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Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods

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Abstract

The effects of microwave cooking, compared with other conventional processes (ordinary and pressure cooking), on phytic acid, tannins, trypsin inhibitor and lectin haemagglutinating activities in vegetable peas were investigated. Protein and amino acid solubilities (in water, saline and HCl solutions) and in vitro protein digestibility (IVPD), as well as HCl-extractable ash and phosphorus, were also studied. Cooking reduced phytic acid and tannin contents. Ordinary cooking, which took longer time, was most effective in reducing phytic acid (47.9%), while microwaving gave the greatest reduction (25.7%) in pea tannins. Trypsin inhibitor and lectins were readily removed by the studied methods. IVPD of raw peas was 73.5% and it was improved upon cooking. Pressure cooking resulted in most the improvement (6.43%). Solubilities of proteins and amino acids were greatly decreased (~50%) by cooking due to thermal modification and loss of fractions soluble in cooking water. Moreover, HCl-extractable ash and phosphorus (as a measure of availability) were enhanced as cooking time increased. Pressure cooking showed the highest percentages of both extractable ash (94.93%) and phosphorus (4.36%).

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1. Introduction

Peas (*Pisum sativum*) are an excellent source of proteins, carbohydrates and essential minerals, but its utility to humans is limited by the presence of anti-nutrients, such as phytic acid, protease inhibitors and tannins (Bishnoi & Khetarpaul, 1994; Savage & Deo, 1989). Several outbreaks of food poisoning due to consumption of foods containing lectins have also been recorded (Noah, Bender, Reaidi, & Gilbert, 1980). In addition, the digestibility of legume proteins is limited by protein structure (Hsu, Vavka, Saterlee, & Miller, 1977) and the presence of antinutrients (Alonso, Aguirre, & Marzo, 2000; Chau & Cheung,1997; Nielsen, 1991). Therefore, the reduction or elimination of these antinutrients is important to improve the biological utilization of legumes, including peas, and to prevent such poisoning. In Egypt, and many other countries, peas are generally processed and consumed in different forms, depending on culture and taste preferences. The most common domestic processing methods include ordinary and pressure cooking. Microwave heating is increasing and its use for cooking is becoming popular, due to the reduction of processing time. The effects of processing vary notably, depending on the techniques and conditions, including time, temperature, moisture content and pH (Nestares et. al., 1996; Singh, 1985). Processing normally affects factors, such as trypsin inhibitors and phytate contents, which in turn can enhance or reduce the bioavailability of proteins and minerals (Nestares, Barrionuevo, Urbano, & Lopez-Frias, 1999). Some antinutrients may exert beneficial health effects at low concentrations. Thus, the manipulation of processing conditions and removal or reduction of certain unwanted components of food may be required (Shahidi, 1997). With this prospect, an attempt has been made to find out the effects of ordinary and pressure cooking, as well as microwave heating, on the level of some anti-nutrients (trypsin inhibitor, phytic acid, tannins, and lectins), in vitro protein digestibility, solubility of amino acids and proteins, and HCl-extractability of ash and phosphorus in vegetable pea seeds.

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2. Materials and methodýs

2.1. Materials

Vegetable peas (*Pisum sativum* L., c.v. Progress No. 9) were bought from a private farm in Ismailia Governorate, Egypt. After removing the extraneous matter, seeds were processed.

2.2. Processing

2.2.1. Ordinary cooking

Pea seeds were cooked in water (seed:water, 1:2) at 100 °C for different intervals (20, 30, and 40 min) which corresponded to three degrees of cooking.

2.2.2. Pressure cooking

Pea seeds were autoclaved in water (seed:water, 1:2) at 120 °C for 10, 15, and 20 min.

2.2.3. Microwave cooking

Pea seeds were microwaved at 2450 MHz. in water (seed:water, 1:2) for 4, 8, and 12 min using a domestic size Moulinex microwave Model Microchef 2335 type 907. After cooking treatments, the seeds were dried at 50 °C for 12 h and the dried seeds were ground into flour to pass a 0.5 mm sieve.

