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Seeds and oil of the Styrian oil pumpkin: Components and biological activities

Cucurbita pepo subsp. *pepo* var. Styriaca is a phylogenetically young member of the *Cucurbita* spp. since the mutation leading to dark green seeds with stunted outer hulls arose only in the 19th century. This mutation defined the so-called Styrian oil pumpkin and facilitated the production of Styrian pumpkin seed oil. It is a regional specialty oil in the south-eastern part of Europe. In this article, we describe the production and economic value of this edible oil as well as its composition on a molecular basis, including fatty acids, vitamins, phytosterols, minerals, polyphenols, and the compounds responsible for its pigments, taste and flavor. We also describe contaminants of Styrian pumpkin seed oil and the most relevant field pests of the Styrian oil pumpkin plant. Finally, we review the putative beneficial health effects of Styrian oil pumpkin seeds and of their products.

Keywords: *Cucurbita pepo*, tocopherols, phenolics, benign prostate hyperplasia, potyvirus.

Review Article

1 Introduction

Styrian pumpkin seed oil is made from seeds of the special pumpkin variety *Cucurbita pepo* subsp. *pepo* var. Styriaca that first emerged in Austria's south-eastern province Styria. This species belongs to the Cucurbitaceae which is a diverse class of plants that consists of at least 119 genera and over 825 species [1]. Within this family there is tremendous genetic diversity both in vegetative and reproductive characteristics. The range of adaptation for cucurbit species includes tropical and subtropical regions, arid deserts, and temperate locations. Although there are some species that have origins in the Old World [2], most of them originated in the New World, and at least seven genera have origins in both hemispheres [3]. Pumpkin plants have been grown since the earliest times of mankind, as evidenced by archeological findings in Mexico, North America and also East Asia [4, 5]. *Cucurbita pepo* subsp. *pepo* belongs to the New World squash family, and recent phylogenetic studies revealed that the common ancestor of all the current *Cucurbita pepo* subsp. *pepo* varieties probably originates from Southern Mexico [6]. It was imported to Europe in the post-Columbian era, and today it is cultivated as food and for decorative purposes in nearly all warm and temperate parts of the globe. Edible oils are produced from various *Cucurbita pepo* species in South Eastern Europe, several African countries and China. When edible oils are pressed from *Cucurbita* spp., usually light yellow and clear oils are obtained. However, the pumpkin seed oil produced in Austria's south eastern province Styria is dark green and shows dichroism.

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Styrian pumpkin seed oil is produced from a special variety within the subspecies *Cucurbita pepo* subsp. *pepo*, which emerged in the first half of the 19th century in this region. This pumpkin variety is called "Styrian oil pumpkin", or *Cucurbita pepo* subsp. *pepo* var. Styriaca, and has been formed by an accidental natural mutation that led to tremendous morphological changes of the seed architecture (Fig. 1A). This mutation, which is the result of a single recessive gene [7], led to a very thin outer hull (naked or hull-less seeds), which highly facilitates the production of this regional specialty oil and also leads to its dark green color. In these seeds, the amounts of lignin and cellulose in the hypodermis, sclerenchyma and parenchyma tissues of the seed coat are reduced [8, 9]. During seed development, initially all tissue layers are completely formed in the testa of both wild type and mutant. However, 20 days after anthesis, lignin, cellulose and hemicellulose amounts are considerably decreased in the mutant [9–11]. In the maturing hull-less seed, the epidermis collapses and together with the reduced lignin and cellulose contents of other seed coat tissues results in a thin papery and nearly translucent seed coat. In addition, the mutant hull-less testa contain higher levels of the polyamines putrescine, spermidine and spermine than the wild-type testa. It was speculated that this was due to a decreased linkage to lignins and/or related to decreased water stress in the mutant testa [12]. In addition, a genetic approach was used to study the expression pattern of genes involved in secondary cell wall bio

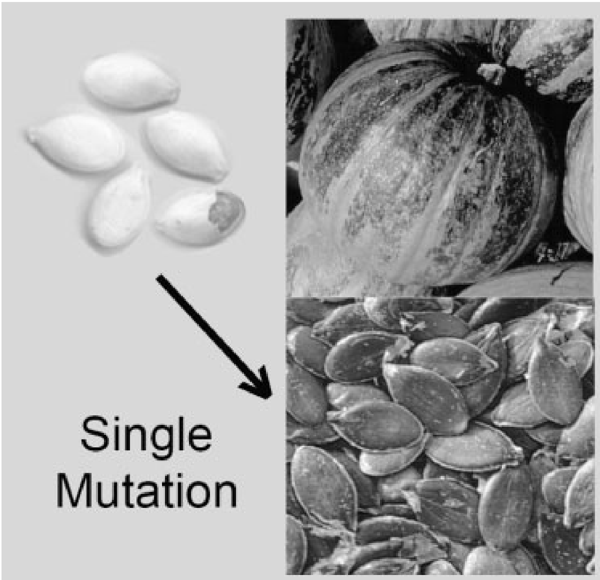

Fig. 1. Morphological differences between wild-type and hull-less pumpkin seeds. A single mutation leads to stunted outer hulls in pumpkin seeds of *Cucurbita pepo* subsp. *pepo* var. Styriaca. Chlorophyll-rich tissues show through the very thin papery outer hull in the mutant seeds and lead to a green rather than a whitish appearance.

synthesis during testa development. Bezold *et al*. used for their study a very close relative of the Styrian oil pumpkin (hull-less) carrying the same single recessive mutation, and compared it to the corresponding wild type. These authors monitored the expression patterns of cellulose synthase, phenylalanine ammonium lyase, 4-coumarate-CoA ligase and cinnamoyl-CoA reductase [13]. Furthermore, these authors report on differential gene expression of glutathione reductase, abscisic acid-responsive protein E, a serine/threonine kinase and a β-ureidopropionase in the two genotypes during testa development [13]. Their results show that the expression levels of genes involved in lignin and cellulose biosynthesis are generally higher in the wild-type testa than in the mutant testa, thus providing a genetic explanation for the earlier anatomical and biochemical results of others. Nowadays, numerous varieties/hybrids have been generated from the original Styrian oil pumpkin in order to increase parameters like yield, seed content, or to introduce resistance against field pests and/or viruses (see Section 4).

