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THE EFFECT OF COOKING ON *IN VITRO* DIGESTIBILITY OF SELECTED LEGUMES

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Abstract

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The aim of this study was to investigate the effect of various cooking methods on nutritional quality by evaluating *in vitro* digestibility of some selected legumes (two cultivars *Pisum sativum* and *Glycine max*). Samples were soaked in 0.2% $NaHCO_3$ for 6 hours and then cooked by normal (20, 25, 30 and 35 min) pressure (8, 10, 12 and 14 min) and microwave (8, 10, 12 and 14 min) cooking. *In vitro* protein and dry matter digestibility were investigated. Pressure cooking and microwave cooking are recommended after soaking with the cooking time between 8–14 mins for *P. sativum* (Xantos and Svit) and *G. max*. Based on *in vitro* protein digestibility of all the cooking treatments, pressure cooking is the most effective.

legumes, normal cooking, pressure cooking, microwave cooking, *in vitro* protein digestibility, *in vitro* dry matter digestibility

Legumes are a good and inexpensive source of dietary proteins, carbohydrates, vitamins and minerals (Kuo *et al.*, 2004). Occurrences of malnutrition have increased in developing countries, with the increase in population and inadequate supply of protein (Adebowale *et al.*, 2005). In this context, legumes can play an important role to offset this trend due to their high content of proteins ranging from 20–40% (Baudoin and Maquet, 1999). Also it is a prestige food item in developed countries due to its health benefits (Pujola *et al.*, 2007).

Many studies on proteins of legumes, explain different reasons for limited digestibility of the seeds, such as the type of proteins present, its limited susceptibility to hydrolysis by digestive proteases, due to its structural characteristics (Tavano and Neves, 2008) and certain antinutritional factors. A variety of processing methods have been practiced including cooking which is the simplest method of processing. Appreciable research was conducted to optimize soaking and cooking treatments of legumes. It has been noted that excessive cooking, however, can result in a decreased nutritive value (Taiwo *et al.*, 1997; Buňka *et al.*, 2009). Protein digestibility is a primary determinant of the availability of amino acids. Therefore, protein digestibility is important in evaluating the nutritive quality. However, studies on quality of protein, by evaluating digestibility for different cooking methods and cooking intervals, after soaking in $NaHCO_3$, for varieties of peas widely grown in Czech Republic and Slovak Republic is minimal. Many authors reported that cooking, significantly improved the protein digestibility (Attia *et al.*, 1994; Habida, 2002) in comparison with the raw samples. Therefore, the present work was undertaken to compare the effects of cooking methods with different cooking times *in vitro* protein and dry matter digestibility.

MATERIALS AND METHODS

Legumes samples of *Pisum sativum* (Xantos, and Svit) and *Glycine max* used for this study were obtained from the Food Research Institute in Bratislava, Slovakia.

Three hundred grams of each of legumes were soaked in 0.2% $NaHCO_3$ (1:5, w/v) for 6 h at room temperature (25 °C) followed methods of past authors (Vijayakumari *et al.*, 2007) and then cooked by the methods below with a few modifications of past authors (Pastuszewska *et al.*, 2004; Mubarak, 2005). Normal cooking: soaked seeds (100 g) were boiled in tap water in the ratio of 1:4 (w/v) on a hot plate and samples were collected in four time intervals (20,

25, 30 and 35 min). Pressure cooking: Soaked seeds (100 g) were pressure cooked in house hold pressure cooker (103.42 kPa) in tap water (1:4, w/v) and Microwave cooking: Soaked seeds (100 g) were placed in a pirex pot with tap water (1:4, w/v), then cooked in a microwave oven (Goldstar, Model ER-50540, 2450 MHz, Czech Republic) and samples were collected in four time intervals for 8, 10, 12 and 14 min. All cooked samples were kept at –80 °C in freezer and lyophilized at –40°C, 12.156 Pa for 48 h (ALPHA 1-4 LSC, Czech Republic). Then samples were ground and analysed.

