Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PROXIMATE ANALYSIS OF SEEDS FROM SOME FIELD BORDER FLOWERING STRIPS

Aman PAUL[1a], Michel FREDERICH[2], Roel UYTTENBROECK[3a], Sandrino FILOCCO[1], Séverin HATT[4a], Priyanka MALIK[1], Arnaud MONTY[3], Frederic FRANCIS[4], Christophe BLECKER[1], Sabine DANTHINE[1]

[1]Food Science and Formulations Unit, Gembloux Agro-Bio Tech, University of Liege, Passage des Deportes-2, Gembloux (5030), Belgium, Phone number- 003281622303, Email- paul.aman@ulg.ac.be
[2]University of Liege, Center for Interdisciplinary Research on Medicines (CIRM), Department of Pharmacy, Laboratory of Pharmacognosy, Building B36, Avenue de l´Hopital 1, Liege (4000), Belgium, Phone number- 003243664330, Email- m.frederich@ulg.ac.be
[3]Biodiversity and Landscape Unit, Gembloux Agro-Bio Tech, University of Liege, Passage des Deportes-2, Gembloux (5030), Belgium, Phone number- 003281622245, Email- roel.uyttenbroeck@ulg.ac.be
[4]Functional and Evolutionary Entomology Unit, Gembloux Agro-Bio Tech, University of Liege, Passage des Deportes-2, Gembloux (5030), Belgium, Phone number- 003281622287, Email- severin.hatt@ulg.ac.be
[a]AgricultureIsLife.be, Gembloux Agro-Bio Tech, University of Liege, Passage des Deportes-2, Gembloux (5030), Belgium, Phone number- 003281622173, Email- paul.aman@ulg.ac.be

Corresponding author email: paul.aman@ulg.ac.be

Abstract

Field border flowering strips are commonly grown throughout the world mainly to enhance biodiversity. However besides their basic function they can also yield numerous compounds which could be interesting for wide range of industries such as food, pharmaceutical, etc. With the aim of valorization, proximate compositional analysis of seeds from some commonly grown flowering strips: Galium verum, Hypericum perforatum, Leontodon hispidus, Lotus corniculatus, Lythrum salicaria, Origanum vulgare and Trifolium pratense was realized. The protein content of residue left after the lipid extraction was also determined for exploring possibilities of its utilization as a protein source. Results suggest that seeds from some of these plants can be a potential source to render food compounds.

Key words: *wildflower strips, proximate composition, valorization.*

INTRODUCTION

Field border flowering strips are grown throughout the world and serve for a wide range of functions in agronomy, environment management, recreation/rural development, nature conservation, and adorn aesthetic value of landscape (Marshall & Moonen, 2002); most important being enhancement of biodiversity (Haaland et al., 2011). In recent years these factors have encouraged the emergence of a new industry engaged in commercial production of seeds from field border flowering strip. This development of agricultural practices to produce seeds in such a pure form will certainly promote other possibilities to utilize these seeds such as their ability to render food or pharmaceutical compounds.

Seeds aid in plant reproduction and for this purpose they carry reserves such as proteins, lipids and carbohydrates which are also an

important part of human diet (Bewley & Black, 1994). Seeds of some plants are directly used in human diets as cereals and legumes, while others such as oilseeds are source of lipids. These lipids are used as food and they also serve for a wide range of industrial purposes.

Earlier reports present reasonable data that seeds from some plants which are commonly grown in the strips can yield a broad spectrum of compounds which could be interesting for food and pharmaceutical industries (Azcan et al., 2004; Kocak et al., 2011). However analysis of seeds for compounds, from a large majority of plants in these strips still needs to be realized. Owing to the fact that seeds from some plants in the strips have already been explored to contain useful compound and the emergence of new sector which has the ability to commercially produce seeds, it could be really interesting to analyze the chemical composition of seeds from other plants in the field border flowering strips.

