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Bioactive Compounds Present in Linseed (Linum Usitatissimum L.) and Millet (Panicum Miliaceum L.) Seeds Under Different Germination Times Reveal Important Sources of Antioxidants for Human Health and Nutrition

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Abstract

The purpose of this study is to evaluate the chemical composition and fatty acids in flax and millet seeds, in addition to exploring the effect of germination time on the seeds, with regard to chlorophyll, carotenoid, and phenol rates, as well as antioxidant capacity. Flax provided the highest lipid percentage (48.3%) when compared to millet (8.7%). The most abundant fatty acid in the flax seed was linolenic acid whereas palmitic, palmitoleic, linoleic and arachidic acids were the main fatty acids in the millet. We detected differences between flax and millet seeds at 72, 96 and 120 hours after germination, with higher rates of total chlorophyll and arytenoids in flax seeds, and higher rates of total phenols and antioxidant capacity in millet seeds. Flax and millet sprout intake may be an alternative to increase bio compounds such as arytenoids and phenols in human diet.

Keywords : phenolic compounds, antioxidant capacity; fatty acids.

Introduction

An excellent alternative for increasing theintake of flax and millet seeds are shoots or sprouts that may be consumed fresh throughout the year (Pająk, Socha, Gałkowska, Rożnowski & Fortuna, 2014;Silva, 2017). The germination of edible seeds for the production of sprouts modifies the food´s texture and nutritional value (Dueñas, Hernández, Estrella & Fernández, 2009; Martinez-Villaluenga et al., 2010; Hung, Hatcher & Barker, 2011). Several studies report high rates of nutrients and reduced anti-nutrients in sprouts, as compared to non-germinated seeds (Oloyo, 2004; Zieliński, Frias, Piskuła, Kozłowska & Vidal-Valerie, 2005; Martinez-Villaluenga et al., 2010). Germinated seeds are actually sources of antioxidant agents such as phenol acids, flavonoids, vitamins, amino-acids and fibers (Paśko, Sajewicz, Gorinstein & Zachwieja, 2008).

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The germination period is a determining factor in the antioxidants' composition , as it affects the accumulation of compounds such as Vitamin C and α-tocopherol (Yang, Basu & Ooraikul, 2001; Frias, Miranda, Doblado & Vidal-Valverde, 2005; Fernandez-Orozco, Frias, Zielinski, Piskula, Kozlowska & Vidal-Valverde, 2008), as well as the composition of fatty acids (Shakuntala, Naik, Jeyarani, Naidu & Srinivas, 2011). Additionally, climate and agronomic conditions during growth, storage conditions and the seed variety used may influence the accumulation of these bioactive compounds (Cevallos-Casals & Cisneros-Zealots, 2010).

Most research mainly focuses on l more common shoots or sprouts, such as wheat (Yang, Basu & Ooraikul, 2001), mung bean, soybean (Fernandez-Orozco, Frias, Zielinski, Pascua, Kozlowska & Vidal-Valerie, 2008) and broccoli (Martinez-Villaluenga et al., 2010;Silva, 2017), all being widely available on the market. Flax and millet sprouts are new vegetable options which may be easily included in one's diet.

Considering the importance of the biocomposites in foods and that the germination process improves the content of the same, the objective was to evaluate in linseed and millet seeds the chemical composition and the profile of fatty acids, and the effect of different germination times of these seeds in the levels of chlorophylls, arytenoids, phenols and antioxidant capacity.

2. Materials and Methods

2.1. Chemical reagents

We obtained β-carotene, Folin-Ciocalteu reagent, gallic acid, 2,2-dyphenyl-1-picrylhydrazyl(DPPH), 2-carboxylic-6-hydroxy-2,5,7,8-tetramethylchromane acid (Trolox) and caproic, caprylic, capric, caproleic, lauric, dodecanoic, muriatic, myristoleic, politic, palmitoleic, margaric, heptadecenoic, stearic, oleic, linoleic, linolenic, arachidic, gadoleico, eicosadienoico, eicosadienoico, eicosatrienoic, tetraenoic, lignoceric and nervonic acids from Sigma-Aldrich, St. Louis, USA. Reagents for spectrophotometric and chromatographic analyses were of HPLC degree; reagents of the highest purity degree were used for all the other analyses (p.a.).

2.2. Samples

In 2012, we acquired flax and millet seeds from commercial producers in the municipalities of Giruá (28°00'10,4" S; 54°13'3,5" W; altitude 343 m) and Novo Machado (27°34'37'' S; 54°30'14'' W; altitude 232 m), in the northwestern region of the state of Rio Grande do Sul, Brazil. According to Köppen and Geiger (1928), the climate is Cfa, or a humid subtropical climate. In 2014, mean annual temperature and rainfall were 19.6°C and 1,847 mm and 20.3°C and 1,742 mm, respectively, for Giruá and Novo Machado.The region´s soil can be classified as typical Red Dystrophic Lactose (Embrapa-CNPS, 2006).

