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

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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 Devrinol) was applied and immediately incorporated into the soil with a tiller. Different doses of nitrogen [corresponding to 0 (N_0), 50 (N_{50}) and 150 (N_{150}) kg N per hectare] were installed in main-plots of 630 m^2 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

- Rapeseed is mainly constituted by fat. The N_0 sample had the highest fat content (47.36%). The fatty acid profile (obtained by GC/FID) is mainly composed by monounsaturated fatty acids, oleic acid being the most abundant (60%).
- The protein content in the samples varied, the greatest value was found in sample N_{150} (17.08%), which possess the highest nitrogen fertilization, while the lower protein content was found in the sample without fertilization (13.93%).
- The content of NDF was similar in all samples (approximately 34%). Moreover, with increasing nitrogen fertilization, the content of ADF was lower.

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 4ºC 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.
- N_0 extract obtained in the extraction at room temperature showed the highest total phenol content, 25.73 mg of GAE/g extract.
- N_{50} 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, N_{50} extract has the greater efficiency (EC_{50} = 1.28 mg/ml).

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