

CHAPTER 5

NUTRITIONAL VALUE

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Abstract: The importance of lentils as important dietary sources of macro and micronutrients essential for human welfare has been recognized since ancient times. Lentils provide sufficient amounts of most essential amino acids to meet the nutrient requirements, although they are deficient in sulfur-containing amino acids like most legumes. Lentils also contain fair amounts of other essential nutrients like minerals, vitamins and complex carbohydrates. In contrast, lentils exhibit a considerable amount of non-nutritional compounds like trypsin inhibitors, tannins or phytic acid that are able to interfere with the availability of several nutrients. Different processing conditions that range from the traditional soaking/cooking to germination, fermentation, or several thermal treatments, are usually employed to improve the organoleptic properties of lentil seed and its nutritional value through reducing the negative effect of the above mentioned non-nutritional components. In addition, technological treatments may significantly enhance the functional and beneficial health properties of the processed lentil food products, making consumption of this legume an appealing alternative for today's world

1. INTRODUCTION

Lentil (*Lens culinaris Medik*) is an important dietary source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds, as well as diverse non-nutritional components like protease inhibitors, tannins, α -galactoside oligosaccharides and phytic acid. Lentil is smaller in size than other pulses like the bean, faba bean or chickpea, with a mean diameter and thickness of 4.45–6.82 and 2.36–2.55 mm, respectively and a volume of 56 mm³ (Fasina et al., 2001; Bhattacharya et al., 2005). Whole lentils have a low weight (13 to 30 seeds to per gram) (Bhatty, 1984; Bhattacharya et al., 2005) and lower seed bulk density than

other legumes such as kidney bean, green peas, black beans or pinto beans (Fasina et al., 2001). In contrast, the kernel density is higher in lentils than all these legumes except for pinto bean.

2. CHEMICAL COMPOSITION OF RAW LENTIL

According with the bibliography the range of values for nutrient composition of lentils is compiled in Table 1.

2.1. Energy

The energy provided by lentil (Table 1) is similar to that of cereals (e.g. wheat) and other pulses (e.g. faba beans, peas or beans). However, it is lower than the crude energy provided by legumes with a higher fat content (e.g. soybeans or lupins).

2.2. Nitrogen

Lentils have an average total nitrogen content of 4.25 g/100 g DM (Table 1), of which nearly 15% is present as soluble non-protein nitrogen mainly composed of free amino acids and small peptides that can be detected by the method of

Table 1. Chemical composition of raw lentil (per 100 grams of dry matter)

	Range	Bibliography
Energy (kJ)	1483–2010	1–3,14,17,30,31,32
Total Nitrogen (g)	3.72–4.88	3,4,10,13,16,18,21,27,30,31
Protein (N × 6.25) (g)	20.6–31.4	1–20, 28–29,30,31,32,33,34,35,36,37
Non-Protein Nitrogen (g)	0.49–1.049	4,21–24
Fat (g)	0.7–4.3	3,11–13,16–19, 21,23,25–27, 29,30,31,33,34,36,37
Carbohydrates (g)	43.4–69.9	17,23,37
Fiber (g)	5.0–26.9	17,23,35
Ash (g)	2.2–4.2	1,11–12,16–18, 21–23,25–27, 28–30,33,34,36,37

1. Kavas and Nehir (1992); 2. Combe et al. (1991); 3. Porres et al. (2002); 4. Sanz et al. (2001); 5. Fasina et al. (2001); 6. Bamdad et al. (2006); 7. Meiners et al. (1976) *J Agric Food Chem* 24, 1122–1126; 8. Manan et al. (1987); 9. Rehman and Shah (2005); 10. Monsoor and Yusuf (2002); 11. Iqbal et al. (2006); 12. Wang and Daun (2006); 13. Jood et al. (1998) *Nahrung* 42, 71–74; 14. Solanki et al. (1999); 15. Bhatta (1984); 16. Kylan and McReady (1975); 17. Ereifeg and Haddad (2001); 18. Candela et al. (1997); 19. Urbano et al. (1995); 20. Lombardi-Boccia et al. (2003); 21. Shekib et al. (1985); 22. El-Mahdy et al. (1985); 23. El-Adawy et al. (2003); 24. Periago et al. (1996) *Food Res Int* 29, 489–494; 25. De Almeida Costa et al. (2006); 26. Valente Mesquita and Reis (2005) *Nutr Food Sci* 35, 264–270; 27. Danisová et al. (1994); 28. Elhardallou et al. (1999) *Food Chem* 67, 113–121; 29. Miller McCurdy et al. (1978); 30. Khan et al. (1987); 31. Sika et al. (1995); 32. Solanki et al. (1999); 33. El-Tinay et al. (1989); 34. Perez-Hidalgo et al. (1997) *J Food Comp Anal* 10, 66–72; 35. Carbonaro et al. (1996); 36. Bartolomé et al. (1995); 37. Cai et al. (2002) *J Food Sci* 67, 1725–1730; 38. Zhao et al. (2005)

Table 2. Distribution of chemical constituents in the different anatomical parts of lentil seed*.

Component	Proportion to whole seed	Protein (g/100 g DM)	Fat (g/100 g DM)	Crude Fiber (g/100 g DM)	Ash (g/100 g DM)	Nitrogen free extract (g/100 g DM)
Seed Coat	8.0–20	14.3	0.6	29.4	1.94	53.7
Cotyledon	80–90.0	26.5–30.1	3.0	1.0	2.45	63.4
Embryo	2.0	71.1	8.2	2.4	3.94	14.4
Whole seed	100.0	29.6	3.1	3.2	2.40	61.7

* Data taken from Adsule et al. (1989) and Cuadrado et al. (2002a)

Lowry in addition to nucleic acids, puric and pyrimidinic bases, and alkaloids. The remaining nitrogen present in lentils is usually classified as protein nitrogen. A minor proportion of nitrogen from legumes (e.g. peas, lupins) and probably lentils also is present in an insoluble form, (Urbano et al. 2005a,b; Porres et al. 2003, 2005) that arises from non-covalent interactions or disulfide bonds between different proteins and remains associated with the insoluble dietary fiber fraction (Martín-Cabrejas et al., 2003). This insoluble nitrogen is related to the appearance of high-molecular-weight protein aggregates that hardly migrate into the resolving gel when the legume proteins are separated by polyacrylamide gel electrophoresis (Urbano et al., 2005a,b; Porres et al., 2003, 2005). Nevertheless, the insoluble nitrogen of legumes did not seem to have a major effect on protein digestibility assessed by *in vitro* or *in vivo* methods (Porres et al., 2005, 2006), although it could play an important role in technological processes like the preparation of protein isolates where protein solubility is a limiting factor to obtain optimal yields of the extraction process.

