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Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*)

Hefnawy¹*, T.H.

a- Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

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Abstract: The effects of microwave cooking and other traditional cooking methods such as boiling and autoclaving on the nutritional composition and anti-nutritional factors of lentils were studied. Cooking treatments caused decreases in carbohydrate fractions was completely eliminated after cooking treatments, antinutritional factors (trypsin inhibitor, tannins and phytic acid) and minerals. Cooking treatments decreased the concentrations of lysine, tryptophan, total aromatic and sulfur-containing amino acids. The losses in minerals in lentil cooked by microwaving were smaller than those cooked by boiling and autoclaving. Based on these results, microwave cooking is recommended for lentil preparation, not only for improving nutritional quality, but also for reducing cooking time.

1. Introduction

Generally, legumes have been reported to have low nutritive value because of low amounts of sulfur-containing amino acids, low protein digestibility and the presence of anti-nutritional factors. Legumes are usually cooked before being used in the human diet. This improves the protein quality by destruction or inactivation of the heat labile anti-nutritional factors (Chau et al 1997 and Vijayakumari et al 1998). However, cooking causes considerable losses in soluble solids, especially vitamins and minerals (Barampama and Sirmard, 1995).

Lentils (*Lens culinaris*) is one of the most important pulse crops in the world because of their nutritional quality. They are rich sources of complex carbohydrates, protein, dietary fiber, vitamins, minerals and high energetic value (Costa et al 2006 and Wang, 2008). Pulses have demonstrated many health benefits: lowering glycemic index for people with diabetes (Viswanathan et al 1989); in cancer prevention (Hangen and Bennink, 2002 and Wang et al 2008) and in protection against cardiovascular diseases due to

its dietary fiber content. Numerous studies have shown that people with high fiber intake have blood pressure lower than those with low fiber intake (Brand et al 1990). Soluble fiber also decreases serum cholesterol and aids in reducing the risk of heart attack and colon cancer (Kelsey, 1978 and Sharma and Kawatra, 1995). A high fiber diet prevents or relieves constipation in human due to the absorption of water from the digestive track (Wang et al 2009).

Pulses contain anti-nutritional components that limit their utilization. These components include trypsin inhibitors, phytic acid, tannins and oligosaccharides. Trypsin inhibitors are low molecular weight proteins capable of binding to and inactivating the digestive enzyme, trypsin (Dave Oomah et al 2011). Phytic acid lowers the bioavailability of minerals (Reddy et al 1988). The chemical composition and oligosaccharides of raw and germinated lentils seeds were reviewed by (Wang et al 2009). One of the major disadvantages of tannins in lentils is a discoloration of the seed. In addition to seed discoloration, tannins bind to proteins through hydrogen binding and hydrophobic interactions, thereby reducing their nutritional quality (Hahn et al 1984). While some efforts have been directed to minimize their contents in the seeds or to minimize their effects through processes, little information

* E-mail Address: hefnawy72@hotmail.com

on the effect of varietal and environmental conditions on these anti-nutritional factors is available.

Cooking of lentil by microwave has not been extensively studied but it has been shown to reduce antinutritive agents in soybean (Rajko et al 1997) and have positive effects on protein digestibility (Khatoun and Prakash, 2004) in eight hole legumes. A study on chickpea cooked by microwave is thus needed to know whether this treatment could improve nutritional quality and eventually replace traditional cooking methods, which are not only costly in energy but also cause important losses in soluble solids. This experimental study was therefore carried out to determine the effect of boiling, autoclaving and microwave cooking on the nutritional composition and nutritive value of lentil seeds (Wang and Dawn 2005).

2. Materials and methods

2.1. Materials

One batch (10 kg) of local lentil were purchased from the local market (Egypt). The seeds were hand-sorted to remove wrinkled, moldy seeds and foreign material, then stored in polyethylene bags in the refrigerator ($4^{\circ}\text{C}\pm 1$) until used.

2.1.1. Processing: cooking treatments

Lentil seed were soaked in distilled water (1:10, w/v) for 12 h at room temperature (25°C). The soaked seeds were drained and rinsed three times with 600 mL distilled water, then cooked by the methods described below:

2.1.2. Boiling: The rinsed soaked seeds were cooked in tap water (100°C) in the ratio of 1:10 (w/v) on a hot plate until they became soft when felt between the fingers (90 min).

2.1.3. Autoclaving: The rinsed soaked seeds were autoclaved using vertical autoclave at 15 lb pressure (121°C) in tap water (1:10, w/v) until they became soft when felt between the fingers (35 min).

