PAPER

THE EFFECT OF INFRARED RADIATION IN MODIFYING NUTRITIONAL AND MECHANICAL PROPERTIES OF GRASS PEA SEEDS

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ABSTRACT

The aim of this work was to evaluate the effect of exposure of grass pea seeds to IR (infrared) radiation applied at varying time intervals on their mechanical properties, trypsin inhibitor activity, and reactive lysine content. Grass pea seeds cv. Derek were exposed to IR radiation at 180°C for 30, 60, 90, 120, and 180 s, respectively.

Compared with that of raw seeds, IR heating of grass pea seeds reduced trypsin inhibitor activity. Reactive lysine proved to be relatively stable in the applied heating conditions. In addition, the process led to the reduction in the value of breaking load required for the destruction of a single seed, which may facilitate further processing, for example, flaking. Therefore, IR heating can be used in processing grass pea seeds.

Keywords: grass pea, infrared radiation, reactive lysine, breaking load

1. INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is one of the oldest legumes cultivated by man. Currently, it is still an important nutritional component in Arabic countries, the Caucasus, and the Indian subcontinent. The advent of cultivation of this species in Poland is related to Tartar settlers who relocated to the Podlasie region in the 17th century. However, nowadays, it can be found only in limited regional cultures. The recent increasing interest in unconventional food products of plant origin also involves grass pea.

The nutritional value of grass pea seeds is primarily associated with their relatively high protein content (24-36%), accompanied by a very rich amino acid composition (MILCZAK *et al*., 2001). The significant amount of oleic, linoleic, and linolenic acids in fat, which is similar to the composition and utility of soybean oil, contributes to the high dietary value of the seeds (GRELA et. al., 2010). It should also be noted that they contain non-starchy polysaccharides (ca. 5%), which can stimulate Bifidobacteria in the digestive tract, enriching the organism with B-group vitamins, with a beneficial effect in absorbing calcium and reducing both the content of putrefactive bacteria and cholesterol absorption (TOMOMATSU, 1994). Grass pea seeds are suitable for human consumption and can be used to manufacture the feed and the production of the so-called "plant casein" (DZIAMBA, 1997).

However, the usability of grass pea seeds may be limited by bioactive components with anti-nutritional activity, such as protease inhibitors, tannins, or neurotoxins. The negative effect of anti-nutrients found in the seeds of leguminous plants, including grass pea, can be minimized through thermal processing (GRELA *et al*., 2001; SZMIGIELSKI and MATYKA, 2004). It seems highly advisable to assess the effect of infrared (IR) radiation on the seeds, including both modifications of the chemical composition and accompanying changes in the mechanical properties. Special attention should be paid to lysine because thermal processing may reduce its reactivity, thus limiting the availability of this essential amino acid (SAGAN and JAŚKIEWICZ, 2011). Lysine is involved in building proteins and producing hormones, enzymes, and antibodies. Lysine is needed for proper bone formation in children, as it helps in absorbing calcium (SARWAR *et al*., 2012).

The aim of the present work was to evaluate the effect of exposure of grass pea seeds to IR radiation, at varying time intervals, on the mechanical properties and selected nutritional components of grass pea seeds.

2. MATERIALS AND METHODS

The research material was seeds of grass pea (*Lathyrus sativus* L.) cv. Derek. The material harvested in 2011 was provided by "Spójnia" Hodowla i Nasiennictwo Ogrodnicze (Breeding and Horticultural Company) in Nochowo, Poland. The seed moisture content was 11±0.2%. Table 1 presents the geometric data and the basic physical properties of grass pea seeds.

Table 1: Basic physical properties of grass pea seeds (cv. Derek) (± standard deviation).

Mass of 1000 seeds (g)	Mean size of seeds (mm)	Angle of tipping over plate (°)	Bulk density ($kg \cdot m^{-3}$)	Bulk density tapped ($kg \cdot m^{-3}$)
116.3±2.49	5.90±0.03	18.3±1.15	810.66±23.09	896.13±10.99

Six 500-g samples of grass pea seeds were prepared. Five of them were exposed to IR radiation at 180°C (the temperature adopted for the process was the temperature of the seed surface exposed to heating) for 30, 60, 90, 120, and 180 s, respectively. Processing was carried out using a laboratory apparatus, which was designed and constructed especially for this purpose, with a wavelength emitted $\lambda = 2.5 - 3.0$ µm (ANDREJKO *et al*., 2011).

