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Compositional and nutritional values of amaranth seeds after pre-sowing He-Ne laser light and alternating magnetic field treatment

A. Sujak, A. Dziwulska-Hunek, and K. Kornarzyński*

Department of Physics, University of Life Sciences, Akademicka 13, 20-033 Lublin, Poland

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A b s t r a c t. Seeds of amaranth plant grown from He-Ne laser light and magnetic field stimulated seeds or from seeds treated with both pre-sowing stimulating methods were analysed for the chemical composition and their nutritive values. Pre-sowing stimulation resulted in increase of dry matter, crude protein, crude fibre and crude ash. Decrease in crude oil and in the level of carbohydrates was generally observed. Use of different methods of presowing stimulation resulted in different response of the seeds in the amino acid composition. Generally, increase in the level of Leu, Lys, Val and Phe + Tyr was observed. At the same time a decrease in the levels of Arg, Glu and Ala was observed. Levels of Cys, Thr, Ile, His and Pro were not affected. The most nutrition-effective was protein from magnetic field stimulated seeds. The energetic values of the flours decreased. The electromagnetic stimulating methods are promising and non-invasive tools in the improvement of the composition and amino acid content of amaranth seeds. Of importance is the observed gain in the level of crude fibre and crude ash, as well as increase in the level of Leu which is normally the limiting amino acid in amaranth.

K e y w o r d s: amaranth, seed, pre-sowing stimulation, chemical components, amino acid composition, nutritive values

INTRODUCTION

Pre-sowing laser and magnetic biostimulation of crop seeds is of growing practical interest due to significant positive effects referring to crop yielding (Pietruszewski 1996; Pietruszewski and Wójcik, 2000; Podleśny *et al.,* 2005) as well as to chemical composition (Koper *et al.,* 1996; Truchliński *et al.,* 2002), mainly in wheat. Nowadays, when rational usage of agricultural production space is promoted, pre-sowing seed treatment with physical factors becomes more important. These factors, which are generally considered harmless for the environment, most often modify the course of some physiological and biochemical processes in the seeds, increasing their nutritive values (Pietruszewski *et al.,* 2007). Increase of sugar content in sugar beet roots after pre-sowing treatment has been reported (Koper *et al.,* 1996), as well as growth in crude protein yield for vetch seeds (Truchliński *et al.,* 2002).

The aim of this study was to check the influence of electromagnetic stimulation of amaranth seeds (*Amaranthus cruentus*). We decided to verify whether such an electromagnetic treatment influences the composition and nutritive values of seeds of mature plants.

Grain amaranth (*Amaranthus* spp.) is an ancient crop originating from Central and South America where it was grown between 5000-7000 years ago (Stallknecht *et al.,* 1993). Amaranth, called pseudo-grain, has been referred to as an herb or even a vegetable (Janick and Simon, 1993; Paredes-Lopez, 1994; Williams, 1995).

It is a relative of the common pigweed. It is a plant extremely adaptable to adverse growing conditions (Bavec and Mlakar, 2002; Berganza *et al.,* 2003). It resists heat and drought, has no major disease problems, and is among plants that are the easiest to grow. There are 60 species of amaranth on the planet. Some of these species are grown for their spinach- like leaves which are eaten as a salad, while other species are grown only for ornamental or decorative purposes. The other species produce seeds that are considered very nutritious.

Amaranth is one of the few species which potentially can become a good source of dietary proteins. Amaranth seed is high in protein (15-18%) and contains respectable amounts of lysine and methionine, two essential amino acids that are not frequently found in grains (Budin *et al.,* 1996). The amino acid composition compares favourably with other crops, since amaranth has a high lysine, threonine and tryptophan content. Lysine and valine are normally the

*Corresponding author's e-mail: agnieszka.sujak@up.lublin.pl

limiting amino acids in most cereal crops. In European conditions, the seed protein concentration ranges from 14 to 18% (Aufhammer *et al*., 1999). In the investigations of Jamriska (1996), done in Slovakia, protein concentration ranged from 18.5 to 20.1% depending on the amaranth species. According to Bejosano and Corke (Bejosano *et al*., 1998), amaranth seeds have proteins with a better balanced content of the essential amino acids than the proteins of cereals and legumes.

It is high in fibre and contains calcium, iron, potassium, phosphorus, and vitamins A and C (Prakash *et al.,* 1995; TingXuan and GuoRui, 2004; Tosi *et al.,* 2001).

Amaranth grain contains between 4.8 and 8.1% of oil (Budin *et al.,* 1996; León-Camacho *et al.,* 2001), although *A. spinosus* and *A. tenuifolius* are reported to contain as much as 17 and 19.3%, respectively (Budin *et al.,* 1996).

