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Review Article

Production technology and characteristics of Styrian pumpkin seed oil

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Cucurbita pepo subsp. *pepo* var. *Styriaca*, the so-called Styrian oil pumpkin, is a phylogenetically young member of the *Cucurbita* spp. A single mutation occurred only in the 19th century and led to dark green seeds with stunted outer hulls. This mutation facilitated the production of Styrian pumpkin seed oil that became a regional specialty oil in the south-eastern part of Europe during the last few decades. We describe in this article the production and economic value of this edible specialty oil as well as the most important parameters relevant for its quality. Furthermore, we report on its molecular composition including fatty acids, vitamins, phytosterols, minerals, polyphenols, and those compounds that are responsible for its color, taste and flavor. Finally, information is provided on potential contaminants of Styrian pumpkin seed oil as well as its putative beneficial health effects.

Keywords: Adulteration / Antioxidants / Cholesterol lowering / *Cucurbita pepo* spp. / Phytosterols

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1 Introduction

Styrian pumpkin seed oil is made from seeds of the special pumpkin variety that first emerged in Austria's south eastern province of Styria. This class of plants 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 [1]. 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 of Styria is dark green and shows dichroism. 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. It is the result of a mutation in a single recessive gene [2] and led to a very thin outer hull (naked or hull-less seeds; Fig. 1A), which highly facilitates the production of this regional specialty oil and also leads to its dark green color. Nowadays, numerous varieties/hybrids have been generated from the original Styrian oil pumpkin in order to improve yield and seed content, or to introduce resistance against field pests and/or viruses. Inferred from the production process (see Section 2.1), Styrian pumpkin seed oil is classified as a natural edible oil; however, it stands between so-called "virgin oils" and refined oils since its production process involves a roasting step but no refining of the product from the first pressing.

Recently, we published a comprehensive review describing and comparing several aspects of Styrian oil pumpkin seeds with the corresponding Styrian pumpkin seed oils [3]. This article here describes the production and economical importance of Styrian pumpkin seed oil for the region, its detailed composition, parameters of its quality, and finally its putative health effects. Therefore, we searched for relevant scientific literature using the Chemical Abstracts and PubMed databases and considered all publications dealing with Styrian pumpkin seed oil. All other pumpkin seed oils made from any other pumpkin family (*e.g.* *C. maxima*, *Telfairia occidentalis*) were excluded since they yield totally different products.

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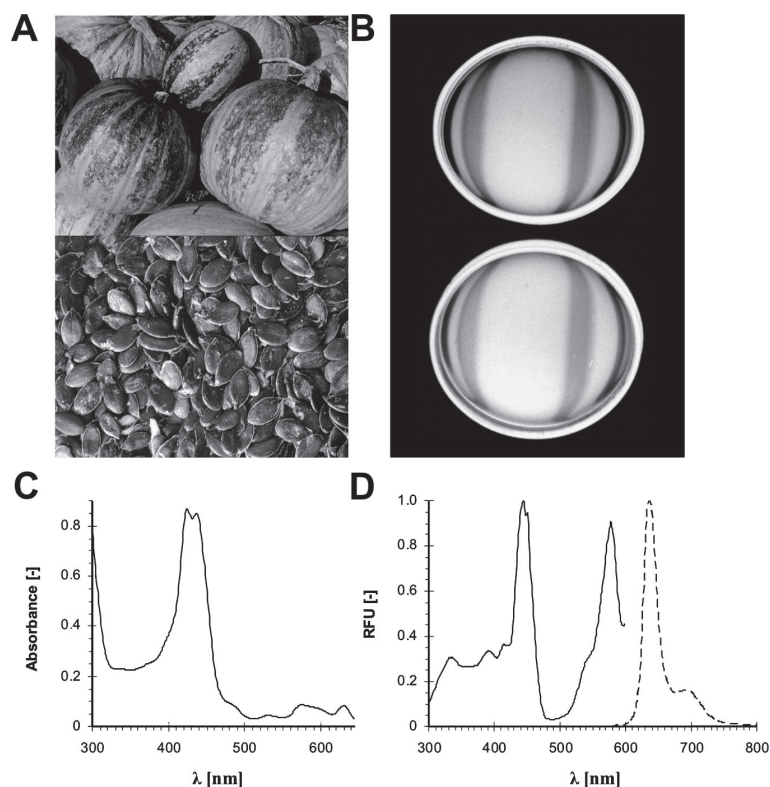


