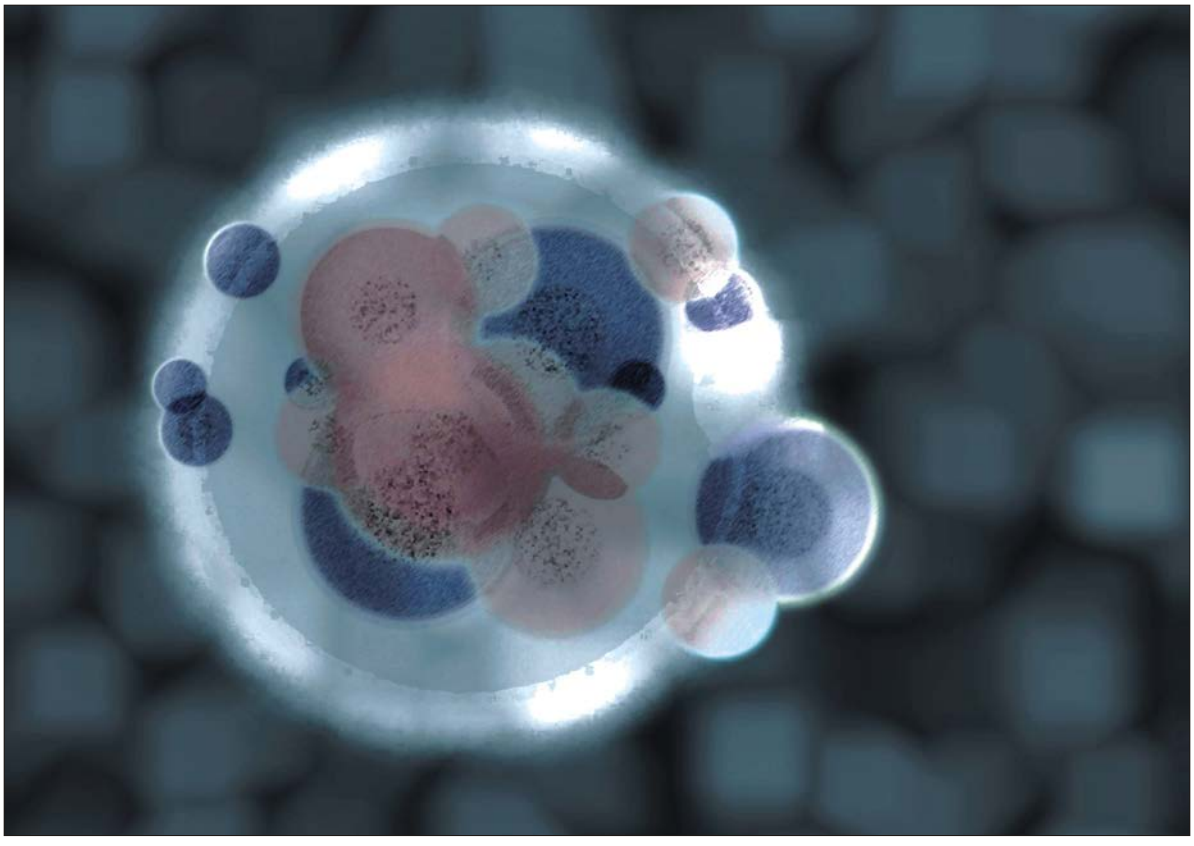

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Changes in Chemical Composition, Phytate, Phytase Activity and Minerals Extractability of Sprouted Lentil Cultivars

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Abstract: The study was conducted to evaluate changes during germination in chemical composition, phytic acid, phytase activity and total and extractable minerals of lentil cultivars. Three Sudanese lentil cultivars (Rubatab, Nadi and Selaim) were germinated for 3 and 6 days. The germinated seeds were dried and milled. Proximate composition, phytic acid content, phytase activity and hydrochloric acid (HCl) extractability of minerals were determined. During germination crude fat and fiber increased, whereas nitrogen free extract (NFE) and food energy decreased. Phytic acid content decreased significantly ($p \leq 0.05$) with an increase in germination time. Germination resulted in a decrease in total phytate phosphorous with correspondingly marked increase in non-phytate phosphorus. Total and extractible minerals (P, Ca, Fe, Mg, Cu and Zn) were positively correlated with duration of germination except Cu and Zn. Germination for 3 days increased phytase activity significantly ($p \leq 0.05$) for all cultivars. Phytase activity of Rubatab cultivar continued to increase up to 6 days of germination, however, for Nadi and Selaim cultivar it slightly decreased. In order to obtain lentil seeds with high phytase activity, low phytic acid and high mineral extractibility, germination process for more than 3 days is recommended.