2 Production and economical importance of pumpkin seed oil

The seeds of the Styrian oil pumpkin are harvested in mid autumn, immediately dried to a remaining water content of 5–7 wt-% and ground. The remaining flesh of the pumpkins is of lower quality as compared to squash and marrow like *Cucurbita moschata* or *Cucurbita maxima* and serves sometimes as addition to animal food. To the dried ground kernels, fresh water and table salt is added to form a soft pulp. This pulp is roasted for up to 60 min at temperatures around 100 °C, which results in coagulation of the protein fraction and permits convenient separation of the lipid fraction by pressing. In addition, this roasting process is responsible for the generation of the typical aroma of the end product (see Section 3.6). The pressing process is performed under isothermal conditions at pressures between 300 and 600 bar. A dark green oil is obtained as an end product and is then stored in dark bottles to avoid light-induced deterioration and sold as specialty oil. The pressing process is not exhaustive, and the remaining pressing cakes still contain a reasonable amount of the valuable oil, but may only be used to produce salad oils of lower quality. After this second pressing, the pressing cakes are currently only used as swill, although they still contain a reasonable amount of vitamins, *e.g.* carotenoids [14]. Only oil from the first pressing, and thus of the highest quality, is allowed to be sold as the brand Styrian pumpkin seed oil. This high-quality regional specialty oil is protected in its geographical origin by European law.

Styrian pumpkin seed oil is also of considerable economical importance for the province of Styria. The so-called oil pumpkin is the third most important field fruit in Styria with 13,000 ha of cultivable land yielding 11,100 tons in the year 2006 [15]. The average yield of pumpkin seeds of this variety strongly depends on the weather conditions, ranging from approximately 400 kg/ha (under drought) up to 1000 kg/ha under optimal conditions, with an average yield of 500–600 kg/ha. For the production of 1 L of this specialty oil, an average of 2.5 kg of pumpkin seeds is required, which corresponds to an amount of 30–40 oil pumpkins. However, this value is heavily influenced by the culture conditions and also by the breeding line itself. The majority of the pumpkin seeds, an estimated 6000 tons in the year 2006, are used for the production of pumpkin seed oil, representing a current market value of more than €30 million.

3 Composition of the pumpkin seeds and the corresponding pumpkin seed oil

Most of the data reported in the literature concerning the composition of pumpkin seeds are lacking a detailed botanical description of the precise species/variety that has been analyzed. In this section, we summarize the most important data from the literature concerning the naked seed variety *Cucurbita pepo* subsp. *pepo* var. Styriaca.

The hull-less seeds of the Styrian oil pumpkin are enriched in their oil content as compared to other *Cucurbita pepo* spp., with crude oil contents ranging from 41 up to 59 wt-%, depending on the genetic diversity [16]. The crude protein content was found to be around 35 wt-%, with albumin and globulin being the most prominent proteins making up about 59% of the total crude protein content [17]. From the seeds of many close relatives of *Cucurbita pepo* spp., low-molecular-weight trypsin inhibitors containing three disulfide bridges were isolated [18]. Although there is no data available about the occurrence of trypsin inhibitors in the hull-less varieties, it seems reasonable to speculate that similar polypeptides are also present due to their close relationship within the family of *Cucurbita pepo*. On the amino acid level, a deficiency in sulfur-containing amino acids (cysteine and methionine) has been described in a close relative of the oil pumpkin [19]. In addition, the occurrence of rare amino acids like citrullin, *m*-carboxyphenylalanine, β-pyrazolalanine, γ-aminobutyric acid, and ethylasparagine has been reported [20]. Minor amounts of carbohydrates, fiber, and ash have also been found in the seeds.

In addition to the primary components listed above, there are several important secondary plant components that have been analyzed only in recent years. Some of them have also been found in Styrian pumpkin seed oils. In the following sections, we describe the distinct chemical compounds found in Styrian oil pumpkin seeds and the corresponding pressed oils.

3.1 Fatty acid composition

The relative fatty acid composition of Styrian oil pumpkin seeds and, as a consequence, of the corresponding Styrian pumpkin seed oils is affected not only by the variety of the cultivars but also by the growth conditions and the degree of ripeness. The seeds of *Cucurbita pepo* subsp. *pepo* var. Styriaca contain more than 80% unsaturated fatty acids, with especially high amounts of polyunsaturated fatty acids (Tab. 1). The predominant fatty acids are linoleic acid, oleic acid, palmitic acid, and stearic acid, which together make up 98.1–98.7% of the total amount of fatty acids. In addition to the fatty acids listed in Tab. 1, Murkovic *et al*. [16] report on traces (<0.5%) of other fatty acids (*e.g.* 16:1, 18:3, 20:4, 22:0, 22:6, and 24:0). Furthermore, Brühl *et al*. [21] reported on the occurrence of traces of *trans* fatty acids (18:1, 18:2, 18:3). However, it is noteworthy that the identity of these very small fatty acid fractions was just inferred from their GC retention times as compared to commercially available standard compounds and not from structural analyses, *e.g.* by mass spectrometry.

Tab. 1. Pattern of main fatty acids in Styrian oil pumpkin seeds. Data represent means of 100 different breeding lines of Styrian oil pumpkin plants, with the 25th and 75th percentiles in italics (modified from [16]).

Fatty acid		25th [wt-%]	Mean [wt-%]	75th [wt-%]
Myristic acid	14:0	*0.1*	1.1	*1.2*
Palmitic acid	16:0	*11.4*	12.0	*13.3*
Stearic acid	18:0	*4.8*	5.7	*6.7*
Oleic acid	18:1	*28.6*	33.3	*38.1*
Linoleic acid	18:2	*43.8*	48.6	*52.4*

The fatty acid pattern of Styrian pumpkin seed oil appears to be very similar to that of Styrian oil pumpkin seeds and thus consists mainly of unsaturated fatty acids. Analysis of several Styrian pumpkin seed oils revealed that the content of polyunsaturated fatty acids (45.6 ± 5% rel) is considerably higher than the content of monounsaturated fatty acids (35.9 ± 10% rel) or saturated fatty acids (18.5 ± 20% rel) [22]. It is noteworthy that Styrian oil pumpkin seeds and also the corresponding oils do not contain erucic acid. This was important as an erucic acid content of more than 0.7% of all fatty acids was generally regarded as a sign for adulteration of Styrian pumpkin seed oil with the cheaper rapeseed oil [23]. However, with the development of rapeseed breeding lines low in content of erucic acid, this method became obsolete. The relative amount of oleic acid is always negatively correlated with the relative amount of linoleic acid [16, 24]. This is due to the precursor-product relationship of these two fatty acids. The activity of the plant-specific microsomal oleoyl desaturase is increased at low temperature, which leads to an increase of linoleic acid and a decrease of oleic acid [25]. Consequently, Styrian oil pumpkins that have been harvested in late autumn, and thus at colder temperatures, contain more linoleic acid and less oleic acid. The high content of linoleic acid is an important nutritional aspect of Styrian pumpkin seed oil. Linoleic acid is an essential fatty acid for humans as it is required for the formation of cellular membranes, vitamin D, and various hormones. Although recently significant differences in the incorporation of fatty acids into various molecular lipid classes have been reported in Japanese squash species [26], there is currently no such data available for the seeds of the Styrian oil pumpkin.