Figure 1. Spectral properties of Styrian pumpkin seed oil. (A) Styrian oil pumpkins and their stunted hull-less green seeds from which the oil is obtained. (B) Transillumination image of high-quality Styrian pumpkin seed oil before (top) and after (bottom) sun light-induced oxidative deterioration (5 days of sun exposure). (C) UV/VIS absorption spectrum of Styrian pumpkin seed oil (recorded in methanol). (D) Styrian pumpkin seed oil shows red fluorescence with an emission maximum of 635 nm: normalized excitation (solid line) and emission spectra (dotted line) in methanol.

2 Production technology and economical importance of Styrian pumpkin seed oil

2.1 Production technology

The seeds of the Styrian oil pumpkin are harvested in mid autumn, immediately dried to a residual water content of 5–7 wt-%, and ground. 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 [4]. The remaining flesh of the pumpkins is of lower quality as compared to squash (*e.g.* *C. moschata*) and marrow (*e.g.* *C. 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. The 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. This roasting process is responsible for the generation of the typical aroma of the end product. The subsequent pressing process is performed under isothermal conditions at pressures between 300 and 600 bar, leading to a dark green oil as the end product. Since the pressing process is not exhaustive, the remaining pressing cakes still contain considerable amounts of the valuable oil. However, the oil from a second pressing step can only be used for lower-quality products, *e.g.* as admixture in salad oils. After

this second pressing, the pressing cakes are merely used as swill, although they still contain a reasonable amount of carotenoids [5].

Styrian pumpkin seed oil production is still predominantly handcraft and is performed mostly by small regional oil mills. The individual small farmers bring in their dried pumpkin seeds and get back the oil as a service of the mill, the latter being either paid or pocketing a small percentage of the product. A few oil mills produce the oil after buying the pumpkin seeds from small farms in the region. Some bigger farms have their own oil production. Automated and continuous production exists only in the very early experimental state and is currently not of any quantitative importance. However, the latter approach is also undesired, since one hallmark of Styrian pumpkin seed oil is its individuality and that it is handcraft. As a consequence, standardization over the dozens of oil mills and the hundreds of different products that are due to individual pumpkin seed batches is very difficult to achieve.

2.2 Quality control of Styrian pumpkin seed oil

Styrian pumpkin seed oil is a regional specialty product that has to undergo several steps of quality control before it is classified as a product protected in its geographical origin by European law (“Protected Geographical Indication”; [6]).

Criteria for awarding the brand Styrian pumpkin seed oil are that the oil is exclusively pressed from Styrian oil pumpkins grown within a protected area in the province of Styria by regional oil mills and, most importantly, that the oil is only from the first pressing and thus of the highest quality. The analytical characterization of the oil is performed by a certified expert laboratory in cooperation with a group of professional food tasters. Currently, the quality control involves measurement of classic oil indices such as acidity and peroxide number as well as markers of adulteration with other edible oils. Randomly selected oil samples are analyzed for chemical contaminants. In a double blind study, an expert sensory panel evaluates the taste and aroma of the best Styrian pumpkin seed oils. Since Styrian pumpkin seed oil is light sensitive, it has to be bottled accordingly, *e.g.* in dark bottles with nozzles. If an oil sample fails to pass only one of the quality criteria indicated above, it does not fulfill the requirements for the label “Protected Geographical Indication”.