Key words: Lentil, phytate, phytase, minerals, extractability, germination

INTRODUCTION

Lentil is a protein/calorie crop packed with nutrients, fibre, complex carbohydrate, folic acid and an important source of iron. Phytate (myo-inositol 1,2,3,4,5,6 hexkis-phosphate) is naturally occurring constituent of plant seeds, roots, tubers and some fruits and vegetable where it acts as a storage form of phosphate. It is account for up to 80% of seed total phosphorus. It is found widely in all cells of legumes (Reddy *et al*., 1982). Germination is a natural biological process of all superior plants by which the seeds comes out of its latency stage, once the minimal environmental condition needs for its growth and development, such as humidity temperature and nutrients are given (Sangronis and Machado, 2007). During germination there are certain changes that could occur as far as the quantity and type of nutrients within the seed. Those changes can vary depending on the type of vegetable, the variety of the seed and the condition of germination (Bau *et al*., 1997; Dhaliwal and Aggarwal, 1999). An increase in the bioavailability of minerals and vitamins has been observed due to germination. Germinated seeds are good sources of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid (Sangronis and Machado, 2007). Because of its high density of negatively charged phosphate groups, phytate forms mixed salts with mineral cations, which are assumed to play an important role in the storage of minerals (Lopez *et al*., 2002). Under gastrointestinal pH conditions, insoluble metal phytate complexes are formed (Gifford and Clydesdale, 1990) which make the metal unavailable for absorption from the intestinal tract of animals and humans (Kratzer and Vohra, 1986). Phytate-degrading enzymes (phytases) catalyse the hydrolysis of phytate. Phytases belong to especial group of phosphatases, which are capable of hydrolyzing phytate to series of lower phosphate esters of myo-inositol and phosphate. Phytates are widely distributed in nature in plants and microorganisms (Engelen *et al*., 1994). The present study was designed to evaluate changes during germination in chemical composition, phytic acid, phytase activity and total and extractable minerals of lentil cultivars.

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MATERIALS AND METHODS

A bulk of healthy and clean seeds of lentil (*Lens culinaris*, Medic) cultivars (Rubatab, Nadi and Selaim) used in this study was obtained from Elhudeeba Research Station, Sudan during the season 2001/2002. The bulk of each cultivar was divided into three equal portions, the first portion was reserved as a control (ungerminated seeds), the second portion was allowed to germinate for 3 days and the third portion was allowed to germinate for 6 days. Prior germination, the seeds were soaked at room temperature in distilled water for 2 h. Germination was carried out in sterile Petri dishes lined with wet filter paper for 3 days and 6 days at 4°C. At the end of respective germination period samples were dried at room temperature, ground to pass a 0.4 mm screen for subsequent chemical analysis. The control groups (ungerminated seeds) were ground and kept at 4°C for further analysis.

The samples of ungerminated and germinated seeds of the cultivars were analyzed for crude protein, fat, crude fiber and ash using the methods of AOAC (1984). Nitrogen free extract (crude carbohydrate) was estimated by difference. The energy content was determined by multiplying the percentages of crude protein, crude fat and nitrogen free extract by factors 4, 9 and 4, respectively (Osborne and Voogt, 1978).

The estimation of phytase activity was based on the estimation of inorganic orthophosphate (Pi) released by hydrolysis of phytic acid, following the method described by Engelen *et al.* (1994). One unit of phytase is defined as the quantity of enzyme, which liberates one micromole of inorganic phosphorus per minute from sodium phytate (0.0051 mol L^{-1}) at optimum pH at 37°C. A standard curve was prepared using sodium phytate of different concentrations as a substrate. The moles of the product formed per second were determined.

The inositol phosphate Ip5 and Ip6 were determined using Sandberg (1986) method. About 0.5 g of the sample was placed in a beaker; 10 mL of HCl (0.5 M) were added and stirred with magnetic stirrer (500 rpm) at room temperature for 2 h; freezed over night at (-20°C) and then stirred for 1 h at room temperature. Thereafter, the contents were centrifuged at 12000 x g for 8 min and the supernatant was taken in microcon YM-30 centrifuge tube and centrifuged in eppendorf-centrifuge (12000 x g) for 25 min. The clear supernatant (0.5 mL) was taken for inositol phosphates (IP5-IP6) analysis using HPLC (Shimadzu, Japan). Phytic acid was expressed as a total of IP5 and IP6.

