lignan was elevated in transgenic flax by more than 30-fold [49]. What is important from the biotechnological point of view is the involvement of polyphenols glycosides in protection of unsaturated fatty acids against oxidation [50]. The significant increase in antioxidant compounds in transgenic flaxseed might protect unsaturated fatty acids, which are essential constituents of flaxoil, but easily undergo peroxidation. Plants extract with abundant flavonoids can protect oil from oxidation. Flax oil received from transgenic flax overexpressing SsGT1 in seeds was examined in gas chromatography with Flame Ionization Detector (FID) detector and the increase in unsaturated fatty acids was observed [49]. The elevated level of antioxidants in flaxseed was accompanied with meaningful changes in fatty acids composition. The additional positive effect of such a manipulation was improved pathogen resistance, which was correlated with increased contents of the glycoside derivates of flavonoid.

Medical and Industrial Applications of Products Based on Genetically Modified (GM) Flax

Transforming flax with various genes was not only aimed at deepening scientific knowledge of flax metabolism but also creating new products for various applications. The challenge of biotechnology is to broaden these applications. In this review, we present some possible use of transgenic flax (Figure 4).

Bandages

Currently the number of patients with serious ulcer wounds is still increasing. This is a consequence of chronic diseases such as diabetes, obesity and atherosclerosis. It is critical for healing ulcer wounds, because as soon as the epidermal cells die, a major barrier to bacteria is breached, and it causes further necrosis to the surrounding tissues [51]. An ulcer that is considered chronic, or non-healing, is one that does not heal in a timely fashion. As human life is increasingly prolonged, the number of difficult-to-heal ulcers is also growing. Their treatment is difficult, complicated and the process of healing uncertain. Around 50% of ulcers heal within four months and there is a high probability of renewal of disease [52]. Unfortunately, there are very few innovative methods for ulcer healing. In this review, we present the very new application of flax fibre in bandages production. Flax fibres with high antioxidant level would be the perfect material for linen wound dressing. Given their high level of antioxidants such as lignans, phenolic acids, kaempferol and quercitin, they can stimulate natural process of wound clearing by macrophages [53]. Such flax bandages can assure the perfect milieu for effective healing by helping the natural process to progress. They are hygroscopic and

Figure 4 Possible applications of flax oil and fibres

thus offer lower infection probabilities. The loose weave of flax tissue enables wound purification from various contaminations. Flax bandages can also be enriched with transgenic oil emulsion or transgenic seedcake extract with significantly high antioxidant level. This can prevent fibroma formation; can also keep optimal humidity that facilitates epithelial cells migration [54]. Simultaneous use of fibres, oil emulsion and seedcake extract from genetically modified flax was expected to promote healing of chronic skin ulcerations. This was the case indeed. The group of 30 patients having long-lasting skin ulceration (from 2 to 23 years) was subjected to treatment with linen dressing for 12 weeks. The treatment was divided into three phases: dry phase with use of highly hygroscopic linen dressing aimed at drying and cleaning up wounds, second stage were linen dressing were wetted with oil emulsion, derived from transgenic flax, in order to supply wounds with polyunsaturated fatty acids (PUFA) and antioxidants, and the third phase were flax bandages wetted with seedcake extract rich in lignans, the anti-inflammatory compounds were used. Such a treatment effectively reduced the wound exudates in almost 67% of the subjects [55]. Moreover, 93% patients exhibited a decrease in the fibrin level, which is one of the steps in wound healing. An important and objective parameter that was assessed was the wound size and it appeared that in the case of 80% of patients, the ulcer size was reduced, among which 23% were totally cured. These results show that linen dressings obtained from GM flax have beneficial effects on wound healing and can be used as an innovative flax biotechnological product.