We conditioned the seeds in 30L opaque plastic bags, and maintained them in a refrigerator at 10ºC until starting of the experiment. We manually selected the seeds, in order to remove any

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damaged seeds or foreign objects. We then sanitized the seeds by immersing them inin a solution of 1% sodium hypochlorite for 3 minutes and afterwards washing them with distilled water, following the Rules for Seed Analysis (RAS) (Brasil, 2009). We analyzed seed quality using germination and vigor tests. The former was performed with 200 seeds (two sub-samples with 100 seeds each) and four replications. We distributed the seeds in gearboxes on two sheets of germ test paper moistened with distilled water (equivalent to 2.5 times the mass of the dry paper) and placed them in a germinator at a constant 25ºC (±2) for the millet seed, and at 21ºC (±2) for the flax seed, with 80% (±5) relative air humidity and 24-h luminosity for the two samples. Three and seven days after the start of the experiment, we counted the percentage of normal seedlings, following the criteria presented by RAS (Brasil, 2009). We executed the vigor test during the first germination test count, on the third day after installation, registering the percentage of normal seedlings following the recommendations outlined by RAS (Brasil, 2009).

2.3. Sprouts

The complete randomized experimental design was arranged in a bi-factor format, with three replications. Factor A was the type of seed (flax or millet), and factor B germination time (0, 24, 48, 72, 96 and 120 hours after germination). We sanitized the seeds by immersing them in a solution of 1% sodium hypochlorite for 3 minutes and then washed them with distilled water, following RAS (Brasil, 2009). They were then distributed in gearboxes on two sheets of germ test paper wetted with distilled water (equivalent to 2.5 times the mass of the dry paper) and placed in a germinator with the same conditions as those established in the germination test.
Sprout harvest was performed 24, 48, 72, 96 and 120 hours after germination, with a control sample (0 h) without any germination. Root protrusion indicated germination and standardized the harvest. Samples of non-germinated flax and millet seeds (control) and seeds at different times of germination were ground in a ball mill (Marconi, MA 350) until becoming first flour and then paste, thus homogenizing the sample. We stored the samples in plastic bags with identification tags and stored them in an ultra freezer (-80ºC) until analysis.

2.4. *Characterization of seeds and sprouts*

2.4.1. *Chemical composition*
We used gravimetric to analyze the humidity of the samples in order to determine their chemical composition, crude fiber was determined by acid and alkaline digestion; lipids were determined by ether extract by Sox let method; protein by micro-Kjedahl system employing the factor 6.25 to convert nitrogen into protein rate; carbohydrates were determined by the difference, subtracting the sum of humidity, protein, lipid, ashes and crude fiber rates from 100 (AOAC, 1995).

2.4.2. *Profile of fatty acids*
We obtained the profiles of the fatty acids of the two types of seeds by the cold extraction of lipids, following the description provided by Bligh and Dyer (1959). After extraction, we

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analyzed the fatty acids according to the method described by Hartman & Lago (1973), adding 500µL of KOH 0.1 N to the sample, which remained in a bain marie at 60ºC for 2 hours. After the mixture cooled, we added 1.5 mL of H_2SO_4 1 M to the sample and once more placed it in the bain marie at 60ºC for 2 hours. After cooling once more, we added 2mL of hexane, and agitated the tubes in order to collect 1 mL from the hexane phase with the methyl esters of the fatty acids We injected 1 µL of this mixtureinto a CG gas chromatograph (Perkin Elmer Clarus500), with a FID detector, capillary column (Phenomenex) with liquid phase made of 5% phenyl and 95 % dimethylpolysiloxane, measuring 15 m x 0.32 mm x 0.1 µm. Data were obtained and processed with Clarus 500. Temperature gradient was employed, with initial column temperature at 90°C for 1 minute; at 160°C with linear increase of 12°C min^{-1}, for 3.5 minutes; at 190°C with linear increase of 1.2°C min^{-1}; and finally, at 230°C with linear increase of 15°C min^{-1}, for 15 minutes. The injector and detector were maintained at 230 and 240°C, respectively. Nitrogen was used as carrier gas at 1.5 mL m^{-1}. Fatty acids were identified by comparing with retention times of methyl esters standards with caproic, caprylic, capric, caproleic, lauric, dodecanoic, myristic, myristoleic, palmitic, palmitoleic, barbaric, heptadecenoic, stearic, oleic, linoleum, linolenic, arachidic, gadoleico, eicosadienoico, eicosadienoico, eicosatrienoic, tetraenoic, lignoceric and nervonic acids. The rate of the samples´ fatty acid was calculated according to the peak area of the chromatograms, multiplied by 100 and divided by total area of the sample´s fatty acids, in percentage.
The other analyses of samples below were performed according to the different germination times of flax and millet seeds.