Amaranthus cruentus L. (bloody amaranth) is used in folk medicine in many countries (Karaseva *et al.,* 2001; Mamedov *et al.,* 2005) as it contains many phenolic compounds as well as flavonoids (Martirosyan *et al.,* 2007). It is worthy to mention that amaranth flour contains no gluten; therefore it is a good source of gluten-free flour for humans suffering from gluten-intolerance (Pasko and Bednarczyk, 2007). Favourable composition of grain amaranth flour helps prevent certain diseases (heart conditions, diabetes, brain stroke) (Martirosyan *et al.,* 2007), and its high content of fibre and starch has a positive effect on digestion disorders (Peterka *et al.,* 2001).

MATERIALS AND METHODS

The experimental material comprised amaranth (*Amaranthus cruentus*) seeds, cultivar Rawa, divided into 4 groups and treated as follows: first group (untreated) remained as control (C), second was subjected to laser simulation (L – in five series during free fall of seeds from the charging chopper chute) with He-Ne light of λ= 630 nm and density power of 6 mW cm^{-2}, third group (F) was stimulated with alternating magnetic field (50 Hz) of an intensity of 30 mT during exposure time of 30 s, and the last group (L+F) was subjected to both laser and magnetic field stimulation. The seeds were then sown on heavy soil (brown soil, class III) in Kłodnica Górna on experimental plots of 1 m^2 each (3.7 g of seeds per each plot). Spacing between the rows was approx. 0.1 m. After the plants were ripe (after 140 days), they were collected, the seeds plucked out, cleaned and rendered free of dust, then placed in tightly closed PVC test tubes at room temperature and subjected to analysis.

Chemicals used in the analysis of dry matter, crude ash, crude protein, oil (crude fat), crude fibre and crude protein (analytical grade) were purchased from POCh, Gliwice, Poland. Amino acid standards (analytical grade) were purchased from ZMBD CHEMIK, Prague, Czech Republic.

Ground amaranth grain (flour) was produced by using a laboratory KNIFETEC 1095 sample mill, Foss Tecator.

Dry matter, crude protein, oil, crude fibre, crude ash and carbohydrates were determined. All determinations were expressed in g kg^{-1}. The defatted flour (with n-hexane in Soxhlet apparatus to a final lipid concentration lower than 1% for further extrusion) had its proximate composition determined by conventional methods for moisture, protein, ash, and lipids, as described in (AOAC, 1990). Flours were analysed for total nitrogen using Kjeldahl procedure (AOAC, 1990) and converted to protein using a factor of 6.25.

The carbohydrates were determined by using a calculation method (Kunachowicz *et al.,* 2006). The available carbohydrates were estimated by subtraction of crude fibre from the carbohydrates.

Amino acids were determined using an AAA 400 automatic amino acid analyser (INGOS, Czech Republic). Prior to analysis, samples were subjected to acid hydrolysis in the presence of 6 M HCl at 105°C for 24 h. Sulphur-containing amino acids were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4°C). Tryptophan was not determined.

The quality of protein was estimated by determination of total amino acids (AA), as well as the fraction of exogenous amino acids (EAA).

Because the nutritional significance of much of the non-peptide nitrogen is unclear, nitrogen analysis of foods is much more precise than the single amino acid analysis and nutritional significance can then be attached to it. Amino acid determinations necessary in nutritive values determination were converted into a g 16 g^{-1} N basis, equivalent to g 100 g^{-1} of protein. All the calculations of nutritional values were based on the amino acid standard (Henley and Kuster, 1994).

The exogenous amino acids (EAA) were estimated in terms of geometric mean of all the concentrations of participating exogenous amino acids compared to the concentration of corresponding standard (in g 16 g^{-1} N) (Sujak *et al.,* 2006):

$$\mathrm{EAA} = \sqrt[10]{a_a / a_{1s} 100 \ldots\ldots. a_n / a_{ns} 100},$$

where: *n* is the number of participating amino acids, *ns* is the number of corresponding amino acids in the standard.

Protein efficiency ratio (PER) was calculated on the basis of protein usability which was expressed in terms of concentrations (in g 16 g^{-1} N) of only two amino acids – leucine and tyrosine, based on experiments on their availability/digestibility (Alsmayer *et al.,* 1974; Sujak *et al.,* 2006):

$$\mathrm{PER} = -\,0.468 + 0.454\mathrm{Leu} - 0.105\mathrm{Tyr}.$$

The energetic value of amaranth flours was estimated by using the calculation method (summarising over the predicted energetic values of all the flour components).

