JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2016, 22(4), 241-246

Journal of Agroalimentary Processes and Technologies

Qualitative investigation on bioactive compounds of alfalfa seeds during germination

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Received: 03 October 2016; Accepted: 15 November 2016

Abstract

Sprouts are widely used as an important source of minerals, vitamins and antioxidants. During germination, the enzymes released nutrients from seed in order to grow a new plant. Digestibility of carbohydrates and proteins increase due to conversion in simple sugars and amino acids. Minerals are absorbed from water and transported in germinated seeds. Also, vitamins C and B are formed during metabolic changes of the seeds. Small amounts of sprouts contain a high concentration of bioactive compounds.

Alfalfa (*Medicago sativa* L.) sprouts contain high levels of nutrients with important impact on human health. This nutrients, especially isoflavones, can reduce the menopause side-effects. In vitro studies shows that isoflavones can decrease the incidence of breast cancer, cardiovascular diseases and metabolic diseases. The aim of this study is to observe the effect of sprouting on some compounds like: carbohydrates, saponins, phenolic compounds, alkaloids, flavonoids. The seed of alfalfa were germinated for 6 days. Hidroalcoholic extracts of the sprouts were subject of qualitative determination. The presences of bioactive compounds was confirmed by FTIR investigation of the extracts.

Keywords: bioactive compounds, alfalfa sprouts, analytical methods, women's disorders, hidroalcoholic extracts.

1. Introduction

The leaves, seeds and sprouts of alfalfa have medicinal use in many metabolic deficiencies, are phytonutrientrich, provide significant amounts of antioxidants [1-3]. Phytochemicals are present in plant seeds and their contents are known to increase during germination in most plant species, from legumes, to oilseeds, to cereals [4-7]. In fact, sprouts (i.e., the young seedlings obtained from seed germination) are becoming more and more popular in western countries as healthy foods, for their positive effects on the prevention of cardiovascular diseases and cancer [8].

Sprouts are manufactured from the seeds of a large number of different plants. Sprouts are believed to be rich in health-promoting phytochemicals compared with their mature counterparts. Germination (sprouting) has been suggested as an inexpensive and effective way to improve the quality of legumes. Sprouting mobilizes polymerized forms, such as concentrated starch and protein, into carbohydrates and free amino acids, respectively. This significantly improves the nutritional value of sprouts, which can be readily used by the human body [9].

Alfalfa sprouts contain high amounts of vitamins A and C, coumestrol, liquiritigenin, isoliquiritigenin,

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loliolide, and saponins [10-12]. Alfalfa sprout saponins interacted with cholesterol to a lesser but significant extent. Sprout saponins also inhibited growth of Trichoderma viride significantly, another measure of saponin-cholesterol interaction [13]. There are many reports on biological activities of bioactive molecules, which could be relevant to the pharmacological effects. Different compounds may be present in different products depending on extraction methods [2]. For e.g., the alcoholic extracts stimulate bile excretion, whereas the aqueous extracts have no such effect [14-16]. With the help of hidroalcoholic extract we conducted to this study that reflects the effect of germination (sprouting) on some compounds. FTIR is a powerful tool for identifying types of chemical bonds in a molecule by infrared absorption spectrum which is a genuine molecular "fingerprint" (FT–MIR) [17]. The capability of hidroalcoholic extracts of alfalfa sprouts powder used as homeopathic mother tinctures has been explored.

2. Materials and Method

1.1.Materials:

Organic alfalfa seeds (*Medicago sativa L.)* - Country of origin Canada - purchased from health stores, Caloris Germinator, Drying device Caloris (39°C), ethanol, ethyl acetate, 1% ferric chloride solution, potassium ferrocyanide, 1% HCl, distilled water, Fehling's reagent A: 69.3g cooper sulfate in 1000ml distilled water, Fehling's reagent B: 250g potassium hydroxide and 346g potassium sodium tartarate was dissolved in 1000ml distilled water.

1.2. Methods:

Seeds were sterilised with an UV Lamp for 1h and after desinfection, seeds were allowed to imbibe wather for 6h at 20^0C. Then water was removed and seeds were dark germinated in the Caloris germinator at 25^0C, after germination the sprouts were dried at $38,9^0$C. This method of drying was chosen for maximum effectiveness in preserving bioactive compounds. The next methods were: powdering, extraction and filtration. After powdering of sprouted alfalfa seed from day 1 to day 6, it was performed an extraction with 40% Ethanol and keeped 10 days at dark and 25°C. Right after filtration, the clear extracts were phytochemical tested and for FTIR analysis, the extracts were kept in the deep freezer until analysis [18]. All the test were done for phytochemical screening using standard procedures [19].