2.3. Analytical methods

Moisture, ash and total nitrogen (according to Kjeldahl method) were determined in treated and raw seeds, as described in the AOAC (1990) and crude protein content was calculated ($N\times6.25$). Phytic acid was extracted with trichloroacetic acid and precipitated as ferric salt according to Wheeler and Ferrel (1971) and tannin contents were determined by the Folin-Denis reagent according to the AOAC (1990). Tannins data were expressed as mg tannic acid per 100 g (dry weight basis).

Trypsin Inhibitor (TI) was determined as described by Kakade et al. (1974). TI activity was calculated as units/mg sample, and one TI unit was defined as a decrease in absorbance of the tested solution at 410 nm by 0.01 in 10 min.

Alpha amylase inhibitor (AI) was extracted by the procedure of Baker, Woo, Throne, and Finny (1991), and assayed according to Huessing, Shade, Chrispeels, and Murdok (1991). One unit of the AI was defined as the amount that gives 50% inhibition of a portion of the amylase that produced one mg maltose monohydrate per min. However, no AI activity against salivary or pancreatic amylase was detected in the tested samples.

Lectins were extracted according to Paredes-Lopez, Schevenin, and Guevara-Lara (1989) and the haemagglutinating activity (HA) of the lectins was measured by a serial dilution procedures using a 4% suspension of trypsinized human red blood cells (type A, B, and O), as described by Lis and Sharon (1972). One unit of HA was defined as the reciprocal of the highest dilution giving a positive agglutination (Kortt, 1984). Proteins soluble in water, saline and HCl (0.1%, pH 3) solutions (0.6 mg pea flour:15 ml extracting solution) were determined according to Lowry et. al. (1951). Bovine serum albumin was used as a standard. Amino acids, extracted with the same solutions, were determined by the ninhydrin method (AOAC, 1990) and calculated as tyrosine.

In vitro protein digestibility (IVPD) was evaluated according to the method of Hsu et al. (1977). Five millilitres of a multienzyme system, with each ml containing 1.6 mg trypsin (14 600 U/g), 3.1 mg of α-chymotrypsin (48 U/g) and 1.3 mg peptidase (102 U/g) were added to 50 ml of sample suspension (each ml containing 6.25 mg protein). The per cent protein digestibility (Y) was calculated from the equation $Y=210.464-18.1x$, where x is the pH change after 10 min. All enzymes were purchased from Sigma Chemical Company.

2.4. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the general linear model procedure of the Statistical Analysis System (SAS, 1988). Means comparisons were performed using Duncan's Multiple Range Test.

3. Results and discussion

3.1. Antinutrients

The effects of various cooking methods on phytic acid, trypsin inhibitor, tannins and lectins in pea seeds are shown in Table 1. Phytic acid in the raw seeds was 11.9 mg/g. Cooking resulted in reducing its content. This reduction varied according to cooking method and time of exposure. The greatest reduction (47.9%) was observed after 40 min of ordinary cooking. This meant that cooking of peas removed considerable amounts of phytic acid which, when present would bind to nutrients such as minerals and proteins, thus halting their utilization (Nestares et. al., 1999). These results agree with the findings of Abu El-Maati (1997); Torre, Rodriguez, and Saura-Calixto (1991), Zdunczyk, Godycka, and Amarowicz (1997).

Trypsin inhibitor activity in raw peas was 2.2 units/mg. The obtained data indicated that cooking treatments completely inactivated the inhibitor. This showed that the trypsin inhibitor of the studied peas was heat-labile. Complete inactivation of trypsin inhibitors by heat was reported by other workers (Bishnoi &