The following energetic values of the compounds have been considered per 1g of component: crude protein – 17 kJ (4 kcal), crude fat – 37 kJ (9 kcal), available carbohydrate – 17 kJ (4 kcal) (Kunachowicz *et al.,* 2006).

The admissible error for determinations of chemical components and amino acids was 5%. One-way analysis of variance was carried out on the experimental results using groups as independent variable. The significance of differences between means was compared by Duncan's multiple range tests. All calculations were performed using the ANOVA package from STATISTICA.pl.6.0.

RESULTS AND DISCUSSION

Although the arithmetical means of the average mass of 200 seeds differed (C – 151.5 ± 1.2 mg, L – 116.6 ± 10.9 mg, F – 133,6±9.8 mg and L+F – 137.4 ± 22.0 mg), no statistical differences were found between the groups. The seeds from the laser-stimulated plants demonstrated the biggest differences in sizes and shapes, together with the lowest average mass, while the seeds from non-stimulated plants were approximately comparable. All the seeds from the plants grown from pre-sowing stimulated seeds demonstrated lower average mass as compared to control. This was the first indication that pre-sowing laser biostimulation influences the final product. This, however, indicates a lower yield in reverse to the other electromagnetically-stimulated plants (Pietruszewski, 1996; Pietruszewski and Wójcik, 2000). The lower average mass suggests that the seeds are smaller as compared to control. This indicates an influence of biostimulation methods on the seed morphological features, consistent with previously reported data on pea (Podleśny *et al.,* 2005).

The chemical composition of amaranth isolates and amino acid composition of the proteins as well as the nutritive values were estimated.

Table 1 shows the chemical composition of the amaranthus isolates reported on a g kg^{-1} of flour. The highest dry matter was found for plants from laser and magnetic-stimulated seeds, as well as from laser stimulated plants (917.1±0.3 and 916.3±0.3 g kg^{-1}) as compared to control (912.6±0.4 g kg^{-1}).

The highest crude protein content (187.0 ± 2.0 g kg^{-1}) was found in laser stimulated amaranth plant ($P \leq 0.01$). Interestingly, the lowest crude protein was found for both laser and magnetic field-stimulated amaranth seeds (172.9 ± 1.9 g kg^{-1}).

Crude protein in this study was slightly higher than reported in (NRC, 1984), but consistent with the previously reported in (Becker *et al.,* 1981; Bejosano and Corke, 1998; Paredes-Lopez, 1994; Stallknecht and Schulz-Schaeffer, 1993). Although stimulation with magnetic field resulted in slender growth in crude protein as compared to control (181.4 ± 1.5 and 180.7 ± 1.2 g kg^{-1}, respectively), statistical difference between samples was found ($P \leq 0.01$). This effect was similar to that previously reported for wheat (Pietruszewski, 1996).

The highest crude fat content (63.9 ± 1.5 g kg^{-1}) was found in control seeds. Although the samples varied in the mean values of crude fat, no statistical differences were found between the samples, except for the statistical difference between L+F sample and other samples ($P \leq 0.01$). In general, crude fat content in all the samples was consistent with previous findings that reported 4.9-8.1% range (Saunders and Becker, 1984), although *A. spinosus* and *A. tenuifolius* are reported to contain as much as 17 and 19.3% of oil, respectively (Budin *et al.,* 1996). The crude oil content in this study was higher than reported in: (NRC 1984). It has been reported previously that the oil from amaranth (especially *Amaranthus cruentus*) has cholesterol-lowering effects (Plate and Areas, 2002).

Increase in crude fibre content was registered for all the samples, except for the laser and magnetic field stimulated seeds (L+F). Although fibre is not considered a nutritive component, especially for humans, it plays a very important physiological role in the correct action of the alimentary tract, and takes part in removing from the organism undesirable substances such as heavy metals or excess cholesterol (Kunachowicz *et al.,* 2006). Fibre belongs to the class of polysaccharides which do not undergo the process of decomposition in the alimentary tract. The consumption of fibre however, accelerates the surpass of the food through the alimentary line and it is reckoned to lower the risk of falling with the tumours of the intestine (Manoj *et al.,* 2001; Rodríguez-Cabezas *et al.,* 2002). Additionally, it has a hypolipemic action because it elevates the viscosity in the small intestine, decreasing lipid absorption, and binds the bile acids in the liver (Escudero *et al.,* 2004).

