

XXIII Encontro Nacional da SPQ



Aveiro 12 a 14 de Junho de 2013



The response of rapeseed cultivar Hydromel to nitrogen fertilization: chemical composition and antioxidant capacity

Dina Costa,^a Clementina M. M. Santos,^{a,b} Artur M. S. Silva,^b Manuel A. Rodrigues^{a,c}

^aDepartamento de Produção e Tecnologia Vegetal, Escola Superior Agrária, Instituto Politécnico de Bragança, 5301-855 Bragança, Portugal; ^bDepartment of Chemistry & QOPNA, University of Aveiro, 3810-193 Aveiro, Portugal; ^cCIMO, Instituto Politécnico de Bragança, Portugal

Winter rapeseed (*Brassic napus* L.) is a species of the Brassicaceae family and an important agricultural crop in many cold temperate countries, where most of other plants do not grow.^[1] It is mainly cultivated in Europe, Asia, North America and Australia, but has a limited acreage in Portugal. The growing demand from rapeseed is due to a progressive increase in its quality for human and animal consumption and mainly because it is being promoted throughout the world as an energy crop for the production of biofuels.^[2] Many studies have shown that both growth and yield of rapeseed are enhanced significantly by high doses of applied nitrogen. However, an excessive nitrogen rate could reduce the nutritional quality of seeds and can negatively affect the farmer economy and environment.^[3,4]

Rapeseed and its products (vegetable oil) contain a high content of bioactive compounds such as phenolic acids, phytosterols, tocopherols, flavonoids, among others. These compounds have demonstrated antioxidant activity, which has been associated to the prevention and treatment of several diseases such as cancer, diabetes, hypertension and degenerative diseases (Alzheimer and Parkinson). In this context, rapeseed compounds have been used as natural antioxidants in several industries such as food, cosmetic and pharmaceutical.^[5]

In this communication we will present and explore the effect of nitrogen fertilization on the chemical and antioxidant properties of rapeseed cultivar Hydromel. Samples of rapeseed were obtained in a field trial held in Bragança, in which rapeseed was grown in the winter / spring season of 2009/2010. It was determined moisture content, ash, protein, fat and fiber (NDF and ADF). The antioxidant activity of the methanolic extracts of rapeseed was measured by DPPH radical scavenging activity, total phenolic content and ferric reducing capacity. Possible correlations between these parameters and industrial applications will be discussed.

Acknowledgments: Thanks are due to Instituto Politécnico de Bragança, Universidade de Aveiro, Fundação para a Ciência e a Tecnologia (FCT), European Union, QREN, FEDER and COMPETE for funding the QOPNA Research Unit (project PEst-C/UII/10062/2011).

References

- [1] Bockisch, M. Fats and oils handbook, *AOCS PRESS*, 1998, p. 251.
- [2] Körbitz, W. Brassica oilseeds. Production and utilization, Eds. Kimber, D.; McGregor, D.I.; Wallingford, UK: Cab International, 1995, p. 195.
- [3] Öztürk, Ö. *Chilean J. Agric. Res.* **2010**, *70*, 132.
- [4] Ozer, H. *Eur. J. Agron.* **2003**, *19*, 453.
- [5] a) Szydłowska-Czerniak, A.; Bartkowiak-Broda, I.; Karlovic, I.; Karlovits, G.; Szlyk, E. *Food Chem.* **2011**, *127*, 556. b) Szydłowska-Czerniak, A.; Trokowski, K.; Karlovits, G.; Szlyk, E. *J. Agric. Food Chem.* **2010**, *58*, 7502. c) Vuorela, S.; Meyer, A. S.; Heinonen, M. *J. Agric. Food Chem.* **2004**, *52*, 8202.

The response of rapeseed cultivar Hydromel to nitrogen fertilization: chemical composition and antioxidant capacity

Dina Costa,^a Clementina M. M. Santos,^{a,b} Artur M. S. Silva,^b Manuel A. Rodrigues^{a,c}

^a Departamento de Produção e Tecnologia Vegetal, Escola Superior Agrária, Instituto Politécnico de Bragança, 5301-855 Bragança, Portugal

^b Departamento de Química & QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal

^c CIMO, Instituto Politécnico de Bragança, 5301-855 Bragança, Portugal



QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL PORTUGAL 2007-2013



COMPETE PROGRAMA OPERACIONAL FACTORES DE COMPETITIVIDADE



UNIÃO EUROPEIA FEDER



INSTITUTO POLITÉCNICO DE BRAGANÇA Escola Superior Agrária



universidade de aveiro



FCT Fundação para a Ciência e a Tecnologia

INTRODUCTION

Winter rapeseed (*Brassica napus* L.) is a species of the Brassicaceae family and an important agricultural crop in many cold temperate countries, where most of other plants do not grow [1]. It is mainly cultivated in Europe, Asia, North America and Australia, but has a limited acreage in Portugal. The growing demand from rapeseed is due to a progressive increase in its quality for human and animal consumption and mainly because is being promoted throughout the world as energy crop for the production of biofuels [2].