By applying uniaxial compression of individual seeds between two parallel plates, mechanical properties were evaluated using a dynamometer (Instron 4302, Instron Ltd., High Wycombe, UK). Individual seeds of grass pea were placed with their seed leaves parallel to the surface of the fixed plate and then they were compressed with the help of the upper plate. The speed of the compression plate shift applied during the test had a constant value of 10 mm/min. Force required for destructing the structure of a single seed was determined. The values of maximal forces were read out using specialist computer software (Instron 12) provided by the Instron Company. The result was reported as the arithmetic mean of 50 replications.

Protein, fat, ash, and raw fiber of the grass pea seeds were determined following AOAC - 984.13, 920.39, 942.05, 962.09, respectively (AOAC, 1990). Amino acids were determined by ion-exchange chromatography, following PN-EN ISO 13903 (2006). Prior to the analysis, the sample was hydrolyzed with 6 M HCl at 105°C for 24 h. Next, automatic amino acid analyzer AAA 400 was used to determine amino acids (Ingos, Praha, Czech Republic). Sulfur-containing amino acids were determined after oxidation to methionine sulfone and cysteic acid with performic acid (prepared by mixing 30% hydrogen peroxide and 99.9% formic acid in a ratio of 1:9) at 0°C for 16 h. Tryptophan was determined after alkaline hydrolysis with a saturated barium hydroxide solution at 105°C for 20 h. According to the procedure of OSER (1951), essential amino acid index (EAA index) was calculated taking into account the ratio of EAA in the test protein relative to their respective amounts in the whole egg protein. The fatty acid composition was determined in conformity with PN-EN ISO 5508 (1996) and PN-EN ISO 5509 (2001). The extracted fat was saponified with a 0.5 M methanolic sodium hydroxide solution at 85-95°C for 10 min. Fatty acid salts were esterified with anhydrous methanol using BF_3 as a catalyst at 100°C for 10 min. The methyl esters of the fatty acids were analyzed using a gas chromatograph with a flame ionization detector (Varian 450-GC, Middelburg, The Netherlands) on 30 m long column Select Biodiesel for FAME (Agilent Technologies, Santa Clara, USA). Based on the study by KAKADE *et al*. (1974), trypsin inhibitor activity (TIA) was determined. The analytical evaluation of TIA is based on the estimation of the part of trypsin activity that is blocked by a buffered extract containing trypsin inhibitors obtained from a seed sample and takes place under conditions ensuring the maximum activity of this enzyme. N-α-Benzoyl-DL-arginine-*p*-nitroanilide (BAPA), a synthetic substrate, was used. The reaction between trypsin and BAPA yields yellow *p*-nitroaniline whose concentration is registered at 410 nm. The calculation of the result is based on a diagnostic reaction between the standard BAPA solution and the standard trypsin solution. The absorbance of this solution corresponds to 40 conventional trypsin units (TU). Extracts obtained from the tested samples containing a trypsin inhibitor characterized by TIA reduce the activity of this enzyme, which is reflected in a proportional decrease in the quantity of produced *p*-nitroaniline and reduced absorbance of the analyzed solution converted into trypsin units inhibited (TUI) per 1 mg dry seed mass ($TUI \cdot mg_{DM}^{-1}$). Reactive lysine was determined by using the HPLC technique according to RAMÍREZ-JIMÉNEZ *et al*. (2004). The method consists in creating a colored complex of ε-DNP-lysine in the reaction with dinitrofluorobenzene (DNFB), and thereafter, hydrolyzing the sample and determining the content of ε-DNP-lysine using a liquid chromatograph with UV-VIS Spectroflow 773 detector (Kratos Analytical, Manchester, UK). Reducing sugars were extracted from the sample with 40% ethanol for 1 h and determined with the Luff-Schoorl method (PN-R-

64784, 1994). The method is based on the reduction of copper salt from the Luff solution (copper citrate with sodium carbonate) by reducing sugars contained in the extract. The reaction was carried out at a boiling point for 10 min. After cooling, the excess of copper ions was reduced with hydrogen iodide generated after the addition of 30% KI solution and 6 M H_2SO_4. Liberated iodine was titrated with a 0.1 M sodium thiosulfate solution using starch as an indicator near the end point. All chemical analyses were performed in three replications.
The results of the determination of the TIA of reactive lysine and reducing sugars in the grass pea seeds were subjected to the analysis of variance (ANOVA). The significance of differences between the mean values was verified using Tukey's test, with $P<0.05$. Calculations were done using Statistica 8.0 software.