In the present research the seeds of some commonly grown plants in field flowering strips i.e. *Galium verum* L., *Hypericum perforatum* L., *Leontodon hispidus* L., *Lotus corniculatus* L., *Lythrum salicaria* L., *Origanum vulgare* L. and *Trifolium pratense* L. were investigated for moisture, ash, protein and lipid content to explore possibilities of utilizing seeds from field border flowering plants to render food compounds.

MATERIALS AND METHODS

Seeds of *G. verum*, *H. perforatum*, *L. hispidus*, *L. corniculatus*, *L. salicaria*, *O. vulgare* and *T. pratense* used for the investigation were procured from ECOSEM, Belgium.

Weight of the seeds, moisture content, protein content, ash content and lipid content was analyzed in the present investigation. Moreover the residue left after lipid extraction was also analyzed for protein content to explore possibilities of its valorization as a protein source.

Weight per 20 seeds was determined using KERN ALT 100-5AM electronic balance (KERN & Sohn GmbH, Germany). Seeds were grinded for 30 s in IKA A-10-B mill (IKA-Werke GmbH & Company, Germany) to obtain a fine powder which was used for further analysis.

Moisture and ash contents were estimated by official methods (AOCS, 2012). Protein content was calculated by multiplying the nitrogen content estimated using the rapid N cube (Elementar Analysensysteme GmbH, Germany) by 6.25.

For lipid extraction a slightly modified previously reported protocol utilizing 2:1 chloroform/methanol (Folch et al., 1957) was adopted. Supernatant was collected three times after repeated cycle of washing 5 g ground seeds with 25 ml of solvent subjected to 10 min of handshaking and then to centrifugation for 10 min at 1800 g in Avanti J-E (Beckman Coulter Inc., Belgium). This supernatant was filtered (5 µm Whatman filter paper, Sigma-Aldrich Company, Belgium) and collected in a 250 ml separation funnel. The washing of the filter paper was done using the same solvent until the solution in separation funnel was 160 ml. Following this 40 ml of 0.58 % NaCl solution was added in the funnel. The mixture was thoroughly mixed and allowed to stand overnight (Figure 1). The lower phase was then collected and 40 ml chloroform was added to the funnel. After standing for 5 hours, again the lower non-aqueous phase was removed. The solvent from this non-aqueous phase was removed by RE-121 Rotavapor (BUCHI Labortechnik GmbH, Netherlands) and nitrogen flushing. The seed residue left after lipids extraction was store at -20 °C and later analyzed for protein content using same method mentioned above.

Figure 1. Lipid Extraction Process

RESULTS AND DISCUSSIONS

The weight per 20 seeds is mentioned in the table 1. Some of the seeds were really small in size specially those of *O. vulgare*, *L. salicaria* and *H. perforatum*, this is also visible from the results. In some cases the standard deviation was high; this indicates that the weight of seeds were quite variable.

Table 1. Weight per 20 seeds. Results are expressed as mean ± standard deviation, n=3

S.NO.	Species	Weight per 20 seed (mg)
1	*G. verum*	8.4 ± 1.2
2	*H. perforatum*	1.7 ± 0.4
3	*L. hispidus*	33.9 ± 3.3
4	*L. corniculatus*	27.9 ± 2.0
5	*L. salicaria*	1.2 ± 0.4
6	*O. vulgare*	0.6 ± 0.1
7	*T. pratense*	37.5 ± 2.8

This seed weight data is in accordance to data previously published in literature i.e. mean seed weight of 0.4 mg for *G. verum*, 0.1 mg for *H. perforatum*, 0.8 mg for *L. hispidus*, 1.2 mg for *L. corniculatus*, 0.1 mg for *L. salicaria*, 0.1 mg for *O. vulgare* and 1.5 mg for *T. pratense* (Kuhn et al., 2004). Moisture content of the seeds is mentioned in Table 2.