2.4.3. *Chlorophylls*

Chlorophyll rate was determined by 1 g samples ground in a mortar with 5 mL acetone 80% (v/v). The material was centrifuged at 10,000 rpm for 10 minutes and the supernatant transferred to a 20 mL flask, completing the volume with acetone 80% (v/v). Rates of chlorophyll *a*, *b*, total (*a*+*b*) were calculated by Lichtenthaler´s formulae (1987) from solution absorbance by spectrophotometry (Jenway, 6700) at 647 and 663 nm. Chlorophyll rates were determined by the equations below:
Total Chlorophyll = 7.15 (A_{663}) + 18.71 (A_{647});
Chlorophyll-a= 12.25 (A_{663}) – 2.79 (A_{647});
Chlorophyll-b= 21.50 (A_{647}) – 5.10 (A_{663}).
Results were given in mg g^{-1} of dry mass (DM) of seeds and sprouts.

2.4.4. Total carotenoids

Total carotenoid rates were determined by AOAC 970.64 method (2000), with modifications. One gram of fresh sample was ground and homogenized with 15 mL of the extracting solvent (hexane:ethane:acetone:toluene, at 10:6:7:7); 1 mL KOH 10 % (v/v) was added and a fast homogenization occurred in a warm bath at 56°C for 20 minutes. After removal from the warm bath, the sample was kept at room temperature for 1 hour. Further, 15 mL hexane aliquots were

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added to the flasks and then calibrated at 50 mL with Na_2SO_4 10 % (m/v), homogenized and kept in the dark for one hour. An aliquot of the supernatant was evaluated by spectrophotometer at wavelength 454 nm. Results were given in mg of β-carotene for 100 gram of DW of seed and sprout.

2.4.5. *Total phenols*

Rate of total phenols was determined by the Folin-Ciocalteu method. Extraction followed method by Khattak, Zeb, Bibi, Khalil and Khattak (2007), with modifications. Three grams of fresh sample, ground in a ball mill (Marconi, MA 350) were placed in a 50 mL Falcon tube, diluted in 20 mL and stirred for 20 minutes. The extracts were centrifuged at 7,000 rpm for 15 minutes. Further, 0.1 mL was removed from the methanol extract at the reaction stage and 0.4 mL of ultrapure water, 2.5 mL of Folin-Ciocalteau solution 0.2 mol L^{-1} and 2 mL of sodium carbonate 7.5 % (m/v) were added. After two hours of incubation, absorbance was compared to control by spectrophotometer at 760 nm (Meda, Lamien, Romito, Millogo & Nacoulma, 2005). Total phenol rate was given in milligrams of gallic acid equivalent (GAE) per gram of DW of seed and sprout.

2.4.6. Antioxidant capacity (DPPH)

The antioxidant capacity was determined by the sequester of free radicals of DPPH (2.2-diphenyl-1-picrylhydrazyl) modified by Moure, Franco, Sineiro, Domínguez, Núñez and Lema (2001). A 3.9 mL aliquot of DPPH 0.1 mol L^{-1} solution was mixed to 0.1 mL of the same methanol extract used for evaluating phenolic compounds. After incubation for 60 minutes, the sample´s absorbance was measured by spectrophotometer at 515 nm and results given in mg of Trolox equivalent antioxidant capacity (TEAC) per gram of DW of seed and sprout.

2.4.7. Statistical analysis

Data were analyzed for normality by Shapiro Wilk´s test; for homoscedasticity by Hartley´s test; for independence of residues by graph analysis. Data were later submitted to analysis of variance by test F ($p \leq 0.05$). When statistical significance was extant, the effects of seed types were compared by test t ($p \leq 0.05$). When the interaction of treatment factors existed, confidence intervals at 95 % were plotted on the graph and the difference was significant when there was no overlaying between the vertical bars. The effects of germination time were evaluated by non-linear regression models ($p \leq 0.05$) as below:
$y = ae^{bx}$, where y = variable response of interest; a = maximum rate for the variable response; b = curve slope; x = germination time (hours); e = constant. Co-relationship between the variables was analyzed by Pearson´s coefficient of co-relation.

3. Results and Discussion

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Germination rates for flax seeds were over 80% and results for millet were over 75%. Vigor rates were 53 and 46% for flax and millet seeds, respectively. Results demonstrate the high quality of the seeds since lowest germination rate for the commercialization of flax seed is 70% (Rio Grande do Sul, 2000). According to Instruction 381/1998 of the Ministry of Agriculture, millet seed lies in the item Other Grasses, with no germination percentage (Brasil, 1998). Since, millet and Panicum grass (*Panicum maximum* Jacq.) are grasses of the same genus and the latter´s germination standard is 40 % (Brasil, 1998), the percentage of millet germination in current assay is almost double the required rate. Consequently, current study analyzed high standard seeds, with great assurance in the studies undertaken.