1.2.1.Test for flavonoids. The flavonoids test was performed from 0.5ml of the extract. The ethyl acetate was added to the extract and observed the formation of yellow organic layer. The formation of yellow organic layer indicates the presence of flavonoids.

1.2.2.Test for phenolic compounds. The phenolic compounds test was performed from 0.5mg of the extract. The 1% ferric chloride solution was added and mixed with potassium ferrocyanide. The formation of bluish green colour indicates the presence of phenolic compounds.

1.2.3.Test for tannins. The tannins test was performed from 1ml of the extract and added 1% HCl. The formation of red precipitate shows the presence of tannins.

1.2.4.Test for carbohydrates. The carbohydrates test was performed from 100mg of the extract dissolved in distilled water and filtered. Fehling's reagent A and Fehling's reagent B were added in 0.5ml filtrate obtained earlier and boiled.

1.2.5.Test for saponins. The saponins test was performed from 0.5ml extract and distilled water. The content was shaken well for few minutes and left for 20 minutes.

1.2.6.FT–IR measurements. The Fourier Transform Infrared Spectrum (FTIR) it is a method of qualitative and quantitative analysis used to analyze and to determine the chemical structure of organic compounds.

The spectra of each sample were obtained using a spectrophotometer Thermo Scientific Nicolet iS50 FT-IR. The samples were analyzed using the technique ATR (Attenuated Total Reflection), cell equipped with a diamond crystal. Spectra of each extract was recorded in the optical region, from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. The spectral data were processed with the Omnic 9.2 Software (Nicolet iS50). The spectra were registered as fluid. The functional groups identification was based on the FTIR bands attributed to stretching and bending vibrations. Frequencies characteristic of the main

classes of organic compounds were taken from literature [10].

3.Results and discussion

This work has been undertaken to gain an understanding of the chemical composition of the alfalfa sprouts powder hidroalcoholic extract at the different stages of seeds germination.

The sprouts extract from day 1 to day 6 were phytochemical analysed with the 5 qualitative test as follows:

Flavonoids test was performed from 0.5ml of the extract with 1ml of ethyl acetate to observe the formation of yellow organic layer. In these samples was identified the formation of yellow organic layer (Table 1).

Phenolic compounds test was performed from 0.5mg of the extract, 3 drops of 1% ferric chloride solution mixed with 1ml of potassium ferrocyanide. The formation of bluish green colour in the samples indicates the presence of phenolic compounds (Table 1)

Tannins test was performed from 1ml extract and added 1% HCl. The mixture was boiled in hot water bath for 10 minutes. The formation of red precipitate shows the presence of tannins. In our samples there were no formation of red precipitate, which indicates the absence of tannins (Table 1).

Carbohydrates test was performed from 100mg of the extract was dissolved in 5 ml of distilled water and was filtred. The filtrate obtained were added 0.5ml of Fehling's reagent A and Fehling's reagent B and boiled until we identify the formation of red precipitate which indicates the presence of carbohydrates (Table 1).

Saponins test was performed from 0.5ml extract and it was added 5ml of distilled water. The content was shaken well for few minutes. The froth formation was observed after 20 minute, which indicates the presence of saponins (Table 1). It was shown that monodesmosidic medicagenic acid glycoside was synthesized after 4 days of germination and subsequently followed by bidesmosidic saponin production. The total saponin concentration increased from 2.12 μmol/g of dry matter at the beginning of germination to around 6 μmol/g after 8−16 days of seedling growth. It was concluded that previous reports on saponin concentration in alfalfa seedlings (8−10% in dry matter) as measured by biological tests were highly overestimated; the real concentration is several times lower [11].

FT–IR fingerprint. The same samples used, which are hydroalcoholic extract from alfalfa sprouts powder, named G1 (day 1), G2 (day 2), G3 (day 3), G5 (day 5), G6 (day 6) in FTIR analysis, gaved the intensity of the bands with no major differences (Fig. 1).

FTIR analysis of the samples revealed the presence of more characteristic bands which are intense, medium or weak - specific functional groups and are characterized by their specific frequencies (Table 2). According to this study, hidroalcoholic extract used as homeopathic mother tinctures appears ideal for extracting a high amount of phenolic compounds and bioactive compounds from *M. sativa* sprouts with potential antioxidant activity content. A total of nine area were observed on FTIR spectra and all are for the compounds followed.

Table 1. Qualitative analysis of hydroalcoholic extract from alfalfa sprouts powders.