Protein content of lentils may change in response to genetic factors and different edaphic and environmental conditions. Table 2 shows that most of protein in lentils is located in the cotyledons (Adsule et al., 1989; Cuadrado et al., 2002a), mainly as storage proteins soluble in salt solution, with a lower proportion as enzymatic proteins soluble in water. It should be noted that the percentage of sulfur-containing amino acids is higher in the enzymatic protein fraction when compared to the storage protein fraction (Bhatty, 1982).

2.2.1. Amino acid composition and nutritional properties

Amino acid composition of lentils is described in Tables 3a and 3b. When compared to the nutrient requirements for the 1-year-old infant (FAO/WHO, 1991), the amino acid profile of lentils protein is deficient in sulfur-containing amino acids methionine and cystine, and in tryptophan. The deficiency is greater for growing rats (NRC, 1995), but less severe for the 2–5 year-old infant or the human adult (FAO/WHO, 1991). The low levels of sulfur containing amino acids in legume proteins and their low digestibility (Sarwar and Peace, 1986; Wu et al., 1996; Porres et al., 2002) has lead FAO/WHO to recommend use of the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) as an index to evaluate the nutritional

Table 3a. Non-essential amino acid composition of lentils (g/16 g N)

Asp	Glu	Ser	Gly	Ala	Arg	Pro	Bibliography
9.29	14.45	4.88	4.82	4.82	7.68	3.52	Shekib et al. (1986)
10.75	15.20	4.80	4.25	4.30	9.25	3.65	Combe et al. (1991)
11.56	14.58	4.38	3.36	3.47	8.68	1.19	Kavas and Nehir (1992)
9.94	16.3	2.86	4.33	2.37	3.90	2.58	Urbano et al. (1995)
13.24	16.70	5.19	4.01	4.52	10.12	4.46	Carbonaro et al. (1997)
10.66	18.51	6.02	5.60	4.69	8.95	7.20	Porres et al. (2002)
11.3–14.1	13.2–16.4	5.5–6.4	3.6–4.3	3.8–4.4	6.5–9.5	3.7–5.9	Canadian Grain Commission (2004)
10.1–15.9	12.8–17.3	5.0–6.3	3.3–4.4	3.6–4.3	6.2–11.1	3.5–6.1	Canadian Grain Commission (2004)
10.6–13.8	14.4–18.3	3.8–5.3	3.7–4.8	3.9–5.0	6.7–7.8	3.6–4.6	Wang and Daun (2006)

quality of a protein for humans. The use of true digestibility of the limiting amino acid ($AAS_{(ASAA)}$) suggested by Wu et al. (1996) may be an even better index. Different lentil varieties have a wide range for protein amino acid content (Tables 3a and 3b). Thus selection of varieties should be possible to improve sulfur-containing amino acids and tryptophan content.

The Leu/Ile and Leu/Lys ratios are useful nutritional quality indices. An excess of leucine impairs the utilization of isoleucine and lysine (Harper et al., 1955; Petterson et al., 1997). The Leu/Ile and Leu/Lys ratios ranges for lentils are, 1.24–1.98 and 1.08–2.03, respectively. These values are indicative of good protein quality and similar to other legumes (Fernandez et al., 1996; Nestares et al., 1996, 2001; Martinez-Villaluenga et al., 2006).

2.2.2. *Non protein amino acid composition*

Lentils possess significant amounts of non-protein amino acids (Table 4; taurine, GABA, γ -hydroxyarginine, γ -hydroxyornithine and trigonelline) with biological effects (Rozan et al., 2001; Kuo et al., 2004).

2.3. Fat

The fat content of lentils is low (0.7–3.6%) (Table 1), and similar to other pulses and cereals (peas, beans, wheat) but less than others (chickpea, lupin, soybean). Unsaturated fatty acids (linoleic, oleic, and linolenic) dominate the lentil profile (Table 5) with small amounts of saturated fatty acids, mainly palmitic acid. The lentil fatty acid profile is similar to that of other legumes, (pea, chickpea, soybean; Canadian Grain Commission, 2004; Kumar et al., 2006).

Table 3b. Essential amino acid composition of lentils (g/16 g N)

His	Ile	Leu	Lys	Thr	Val	Tyr	Phe	Met	Cys	Trp	Bibliography
3.36	4.96	7.28	7.09	3.78	4.92	3.23	4.72	0.94	0.96	0.72	Shekib et al. (1986)
2.45	4.05	6.65	6.55	3.40	4.55	3.55	3.60	0.90	1.40	–	Combe et al. (1991)
–	3.44	6.13	6.35	2.69	4.19	2.24	4.34	0.22	1.12	0.74	Kavas and Nehir (1992)
1.33	4.04	7.24	4.27	2.49	3.04	1.13	5.52	1.10	1.46	–	Urbano et al. (1995)
2.56	4.48	7.79	6.84	4.03	5.29	3.06	4.43	0.83	1.04	–	Carbonaro et al. (1997)
1.33	4.31	7.72	5.79	4.23	5.09	1.13	5.77	1.25	0.94	–	Porres et al. (2002)
1.8–3.0	2.6–4.2	5.7–7.1	4.4–7.0	3.6–4.9	3.3–4.9	1.18–3.4	3.7–5.0	0.8–1.2	0.7–1.4	0.9–2.6	Canadian Grain Commission (2004)
2.0–2.8	2.6–4.0	5.8–7.1	4.0–6.7	3.8–4.7	3.4–4.7	1.6–3.3	3.7–4.8	0.8–1.1	0.8–1.4	0.6–0.9	Canadian Grain Commission (2004)
2.6–3.3	4.4–5.5	6.8–8.7	6.3–8.2	3.4–4.4	4.7–6.1	7.4–9.4*			2.2–4.2 [‡]	0.6–0.9	Wang and Daun (2006)
Recommended intakes											
2.6	4.6	9.3	6.6	4.3	5.5	7.2*			4.2 [‡]	1.7	FAO/WHO 1-year-old
1.9	2.8	6.6	5.8	3.4	3.5	6.3*			2.5 [‡]	1.1	FAO/WHO Pre-school (2–5 years old)
1.9	4.1	7.1	6.1	4.1	4.9	6.8*			6.5 [‡]	1.3	NRC (1995) growing rat