3.2 Vitamins

Tocopherols are the major lipophilic antioxidants found in Styrian oil pumpkin seeds and their corresponding oils. The seeds of the Styrian oil pumpkin contain considerable amounts of the vitamin E derivatives tocopherol and tocotrienol. The predominant isomers of both tocol classes are the γ- and α-isomers, with tocopherols at about 5–8 times higher levels than tocotrienols [27]. Due to the physiological importance of tocopherols (antioxidants; for α-*R*,*R*,*R*-tocopherol see [28]), a high content of vitamin E in the seeds was defined as an objective for the generation of new breeding lines. Murkovic *et al*. [29] analyzed 100 different breeding lines and found γ-tocopherol contents ranging from 41 to 620 μg/g dry seeds and α-tocopherol contents between 0 and 91 μg/g dry seeds. The contents of the β- and δ-tocopherols were found to be very low, but sporadically (10 and 18 out of 100 breeding lines) reached 16 and 49 μg/g dry seeds, respectively [29]. The ratios of γ-tocopherol and α-tocopherol varied widely over the breeding lines and were found in the range between 5 and 10, indicating that the biosynthesis of these two tocopherols is not strictly coupled to one another.

The corresponding Styrian pumpkin seed oils also contain reasonably high amounts of tocopherols showing the same trends as described above for the pumpkin seeds. As expected, various Styrian pumpkin seed oils from different production lots have been reported to contain widely differing amounts of tocopherols, with γ-tocopherol being always the predominant isomer. The high-quality pumpkin seed oils are obtained from seeds containing γ-tocopherol concentrations of up to 800 μg/g oil [22]. α-Tocopherol concentrations were found in the range between 18 and 282 μg/g oil, but the minor β- and δ-isomers were not detected in these pumpkin seed oils [22, 30].

In addition to vitamin E derivatives, vitamin A and various carotenoids have also been detected in Styrian oil pumpkin seeds and in Styrian pumpkin seed oils as well as in the pressing residues of Styrian pumpkin seed oils. The predominant carotene found in Styrian pumpkin seed oil was lutein (71%) followed by β-carotene (12%) and cryptoxanthin b (5.3%) [31]. In the pressing residue of pumpkin seed oils, similar trends have been found, with lutein being the major carotenoid followed by β-carotene [14]. Although various vitamin B compounds were found in whole pumpkins [19], there are currently no data about their occurrence in the seeds or even the corresponding oils.

3.3 Minerals

In Styrian oil pumpkin seeds various minerals have been analyzed, and their concentrations did not only depend on the culture conditions but also on the breeding line. The seed contents of potassium, magnesium, calcium, and sodium were reported to be 183, 105, 27 and 3.6 μg/g dry seeds, respectively. Furthermore, it was found that magnesium, potassium and phosphorus correlated positively with one another and that the concentrations of both potassium and phosphorus depended on the variety and the culture location [24]. The total phosphorus content in Styrian pumpkin seeds was found to be around 211 μg/g dry seeds. However, no correlation was found between the phosphorus content of the seeds and the concentration of phospholipids (determined as phosphatidic acid) in the Styrian pumpkin seed oils in this study. The total phospholipid concentration in Styrian pumpkin seed oil showed considerable variation (0.5–1.04 wt-%) [24]. It is noteworthy that the concentrations of sodium, calcium, magnesium, potassium and phosphorus are higher in the Styrian pumpkin seed oil than can be expected from the analysis of the seeds, because during the production process salt is added to improve the yield of the oil [32]. The physiologically relevant trace element selenium was found in hull-less Styrian oil pumpkin seeds grown in Northern Slovenia at concentrations between 23 and 37 ng/g dry seeds. In the pressing residue the selenium content was enriched, while in the corresponding oil its concentration was below the detection limit (1 ng/g oil) in this study [33]. In another study, the same pumpkin plants were enriched with selenium during growth. It was found that selenium was predominantly incorporated as selenomethionin (81% [34]) providing a reasonable explanation of why this protein-bound mineral is effectively retained in the pressing residue. The iodine content of Styrian oil pumpkin seeds was found in the range between 5 and 13 ng/g. Iodine was poorly retained in the pressing residue (7–32 ng/g dry seeds) and was found in the corresponding pumpkin seed oils at concentrations between 2 and 3 ng/g oil [33]. Thus, Styrian pumpkin seed oil is a moderate source of iodine and could help increasing the iodine intake in iodine-deficient regions.

3.4 Phytosterols

Styrian oil pumpkin seeds contain about 1.5–1.9 mg/g dry seeds of phytosterols which become enriched in the corresponding Styrian pumpkin seed oils (3.5–4.0 mg/g oil) [27]. In contrast to most other edible seed oils, the main phytosterols in Styrian pumpkin seed oils are Δ7-sterols rather than Δ5-sterols (Fig. 2). In Styrian pumpkin seed oil, higher levels of the Δ7-sterols α-spinasterol (Δ7,22-stigmastadien-3β-ol), Δ7,22,25-stigmastatrien-3β-ol, Δ7,25-stigmastadien-3β-ol, Δ7-stigmasten-3β-ol and Δ7-avenasterol (Δ7,22-stigmastadien-3β-ol) as compared to the major Δ5-sterol β-sitosterol (24a-ethylcholeste-5-en-3β-ol) (Tab. 2) are found [35]. Analyses of 19 genuine Styrian pumpkin seed oil samples obtained through pressing led