2.3 Economical importance

Styrian pumpkin seed oil is also of considerable economical importance for the province of Styria. The Styrian oil pumpkin is the third most important field fruit in Styria with 12,500 ha of cultivable land yielding 11,100 tons in 2006 [7]. 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 Styrian pumpkin seed oil, an average of 2.5 kg of pumpkin seeds are required, which corresponds to an amount of 30–40 Styrian 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 2006, are used for the production of pumpkin seed oil representing a current market value of more than 30 million Euros. In the year of 2006, approximately 20% of the oil was exported, with Germany being the main importing country followed by other European Union members and the USA.

3 Composition of Styrian pumpkin seed oil

Most of the data reported in the literature concerning the composition of pumpkin seeds or pumpkin seed oils are lacking detailed botanical descriptions of the species/variety that has been analyzed. In this chapter, we summarize the most important data from the literature concerning Styrian pumpkin seed oil pressed from the naked seed variety *Cucurbita pepo* subsp. *pepo* var. *Styriaca* and we describe the distinct chemical compounds found in Styrian pumpkin seed oil. Triacylglycerols represent the largest fraction of the oil, which also contains several important secondary plant components that have been analyzed only recently.

3.1 Fatty acid composition

The fatty acid pattern of Styrian pumpkin seed oil appears to be very similar to that of Styrian oil pumpkin seeds. Analysis of several Styrian pumpkin seed oils revealed that the content of polyunsaturated fatty acids ($45.6 \pm 5\%$ rel) is considerably higher than the content of monounsaturated fatty acids ($35.9 \pm 10\%$ rel) or saturated fatty acids ($18.5 \pm 20\%$ rel) [8], with linoleic acid, oleic acid, palmitic acid, and stearic acid (together $\geq 98\%$) being the predominant fatty acids. The relative amount of oleic acid is always negatively correlated with the relative amount of linoleic acid [4, 9], which is due to the substrate-product relationship of these two fatty acids [10]. The high content of linoleic acid is an important nutritional aspect of Styrian pumpkin seed oil since linoleic acid is an essential *n*-6 fatty acid. In addition to these fatty acids, traces ($<0.5\%$) of other fatty acids (*e.g.* 16:1, 18:3, 20:4, 22:0, 22:6, 24:0, *trans* fatty acids) were found in the seeds [4, 11], yet their identity 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.

3.2 Vitamins

Tocols are the major lipophilic antioxidants found in Styrian oil pumpkin seeds and in the corresponding oils in which they become enriched during oil production. Tocopherols and tocotrienols, especially their γ - and α -isomers, are the most abundant tocol derivatives in Styrian pumpkin seed oil. The tocopherol content is about 5–8 times higher as compared to the tocotrienols [11, 12]. Because of the physiological importance of tocopherols (antioxidants, signaling molecules [13]), a high content of vitamin E in the seeds was defined as an objective for the generation of new breeding lines [14]. The ratios of γ -tocopherol to α -tocopherol in over 100 new breeding lines varied widely and were found to be in the range between 5 and 10, indicating that the biosynthesis of these two tocopherols is not strictly coupled to one another. Various Styrian pumpkin seed oils from different production lots contained widely differing amounts of tocopherols, with γ -tocopherol being always the predominant isomer. High-quality pumpkin seed oils contained γ -tocol isomer concentrations up to 800 mg/kg [8] and α -tocopherol concentrations in the range between 18 and 282 mg/kg. β - and δ -isomers were occasionally found in the seeds, but they could not be detected in the corresponding oils [8, 15]. The tocopherol content is sufficiently high to contribute significantly to the recommended daily intake of the German Society for Nutrition, *i.e.* 48 mg for γ -tocopherol and 8 mg for α -tocopherol [16]. These recommended daily amounts can be reached by a daily consumption of approximately 40 g of high-quality pumpkin seed oil.