There was no statistical significance in chemical composition for the treatment type of seed with regard to the variable ashes. In the case of the other variables, millet seeds had a higher percentage in humidity and carbohydrates, whereas percentage rates of lipids, proteins and fibers were higher for flax seed (Table 1).

Table 1

Chemical composition of flax and millet seeds.

Type of seed	Chemical composition (%)[1/]					
	Humidity	Lipids	Proteins	Ashes	Fibers	Carbohydrates
Flax	5.06±0.12 *	48.3±2.93 *	18.7±0.60 *	3.72±0.06 NS	8.35±0.55 *	15.8±3.68 *
Millet	9.50±0.06	8.70±0.17	12.5±0.32	3.83±0.05	5.22±0.20	60.3±0.38

[1/] Mean of three determinations ± standard deviation.

* Significant by test t ($p \leq 0.05$) when types of seeds are compared. NS: not significant by test F ($p \leq 0.05$).

Flax and millet seeds had low humidity rates, propitious for storage during a long period of time, since the higher the humidity rate the greater is the decomposition by microbial activities (Bozan& Temelli, 2008). High protein levels of the seeds under analysis reveal their excellent protein reserves which, in their turn, provide amino-acids used in the germination and growth of the seedlings (Shewry, Napier & Tatham, 1995). As expected, the oleaginous flax seed had a high lipid percentage (40 – 60%) within the range conceived by Dorrel (1970).

Results of the main chemical components of flax and millet seeds were similar to rates given in the literature (Mueller, Eisner, Yoshie-Stark, Nakada & Kirchhoff, 2010; Devisetti, Yadahally&Bhattacharya, 2014).

There was no statistical significance for seed type with regard to stearic, oleic, erucic and total unsaturated fatty acids. Linolenic acid was the major fatty acid, whereas palmitic, palmitoleic, linoleic and arachidic acids were the main fatty acids in the millet seeds (Table 2). It is a well-known fact that oil composition of plants cultivated at low temperatures (in this case, the seeds under analysis) has a high concentration of unsaturated fatty acids, and thus the best temperatures of the fusion point (Castro, Klugue & Peres, 2005).

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The difference in the composition of fatty acids in vegetal lipids varies according to the species (Taiz & Zeiger, 2004). In fact, plants are renewable sources of fatty acids. In fact, several species accumulate then as triacylglycerols, or rather, the main reserve components in the seeds (Thelen&Ohlrogge, 2002).

Fatty acids are important for human health since they are the precursors of eicosanoid biosynthesis, or rather, the bio-regulators of several cell metabolic processes (Khotimchenko & Yakovleva, 2005; Gressler et al., 2010). Moreover, fatty acids Omega-3, a major component in flax seeds, and Omega-6, a major component in millet seeds, are not merely a source of energy but also provide the essential biological functions required by the human body. It has been suggested that ingestion of fatty acids Omega-3 and Omega-6 is associated with decrease in risks for cardiovascular diseases and benefits people suffering from chronic diseases (Simopoulos, 2008). The incorporation of flax and millet seeds in the diet may enhance human health.

Table 2

Fatty acids (%) in flax and millet seeds

Type of seed	Fatty acids (%)**				
	C16:0	C16:1	C18:0	C18:1	C18:2
Flax	5.67±0.38 *	0.00±0.00[1/] *	2.58±0.49 [NS]	22.20±1.55 [NS]	13.20±0.25 *
Millet	7.14±0.06	0.14±0.00	2.00±0.00	23.09±0.00	65.10±0.26

Type of seed	C18:3	C20:0	C22:1	Totally saturated	Totally unsaturated
Flax	54.40±1.57 *	0.07±0.10 *	1.36±0.72 [NS]	8.32±0.21 *	91.16±0.50 [NS]
Millet	1.18±0.02	0.51±0.02	0.00±0.00	9.65±0.04	89.51±0.28

* Significant by test t ($p \leq 0.05$) when types of seed are compared. [NS]: not significant by test F ($p \leq 0.05$). [1/] Mean of three determinations ± standard deviation. ** Fatty acids: C16:0: palmitic; C16:1: palmitoleic; C18:0: stearic; C18:1: oleic; C18:2: linoleic; C18:3: linolenic; C20:0: arachidic; C22:1: erucic.