*Sum of Phe + Tyr

[‡]Sum of Met + Cys

Table 4. Free non protein amino acids and trigonelline in lentils (mg/100 g DM)*

Amino acids	<i>L. culinaris</i>	<i>L. ervoides</i>	<i>L. nigricans</i>	<i>L. odemensis</i>	<i>L. orientalis</i>
γ -hydroxy-arginine	2.52–1.58	trace	2.04	0.75	5.73
γ -hydroxy-orнитine	0.01-ND	0.01	0.04	0.02	0.04
α -aminobutyric acid	0.01-ND	0.01	0.02	0.02	0.03
Taurine	0.46–0.03	0.01	0.21	0.019	0.05
Homoarginine	Trace	0.06	0.01	trace	ND
Trigonelline	11.85–0.40	ND	8.31	1.80	18.15

* Results taken from Rozan et al. (2001) and Kuo et al. (2004)

Table 5. Fatty acid composition of lentils

Fatty acid (% in oil)	<i>Lens culinaris</i> cv. Trebisowska [△]	Canadian green lentils*	Canadian red lentils*	Australian lentils*
Lauric (C12:0)		ND	ND	–
Myristic (C14:0)	0.40	0.00–0.93	0.42–0.73	0.60–0.60
Palmitic (C16:0)	17.90	10.79–15.36	13.25–15.77	12.70–13.70
Palmitoleic (C16:1)		0.00–0.61	0.00–0.33	–
Stearic (C18:0)	2.00	1.27–1.82	1.34–1.65	1.80–2.10
Oleic (C18:1)	20.10	17.04–25.63	17.05–22.17	22.70–28.00
Linoleic (C18:2)	37.60	40.97–46.14	42.91–45.23	41.90–57.14
Linolenic (C18:3)	6.90	11.93–16.23	12.68–14.66	11.60–12.70
Arachidic (C20:0)		0.77–1.11	0.80–0.92	–
Gadoleic (C20:1)		1.21–1.58	1.12–1.24	1.20–1.30
Eicosadienoic (C20:2)		0.00–0.22	0.00–1.86	–
Behemic (C22:0)		0.81–1.13	0.81–0.91	–
Erucic (C22:1)		0.36–0.74	0.80–0.92	–
Lignoceric (C24:0)		0.47–1.99	0.56–0.70	–
Nervonic (C24:1)		ND	ND	–
Other [‡]	15.10			

[△]Grela and Günter (1995); * Canadian Grain Commission (2004)

[‡] Includes C10:0, C12:0, C20:0, C20:1, C20:2, C22:0, C22:1.

2.4. Carbohydrates

Carbohydrate concentration in whole lentils ranges from 43 to 70% (Table 1). The available carbohydrate fraction includes individual soluble sugars such as fructose, glucose and sucrose (1 to 2.5%), galacto-oligosaccharides such as raffinose, ciceritol, stachyose and verbascose (2 to 8%), non-starch polysaccharides (~ 20%), and starch (35 to 63%) with an amylose content of 20–45.5% (Table 6).

Table 6. Carbohydrate composition of lentils

	(g/100 g DM)	References
Total available soluble sugars	1.1–3.2	1–7
Fructose	0.01–0.17	1, 2, 3, 5, 6
Sucrose	1.09–2.97	1, 2, 3, 5, 6, 8
Total α -galactosides	1.8–6.8	1–3, 5–7
Raffinose	0.16–1.49	1–3, 5–10
Ciceritol	0.24–1.99	2, 3, 5, 6
Stachyose	1.1–3.1	1–3, 5–10
Verbascose	ND-1.35	1–3, 5–9
Starch	34.7–65	1, 3, 4, 7–13

1. Frias et al. (1994); 2. Frias et al. (1996a); 3. Frias et al. (1995a); 4. Vidal-Valverde and Frias (1992); 5. Vidal-Valverde et al. (1993a); 6. Vidal-Valverde et al. (1993b); 7. Reddy et al. (1984); 8. Wang and Daun (2006); 9. Fasina et al. (2001); 10. El-Adawy et al. (2003); 11. Jood et al. (1998) *Nahrung* 42, 71–74; 12. Cai et al. (2002) *J Food Sci* 67, 1725–1730; 13. Sotomayor et al. (1999)

2.5. Dietary Fiber

Lentil dietary fiber ranges from 9.7 to 24.1% (Table 1). It is mainly cellulose, hemicellulose, pectic substances and lignin (Table 7) (Vidal-Valverde and Frias, 1991; Vidal-Valverde et al., 1992a; Ramulu and Udayasekhara Rao, 1997; Urbano et al., 1999; De Almeida Costa et al., 2006).