Table 2. Moisture content (%) of seeds. Results are expressed as mean ± standard deviation, n=2

S.NO.	Species	Moisture content (%)
1	*G. verum*	9.21 ± 0.03
2	*H. perforatum*	6.51 ± 0.26
3	*L. hispidus*	7.85 ± 0.21
4	*L. corniculatus*	8.04 ± 0.04
5	*L. salicaria*	7.37 ± 0.26
6	*O. vulgare*	7.94 ± 0.72
7	*T. pratense*	8.70 ± 0.60

Table 2 indicates that moisture content of all the seven variety of seeds analyzed was roughly between 6-9 %. This moisture content should not be confused with the moisture in fresh seeds. This is the moisture content which is present in seeds after being subjected to different treatments by the supplier followed by packaging and storage in optimum conditions (one year in a cool and dry place in this case). Moisture content of oilseeds is an important parameter; it plays an important role not only in storage but also in industrial processes such as drying, milling and oil extraction (Lubatti & Bunday, 1960). It is really important to maintain optimum moisture content in seeds during storage which will cause minimum damage to the seed components and maintain seed viability. For oilseeds like rape the recommended moisture content during storage is between 7 to 10 % (Gawrysiak-Witulska et al., 2012) which is quite comparable to the moisture content in seed investigated. Ash content of the seeds on dry basis is mentioned in table 3.

Table 3. Ash content (%) of seeds on dry basis. Results are expressed as mean ± standard deviation, n=2

S.NO.	Species	Ash content (%)
1	*G. verum*	6.65 ± 0.05
2	*H. perforatum*	4.99 ± 0.18
3	*L. hispidus*	4.56 ± 0.06
4	*L. corniculatus*	4.67 ± 0.13
5	*L. salicaria*	5.46 ± 0.03
6	*O. vulgare*	3.03 ± 0.15
7	*T. pratense*	3.88 ± 0.08

Ash content of a sample is the measure of minerals present in the sample. It is well known that some minerals have special significance in proper body functioning. In the present investigation ash content of seeds from all the species were between 3-7 %. Further it could be interesting to analysis individual minerals present in the seeds.

Protein content of the seeds on dry basis is mentioned in figure 2, which clearly indicates that seeds from some plants in field border flowering strips offer an interesting source of proteins. The protein content of *T. pratense* and *L. corniculatus* were quite comparable to the proteins content of other high protein commonly consumed legume such as soybean i.e. 35.8 % (Christensen, 2009). Both of these plants belong to Leguminosae (Fabaceae) family which is known to have nitrogen fixing bacteria in their roots. These bacteria uptake atmospheric nitrogen and convert it into more usable form for the plants (Mafongoya et al., 2004), which maybe one of the reason behind the high protein content of the seeds from these plants. Seeds of *T. pratense* were found to have highest amount of proteins amongst all investigated. Previous

research reported maximum protein content of 17.3 % on dry basis in the seeds of *T. pratense* (Kratovalieva et al., 2012) which is much lower than the findings of current research. However analysis on other species in *Trifolium* family have supported the fact that protein content in plants of this family could even exceed 36 % (Kokten et al., 2011).

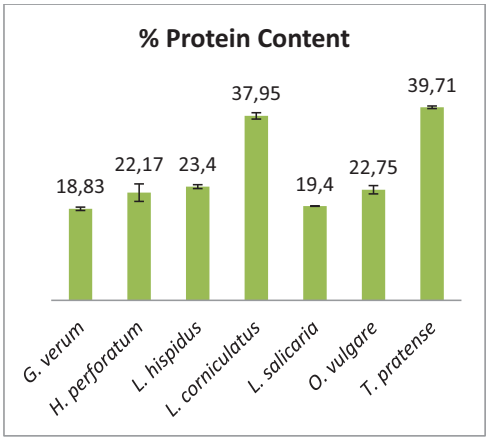

Figure 2. Protein content (%) of seeds on dry basis. Results are expressed as mean ± standard deviation, n=2

The chemical composition of seeds is related to the genetics, environment, climate and minerals in the soil (Mayer & Poljakoff-Mayber, 1982). This could also be one of the reasons behind variation of present results from the earlier published literature. The protein content of *L. corniculatus* seeds was in close proximity to the earlier investigation published (Kocak et al., 2011). However in order to introduce them as novel protein sources it is important to investigate their amino acid profile, protein digestibility and even the presence of antnutritional factors.