In addition, vitamin A and various carotenoids have been detected in Styrian oil pumpkin seed products. The most

which involves a roasting step. The rather high temperatures and a long roasting time (>45 min) are required for the development of the characteristic aroma [26]. The odorants in the oil were analyzed using aroma extract dilution analysis and gas chromatography-olfactometry of the headspace of oil samples. The two techniques led to the identification of at least 27 and 24 main odorants, respectively [27]. 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 is also described as “fatty”, for which mainly oxidation products of (poly)unsaturated fatty acids such as (*E,E*)-2,4-decadienal and (*E,E*)-2,4-nonadienal are responsible [27]. In addition, several other compounds have been identified in Styrian pumpkin seed oil that are associated with impressions like “green” (e.g. hexanal) or “malty” (e.g. 2-methylpropanal or 3-methylbutanal), which are important for its overall flavor. Oxidative deterioration of fatty acids leads to the formation of aldehydic oxidation products which are the predominant odorants associated with a rancid and acidic impression.

3.7 Phenolic compounds

We found that polar extracts of Styrian oil pumpkin seeds and the corresponding oils possessed considerable antioxidative capacities [8, 28], which were higher when the solvents used for extraction were more polar. As a consequence, a considerable fraction (up to 40%) of the apparent total antioxidant capacity of Styrian pumpkin seed oil was found to be due to its polar components. This “polar antioxidant capacity” correlated very well with the total content of phenolics in Styrian pumpkin seed oil ($r = 0.91$, $p < 0.001$) [8]. These polar components are found in the oil because its production is based on pressing of an aqueous seed suspension without further processing. Improvement of phase separation during the production process or refining of the oil would lead to a decrease in antioxidant capacity, which was observed in the case of olive oil production. Refined olive oils showed significantly lower antioxidant capacities as compared to fresh virgin olive oil (Fig. 3). 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 natural oils in this study (Fig. 3, [8]). The quality differences between the pumpkin seed oils investigated in a comparative study were supposed to be mainly caused by the different production processes, which were essentially handcraft. It has to be emphasized that the detailed chemical structures of these polar antioxidants are still unknown. Eight different acylated phenolic glycosides (cucurbitosides F to M) were recently found in the seeds of a Japanese variety of *Cucurbita pepo* spp. after hydrolytic degradation followed by analysis using NMR

spectroscopic methods [29]. It is unclear whether these or similar compounds are also present in the seeds of the Styrian oil pumpkins, and further studies are required. Other phenolic compounds found in the seeds of *Cucurbita pepo* spp., like secoisolariciresinol [12, 30], lariciresinol [31], genistein, and daidzain [30], have not been detected in Styrian pumpkin seed oil. In case these compounds were in the seeds and not destroyed during the roasting steps, they are very likely to be retained in the pressing residue.

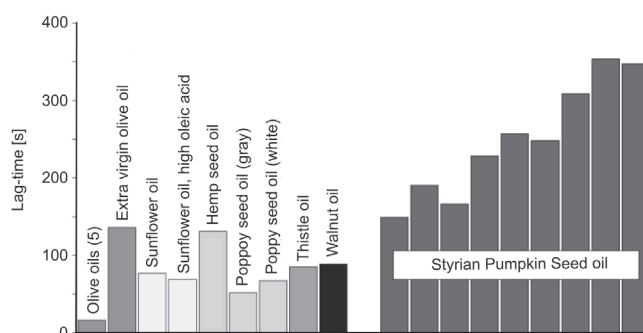


Figure 3. Comparison of the total antioxidant capacities of various Styrian pumpkin seed oils and other natural edible oils. The indicated lag times were determined as a measure of the antioxidant capacities of the oils using an established fluorescence method (modified from [8]).