There was a significant interaction between treatment factors type of seed and germination time for the variables chlorophyll *a* and *b* and total chlorophyll, carotenoids, total phenols and antioxidant capacity (Figures 1 and 2). There was a difference between flax and millet seeds at germination times 72, 96 and 120 hours when chlorophyll *a* and *b* andtotal chlorophyll of the two types of seeds were compared. The variable was adequately adjusted to the equation of exponential regression, featuring rates of the coefficient of determination (R^2) with 0.90 and 0.76 (Fig. 1A), 0.94 and 0.93 (Fig. 1B) and 0.94 and 0.89 (Fig. 1C) respectively for flax and millet seeds, and thus a satisfactory adjustment of the data to the model. The seed revealed increasing

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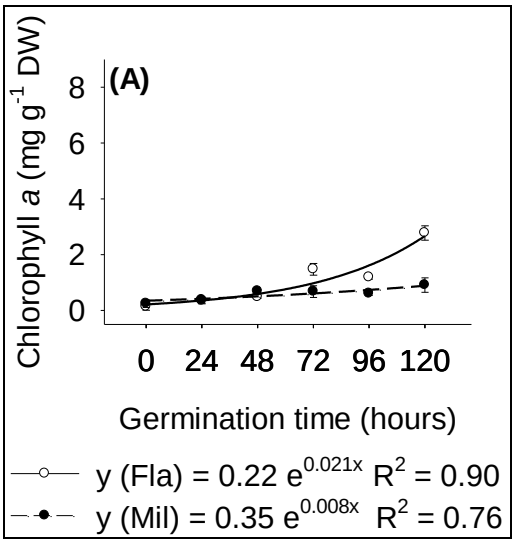
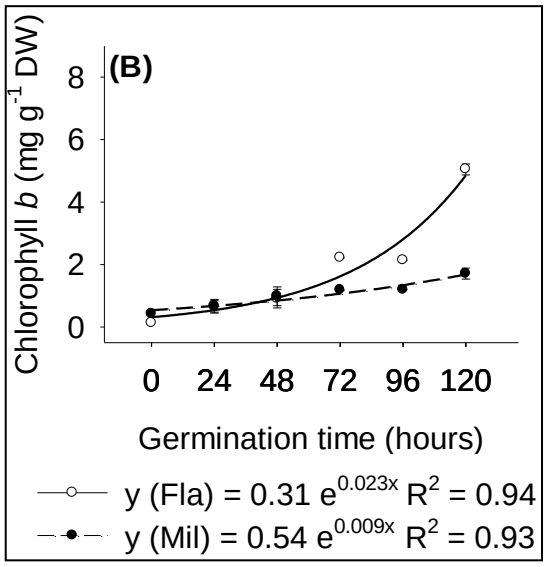
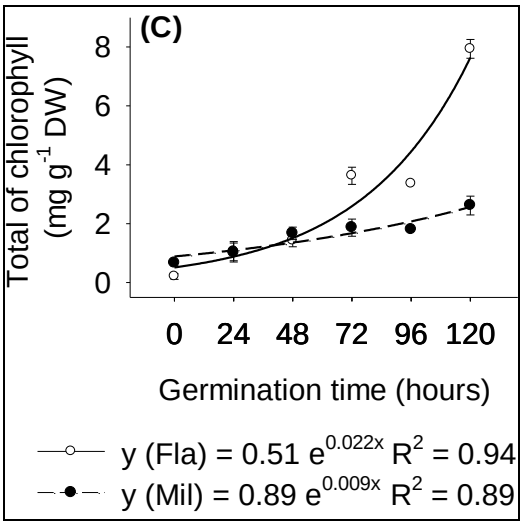
rates for chlorophyll *a* and *b* and total chlorophyll with an increase in germination time, whose maximum rates estimated by the model were respectively 2.73 and 0.91; 4.90 and 1.59; 7.15 and 2.62 mg g^{-1} DW for flax and millet seeds.