T a b l e 1. Chemical composition (g kg^{-1} ± S.D.) and energetic values of amaranth seeds

Specification	Dry matter (g kg^{-1})	Crude protein	Oil	Crude fiber	Crude ash	Carbohydrate	Available carbohydrate
C	912.6 ± 1.0^{a}	180.7 ± 1.2^{a}	63.9 ± 1.5^{a}	39.8 ±2.6^{a}	38.1 ± 0.7^{a}	629.9 ± 2.0^{a}	590.1 ± 4.4^{a}
L	916.3 ± 2.4^{b}	187.0 ± 2.0^{b}	61.8 ± 0.9^{a}	47.1 ± 1.0^{b}	47.5 ± 0.9^{b}	620.6 ± 2.8^{b}	573.5 ± 2.8^{b}
F	915.6 ± 0.8^{a}	181.4 ± 1.5c	59.5 ± 0.9^{a}	44.2 ± 1.0^{a}	46.2 ± 1.0^{bc}	628.4 ± 3.0^{a}	584.2 ± 3.0^{ac}
L+F	917.1 ± 2.1^{ac}	172.9 ± 1.9A^{ab}	62.3 ± 0.5^{ab}	39.8 ± 1.5^{ac}	45.0 ± 1.4^{ad}	636.9 ± 2.8^{ac}	597.1 ± 3.3^{Ab}

C – control, untreated seeds; L – seeds subjected to laser simulation (in five series during free fall of seeds from the charging chopper chute) with He-Ne light of λ =630 nm and density power of 6 mW cm^{-2}; F – seeds stimulated with alternating magnetic field (50 Hz) of an intensity of 30 mT during the exposure time of 30 s; L+F - seeds subjected to both laser and magnetic field stimulation.
Means in the same column with different letters are significantly different; a-d – $P \leq 0.01$, A – $P \leq 0.05$.

The samples varied in the level of carbohydrates. The samples examined showed a different response to the pre-sowing seed treatment. As seen from Table 1, the level of carbohydrates decreased in the laser stimulated and magnetic field-stimulated samples and increased in the samples treated with both the magnetic stimulating methods. Carbohydrates provide a majority of energy in the diets of most people. There are many reasons why this is desirable. In addition to providing easily available energy for oxidative metabolism, carbohydrate-containing foods are vehicles for important micronutrients and phytochemicals. Dietary carbohydrates are important to maintain glycemic homeostasis and for gastrointestinal integrity and function. Unlike fat and protein, high levels of dietary carbohydrates, provided they are obtained from a variety of sources, is not associated with adverse health effects. Finally, diets high in carbohydrates, as compared to those high in fat, reduce the like-lihood of developing obesity (Kunachowicz *et al.,* 2006; Moore, 2004).

A significant growth in crude ash content was registered (*ca*. 20%) in the samples treated previously with electromagnetic stimulating methods. Even in the control the ash content was higher than reported previously for *Amaranthus cruentus* in (NRC, 1984). The mineral content in plants depends strictly on the soil (Lubecka and Pogorzelski, 2006).

Unfortunately, the seed is an extremely complex system which can not be controlled. Most probably biological processes proceed in parallel when the seed is stimulated with electromagnetic fields. Although at this stage of our study we cannot explain this effect, we can suppose that the pre-sowing electromagnetic stimulating methods may influence the minerals uptake from the soil. In the case of vetch seeds, an increase of Fe in seed yield has been reported (Truchliński *et al.,* 2002).

Minerals such as P, K, Ca, Mg and Cl are the main constituents of ash. Apart from them, ash can contain Fe, Zn, Cu, Mn and Mo. Minerals determine animal growth, development, reproduction and health. They are part of the tissues and body fluids. They are components or activators of numerous hormones, enzymes and vitamins. When combined with proteins, they allow maintaining cellular oxidation activity and oxygen transport, determining the right metabolic processes (Valaja *et al.,* 2000) and can also influence the cardiovascular risk factors (Vaskonen, 2003). The absorption of bioelements depends on their chemical form as well as on many other factors activating or inhibiting absorption (Liu *et al.,* 2000). It has been reported that although high mineral content can have a positive effect according to the supply with minerals, it can also decrease fat digestibility (Rouvinen and Kiiskinen, 1991).