The *in vitro* digestibility of ground seed legumes were determined by using pepsin (3 g/1.5l of 0.1M HCl in to one jar; pepsin EC 3.4.23.1. from porcine gastric mucosa, Merck, Darmstadt, Germany) by using the Daisy^{II} Incubator at 39 ± 1 °C 24 h. Then the Jars kept at 80 ± 1 °C for 30 min to dissolve starch and bags are washed thrice with distilled water and kept in the oven at 105 ± 1 °C for 24 h and record the dry weight and undigested protein was measured by the micro Kjeldahl method and calculated *in vitro* digestibility (IVPD). Same method was carried out to analyze the digestibility of organic matter by keeping all bags in muffle furnace and the weight of ash after drying was recorded and i*n vitro* dry matter digestibility (IVDDM) was calculated (Anonym, 2008a).

Ground samples were dried in an oven at 105 ± 1 °C for 24 h to constant weight for dry matter (DM). Crude protein (CP) was determined by micro Kjeldhal method and protein content was calculated by multiplying N factor 6.25 (Anonym, 2008b).

RESULTS AND DISCUSSION

With regard to the raw seeds (Table I), the CP contents are fairly high and it ranged from 21.9–34.9% which is in agreement with past investigations (Baudoin and Maquet, 1999). *G. max* had the highest value CP. The DM content was above 91.3% and the highest (93.5%) was noted in *G. max.* IVPD was ranged from 62.6–75.0% while IVDDM was ranged from 60.8–71.5%.

According to the results of cooked samples after lyophilization the DM content was above 95% in all cooking times, irrespective of the method of cooking, for all legumes under study. The value of CP was in the range from 22.6–42.0%. The slight decrease of DM% and CP% with the increasing of cooking time may be due to leaching of compounds to the cooking water (Pujola, Farreras and Casanas, 2007).

As shown in Table II and III the values of IVDDM and IVPD were increased for all three legumes under study after cooking for all cooking times and methods of cooking when compared with respective raw seeds Table I.

After cooking the maximum value of IVDDM of *P. sativum* (Xantos), (Table II) in comparison with its respective value for raw seeds (60.8% as in Table I), was 81.7% in 14 min of pressure cooking. However, it did not differ significantly ($P \geq 0.05$) for 12–14 min of pressure cooking, for 10–14 min microwave cooking and for 35 min of normal cooking. Similarly the maximum value for IVDDM in *P. sativum* (Svit), of 86.8% was obtained in comparison with its respective value for raw seeds (69.5% as in Table I) for 14 min of microwave cooking and it did not differ significantly ($P \geq 0.05$) for 20–35 min of normal cooking, 10–14 min of pressure cooking and 12–14 min of microwave cooking. IVDDM values were always above 80% for all cooking methods and for all times of cooking. The maximum value for IVDDM of *G. max*, of 87.9% was obtained in comparison with its respective value for raw seeds (71.5% as in Table III) was for 14 minutes of pressure cooking and it did not differ significantly ($P \geq 0.05$) 35 min of normal cooking, 12–14 min of pressure cooking and 8–14 min of microwave cooking. The results of IVDDM above are in agreement with the studies of other workers (Mubarak, 2005).

As shown in Table III, the IVPD values of *P. sativum* (Xantos), in comparison with its respective value for raw seeds (62.6% as in Table I), was increased up to a maximum of 85.8% after cooking 14 min of pressure cooking and it was significant ($P < 0.05$). However, for 35 min of normal cooking, or for 12 min of pressure cooking or for 10–14 min of microwave cooking resulted in IVPD values not significantly varying with 84.3% ($P \geq 0.05$).

The IVDP values of *P. sativum* (Svit), in comparison with its respective value for raw seeds (75.0% as in Table I), was increased up to a maximum of 90.1% after 35 min of normal cooking. This value is significant ($P < 0.5$). The IVPD values of all cooking times ranging from 8 to 14 min for pressure cooking and microwave cooking did not differ significantly ($P \geq 0.05$).

The IVDP values of *G. max*, in comparison with its respective value for raw seeds (74.9% as in Table I), was increased up to 91.8% being the maximum value, after 14 min of microwave cooking. The IVPD values of *G. max* (86.5–87.3%) did not significantly differ ($P \geq 0.05$) for 25–30 min of normal cooking and 8–12 min of pressure cooking.