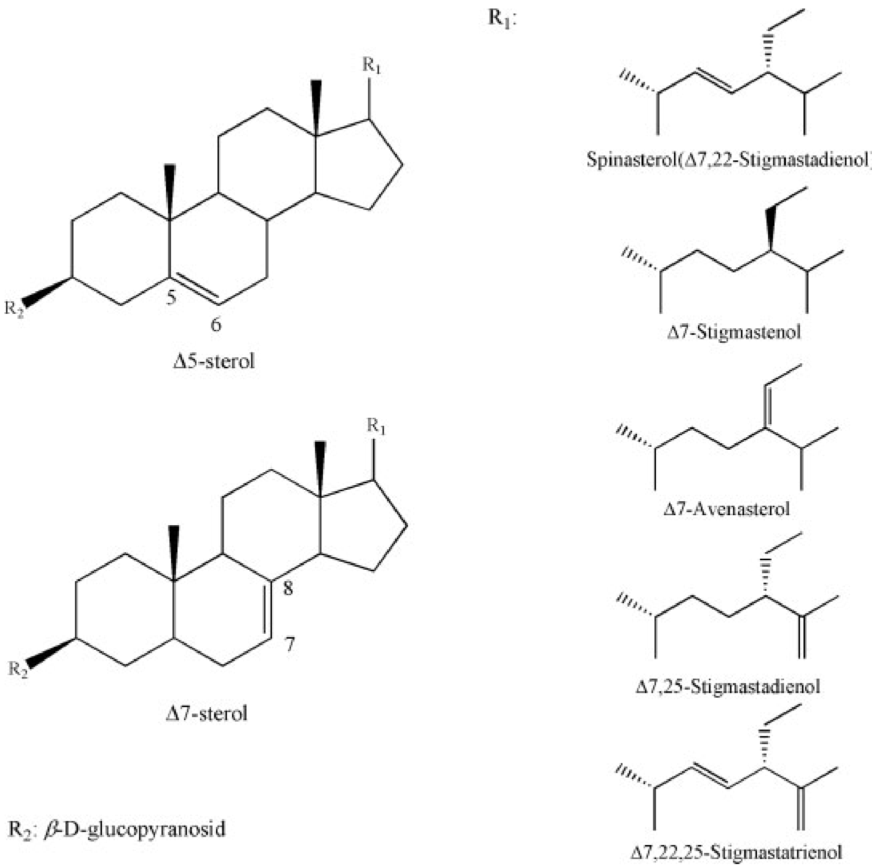

Fig. 2. Phytosterols found in Styrian oil pumpkin seeds and its products. The phytosterol composition of Styrian pumpkin seed oil is unique and consists mainly of Δ7-phytosterols. A comparison of the backbones of Δ7- and Δ5-phytosterols is shown as well as the side chains of the predominant Δ7-phytosterols found in Styrian pumpkin seeds.

Tab. 2. Phytosterol content of a typical Styrian pumpkin seed oil (modified from [35]). The amount of Δ7-phytosterols is compared to the amount of the most prominent Δ5-phytosterol, which is β-sitosterol.

Phytosterol	[μg/mL]
Spinasterol (Δ7,22-stigmastadienol)	447.8
Δ7,22,25-Stigmastatrienol	427.5
Δ7,25-Stigmastadienol + Δ7-stigmastenol	395.4
Δ7-Avenasterol	230.1
β-Sitosterol (Δ5)	84.6

to similar results. Although the absolute phytosterol content varied to some extent, the ratios of the different phytosterols were almost the same. A similar result was found for 147 Styrian oil pumpkin seed extracts [35]. However, these authors did not analyze the carbohydrate-bound phytosterols that have also been found in the seeds of *Cucurbita pepo* spp. Δ7-Sterols exist also bound to β-D-glucopyranosides from which they can be released by acid hydrolysis [36].

It is noteworthy that an improved liquid chromatographic method allows separating nearly all Δ7- and Δ5-sterols of pumpkin seed oils [37]. This method proved to be very useful for the detection of minor adulterations (<5%) of this high-quality product with cheaper edible oils, *e.g.* sunflower oil, rapeseed oil or soybean oil. The phytosterol-based method is a major improvement for detection as compared to former techniques relying on fatty acid patterns. An isotope ratio-mass spectrometric method was also developed to detect adulterations based on the geographical origin of pumpkin seeds and the corresponding oils (characteristic ^{13}C distribution) [38]. However, the phytosterol-based approach is still the state-of-the-art technique to date.

3.5 Pigments in pumpkin seed oil

High-quality Styrian pumpkin seed oil is obtained from its hull-less green seeds (Fig. 1) and has a dark green color and concomitantly shows orange to red fluorescence (Fig. 3). The green color and the reddish fluorescence of the oil are due to various tetrapyrrol-type compounds like protochlorophyll (a and b) and protopheophytin (a and b), the latter being a protochlorophyll lacking the magnesium ion. These compounds are located in the inner seed-coat tissues of all *Cucurbita pepo* seeds, but only get into the

