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EFFECT OF MICROWAVE FIELD ON TRYPSIN INHIBITORS ACTIVITY AND PROTEIN QUALITY OF BROAD BEAN SEEDS (*VICIA FABA* VAR. *MAJOR*)

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ABSTRACT

Background. In human nutrition legume seeds are usually subjected to soaking and thermal processes, mainly by using traditional cooking method. This method which has been used for decades, does not allow to control and adjust the parameters of this process. Therefore it does not seem to be the optimal method. Undoubtedly, microwave field is an alternative thermal process to conventional technique. The aim of this study was to assess the impact of microwave field on the activity of trypsin inhibitors and protein quality of three varieties of broad bean seeds.
Material and methods. The study was performed on dry seeds of broad bean varieties *Windsor White*, *Bachus* and *Basta*. The seeds were soaked and heated in a microwave. The seeds absorbed different energy doses from 500 J/g, through 750, 1000, 1250, 1500, 1750 to 2000 J/g. The study material prepared in this way was tested for trypsin inhibitor activity, protein solubility and *in vitro* protein digestibility. The results were analysed by the one-way analysis of variance.
Results. Microwave heating resulted in decreased activity of trypsin inhibitors and protein solubility and increased digestibility of protein in all tested varieties of broad bean seeds. With increasing doses of the microwave field energy a decrease in protein solubility was observed. Satisfactory reduction in trypsin inhibitors at the level of 70-75% and highest protein digestibility were obtained by using a microwave field with energy dose of 1000 J/g of seeds.
Conclusion. It can be concluded that the optimal dose of microwave energy field which will produce a relatively low activity of trypsin inhibitors and the highest protein digestibility together with maintaining solubility of broad been seeds was 1000 J/g seed.

Key words: trypsin inhibitor, *in vitro* protein digestibility, microwave field, broad bean seeds

INTRODUCTION

Modern heating technologies currently used in the food industry are intended to primarily reduce the time of thermal processing and also decrease the cost of the entire process through specific energy savings. Obtaining such an effect is difficult or even impossible by using conventional heating, therefore microwave heating technique is being currently used on big scale in the food and catering technology.

In contrast to conventional heating, capacitive heating with microwaves is almost immediate. This technique also allows precise adjustment of the dosage of energy, creating the opportunity to give the optimum

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amount of energy, which positively affects the nutritional value of fixed food product [Hardy et al. 1999]. The depth of microwaves penetration depends mainly on the microwave frequency. The lower the frequency, the deeper the penetration. The type of product is also crucial, as well as its specific density, water and salt content. For heating efficiency the shape of the product is also important [Hill 1998, Lewicki 1999].

In human nutrition legume seeds are usually subjected to soaking and thermal processes, mainly by using traditional cooking method [Nasar-Abbas et al. 2008, Pugalenthi et al. 2006, Taiwo et al. 1997, Xie 2000]. This method which has been used for decades, does not allow to control and adjust the parameters of this process. Therefore it does not seem to be the optimal method. Undoubtedly, microwave field is an alternative thermal process to conventional technique, which allows precise dosage of energy and control of the thermal process [Negi et al. 2001]. Many authors have reported that the microwave field increases protein digestibility and availability while reducing antinutritive labile components, such as trypsin inhibitors and hemagglutinin [In-Hwa-Han et al. 2007, Pysz et al. 2006, Khatoon and Prakash 2004, Rehman and Shah 2005]. Operation of microwave radiation also leads to dehydration and coagulation of food protein and decrease of their solubility, which results directly from the applied radiation dose [Dolińska and Warchlewski 2003, Duranti and Gius 1997].

In this context, the objective of this study was to assess the impact of microwave field, depending on the dose of energy, on the activity of trypsin inhibitors and protein quality of three varieties of broad bean seeds by determining the solubility and digestibility.

MATERIAL AND METHODS

Material consisted of three dry, field bean varieties (*Vicia faba* var. major), *Windsor White* obtained from the company, "Polan" Krakowska Horticultural Breeding and Seed Production, *Bacchus*, and *Basta* with the company "Spójnia" Nochowo, Breeding and Seed Production Horticulture.