Phytate phosphorus was determined by using Reddy (1982) relationship:

$$\text{Phytate phosphorus (mg/100 g)} = \frac{A \times 28.18}{100}$$

Where A is the phytic acid content (mg/100 g).

Non-phytate phosphorus was calculated by difference between the total phosphorus and phytate phosphorus.

Minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt (1982). About 1.0 g sample was acid-digested with diacid mixture (HNO_3: $HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and was used for the determination of total minerals. The amount of iron, zinc, manganese, cobalt and copper were determined using atomic absorption spectroscopy (Perkin-Elmer 2380). Ammonium vandate was used to determine phosphorus along with the ammonium molybdate method of Chapman and Pratt (1961). Calcium and magnesium were determined by the titration method described by Chapman and Pratt (1961). Sodium and potassium were determined using a flame photometer (CORNIG EEL) according to the AOAC (1984) method.

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). About 1.0 g sample was shaken with 10 mL of 0.03 M HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven-dried at 100°C and then the sample was acid-digested with diacid mixture (HNO_3: $HClO_4$, 5:1, v/v). The amount of the extractable minerals was determined as milligrams per 100 g sample by the methods described above.

Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) as described by Snedecor and Cochran (1987).

RESULTS AND DISCUSSION

The data obtained showed remarkable changes in proximate composition and food energy values of lentil due to germination (Table 1). As germination progressed crude fat and crude fiber increased slightly for the three cultivars. Ash content was slightly increased for Selaim cultivar while for Rubattab and Nadi it slightly decreased with the germination time. The protein content of Nadi and Selaim cultivars slightly increased while that of Rubattab it decreased with the germination time. The Nitrogen Free Extract (NFE) and food energy for all cultivars decreased with the germination time. The slight increment in fat content during germination for all

Table 1: Proximate composition and energy value of lentil cultivars as affected by germination

Cultivars	Germination time (days)	Crude fibre (%)	Ash (%)	Crude protein (%)	Fat (%)	Nitrogen free extract (%)	Energy (kcal/100 g)
Rubatab	0	3.7 (±1.0)^{b}	2.5 (±0.03)^{a}	28.2 (±0.03)^{a}	0.60 (±0.0)^{b}	67.5 (±0.01)^{a}	388.2 (±0.10)^{a}
	3	5.2 (±0.5)^{a}	2.5 (±0.05)^{a}	27.0 (±0.34)^{a}	0.60 (±0.01)^{b}	64.7 (±0.02)^{b}	372.2 (±0.15)^{b}
	6	5.8 (±0.07)^{a}	2.4 (±0.01)^{b}	27.6 (±0.54)^{a}	0.67 (±0.0)^{a}	63.5 (±0.208)^{c}	370.7 (±0.208)^{a}
Nadi	0	1.9 (±0.1)^{b}	3.9 (±0.06)^{b}	24.5 (±0.00)^{b}	0.65 (±0.06)^{c}	70.0 (±0.136)^{a}	383.0 (±0.10)^{a}
	3	5.7 (±0.15)^{a}	3.1 (±0.07)^{a}	26.5 (±1.60)^{a}	0.80 (±0.01)^{b}	64.0 (±0.15)^{c}	368.9 (±0.20)^{c}
	6	5.8 (±0.1)^{a}	3.0 (±1.0)^{b}	25.7 (±0.04)^{a}	0.97 (±0.01)^{a}	64.6 (±0.01)^{b}	369.6 (±0.15)^{b}
Selaim	0	1.6 (±0.03)^{b}	2.80 (±1.0)^{b}	27.9 (±0.15)^{a}	0.45 (±0.01)^{c}	67.3 (±0.15)^{a}	384.9 (±0.15)^{a}
	3	5.0 (±0.11)^{a}	3.0 (±1.0)^{b}	28.1 (±0.13)^{a}	0.52 (±0.05)^{b}	63.4 (±0.20)^{c}	370.4 (±0.15)^{c}
	6	6.4 (±0.076)^{a}	3.0 (±1.0)^{b}	28.4 (±0.08)^{a}	0.75 (±0.01)^{a}	64.5 (±0.10)^{b}	378.4 (±0.15)^{b}