2.6. Minerals

Lentils are an important source of dietary essential minerals. These include macronutrients (K, P, Ca, Mg, Na), micronutrients (Fe, Zn, Cu, Mn) and trace elements (Al, Cr, Ni, Pb, Co, Se, Mo) (Tables 8a and 8b). However, the bioavailability of minerals from lentils may be low due to the presence of non-nutritional compounds (phytic acid, tannins, oxalate) that interfere with their nutritive utilization. Most of

Table 7. Dietary fiber in lentils (in grams per 100 g DM)

	Range	Bibliography
Dietary fiber (as NDF)	16.2–21.3	1–3
Cellulose	4.1–5.33	1, 2, 4
Hemicellulose	6.0–15.74	1, 2, 4
Total dietary fiber	11.0–26.9	5–7
Soluble dietary fiber	1.2–6.7	5–10
Insoluble dietary fiber	8.8–31.4	5–10

1. Vidal-Valverde and Frias (1991); 2. Vidal-Valverde et al. (1992a); 3. Arntfield et al. (2001); 4. Reddy et al. (1984); 5. Bartolomé et al. (1995); 6. Perez-Hidalgo et al. (1997) *J Food Comp Anal* 10, 66–72; 7. Ramulu and Udayasekhara Rao (1997); 8. Elhardallou and Walker (1999) *Food Chem* 67, 113–121; 9. De Almeida Costa et al. (2006); 10. Candela et al. (1997)

Table 8a. Mineral composition of lentils (mg/100 g DM)

Ca	Total-P	Phytate-P	Inorg.-P	K	Mg	Na	Zn	Fe	Bibliography
33	305.2-409.7	123.5-175.9					4.6	12.8	Kylen and McReady (1975) Gad et al. (1982) Bhatty (1984)*
70	450			1160	100	40			
40-160	280-630			880-1440	80-140	20-180			
40; 40	153; 247	120; 195		6.9; 4.3		6.9; 4.3	2.5; 3.0	8.9; 11.0	El-Mahdy et al. (1985) Shekib et al. (1985)
42	380			753		4.61	5.11	7.2	Khan et al. (1987)
190.1	282.9	46.8					6.1	9.6	Manan et al. (1987) Bhatty (1989)
60	331; 467; 389	151; 203; 168		970	100				
90	360	179.8		900	100	40	2.9	12.6	Combe et al. (1991)
	380						3.7	7.9	Kavas and Nehir (1992)
78.6	239.3								
81.2; 102	282; 308								
115-165	250-460			55.8			3.7; 3.3	7.7; 14.6	Danisová et al. (1994) Sika et al. (1995)
128	373	184.3						8.0-9.2	Solanki et al. (1999)
77							3.57	7.65	Urbano et al. (1999)
42.3; 97.9;	458.5; 315;			548	129; 119	78.6; 30.4;	6.2; 3.7; 4.9	13.3; 11.9; 9.2	Sebastiá et al. (2001) Ereifeq and Haddad (2001)
60.6	441.6					68.3			
	338						4.2	8.2	Kopfk et al. (2002)
76	372	338		240	48.5	11			El-Adawy et al. (2003)
							3.4	6.8	Lombardi-Boccia et al. (2003)
53.4							4.1	9.3	Sahuquillo et al. (2003)
69.1	387.6	205.7	57.9	2370	105.1				Porres et al. (2003, 2004)
210	340				220		3.8	6.5	Demirbas (2005)
							2.5	8.2	Erdogan et al. (2006)
120	294			874		79	4.4	9.9	Iqbal et al. (2006)
79.7	509.4	205.5-313.9		1055	138		4.0	7.9	Wang and Daun (2006)*
48.4-107	344-725			550-1268	121-167		2.9-5.9	6.6-9.8	

* The means and range of values have been taken from the bibliographic reference and included in the table.

Table 8b. Mineral composition of lentils (per 100 grams of dry matter)

	Khan et al. (1987)	Combe et al. (1991)	Sika et al. (1995)	Ereifeq and Haddad (2001)	Kopflik et al. (2002)	El-Adawy et al. (2003)	Demirbas (2005)	Iqbal et al. (2006)	Erdogan et al. (2006)
S (mg/100 g)							100		
Mn (mg/100 g)	3.4	1.4		1.3; 1.48	1.43	1.8	5.38	1.6	1.17
Cu (mg/100 g)	2.3	3.2		1.1; 0.88	0.85		1.8	3.1	1.01
B (mg/100 g)		0.86	0.992				1.05		
Al (μ g/100 g)			10						
Cr (μ g/100 g)			27.2						
Ni (μ g/100 g)	300		115		189				
Pb (μ g/100 g)			37						
Co (μ g/100 g)					7.1				
Se (μ g/100 g)					103				
Mo (mg/100 g)					1.27		0.73		

the minerals are located in the cotyledons, except for Ca and Fe that are present in a considerable proportion in the lentil seed coat (Adsule et al., 1989). As for other legumes (e.g. peas, lupin, beans; Porres et al., 2003, 2005; Urbano et al., 2006), potassium is the mineral present in quantitatively highest levels, followed by phosphorus, calcium, magnesium and sodium. A high proportion of phosphorus is found in phytic acid with potentially low availability for simple-stomached animals. Less is present as free inorganic phosphate (5.6–14.9%, El-Mahdy et al., 1985; Porres et al., 2004) or other organic phosphorus components (8.6–15.4%, El-Mahdy et al., 1985).

2.7. Vitamins

Lentils is a rich source of water soluble vitamins (Table 9) and, as with most species of legumes contains only small amounts of vitamin C, carotene and retinol.

2.8. Non-nutritional Components

The composition of non-nutritional components found in *Lens culinaris* and their physiological effects are described in Tables 10 and 11. Protease (trypsin and chymotrypsin) inhibitors found in lentil can decrease the effectiveness of pancreatic enzymes and interfere with the digestive utilization of protein, causing an enlargement of pancreas. Lentils contain amylase inhibitors in lower values than *Phaseolus* which may contribute to the lower nutritive value of uncooked seeds. While lentils contain lectins Grant et al. (1983) have included lentil in the group of pulses with low reactivity to the erythrocyte agglutination test and low toxicity

Table 9. Vitamin content of lentils

	(mg/100 g DM)	References
Thiamin	0.2–0.72	2, 5
Riboflavin	0.03–0.41	1, 2, 5
Niacin	1.24–1.29	2, 7
B ₆	0.55–0.60	3, 5
Retinol	17–112*	4, 5
β-carotene	0.10	5
Biotin	0.132	4
Folic acid	0.03–1.5	4, 5, 8
Pantothenic acid	1.4–1.8	4, 5
Vitamin C	Nd-7	5, 6

*International Units (I.U.).