3. RESULTS AND DISCUSSIONS

The value of the physical parameters determined for the grass pea seeds (Table 1) were similar to those presented by other authors (ALTUNAS and KARADAG, 2006). The mass of 1000 seeds (116.3±2.49 g) was similar to the average quoted by DZIAMBA (1997).
Seed resistance to compression plays an important role in many technological processes. Thus, an attempt has been made to determine the changes caused by the thermal activity of IR radiation in the mechanical resistance of the grass pea seeds. The data presented in Fig. 1 show that the thermal effect of IR radiation on the grass pea seeds led to a decrease in breaking load, in accordance with the exposure time. The highest values of breaking load were registered for seeds that had not been thermally processed (1.019 kN), while heating the seeds for even a short period of 30 s with IR radiation reduced the value of breaking the load to 0.885 kN. Furthermore, increasing the time of the seed exposure to IR radiation led to a reduction in the measured values. The lowest value, i.e., 0.463 kN, was obtained from the seeds subjected to heating for 180 s. The dependence presented in Fig. 1 has a nearly linear character; hence, it was described by means of the first-degree regression equation. The good correspondence of the experimental data and the equation is confirmed by the high value (close to 1) of the determination coefficient R^2.

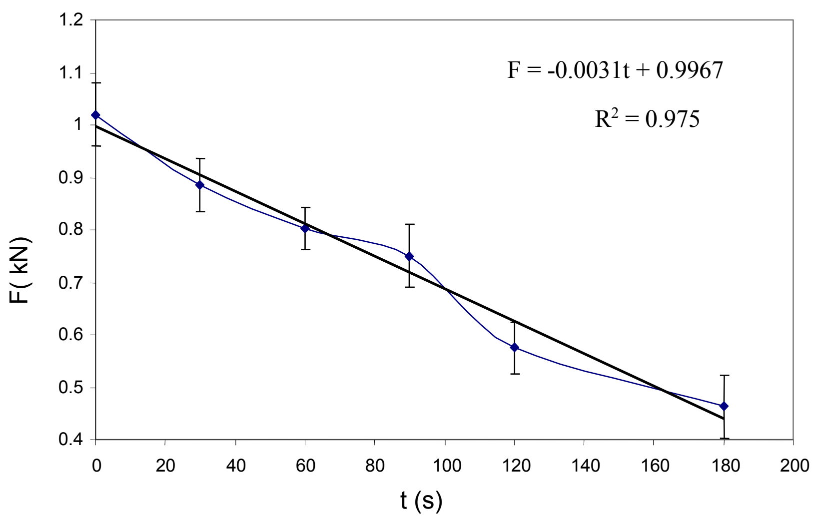

Figure 1: Effect of heating time (t) with infrared radiation on the value of breaking load (F) of the grass pea seeds.

The dependencies obtained are not characteristic of one type of material only, namely, grass pea seeds. On the basis of the results recorded previously (ANDREJKO *et al*., 2011), it was concluded that the values of the breaking load causing destruction of wheat cv. Zawisza were lower following IR radiation. FASINA *et al*. (2001) recorded the similar observations as follows: after the thermal processing of beans, peas, and lentils, reduced forces destroying the seeds were noted and were found to be different for each individual material, despite the same conditions of processing.

The content of the chemical components in the grass pea seeds (Table 2) was similar to the values quoted in the available sources (GRELA *et al*., 2010; SMULIKOWSKA *et al*., 2008; TAMBURINO *et al*., 2012). The analyzed seeds contained 24.35% of protein with a high nutritional value, resulting from their amino acid composition (Table 3).

Table 2: Chemical composition of grass pea seeds (± standard deviation).

Basic components (%)	
Protein	24.35±0.30
Fat	0.88±0.18
Ash	2.62±0.08
Raw fiber	5.16±0.42
Fatty acids (% of FA total)	
C 16:0	9.09±0.31
C 18:0	4.41±0.22
C 18:1	12.87±0.42
C 18:2	57.30±1.02
C 18:3	14.53±0.21
SFA	14.87
MUFA	13.09
PUFA	72.09
n-3	14.42
n-6	57.41
PUFA *n*-6/n-3	3.98

Table 3: Content of amino acids in grass pea seeds (± standard deviation).

Amino acids (g·kg^{-1})	
Phenylalanine	10.7±0.35
Tryptophan	4.2±0.05
Methionine	2.0±0.03
Threonine	9.3±0.10
Leucine	16.3±0.11
Isoleucine	10.1±0.10
Valine	11.2±0.18
Lysine	15.7±0.20
EAAI[1]	72.0

[1]Essential Amino Acid Index.

Among the essential amino acids, lysine and leucine were the most abundant, which is consistent with the results obtained for an Italian variety (TAMBURINO *et al*., 2012). The content of the essential amino acids in the grass pea seeds was similar to soybean, except for methionine, which was definitely less abundant in grass pea than in soybean. The index of essential amino acids, EAAI, amounting to 72.0, remained within the range of the values obtained for Polish varieties of bean seeds (SAGAN and JAŚKIEWICZ, 2011).