The amino acid composition of amaranth flour compa- res favourably with other crops, since amaranth has a high lysine, threonine and tryptophan content. Lysine and valine are normally the limiting amino acids in most cereal crops. Level of particular essential as well as non-essential amino acids is high as compared to a standard calculated by Henley and Kuster (1994), based on the 1984 FAO/ WHO suggested patterns for amino acid requirements. In the present study we found slightly higher levels of Lys, Met + Cys, Thr, Leu,

T a b l e 2. Amino acid composition (g kg^{-1}) and nutritional values of amaranth seeds

Specification	C	L	F	L+F	Standard*
Essential amino acids (g 16 g^{-1} N)					
Lys	5.9	6.1	6.1	6.1	5.8
Met +Cys	4.5	4.3	4.4	4.6	2.5
Cys	2.3	2.2	2.2	2.3	-
Thr	3.6	3.6	3.6	3.5	3.4
Ile	3.4	3.4	3.5	3.5	2.8
Val	4.2	4.3	4.4	4.3	3.5
Leu	5.4	5.5	5.6	5.5	6.6
His	3.0	3.1	3.1	3.0	1.9
Phe + Tyr	7.1	7.3	7.3	7.4	6.3
Tyr	3.3	3.5	3.4	3.4	-
Non-essential amino acids (g 16 g^{-1} N)					
Arg	10.8	10.5	10.4	10.4	
Asp	8.5	8.2	8.5	9.3	
Ser	5.7	6.3	5.9	5.3	
Glu	14.4	14.2	14.3	14.2	
Pro	4.2	3.8	4.3	4.3	
Gly	6.8	6.8	6.3	6.5	
Ala	4.2	4.2	4.1	4.1	
Nutritional values					
AA (g 16 g^{-1} N)	91.7	91.6	91.8	92.0	
EAA (g 16 g^{-1} N)*	28.7	30.9	29.1	29.3	
PER	1.64	1.65	1.70	1.69	
Energetic value (kJ 100 g^{-1})	1546.90	1521.51	1521.85	1539.52	
Energetic value (kcal 100 g^{-1})	365.86	359.82	359.83	364.07	

Explanations as in Table 1. AA, amino acid participation; EAA, essential amino acid participation, *Henley and Kuster, 1994; PER, protein efficiency ratio.

His, Phe + Tyr, Arg Asp, Ser, Pro and Ala; lower levels of Ile, and Gly, and the same levels of Val and Glu as compared to those previously reported (Becker *et al.,* 1981; Saunders and Becker, 1984).

The use of different methods of pre-sowing stimulation resulted in different response of the seeds in the amino acid composition (Table 2). Generally, a growth in the level of Leu, Lys, Val and Phe + Tyr was observed. At the same time, a decrease in the levels of Arg, Glu and Ala was observed. Pre-sowing stimulation did not affect the levels of Cys, Thr, Ile, His and Pro (Table 3).

However, Leu was found to be the limiting amino acid (*ca*. 16% shortage), see also Bejosano and Corke (1998).

Amino acids that are not used as 'building blocks' in the body's protein synthesis are catabolised and release nitrogen waste. The nutritional value of protein is, therefore, represented by a fraction of essential amino acids (EAA). For all the samples examined, EAA values were below the level of 36 g 16 g^{-1} N, recommended by (Favier *et al.,* 1995), based on nine exogenous amino acids (Lys, Met, Cys, Thr, Ile, Trp, Val, Leu and His). In the present study we did not measure the Trp content. As seen from comparison of AA and EAA indices, the best results of pre-sowing stimulation were obtained from laser stimulation (L). Considering the availability of EAA, the most nutrition-effective is the protein from magnetic field (F) stimulated seeds.

The energetic values of the flours were affected by the stimulation applied. In all the cases a decrease in energetic value was observed.

Table 3. Results of analysis of variance carried out on the experimental results using the group as an independent variable

Specification	C	L	F	L+F
Essential amino acids				
Lys	a	b	A	c
Met +Cys	a	ab	bc	Abd
Cys	a	b	bc	bd
Thr	a	ab	ac	d
Ile	a	a	A	b
Val	a	b	c	d
Leu	a	b	b	c
His	a	b	Aa	c
Phe + Tyr	a	b	a	c
Tyr	a	b	a	c
Non-essential amino acids				
Arg	a	b	a	c
Asp	a	b	bc	a
Ser	a	b	Ac	d
Glu	a	a	b	c
Pro	a	ad	a	Aab
Gly	a	a	c	d
Ala	a	b	Aab	a
Nutritional values				
Energetic value	a	b	Aa	bc

Explanations as in Table 1. Means in the same row with different letters are significantly different a-d P ≤0.01; A-D P ≤ 0.05.

CONCLUSIONS

1. Electromagnetic stimulation methods are promising and non-invasive tools in the improvement of the composition and amino acid content of amaranth seeds.

2. Of importance is the observed growth in crude fibre and crude ash content, as well as the increase in the level of Leu which is the limiting amino acid in amaranth.

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