Seeds were soaked by the reduced hydration method in heat in the conditions of changing temperature. The ratio of seeds to the water during soaking was 1:4, seeds were spilled with hot water and left for 3 hours, yielding a final soaking temperature at 22°C. After soaking of seeds in hot temperature in a microwave oven (Panasonic 900 W power), 7 different times were applied in which, the seeds were given different doses of energy from 500 J/g to 750, 1000, 1250, 1500, 1750 up to 2000 J/g. Samples were lyophilised and ground. In the so-prepared material, the activity of trypsin inhibitors was determined by using the method by Kakade et al. [1974], the solubility of proteins by Klepacka et al. [1997] and in vitro protein digestibility method elaborated by Hsu et al. [1977]. For determination of in vitro digestibility, multienzymatic kit was used (peptidase, trypsin, chymotrypsin), Sigma Aldrich.

The results were analysed by univariate analysis of variance, significance of differences between means was verified by using Duncan test at $P < 0.05$.

RESULTS AND DISCUSSION

The influence of microwave fields at various energy doses on the antitrypsin activity of broad bean seeds is presented in Table 1.

The highest antitrypsin activity of seed varieties were characterised by *Winsor White* at 6.03 TIU/mg

Table 1. Effect of microwave field delivering different energy doses on the activity of trypsin inhibitors of broad bean seeds, TIU/mg

Microwave field J/g	Cultivar		
	Windsor White	Bacchus	Basta
0	6.03 g	4.75 g	4.53 g
500	4.82 f	2.26 f	3.54 f
750	2.29 e	1.85 e	2.20 e
1000	1.44 d	1.21 d	1.39 d
1250	1.22 c	0.97 c	1.18 c
1500	1.01 b	0.86 b	0.94 b
1750	0.85 a	0.81 b	0.74 a
2000	0.83 a	0.75 a	0.72 a

Mean values in the same column with the different letters are statistically significantly different at the level ($P < 0.05$).

seeds. The other two tested varieties of broad bean seeds showed significantly lower activity of trypsin inhibitors, respectively 4.75 TIU/mg for *Bacchus* and 4.53 for *Basta*. The use of microwave field resulted in statistically significant ($P < 0.05$) decrease in antitrypsin activity of soaked broad bean seeds. The lowest dose of energy (500 J/g) caused inactivation of IT for *Windsor White* and *Basta,* by 20% and 50% in the case of *Bacchus*. Increasing the dose of energy to the level of 2000 J/g led to further declines of IT activity by 86% for broad bean varieties *Windsor White* and 84% for the varieties *Bacchus* and *Basta*. However, 1000 J/g was the optimal energy dose at which approximately 75% reduction of antitrypsin activity was obtained.

Seeds of broad beans are not with the highest activity of IT among the legumes plants. The level of these compounds in soybeans is on average 20-30 times higher than in seeds of field bean [Grela et al. 2003, Lampart-Szczapa 1997]. However, their presence in plants poses a real danger associated with the inhibition of enzymatic processes, mainly digestive enzymes. Trypsin inhibitors contribute to reduced protein proteolysis in the digestive tract, and this can lead to hypertrophy of the pancreas. Therefore, a significant reduction in IT under applied of microwave field substantially increases the protein digestibility and availability of legume seeds [Khatoon and Prakash 2004, Pisulewski et al. 2000].

A similar level of reduction of trypsin inhibitors was obtained by other authors. Morrison et al. [2007] showed approximately 90% reduction in IT level in the soaked and cooked peas. Also 70-80% reduction of trypsin inhibitors in seeds of beans was obtained by Pisulewski et al. [2000]. A similar level of trypsin inhibitors reduction in the boiled and autoclaved pea seeds, was demonstrated by Khalil and Mansour [1995], while Alajaji and El-Adawy [2006] reported that thermal treatment of chickpea seeds reduces the inhibitory activity by 80-84%.

Influence of various energy doses of microwave fields on the proteins solubility of broad bean seeds is presented in Table 2.

Levels of protein solubility of three broad bean seed cultivars, *Windsor White*, *Bacchus* and *Basta* after soaking process were at 62.5%, 63.1%, 69.1%, respectively. Application of the thermal factor in the form of microwave radiation leads to denaturation of proteins and their limited solubility. This parameter has been changing with increasing microwave field intensity. The lowest energy dose at 500 J/g resulted in statistically significant ($P < 0.05$) decrease in protein solubility in all tested varieties. The biggest falls were observed in the case of *Basta* varieties, to the level of 42.5%, despite the highest protein solubility in the seeds of this variety. Further increase in the microwave field intensity did not cause meaningful decline in protein solubility of *Basta* bean seed varieties, but these declines were statistically significant ($P < 0.05$). At a dose of energy at 1250 J/g, protein solubility of *Basta* bean was lowest and amounted to 28.7%. Increasing the dose of energy to 2000 J/g did not result in statistically significant ($P > 0.05$) decrease in protein solubility for this variety of broad bean seeds.