1. Vidal-Valverde and Frias (1993b); 2. Frias et al. (1995a); 3. Sierra and Vidal-Valverde (1997); 4. Savage (1988) Nutr Abs Rev 5, 319–343; 5. Souci et al. (2000) Food Composition and Nutrition Tables, CRC Press; 6. Frias et al. (2002); 7. Urbano et al. (1995); 8. Han and Tyler (2003) J Agric Food Chem 51, 5315–5318

Table 10. Non-nutritional content of lentils (expressed in dry matter)

	Range	it References
Trypsin and chymotrypsin inhibitors (U/mg)	2.7–6.1	1–9,36
α -amylase inhibitors (U/g)	2–18	10
Lectins (U/mg)	0.20–7.7	1,6,11,12
Tannins (mg/g)	< 0.5–10.9	1,5,8,9,11,12,13,14,15
Oxalate (g/Kg)	1.18–5.4	11,16,17
Phytic acid (g/100 g)	0.15–2.34	1,9,12,13,15,18–32
α -galactosides (g/100 g)	1.8–7.5	9,32
Saponins (mg/100 g)	40–127	33–35

1. El-Mahdy et al. (1985); 2. Vidal-Valverde et al. (1994); 3. Urbano et al. (1995); 4. Frias et al. (1995b); 5. Tabera et al. (1995); 6. Hernández-Infante et al. (1998); 7. Fasina et al. (2001); 8. Porres et al. (2003); 9. Wang and Daun (2006); 10. Jaffé et al. (1973) *Nutr Rep Int* 7, 169–173; 11. Savage (1988) *Nutr Abs Rev* 5, 319–343; 12. El-Adawy et al. (2003); 13. Khan et al. (1987); 14. Carbonaro et al. (1996); 15. Rehman and Shah (2005); 16. Massey et al. (2001); 17. Quinteros et al. (2003) *Int J Food Sci Nutr* 54, 373–377; 18. Bhatta (1989); 19. Bhatta (1989) *Can Inst Food Sci Technol J* 22, 137–142; 20. El-Tinay et al. (1989); 21. Lombardi-Boccia et al. (1991); 22. Ravindran et al. (1994) *Food Chem* 50, 133–136; 23. Bhatta (1995); 24. Sharma et al. (1996) *Nahrung/Food* 40, 182–184; 25. Morris and Hill (1996) *J Food Comp Anal* 9, 2–12; 26. Agte et al. (1998) *J Food Sci Technol Mys* 35, 330–332; 27. Urbano et al. (1999); 28. Arntfield et al. (2001); 29. Egli et al. (2002); 30. Vidal-Valverde et al. (2002a); 31. Frias et al. (2003b); 32. Porres et al. (2004); 33. Frenwick and Oakenfull (1983) *J Sci Food Agric* 34, 186–191; 34. Ruiz et al. (1996) *J Agric Food Chem* 44, 1526–1530; 35. Ruiz et al. (1997); 36. Zhao et al. (2005) *J Food Sci* 70, S371–S376

when compared to other highly reactive legumes like *Phaseolus vulgaris*, *Phaseolus coccineus* or *Phaseolus acutifolius*. Cuadrado et al. (2002a) purified a lentil lectin extract and fed it to rats at a 5-fold higher dose than the level in lentils without observing any detrimental effect on growth, digestion or metabolism of protein compared to controls. It appears lentil lectin, like other mannose/glucose specific lectins, has a limited effect on the metabolism of experimental animals.

The detrimental effect of phytic acid arises from it forming complexes with protein and minerals in the small intestine making these nutrients poorly available for absorption (Cheryan, 1980). Tannins are polyphenolic compounds that form complexes with salivary and dietary protein and other food components, interfering with their availability and reducing the nutritive utilization of foodstuffs (Bartolomé et al., 1995; Carbonaro et al., 1996).

α -Galactosides are non-nutritional constituents of legumes formed mainly by raffinose, ciceritol, stachyose and verbascose. They are an important contributor to flatulence (Granito et al., 2005). Reddy et al. (1984) reported that mung beans and green lentils are less flatulent than navy, kidney, red kidney, chickpea and peas. α -Galactosides are not digested by monogastric animals because the intestinal mucosa lack the hydrolytic enzyme α -galactosidase. These sugars are unable to pass through the intestinal wall (Rackis, 1975). The microflora in the lower intestinal-tract ferments them and produces large amounts of carbon dioxide, hydrogen and small quantities of methane and short chain fatty acids, thus lowering the

Table 11. Physiological effects of non-nutritional components

	Food Intake	Growth	Pancreas	Digestive utilization of protein	Mineral and vitamin bioavailability	Other effects
Trypsin and chymotrypsin inhibitors	ND	↓ (1, 2, 3, 4)	Hyperplasia (1, 2); pancreatic tumors (3, 4) ↑ pancreatic secretion (5)	↓ <i>In vitro</i> and <i>In vivo</i> (6, 7)	ND	Anti cancer
Lectins	↓ (8)	↓ (2)	Hypertrophy (4, 9, 10)	↓ <i>In vitro</i> and <i>In vivo</i> (11, 12)	ND	Intestinal Hypertrophy, Toxicity, Decreased activity of brush border enzymes
Tannins	↓ (14)	↓ (14, 15)		↓ nutritive utilization of protein and other nutrients (16–18)	↓	↑ production and secretion of salivary proteins (24), Antioxidant effects

Phytic acid	↓ <i>In vitro</i> (25,26)	↓ Fe; Zn; Ca; Mg; Cu; P; Phytate/Zn molar ratios (27–31)	↓ Glycemic index, Antioxidant, Anti cancer activity, Hypocholes- terolemic (32)
α-galactosides	↓ (33,34) flatul production		
Saponins			Hypocholes terolemic Anti cancer (35,36)