2.1.4. Microwave cooking: The rinsed soaked seeds were placed in a glass beaker with tap water (1:10 w/v), then cooked in a microwave oven on high for 15 min until they became soft when felt between the fingers. The cooked seeds were dried in an electric air draught oven at 50°C for 20 h

2.1.5. Cooking treatments: were replicated three times. Raw and processed lentil were ground in an electric mill equipped with stainless steel blades to pass through a 60 mesh (British standard screen) nylon sieve.

2.2. Chemical analysis

2.2.1. Chemical analysis: of nutritional constituents. Nitrogen (N) was determined by the Dumas combustion method calibrated with EDTA (AOAC, 2000). Crude protein content was calculated using ($\text{N} \times 5.75$). Moisture and ash content were determined gravimetrically in accordance with AACC methods 44-17 and 08-16, respectively (AACC, 2000). Starch was determined colorimetrically as described by the method AACC 76-13 (AACC, 2000). Resistant starch was measured by the AACC method 32-40 (AACC,

2000). Soluble, insoluble and total dietary fiber contents were determined by sequential enzymatic digestion according to AACC method 35-05 (AACC, 2000). Minerals were determined by atomic absorption spectrophotometry (Gawalko et al 1997).

2.2.2. Chemical analysis of anti-nutritional constituents

Trypsin inhibitor activity (TIA) was determined colorimetrically using an Ultraspec 3000 spectrophotometer (Biochrom Ltd., Cambridge, England) at 410 nm (Smith et al 1980) with benzoyl-DL-arginine-p-nitroanilide hydrochloride as the substrate. Tannins were assayed in accordance with the modified vanillin-HCl method of (Price et al 1978) and (+)-catechin was used as the reference standard. Phytic acid was extracted and separated by ion-exchange chromatography according to the method of AOAC (2000) before being quantified colorimetrically using an Ultraspec 3000 spectrophotometer (Biochrom Ltd., Cambridge, England) at 500 nm (Latta and Eskin, 1980).

2.2.3. Oligosaccharides were determined by high performance anion exchange (HPAE) chromatography with pulsed amperometric detection (PAD) (Wang and Daun, 2006). The HPAE system is comprised of an ICS-3000 solvent delivery pump, an ED40 chemical electrode detector, an AS50 autosampler and an AS50 thermal compartment. One gram of lentil flour was extracted with 20 ml of 80% EtOH at 70°C for 30 min. Lactose was added as an internal standard in the sample before extraction. The suspension was then centrifuged at 12,000 rpm at 15°C for 15 min. The supernatant was decanted, and an aliquot was centrifuged at 12,000 rpm for 5 min. A sample of the centrifuged supernatant was diluted with deionized water and passed through a 0.45 mm filter. The filtrate (25 ml) was injected on a PA1 analytical anion exchange column (4 x 250 mm). The mobile phase used was 0.15 mol/L sodium hydroxide at the flow rate of 1.0 ml/min.

2.2.4. Amino acids

Total amino acids were determined on a Beckman 7300 High Performance Amino Acid Analyzer from hydrolysates obtained by hydrolysis of 15–25 mg sample with 2.0 ml of 6.0 N HCl in an evacuated sealed tube at 110°C for 24 h. Cystine was measured as cysteic acid and methionine as methionine sulfone after performic acid oxidation prior to hydrolysis in 6 N HCl, and tryptophan analysis was performed on a hydrolysate obtained by alkaline hydrolysis of a sample as described by (Tkachuk and Irvine, 1969).

2.3. Statistical analyses

Data were assessed by analysis of variance (ANOVA) (SAS, 2002). The Duncan multiple range test was used to separate means and significance was accepted at $P \leq 0.05$.

3. Results and discussion

3.1. Chemical composition

Chemical compositions of raw and treated lentils seeds are presented in Table (1). No significant ($P > 0.05$) differences in total protein and moisture contents were observed between cooked treatments of lentils seeds. These observations are in agreement with those reported by (Barampama and Simard, 1995) for

Table 1. Effect of different cooking methods on the chemical composition of lentil seeds (g/100 g dry weight basis)*

Treatment	Total protein	Non-protein nitrogen	Ash	Fat	Crude fiber	Moisture
Raw	26.6 ± 0.50	2.4 ± 0.09	3.4 ± 0.04	1.0 ± 0.08	6.3 ± 0.13	8.51 ± 0.31
Boiling	26.2 ± 0.36	2.1 ± 0.12	3.3 ± 0.07	0.9 ± 0.09	6.2 ± 0.12	8.63 ± 0.29
Autoclaving	26.1 ± 0.47	2.0 ± 0.07	3.3 ± 0.06	0.9 ± 0.06	6.4 ± 0.10	8.55 ± 0.33
Microwave cooking	26.1 ± 0.50	2.2 ± 0.10	3.2 ± 0.10	0.9 ± 0.07	6.4 ± 0.12	8.4 ± 0.35

*Means in the same column with different letters are significantly ($P < 0.05$) different.

