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Changes in Total Protein Digestibility, Fractions Content and Structure During Cooking of Lentil Cultivars

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Abstract: The effect of cooking on total protein digestibility and fractions content and structure of lentil cultivars was investigated. Cooking significantly (P = 0.05) reduced the protein digestibility using pepsin and/or pancreatin. Pepsin digestibility of raw seeds ranged from 44.6 to 52.1% and that of both pepsin and pancreatin ranged from 81.8 to 99.9%. Cooking reduced the protein digestibility of the cultivars and was found to be range from 22.3 to 19.7% when pepsin was used and ranged from 77.1 to 88.2% when both pepsin and pancreatin were used. The major protein in lentil was albumin followed by globulin. Cooking significantly (P = 0.05) decreased the albumin content. The decrease was accompanied by significant increment in the glutelin fractions. SDS-PAGE of cooked lentil protein fractions showed that lentil protein was altered quantitatively and qualitatively due to cooking. The number of subunits of total protein in lentil cultivars before cooking was found to range from 17 to 19 bands. However, after cooking they decreased and ranged from 13 to 16 bands. The effect of cooking was most pronounced in the prolamins fractions and its subunits were reduced from 4 to 2 with a higher molecular weight of 56.0kDa.

Key words: Protein digestibility, fractionation, cooking, lentils, cultivars

Introduction

Food legumes, distinctively termed "poor man's meat" appear to be the most potential solution to overcome the crisis of protein-calorie malnutrition in the less developed countries (Zahary, 1972). Lentil is probably the oldest grain legume to be domesticated (Bahl *et al.*, 1993). It is now cultivated in most subtropical and also in the northern hemisphere such as Canada and Pacific Northwest regions. Protein concentration of lentils reportedly ranges from 22 to 34.6% (Adsule *et al.*, 1989). About 90% of lentil protein is found in the cotyledons, with albumins and globulins being the major fractions. The digestibility of legume protein is dependent on protein structure (Deshpande and Damodaran, 1989). Digestibility coefficients for lentil are relatively high and range from 78 to 93%, while biological values range from 32 to 58% (Husle, 1990). Heating is responsible for protein denaturation, eventually followed by aggregation of the unfolded molecules, which results in loss of solubility. Thermal denaturation involves an initial stepwise dissociation of subunits and subsequent reassociation of only partially unfolded molecules with formation of either soluble or insoluble complexes (Kinsella *et al.*, 1985). The protein quality of a food or feed depends on its amino acid composition and digestibility; protein digestibility primarily determines the availability of its amino acid (Hahn *et al.*, 1981).The existence of folding of polypeptide chains in the native protein structure as well as the subunits in some proteins facilitates studying the denaturation phenomena. Accordingly, changes in molecular size or shape serve for characterization of the denatured proteins (Monteiro *et al.*, 1982). Also they reported that separation of protein components on polyacrylamide gels depends not only on the charge but also very strongly on the size of the molecule. They concluded that binding of dodecyl sulphate ion to protein was the basis of electrophoretic separation. The present study was undertaken to study the effect of cooking on total protein digestibility and fractions content and structure of lentil cultivars.

Materials and Methods

Three Sudanese lentil cultivars were obtained from Elhudaiba Research Station, Northern State and the fourth one (Indian) was obtained from Khartoum North market. Samples were carefully cleaned and freed from foreign materials and then ground to pass a 0.4mm screen. For cooking, the flour was suspended in water (1:10 w/v) and boiled in a boiling water bath for 20 min., the cooked gruel was then dried at 65°C and ground to pass a 0.4mm screen. All reagents used in this study were reagent grade.

Determination of *in vitro* protein digestibility

Pepsin digestibility: The *in vitro* protein digestibility was carried out according to the method of Maliwal (1983) as described by Monjula and John (1991) with a minor modification. A known weight of the sample containing 16mg nitrogen was taken in triplicate and digested with 1mg pepsin in 15 ml of 0.1 M HCl at 37°C for 2 h. The reaction was stopped by addition of 15ml of 10% trichloroacetic acid (TCA), the mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA .soluble fraction was assayed for nitrogen using the micro-Kjeldahl method (AOAC, 1984). Digestibility was obtained by using the following equation:

$$\text{Protein digestibility \%} = \frac{\text{N in supernatant-enzyme N}}{\text{N in sample}} \times 100$$

Pepsin-pancreatin digestibility: The method was carried according to Saunders *et al.* (1973). About 250 mg sample was suspended in 15ml of 0.1 NHCl containing 1.5mg pepsin, the mixture was incubated at 37°C for 3 h, then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 ml 0.2 M phosphate buffer (pH 8.0)., containing 0.005 M sodium azide, then the mixture was incubated at 37°C for 24 h. 10 ml of 10% trichloroacetic acid (TCA) were added to stop the reaction then centrifuged at 5000rpm for 5 minute. 5ml of the supernatant were taken for nitrogen analysis using the micro-Kjeldahl method (AOAC, 1984).