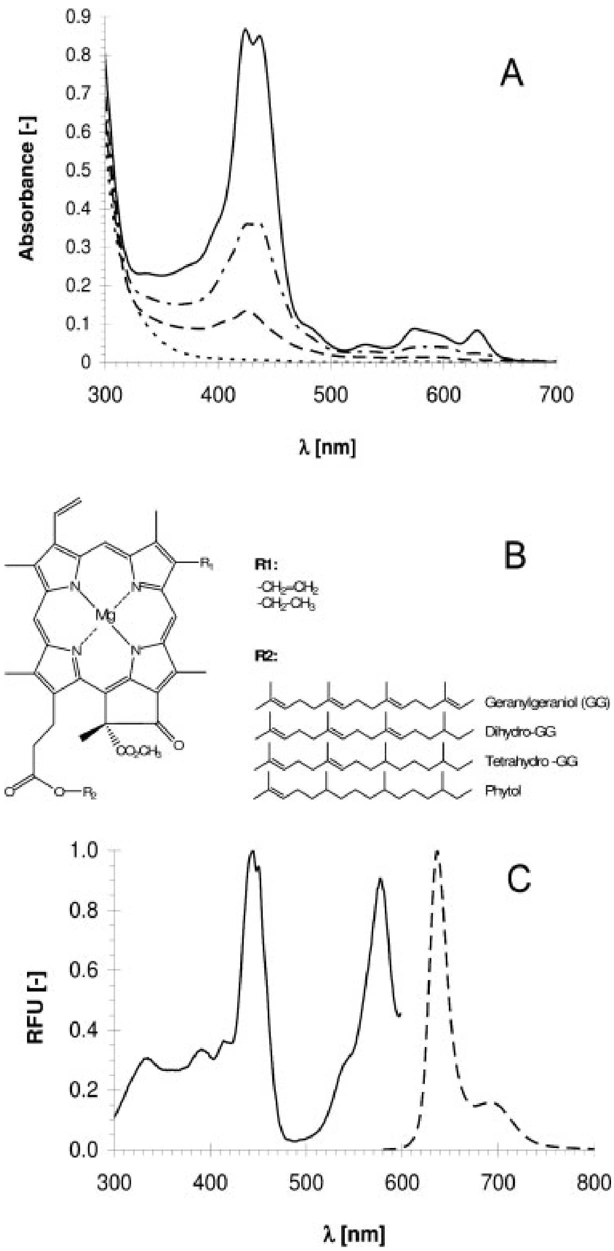

Fig. 3. Spectral properties of Styrian pumpkin seed oil. (A) UV/VIS absorption spectrum of Styrian pumpkin seed oil (solid). Exposure of Styrian pumpkin seed oil to sunlight destroys the characteristic color of the oil. UV/VIS spectra of the same Styrian pumpkin seed oil after different exposure to sunlight: 3 days (dash-dotted), 5 days (dashed), and 14 days (dotted). All spectra were recorded in methanol. (B) Chemical structure of protochlorophyll a. The structures detected in Styrian pumpkin seed oil have either an ethyl or a vinyl group (R_1) and differ in their isoprenoid side chains (R_2). (C) Emission and absorption spectra. Styrian pumpkin seed oil shows red fluorescence with an emission maximum of 635 nm; Normalized excitation (solid) and emission (dotted) spectra of a methanolic solution of Styrian pumpkin seed oil are shown.

oil during the production of pumpkin seed oils from mutants with stunted outer hulls (Styrian oil pumpkin seeds). Analysis of protochlorophyll a esters from inner seed-coat tissues of an American *Cucurbita pepo* variant (not hull-less) *via* gas chromatography-mass spectrometry suggested that the tetrapyrrol backbone is esterified to virtually all C_{20} isoprenoid alcohols between and including geranylgeraniol and phytol (Fig. 3B, [39]). The predominant alcohol is phytol making up 90% of all alcohols esterified to protochlorophyll a. These authors also report on the occurrence of 4-vinyl-(4-desethyl)-protochorophyll a, which is esterified to the same isoprenoid alcohols as protochlorophyll a as well as farnesol and phytanol. The predominant alcohol esterified to 4-vinyl-(4-desethyl)-protochorophyll a is again phytol, which in this case represents only 50% of the total alcohols. Only one study was performed to separate the pigments of the hull-less seed variant *Cucurbita pepo* subsp. *pepo* var. Styriaca using thin-layer chromatography. It is not surprising that much less different compounds have been identified by this method [40]. In addition to the green color, there are also other, yellowish, pigments in pumpkin seed oil. They are carotenoids, with lutein being the predominant component. These compounds have already been discussed above (see Section 3.2).

When pumpkin seed oil deteriorates, *e.g.* under the influence of sunlight and oxidation, the intense green pigments are destroyed (Fig. 3A). This disappearance of the green pigments is used as a criterion for rapid and simple optical quality control of pumpkin seed oils. Recently, extensive spectroscopic characterization of various sets of Styrian pumpkin seed oils (high- and low-quality oils) led to the suggestion to use the UV/VIS spectroscopic fingerprints as a chemometrical means of quality control [41].

3.6 Compounds responsible for the aroma of pumpkin seed oil

Styrian pumpkin seed oil is well appreciated as edible oil because of its taste and flavor. It has a very characteristic nut-like roast aroma that is tightly connected to its production process (see Section 2), which involves a roasting step of the ground pumpkin seeds. The relatively high temperatures and a certain roasting time (>45 min) are necessary for the development of the characteristic aroma of Styrian pumpkin seed oil [42]. The odorants in Styrian pumpkin seed oils were analyzed using aroma extract dilution analysis and gas chromatography-olfactometry of the headspace of oil samples. Both techniques led to the identification of at least 27 and 24 odorants, respectively [43]. The compounds responsible for the flavor of Styrian pumpkin seed oil are various pyrazine derivatives. The highest flavor dilution factors for compounds connected with the impression of a "roasty" aroma were reported for 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine. The aroma of Styrian pumpkin seed oil is also described as "fatty", for which mainly the oxidation products of (poly)-unsaturated fatty acids are responsible. The predominant compounds in this class are (*E*,*E*)-2,4-decadienal and (*E*,*E*)-2,4-nonadienal [43]. With ongoing oxidative deterioration of Styrian pumpkin seed oil, aldehydic fatty oxidation products become the predominant odorants, leading to a rancid and acidic impression. In addition, several other compounds have been identified in Styrian pumpkin seed oil that are connected with impressions like "green" (*e.g.* hexanal) or "malty" (*e.g.* 2-methylpropanal or 3-methylbutanal), which are important components of its unique flavor.

3.7 Phenolic compounds

Various plants synthesize a plethora of different phenolic compounds, and the phenolic pattern can also be characteristic for the species. Different plant-derived classes of phenolic compounds have been isolated and characterized. They show manifold interesting properties including antioxidant, estrogenic or anti-estrogenic effects. In addition, amongst them are potential anticancer and cardioprotective drugs, and some that possess antimicrobial and antiviral activities. Pumpkin plants and their seeds as well as their products have long been overlooked in this respect, and data about their phenolic composition are scarce. Searching for phytoestrogens, Adlercreutz and Mazur found trace amounts of the isoflavones genistein (15.3 μg/kg dry seeds) and daidzain (5.6 μg/kg dry seeds) in pumpkin seeds [44]. Furthermore, these authors have detected the lignan secoisolariciresinol in pumpkin seeds at a concentration of 214 mg/kg dry seeds. However, neither details about the precise cultivar nor the exact analytical procedure were given. A later study using HPLC separation and coulometric detection reports on much lower amounts of secoisolariciresinol (3.8 mg/kg dry seeds) in Styrian oil pumpkin seeds, even after chemical and enzymatic hydrolysis [27]. In addition, it was found that after 20 min of the roasting process, which is mandatory for the production of Styrian pumpkin seed oil, secoisolariciresinol was no longer detectable. Secoisolariciresinol is of interest because in its glycosylated form it shows antitumor activity in the early promotion stage of tumorigenesis [45]. Recently, another lignan, lariciresinol, has been detected in the seeds of *Cucurbita pepo* spp. using a gas chromatography-mass spectrometry approach [46].