*Means ± standard deviation of three determinations.

cooked common beans (*Phaseolus vulgaris*). Also, (Khatoon and Prakash, 2004 and Wang, 2005) reported that microwave cooking and pressure cooking do not affect the nutrient composition of eight legumes. Cooking treatments significantly ($P < 0.05$) decreased the non-protein nitrogen, ash and fat contents. These decreases might be attributed to their diffusion into cooking water. Crude fiber was significantly ($P < 0.05$) increased by cooking treatments. This increase could have been due to protein-fiber complexes (Bressani, 1993) formed after possible chemical modification induced by the soaking and cooking of dry seeds.

3.2. Oligosaccharides

Table (2) were shows oligosaccharides of raw and treated chickpea seeds. Reducing sugars, sucrose, raffinose and stachyose were significantly ($P < 0.05$) reduced, while verbascose was completely eliminated after cooking treatments. These reductions are presumably due to their diffusion into cooking water. No publications were found regarding the effect of microwave cooking on the flatulence factors (raffinose, stachyose and verbascose) content of legumes. These observations are in agreement with that reported by (Khalil and Mansour, 1995).

3.3. Antinutritional factors

The antinutritional factors of raw and treated lentils seeds are shown in Table (3). Trypsin inhibitor activity was significantly ($P < 0.05$) decreased by cooking treatments. The highest reduction was noted after autoclaving (80.87%), followed by boiling (80.27%) and microwave cooking (81.50%). Wang et al (1997) reported that steam blanching of cowpea resulted in higher reduction in trypsin inhibitor activity than using water blanching. However, (Hernandez-Infante et al 1998) reported that microwave cooking destroyed trypsin inhibitors to a degree similar to that observed in six legumes cooked using the conventional method.

Tannins (46.04–49.10%) and phytic acid (30.93–41.32%) in lentils were significantly ($P < 0.05$) reduced by cooking. Similar results were obtained by (Vijayakumari et al 1998 and Wang et al (2009).

3.4. Minerals

Mineral contents of raw and cooked lentils seeds are presented in Table (4). The minerals leached from the lentils seeds into the distilled water at different rates during cooking treatments. However, microwave cooking resulted in the greatest retention of all minerals, followed by autoclaving, then boiling. (Haytowitz and Matthews, 1983) reported that cooking in boiling water caused great losses of K (30%), Cu (17%) and Fe (10%). (Longe, 1983) reported losses of 30% Cu and 23% Mg from mature cowpeas when cooked by autoclaving.

3.5. Amino acid composition

Data presented in Table (5) show the amino acid composition of raw and treated lentils seeds. Lentils protein was rich in essential amino acids such as isoleucine, lysine, total aromatic amino acids and tryptophan compared with the (Ereifej and Haddad, 2001) reference. Therefore, lentils protein could very well complement those protein sources that are low in lysine and tryptophan. However, leucine, total sulfur amino acids, threonine and valine were slightly deficient in lentils protein compared with the reference pattern. Boiling and microwave cooking caused a slight increase in total essential amino acids, but they were not influenced by autoclaving. Cooking treatments decreased the concentration of lysine (except microwave cooking), tryptophan, and total aromatic and sulfur amino acids. However, cooked lentils seeds were still higher in lysine, isoleucine (except autoclaving) and total aromatic amino acid contents than the (Ereifej and Haddad, 2001) reference pattern. These results confirmed those reported by (Khalil and Mansour, 1995), who found that cooking reduced sulfur-containing amino acids and tryptophan in faba bean. All treatments increased the concentration of leucine, but valine was not affected. The leucine:isoleucine ratios of all treated lentils seeds were typical, with an ideal ratio of 1.8:1 suggested by (Ereifej and Haddad, 2001).

4. Conclusions

As shown in this study, boiling, autoclaving and microwave cooking affect the composition, antinutritional factors, flatulence factors and nutritional quality of chickpeas. However, microwave cooking caused slight losses in minerals, while boiling and autoclaving caused significant losses. All cooking treatments improved the in-vitro protein digestibility and protein efficiency ratio of lentils. It is quite clear that cooking lentils by microwave not only saves time but also retains the most nutritive value.