Lipid content of the seeds on dry basis is mentioned in figure 3. Some seeds have been extensively investigated and utilized by humans for edible and industrial oils. The seeds from some plants commonly grown in field border flowering strips are really interesting sources of lipids as indicated in figure 3. Particularly *H. perforatum*, *O. vulgare* and *L. salicaria* have considerably high amount of lipids.

Seeds from all three species contain more lipids then proteinaceous seeds such as soybean i.e. 18.4 % (Christensen, 2009) but less then oleaginous seeds such as rapeseed i.e. 41% (Wang, 2010). The lipids from *H. perforatum* were similar to the results documented earlier i.e. 27.2-30.6 % (dry basis) using diethyl ether and methyl-tert-butyl ether as solvent (Fontanel, 2013). For *O. vulgare* the lipid content was higher than the earlier reports (20.09 % on dry basis) where hexane was used for extraction (Azcan et al., 2004).

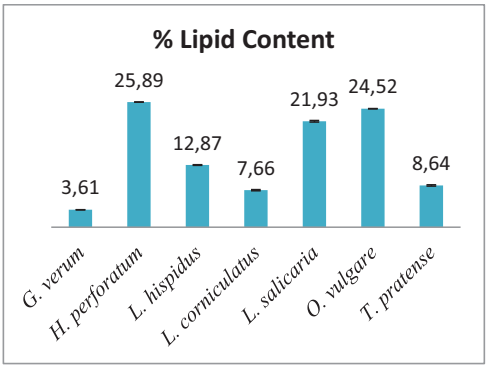

Figure 3. Lipid content (%) of seeds on dry basis. Results are expressed as mean ± standard deviation, n=2

In the above statement, the value was converted from wet basis (as reported in literature) to dry basis considering the experimental results of moisture content in *O. vulgare* seeds of 7.94 %. Further it could be interesting to investigate the lipid content of some other plants in the flowering strips and also the composition (fatty acid composition, triacylglycerol profile and other minor compounds) of these lipids to explore their food or industrial usage. Protein content of seed residue left after lipid extraction on wet basis is mentioned in the Table 4.

Table 4. Protein content (%) of seed residue left after lipid extraction on wet basis. Results are expressed as mean ± standard deviation, n=2

	Species	Protein content (%) in seed residue left after lipid extraction
1	*G. verum*	17.49 ± 0.20
2	*H. perforatum*	19.08 ± 0.32
3	*L. hispidus*	14.63 ± 1.24
4	*L. corniculatus*	32.63 ± 0.19
5	*L. salicaria*	18.69 ± 0.28
6	*O. vulgare*	23.63 ± 1.60
7	*T. pratense*	37.07 ± 0.51

Defatted seeds cakes are by-products of oil extraction industry. They have been utilized in a wide range of application such as food, feed, fuel and raw material of biotech products (Ramachandran et al., 2007). The seed residue left after lipid extraction from *T. pratense* and *L. corniculatus* has protein in considerably high quantities.

These results are in accordance to the protein content in non-defatted seed powder reported earlier in this paper. Protein content of defatted seed residue from *T. pratense* and *L. corniculatus* are quite comparable to the protein content of mustard and sunflower seeds press-cakes i.e. 38.5 % and 34.1 %. (Ramachandran et al., 2007). It is important to note that during the process of lipid extraction some non-lipid compounds (soluble in chloroform and methanol) are washed away. The residue left after lipid extraction from some of the high lipid containing seeds could be used to prepare protein concentrates, animal feeds, etc.

CONCLUSIONS

Field border flowering strips play important roles in agronomic practices, environmental management and other functions. Seeds from some of these field border flowering strips could be an interesting source of food compounds. Investigation in the present research revealed that seeds from *H. perforatum*, *O. vulgare* and *L. salicaria* contains considerable high amount of lipids, while the seeds of *T. pratense* and *L. corniculatus* are rich in proteins.

Results indicate that seeds from some of these plants could be potential alternate to commercially used oil source such as soybean. However besides these quantitative parameters, it is also important to study the quality of proteins (amino acid profile, digestibility and antnutritional factors) and lipids (fatty acid profile, triacylglycerol profile and minor components) in these seeds. Further, more research is required on compounds from seeds of these plants and some other plants which are grown in wildflower strips to explore their food and industrial potential.