Calculation:

$$\text{Protein digestibility \%} = \frac{(T\text{-}B)\times N\times 14\times 00\times TV}{(X)\times a}$$

Where:

$$X = \frac{250 \times CP\,\%}{100 \times 6.25}$$

N = Normality of HCl, T = ml of titer, B = ml of banck, a = Number of ml of aliquot, TV = Total volume of the mixture, 14 = Equivalent weight of nitrogen, 250 = Sample weight in mg, CP%= Percent crude protein.

Protein fractionation: The protein fractions were extracted according to solubility in different solvents as described by Osborne and Mendel (1914) with a minor modification.2gm of cooked and uncooked lentil flour were extracted twice with 30ml IM NaCl for 30 min at room temperature using a mechanical shaker, then centrifuged at 3000rpm for 30 min. About 10ml of the liquor was taken for protein determination using micro-Kjeldahl method (globulin). The residue was then extracted successively in a similar manner with distilled water (albumin), 70% ethanol (prolamin), 0.2% NaOH (glutelin), the residue represents the insoluble protein.

SDS-polyacrylamide gel electrophoresis: SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was done, using the method of Laemmli (1970), with 15% acrylamide separating gel and 3% acrylamide stacking gel containing 0.1% SDS. Samples (20 µl, 0.2%) were prepared in a Tris-glycine buffer at pH 8.8 containing 1% SDS. Electrophoresis was done at a current of 10m A for 5 h in electrophoretic Tris-glycine buffer containing 0.1% SDS. After electrophoresis, the gel sheets were stained for proteins with 0.2% Coomassie brilliant blue-R250. Protein stain was destained with 10% acetic acid containing 20% methanol.

Table 1: Effect of cooking on *in vitro* protein digestibility (IVPD) of lentil cultivars using pepsin and pepsin with pancreatin

		IVPD (%)	
Cultivars	Treatments	Pepsin	Pepsin with pancreatin
Nadi	Uncooked	48.61 (±0.60)^a	81.76 (±1.69)^a
	Cooked	20.93 (±1.21)^b	77.05 (±1.42)^b
Rubatab	Uncooked	50.21 (±1.74)^a	94.46 (±1.06)^a
	Cooked	24.02 (±0.53)^b	81.55 (±1.31)^b
Seliam	Uncooked	44.57 (±2.11)^a	99.88 (±1.19)^a
	Cooked	22.32 (±0.16)^b	88.16 (±2.60)^b
Indian	Uncooked	51.96 (±0.82)^a	99.72 (±2.74)^a
	Cooked	19.74 (±0.29)^b	88.65 (±1.02)^b

Values are means (±SD). Means not sharing a common letter in a column are significantly different at P = 0.05 as assessed by Duncan multiple range test

Results and Discussion

***In vitro* protein digestibility (IVPD):** The effect of cooking on IVPD of lentil cultivars is shown in Table 1. When the cultivars flour was digested with pepsin, uncooked flour gave a range of 44.6-51.9. Cooking of the flour significantly (P = 0.05) decreased the IVPD and it was found to range from 19.7 to 24.0%. However, when pepsin together with pancreatin were used the IVPD of raw flour was significantly (P = 0.05) increased and was found to range from 81.8% to 99.7%. Cooking significantly (P = 0.05) decreased the IVPD and was found to range from 77.1% to 81.60%. It was clear that the IVPD obtained was significantly (P = 0.05) affected by cooking even when the flour was digested with both pepsin and pancreatin. Similar results were observed by Carbonaro *et al.* (1997) who attributed the lack of improvement in digestibility of faba bean and lentil to be related in part to protein aggregation that is a consequent to the thermal treatment. Carbonaro *et al.* (1993) suggested formation of aggregated protein on heat treatment through oxidation of sulfhydryl groups and through interactions between acidic and basic residues and would be more resistant to proteases as reported by Darcy, (1984) and Desrosiers *et al.* (1987). Moreover, Otterburn *et al.* (1977) suggested the formation of a three dimensional network on severe heating of proteins, as a result of Ca^{+2} mediated electrostatic bonds, hydrophobic interactions and the involvement of cross links, preventing enzyme penetration or masking the sites of the enzyme attack. The negative effect of cooking on the IVPD also observed by Abdel Rahim