Biocomposites

Flax with overexpression of three genes crucial for PHB synthesis could have many possible applications. Transgenic flax fibres enriched with biodegradable plastic – PHB are useful in biomedical and technical applications. These fibres can be used for biocomposites production as enforcement of biodegradable composite materials. Great disadvantages of currently produced composites are their poor biomechanical properties and poor bioactive actions. PHB is a very brittle material and requires another polymer to compensate elasticity, stiffness or durability. At present, composites are composed of biodegradable polymers such as PHB and non-organic phase (hydroxyapatite, bioactive glass fibres or glass-ceramic fibres) for medical use [56] or plant fibres (cellulose polymers) with synthetic polymers (polystyrene, polypropylene and glass fibres) for automotive industry [57]. In the last decade, bio-fibres were applied by European car-makers for constructing door panels, seat backs, headlines, package trays, dashboards and trunk liners [58, 59]. The interesting issue would be to not only produce biocomposites that would be biodegradable but also have favourable mechanical properties such as elasticity, plasticity, durability and have some bioactive features (antioxidative). Between the warp phase, which is a constituent of a composite that is thrown across (e.g. PHB) and filling (e.g. cellulose fibres or glass fibres), discontinuous fragments are formed. They weaken the integrity of the biocomposite, which is a significant disadvantage. The possible solution to this problem would be production of a natural biocomposite from PHB-enriched flax fibres. This would definitely improve its biomechanical properties. Moreover, flax fibres are a great source of lignans, which possess antioxidative properties, and are thus bioactive. So far, only plant fibres without chemical competence with warp have been used. A great innovation would be introduction to flax fibres of a biodegradable polymer that would make the compatibility of biopolymers constituents better. Use of transgenic flax fibres with PHB would enforce traditional mechanical bond in composites with an additional chemical bond (hydrogen bond) between warp and polymeric fibres. This additional bond is said to improve mechanical properties of composites. Application of transgenic flax fibres could make the economy independent of synthetic material derived from crude oil processing.

Another important and promising application of flax fibres with PHB is packaging of biomedical products. The plastics used nowadays (polypropylene – PP and polystyrene – PS) are produced from crude oil polymers and are used for packaging products. Alarming increase of plastic packaging and their non-biodegradability contribute to environmental damage. Annual plastic production is estimated to be about 140 million tonnes and its significant part is introduced into the ecosystem as industrial wastes [60]. Application of flax biocomposites in packaging and replacing only 20% of polypropylene in such products would eliminate 28 million tonnes of polypropylene from the environment per year. Transgenic plastic flax could also be used in many other medical products such as surgery thread or implants. Previous experiments showed that composites of flax plastic fibres do not promote platelet aggregations in contrast to pure polypropylene. *In vitro* experiments indicated that flax plastic fibres have bacteriostatic properties (data not published). Biocomposites with bioplastic flax fibres give the new possibilities of their application as implants in orthopaedic, tissue engineering and other branches of medicine.

Nutritional Supplements

Since the flaxseed is a rich source of PUFA, it has beneficial health effects. However, flaxseed oil easily undergoes oxidation. The energy necessary to initiate peroxidation is lower, the more unsaturated bounds are present in fatty acids chains. The reaction of reactive oxygen species (ROS) with PUFA has been studied as it promotes development of undesirable flavour and odour in food. Lipid peroxidation in plant membranes can degrade unsaturated fatty acids, which makes flax oil very unstable and even toxic. This process leads to free radical production, which is associated with carcinogenesis, ageing, inflammation and atherosclerosis [61]. To preserve flax oil it is necessary to store it quickly in cold, dark conditions with limited light access. These conditions make flaxseed oil an unpopular industrial product. A possible solution to these problems would be to use a transgenic flaxseed with increased antioxidant content for flax oil production. Both transgenic lines would be suitable for this purpose: flax with overexpression of genes coding for flavonoids synthesis and also with overexpression of glycosyltransferase gene as it makes flavonoids more stable. Since flavonoid compounds protect lipids against peroxidation and have hydroxyradical scavenging activity [62], transgenic flax oil could be the great source of precious flax oil. Fatty acid composition analysis of transgenic flax revealed increase in unsaturated fatty acids level, which suggests that there is a positive effect of flavonoid compound. It is also reported that other components such as lignans, fibres and proteins protect oil against lipid peroxidation [63]. Seedcake from transgenic flaxseed is rich in strong antioxidant secoisolariciresinol (SDG). This lignan also contributes to health-promoting actions [64]. Transgenic flax oil can be directly used in food, medical and cosmetic products. Flax products are widely used and available as flax seed, flax seed oil or flax seed oil supplements, in liquid form or soft gel capsules. Flax is a constituent in cream, ointments and masks; it contains vitamins F, A, E and microelements, so it can provide the essential nutritional elements, proper humidity of skin, slow effects of ageing and protect it from unfavourable external factors [65]. As a dietary supplement, flax is mainly used for its high unsaturated fatty acid content and can be found in many different products. A great innovation would be the introduction flax with improved antioxidant properties and better nutritional parameters to this wide range of products.