Table 2. Effect of microwave field delivering different energy doses on the solubility of broad bean seed protein, %

Microwave field J/g	Cultivar		
	Windsor White	Bacchus	Basta
0	62.5 d	63.1 d	69.1 d
500	53.6 c	54.4 c	42.5 c
750	52.0 c	44.4 b	37.1 c
1000	50.5 c	39.5 ab	33.9 b
1250	49.1 c	34.6 a	28.7 a
1500	40.3 b	38.5 ab	36.1 b
1750	35.8 a	35.3 a	36.5 b
2000	35.6 a	35.3 a	35.8 b

Mean values in the same column with the different letters are statistically significantly different at the level ($P < 0.05$).

Somewhat differently behaved other two varieties of the examined broad bean seeds. The field of microwave energy dose at 500 J/g reduced the solubility of protein variants Windsor White to the level of 53.6% while the variety *Bacchus* to 54.4%. Further enhancing the intensity of the microwave field to the level of 1250 J/g did not result in significant ($P > 0.05$) decrease in protein solubility of bean seed varieties *Windsor White*. Only the intensity of the microwaves at 1500 and 1750 J/g caused a further significant (P < 0.05) decrease in solubility of the protein level, respectively by 40.3% and 35.8%. In the case of *Bacchus* variety lowest value of protein solubility (34.6%) was observed at a dose of energy 1250 J/g, as compared to the a variety of *Basta.* Further increase did not bring back the changes of this parameter.

The applied technology process (microwave heating) caused a decrease in protein solubility in all tested varieties of broad beans. This decrease resulted from the progressive protein denaturation with increasing energy dose and dehydration caused by thermal factor. Probably tertiary protein structure was blocked and hydrophobic groups were exposed, which were previously within the spatial structure of the protein. At the same time, the polar groups have been moved to the interior of the molecule. As a result of this protein change, aggregation or coagulation occurred in the degree depending on the intensity of the applied thermal factor, what decreased the hydration of the protein molecule and thus its solubility [Dolińska and Warchlewski 2003, Duranti and Gius 1997].

The effect of microwave field with different doses of energy on protein *in vitro* digestibility of broad bean seeds is presented in Table 3.

Table 3. Effect of microwave field delivering different energy doses on the *in vitro* digestibility of broad bean seed protein, %

Microwave field J/g	Cultivar		
	Windsor White	Bacchus	Basta
0	46.0 a	52.2 a	51.5 a
500	57.1 b	68.0 b	53.2 b
750	69.4 c	73.2 c	64.1 c
1000	76.5 d	76.1 d	78.2 d
1250	78.7 d	78.4 d	78.8 d
1500	79.0 d	78.9 d	79.8 d
1750	78.8 d	79.0 d	81.2 d
2000	78.8 d	78.8 d	81.0 d

Mean values in the same column with the different letters are statistically significantly different at the level (P < 0.05).

In vitro protein digestibility of three field broad bean seeds cultivars increased at different levels, by 46.0%, 52.2%, 51.5% for *Windsor White*, *Bacchus* and *Basta*, respectively. *Windsor White* variety was characterised by a significantly lower digestibility compared to other varieties of seeds. The use of microwave radiation has led to an increase in protein digestibility of all broad beans examined. The lowest amount of energy (500 J/g) caused a significant (P < 0.05) increase in protein digestibility, by 57.1%, 68.0% and 53.2%. Further increase in energy doses up to 1000 J/g also increased significantly (P < 0.05) protein digestibility of all examined beans, reaching values of 76.5% for the varieties of *Windsor White*, 76.1% for the *Bacchus* and 78.2% for a change *Basta*. Increasing energy up to 2000 J/g did not result in longer statistically significant (P > 0.05) changes in the protein digestibility of broad bean cultivars. Varieties of *Windsor White* and *Basta* react similarly to energy microwave field changes in all its range, while the variety *Bacchus* showed a different response to lower doses of energy. These results allow to conclude that the optimal dose of microwave energy field which will produce the appropriate protein digestibility, is a dose of 1000 J/g seed.

The applied microwave field at the optimal dose resulted in a significant increase in protein digestibility by about 66% for *Windsor White* broad bean, 45% for the *Bacchus* variety and 52% for *Basta* variety. These results are consistent with the literature [Alonso et al. 2000, Dolińska and Warchalewski 2003, Khalil and Mansour 1995, Negi et al. 2001].