SI No	Phytochemical test	G1	G2	G3	G4	G5	G6
1	Flavonoids test	+	+	++	++	++	++
2	Phenolic compounds test	+	+	++	++	++	++
3	Tannins test	-	-	-	-	-	-
4	Carbohydrates test	+	+	++	++	++	++
5	Saponins test	+	+	+	++	+	++
Present ++ Moderately present + Absent -							
Hydroalcoholic extract from alfalfa sprouts powder, named G1 (day 1), G2 (day 2), G3 (day 3), G5 (day 5), G6 (day 6)							

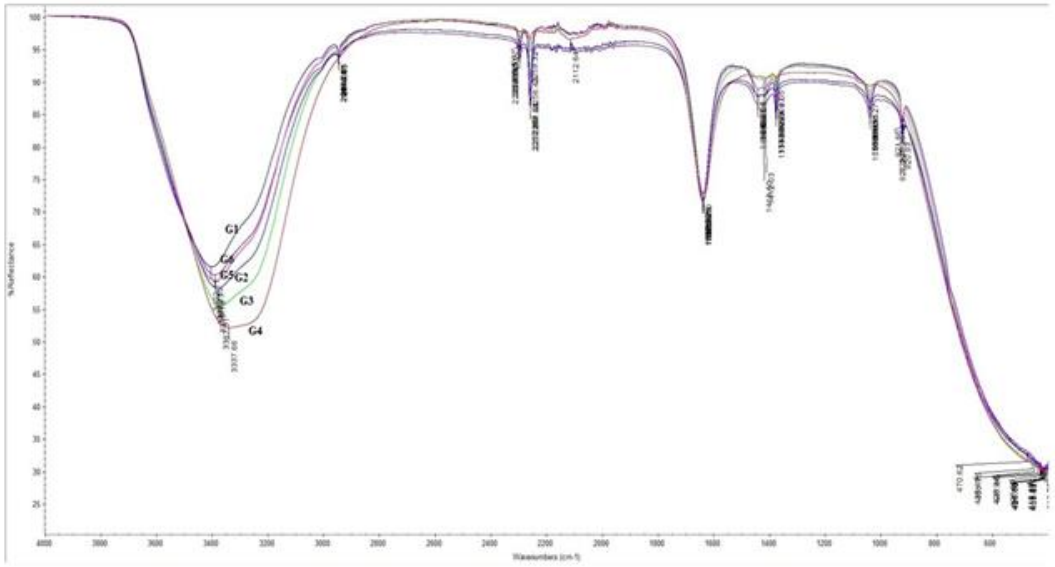

Figure 1. IR Spectra of the samples G1, G2, G3, G4, G5 and G6 (alfalfa sprouts powder extract from day 1 to day 6 of germination).

Table 2. Frequencies and possible groups present in alfalfa sprouts powder extract

The Functional Group		**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
The group -O-H (phenol alcohols), and hydrogen bond O-H ... OH- amide band specific protein and nucleic acid	Frequency (ν, cm^{-1}) Type band	3379.18 wide	3389.19 wide	3367.31 wide	3367.66 wide	3387.75 wide	3402.11 wide
Unsaturated groups, triple bond	Frequency (ν, cm^{-1}) Type band	2295.92 medium	2295.57 medium	2295.97 medium	2295.89 weak	2295.51 medium	2295.11 medium
Unsaturated groups, triple bond	Frequency (ν, cm^{-1}) Type band	2258.21 medium	2257.69 medium	2258.43 medium	2259.32 weak	2257.64 medium	2257.04 medium
Saturated groups, C-H ($C\ sp^3$)	Frequency (ν, cm^{-1}) Type band	2945.24 weak	2944.78 weak	2946.05 weak	- -	2944.81 weak	2944.55 weak
Aldehyde and ketone groups with saturated character, C=O; **Unsaturated groups, double bonds -C=C-;** **Amide group (α-helix structure and -β)**	Frequency (ν, cm^{-1}) Type band	1637.32 intense	1637.05 intense	1636.99 intense	1635.97 intense	1636.75 intense	1636.70 intense
Unsaturated groups -C=C-H, aromatic	Frequency (ν, cm^{-1}) Type band	1440.40 weak	1440.09 weak	1418.60 weak	1412.03 weak	1440.06 weak	1440.97 weak
Saturated groups, C-H ($C\ sp^3$)	Frequency (ν, cm^{-1}) Type band	1374.22 medium	1374.35 medium	1374.07 medium	1374.05 weak	1374.33 medium	1374.49 medium
Amine groups, C-N; **Hysterical groups, –COOR;** **Phosphodiester bonds.**	Frequency (ν, cm^{-1}) Type band	1038.45 medium	1038.34 medium	1038.27 medium	- -	1038.15 medium	1038.16 medium
Carboxyl group, -COOH; Unsaturated groups, =C-H	Frequency (ν, cm^{-1}) Type band	921.47 medium	921.24 medium	921.65 weak	- -	921.26 medium	920.93 medium