The increase of IVPD of legumes investigated in this study i.e. *P. sativum* (Xantos and Svit) and *G. max* may attributed to the increase in permeability of the seed coat caused by the ionic strength of the soaking in $NaHCO_3$ and heat under pressure may further enhance the leaching out of oligosaccharides into the medium by increasing the permeability of the seed coat (Vijayakumari *et al.*, 2007).

However, the reduction in IVPD with increasing cooking time was not observed in all three methods of cooking used in this study. As shown in Table III, The IVPD increase in cooked peas, in comparison with raw seeds (Table I), can be explained that the cooking times used in cooking methods may be adequate, not only to complete elimination of trypsin inhibitor, reduction of tannins and phytic acid contents, but also by the effect of heat on the three dimensional structure of pea proteins, and this reason was noted previous author (Sulieman *et al.*, 2008).

I: *Values and digestibility of dry matter and crude protein of raw seeds*

Legume	Cultivar	DM (% w/w)	IVDDM (%) *	CP (% w/w)	IVPD (%) *
P. sativum	Xantos	91.5 ± 0.08	60.8 ± 1.81^{a} A	21.9 ± 0.47	62.6 ± 4.40^{a} B
	Svit	91.3 ± 0.09	69.5 ± 4.71^{a} B	23.1 ± 0.35	75.0 ± 3.55^{a} A
G. max		93.5 ± 0.11	71.5 ± 2.31^{a} B	34.9 ± 0.40	74.9 ± 3.95^{a} B

Data shown are mean ± SD; n =10
DM – Dry matter; IVDDM – *In vitro* digestibility of Dry matter
CP – Crude protein; IVPD - *In vitro* protein digestibility
* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a raw with the same capital letter do not differ significantly ($P \geq 0.05$)

II: *Values of in vitro digestibility of dry matter (in %) with different cooking method and in different cooking times (lyophilizated samples)*

Cooking		***P. sativum*** (Xantos)	***P. sativum*** (Svit)	***Glycine max***
Method	**Time (min)**			
Normal	20	72.3 ± 0.82^{a} A	84.5 ± 2.29^{a,c} B	82.7 ± 0.56^{a} B
	25	72.6 ± 0.46^{a} A	84.5 ± 1.32^{a,c} B	82.6 ± 0.27^{a,b} B
	30	75.2 ± 0.48^{b} A	86.3 ± 0.64^{a} B	84.7 ± 0.40^{b} B
	35	80.7 ± 1.04^{c} A	86.7 ± 0.99^{a} B	87.5 ± 1.10^{c} B
Pressure	8	73.5 ± 1.18^{a} A	80.5 ± 3.77^{b,c} B	81.6 ± 0.43^{a} B
	10	75.1 ± 3.17^{a,b}A	85.8 ± 2.87^{a} B	83.4 ± 1.06^{a,b} B
	12	80.7 ± 0.38^{c} A	85.1 ± 2.14^{a} B	85.1 ± 1.56^{b,c} B
	14	81.7 ± 1.21^{c} A	86.4 ± 0.23^{a} B	87.9 ± 2.65^{c} B
Microwave	8	72.7 ± 0.93^{a} A	80.1 ± 1.39^{c} B	84.7 ± 1.10^{b,c} C
	10	78.7 ± 0.92^{b,c}A	80.7 ± 1.45^{c,d} B	85.6 ± 1.26^{b,c} C
	12	80.5 ± 2.68^{c} A	83.3 ± 1.37^{a,d} B	86.3 ± 1.46^{b,c} C
	14	80.2 ± 4.27^{b,c}A	86.8 ± 1.33^{a} B	84.6 ± 2.17^{b,c} B

Data shown are mean ± SD; n =10
* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a raw with the same capital letter do not differ significantly ($P \geq 0.05$)

III: *Values of in vitro protein digestibility (in %) with different cooking method in different cooking times (lyophilizated samples)*