Values are means±SD. Means not sharing a common superscript letter (a, b, c or d) in a column are significantly ($p \leq 0.05$) different as assessed by Duncan's multiple range tests

Table 2: Phytase activity, phytic acid, phosphorus, phytate and non-phytate phosphorus of germinated lentil cultivars

					Phytate phosphoms		Non phytate phosphorus	
Cultivars	Germination time (days)	Phytase activity FTU kg^{-1}	Phytic acid (mg/100 g)	Phosphorus (mg/100 g)	Total (mg/100 g)	Total phosphoms (%)	Total (mg/100 g)	Percentage of total
Rubatab	0	29.30 (±3.2.01)^{c}	848.9 (±0.01)^{a}	363.3 (±1.53)^{a}	239.3 (±0.10)^{a}	65.8 (±0.29)^{a}	124.1 (±1.51)^{c}	34.2 (±0.46)^{c}
	3	82.00 (±4.41)^{b}	617.1 (±0.00)^{b}	377.3 (±22.5)^{b}	137.9 (±0.70)^{b}	46.5 (±0.27)^{b}	203.6 (±22.0)^{b}	54.0 (±6.00)^{b}
	6	128.00 (±3.50)^{a}	413.8 (±0.00)^{c}	388.0 (±5.10)^{a}	116.6 (±0.05)^{c}	301.0 (±0.60)^{c}	271.4 (±0.90)^{a}	70.0 (±1.30)^{a}
Nadi	0	34.70 (±4.20)^{a}	1487.4 (±0.00)^{a}	464.0 (±4.20)^{b}	419.1 (±0.80)^{a}	90.3 (±0.80)^{a}	44.9 (±1.40)^{a}	9.7 (±0.40)^{c}
	3	132.00 (±12.1)^{a}	1106.8 (±0.06)^{b}	497.3 (±5.51)^{a}	311.7 (±0.20)^{b}	62.7 (±0.70)^{b}	185.6 (±0.29)^{c}	37.4 (±1.10)^{b}
	6	106.00 (±5.01)^{a}	806.2 (±0.50)^{c}	502.3 (±4.51)^{a}	227.3 (±2.60)^{c}	45.2 (±0.40)^{c}	275.0 (±5.51)^{b}	54.8 (±0.90)^{a}
Selaim	0	68.30 (±4.01)^{a}	941.7 (±0.90)^{a}	432.7 (±4.51)^{b}	265.4 (±3.10)^{a}	61.3 (±0.66)^{a}	167.3 (±4.51)^{c}	38.6 (±1.00)^{c}
	3	108.30 (±9.01)^{a}	548.6 (±0.80)^{b}	435.0 (±2.00)^{b}	164.8 (±4.30)^{b}	37.9 (±0.20)^{b}	270.2 (±2.00)^{b}	62.1 (±0.45)^{b}
	6	80.00 (±8.07)^{a}	234.7 (±0.06)^{c}	450.0 (±1.00)^{a}	66.1 (±1.30)^{c}	14.7 (±0.01)^{c}	383.9 (±1.00)^{a}	66.1 (±1.00)^{a}

Values are means (±SD). Means not sharing a common superscript letter (a, b, c or d) in a column are significantly ($p \leq 0.05$) different as assessed by Duncan's multiple range tests

Table 3: Total and extractable (mg/100 g) phosphorus and calcium of germinated lentil cultivars

		P		Ca	
Cultivars	Germination time (days)	Total	Extractable	Total	Extractable
Rubatab	0	363.3 (±1.53)^{b}	246.6 (±0.00)^{c}	63.5 (±9.7)^{b}	41.5 (±0.45)^{a}
	3	377.3 (±22.5)^{b}	269.0 (±2.50)^{b}	107.3 (±1.5)^{a}	86.2 (±0.10)^{b}
	6	388.0 (±2.30)^{c}	287.5 (±3.40)^{a}	111.0 (±7.0)^{a}	93.2 (±1.20)^{a}
Nadi	0	464.0 (±5.50)^{b}	354.5 (±6.10)^{b}	72.0 (±7.3)^{b}	48.6 (±4.60)^{b}
	3	497.3 (±5.51)^{b}	395.5 (±7.20)^{a}	119.5 (±2.5)^{a}	104.2 (±2.20)^{a}
	6	502.3 (±4.51)^{a}	398.0 (±3.10)^{a}	118.0 (±4.0)^{a}	98.6 (±2.60)^{b}
Selaim	0	432.7 (±4.51)^{b}	331.0 (±3.40)^{c}	68.6 (±0.60)^{b}	65.5 (±2.60)^{b}
	3	435.0 (±2.00)^{b}	349.0 (±1.30)^{b}	100.3 (±1.8)^{a}	83.1 (±3.10)^{a}
	6	450.0 (±1.00)^{a}	363.0 (±2.80)^{a}	104.0 (±1.0)^{a}	83.2 (±2.20)^{a}