4 Parameters of oil quality, adulteration and chemical contaminants

The shelf life of Styrian pumpkin seed oil is mainly determined by its light sensitivity, and the light-induced oxidation of the oil can efficiently be prevented by storing the product in the dark at low temperatures. Under optimal storage conditions, the shelf life of Styrian pumpkin seed oil can be around 12 months. Its green pigments act as efficient sensitizers of light-induced fatty acid oxidation. The high oxidation susceptibility of Styrian pumpkin seed oil is mainly due to its high content of polyunsaturated fatty acids. Although Styrian pumpkin seed oil contains considerable amounts of lipophilic and polar antioxidants, the latter are not capable of stopping a once initiated radical chain reaction within the bulky lipid phase. On the contrary, certain tocopherol isomers can be rather pro-oxidative molecules within bulky lipophilic phases, especially if they cannot transfer their unpaired electrons to less reactive molecules, which is a process that normally happens under physiological conditions. When Styrian pumpkin seed oil deteriorates under the influence of sunlight-induced oxidation, it quickly loses its dichroism due to destruction of its pigments and becomes a light yellow, rancid lipid mixture. Thus, its color is used as a criterion for a rapid and simple optical quality control of Styrian pumpkin seed oils (Fig. 1B). Recently, various Styrian pumpkin seed oils (high- and low-

quality oils) were characterized by optical spectroscopy, leading to the recommendation that UV/VIS spectroscopic fingerprints should be used for chemometrical quality control [32].

4.1 Adulteration

Adulteration of Styrian pumpkin seed oil with different cheaper edible oils, *e.g.* sunflower oil, rapeseed oil or soybean oil, is regarded as professional fraud. Therefore, analytical methods for the measurement of adulterations are continuously being improved. Styrian pumpkin seed oil does not contain erucic acid and, as a consequence, an erucic acid content of more than 0.7% of all fatty acids was regarded as a sign of adulteration with the cheaper rapeseed oil [33]. However, with the development of rapeseed breeding lines low in content of erucic acid, this method became less important. Determination of erucic acid cannot be used to detect adulteration with soybean oil which does not contain this fatty acid. In this case, α -linolenic acid is a better indicator for contamination. With the exception of sunflower oils, all other main seed oils for human consumption contain this fatty acid. A more recent method is based on characteristic ^{13}C patterns depending on the geographical origin of pumpkin seeds and the corresponding oils. These measurements can be performed using an established isotope ratio-mass spectrometric method [34]. Furthermore, an improved liquid chromatographic method was developed that allows separating nearly all Δ^7 - and Δ^5 -sterols of pumpkin seed oils [35] in one HPLC run. This method proved to be very useful for the detection of minor adulterations (<5%) and has become the state-of-the-art technique in the analysis of adulteration of Styrian pumpkin seed oil.

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 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 content of heavy metals in the soil. A chronopotentiometric stripping method has recently been developed for the determination of As(III) and As(V) in oils and oilseeds [36]. Several other toxic heavy metal contaminants such as As, Cd, Cu, Hg, Mn, Pb, Se and Zn have been determined reliably using inductive-coupled plasma-MS after microwave-assisted pressure decomposition of Styrian oil pumpkin seeds and Styrian pumpkin seed oil samples [37].

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 enriched 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 was found between the concentration of hexachlorbenzene (HCB, a formerly used fungicide) in Styrian pumpkin seed oil and the corresponding agricultural soil samples [38]. However, only as long as Styrian oil pumpkins were grown on soils containing less than 1 μg HCB/kg soil, the HCB level in the respective oils was lower than the current Austrian intervention limit (20 μg /kg oil). On the contrary, the concentration of lindane (γ -hexachlorocyclohexane, a carcinogenic insecticide) in the oils was independent of its concentration in the soil, since this compound is hardly enriched in the pumpkin seeds (the Austrian intervention limit is 10 μg /kg oil).

The concentrations of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), a breakdown product of the formerly used pesticide DDT, were approximately tenfold higher in various *Cucurbita pepo* subsp. *pepo* cultivars than in other species [39–41]. The ability of these pumpkins to extract DDE from soil makes monitoring of these compounds in Styrian pumpkin seed oil mandatory. The current Austrian intervention limits for DDT (2,4'-DDT and isomeric 4,4'-DDT) and its breakdown products (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD) is 50 μg /kg (sum of all compounds).

