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Spectral analysis and characterisation

IDENTIFICATION OF PHYTOSTEROLS IN *Coriandrum sativum* L. SEEDS AS FUNCTION OF THEIR GERMINATION

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ABSTRACT

Quantitative and qualitative changes in the composition of some phytosterols (β-sitosterol, stigmasterol, campesterol) and non-steroidal compounds such as β-amyrin and betulin obtained from seeds of *Coriandrum sativum* L. Armenian population in relation to plant germination time were studied. Non-germinated seeds (Group I), seeds germinated for two days (Group II), four days (Group III) and eight days (Group IV) were used. The qualitative and quantitative composition of the biological mixtures obtained was studied by gas chromatography combined with mass spectrometry. A certain dependence on the duration of the plant growth and development processes was observed in the chromatographic indices of the compounds, in particular the peak area.

Keywords: *Coriandrum sativum* L., seed germination, phytosterols, non-steroidal compounds, gas chromatography-mass spectrometry.

AIMS AND BACKGROUND

Plant sterols (phytosterols / phytosterins) are plant steroid compounds (sterols and stanols) with a structure similar to cholesterol, but a structure of the side chain of the main backbone. Phytosterols (PS) are fatty compounds of plant origin (steroids), most of which occur as non-saponifiable substances in plant lipids. They consist of a steroid skeleton characterised by a saturated C-5 and C-6 bond within a sterol[1]. Today

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the desire for an optimally balanced diet based on the development of recommendations rich in bioactive constituents is dictating a modern healthy lifestyle[2]. For this reason, research into the discovery and identification of biologically active chemical compounds in plant constituents has been of particular interest.

PS are natural components of plant fats and oils, which are non-toxic and inexpensive by-products of the food industry. The end products are often not organoleptically pleasing in structure and taste. Such applications require the use of dispersions of PS in aqueous media such as dairy drinks, water-based drinks, and fruit juices, or in lipid media such as margarine and mayonnaise[3]. PS are widely known to have health benefits when taken orally. However, their physicochemical properties make it difficult to incorporate these molecules into standard food or pharmaceutical matrices. The use of a dispersion of PS in oil containing at least 3% by weight of PS is known for the manufacture of medicaments or nutritional compositions for treating or preventing any of the following: hypercholesterolaemia, coronary heart disease, diabetes, atherosclerosis, inflammation, osteoarthritis, Alzheimer's disease, breast cancer, colon cancer and benign prostatic hyperplasia[2].

The ability of PS to inhibit the absorption of dietary cholesterol has sparked interest in the food industry to develop products containing PS. As a result, people who consume phytosterols with cholesterol-containing foods have lower serum cholesterol levels than people whose diets do not contain PS with the estimated daily intake of its as cholesterol-lowering agents is 0.8 to 2.0 g (Ref. 3). There are many ways to prepare aqueous PS suspensions or dispersions and use them as an ingredient in low-cholesterol foods[3,4]. Plant sterols can be derived from tallow or vegetable oils. The latter can be derived from a variety of oils. These include coconut, corn, cotton, olive, palm, peanut, rapeseed, safflower, linseed, soya and sunflower[5].

Coriander (*Coriandrum sativum* L.) is an herb that has been used for over three thousand years in both traditional medicine and the national cuisine of many Asian and Mediterranean peoples. In Armenia, this essential oil plant's seeds, roots and herbs are also used. To date, it has been established that extracts from coriander leaves and seeds have pronounced antioxidant activity[6,7], which allows them to be recommended in anti-aging therapy of the skin caused by exposure to UV radiation[8]. For the prevention and treatment of COVID-19 (Refs 9 and 10), *Coriander sativum* L. has recently been proposed using a network pharmacology approach[11]. They also protect keratinocytes from induced peroxidative stress[12]. Campesterol, stigmasterol and β-sitosterol are the most abundant PS in almost all plant products[13,14]. The latter are integral natural components of plant cell membranes. They determine the properties of the plasma membrane and the membranes of the endoplasmic reticulum and mitochondria. They have been shown to play a role in the adaptation of membranes to temperature changes[15–17].

Based on the above, the research in the presented article aims to obtain information to identify quantitative and qualitative changes in the composition of phytosterols and some non-steroidal compounds such as β-amyrin and betulin, obtained from seeds of

Coriandrum sativum L. of the Armenian population, depending on the time of plant germination, and to identify these compounds using the method of gas chromatography combined with mass spectrometry (GC-MS).