The fatty acid profile in the analyzed seeds was similar to that quoted by other authors (GRELA *et al*., 2010). The high share of PUFA in the sum of fatty acids is notable, as well as the high ratio of *n*-6:*n*-3. The high level of polyunsaturated fatty acids is extremely important in preventing cardiovascular disease.

The content of trypsin inhibitors was at a level of 37.09±1.41 TUI/mg of dry mass of raw seeds. GRELA *et al*. (2001) and SZMIGIELSKI and MATYKA (2004) noted a lower activity of trypsin inhibitors in raw grass pea seeds (19.64 and 23.13 TUI/mg of dry mass). Heating grass pea seeds with IR radiation led to a reduced TIA, which becomes more significant with the longer time of processing. Statistically significant changes were already observed after 60 s exposure of the seeds to IR radiation (Table 4). After 60 s of radiation, the TIA was 90% of its value in the raw samples and was only 41% after 180 s. This confirms some previous analyzes, which revealed that thermal processing reduces the content of anti-nutrients in the seeds of grass pea. The reduction in the activity of trypsin inhibitors is extremely important because of their anti-nutritional effects. These inhibitors block trypsin, which leads to the inhibition of some processes, thereby restricting the use of the protein and reducing proteolysis in the gastrointestinal tract; in turn, this may result in the inhibition of the growth of the organism (SARWAR *et al*., 2012).

Table 4: Trypsin inhibitor activity, content of reactive lysine, and reducing sugars in grass pea seeds (± standard deviation).

Time of processing (s)	Trypsin inhibitors activity ($TUI·mg_{DM}^{-1}$)	Reactive lysine ($g·kg_{DM}^{-1}$)	Reducing sugars ($g·kg_{DM}^{-1}$)
0	37.09^{a}±1.41	15.21^{a}±0.53	7.00^{a}±0.54
30	35.93^{a}±0.89	15.18^{a}±0.25	6.89^{a}±0.42
60	33.49^{b}±1.39	15.20^{a}±0.04	6.72^{ab}±0.39
90	27.57^{c}±0.96	14.84^{ab}±0.76	5.95^{ab}±0.25
120	22.80^{d}±0.68	14.86^{ab}±0.42	5.71^{b}±0.14
180	15.34^{e}±0.74	13.95^{b}±0.14	5.65^{b}±0.32

a,b,c Statistically significant differences in columns ($P<0.05$).

In order to determine the influence of the processing procedures on the nutritional value of the food, the content of reactive lysine may be used as an indicator. Exposing grass pea seeds to high temperature may pose a risk of lowering the nutritional value of protein, for example, by reducing the content of reactive lysine, which at higher temperatures reacts with other native compounds. This amino acid becomes inaccessible for the digestive processes after blocking the free ε-amino group. In this process, the susceptibility of other leguminous seeds occurring during the thermal processing was also observed by SAGAN and JAŚKIEWICZ (2011), and ŽILIĆ *et al*. (2006), among others. Reactive lysine in the analyzed seeds was relatively resistant to the IR radiation. Statistically significant losses of this compound were noted only as a result of the 180 s exposure to the IR radiation (Table 4). The retention of reactive lysine in the sample heated in this manner amounted to 92%,

and it was higher in comparison with soybean seeds of Serbian varieties subjected to heating with IR radiation at a temperature of 150°C and flaking for 2-3 min (retention after the process: 79% and 55%) (ŽILIĆ *et al*., 2006). The results may confirm earlier studies on the influence of thermal processes on the content of reactive lysine in bean seeds (SAGAN and JAŚKIEWICZ, 2011), which suggest that prolonged heating may lead to a reduction in the content of this amino acid.
The content of reducing sugars exceeded the sum of glucose and fructose determined by PIOTROWICZ-CIEŚLAK *et al*. (2008) in the seeds of Derek and Krab lines and was lower than that in Ethiopian varieties (URGA *et al*., 1995). A significant reduction was noted in the content of reducing sugars after 120 and 180 s of heating (Table 4). After this period, the content of reducing sugars in the grass pea seeds was 81% of the initial amount. This may result from the reaction of these compounds with other components occurring at higher temperature, for example, with amino acids (Maillard's reactions), including reactive lysine (NARANJO *et al*., 1998).

4. CONCLUSIONS

IR heating of grass pea seeds resulted in a decreased TIA, compared with that of raw seeds. Reactive lysine proved to be relatively stable in the applied heating conditions. In addition, the process reduced the value of breaking load required for destructing a single seed. This may facilitate further processing, for example, flaking. Therefore, IR heating can be applied in processing of grass pea seeds.

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