Values are means (±SD). Means not sharing a common letter (a, b, c or d) in column are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range tests

cultivars compared to other constituents may be due to the fact that fat contain about twice the food energy values of protein and carbohydrate (Osborne and Voogt, 1978), the reduction in food energy value of the germinated seeds might be attributed to the very slow increment in fat content. Nielson and Liener (1984), Ologhobo and Fetuge (1986) and Shastry and John (1991) attributed the reduction of both nutrients to their utilization during germination process. Reduction of some storage nutrients of lentil seeds resulted in a concomitant increase in other nutrients.

Phytase activity of ungerminated lentil seeds was found to be 29.30, 34.70 and 68.30 FTU kg^{-1} for Rubattab, Nadi and Selaim cultivars, respectively (Table 2). Germination of the seeds for 3 days increased phytase activity significantly ($p \leq 0.05$) to 82.0, 132.0 and 108.0 for the cultivars, respectively. For Rubatab cultivar, phytase activity continued to increase progressively with an increase in the period of germination to 6 days. Results reported by Viresos *et al.* (2000) showed that phytase activity in soybean and peas are 32 and 86 FTU kg^{-1}, respectively. Different authors have reported that processes, such as soaking and germination, activate the endogenous phytases, which are able to hydrolyse Ip6 releasing lower inositol phosphates (Beal and Mehta, 1985; Honke *et al.*, 1998; Kozlowska *et al.*, 1996). Phytic acid content of ungerminated lentil seeds was found to be 848.9, 1487.4 and 941.7 mg/100 g for Rubattab, Nadi and Selaim cultivars, respectively. For all cultivars germination of the seeds for 3 days reduced 26-41% of the total

Table 4: Total and extractable trace minerals (mg/100 g) of germinated lentil cultivars

		Fe		Mg		Cu		Zn	
Cultivars	Germination time (days)	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
Rubatab	0	5.4 (±0.05)^{b}	3.1 (±0.10)^{c}	87.9 (±0.60)^{c}	80.0 (±5.0)^{c}	1.5 (±0.02)^{b}	0.9 (±0.05)^{a}	5.30 (±0.30)^{a}	5.34 (±1.40)^{a}
	3	11.0 (±0.80)^{a}	5.1 (±0.60)^{b}	110.0 (±7.0)^{b}	97.0 (±4.0)^{b}	1.4 (±0.20)^{b}	1.0 (±0.04)^{a}	5.60(±0.32)^{a}	5.18 (±1.80)^{a}
	6	11.2 (±2.30)^{a}	5.6 (±0.60)^{a}	118.8 (±3.0)^{a}	106.0 (±.2.0)^{a}	1.8 (±0.08)^{a}	1.3 (±0.09)^{a}	5.50 (±0.1)^{a}	5.00 (±.0.00)^{a}
Nadi	0	5.5 (±0.70)^{b}	3.0 (±0.50)^{b}	99.9 (±10.0)^{b}	91.5 (±1.5)^{b}	2.8 (±0.15)^{a}	1.6 (±0.03)^{a}	6.10 (±0.00)^{a}	5.80 (±0.30)^{a}
	3	8.9 (±0.08)^{a}	4.4 (±0.20)^{a}	115.0 (±0.5)^{a}	109.3 (±2.3)^{a}	2.1 (±0.26)^{b}	1.8 (±0.05)^{a}	6.20 (±0.60)^{a}	5.80 (±0.1)^{a}
	6	8.9 (±0.03)^{a}	4.4 (±0.70)^{a}	118.0 (±0.4)^{a}	107.0 (±3.0)^{a}	1.7 (±0.00)^{c}	1.2 (±0.02)^{b}	5.80 (±0.90)^{a}	5.00 (±0.50)^{b}
Selaim	0	6.5 (±0.60)^{c}	2.7 (±0.30)^{c}	95.8 (±0.5)^{c}	86.3 (±2.3)^{b}	2.0 (±0.08)^{a}	1.5 (±0.04)^{a}	6.80 (±0.10)^{a}	6.44 (±0.20)^{a}
	3	10.3 (±0.90)^{b}	5.6 (±0.60)^{b}	112.5 (±0. 5)^{b}	100.9 (±0.9)^{a}	1.8 (±0.14)^{a}	1.2 (±0.01)^{a}	7.03 (±0.3)^{a}	6.60 (±0.00)^{a}
	6	13.5 (±0.06)^{a}	6.0 (±0.00)^{a}	119.0 (±1.0)^{a}	103.1 (±2.1)^{a}	2.0 (±0.11)^{a}	1.5 (±0.00)^{a}	6.88 (±0.07)^{a}	6.20 (±0.20)^{b}