EXPERIMENTAL

Sterile, pre-moistened cotton wool, 1–1.5 cm thick, was placed on the bottom of a metal tray, over which a gauze handkerchief was placed. Then 5 g of weighted coriander seeds (*Coriandrum sativum* L.) were distributed on each of the gauze handkerchiefs and germinated at 20–25°C in a dimly lit chamber. The following experimental design was used to obtain biological material: non-germinated seeds (Group I), seeds germinated for two days (Group II), four days (Group III) and eight days (Group IV). The GC-MS method was then used to determine the qualitative and quantitative composition of the biological mixtures obtained[18]. Sample preparation is limited to isolating the sterol fraction and converting any conjugated or esterified phytosterols to free phytosterins suitable for GC analysis, as the phytosterol content is usually less than 1% of the matrix weight. Direct alkaline or acid hydrolysis has been used successfully for many matrices[19,20]. The biological material obtained from each group was weighed and crushed in a porcelain mortar, gradually adding 7.5% KOH and grinding to a paste-like consistency. The material was then washed again with 7.5% potassium hydroxide. The precipitate was transferred to a flask so that the total volume of alkali was 50 ml. Finally, the flask was placed in a boiling water bath with a reflux condenser and boiled for one hour (saponification occurred during this process). After cooling, the contents of the flask were transferred to a separating funnel. Three extractions were made with 50 ml light petroleum and hexane. To the organic layer obtained, 40 ml of water was added and shaken. The water was then discarded and the organic layer was washed with water to pH 7.0. The resulting mixture was filtered through 20 g of Na_2SO_4, then it was evaporated to dryness and 2 ml of tetrahydrofuran was added. The chromatographic system consisted of a GC-MS gas chromatograph (Bruker 450 GC-MS/USA) using an Optima-5 30 m column with a heating temperature of 260°C and a temperature raise rate of 20°C/min. The injector temperature was 250°C, the first mass was 35 *m*/*z*, the last mass was 500 *m*/*z*, the carrier gas flow rate was 2 ml/min, the start time was 2 min and the end time was 20 min. The experiments were performed with up to 5 technical replicates and up to 3 biological replicates.

RESULTS AND DISCUSSION

The chromatographic values of the peak area of compounds formed during germination of coriander seeds obtained by us increase and show a certain dependence on the duration of plant processes (Table 1).

Table 1. Some chromatographic indices of compounds formed during the germination of seeds of the coriander plant (*Coriandrum sativum* L.)

Compounds	Match	Germination period for the seeds								RSD (%)
		Unsprouted seeds (Group I)		Two days old (Group II)		Four days old (Group III)		Eight days old (Group IV)		
		T	S	T	S	T	S	T	S	
Campesterol	823	–	–	–	–	7.947	1.884e+7	7.884	5.068e+7	0.563
Stigmasterol	893	8.446	3.149e+7	8.396	5.389e+7	8.434	1.845e+8	8.382	2.319e+8	0.611
β-Sitosterol	853	9.361	2.729e+7	9.321	6.676e+7	9.373	9.392e+7	9.268	1.840e+8	0.498
β-Amirin	841	–	–	9.944	1.986e+7	9.870	2.219e+7	9.892	8.276e+7	0.413
Betulin	650	–	–	13.373	2.646e+7	13.441	4.392e+7	13.673	8.076 e+7	0.233

Note: T – retention time, min; S – peak area; RSD – relative standard deviation not exceeding 2%, indicating a high degree of precision of the analyses.

Campesterol, for example, is registered in Group III seedlings, which may be related to its synthesis during photosynthesis. In addition, an increase in the contents of stigmasterol and β-sitosterol is observed, which is not related to the process of inter-conversion of one type of sterol into another. In this case, the appearance of stigmasterol cannot be related to other sterols. During seed germination, a new sterol is formed that was not detected in the earlier stages of germination. The *m*/*z* values of the fragments of these substances generated during the mass spectrometric analyses were used to identify the chromatographic peaks by GC-MS in full scan mode.

Figure 1 shows typical masses of extracted sterols. The presence of a given substance in the substrate studied can be judged from the coincidence of *m*/*z* values. For example, for β-sitosterol the characteristic *m*/*z* = 414 with confirming *m*/*z* = 43 and 55, Match 853; for stigmasterol the feature *m*/*z* = 412 with confirming *m*/*z* = 55 and 83, Match 893; for campesterol the characteristic *m*/*z* = 400 with confirming *m*/*z* = 55 and 43, Match 823; for β-amyrin the characteristic *m*/*z* 218, with confirming 203, Match 841; for betulin, the characteristic *m*/*z* 189, with confirming 95, 207, Match 650. If the match is greater than Match 700, this confirms the identity of the substance.