The absorption bands between 921–1039 cm^{-1} may be attributed to C–H bending vibrations from isoprenoids with signals at 921.47 – 920.93, to C–O bending stretching vibrations of mono–, oligo – and carbohydrates, with signals at 1038.45 (G1), 1038.34 (G2), 1038.27 (G3), 1038.15 (G5) and 1038.16 cm^{-1} (G6). The signals between 1440.40 – 1500 cm^{-1} may be assigned to aromatic parts and to N–H bending vibrations (amino acids), while the absorption situated between 1500 - 1637.32 (G1), 1637.05(G2), 1636.99 (G3), 1635.97 (G4), 1636.75 (G5) and 1636.70 cm^{-1} (G6) corresponds to a complex of stretchings vibrations C-O (amide) and C–C stretching vibration of the phenyl groups, C=O stretching vibrations (aldehydes, ketones and esters) [10,20].

These absorption studied, followed by the domain 2800–2950 cm^{-1}, corresponds to C–H stretching vibrations, specific to CH_3 and CH_2 in lipids, methoxy derivatives [21]. The domain 3379.18 (G1), 3389.19 (G2), 3367.31 (G3), 3367.66 (G4), 3387.75 (G5) and 3402.11 cm^{-1} (G6) corresponds to stretching vibrations of OH groups (water, alcohols, phenols, carbohydrates, peroxides) as well as to amides (3650 cm^{-1}) [22,23]. It can be considered in this case that measurements, based on the FTIR absorption spectrum, frequency bands belong to 3300 cm^{-1}, 1636 cm^{-1} and 1038 cm^{-1}specific functional groups of organic compounds such as polyphenols, carbohydrates, fatty acids and proteins.

4. Conclusions

The study showed that a simply and effective method, as FTIR analysis, reveals in homeopathic mother tinctures that the fingerprints regions followed are located between 1038, 1636 and 3300 cm^{-1} and the specific functional groups involved have been identified. It gaves clear results on analysis, can be used to control and monitor the composition of extracts and to be as clear as possible. We realized further investigation, for every FTIR data, which were correlated with UHPLC-MS screening method for direct analysis of polyphenols and flavonoids in alfalfa sprouts powder extract.

The most popular methods of standardization of plant pharmaceutical products, including homeopathic tinctures, involve the determination of the content of flavonoids [24-26] and the presented work is an example of such a procedure which can be applied to tinctures.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Dong, X.F., Gao, W.W., Su, J.L., Tong, J.M., Zhang, Q., Effects of dietary polysavone (Alfalfa extract) and chlortetracycline supplementation on antioxidation and meat quality in broiler chickens, *Br Poult Sci*, **2011**, *52*, 302–309
2. Butnariu, M., An analysis of Sorghum halepense behavior in presence of tropane alkaloids from Datura stramonium extracts, *Chem. Cent. J*, **2012**, *6*,75
3. Stadnik, J., Dolatowski, Z.J., Grela, E.R., Karwowska, M., Effect of proteinxanthophylls (PX) concentrate of alfalfa supplementation on physicochemical properties of turkey breast and thigh muscles during ageing *Meat Sci*, **2010**, *86*, 486–490
4. Alvarez-Jubete, L., Wijngaard, H,, Arendt, E.K., Gallagher, E., Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa, buckwheat and wheat as affected by sprouting and baking, *Food Chemistry*, **2010**, *119*, 770–778
5. Baenas, N., Moreno, D.A., Garcia-Viguera, G.C., Selecting sprouts of Brassicaceae for optimum phytochemical composition, *Journal of Agricultural and Food Chemistry*, **2012**, *60*, 11409–11420
6. Benincasa, P., Galieni, A., Manetta, A.C., Pace, R., Guiducci, M., Pisante, M., Stagnari, F., Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species, *Journal of the Science of Food and Agriculture*, **2015**, *95*(9), 1795–1803
7. Marton, M., Mándoki, Z., Csapo-Kiss, Z., Csapó, J., The role of sprouts in human nutrition. A review, *Acta Universitatis Sapientiae*, **2010**, *3*, 81–117
8. Ma, D., Zhang, Y. Xue, Y., Wang, P., Yang, T., Shao, X., Isoflavone and its metabolite equol inhibit the development of 7, 12-dimethylbenz (a) anthracene (DMBA) - induced mammary tumours in ovariectomised rats, *Journal of Functional Foods*, **2014**, *7*, 580–589

