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ANALYSIS OF *TRANS*-RESVERATROL IN OILSEEDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Oilseeds are very popular edibles that are often used to enhance the fibre content of baked goods, and specific types are used for preserving and seasoning. Polyphenol-related researches have been receiving growing attention in the last 20 years, especially the ones concentrating on stilbenoids. In previous studies, resveratrol concentrations have been determined from oilseeds such as peanut.

The aim of our research was to define the composition of oilseeds with a focus on the bioactive compounds, more specifically the resveratrol.

Research took place in 2010–2011 at the University of Pécs, Medical School, using non-random, convenience sampling. Oilseeds studied in the research were: sunflower seed, roasted peanut, un-roasted peanut, sesame seed, pumpkin seed, almond, linseed, bio white mustard seed, bio black mustard seed, mustard seed of foreign provenance, and wild black mustard seed. All of these oilseeds can be purchased from trade. Samples used in the research were obtained from the producers and collectors. High Performance Liquid Chromatography (HPLC) was used for the measurements.

Summarising our results, it can be stated that each type of oilseed analysed in our research can be regarded as good sources of resveratrol. The highest level of resveratrol was detected in the sunflower seeds (0.00398±0.0001 mg g^{-1}), almonds (0.00176±0.00021 mg g^{-1}), roasted peanut (0.00206±0.00013 mg g^{-1}), and wild black mustard seeds (0.0023±0.0007 mg g^{-1}).

Keywords: HPLC, oilseed, resveratrol, stilbene

In the past decade, intensive research focusing on free radicals' damage and antioxidant compounds has led to the conclusion that dietary antioxidants play a role in the prevention of various diseases, for example the cardiovascular- and tumour-related diseases (Margaill et al., 2005; Cheung et al., 2007; Pazdro & Burgess, 2010). Chemical and biochemical research reports the exact physiological effects of the polyphenols and flavonoids, and also their level in food and edibles (Kirkham & Rahman, 2006; Bouayed & Bohn, 2010). The aforementioned flavonoids experience transformation in their structure while in the gastrointestinal tract, thus their effect in terms of nutritional physiology does not come from the molecules, but from their metabolites (Limón et al., 2009). Polyphenol-related researches have been receiving growing attention in the last 20 years, especially the ones concentrating on stilbenoids (Seifried, 2007; Aparicio, 2010). In our research we have focused on resveratrol, which also belongs to the group of stilbenoids, and was defined in Europe for the first time in 1976 using

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Vitis vinifera (common grape vine) (Cai et al., 2011; Weidner et al., 2013). Moreover, resveratrol concentrations have been determined from oilseeds such as peanut (Mainer & Andreas, 2003; Tokuşoğlu et al., 2005; Chukwumah et al., 2011; Sobolev et al., 2011; Wang et. al., 2013). Although these studies have all used various oilseeds to detect resveratrol, no previous research has analysed oilseeds in general to examine their level of resveratrol. Oilseeds are very popular edibles that are often used to enhance the fibre content of baked goods, and specific types are used for preserving and seasoning. Often these seeds are eaten as snacks, since they have pleasurable taste and high enjoyment value, so the question seems important to be considered: what nutrients pass into the human digestive system through oilseeds?

The aim of our research was to define the composition of oilseeds with focus on the bioactive compounds, more specifically the resveratrol these seeds contain. The two main questions to be studied were:

i) Does the heat used during processing have any effect on the level of bioactive materials in the seed? Since the intake of certain oilseeds (such as peanuts) may take place in roasted or unroasted forms, the heat used during manufacturing may alter the composition of the seeds, thus their resveratrol-level.

ii) Does the technique of cultivation have any effect on seed composition? Is there any difference in the levels of resveratrol in mustard seeds coming from regular or bio cultivation?

1. Materials and methods

1.1. Investigated samples

Oilseeds observed in the study were: sunflower seed, roasted peanut, un-roasted peanut, sesame seed, pumpkin seed, almond, linseed, bio white mustard seed, bio black mustard seed, mustard seed of foreign provenance, and wild black mustard seed. All of these oilseeds can be purchased from trade. Samples used in the research were obtained from the distributor, except mustard seeds that were obtained from the producers and collectors (Table 1).

1.2. Sample preparation

Treatment of the collected seeds was the following: 5 g of oilseeds were chopped using a pestle and mortar, transferred into beakers, and placed in a vacuum desiccator for 24 hours. The next day, 1 g was measured out from each sample. A 1:1 ratio methanol–water mixture was blended, 5 cm^3 of the mixture was transferred by a pipette onto the prepared oilseed samples. Ultrasonic treatment was carried out for 10 minutes at room temperature, after which the tincture was strained. Next, the extract was filtered and stored at 4 °C for further HPLC investigations.