Table 2: Effect of cooking on protein fractions (%) of lentil cultivars

Cultivars	Treatments	Globulin	Albumin	Prolamin	Glutelin	Insoluble protein	Protein recovery
Nadi	Uncooked	28.17 (±0.00)^a	56.26 (±1.03)^a	1.96 (±0.08)^a	2.53 (± 0.60)^b	8.23 (±0.00)^b	97.15
	Cooked	22.77 (±0.00)^b	35.23 (±2.03)^b	1.64 (±0.19)^b	27.35 (±1.52)^a	12.2 (±0.01)^a	99.19
Rubatab	Uncooked	26.47 (±0.00)^a	64.00 (±0.00)^a	1.50 (±0.00)^a	3.50 (±0.00)^b	7.89 (±10.00)^b	103.36
	Cooked	24.59 (±0.00)^b	37.95 (±0.00)^b	1.31 (±0.09)^b	26.94 (±0.46)^a	12.37 (±0.00)^a	103.16
Seliam	Uncooked	29.22 (±0.01)^a	61.87 (±2.23)^a	1.64 (±0.00)^a	2.10 (±0.00)^b	8.78 (±0.01)^b	100.67
	Cooked	26.28 (±0.01)	39.87 (±1.54)^b	1.12 (±0.00)^b	20.70 (±0.63)^a	12.28 (±0.00)^a	103.21
Indian	Uncooked	29.50 (±0.02)^a	61.50 (±0.46)^a	1.43 (±0.12)^a	3.20 (±0.26)^b	7.10 (±0.01)^b	102.73
	Cooked	26.72 (±0.01)^b	30.19 (±1.10)^b	1.00 (±0.00)^b	27.65 (±0.50)^a	13.33 (±0.00)^a	98.89

Values are means (±SD). Means not sharing a common letter in a column are significantly different at P = 0.05 as assessed by Duncan multiple range test.

(2004) for faba bean and for corn as reported by Yousif (2000) who attributed the reduction in IVPD to the formation of disulphide bonds resulting in folding of protein molecule and hence decreasing its susceptibility to digestive enzymes.

Protein fractions: Table 2 shows the effect of cooking on total protein fractions of lentil cultivars. The total protein of lentil was fractionated on the basis of solubility for each cultivar into albumins, globulins, prolamins and glutelins. For all cultivars the albumins content of uncooked flour ranged from 56.26 to 64.00% and when the cultivars were cooked it decreased significantly (P = 0.05) and was found to range from 30.19 to 39.87%.Similar results were obtained by Yagoub (2003) for cooked karkade seed, who attributed this loss on cooking to high susceptibility of albumin to heat treatment. For all cultivars globulins content of uncooked flour ranged from 26.28 to 29.50% and after cooking it was ranged from 22.77 to 29.22%. The prolamins content of uncooked flour was ranged from 1.43 to 1.96 and after cooking it was ranged from 1.00 to 1.64% for all cultivars. Glutelins content of uncooked cultivars was ranged from 2.10 to 3.50 and it was significantly (P = 0.05) increased after cooking and was found to be range from 20.70 to 27.65%.It was clear that cooking increased glutelins fraction by about more than 10 fold. The increment in glutelin after cooking was reported in cereals by Fageer and El Tinay (2004); Arbab and El Tinay (1997); Yousif (2000).

Structural changes in protein fraction: Figures 1-3 show the SDS-PAGE pattern of some lentil protein fractions before and after cooking of four cultivars. For all cultivars the number of bands of globulin fraction ranged from 2 to 4 for cooked and uncooked samples, respectively with low molecular weight ranging from 12-45.5kDa (Fig. 1). Number of bands for the prolamin fractions was greatly affected by cooking for the cultivars Indian, Selaim and Rubatab and they were found to have four bands before cooking and after cooking showing only two bands reaching a molecular weight of ~56.0kDa (Fig. 2). Gultelin fractions had bands with high molecular weight reaching 71 kDa. The number of bands of this