Many studies have shown that both growth and yield of rapeseed are enhanced significantly by high doses of applied nitrogen. However, an excessive nitrogen rate could reduce the nutritional quality of seeds and can negatively affect the farmer economy and environment [3,4].

Rapeseed and its products (vegetable oil) contain a high content of bioactive compounds such as phenolic acids, phytosterols, tocopherols, flavonoids, among others. These compounds have demonstrated antiradical activity, which has been associated to the prevention and treatment of several diseases such as cancer, diabetes, hypertension and degenerative diseases (Alzheimer and Parkinson). In this context, rapeseed compounds have been used as natural antioxidants in several industries such as feed, cosmetic and pharmaceutical [5].

MATERIAL AND METHODS

Rapeseed samples

Field experiments with rapeseed winter cultivar were carried out during the growing season of 2009/2010 in the Santa Apolónia farm in Bragança, NE Portugal. In pre-sowing, a herbicide (trademark Devrindl) was applied and immediately incorporated into the soil with a tiller. Different doses of nitrogen [corresponding to 0 (N₀), 50 (N₅₀) and 150 (N₁₅₀) kg N per hectare] were installed in main-plots of 630 m² with mechanical sowing. Each main-plot was harvested three different samples of rapeseeds in different locations. Rapeseed samples were then stored in the dark at room temperature, until treatment and further analysis.

It was determined moisture, ash, protein and fat were determined according to the methodologies described in "Association of Official Analytical Chemists (AOAC)". Fiber content were obtained by the Van Soest method: neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Table 1).

Table 1. Chemical composition of rapeseed cultivar Hydromel with different doses of applied nitrogen (mean ± standard deviation).

| | N ₀ | N ₅₀ | N ₁₅₀ |
|------------------------------|----------------|-----------------|------------------|
| Moisture (%) | 4.64±0.04 | 4.78±0.09 | 4.54±0.25 |
| Ash (%) | 4.10±0.10 | 4.21±0.10 | 3.95±0.05 |
| Crude protein (%) | 13.93±0.42 | 15.74±0.15 | 17.08±0.65 |
| Crude fat (%) | 47.36±0.61 | 45.78±0.51 | 44.62±0.89 |
| Carbohydrates (%) | 29.97±1.83 | 29.49±1.34 | 29.81±2.78 |
| Energy (kcal/ 100g of seeds) | 602 | 593 | 589 |
| NDF (% d.m.) | 34.81±1.68 | 34.47±1.69 | 34.06±1.08 |
| ADF (% d.m.) | 31.08±3.76 | 27.38±2.81 | 25.45±2.84 |
| ADL (% d.m.) | 18.01±3.37 | 16.57±1.44 | 13.24±1.76 |
| Celulose (% d.m.) | 13.1 | 10.8 | 12.2 |
| Hemicelulose (% d.m.) | 3.7 | 7.1 | 8.6 |

Important to consider in the production of feed!

Important to consider in the production of biofuels!

Feed consumption
Digestibility
Lignin

Fiber content

Antioxidant assays

Preparation of the methanolic extracts

Room temperature: Rapeseed samples (typically 5 g) were extracted by stirring with 100 ml of methanol at 25 °C at 150 rpm for 24 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol. The combined extracts were evaporated at 40 °C to dryness and weighed to determine the yield (Table 2).

Reflux temperature: Rapeseed samples (typically 5 g) were extracted with methanol using a soxhlet apparatus for 8 h and filtered through Whatman No. 4 paper. The residue was evaporated at 40 °C to dryness and weighed to determine the yield (Table 2).

Methanolic stock solutions at a concentration of 50 mg/ml were prepared and stored at 4°C for further use.