1.3. High Performance Liquid Chromatography (HPLC)

The chromatographic separation was carried out as previously mentioned in our paper (Mark et al., 2005). The HPLC system is made up of a pump type Dionex M 480 GT, an injector type Rheodyne 8125 (with a 20 µl sample loop), and a Dionex M 340S UV diode array detector. A 250×4.6 mm column, packed with 5 µm particle size C18 material has been used for the separations (Jambor et al., 2013). A Chromeleon ver. 6.8 SR10 (Softron GmbH, Germering, Germany) data management software was used for the control of the equipment

Table 1. Names and resources of oilseeds used in the research

No.	Name	Species	Distributor / Place of origin	Year of the collection	Technique of cultivation	Harvested quantity
1	Almond	*Prunus amigdalus*	Rapulzel	2006	Not known	200 g
2	Sunflower seed	*Helianthus Annuus* L.	Spar	2006	Not known	200 g
3	Linseed	*Linum usitatissimum*	Biopont	2006	Not known	150 g
4	Roasted peanut	*Arachis hypogaea* L.	Biopont	2006	Not known	200 g
5	Non-roasted peanut	*Arachis hypogaea* L.	Rapulzen	2006	Not known	200 g
6	Sesame seed	*Sesanum indicum*	Biopont	2006	Not known	150 g
7	Pumpkin seed	*Cucurbita*	Rapulzel	2006	Not known	200 g
8	Bio white mustard seed	*Sinapis alba*	Hungary, Mohács	2006	Eco (without chemicals)	500 g
9	Bio black mustard seed	*Brassica nigra*	Hungary, Mohács	2006	Eco (without chemicals)	500 g
10	Mustard seed of foreign provenance	*Sinapis alba*	Slovakia, Raca	2006	Conventional (chemical used)	500 g
11	Wild black mustard seed	*Brassica nigra*	Hungary, Mosonmagyaróvár	2006	Not regulated	500 g

and for data evaluation. A multi-step gradient method has been applied using methanol–water–acetic acid (10/90/1; v/v/v) mixture as solvent A and methanol–water–acetic acid (90/10/1; v/v/v) mixture as solvent B at a flow rate of 1.5 ml min^{-1}. The gradient profile was: 0.0–18.0 min from 0% to 40% B; 18.0–25.0 min from 40% to 100% B; 25.0–27.0 min 100% B. Chromatographic separations were monitored at 306 nm (Mark et al., 2005; Montsko et. al., 2008). Peaks of the components in focus were looked up based on retention time, and identified based on the spectrum, quantification was carried out using peak areas method. Measurement evaluation was completed using a calibration curve. All data were analysed by *t*-tests (n=5), using Microsoft Excel 2003.

2. Results and discussion

Levels of *trans*-resveratrol can be observed on the chromatograms of the analysed samples (Fig. 1). *Trans*-resveratrol is a more biologically active type of stilbenoids, thus depicting these materials served our research-aims better. The highest level of resveratrol was detected

in the sunflower seeds (0.00398±0.0001 mg g^{-1}), almonds (0.00176±0.00021 mg g^{-1}), roasted peanut (0.00206±0.00013 mg g^{-1}), and wild black mustard seeds (0.0023±0.0007 mg g^{-1}). Other types of oilseeds analysed in this study contained only minor level of *trans*-resveratrol.

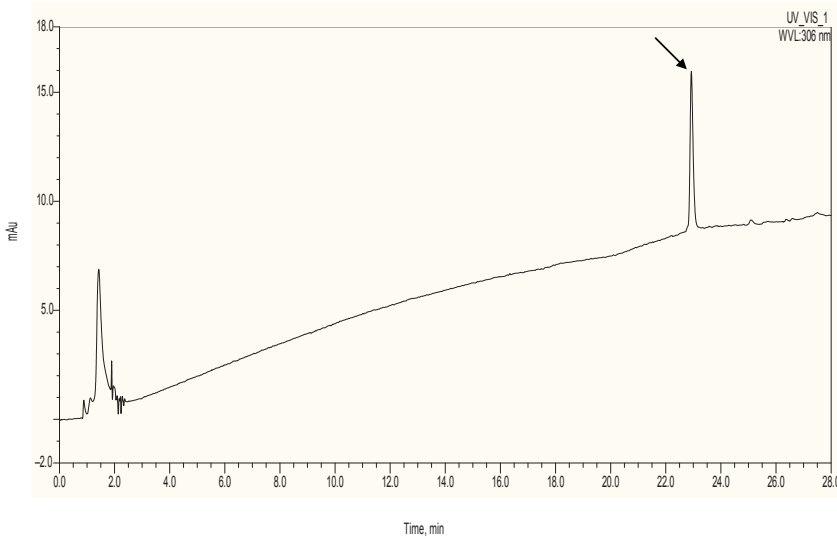

Fig. 1.: Representative chromatogram of the sunflower seeds extract at 306 nm. Trans-resveratrol indicated by an arrow. The detailed chromatographic parameters were mentioned in the text. Briefly, a 250 × 4.6 mm column packed with 5 μm particle size C18 material has been used for the separations. A multi-step gradient method has been applied using methanol–water–acetic acid (10/90/1; v/v/v) mixture as solvent A and methanol–water–acetic acid (90/10/1; v/v/v) mixture as solvent B at a flow rate of 1.5 ml min^{-1}. The gradient profile was: 0.0–18.0 min from 0% to 40% B; 18.0–25.0 min from 40% to 100% B; 25.0–27.0 min 100% B.