1. Pusztaí et al. (1992) *Br J Nutr* 68, 783–791; 2. Grant et al. (1993) *J Nutr* 123, 2207–2215; 3. Grant et al. (1995) *Br J Nutr* 73, 17–29; 4. Oliveira et al. (2000) *Food Chem* 70, 185–191; 5. Laporte and Tremolieres (1973) *Nutr Metab* 15, 192–206; 6. Leterme et al. (1990) *Anim Feed Sci Technol* 29, 45–55; 7. Rani et al. (1996) *Nahrung/Food* 40, 145–146; 8. Grant and Van Driessche (1993) *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, Wageningen Pers; 9. De Oliveira et al. (1988) *Nutr Res* 8, 943–947; 10. Bardeoz et al. (1989) *Med Sci Res* 17, 309–311; 11. Jaffé and Brucher (1972) *Arch Latinoam Nutr* 22, 267–281; 12. Thompson et al. (1986) *J Food Sci* 51, 150–153; 13. Deglaire et al. (2006) *J Agric Food Chem* 54, 5197–5202; 14. Mole et al. (1993) *Biochem Systemat Ecol* 21, 667–677; 15. Jambunatham and Mertz (1973) *J Agric Food Chem* 21, 692–696; 16. Jansman et al. (1995) *J Anim Sci* 73, 118–127; 17. Yoneda and Nakatsubo (1998); 18. Mateus et al. (2004) *Anal Chim Acta* 513, 135–140; 19. Brown et al. (1990) *Nutr Res* 10, 343–353; 20. Matuschek et al. (2001) *J Agric Food Chem* 49, 5630–5638; 21. Matuschek and Svanberg (2002) *J Food Sci* 67, 420–424; 22. Matuschek and Svanberg (2005) *Food Chem* 90, 765–771; 23. Towo et al. (2006) *Food Chem* 94, 369–376; 24. Bacon and Rhodes (2000) *J Agric Food Chem* 48, 838–843; 25. Singh and Krikorian (1982); 26. Kies et al. (2006) *J Agric Food Chem* 54, 1753–1758; 27. Cheryan (1980); 28. Morris and Ellis (1980) *J Nutr* 110, 1037–1045; 29. Fordyce et al. (1987) *J Food Sci* 52, 440–444; 30. Zhou et al. (1992) *J Nutr* 122, 2466–2473; 31. Porres et al. (2005); 32. Rickard and Thompson (1977) *ACS Symp Ser* 662, 294–312; 33. Fleming (1981); 34. Granito et al. (2005); 35. Sidhu and Oakenfull (1986); 36. Konoshima et al. (1992)

pH (Rackis, 1975). Fleming (1981) reported a significant positive correlation between intestinal gas production and the content of α -galactosides in legume seeds. However, α -galactosides are considered as prebiotics since as they resist hydrolysis by digestive enzymes and are not absorbed in the upper part of the gastrointestinal tract, they pass into the large bowel and promote the growth of *Bifidobacterium* and *Lactobacillus* (Roberfroid, 2002; Martínez-Villaluenga et al., 2007).

Lentil has a lower saponin content (75–127mg/100g) than soybean or kidney bean (650 and 350 mg/100g, respectively) but a higher level than lupin (38–74 mg/100g) (Ruiz et al., 1997). Saponins have a bitter taste, foam in aqueous solutions and haemolyze red blood cells. However, they also have a benefit in lowering plasma cholesterol levels in humans (Sidhu and Oakenfull, 1986) and have anticancer activity (Konoshima et al., 1992).

3. EFFECT OF DIFFERENT PROCESSING CONDITIONS ON THE CHEMICAL COMPOSITION OF *LENS CULINARIS*

To improve nutritional quality and utilization of lentils as food products, seeds are processed by several methods to remove the undesirable compounds. Physical and chemical methods are used (e.g. soaking, cooking, germination, fermentation, selective extraction, membrane filtration, irradiation and enzyme treatments). These treatments lead to a significant reduction or total elimination of non-nutritional compounds. Breeding programs have also been used, however, progress is slow as there are many different non-nutritional compounds at high levels in raw seeds (Table 10 and 11).

Germination and fermentation processes lead to catabolism of the lentil seed components whereas other processes (e.g. cooking) may cause thermal degradation or may involve extraction of non-nutritional components.

3.1. Soaking and Cooking Processes

Soaking and cooking are employed at both household and industry levels. They improve palatability and destroy heat-labile and heat-resistant non-nutritional components that are able to interfere with the nutritive utilization of protein (Vidal-Valverde et al., 1994). These processes may also reduce total nitrogen, non-protein nitrogen, and minerals, due to leaching into the soaking or cooking solution (Shekib et al., 1985; Rehman and Shah, 2005) (Table 12). Other authors did not observed any substantial loss and have described a considerable increment in total nitrogen (Manan et al., 1987; De Almeida Costa et al., 2006) and ash (Candela et al., 1997) content of lentils after the soaking/cooking process. Candela et al. (1997) studied the effect on chemical composition due to holding cooked lentils at 65 °C for 3 hours. They observed no considerable changes in the nutrient composition with the exception of certain amino acids like Hys, Leu, Lys, Phe, Thr and Val. Hernández-Infante et al. (1998) reported that both traditional and microwave cooking caused a significant reduction in the content of available lysine

Table 12. Effect of thermal processes on the nutrient composition of lentils (expressed in dry matter)

Reference	Shekib et al. (1985) Decorticated/cooked 30 min	Candela et al. (1997) Soak 12 h/cook 3 h	Urbano et al. (1995, 1999) Dry heat	Rehman and Shah (2005) Normal/Autoclave cooking	De Almeida Costa et al. (2006)
TN (%)	Raw 4.88 Cooked 4.52	Raw 4.27 Cooked 4.05	Raw 4.05 Dry heat 4.01	Raw 3.70 Norm. 3063	Raw 3.81 Soaked/cooked 4.34
NPN (%)	0.58 0.45				
Starch (%)	67.0 69.5 [‡]	33.8 27.2	48.7 38.6	42.8 42.4	56.4 [‡] 61.8 [‡]
Fat (%)	1.23 0.86	3.59 10.11	1.3 1.3		2.15 2.36
Fiber (%)	2.45 2.33	35.11 [@] 24.38	20.6* 15.5		19.0# 21.4
		3.75 7.65	4.8 11.7		1.44 1.37
Ash (%)	2.46 2.41	31.36 16.63	1.3 3.1		
		3.21 6.12	5.34		

[‡] Carbohydrate content.

TN = Total Nitrogen.

NPN = Non-Protein Nitrogen

[‡] Nitrogen-free extract

[@] Total; soluble; insoluble.

* neutral detergent fiber (NDF), acid detergent fiber (ADF), Lignin # insoluble dietary fiber (IDF) and soluble dietary fiber (SDF)

from lentils. This was in spite of a considerably reduced amount of trypsin inhibitor activity and lectins. Pirman et al. (2001) observed an increase in the content of all essential and non essential amino acids with the exception of Thr as a result of cooking lentils (Table 13).