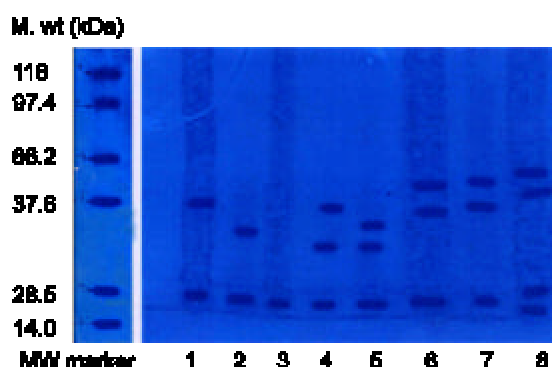

Fig. 1: SDS-PAGE pattern of globulin fraction of cooked and uncooked lentil cultivars. Lane 1, Uncooked Indian; lane 2, Cooked Indian; lane 3, Uncooked Selaim; lane 4, Cooked Selaim ; lane 5, Uncooked Rubatab; lane 6, Cooked Rubatab; lane 7, Uncooked Nadi; lane 8, Cooked Nadi.

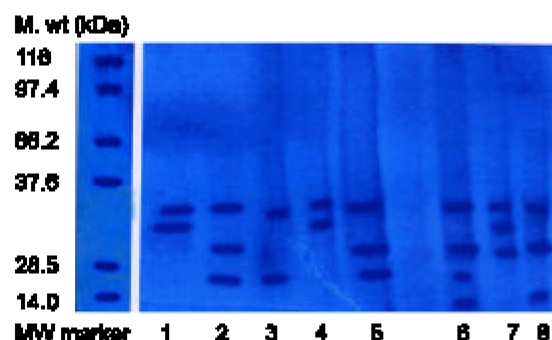

Fig. 2: SDS-PAGE pattern of albumin fraction of cooked and uncooked lentil cultivars. Lane 1, Uncooked Indian; lane 2, Cooked Indian; lane 3, Uncooked Selaim; lane 4, Cooked Selaim ; lane 5, Uncooked Rubatab; lane 6, Cooked Rubatab; lane 7, Uncooked Nadi; lane 8, Cooked Nadi.

fraction ranged from 3 to 6 (Fig. 3). Protein residue showed 4-5 bands with high molecular weight reaching 78.4 kDa. Within one cultivar, the number of bands (subunits) of the total protein was affected by cooking. Uncooked samples showed a high number of bands of total protein fractions reaching 19, 17, 17 and 17 bands

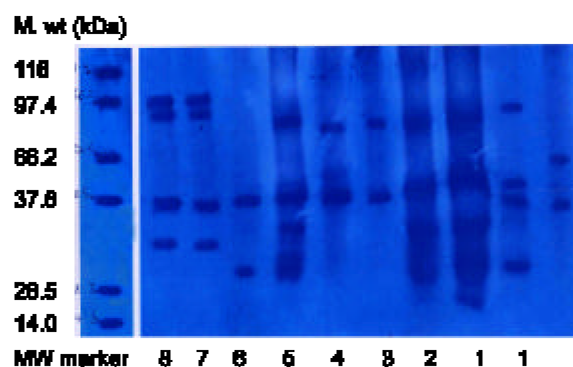

Fig. 3: SDS-PAGE pattern of prolamin fraction of cooked and uncooked lentil cultivars. Lane 1, Uncooked Indian; lane 2, Cooked Indian; lane 3, Uncooked Selaim; lane 4, Cooked Selaim ; lane 5, Uncooked Rubatab; lane 6, Cooked Rubatab; lane 7, Uncooked Nadi; lane 8, Cooked Nadi.

for cultivars Indian, Selaim, Rubatab and Nadi, respectively. Cooking decreased these numbers to 16, 15, 16 and 13 for the cultivars Indian, Selaim, Rubatab, Nadi, respectively (data not shown). Results obtained in this study are similar to those reported by Ahmed *et al.* (1995) on three types of legumes. They found that chickpea seed protein containing 19 bands with a molecular weight ranging from 12 to 89kDa. Faba bean contained 25 bands with a molecular weight ranging from 12 to 78kDa and terms seeds contained 16 bands with a molecular weight ranging from 12 to 98kDa. Chiou *et al.* (1997) reported that the molecular weight of peanut cultivars proteins (untreated samples) ranged from 14 to 67kDa.

Conclusion: Cooking resulted in a significant reduction in IVPD using pepsin or pepsin with pancreatin and also reduced the level of albumin fraction while glutelin level increased. The major protein in lentils was albumin followed by globulin. SDS-PAGE of cooked and uncooked proteins fractions showed that lentil protein was altered quantitatively and qualitatively due to cooking, this effect was most pronounced in prolamin fractions.

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