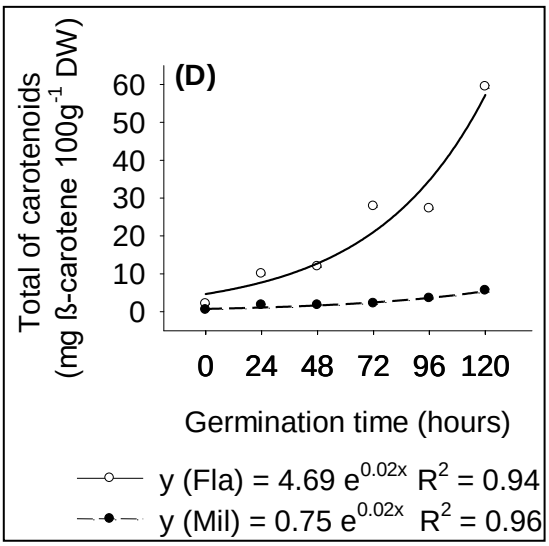


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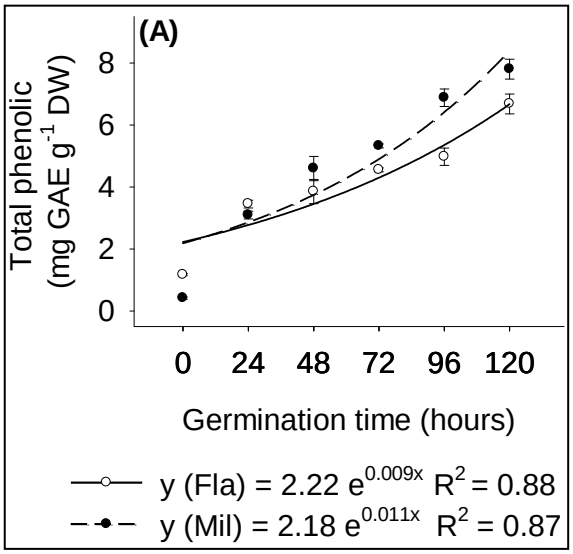
Fig. 1. Chlorophyll *a* (A), *b* (B) and total (C) (mg g^{-1} DW) and total carotenoids (mg β-carotene $100g^{-1}$ DW - D) of flax (Fla) and millet (Mil) seeds at different germination times (0, 24, 48, 72, 96 and 120 hours).(Vertical bars represent confidence intervals at 95%).

Rate of total carotenoids had a behavior similar to chlorophyll *a*, *b* and total chlorophyll when the two seeds were compared. The variable adjusted itself adequately to the exponential regression equation, with coefficient of determination rates (R^2) 0.94 and 0.96, respectively, for flax and millet seeds. Adjustment of data to the model was satisfactory. The seeds had an increasing rate of total carotenoids in proportion to increase in germination time whose maximum rates estimated by the model were respectively 51.70 and 8.27 mg of β-carotene $100g^{-1}$ DW for flax and millet seed (Fig. 1D). β-carotene and chlorophyll a increases were also reported after the second day of germination in sprouts of two soybean varieties (Lee, Hwang, Lee, Chang & Choung, 2013), similar to results in current study with sprouts of flax and millet seeds.

There was a significant difference at 0, 72, 96 and 120 hours of germination for the variable total phenols when the two seeds were compared. The variable adjusted itself adequately to the exponential regression equation, with rates of the coefficient of determination (R^2) 0.88 and 0.87 respectively for flax and millet seeds. Adjustment of data to the model was satisfactory. It should be enhanced that there was an increase in the concentration of total phenols for both types of seeds due to germination time, whose maximum rates estimated by the model were 6.54 and 8.16 mg EAG $100g^{-1}$ DM (Fig. 2A).

Paśko, Bartoń, Zagrodzki, Grinstein, Fołta & Zachwieja (2009) registered a higher rate in total phenols in sprouts when compared to seeds, and suggested that the synthesis of phenolic antioxidants occurred throughout germination. Germination also changes the phenolic composition, mainly due to the activation of endogenous
enzymes and biochemical metabolism of the seeds during the process (Dueñas, Hernández, Estella & Fernandez, 2009).



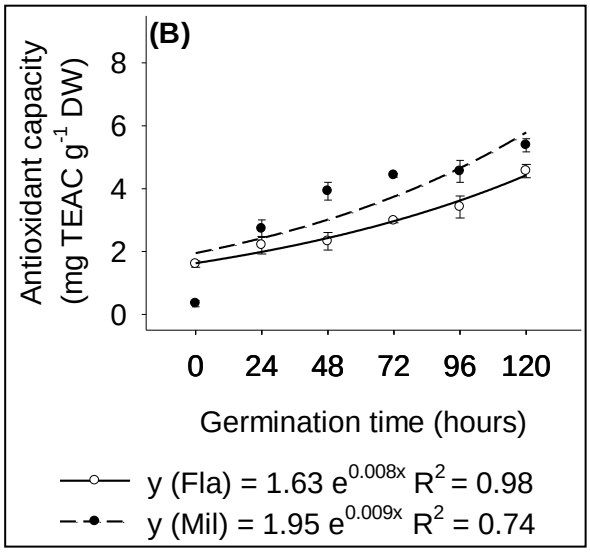


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Fig. 2. Total phenols (mg GAE g^{-1} DW - A) and antioxidant capacity - C A (mg TEAC g^{-1} DW - B) of flax (Lin) and millet (Pai) seeds at different times of germination (0, 24, 48, 72, 96 and 120 hours) (Vertical bars represent confidence intervals at 95%).

There was significant difference between flax and millet seeds at times 0, 48, 72, 96 and 120 hours of germination when their Trolox-equivalent antioxidant capacity was compared. The variable adjusted itself adequately to the exponential regression equation, with rates of coefficient of determination (R^2) respectively 0.98 and 0.74 for flax and millet seeds. Adjustment of data to the model was satisfactory. There was an increasing antioxidant capacity for both seeds due to germination time whose maximum rates estimated by the model were 4.26 and 5,74 mg TEAC g^{-1} DW (Fig. 2B). Differences between the samples were similar to those for total phenols, excepting 48 hours. The above was expected due to the high co-relationship between the variables (Table 3).