Carbohydrates.- Soaking and cooking decrease the content of soluble available sugars and α -galactoside oligosaccharides from lentils (Vidal-Valverde et al., 1993a; Vidal-Valverde et al., 2002b). The degree of changes after soaking depend upon several variables including temperature, length, illumination, lentil/water ratio, elimination of the soaking liquid and milling of the lentil seeds. After soaking of lentils for 9 hours in water, citric acid, or bicarbonate solution, Vidal-Valverde et al. (1992b) and Frias et al. (1995a) reported metabolic changes similar to those taking place during germination that led to a significant decrease in α -galactoside oligosaccharides and increments in fructose and glucose, but not sucrose. These authors also reported an increase in the content of riboflavin and the ratio of available to total starch, observations that could imply the higher nutritional value of soaked lentils. The presence of light during the soaking process had a significant effect on the changes of some components like sucrose, raffinose, ciceritol and stachyose, but no effect on total and digestible starch, hydrosoluble vitamins, or dietary fiber content. Sugars such as fructose, glucose and sucrose decreased significantly during cooking. Autoclaving caused a slight but significant decrease in total starch content of cooked lentils (Rehman and Shah, 2005) and improved significantly the *in vitro* digestibility.

Table 13. Effect of cooking process on the essential and non essential amino acid composition of lentils (g/16 g N)*

Amino Acids	Raw Lentil	Cooked Lentil
Non Essential		
Ala	3.65	3.90
Arg	6.36	7.68
Asp	9.46	10.00
Glu	12.92	13.40
Gly	3.59	3.83
His	2.09	2.20
Pro	3.50	3.75
Ser	3.90	4.01
Essential		
Cys	0.07	0.09
Ile	3.64	4.08
Leu	6.57	7.10
Lys	5.84	6.34
Met	0.59	1.24
Phe	4.67	5.06
Thr	3.33	2.95
Tyr	1.40	2.73
Val	4.02	4.53

*Taken from Pirman et al. (2001)

Frias et al. (2003a) established the optimal soaking conditions of lentils for an adequate action of endo and exo α -galactosidase activity and studied the kinetics of soluble carbohydrates as a result of the activity of these enzymes. Soaking led to a significant reduction in the content of sucrose and α -galactoside oligosaccharides matched by an increment in the levels of fructose, glucose and galactose. Therefore, soaking could result in the development of lentil-derived flours with low flatulence and high functionality.

Fat.- Cooking can significantly reduce total fat and fatty acid content of lentils (Shekib et al., 1985; Pirman and Stibilj, 2003) (Tables 12 and 14). The reported losses can be of greater nutritional importance if lentils are consumed after discarding the soaking/cooking solutions. However, other normal additives during cooking (e.g. olive oil replacing the fat content, Candela et al., 1997) may overcome processing losses (Table 12).

Fiber.- Vidal-Valverde and Frias (1991) have reported a significant decrease in the levels of Neutral Detergent Fibre (NDF) and hemicellulose and no major effect of normal and pressure cooking on the Acid Detergent Fiber (ADF), cellulose or lignin content of lentils when expressed on a dry matter basis. Vidal-Valverde et al. (1992a), found soaking lentils in water prior to cooking did not result in any major alteration in the NDF or ADF fractions of lentils, whereas soaking in 0.1% citric acid or 0.07% sodium bicarbonate caused a significant increase in NDF and hemicellulose, with hardly any effect in ADF, cellulose or lignin content. In

Table 14. Effect of cooking process on the fatty acid composition of lentils*

Fatty acid (mg/100 g DM)	Raw lentil	Cooked lentil
14:0	6.3	2.5
15:0	3.8	1.6
16:0	343	161
16:1, n-7	1.5	0.7
17:0	3.4	1.7
17:1, n-7	2.1	0.5
18:0	34.3	17.9
18:1, n-9	628	324
18:2, n-6	1172	619
18:3, n-3	382	198
20:0	10.6	5.9
20:1, n-9	21.3	12.1
22:0	—	—
Total fatty acids	2608	1344
Total saturated	401	191
MUFA	653	337
PUFA	1554	816
Proportion n-6: n-3	1:0.33	1:0.32
Total fat (g/100 g DM)	3.26	1.68

*Taken from Pirman and Stibilj (2003)

contrast, cooking after either one of the soaking processes led to a significant decrease in the levels of NDF and hemicellulose, and significantly enhanced the content of ADF, cellulose and lignin. Ramulu and Udayasekhara Rao (1997) have reported a considerable reduction in total and insoluble dietary fiber content of lentils as a result of dehusking, and a considerable increase in the afore mentioned dietary fiber fractions as a consequence of cooking, whereas none of the processing techniques mentioned caused any major effect on soluble dietary fiber content of lentils. In contrast, Candela et al. (1997) reported a considerable increase in soluble dietary fiber and a significant reduction in the insoluble dietary fiber content of lentils as a result of cooking and warm-holding.

Vitamins.- The content of hydrosoluble vitamins in lentils tended to be decreased or did not change after a short soaking process with the exception of the levels of vitamin B₂ and available niacin under some experimental conditions with ground lentils (Vidal-Valverde et al., 2002b). The effect of different pH of the soaking solution and cooking process on the content of thiamin, riboflavin and available niacin of lentils was studied by Prodanov et al. (2004). These authors did not observe any effect of the different soaking solutions on thiamin content, whereas the levels of riboflavin increase in soaked lentils irrespective of the solution pH and available niacin levels decreased more pronouncedly in water-soaked than in citric acid or bicarbonate-soaked lentils. Cooking caused a significant reduction in thiamin, riboflavin and available niacin of soaked lentils with the exception of the latter vitamin in citric acid or bicarbonate-soaked lentils.