Table 1
Effect of cooking on antinutrients in vegetable pea seeds

Attribute	Phytic acid mg g^{-1}	Trypsin inhibitor units mg^{-1}	Tannins mg g^{-1}	Lectins (units)		
				A	B	O
Raw (control)						
	11.9 a	2.2	2.06 a	8	8	4
Ordinary cooked						
20 min	9.1 b (23.5)	n.d (100)	1.81 b (12.1)	n.d (100)	n.d (100)	n.d (100)
30 min	6.9 c (42.0)	n.d (100)	1.75 b (15.0)	n.d (100)	n.d (100)	n.d (100)
40 min	6.2 c (47.9)	n.d (100)	1.70 b (17.5)	n.d (100)	n.d (100)	n.d (100)
Autocalved 121 °C						
10 min	11.6 a (2.5)	n.d (100)	1.70 b (17.5)	n.d (100)	n.d (100)	n.d (100)
15 min	10.4 ab (12.6)	n.d (100)	1.63 b (20.9)	n.d (100)	n.d (100)	n.d (100)
20 min	9.5 b (20.2)	n.d (100)	1.63 b (20.9)	n.d (100)	n.d (100)	n.d (100)
Microwaved						
4 min	11.9 a (0.0)	n.d (100)	2.00 a (2.9)	n.d (100)	n.d (100)	n.d (100)
8 min	10.2 ab (14.3)	n.d (100)	1.54 b (25.2)	n.d (100)	n.d (100)	n.d (100)
12 min	7.3 c (38.7)	n.d (100)	1.53 b (25.7)	n.d (100)	n.d (100)	n.d (100)

Values in the same column with different letters are significantly different ($P=0.5$); four replicates, each performed three times were carried out. Figures in the parentheses indicate the percentage decrease over the control values.

Khetarpaul, 1994; Liener, 1975; Trugo, Monanglo, Trugo, & Bachknudsen 2000) for legume seeds.

Tannins in peas were reduced from 2.06 mg/g in raw seeds to 1.53 mg/g in microwave cooked peas. Pressure cooking brought about a higher reduction (17.5–20.9%) in tannins than that observed by ordinary cooking (12.1–17.5%). As noticed with phytic acid, cooking partly eliminated tannins. These results are in agreement with those of Egbe and Akinyele (1990) in lima bean and Abd El-Rahman and Abd El-Aleem (1996) in black bean.

Lectin HA in raw peas, against human red blood cells type A, B, and O, were 8,8 and 4 units, respectively. Fortunately, all the studied cooking regimes totally destroyed pea lectins in the studied cultivar. Kotaru, Saito, Yoshikawa, Icheuchi, and Ibuki (1987) observed that HA in winged bean (with respect to human erythrocytes type A, B and O) had completely disappeared after heating in a boiling water bath for 10 min. Also, Abu El-Maati (1997) revealed that canning totally inactivated HA in mung bean and peas. Lectins would result in harmful effects, including depleting body proteins, rapid weight loss and ultimately death (Lalles & Jansman, 1998).

3.2. Protein digestibility

Total crude proteins and IVPD are sown in Table 2. A slight decrease in the crude proteins was observed in the cooked peas. The raw seeds contained 27.2%, while cooked peas contained from 25.6 to 26.3% (dry weight basis) according to the time and method of cooking. This decrease was probably due to leaching of water-soluble proteins into cooking water. Similar results were reported by Manan, Hussain, Alli, and Iqbal (1987), Periago, Vidal, Ros, Rincon, and Martinez (1998) and Zdunczyk et al. (1997).

Table 2
Protein content and in vitro protein digestibility of vegetable pea seeds, as affected by cooking method

Attribute	Total crude protein, (N×6.25) (%)	In vitro protein Digestibility (%)
Raw		
	27.2±1.0	73.5±1.3
Ordinary cooked		
20 min	26.0±0.9	76.0±1.4 (3.37)
30 min	25.9±1.1	77.2±1.6 (4.91)
40 min	25.6±1.0	78.3±1.2 (6.43)
Autocalved 121 °C		
10 min	25.8±1.1	77.4±1.2 (5.21)
15 min	25.7±1.2	78.3±1.4 (6.43)
20 min	25.7±1.0	78.12±1.5 (6.23)
Microwaved		
4 min	26.3±1.1	74.2±1.4 (0.91)
8 min	26.2±1.0	75.1±1.3 (2.15)
12 min	26.2±0.9	75.5±1.2 (2.69)

Data are expressed as average±standard deviation of four replicates, each performed three times. Figures in the parentheses indicate the percentage increase over the control values.