Figure 2 shows that the level of resveratrol in sunflower seed is significantly higher ($P<0.005$) than in other oilseeds. The levels of resveratrol in almond and mustard seeds are still noteworthy, but less than that of the sunflower seed.

To measure the effect of heat on the seed during preparation process, we have analysed various forms of peanuts. There are two types of intake known in this case: roasted or non-roasted. Both have been analysed in our study and it was found that the level of resveratrol is significantly different ($P<0.005$) in case of roasted and un-roasted peanuts. The level of resveratrol is minor in non-roasted peanut, however, it is as high as 0.0021±0.00116 mg in the roasted form. As the reason for the difference, we assume that resveratrol-release is higher in the seed when it is heated.

Results of our study make it clear that the method of cultivation has lower effect on the seed's resveratrol level, than the heat it is treated with. To analyse the effect of growing method on the seed's resveratrol level, we have compared mustard seeds from various resources: regular to bio cultivating method, and bio to wild growing method. It is unambiguous from our results that the wild black mustard seed has the highest level of resveratrol, followed by the somewhat lower level of resveratrol in the bio black mustard seed, however, the difference is not significant ($P>0.005$). The difference is even smaller between growing methods in case of the white mustard seed's regular and bio grown types.

All plants were cultivated in the same year (2006), using generally resembling nutrients. Level of rainfall and number of sunny days were approximately the same in all cases. Based on our analysis we may also add that there is no difference in the level of resveratrol between the black and white types of mustard seeds.

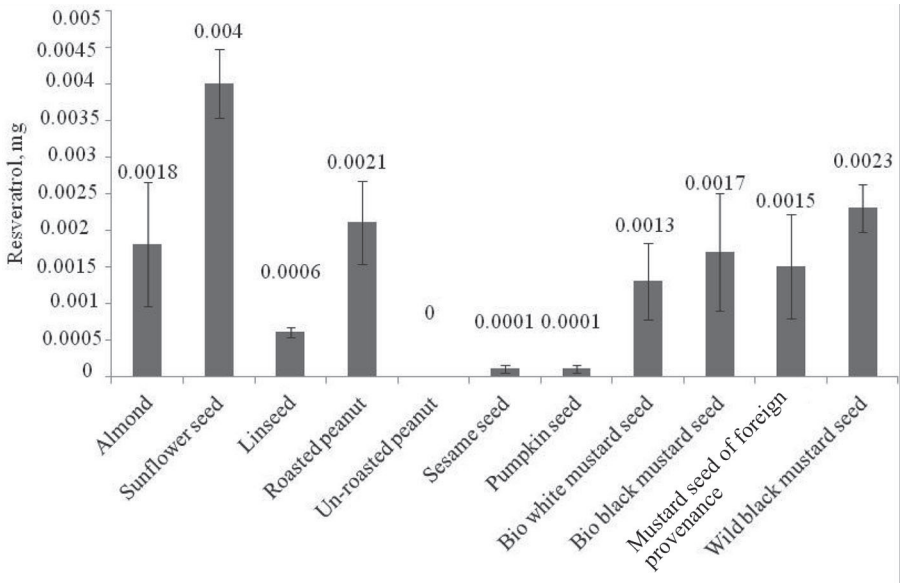

Fig. 2.: Level of resveratrol in the examined oilseeds, in 1 g dry matter

3. Conclusion

Epidemiological and clinical investigations showed that the polyphenolic phytoalexin resveratrol is associated with reduced cardiovascular disease and reduced cancer risk. Quantitative amounts of *trans*-resveratrol were determined as 0.03–14.34 ppm in grapes, grape vine, red wines, and peanuts by HPLC- and GC-MS–based chromatographic methods. Stilbene contents in field-grown fruit may also be affected by a wide range of environmental effects including temperature, soil, light levels, the nutritional condition of the plant, and pathogen attack (Mark et al., 2005, Tokuşoğlu et al., 2005).

Summarising our results, it can be stated that each type of oilseed analysed in our research can be regarded as a good source of resveratrol. In comparison with red wine (which is claimed to be a good source of resveratrol), it can be stated that the highest resveratrol-containing oilseed, sunflower seed, has the same level of *trans*-resveratrol as merlot and pinot noir, the highest resveratrol-containing types of red wine (Mark et al., 2005).

Furthermore, we should not forget the level of resveratrol that almonds, roasted peanut, and various mustard seeds contain.

To answer our research question i), we can declare that the level of resveratrol in an oilseed can be raised by roasting, as roasted peanuts exhibited significantly higher level of resveratrol, than non-roasted peanuts.

The answer to our research question ii) is that there is no difference in resveratrol-level in case of black or white mustard seeds, and our results have also shown that the method of cultivation does not affect significantly the detectable level of resveratrol. As an implication to everyday usage, we may state that due to their positive physiological effect, moderate intake of oilseeds can have a role in preventing various diseases.

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