Values are means (±SD). Means not sharing a common letter (a, b, c or d) in column are significantly different ($p \leq 0.05$) as assessed by Duncan multiple range tests

phytate of the seeds. Further reduction (46-75%) in phytate of the cultivars was observed when the seeds were germinated for 6 days. Results showed that germination is an effective mean that caused a significant ($p \leq 0.05$) loss of more than 50% of total phytate of the seeds. Similar results were observed by Duhan *et al.* (2002) who found that after 48 h germination a loss of up to 45% was noticed in pigeon pea. Loss of phytic acid during germination was found to be due phytase activity, which is reported to be present in various plant foods (Lolas and Markakis, 1975). About 61.3 to 90.3% of the total phosphorus was present as phytate phosphorus in the ungerminated seeds of the cultivars. Germination resulted in a significant ($p \leq 0.05$) decrease in total phytate phosphorus with correspondingly marked increase in non-phytate phosphorus and HCl extractable phosphorus. Cleavage of phosphorus from phytic acid may explain the increase in the level of non-phytate phosphorus.

Table 3 and 4 show the effect of germination of lentil cultivars on total and extractable minerals. For all cultivars P content increased with germination time with a maximum value (502.2 mg/100 g) obtained for Nadi cultivar after germination for 6 days (Table 3). Extractable P for all cultivars also significantly ($p \leq 0.05$) increased with the germination time with a maximum extractable value (398.0 mg/100 g) recorded for Nadi cultivar, which represent about 79% of the total P of the cultivar. Ca content and extractability for all cultivars were significantly ($p \leq 0.05$) increased with the germination time with a maximum value (119.5 mg/100 g) obtained for Nadi cultivar after germination for 3 days and out of this amount about 87% was found to be extractable.

Fe content and extractability for all cultivars were significantly ($p \leq 0.05$) increased with germination time with maximum total (13.5 mg/100 g) and extractable (6.0 mg/100 g) were observed for Selaim cultivar germinated for 6 days (Table 4). Total and extractable Mg for all cultivars followed a trend similar to that obtained for Fe. However, Cu and Zn content and extractability were fluctuating with the germination time. The results obtained clearly indicated that germination of lentil seeds for a certain period greatly improved the availability of minerals. The increment in both total and extractable minerals after germination also indicated the strong correlation between phytate content and minerals extractability. Similar results were observed by Oloya (2004) who found that germination of pigeon pea seeds for up to 4 days resulted in significantly higher contents of iron, calcium, magnesium and phosphorus. Samia *et al.* (2007) reported that germination of pearl millets greatly reduced phytic acid content with a concomitant increase in extractable minerals.

CONCLUSION

Germination of various lentil cultivars caused markedly improvement in some valuable nutrients of the seeds. It also increased significantly the HCl-extractable parts of both major and trace minerals and reduced significantly phytic acid content of the cultivars.

REFERENCES

AOAC, 1984. Official Methods of Analysis. 14th Edn., Washington, DC: Association of Official Agricultural Chemists.

Bau, H., C. Villaume, J. Nicolas and L. Mejean, 1997. Effect of germination on trypsin inhibitor activity in lentil and pigeon pea. J. Food Sci. Techol., 23: 260-263.

Beal, L. and T. Mehta, 1985. Zinc and phytate distribution in peas: Influence of heat treatment, germination, pH, substrate and phosphorus on pea phytate and phytase. J. Food Sci., 50: 96-100.