In our laboratory, we found that polar Styrian oil pumpkin seed extracts and also polar extracts of Styrian pumpkin seed oils possess considerable antioxidative capacities [22, 47], which were higher when the solvents used for extraction were more polar. Consequently, a considerable fraction (up to 40%) of the apparent antioxidant capacities of Styrian pumpkin seed oil was found to be due to polar components in the oil. This "polar antioxidant capacity" correlated very well with the total content of phenolics in Styrian pumpkin seed oil ($r = 0.91$, $p < 0.001$). The polar components are enriched in Styrian pumpkin seed oil mainly because the oil production is based on pressing of an aqueous seed suspension without further processing of the obtained oil (see Section 2). Perfection of separation during the production process or refining of the oil leads to a decrease in the antioxidant capacity, as it was observed for refined olive oil (standard deviation between different refined olive oils was 10%) as compared to fresh virgin olive oil from the first pressing [22]. Although Styrian pumpkin seed oils showed considerable differences in their antioxidant capacities, it is noteworthy that all Styrian pumpkin seed oils were at least equal, if not superior, in their antioxidant capacities to all other edible oils in this study (Fig. 4, [22]). The differences between the pumpkin seed oils were most likely due to the production process, which is mainly handcraft. Thus, we suggest the antioxidant capacity of Styrian pumpkin seed oil as a novel and important criterion for quality control with respect to the stability and shelf life of this oil. Very recently, for the first time, eight different acylated phenolic glycosides were found in the seeds of a Japanese variety of *Cucurbita pepo* and named cucurbitosides F to M [48]. These compounds were isolated *via* various hydrolytic approaches and identified using different spectroscopic methods. However, the question as to whether these or similar compounds are also present in the seeds of the Styrian oil pumpkin is still unsolved, and further studies with this cultivar are necessary.

4 Field pests and chemical contaminants

4.1 Field pests of the Styrian oil pumpkin plant

In the early seventies, for the first time a potyvirus was identified in Northern Italy that caused yellow mosaics on zucchini plants [49]. This virus was named zucchini yellow mosaic virus (ZYMV) and has spread over the globe infecting nearly all economically important *Cucurbitaceae* spp. including the Styrian oil pumpkin. The economic impact of this virus became apparent in Styria in 1997 when more than 60% of the Styrian oil pumpkin yield was lost [50]. This also led to a dramatic decrease in Styrian pumpkin seed oil production and, as a consequence,

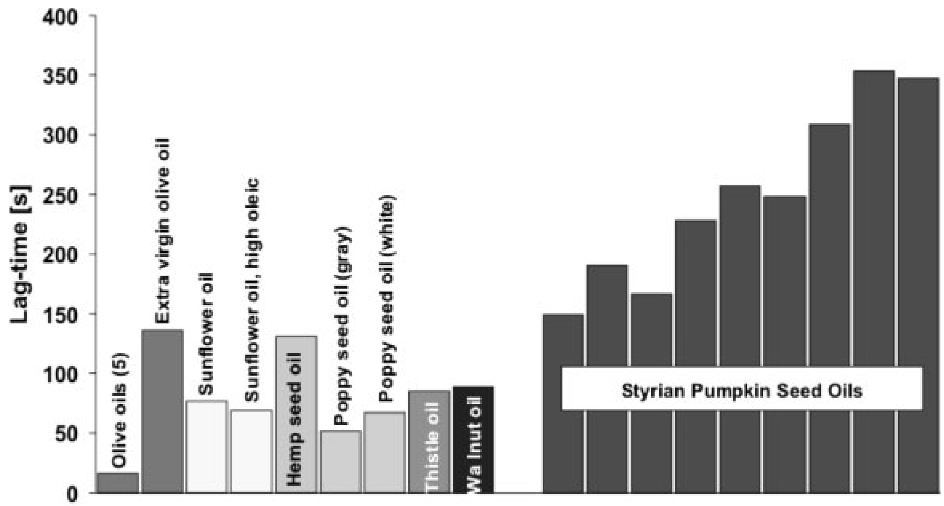

Fig. 4. Antioxidant capacities of a selection of Styrian pumpkin seed oils and other edible oils. The indicated lag times were determined as a measure of the antioxidant capacities of the oils, using an established fluorescence method [22]. Although the antioxidant capacities of the individual pumpkin seed oils differ to a great extent, all pumpkin seed oils were at least as good, if not superior, as compared to the other edible oils in this study.

caused an economic damage of approximately €14.5 million. ZYMV isolates show a high biological variability, especially in the N-terminal region of their coat protein [51]. The efficient spread of the virus within one field depends on occurrence and number of various aphid species [52]. In addition, ZYMV can be transmitted by the seeds of the cultivars, which seems to be the major cause for intercontinental spread of ZYMV. In the last years, this has been proven for hull-less pumpkin seed varieties, buttercup squash, and zucchini [53–55]. Obvious symptoms of ZYMV infection are yellowing and deformation of the leaves, reduction of leaf size, and the development of a yellow mosaic with dark green blisters. Furthermore, the fruit development is impaired and the typical marks of the fruits are lost. Recently, cytological modifications of ZYMV-infected Styrian oil pumpkin plants were studied using electron microscopy. Infected plant cells were found to contain cylindrical inclusions, proliferated endoplasmatic reticula and filamentous viral particles. In addition, while the amount of plastoglobuli and starch was increased, the number of chloroplasts and thylakoids was significantly decreased [56]. The latter is probably responsible for the leaf yellowing after ZYMV infection. Pathogen infection of plants frequently leads to increased oxidative stress and, as a consequence, to a higher production of antioxidants like ascorbate, glutathione and antioxidant enzymes as a plant response [57]. This was also reported for systemically ZYMV-infected cultivars of the Styrian oil pumpkin plant, in which the activities of antioxidant enzymes were increased [58]. Interestingly, the total glutathione content of Styrian oil pumpkin plants did not change after ZYMV infection, whereas the subcellular distribution of glutathione was tremendously altered after ZYMV infection [59]. In order to reduce future damage by ZYMV and related potyvirus strains, considerable efforts have been undertaken in Austria to select for mosaic virus resistance and tolerance. This program is based on a conventional backcross selection strategy using the ZYMV-resistant species *Cucurbita moschata* and DNA marker-based selection analysis, with the aim of generating dominant virus resistance/tolerance. In addition, selection programs for cultivars tolerating bacterial and fungal pests like mildew have been started in Austria.