Protein digestibility is a primary determinant of the availability of amino acids and, therefore, protein digestibility is important in evaluating the nutritive quality of a food protein. The IVPD of raw peas was 73.5% and was improved by cooking. The highest

Table 3
Amino acid (AA) and protein solubilities (in water, saline and HCl) of vegetable peas, as affected by cooking method

Attribute	Water-extractable		Saline-extractable		HCl-extractable	
	Protein (%)	Total AA (%)	Protein (%)	Total AA (%)	Protein (%)	Total AA (%)
Raw						
	3.13 a	4.37 a	10.80 a	3.75 a	4.2 a	6.87 a
Ordinary cooked						
20 min	1.63 c	0.68 c	5.60 c	0.75 c	1.68 c	3.13 b
30 min	1.55 c	0.71 c	5.60 c	0.56 c	1.80 c	1.82 b
40 min	1.56 c	0.84 c	5.70 c	0.31 c	1.85 c	0.88 c
Autocalved 121 °C						
10 min	1.72 c	0.90 c	5.50 c	0.62 c	1.68 c	0.88 c
15 min	1.72 c	0.94 c	5.56 c	0.50 c	1.72 c	0.63 c
20 min	1.75 c	1.16 c	5.52 c	0.37 c	1.85 c	0.63 c
Microwaved						
4 min	2.33 b	3.34 b	8.20 b	3.31 a	3.25 b	6.25 a
8 min	1.55 c	1.94 bc	5.76c	1.13 b	1.72 c	2.56 b
12 min	1.60 c	2.50 b	5.68 c	1.06 b	1.63 c	2.31 b

Values in the same column with different letters are significantly different ($P = 0.5$). Four replicates, each performed three times, were carried out.

Table 4
Total and HCl-extractable ash and phosphorus in raw and cooked vegetable peas

Attribute	Ash (%)		Phosphorus mg 100 g^{-1}	
	Total	HCl-extractable	Total	HCl-extractable
Raw				
	3.55±0.10	2.82±0.09 (79.4)	475±18	25.0±0.8 (5.26)
Ordinary cooked				
20 min	2.90±0.08	2.01±0.06 (69.3)	419±16	8.75±0.3 (2.09)
30 min	2.71±0.07	2.02±0.06 (74.5)	394±15	10.1±0.3 (2.51)
40 min	2.62±0.07	2.30±0.08 (87.8)	388±15	13.1±0.4 (3.38)
Autocalved 121 °C				
10 min	2.84±0.09	2.14±0.07 (75.4)	394±15	8.80±0.3 (2.23)
15 min	2.84±0.08	2.55±0.08 (89.8)	351±14	13.8±0.4 (3.92)
20 min	2.76±0.07	2.62±0.08 (94.9)	346±14	15.1±0.5 (4.36)
Microwaved				
4 min	3.22±0.10	2.53±0.08 (78.6)	463±17	10.0±0.3 (2.16)
8 min	3.07±0.09	2.76±0.09 (89.9)	395±16	13.1±0.4 (3.32)
12 min	3.00±0.08	2.80±0.10 (93.3)	388±14	13.1±0.4 (3.39)

Data are expressed as average±standard devietion of four replicates, each performed three times. Figures in the parentheses indicate the percentages of HCl-extractable ash and phosphorus.

IVPD (78.3%) was obtained by pressure cooking (15 min) or by ordinary cooking (40 min), whilst the least improvement was noticed in the microwave-cooked peas (4 min). Similar values were reported for the Pakistani (Manan et. al., 1987) and Polish (Abd El-Moniem, Honke, & Bednorska, 2000) pea cultivars.