Table 2. Extracts yields (%) obtained from methanolic extraction at room temperature and reflux of rapeseed cultivar Hydromel (mean ± standard deviation).

| | N ₀ (%) | N ₅₀ (%) | N ₁₅₀ (%) |
|------------------|--------------------|---------------------|----------------------|
| Room temperature | 16.0±1.0 | 18.4±1.3 | 20.1±2.3 |
| Reflux | 27.4±0.9 | 26.1±2.9 | 24.2±3.0 |

The antioxidant activity of the methanolic extracts of rapeseed was measured by total phenolic content, DPPH radical scavenging activity and ferric reducing power (Table 3).

Table 3. Antioxidant activity of methanolic extracts of rapeseed cultivar Hydromel at room temperature and reflux (mean ± standard deviation).

| Antioxidant activity | Temperature | N ₀ | N ₅₀ | N ₁₅₀ |
|---|------------------|----------------|-----------------|------------------|
| Total phenols (mg GAE /g extract) | Room temperature | 25.73 | 18.34 | 17.07 |
| | Reflux | 20.48 | 19.39 | 22.40 |
| DPPH scavenging activity EC ₅₀ (mg/ml) | Room temperature | 0.93±0.05 | 0.92±0.01 | 1.07±0.03 |
| | Reflux | 1.34±0.02 | 1.07±0.06 | 1.13±0.04 |
| Reducing power EC ₅₀ (mg/ml) | Room temperature | 0.99±0.02 | 1.03±0.02 | 1.00±0.01 |
| | Reflux | 1.36±0.13 | 1.28±0.07 | 1.33±0.04 |

- * Rapeseed extracts obtained from extraction at room temperature showed the highest antioxidant efficiency than those obtained from extraction at boiling temperature, for all methodologies tested.
- * N₀ extract obtained in the extraction at room temperature showed the highest total phenol content, 25.73 mg of GAE/g extract.
- * N₅₀ extracts presented the highest DPPH scavenging activity at room temperature and at reflux.
- * Similar reducing power were obtained in the extracts at room temperature. At reflux, N₅₀ extract has the greater efficiency (EC₅₀ = 1.28 mg/ml).

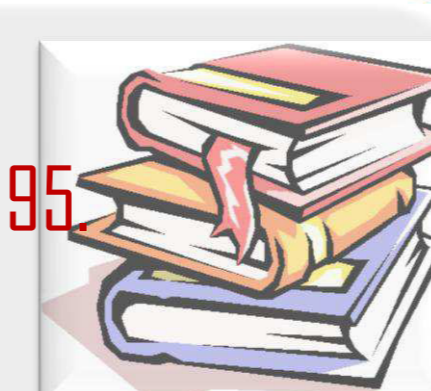
1. Bockisch, M. Fats and oils handbook, *AOCS PRESS*, 1998, p. 251.

2. Körbitz, W. Brassica oilseeds. Production and utilization, Eds. Kimber, D.; McGregor, D.I.; Wallingford, UK: Cab International, 1995, p. 195.

3. Öztürk, Ö. *Chilean J. Agric. Res.* 2010, 70, 132.

4. Ozer, H. *Eur. J. Agron.* 2003, 19, 453.

5. a) Szydłowska-Czerniak, A.; Bartkowiak-Broda, I.; Karlovic, I.; Karlovits, G.; Szlyk, E. *Food Chem.* 2011, 127, 556. b) Szydłowska-Czerniak, A.; Trokowski, K.; Karlovits, G.; Szlyk, E. *J. Agric. Food Chem.* 2010, 58, 7502. c) Vuorela, S.; Meyer, A. S.; Heinonen, M. *J. Agric. Food Chem.* 2004, 52, 8202.



Acknowledgments

Thanks are due to Instituto Politécnico de Bragança, Universidade de Aveiro, Fundação para a Ciência e a Tecnologia (FCT), European Union, QREN, FEDER and COMPETE for funding the QOPNA Research Unit (project PEst-C/QUI/UI0062/2011).



XXIII ENCONTRO NACIONAL DA SPQ

DESAFIOS EM QUÍMICA

Universidade de Aveiro
12-14 de Junho de 2013



Certificado de Participação

Certifica-se que Clementina Maria Moreira dos Santos apresentou a Comunicação em painel “The response of rapeseed cultivar Hydromel to nitrogen fertilization: chemical composition and antioxidant capacity”, durante o XXIII Encontro Nacional da SPQ, que decorreu de 12 a 14 de junho de 2013, na Universidade de Aveiro.

Artur Silva

Presidente da Comissão Organizadora e Científica

Aveiro 12 a 14 de junho de 2013

Data