Very recently, another pest has been identified endangering the fields of pumpkin cultivars in South Eastern Europe. In the early nineties, the American Chrysomelid beetle *Diabrotica virgifera virgifera* LeConte (Dvv), the so-called western corn rootworm, spread from Belgrade, the site of its introduction into South Eastern Europe, in nearly all directions. First appearances of Dvv in Slovenia nearby the Austrian borders were reported in 2004. Adult Dvv females deposit eggs in the soil nearby the base of plants. In spring, the newly hatched larvae begin feeding from the plant roots, thereby heavily damaging the plants [60]. Rootworm feeding is associated with decreased water and nutrient uptake, and due to weakening of the root system, the plants become prone to mechanical damage by winds or heavy rain. Although Dvv is especially dangerous for corn, Dvv has been found to infest also various *Cucurbita pepo* cultivars [61]. Currently, there is no data available about the impact of Dvv on the Styrian oil pumpkin and its products. However, the danger must not be underestimated since this pest causes economic losses in the $1 billion range in the US per year. Thus, monitoring presence and advance of Dvv using improved traps in South Eastern Europe will be a first step in controlling this pest [62].

4.2 Chemical contaminants

Environmental pollutants can be divided into two main groups, heavy metal contaminants and persistent organic pollutants, both being predominantly of anthropogenic origin. Although some heavy metals are essential trace elements, their concentrations in plant-derived materials (*e.g.* seeds) can by far exceed the upper limits for dietary food components. There is considerable regional variation of heavy metal contamination of *Cucurbita pepo* cultivars, depending on the extent of pollution and, to a minor degree, the natural occurrence of heavy metals in the soil. For the determination of As(III) and As(V) in oilseeds, a chronopotentiometric stripping method has recently been developed [63]. In addition, several toxic heavy metal contaminants like As, Cd, Cu, Hg, Mn, Pb, Se and Zn have been determined reliably using inductive coupled plasma (ICP) mass spectrometry following microwave-assisted pressure decomposition of Styrian oil pumpkin seeds and Styrian pumpkin seed oil samples [64].

Furthermore, there are many ubiquitous toxic organic compounds that can be found in soils and sediments at various concentrations due to regionally differing immission. Persistent organic pollutants are mostly hydrophobic and thus potentially accumulate in fatty body tissues. However, uptake of these pollutants as well as accumulation, *e.g.* in fat-rich seeds, can be very different between various plant species. A strong positive correlation between the concentration of hexachlorbenzene (HCB), a formerly used fungicide, in Styrian pumpkin seed oil and the corresponding agricultural soil samples was reported [65]. Interestingly, the same study found that the concentration of lindane (γ-hexachlorcyclohexane), a carcinogenous insecticide, was independent of its concentration in the soil. Although the contamination levels of the agricultural soils with lindane were higher as compared to the HCB levels, the amounts of lindane found in Styrian pumpkin seeds were significantly lower. This effect could be explained by the much higher uptake rates of HCB compared with lindane [65]. It is noteworthy that, as long as Styrian pumpkin plants were grown on soils containing less than 4 ng HCB/g soil, the contamination level of HCB in Styrian pumpkin seed oils was lower than the Austrian intervention limit (250 ng HCB/g oil). At the same time, the lindane concentrations found in Styrian pumpkin seed oils were in the range of 20–40 ng/g oil.

In addition to the above-mentioned compounds, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), a breakdown product of the pesticide DDT, has been found in approximately tenfold higher concentration in various *Cucurbita pepo* subsp. *pepo* cultivars than in other species [66–68]. The ability of these plants to extract this carcinogenic compound from soil was also studied in a project aiming to reduce contamination in strongly affected soils [69]. A higher plant density and higher soil moisture heavily increased the accumulation of the contaminant and thus phytoremediation of DDE [70].

Furthermore, polycyclic aromatic heterocycles (PAH) are ubiquitous environmental pollutants that can be strongly enriched in edible oils. PAH can be incorporated into Styrian pumpkin seed oils either *via* absorption from polluted soil or *via* production of the oil, which involves a roasting step. Sixteen different PAH, including highly cytotoxic compounds like benzo[a]pyrene or chrysene, were considered by the German Society for Fat Science to be most important for analysis. Recently, a novel gas chromatography-mass spectrometry method using temperature-controlled microwave-assisted saponification was developed for the determination of PAH in Styrian pumpkin seed oil [71]. The application of this method revealed significant differences in the PAH contents of various Styrian pumpkin seed oils (ng/g range). It was found that these differences were mainly due to the roasting step of Styrian pumpkin seed oil production. As a consequence, the authors recommend monitoring the PAH content of Styrian pumpkin seed oils produced for the consumer market [71], since levels in the very low µg/kg range (various intervention limits within the EC) are generally considered to be precarious to human nutrition.

5 Pumpkin seed products in health and disease

5.1 Cholesterol-lowering effects

Many plant-derived homologs of cholesterol (phytosterols and phytostanols) have been reported to have a cholesterol-lowering effect. Several studies have investigated the cholesterol-lowering mechanisms of phytosterols, which were reviewed only recently [72–74]. Dietary uptake of phytosterols appears to be as important in cholesterol lowering as reduction in consumption of saturated fats. Since phytosterols are in the seeds of the Styrian oil pumpkin as well as in the corresponding pumpkin seed oils (see Section 3.4), it can be speculated that these components together with the high content of linoleic acid can exert beneficial health effects in lipid-associated disorders like atherosclerosis.

5.2 Benign prostate hyperplasia

Urinary incontinency is an age-related disorder of various origins that may affect both human genders. For example, male patients with prostate hyperplasia (BHP) frequently suffer from overflow incontinence, while women are more likely to develop symptoms of stress incontinence, especially in post-menopausal ages. These two forms of incontinence generally are in both genders due to changes in the hormonal equilibrium between androgens and estrogens [75–77]. Polar extracts of *Cucurbita pepo* subsp. *pepo* var. Styriaca seeds might exert physiologically relevant effects on this level. It is known that these extracts inhibit aromatase, the key enzyme of estrogen biosynthesis, which converts testosterone into 17β-estradiol [78, 79]. The chemical nature of the polar pumpkin seed components is currently unknown. Adlercreutz *et al*. suggested that phenylglycosides were responsible for the phytoestrogenic effects [44]. However, more research is needed to elucidate the precise chemical structures of the bioactive seed components. In addition, controlled clinical studies are required to assess the putative beneficial effects of these extracts before they can be recommended for the treatment of stress incontinence.