Conclusions

Flax is a plant widely distributed with a long cultivation history as industrial oil and fibre crop. There is a still growing interest in the medicinal, nutraceutical and industrial value of flax. The interest is mainly concentrated on biological activity of unsaturated fatty acids but also on lignans (SDG) and soluble dietary fibres. Flax is also of much interest for scientists who try to improve its favourable properties by the tools of genetic engineering tools. Increasing the expression level of the heterologous genes presented in this review contributes to improved pathogen resistance, oil stability, fibre quality and elevated antioxidant level. As the transformation protocol of flax is well established and results are very promising, it is clear that genetically modified flax has a future in industrial and medical applications.

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References

1. Muir AD, Westcott Neil D, editors. Flax: The Genus Linum. Taylor and Francis, Saskatchewan, Canada; 2003.
2. Zhan ZP, Huang FR, Luo J, Dai JJ, Yan XH, Peng J. Duration of feeding linseed diet influences expression of inflammation-related genes and growth performance of growing-finishing barrows. Journal of Animal Science 2009;87:603–11.
3. Pellizzon MA, Billheimer JT, Bloedon LT, Szapary PO, Rader DJ. Flaxseed reduces plasma cholesterol levels in hypercholesterolemic mouse models. Journal of the American College of Nutrition 2007;26(1):66–75.
4. Rodríguez-Cruz M, del Prado M, Torres N. Molecular mechanisms of action and health benefits of polyunsaturated fatty acids. Revista de Investigacion Clinica 2005;57(3): 457–72.
5. Doughman SD, Krupanidhi S, Sanjeevi CB. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Current Diabetes Reviews 2007;3(3):198–203.
6. De Spirt S, Stahl W, Tronnier H, Sies H, Bejot M, Maurette JM, *et al.* Intervention with flaxseed and borage oil supplements modulates skin condition in women. British Journal of Nutrition 2009;101:440–5.
7. Wang L, Chen J, Thompson LU. The inhibitory effect of flaxseed on the growth and metastasis of estrogen receptor negative human breast cancer xenograftsis attributed to both its lignan and oil components. International Journal of Cancer 2005;116(5):793–8.
8. Bozan B, Temelli F. Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioresource Technology 2008;99(14):6354–9.
9. Ilvessalo-Pfaffli M-S. Fiber Atlas: Identification of Paper-making Fibers. Springer-Verlag, Berlin; 1995. p. 292–337.
10. Antonov V, Marek J, Bjelkova M, Smirous P, Fischer H. Easily available enzymes as natural retting agents. Biotechnology Journal 2007;2:342–6.
11. Zhang J, Henriksson G, Johansson G. Polygalacturonase is the key component in enzymatic retting of flax. Journal of Biotechnology 2000;81(1):85–9.
12. Zhang J, Henriksson H, Szabo IJ, Henriksson G, Johansson G. The active component in the flax-retting system of the zygomycete *Rhizopus oryzae* sp. is a family 28 polygalacturonase. Journal of Industrial Microbiology and Biotechnology 2005;32(10):431–8.
13. de la Riva GA, González-Cabrera J, Vázquez-Padrón R, Ayra-Pardo C. *Agrobacterium tumefaciens*: a natural tool for plant transformation. EJB Electronic Journal of Biotechnology 1998;1(3):18–133.
14. Gelvin SB. *Agrobacterium*-mediated plant transformation: the biology behind the 'gene-jockeying' tool. Microbiology and Molecular Biology Reviews 2003;67(1):16–37.
15. Jordan MC, McHughen A. Transformed callus does not necessarily regenerate transformed shoots. Plant Cell Reports 1988;7:285–7.
16. Dong JZ, McHughen A. Transgenic flax plants from *Agrobacterium* mediated transformation: incidence of chimeric regenerants and inheritance of transgenic plants. Plant Science 1993;91:139–48.
17. Dong JZ, McHughen A. An improved procedure for production of transgenic flax plants using *Agrobacterium tumefaciens*. Plant Science 1993;88:61–71.
18. Yildiz M, Er C. Increasing the injured area on hypocotyl explants of flax (*Linum usitatissimum* L.) leads to high frequency callus-based shoot regeneration. Turkish Journal of Biology 2002;26:95–8.
19. Beranova M, Slavomír R, Vávrová Z, Skalicky T. Sonication assisted *Agrobacterium*-mediated transformation enhances the transformation efficiency in flax (*Linum usitatissimum* L.). Plant Cell, Tissue and Organ Culture 2008;94:253–9.
20. Harborne JB, Williams CHA. Advances in flavonoids research since 1992. Phytochemistry 2000;55:481–504.
21. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine 2005;26(6):459–516.
22. Wróbel-Kwiatkowska M, Lorenc-Kukula K, Starzycki M, Oszmiański J, Kepczyńska E, Szopa J. Expression of β-1,3-glucanase in flax causes increased resistance to fungi. Physiological and Molecular Plant Pathology 2004;65:245–56.
23. Cushnie PT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005;26:343–56.
24. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflammatory Research 2009;58(9):537–52.
25. Lorenc-Kukuła K, Amarowicz R, Ski JA, Doermann P, Starzycki M, Skała J, *et al.* Pleiotropic effect of phenolic compounds content increases in transgenic flax plant. Journal of Agriculture and Food Chemistry 2005;53:3685–92.
26. Harborne JB, Williams CHA. Advances in flavonoids research since 1992. Phytochemistry 2000;55:481–504.
27. Lorenc-Kukuła K, Wróbel-Kwiatkowska M, Starzycki M, Szopa J. Engineering flax with increased flavonoid content and thus *Fusarium* resistance. Physiological and Molecular Plant Pathology 2007;70:38–48.
28. Fujisawa M, Watanabe M, Choi S-K, Teramoto M, Ohyama K, Misawa N. Enrichment of carotenoids in flaxseed (*Linum usitatissimum*) by metabolic engineering with introduction of bacterial phytoene synthase gene *crtB*. Journal of Bioscience and Bioengineering 2008;105(6): 636–41.
29. Postel S, Kemmerling B. Plant systems for recognition of pathogen-associated molecular patterns. Seminar on Cell and Developmental Biology 2009 in press, doi:10.1016/j.semedb.2009.06.002.
30. Dangl JL, Jones JDG. Plant pathogens and integrated defence responses to infection. Nature 2001;411:826–33.
31. D'Ovidio R, Mattei B, Roberti S, Bellincampi S. Polygalacturonases, polygalacturonase-inhibiting proteins and pectic oligomers in plant–pathogen interactions. Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics 2004;1696(2):237–44.
32. Esquerré-Tugayé MT, Boudart G, Dumas B. Cell wall degrading enzymes, inhibitory proteins, and oligosaccharides participate in the molecular dialogue between plants pathogens. Plant Physiology and Biochemistry 2000;38: 157–63.

