

**The effects of growing and storage conditions on the content of
biologically active compounds and antioxidant activity of clover
sprouts (*Trifolium L.*)**

Ewelina Grygiel, MSc

Supervisor

Grażyna Budryn, Prof.

Auxiliary supervisor

Ilona Gałązka-Czarnecka, PhD

Abstract

Sprouted seeds, including clover sprouts, can be an important source of phytochemicals, demonstrating multiway pharmacological, health-promoting and preventive effects. The profile and level of these compounds are strongly dependent on the plant genotype but also on the seed germination conditions, which are important for increasing the nutritional and bioactive value, as well as better tastiness of the sprouted seeds.

The main aim of the study was to compare the quantitative and qualitative composition of isoflavones, polyphenolic compounds and L-ascorbic acid in selected varieties of clover sprouts (*Trifolium* L.) and to determine the antioxidant activity during growth and germination in various conditions. Additionally, the impact of cold storage and modified atmosphere storage on the content and stability of selected biologically active compounds and the antioxidant activity of clover sprouts of various varieties was assessed. At the same time, an analysis of the sensory characteristics of sprouts during storage was performed.

The research material consisted of seeds of five varieties, available in specialized commercial establishments and sprouts obtained from them: red clover (*Trifolium pratense* L.) *Rozeta* and *Nike*, white clover (*Trifolium repens* L.), egyptian clover (*Trifolium alexandrinum* L.) and pale pink clover (*Trifolium hybridum* L.). In the initial stage, the extraction efficiency of polyphenolic compounds and isoflavones was determined depending on the solvent and its concentration using a pressure extractor. The second stage of the research included analyzes during the cultivation and growth of clover sprouts in conditions with access to diffused daylight and in the dark. The cultivation was carried out in Mikrofarm EasyGreen automated sprouters. The content of total polyphenols in the extracts from seeds and sprouts was determined using the Folin Ciocalteu method, the content of L-ascorbic acid using the Tillmans method, the ability to scavenge DPPH free radicals, as well as the quantitative and qualitative composition of isoflavones and coumestrol using the LC-ESI-MS method. The basic composition of sprouts of individual varieties was also compared, including the content of water, protein and fat.

The results obtained during the experiments confirmed that growth conditions, and in particular access to daylight, influence the synthesis of isoflavones, polyphenolic compounds, L-ascorbic acid and the antioxidant activity of clover sprouts. Moreover, based on the conducted research, it can be concluded that the content of the above-mentioned ingredients and the antioxidant capacity of sprouts are significantly determined by the variety of sprouts and the length of germination period. In all analyzed samples, sprouts growing with access to diffused daylight showed higher contents of L-ascorbic acid and polyphenolic compounds and stronger antioxidant properties. Under the compared growth conditions, the general content of tested polyphenolic compounds during

cultivation increased successively, reaching maximum values between the 7th and 9th day of sprout collection, depending on the variety. The scavenging ability of DPPH free radicals also changed significantly during germination, and the maximum scavenging effect was achieved on days 6 and 7. In turn, to maintain the beneficial content of L-ascorbic acid in clover sprouts, it is best not to exceed 5 to 6 days of germination.

The results of the conducted research also showed that the content of both isoflavones and coumestrol in clover seeds and sprouts varied significantly depending on the variety. The highest concentration of the total analyzed isoflavones was found in red clover sprouts and seeds of the *Rozeta* variety, while the lowest amounts of these compounds were determined in white clover sprouts and seeds. The dominant isoflavone in red clover seeds were ononin and formononetin. The highest amounts of genistein and biochanin A were determined in egyptian clover. White clover seeds contained the highest amounts of genistin, and pink clover seeds were rich in genistein and formononetin. Significant differences were also observed in the content of coumestrol depending on the tested seed variety. This compound was present in 5 of the 3 clover varieties tested, and its richest source turned out to be the seeds of white pink (swedish) and white clover, the smallest amounts were determined in the red clover of the *Rozeta* variety. Coumestrol was not detected in the seeds of red clover *Nike* and egyptian clover, but it was present in their sprouts. The results of the analyzes showed that both the qualitative profile of isoflavones and the quantitative dynamics of the accumulation of these compounds in clover sprouts depended on the cultivation conditions and changed significantly during germination (at the level of $p < 0.05$). Moreover, based on the results obtained, it is not possible to determine one common day that could be the most favorable day for harvesting clover sprouts in terms of the highest concentration of all tested isoflavones. The maximum content of individual substances occurs on different days, depending on the variety, species and cultivation conditions and is an individual feature of each compound.

Based on the observations made, it can be concluded that access to daylight during red clover germination favors the accumulation of free isoflavones (aglycones), while germination in the dark is beneficial for the accumulation of bound forms of isoflavones (glycosides). Similar relationships occurred in changes in the concentration of biochanin A and sissotrin, genistein and genistin, as well as daidzein and daidzin in the sprouts of both red clover varieties.

The last stage of the research included the analysis of red clover sprouts from the *Rozeta* variety seeds stored in refrigerated conditions in LDPE foil and packaging in a modified atmosphere composition (MAP 1 and MAP 2). The obtained results indicate that low temperature does not cause the degradation of polyphenols, and in order to maintain a relatively high level of polyphenol compounds, it is best to store it under refrigerated conditions for no more than 10 days and to use MAP with a reduced oxygen content (5%) and an increased carbon dioxide content (also 5%), during

storage of red clover sprouts is a more favorable concentration for maintaining antioxidant properties than increasing it to 10%. The best variant of storing clover sprouts to slow down the degradation of L-ascorbic acid was a combination of low temperature and MAP with a composition of 5% O₂, 5% CO₂, 90% N₂. However, an increase in CO₂ content to 10% did not result in better stabilization of this ingredient. Moreover, based on the experiments conducted, it can be concluded that storing red clover sprouts under the MAP conditions used increases their bioactivity. The content of isoflavones increased several times during 14 days of storage. The obtained results also prove that storing red clover sprouts in refrigerated conditions at 2±1 °C wrapped in LDPE foil, has a positive effect on the content of chlorophyll and carotenoid pigments for up to 10 days.

The sensory quality assessment showed that sprouts stored in MAP 2 had more favorable sensory characteristics compared to those stored only in refrigerated conditions (K) and in MAP 1. It can also be stated that storing red clover sprouts in a modified atmosphere composed of 5% O₂, 5% CO₂, 90% N₂, where the oxygen content is reduced while the carbon dioxide concentration is increased has a positive effect on their sensory properties over time. up to 9 days.

The presented research results confirm the influence of daylight on the synthesis of isoflavones and vitamins as well as on the antioxidant activity during clover seed germination. Later storing red clover sprouts in various conditions affects the stability of selected isoflavones, vitamins, chlorophylls, carotenoids, antioxidant activity and their sensory properties in different ways. Therefore, it is worth analyzing different conditions of sprout growth and storage to ensure the highest possible levels of bioactive compounds.

Due to the high content of bioactive compounds such as isoflavones, clover sprouts of the tested varieties may be health-beneficial dietary supplement and be used in the food industry to obtain complex products. Seed germination and subsequent storage of sprouts can be a good way to effectively modify the nutraceutical and nutritional values.