Cooking		***P. sativum*** (Xantos)	***P. sativum*** (Svit)	***Glycine max***
Method	**Time (min)**			
Normal	20	73.2 ± 1.12^{a} A	85.7 ± 0.78^{a} B	75.1 ± 0.33^{a} A
	25	74.5 ± 0.03^{a} A	86.7 ± 0.97^{a} B	87.3 ± 4.53^{b} B
	30	77.8 ± 0.06^{b} A	87.7 ± 0.54^{a} B	86.5 ± 0.43^{b} B
	35	84.3 ± 0.03^{c} A	90.1 ± 0.63^{b} B	91.1 ± 0.63^{c} B
Pressure	8	73.0 ± 0.14^{a} A	84.9 ± 0.28^{a} B	87.1 ± 0.45^{b} C
	10	76.1 ± 1.49^{b} A	87.8 ± 0.44^{a} B	87.5 ± 0.52^{b} B
	12	84.0 ± 0.65^{c} A	87.9 ± 1.75^{a} B	87.5 ± 0.48^{b} B
	14	85.8 ± 0.10^{d} A	89.8 ± 0.58^{a,b} B	90.4 ± 0.14^{c} B
Microwave	8	74.2 ± 0.15^{a} A	83.0 ± 1.44^{a} B	90.6 ± 0.14^{c} C
	10	83.2 ± 0.45^{c} A	85.8 ± 0.20^{a} B	90.8 ± 0.53^{c} B
	12	83.9 ± 0.18^{c} A	85.4 ± 1.27^{a} A	90.7 ± 0.68^{c} B
	14	84.3 ± 0.14^{c} A	87.5 ± 0.33^{a,b}B	91.8 ± 0.64^{c} C

Data shown are mean ± SD; n =10
* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a raw with the same capital letter do not differ significantly ($P \geq 0.05$)

It was noted that microwave cooked legume seeds of every variety under study, had not gained the same softness in texture as in pressure cooked samples. This is in agreement with (ABD EL-Moniem, 1999). This study further confirmed the previous research that salts may have an effect on improve the textural qualities of legumes, and also affect the protein content and this has been noted by past investigators (Onwuka and Okala, 2003).

Based on the results of IVDDM, IVPD of all the cooking treatments, pressure cooking seems to be the most effective in improving IVDDM and IVDP with highest sensory quality.

Further, it is clear that cooking can be used for the improvement of protein quality of peas and this is in agreement with Nagra and Bhatty (2003). Cooking is particularly important in the preparation of legumes for consumption, from the point of view not only of acceptability but also of improvement on protein digestibility.

CONCLUSION

Values of *in vitro* dry matter and protein digestibility increased for *P. sativum* (Xantos and Svit) and *G. max* after cooking in all cooking times for normal cooking, pressure cooking and microwave cooking after soaking in 0.2% $NaHCO_3$ for 6 h. Pressure cooking (8–12 min) is the most effective in improving *in vitro* dry matter and protein digestibility after soaking with 0.2% $NaHCO_3$. Cooking can be used for the improvement of protein quality of *P. sativum* (Xantos and Svit) due to higher IVPD values after cooking and therefore, these legumes can be used as an alternative to *G. max*.

SOUHRN

Vliv způsobu kulinární úpravy na *in vitro* stravitelnost u vybraných luštěnin

V práci byl sledován účinek tří metod kulinární úpravy (klasické vaření, tlakové vaření a mikrovlnný ohřev) na *in vitro* stravitelnost sušiny a dusíkatých látek u luštěnin *Pisum sativum* (odrůd Xantos a Svit) a *Glycine max*. Zrna luštěnin byla nejprve ponořena do roztoku 0,2% $NaHCO_3$ na dobu šest hodin. Při klasické metodě vaření byla nabobtnaná semena vařena 20, 25, 30 a 35 minut, při tlakovém vaření a při mikrovlnném ohřevu 8, 10, 12 a 14 minut. *In vitro* stravitelnost sušiny vybraných luštěnin se pohybovala v rozpětí 60,8–71,5 %, dusíkatých látek v rozpětí 62,6–75,0 %. Optimální dobu vaření při klasickém a mikrovlnném ohřevu doporučujeme 8–14 minut. Rovněž tlakový ohřev lze doporučit jako vhodnou metodu kulinární úpravy luštěnin.

luštěniny, kulinární úprava, klasické vaření, tlakové vaření, mikrovlnné vaření, *in vitro* stravitelnost

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