Styrian oil pumpkin seed extracts are considered important phytotherapeutical agents for the treatment of BHP and have been used in the treatment of symptomatic micturition disorders. The beneficial effects of these extracts on BHP may likely be ascribed to its inhibitory effect on 5α-reductase which converts testosterone to the more physiologically active dihydrotestosterone [80, 81]. However, it was a matter of controversy for a long time whether the phytotherapeutic approach in the therapy of BHP was superior to placebo treatment. This was mainly due to a lack of systematic placebo-controlled clinical double-blind studies, which were performed only during the last 15 years. Nevertheless, clinical studies with pumpkin seeds and/or pumpkin seed extracts are still scarce.

The seeds of the Styrian oil pumpkin contain considerable amounts of Δ7-phytosterols, either in free form or bound to sugar molecules (see Section 3.4). A lipid-steroidal extract of hull-less seeds was also found to have an inhibitory effect on 5α-reductase in cultured human prostate fibroblasts [80, 81]. Furthermore, the same authors reported on an anti-inflammatory effect of this extract in carrageenin- or dextran-induced edema models. In a human trial, it was found that intake of a whole extract of Styrian oil pumpkin seeds correlated with reduced BHP-associated symptoms [82]. Schilcher *et al*. reported that orally administered Δ7-phytosterol-rich *Cucurbita pepo* subsp. *pepo* seeds (3–4 days before prostatectomy) decreased the amount of dihydrotestosterone in prostate tissue of patients [83]. In addition, the same authors detected Δ7-phytosterols in the prostate tissue of these patients. In castrated rats, a lipid-steroidal extract antagonized testosterone-induced prostate development in a dose-dependent manner [84].

Data from placebo-controlled, double-blind studies with Styrian oil pumpkin seed extracts became available only in recent years. The first randomized double-blind study was performed with a formulation called "Curbicin", which is a preparation obtained from both *Cucurbita pepo* seeds and *Sabal serrulata* (dwarf palm) fruit containing 80 mg of each of the standardized extracts. In this study including 53 patients, a significant improvement of standardized objective parameters like urinary flow, micturition time, and residual urine was reported [85]. However, the preparation did not solely contain a pumpkin seed extract but also a dwarf palm extract, which shows inhibitory effects on human 5α-reductase on its own [80, 81]. A much larger multicentric, but not placebo-controlled, clinical trial involving 2245 patients with BHP in stages I and II was performed only recently. In this study, the patients received a commercially available pumpkin seed extract (detailed cultivar and extraction method were not disclosed) over a period of 3 months. Urinary symptoms were scored by the International Prostate Symptom Score, and the study reported on a reduction of 41.4% as well as hardly any undesired side effects ($<$4%) [86]. Very recently, a placebo-controlled double-blind study was performed with Wistar rats treated with testosterone and prazosim in order to induce prostate growth. This model has been widely used and validated as an animal model of urinary tract obstruction due to benign prostate enlargement [87, 88]. It was found that phytosterol-rich pumpkin seed extracts and an artificial phytosterol mixture proved to have a highly significant anti-proliferative effect on the prostate in this model [89]. Unfortunately, no details about the precise variety of the pumpkin seeds and no analysis of the phytosterol content, neither of the pumpkin seed extract nor of the artificial phytosterol mixture, was undertaken.

Recent studies found that Δ5-phytosterols and phytostanols in free form are not efficiently absorbed in the human gastrointestinal tract [90]. These results do not strengthen the hypothesis that free Δ7-phytosterols are effective agents for the treatment for BHP. However, it is very important to emphasize that Δ7-phytosterols in pumpkin seeds are also esterified to various sugar molecules [36], which could improve their bioavailability. As a consequence, (polar) Styrian oil pumpkin seed extracts can be expected to have more pronounced effects than lipid extracts of Styrian pumpkin seed oil. However, it can be anticipated that the polar seed components including Δ7-phytosterol-glycosides are very likely to be retained in the pressing residue and thus are not found in the corresponding oil. Although the results of the available studies suggest the use of Styrian oil pumpkin seed extracts for the treatment of BHP in the stages I an II, it is important to emphasize that the active compound(s) and mechanisms responsible for these effects still remain to be identified.

6 Outlook

New World *Cucurbita* spp. are known in Europe since the early 16th century [91] and acquired the status of a "healthy oil" in the consumer's perception. The seeds of the Styrian oil pumpkin and its oil are rich in antioxidants (lipophilic tocopherols and polar compounds). The German Society for Nutrition recommends a daily intake of 48 mg of γ-tocopherol and 8 mg of α-tocopherol [92]. These recommended daily amounts (RDA) could be reached by a daily consumption of 77 g and 88 g of pumpkin seeds with the highest amount of γ-tocopherol and α-tocopherol, respectively [29]. Due to the enrichment of tocopherols in Styrian pumpkin seed oil, the daily intake of the oil can be lowered by a factor of two to three in order to reach the RDA. There are also polar antioxidants in the seeds and, due to the production process, these compounds are in the corresponding dark green oils as well [22]. However, more research is necessary to reveal the precise molecular structures of these polar compounds. Thus, the potential beneficial health effects and the nutritional values of Styrian oil pumpkin seeds and its products need to be proven yet.

The Styrian oil pumpkin is a plant/crop that possesses an enormous potential for future research. Improvement of Styrian oil pumpkin breeding lines is certainly one of the main objectives in this context. It will no longer be based solely on classic farming methods but will take advantage of the modern techniques of molecular biology. Such modern plant engineering could lead to improved cultivars with even higher contents of lipophilic and, more importantly, hydrophilic antioxidants. Furthermore, once molecular candidates useful for BHP treatment are elucidated, breeding lines could be improved with respect to these components. Finally, heterologous expression of enzymes generating α-linolenic acid of the *n*-3 family of polyunsaturated fatty acids would lead to a modified crop of higher nutritional quality.

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