Chapman, H.D. and F.P. Pratt, 1961. Ammonium Vandate-Molybdate Method for Determination of Phosphorus. Methods of Analysis for Soils, Plants and Water. 1st Edn., USA: California University, Agriculture Division, pp: 184-203.

Chapman, H.D. and F.P. Pratt, 1982. Determination of Minerals by Titration Method. In: Methods of Analysis for Soils, Plants and Water. 2nd Edn., Oakland, CA: Agriculture Division, California University, pp: 169-170.

Chauhan, B.M. and L. Mahjan, 1988. Effect of natural fermentation on the extractability of minerals from pearl millet flour. J. Food Sci., 53: 1576-1577.

Dhaliwal, Y. and R. Aggarwal, 1999. Composition of fat in soybean as affected by duration of germination and dry temperature. J. Food Sci. Technol., 36: 266-267.

Duhan, A., N. Khetarpaul and S. Bishnoi, 2002. Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. Food Chem., 78: 9-14.

Engelen, A.J., F.C. Vander-Heeft, P.H.G. Randsddrop and E.L.C. Simt, 1994. Simple and rapid determination of phytase activity. J. AOAC Int., 77: 760-764.

Gifford, S.R. and F.M. Clydesdale, 1990. Interactions among calcium, zinc and phytate with three different sources. J. Food Sci., 55: 1720-1723.

Honke, J., H. Kozlowska, C. Vidal-Valverde, J. Frras and R. Gorecki, 1998. Changes in quantities of inositl phosphates during maturation of legume seeds. Zeitschrift furLebensmittel Untersuchung und Forschung, 26: 279-283.

Kratzer, F.H. and P. Vohra, 1986. Role of Phytic Acid and Other Phosphates as Chelating Agents. In: Chelates and Nutrition, Boca Raton, FL: CRC Press, pp: 49-61.

Kozlowska, H., J. Honke, J. Sadowska, J. Frias and C. Vidal-Valverde, 1996. Natural fermentation of lentils: Influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphorus. J. Sci. Food Agric., 71: 367-375.

Lolas, G.M. and P. Markakis, 1975. Phytic acid and other phosphorus compound of beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem., 23: 13-15.

Lopez, H.W., F. Leenhard and C. Coudray, 2002. Minerals and phytic acid interaction: Is it a real problem for human nutrition? Inter. J. Food Sci. Techol., 37: 727-739.

Nielson, S.S. and I.E. Liener, 1984. Degradation of the major storage proteins of *phaseolus vulgaris* during germination. Role of endogenous proteases and protease inhibitor. Plant Phys., 74: 494-502.

Ologhobo, A.D. and B.L. Fetuge, 1986. Change in carbohydrate contents of germinated cowpea seeds. Food Chem., 20: 117-125.

Oloya, R.A., 2004. Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L. Food Chem., 85: 479-502.

Osborne, D.R. and P. Voogt, 1978. Calculation of Caloric Value. In: Analysis of Nutrients in Foods. New York, Academic Press, pp: 24-34.

Reddy, N.R., S.K. Sathe and D.K. Salunkhe, 1982. Phytase in legume and cereals. Adv. Food Res., 28: 1-9.

Samia, M.A., B.E. Hagir, H.L. Wisal, B.H. Amro, E.B. Elfadil and H.E. Abdullahi, 2007. Antinutritional factors content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination. Int. J. Food Sci. Nutr., 58: 6-17.

Sandberg, A.S. and R. Ahderinne, 1986. HPLC method for determination of inositol tri-, tetra-, penta- and hexa phosphates in food and intestinal contents. J. Food Sci., 51: 547-550.

Sangronis, E. and C.J. Machado, 2007. Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. LWT J. Sci. Techol., 40: 116-120.

Shastry, M. and E. John, 1991. Biochemical changes and *in vitro* protein digestibility of the endosperm of germinating *Dolichos lablab*. J. Sci. Food Agric., 52: 529-538.

Snedecor, G.W. and W.G. Cochran, 1987. Statistical Methods. 17th Edn., Ames, IA, USA: The Iowa State University Press, pp: 221-222.

Viresos, A., C. Centeno, A. Brenes, R. Canales and A. Lazano, 2000. Phytase and acid phosphataes activities in plant feedstuffs. J. Agric. Food Chem., 48: 4009-4013.