33. Kim ST, Kim SG, Hwang DH, Kang SY, Kim HJ, Lee BH, *et al.* Proteomic analysis of pathogen responsive proteins from rice leaves induced by rice blast fungus. *Magnaporthe grisea*. Proteomics 2004;4(11):3569–78.

34. Cui Y, Bell AA, Joost O, Magill C. Expression of potential defense response genes in cotton. Physiological and Molecular Plant Pathology 2000;56:25–31.

35. Rabe E. Stress physiology: the functional significance of the accumulation of nitrogen-containing compounds. Journal of Horticultural Science 1990;65:231–43.

36. Wang S, Song C, Mizuno W, Sano M, Maki M, Yang C, *et al.* Estimation on biodegradability of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/V) and numbers of aerobic PHB/V degrading microorganisms in different natural environments. Journal of Polymers and the Environment 2005;13(1):39–45.

37. Steinbuchel A, Fuchtenbusch B. Bacterial and other biological systems for polyester production. TIBTECH 1998;16:419–27.

38. Bohmert K, Balbo I, Kopka J, Mittendorf V, Nawrath C, Poirier Y, *et al.* Transgenic *Arabidopsis* plants can accumulate polyhydroxybutyrate to up to 4% of their fresh weight. Planta 2000;211:841–5.

39. Wróbel M, Zebrowski J, Szopa J. Polyhydroxybutyrate synthesis in transgenic flax. Journal of Biotechnology 2004;107:41–54.

40. Wróbel-Kwiatkowska M, Szopa J, Dymińska L, Mączka M, Hanza J. Spectroscopic characterization of genetically modified flax fibres enhanced with poly-3-hydroxybutyric acid. Journal of Molecular Structure 2009;920:214–9.

41. Amthor JS. Efficiency of lignin biosynthesis: a quantitative analysis. Annals of Botany 2003;91:673–95.

42. Barakat A, Bagniewska-Zadworna A, Choi A, Plakkat U, DiLoreto DS, Yellanki P, *et al.* The cinnamyl alcohol dehydrogenase gene family in *Populus*: phylogeny, organization, and expression. BMC Plant Biology 2009;9:26.