ACKNOWLEDGMENTS

This research was made possible from the financial support of the CARE Agriculture Is Life, Gembloux Agro-Bio Tech, University of Liege, Belgium.

REFERENCES

AOAC, 2012. Official Methods of Analysis of AOAC International 19th ed., AOAC International, Maryland.

Azcan N., Kara M., Demirci B., Baser K., 2004. Fatty Acids of the Seeds of *Origanum onites L.* and *O. vulgare L.* Lipids, 39, 487-489.

Bewley J.D., Black M., 1994. Seeds. In: Seeds: Physiology of Development and Germination. Springer USA, New York, 1-33.

Christensen T., 2009. Nutritional Information, The Danish Food Composition Databank. Available at: http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=0032 [Accessed February 7, 2015].

Folch, J., Lees M., Stanley G.H.S., 1957. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues.

Fontanel D., 2013. Exceptionally High Content of Unsaponificable Matter in Plant Species with High Fat Content. In: Unsaponificable Matter in Plant Seed Oils. Springer, Heidelberg, 317-318.

Gawrysiak-Witulska M., Rudzinska M., Wawrzyniak J., Siger A., 2012. The Effect of Temperature and Moisture Content of Stored Rapeseed on the Phytosterol Degradation Rate. Journal of the American Oil Chemists' Society, 89, 1673-1679.

Haaland C., Naisbit R.E., Bersier L.-F., 2011. Sown Wildflower Strips for Insect Conservation: A Review. Insect Conservation and Diversity, 4, 60-80.

Kocak A., Kokten K., Bagci E., Akcura M., Hayta S., Bakoglu A., Kilic O., 2011. Chemical Analysis of Seeds of Some Forage Legumes from Turkey. A Chemotaxonomic Approach. Grasas y Aceites, 62, 383-388.

Kokten K., Kocak A., Kalpan M., Akcura M., Bakoglu A., Bagci E., 2011. Tannin, Protein Content and Fatty Acid Composition of the Seeds of Some *Trifolium L*. Species from Turkey. Asian Journal of Animal and Verterinary Advances, 6, 88-95.

Kratovalieva S., Popsimonova G., Ivanovska S., Jankuloski L., Meglic V., 2012. Macedonian Genebank: Seed Protein Content of Wild Red Clover (*Trifolium pratense L.*) Accessions. Agriculturae Conspectus Scientificus, 77, 199-202.

Kuhn I., Durka W., Koltz S., 2004. BiolFlor – A New Plant-trait Database as a Tool for Plant Invasion Ecology. Diversity and Distribution, 10, 363-365.

Lubatti O.F., Bunday G., 1960. The Water Content of Seeds. I.- The Moisture Relations of Seeds Peas etc. Journal of the Science of Food and Agriculture, 11, 685-690.

Mafongoya P.L, Giller K.E., Odee D., Gathumbi S., Ndufa S.K., Sitompul S.M., 2004. Benefiting from N_2-Fixation and Managing Rhizobia. In: van Noordwijk M., Cadisch G., Ong C. (Eds.). Below-Ground Interactions in Tropical Agroecosystems 1st ed. CABI, Oxfordshire, 227-242.

Marshall E.J.P., Moonen A.C., 2002. Field Margins in Northern Europe: Their Functions and Interaction with Agriculture. Agriculture, Ecosystems and Environment, 89, 5-21.

Mayer A.M., Poljakoff-Mayber a., 1982. Chemical Composition of Seeds. In: The Germination of Seeds 3rd ed. Pergamon Press, Oxford, 10-21.

Ramchandran S., Singh S.K., Larroche C., Soccol C.R., Pandey A., 2007. Oil Cakes and their Potential Biotechnological applications- A Review. Bioresource Technology, 98, 2000-2009.

Wang H., 2010. Review and Future Development of Rapeseed Industry in China. Chinese Journal of Oil Crop Sciences, 2, 300-302.