Several studies registered increase in antioxidant capacity after germination (Dueñas, Hernández, Estrella & Fernández, 2009; Paśko, Bartoń, Zagrodzki, Grinstein, Fołta & Zachwieja, 2009; Martinez-Villaluenga et al., 2010). The process has been attributed to the biochemical metabolism of the seeds during germination (Dueñas, Hernandez, Estrella & Fernández, 2009). The spouts´ higher antioxidant capacity when compared to that of seeds has been caused by the differences in polyphenol contents, anthocyanins and other compounds (Paśko, Bartoń, Zagrodzki, Gorinstein, Fołta & Zachwieja, 2009).

In the case of co-relationships (Table 3), the variables β-chlorophyll and total chlorophyll had the highest coefficient of positive co-relationship (r = 0.99; p<0.0001). When there is an increase in β-chlorophyll rates, there is also an increase in total chlorophyll. Other positive co-relationships between antioxidant capacity and total phenols (r = 0.96; p<0.0001); total carotenoids (r = 0.77; p<0.0001) and total chlorophyll (r = 0.56, p = 0.008) were also relevant. It is thus supposed that an increase in antioxidant level, similar to carotenoids and total phenols in sprouts, directly affect their increase in antioxidant capacity. Relevant co-relationships between the bioactive compounds and their antioxidant capacity are also reported in the literature (Xu & Chang, 2007; Aguilera et al., 2015).

Table 3

Coefficients of Pearson´s correlation and *p* rates between dependent variables.

Dependent variables	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Total carotenoids	Total phenols	Antioxidant capacity
	(1)	(2)	(3)	(4)	(5)	(6)
(1)	1.000	0.994* <0.0001**	0.997 <0.0001	0.884 <0.0001	0.612 0.0003	0.582 0.001
(2)		1.000	0.999 <0.0001	0.907 <0.0001	0.621 0.001	0.570 0.007
(3)			1.000	0.900 <0.0001	0.613 0.001	0.560 0.008

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(4)		1.000	0.821 <0.0001	0.773 <0.0001
(5)			1.000	0.957 <0.0001
(6)				1.000

* Coefficient of Pearson´s correlation. ** Rate of *p*.

Conclusions

Flax seed had a higher lipid percentage when compared to that of millet seed. Linolenic acid was the major fatty acid in flax seed, whereas palmitic, palmitoleic, linoleic and arachidic acids were the main acids in the millet seed. Flax and millet seeds differed at 72, 96 and 120 hours after germination, with higher total chlorophyll and carotenoid rates for flax seed and higher total phenol and antioxidant rates for millet seeds. Germinated flax and millet seeds have several compounds good for human health, such as some types of carotenoids and phenolic compounds. The intake of flax and millet sprouts may be a good alternative to increase the supply of these compounds in human diet.

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References

Aguilera, Y., Herrera, T., Benítez, V., Arribas, S. M., López de Pablo, A. L., Esteban, R. M., Martin-Cabrejas, M. A. et al. (2015). Estimation of scavenging capacity of melatonin and other antioxidants: Contribution and evaluation in germinated seeds. *Food Chemistry*, 170, 203-211.

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis. 16th Edition, Arlington.

Associationof Official Analytical Chemists (AOAC) (2000). Official methods of analysis of the Association of Official Analytical Chemists. 17th Edition AOAC *International*, Gaithersburg MD.

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal Biochemistry and Physiology*, 37, 911-917.

Bozan, B., & Temelli, F. (2008). Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource Technology*, 99, 14, 6354-6359.

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ISSN: 2581-3366

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes* / Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS, 399.

Brasil. (1998). *Portaria nº 381*, de 05 de Agosto de 1998. Diário Oficial da União, Brasília, DF, Seção 1, 43.

Castro, P. R. C., Klugue, R. A., & Peres L. E. P. (2005). *Manual de fisiologia vegetal: teoria e prática*. Piracicaba: Editora Agronômica Ceres, 650.

Cevallos-Casals, B.A., & Cisneros-Zevallos, L. (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, 119, 1485-1490.

Devisetti , R., Yadahally , S. N., &Bhattacharya, S. (2014). Nutrients and antinutrients in foxtail and proso millet milled fractions: Evaluation of their flour functionality. *LWT – Food Science and Tecnology*, 59, 889-895.

Dorrel, D.G. (1970). Distribution of fatty acids within the seed of flax. *Canadian Journal of Plant Science*, 50, 71-75.

Dueñas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chemistry*, 117, 599-607.

Embrapa - Centro Nacional de Pesquisa de Solos (CNPS) (2006) *Sistema Brasileiro de Classificação de Solos*. (2 ed.) Rio de Janeiro: Embrapa Solos. 412.

Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M. K., Kozlowska, H., & Vidal-Valverde, C. (2008). Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emmerald, *Glycine max* cv. jutro and *Glycine max* cv. Merit. *Food Chemistry*, 111, 622-630.

Frias, J., Miranda, M. L., Doblado, R., & Vidal-Valverde, C. (2005). Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa. *Food Chemistry*, 92, 211-220.

Gressler, V., Yokoya, N. S., Fujii, M. T., Colepicolo, P., Torres, R. P., Pinto, E. et al. (2010). Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chemistry*, 120, 585-590.