43. Kim H, Ralph J, Lu F, Pilate G, Leple JC, Pollet B, *et al.* Identification of the structure and origin of thioacidolysis marker compounds for cinnamyl alcohol dehydrogenase deficiency in angiosperms. Journal of Biological Chemistry 2002;277:47412–9.

44. Tobias CM, Chow EK. Structure of the cinnamyl alcohol dehydrogenase gene family in rice and promoter activity of a member associated with lignification. Planta 2005; 220:678–88.

45. Wróbel-Kwiatkowska M, Starzycki M, Zebrowski J, Oszmiański J, Szopa J. Lignin deficiency in transgenic flax resulted in plants with improved mechanical properties. Journal of Biotechnology 2006;128:919–34.

46. Cheng GW, Malencik DA, Breen PJ. UDP-glucose: Flavonoid-O glucosyltransferase from strawberry fruit. Phytochemistry 1994;6:1435–9.

47. Rorat T, Grygorowicz WJ, Berbezy P, Irzykowski W. Isolation and expression of cold specific genes in potato (*Solanum sogarandinum*). Plant Science 1998;133(1):57–67.

48. Aksamit-Stachurska A, Korobczak-Sosna A, Kulma A, Szopa J. Glycosyltransferase efficiently controls phenylpropanoid pathway. BMC Biotechnology 2008;5(8):25.

49. Lorenc-Kukuła K, Żuk M, Kulma A, Czemplik M, Kostyń K, Skała J, *et al.* Engineering flax with the GT family 1 *Solanum sogarandinum* glycosyltransferase SsGT1 confers increased resistance to *Fusarium* infection. Journal of Agriculture Food and Chemistry 2009;57(15):6698–705.

50. Aksamit-Stachurska A, Korobczak-Sosna A, Kulma A, Szopa J. Glycosyltransferase efficiently controls phenylpropanoid pathway. BMC Biotechnology 2008;5:8–25.

51. Abbade LP, Lastória S. Venous ulcer: epidemiology, physiopathology, diagnosis and treatment. International Journal of Dermatology 2005;44(6):449–56.

52. Nicolaides AN. Investigation of chronic venous insufficiency: a consensus statement. Circulation 2000;102:126–63.

53. Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M, Roy S. Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm. Annals of New York Academy of Sciences 2002;957:239–49.

54. Dyson M, Young S, Pendle CL, Webster DF, Lang SM. Comparison of the effects of moist and dry conditions on dermal repair. Journal of Investigative Dermatology 1988;91:434–9.

55. Skórkowska-Telichowska K, Żuk M, Kulma A, Bugajska-Prusak A, Ratajczak K, Gąsiorowski K, *et al.* FlaxAid, the new bandage based on transgenic flax products. Biotechnology Progress In Print, 2009.

56. Martin DP, Williams SF. Medical applications of poly-4-hydroxybutyrate: a strong flexible absorbable biomaterial. Biochemical Engineering Journal 2003;16(2):97–105.

57. Suddell BC, Evans WJ. Natural fiber composites in automotive industry. In Mohanty AK, Misra M, Drzal LT, editors. Natural Fibers, Biopolymers and Biocomposites. Taylor and Francis Group, CRC Press, Boca Raton, FL; 2005. p. 231–259.

58. Suddel BC, Evans WJ. The increasing use and application of natural fiber Composite materials within the automotive industry. In Seventh Composite Conference on Woodfiber–Plastic Composites, 19–20 May 2003, Madison, WI, USA; 2003. p. 7–14.

59. Bledzki AK, Faruk O, Sperber VE. Cars from bio-fibers. Macromolecular Materials and Engineering 2006;291:449–57.

60. Kopeliovich D. Plastics Recycling. SubsTech. Available from: URL: http://www.substech.com/dokuwiki/doku.php?id=plastics_recycling.

61. Choi ChW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, *et al.* Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Science 2002;163:1161–8.

62. Kim YJ, Yokozawa T. Modulation of oxidative stress and melanogenesis by proanthocyanidins. Biological and Pharmaceutical Bulletin 2009;32(7):1155–9.

63. Newairy AS, Abdou HM. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food and Chemical Toxicology 2009;47(4):813–8.

64. Prasad K. Flaxseed and cardiovascular health. Journal of Cardiovascular Pharmacology 2009. doi:10.1097/FJC.0b013e3181af04e5.

65. De Spirt S, Stahl W, Tronnier H, Sies H, Bejot M, Maurette J-M, *et al.* Intervention with flaxseed and borage oil supplements modulates skin condition in women. British Journal of Nutrition 2009;101(3):440–445.