9. Randhir, R., Lin, Y., and Shetty, K., Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors, *Process Biochemistry*, **2004**, *39*, 637–646
10. Hong, Y.H., Wang, S.C., Hsu, C., Lin B.F., Kuo, Y.H., Huang, C.J., Phytoestrogenic compounds in alfalfa sprout (*Medicago sativa*) beyond coumestrol, *Journal of Agricultural and Food Chemistry*, **2011**, *1*, 131–137
11. Oleszek, W.A., Composition and quantification of saponins in alfalfa (*Medicago sativa L.*) seedlings, *Journal of Agricultural and Food Chemistry*, **1998**, *3*, 960–962
12. Plaza, L., Ancos De, B., Cano, P.M., Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycine max*), wheat (*Triticum aestivum L.)* and alfalfa (*Medicago sativa*) treated by a new drying method, *European Food Research Technology*, **2003**, *216*, 138–144
13. Story, J.A., Lepage, S.L., Petro, M.S., West, L.G., Cassidy M.M, et al., Interactions of alfalfa plant and sprout saponins with cholesterol in vitro and in cholesterol-fed rats, *Am J Clin Nutr*, **1984**, *39*(6), 917– 929
14. Coblentz, W.K., Bertram, M.G., Effects of a propionic acid–based preservative on storage characteristics, nutritive value, and energy content for alfalfa hays packaged in large round bales, *J Dairy Sci*, **2012**, *95*, 340–352
15. Han, Y., Khu,D.M., Monteros. M.J., High–resolution melting analysis for SNP genotyping and mapping in tetraploid alfalfa (*Medicago sativa L.), Mol Breed*, **2012**, *29*, 489–501
16. Butnariu,M., Simuleac, A., Dehelean, C., Chirita, R., Saratean, V., Studies concerning fertilizer influence (NPK in different doses) on quantity of corn plants chlorophyll, *Rev Chim-Bucharest* **2006,** *57*, 1138–1143
17. Chon, S.U., Kim, Y.M., Park, Y.J., Heo, B.G., Park, Y.S., Gorinstein S., Antioxidant and antiproliferative effects of methanol extracts from raw and fermented parts of mulberry plant (*Morus alba L.*), *Eur Food Res Technol.*, **2009**, *230*, 231–237
18. Sun,Y., Xu, W., Zhang, W., Hu, Q., Zeng, X., Optimizing the extraction of phenolic antioxidants from kudingcha made from Ilex kudingcha C.J. Tseng by using response surface methodology, *Sep Sci Technol*, **2011**, *78*, 311–320
19. Harborne, J.B., Phytochemical methods - A guide to modern techniques of plant analysis. Chapman and Hall, London, 1998
20. Ragavendran, D., Sophia, C., Raj, A., Gopalakrishnan, V.K., Functional group analysis of various extracts of aerva lanata (l.,) by FTIR spectrum, *Pharmacologyonline*, **2011**, *1*, 358-364
21. Pramila,D.M., Xavier, R., Marimuthu,K., Kathiresan, S., Khoo, M.L., Senthilkumar, M., Sathya, K. and Sreeramanan, S., Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: Lamiaceae). *Journal of Medicinal Plants Research*, **2012,** *6*(2), 331-335, DOI: 10.5897/JMPR11.1232
22. Du, F.Y., Xiao, X.H., Luo, X.J., Li, G.K., Application of ionic liquids in the microwave-assisted extraction of polyphenolic compounds from medicinal plants, *Talanta*, **2009**, *78*(3), 1177–1184, doi:10.1016/j.talanta.2009.01.04
23. Hirun, N., Dokmaisrijan, S., Tantishaiyakul. V., Experimental FTIR and theoretical studies of gallic acid-acetonitrile clusters, *Spectrochim Acta A Mol Biomol Spectrosc*, **2012**, *86*, 93–100
24. Selivantchikova, I.B., Lyakina, M.N., Kostennikova, Z.P., *Farmatsija*, **2001**, *6*, 14–15
25. Bilia,A.R., Bergonzi, M.C., Gallori, S., Mazzi, G., Vinceri, F.F., *J Pharm Biomed Anal* **2002a**, *30*, 613–624
26. Bilia, A.R., Bergonzi, M.C., Mazzi, G., Vinceri, F.F., *Drug Dev Ind Pharm* **2002b**, *28*, 609–619