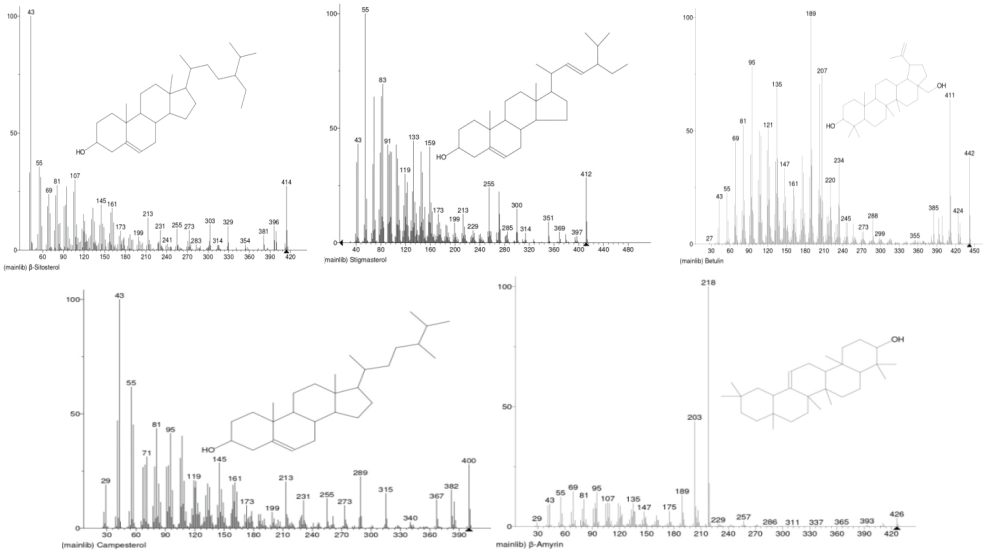

Fig. 1. Masses of sterols (sitosterol, stigmasterol, campesterol), betulin and β-amyrin

In fact, three sterols, β-amyrin and betulin, formed during germination of coriander seeds were identified by GC-MS and the increase of β-sitosterol and stigmasterol in coriander seeds depending on the duration of germination (Fig. 2).

The increase in betulin continued after eight days of germination according to the results shown in Fig. 2. This fact could be an expression of the plant's defense response to external changes (fungi, viruses, bacteria, insects)[18,19]. Almost linear change in β-amyrin content was obtained due to an increase in its rate of formation. This

indicates the involvement of sterols in the production of plant defense mechanisms against harmful external influences.

GC-MS has therefore been used to identify the active compounds whose content increases during germination of *Coriandrum sativum* L. seeds. These compounds include not only steroids (β-sitosterol, stigmasterol, campesterol). Non-steroidal compounds (betulin and β-amyrin) were also identified. The quantitative increase of sterols slowed down dramatically after eight days. However, betulin and beta-amyrin continued to increase.

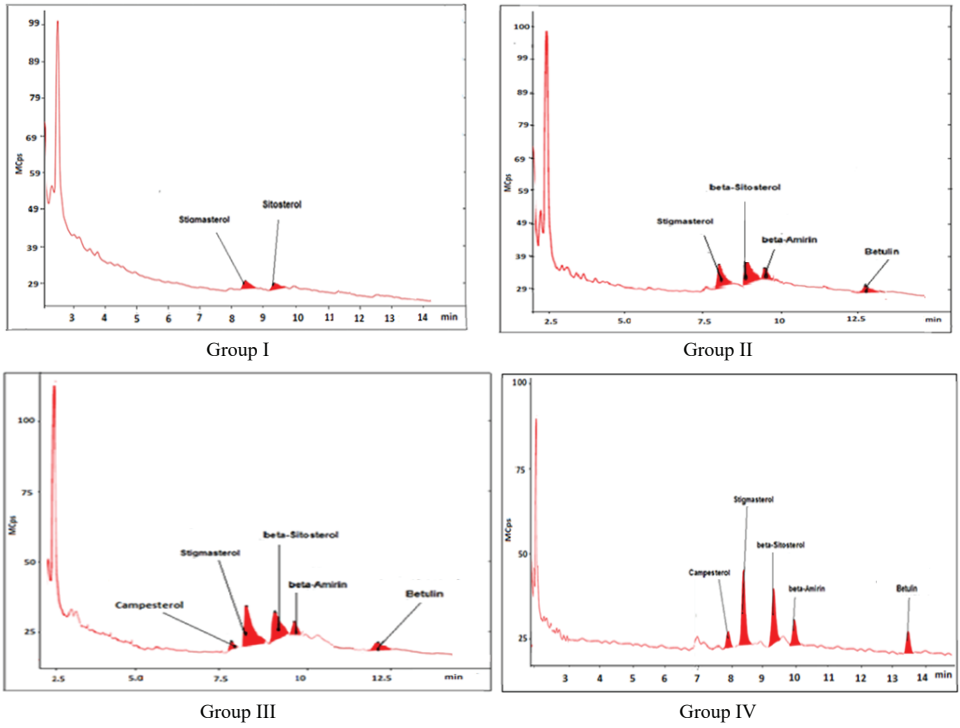

Fig. 2. Chromatograms of phytosterols obtained by extraction from eight-day-old seedlings of *Coriandrum sativum* L.

CONCLUSIONS

During seed germination of *Coriandrum sativum* L., an increase in stigmasterol and β-sitosterol is detected. In addition, a new sterol was formed which was not detected in the earlier stages of germination. Campesterol was detected in seedlings after six days of germination. The nature of the increase in stigmasterol and β-sitosterol content indicated that the appearance of stigmasterol could not be attributed to other sterols. The dynamics of β-sitosterol and stigmasterol increase in coriander seeds showed a dependence on the duration of their germination. The induced increase in stigmasterol was insignificant after eight days of germination. In the case of β-sitosterol, a

strong increase in its content was observed. At the same time, the increase in betulin content continued after eight days of germination, which may be a manifestation of the plant response.

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