The IVPD increase in cooked peas, as compared with raw, may be explained, not only by the complete elimination of trypsin inhibitor (Gad et al., 1982), the reduction of tannins and phytic acid contents (El-Shami, 1993), but also by the effect of heat on the three dimensional structure of pea proteins (Chau & Cheung, 1997; Hsu et al., 1977). However, processing can also cause a decrease in protein digestibility, via non-enzymatic browning, thermal cross-linking (Tannenbaum, 1974), and the formation of complexes between proteins and tannins or phytates (Burbano, Muzquiz, Osagie, Ayet, & Cuadrado, 1995; Trugo & Van Baer, 1998). Moreover, some proteins, such as globulins, the major storage proteins in legumes, are intrinsically more resistant to proteolysis (Chau & Cheung, 1997) even after thermal treatment (Alonso et al., 2000).

3.3. Amino acids and protein solubility

Solubilities of pea proteins and amino acids are shown in Table 3. Solubilities of proteins of the uncooked peas in water, saline and HCl solutions were 3.13, 10.8 and 4.2%, respectively. Variation in protein solubility could be attributed to the type of proteins in peas and the solvents applied. It is established that globulins are the major storage proteins in legumes. The pH is also an important factor. Carbonaro, Capelloni, Nicoli, Lucarini, and Carnovale (1997) observed a marked reduction in protein solubility after cooking all legumes (faba bean, lentil, chickpea and dry bean) in water and saline solutions in a pH range of 1–13. At pH 10, solubilization occurred. In the present study, cooking generally reduced protein solubility. Also, the three cooking methods gave comparable effects, except microwave heating for a short time (4 min), which showed a

relatively higher solubility than other heat treatments. This observation was true in all studied solvents. Sakla, Ghali, El-Farra, and Rizk (1988) found that water-soluble proteins of soy bean decreased from 13.1% (raw) to 4.57% after 6 min of microwave treatment.

Also, amino acid solubility was significantly decreased by cooking, regardless of the method used. Unlike proteins, the amino acids of pea seeds were most soluble in HCl solution (6.87%), followed by water (4.37%). This could be attributed to the proportion of charged amino acids, as compared with the net charge on the pea proteins in the HCl solution.

3.4. Ash and phosphorus availability

HCl-extractable minerals were used as a measure of mineral bioavailability (Duhan, Khetarpaul, & Bishnoi, 1998, 1999).

As shown in Table 4, cooking resulted in decreasing total and HCl-extractable ash (HClA), as well as total and HCl-extractable phosophorus (HClP) in pea seeds. This could be due to the fact that most minerals were readily soluble in aqueous solution and leach out into cooking water. However, cooking especially for a longer time, increased the percentage of HClA (HclA × 100/total ash). Autoclaving for 20 min showed the highest percentage (94.93%) followed by microwaving (93.33%).

Total phosphorus was also reduced by cooking as part of the element was transferred into the cooking water. Raw seeds contained 475 mg/100 g, while the cooked seeds contained from 346 to 463 mg/100 g. Pressure cooking (more than 10 min) showed the lowest total phosphorus content. However, HClP showed a different trend. As cooking time increased, the amount and percentage of HClP increased. This observation may be attributed to the reduction in the level of phytic acid upon cooking (as shown in Table 1). It is notable that the tabulated value of phytic acid was the amount of extractable phytate, as some of the phytate may form unextractable complex with proteins or with other macromolecules (Gad, Mohamed, El-Zalaki, & Mohasseb, 1982; Reddy & Salunkhe, 1981, Reddy et al., 1988). Also, Duhan et al. (1999) ascribed the improvement of HCl extractability of calcium and phosphorus of cooked pigeon peas to the reduction in phytic acid and polyphenols.

In conclusion, the studied cooking methods resulted in a reduction in phytic acid and tannin contents, and completely eliminated trypsin inhibitors and lectins in the cooked peas. Also, cooking improved the IVPD of pea samples. The solubilities of proteins and amino acids of the cooked peas were also greatly decreased due to thermal modification and loss of fractions soluble in cooking water. Moreover, HCl-extractable ash and phosphorus were enhanced as cooking time increased, due to the reductions in phytic acid and tannin contents. Pressure cooking showed the most improvement in the IVPD of peas, while microwave cooking showed the least.

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