Table 2. Effect of different cooking methods on the carbohydrate fractions of lentil seeds (g/100 g dry weight basis)*

Treatment	Reducing sugars	Sucrose	Raffinose	Stachyose	Verbascose	Starch
Raw	0.75 ± 0.04	1.79 ± 0.08	0.40 ± 0.07	1.81 ± 0.08	0.48 ± 0.06	42.0 ± 0.60
Boiling	0.60 ± 0.05	1.4 ± 0.08	0.20 ± 0.05	1.0 ± 0.07	0.31 ± 0.00	41.0 ± 0.40
Autoclaving	0.56 ± 0.04	1.3 ± 0.11	0.10 ± 0.09	1.1 ± 0.06	0.22 ± 0.00	41.5 ± 0.29
Microwave cooking	0.59 ± 0.05	1.36 ± 0.11	0.0 ± 0.08	0.9 ± 0.07	0.22 ± 0.00	41.2 ± 0.50

*Means in the same column with different letters are significantly (P<0.05) different.

*Means ± standard deviation of three determinations

Table 3. Effect of different cooking methods on the antinutritional factors of lentil seeds (dry weight basis)*

Treatment	Trypsin inhibitor	Tannins	Phytic acid
Raw	2.83 ± 0.10	1.28 ± 0.05	4.11 ± 0.09
Boiling	0.15 ± 0.09	0.91 ± 0.07	2.6 ± 0.06
Autoclaving	0.19 ± 0.10	0.82 ± 0.10	2.4 ± 0.08
Microwave cooking	0.19 ± 0.08	0.84 ± 0.09	2.5 ± 0.05

*Means in the same column with different letters are significantly (P<0.05) different.

*Means ± standard deviation of three determinations.

Table 4. Effect of different cooking methods on selected mineral contents of lentil seeds (mg/100 g dry weight basis)*

Treatment	Ca	K	Mg	P	Na	Fe	Cu	Zn	Mn
Raw	97.3 ± 0.12	960 ± 0.66	138 ± 0.32	541 ± 0.20	78 ± 0.08	7.3 ± 0.06	1.0 ± 0.89	4.3 ± 0.04	2.4 ± 0.07
Boiling	50.21 ± 0.11	420 ± 0.51	118 ± 0.28	462 ± 0.14	72 ± 0.07	6.1 ± 0.01	0.73 ± 0.55	3.4 ± 0.05	1.8 ± 0.04
Autoclaving	54.6 ± 0.23	512 ± 0.23	122 ± 0.21	480 ± 0.10	73 ± 0.09	6.9 ± 0.03	0.81 ± 0.48	3.8 ± 0.04	1.9 ± 0.08
Microwave cooking	56.7 ± 0.10	520 ± 0.21	124 ± 0.21	488 ± 0.90	75 ± 0.09	7.0 ± 0.06	0.94 ± 0.44	3.9 ± 0.02	2.0 ± 0.02

*Means in the same column with different letters are significantly (P<0.05) different.

*Means ± standard deviation of three determinations.

Table 5. Effect of different cooking methods on the amino acid composition of lentil seeds (g/16gN)

Treatment	Raw	Boiling	Autoclaving	Microwave cooking
Alanine	4.3	4.0	4.2	3.9
Arginine	7.2	7.0	7.0	7.0
Aspartic	11.5	11.4	11.4	11.5
Glutamic	15.5	16.6	16.0	16.3
Glycine	4.1	4.5	3.9	4.0
Histidine	3.0	2.8	2.9	2.9
Isoleucine	4.7	4.6	4.5	4.6
Leucine	7.4	8.0	7.6	7.6
Lysine	7.0	6.8	6.6	6.9
Methionine	1.4	1.2	1.2	1.3
Cystine	1.2	1.1	1.0	1.2
Phenylalanine	6.1	5.8	6.0	6.0
Tyrosine	2.3	3.1	2.2	2.3
Proline	4.0	3.7	4.5	4.5
Serine	4.6	4.2	4.5	4.3
Threonine	3.8	4.6	4.8	4.5
Tryptophan	0.7	0.5	0.6	0.6
Valine	5.2	5.1	5.1	5.1

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تأثير طرق معاملات الطهي على المكونات الغذائية وعوامل مضادات الغذاء على العدس

[٦]

حفاوى طه منصور حفاوى^١

١ - قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة الزقازيق - الزقازيق - مصر

الموجز

أدت الى انخفاض تركيز الاحماض الامينية العطرية والكبريتية وكذلك الليسين. الفقد في المكونات الغذائية في العدس المطبوخ بواسطة الميكرويف كان اقل من الفقد باستخدام الغليان والأتوكيلاف استنادا الى هذه النتائج فإننا نوصى بطبخ العدس بواسطة الميكرويف لانه يقلل فترة الطهي ويحسن من الجودة

دراسة تأثير الطهي بالميكرويف وغيرها من اساليب الطهي التقليدية مثل الغليان والتعقيم على المكونات الغذائية وعوامل مضادات الغذاء على العدس - معاملات الطهي ادت الى انخفاض فى اقسام السكريات وعوامل مضادات الغذاء (التريبسين والتانينات وحمض الفايترك)، وكذلك معاملات الطهي