Hartman, L., & Lago, B.C. (1973). A rapid preparation of fatty methyl esters from lipids. *Laboratory Practice*, 22, 475-477.

Hung, P. V., Hatcher, D. W., & Barker, W. (2011).Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chemistry*, 126, 1896–1901.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, S. A., & Khattak, M. S. (2007). Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts. *Food Chemistry*, 104, 1074-1079.

Khotimchenko, S. V., & Yakovleva, I. M. (2005). Lipid composition of the red alga Tichocarpus crinitus exposed to different levels of photon irradiance. *Phytochemistry*, 66, 73-79.

Köppen, W., & Geiger, R. (1928). *Klimate der Erde*. Gotha: Verlag Justus Perthes, Wall-map, 150 x 200 cm.

Vol. 2, No. 04; 2018

ISSN: 2581-3366

Lee, J., Hwang, Y.-S., Lee, J.-D., Chang, W.-S., Choung, M.-G. (2013). Metabolic alterations of lutein, b-carotene and chlorophyll a during germination of two soybean sprout varieties. *Food Chemistry*, 141, 3177-3182.

Lichtenthaler, H. K. (1987). *Chlorophyll and carotenoids: pigments of photosynthetic biomembranes.* In: Colowick, S. P., Kaplan, N. O. Methods in enzymology. San Diego: Academic Press, 350-382.

Martinez-Villaluenga, C., Peñas, E., Ciska, E., Piskula, M. K., Kozlowska, H., Vidal-Valverde, C., & Frias, J. (2010). Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds. *Food Chemistry*, 120, 710-716.

Meda, A., Lamien, E. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, 91, 571-577.

Moure, A., Franco, D., Sineiro, J., Domínguez, H. Núñez, M. J., & Lema, J. M. (2001). Antioxidant activity of extracts from *Gevuina avellana* and *Rosa rubiginosa* defatted seeds. *Food Research International*, 34, 103-109.

Mueller, K., Eisner, P., Yoshie-Stark, Y., Nakada, R., & Kirchhoff E. (2010). Functional properties and chemical composition of fractionated brown and yellow linseed meal (*Linum usitatissimum* L.). *Journal of Food Engineering*, 98, 453-460.

Oloyo, R.A. (2004). Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L. *Food Chemistry*, 85, 497-502.

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., & Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry*, 143, 300-306.

Paśko, P., Bartoń, H., Zagrodzki, P., Gorinstein, S., Fołta, M., & Zachwieja, Z. (2009). Anhocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry*, 115, 994-998.

Paśko, P., Sajewicz, M., Gorinstein, S., & Zachwieja, Z. (2008). Analysis of the selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC method. *Acta Chromatographica*, 20, 661-672.

Rio Grande do Sul. Secretaria da Agricultura e Abastecimento. Departamento da Produção Vegetal. Comissão Estadual de Sementes e Mudas do Estado do Rio Grande do Sul (P. Alegre, RS). *Normas e padrões de produção de sementes para o Estado do Rio Grande do Sul*. Porto Alegre. 4 ed., 2000, 160 p. Semente; Produção; Normas; Padrões; Rio Grande do Sul; Brasil; CESM/RS CDD: 631.52108165.

Shakuntala, S., Naik, J. P., Jeyarani, T., Naidu, M. M., & Srinivas, P. (2011). Characterisation of germinated fenugreek (*Trigonella foenum-graecum* L.) seed fractions. *International Journal of Food Science & Technology*, 46, 2337-2343.

Shewry, P.R., Napier, J.A., & Tatham, A.S. (1995). Seed storage proteins: Structures and biosynthesis. *The Plant Cell*, 7, 945-956.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases.*Experimental Biology and Medicine*, 233, 674-688.

Vol. 2, No. 04; 2018

ISSN: 2581-3366

Silva, A. C. (2017). Linhaça na redução do colesterol: uma prospecção tecnológica. *Revista Brasileira de Nutrição Esportiva*, v.11, n 65.

Taiz, L., & Zeiger, E. (2004). *Fisiologia Vegetal*. Tradução: Santarém, E. R. et al. 3 edição. Porto Alegre: Editora Artmed.

Thelen, J. J., & Ohlrogge, J. B. (2002). Metabolic Engineering of Fatty Acid Biosynthesis in Plants. *Metabolic Engineering*, 4, 12-21.

Xu, B. J., & Chang, S. K. C. (2007). Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Science*, 72, 167-177.

Yang, F., Basu, T.K., & Ooraikul, B. (2001). Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Sciences and Nutrition*, 52, 319-330.

Zieliński, H., Frias, J., Piskuła, M. K., Kozłowska, H., & Vidal-Valverde, C. (2005). Vitamin B_1 and B_2, dietary fiber and minerals content of Cruciferae sprouts. *European Food Research and Technology*, 221, 78-83.