However, Khatoon and Prakash [2004] found that protein digestibility of legumes cooked in the microwave is lower than the steam pressure cooking. Higher protein digestibility was also obtained when the seeds were soaked in water before heat treatment [In-Hwa-Han et al. 2007, Rehman and Shah 2005].

The increase in protein digestibility obtained after the application of the thermal factor is primarily due to loosening of cell structure and the opening of the tertiary structure of proteins leading to its denaturation and protein becomes more susceptible to proteolytic enzymes. The applied high temperature also leads to inactivation of antinutritive thermolabile factors occurring in seed beans, such as trypsin inhibitors and lectins, but also causes a reduction in tannins and poliphenols [Siddhuraju and Becker 2005, Vijayakumari et al. 2007]. Prolonged heating or keeping food in the hot state leads to loss in content and digestibility of amino acids, causing loss of nutritional value. In this situation, disadvantageous connections are formed, both between the same amino acids and also between amino acids and carbohydrates that are resistant to enzyme digestion [Alonso et al. 2000, Dolińska and Warchalewski 2003, Khalil and Mansour 1995, Mitrus 2000, Negi et al. 2001].

CONCLUSION

1. The applied microwave field resulted in statistically significant ($P < 0.05$) decrease in antitrypsin activity of soaked broad bean seeds. Satisfactory reduction of trypsin inhibitors at the level of 70-75% was obtained for the microwave field at 1000 J/g.

2. Microwave heating resulted in a decrease of protein solubility in all tested varieties of broad beans. With increasing energy dose of microwave field, a decrease in protein solubility was observed.

3. Microwave heating increased the digestibility of protein in all tested varieties of broad bean seeds. The highest protein digestibility was obtained using a microwave field of energy dose at 1000 J/g of seeds.

4. It can be concluded that the optimal dose of microwave energy field which will produce a relatively low activity of trypsin inhibitors and the highest protein digestibility, together with maintaining solubility of broad bean seeds was 1000 J/g of seeds.

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WPŁYW POLA MIKROFALOWEGO NA AKTYWNOŚĆ INHIBITORÓW TRYPSYNY ORAZ JAKOŚĆ BIAŁKA NASION BOBU (*VICIA FABA* VAR. *MAJOR*)

STRESZCZENIE

Wstęp. W żywieniu człowieka nasiona roślin strączkowych z reguły są poddawane moczeniu i procesom termicznym, głównie gotowaniu metodą tradycyjną. Wspomniana metoda, stosowana od dziesięcioleci, nie pozwala na kontrolowanie i regulowanie parametrów procesu. Nie wydaje się więc metodą optymalną. Niewątpliwą alternatywą konwencjonalnych procesów termicznych jest wykorzystanie techniki pola mikrofalowego. Celem pracy była ocena wpływu pola mikrofalowego na aktywność inhibitorów trypsyny oraz jakość białka trzech odmian nasion bobu.

Materiał i metody. Materiałem badawczym były suche nasiona bobu odmiany *Windsor Biały, Bachus* oraz *Basta.* Nasiona moczono, a następnie ogrzewano w kuchence mikrofalowej. Otrzymały one różne dawki energii, począwszy od 500 J/g, poprzez 750, 1000, 1250, 1500, 1750 aż do 2000 J/g. W tak przygotowanym materiale badawczym oznaczono aktywność inhibitorów trypsyny, rozpuszczalność białka oraz strawność białka *in vitro*. Wyniki poddano jednoczynnikowej analizie wariancji.

Wyniki. Ogrzewanie mikrofalowe spowodowało zmniejszenie aktywności inhibitorów trypsyny i rozpuszczalności białka oraz wzrost strawności białka we wszystkich badanych odmianach nasion bobu. Wraz ze wzrostem dawki energii pola mikrofalowego obserwowano zmniejszenie rozpuszczalności białka. Zadowalającą redukcję inhibitorów trypsyny na poziomie 70-75% oraz największą strawność białka uzyskano z zastosowaniem pola mikrofalowego o dawce energii 1000 J/g nasion.

Wnioski. Na podstawie uzyskanych wyników można stwierdzić, że optymalną dawką energii pola mikrofalowego pozwalającą uzyskać stosunkowo małą aktywność inhibitorów trypsyny przy jak największej strawności białka nasion bobu i zachowaniu jego rozpuszczalności było 1000 J/g nasion.

Słowa kluczowe: inhibitory trypsyny, strawność białka *in vitro*, pole mikrofalowe, nasiona bobu

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