Polycyclic aromatic hydrocarbons (PAH) are another class of ubiquitous environmental pollutants that can be strongly enriched in edible oils. PAH can be incorporated in 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 important analytes. Currently, the intervention limits for PAH are subdivided in a total limit and a limit for the so-called heavy fraction including compounds with five and more aromatic rings like benzo[a]pyren. The Austrian intervention limit for benzo[a]pyren is 2 μg /kg. Recently, a novel GC-MS method in combination with temperature-controlled, microwave-assisted saponification of the oil components was developed for the determination of PAH in Styrian pumpkin seed oil [42]. The application of this method revealed significant differences in the PAH contents of various Styrian pumpkin seed oils (μg /kg range). In a set of Styrian pumpkin seed oils that were roasted under unfavorable conditions (too high temperature), PAH concentrations of up to 120 μg /kg for the light-fraction (2- and 3-ring PAH) and up to 3 μg /kg for the heavy-fraction PAH were found [42]. Optimization of the roasting conditions led to a decrease of the heavy-fraction PAH to under the limits of detection and a decrease of the remaining light-fraction PAH to less than 40 μg /kg. PAH concentrations in this latter range were frequently found in edible oils [43, 44]. As a consequence, monitoring of the PAH content of Styrian pumpkin seed oils pro-

duced for the consumer market is recommended, since levels in the very low $\mu\text{g/kg}$ range, especially of the heavy-fraction PAHs, are generally considered to be hazardous to human health.

5 Styrian pumpkin seed oil in health and disease

It was reported that many plant-derived homologues of cholesterol (phytosterols and phytostanols) showed a plasma cholesterol-lowering effect. Several studies have investigated the cholesterol-lowering mechanisms of phytosterols, which were reviewed only recently [45–47]. Dietary uptake of phytosterols appears to be as important in cholesterol lowering as a reduction in consumption of saturated fats. Since glycosylated and free phytosterols were found in the seeds [48], but only the more lipophilic free phytosterols have been detected in the oil, it can be speculated that the latter, together with the high content of linoleic acid, can exert beneficial health effects in lipid-associated disorders like atherosclerosis.

Although there are certain indications of beneficial effects of Styrian pumpkin seeds in the treatment of benign prostate hyperplasia (BHP) stages I and II (reviewed in [3]), it is highly unlikely that there is any contribution of Styrian pumpkin seed oil as inferred from its composition.

6 Perspectives

New World *Cucurbita* spp., known in Europe since the early 16th century [49], are appreciated for their nutritional qualities and acquired the status of a “healthy oil” in the consumer’s perception. As for Styrian pumpkin seed oil, which is rich in linoleic acid but also in antioxidants (lipophilic tocopherols and polar compounds) and phytosterols, potential beneficial health effects due to its minor secondary components need to be proven yet. However, its vitamin E content is sufficiently high to contribute significantly to the recommended daily intake of the German Society for Nutrition [16]. In future, more research is necessary to reveal the detailed molecular structures and the biological effects of the polar secondary plant components of the Styrian oil pumpkin seeds and their corresponding oils.

The Styrian oil pumpkin is a plant/crop that possesses an enormous potential for future research. Improvement of Styrian oil pumpkin breeding lines and, as a consequence, of their oils is certainly a main objective 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. For instance, once molecular candidates useful for BHP treatment are elucidated, breeding lines can be improved with respect to these components. Another ambitious goal that could be achieved in a not too distant future is the heterologous expression of enzymes generating α -linolenic acid of

the *n*-3 family of polyunsaturated fatty acids, leading to a modified crop and an oil of even more interesting nutritional properties.

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Conflict of interest statement

The authors have declared no conflict of interest.

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