Minerals.- Shekib et al. (1985) have observed a considerable loss of crude ether extract, Ca, P, Fe, Na, K, and Zn content after decorticating and cooking lentils in boiling water for 30 min, whereas hardly any change was observed in the total and non protein nitrogen, crude fiber, ash or nitrogen-free extract content. El-Tinay et al. (1989) studied the effect of soaking and cooking on the nutrient composition of lentil (Table 15). Soaking led to leaching losses of all the minerals studied that

Table 15. Effect of soaking and cooking processes on mineral retention from lentils

	Shekib et al. (1985) mg/100 g DM		El-Tinay et al. (1989) % retention	
	Raw	Cooked	Soaking	Cooking
Ca	42	15.4	78	56
P	380	204	75	48
Fe	7.2	2.4	78	58
Na	4.6	3.8	61	45
K	753	213	75	42
Zn	5.11	3.13	84	42
Cu			91	64
Mg			82	56
Mn			80	60
Phytate			100	67

were further aggravated by cooking. Retention of Cu after the soaking/cooking tended to be higher than that of other minerals while Na showed the greatest loss. Soaking did not cause a major loss of phytic acid, whereas cooking did result in a considerable loss of this non-nutritional component. Similar results regarding the effect of cooking on phytic acid content of lentils have been reported by other authors (Gad et al., 1982; Manan et al., 1987; Vidal-Valverde et al., 1994). Loss of phytic acid with soaking and cooking has also been reported in bean and faba bean (Chang et al., 1977; Fernández et al., 1997). Frias et al. (2003b) studied the phytase-catalyzed inositol phosphate degradation in lentils, and found processing conditions which caused a marked reduction in inositol hexa- and pentaphosphate, but had no major effect on inositol tetra- and triphosphate content. Candela et al. (1997) observed a significant increase in the ash content of lentils ascribed to contamination with minerals from the equipment/water used.

3.2. Canning

Martin-Bellosillo Llanos-Barriobero (2001) reported that proximate composition of canned lentils did not vary significantly with processing time and location where the processing was done with the exception of Na, Ca, (due to brine addition), K (due to soaking in potassium metabisulfite) and ascorbic acid content. There was considerable retention of most nutrients (protein, carbohydrates, fiber, minerals and vitamins) during the canning process (Table 16). Vitamin B₂ was the most sensitive soluble vitamin to canning followed by vitamin B₁, vitamin C, and vitamin B₆.

3.3. Cooking Quality of Lentils

Cooking time is an important factor used to define cooking quality. Others include texture, flavor and appearance of lentil-based foods. Cooking time is affected by genetic, storage, environmental and sowing factors. Long storage periods at relatively high moisture/temperature conditions resulted in increased cooking times. Treatments like soaking overnight, irradiation or tempering can improve cooking characteristics (Khan et al., 1987; Arntfield et al., 2001; Çelik et al., 2004). Iliadis (2001; 2003) found cooking time can be affected by lentil genotype, harvesting process (early harvesting was more desirable for shorter cooking time and lower seed loss due to pods shattering), climatic conditions (rainy years induced longer cooking times than dry years), and by storage conditions, with an increase in the cooking time of stored lentils that was more pronounced in the late harvested lentils. Wet conditions during storage tended to further lengthen the cooking time, especially of those lentils genetically prone to need a longer cooking time. Finally, the influence of genotype was stronger than the influence of soil type for cooking quality of lentils.

The texture of micronization-cooked legumes softened as tempering moisture increased. This was related to increased starch gelatinization and decreased protein

solubility during micronization. A reduction in phytic acid and NDF was observed, and the strength of cell walls was also affected. Changes in pectic substances did not appear to influence texture. Addition of different salts to the tempering solution (2% sodium tripolyphosphate, 150 ppm sodium EDTA, mixture of 1% citric acid/2% ascorbic acid) reduced cooking time of lentil when tempering at 40% moisture was carried out, but not when the moisture content was 20%. Arntfield et al. (2001) studied the effect of internal temperatures reached inside the lentil seed during the micronizing process. If micronizing temperature is too high (170°C), an increase in cooking time and darkening of the seed is also observed relative to a lower micronization temperature (138°C).

Bhatty (1984) studied the relationship between physical and chemical characteristics and different environmental conditions on the cooking quality of lentils. Location and season of growth and cultivar all had a major influence on the cooking quality of lentil. Correlations were obtained between cooking quality and $\text{Ca}^{2+} + \text{Mg}^{2+}/\text{P}$ ratios. This ratio will depend upon the fertilization, availability and uptake of soil P by the lentil plants. The implications of the correlations are not clear but may relate to some role that P, particularly phytic acid-P, may play in determining the cooking quality of lentil. Higher peak and set-back viscosities of good cooking lentil meals indicated some role of starch and/or other components in affecting the cooking quality of lentil. Erskine et al. (1985) found a positive genetic correlation (0.919) between cooking time and seed size suggesting seed size can be used to predict cooking quality.

Table 16. Effect of canning process on the nutrient composition of lentils*

	Nutrient composition of canned lentils	% nutrient retention and uptake
Moisture (%)	79.4 ± 1.9	—
Protein (%)	6.4 ± 0.7	103
Carbohydrate (%)	10.4 ± 1.0	85
Fat (%)	0.20 ± 0.02	—
Fiber (%)	2.4 ± 0.3	101
Ash (%)	1.26 ± 0.16	232
Na (%)	0.58 ± 0.14	1030
K (%)	0.15 ± 0.002	142
Fe (mg/100 g)	1.24 ± 0.15	101
P (mg/100 g)	58 ± 6	—
Ca (mg/100 g)	17 ± 3	220
Mg (mg/100 g)	14.3 ± 2.2	101
Vitamin B ₁ (mg/kg)	0.92 ± 0.13	71
Vitamin B ₂ (mg/kg)	0.892 ± 0.009	68
Vitamin B ₆ (mg/kg)	0.95 ± 0.12	82
Vitamin C (mg/kg)	10.79 ± 0.5	74

*Taken from Martin-Belloso and Llanos-Barriobero (2001)

Bhatty (1989), concluded that phytic acid was the major determinant of shear force and, hence, cooking quality of lentil. There seems to be a strong positive relationship between phytic acid expressed either as a percentage of the seed weight or seed P, and cooking quality of lentils grown under diverse conditions. Therefore, any condition that lowers the phytic acid content of lentil would likely result in a decrease of their cooking quality. Soaking of poor cooking lentils in phytic acid or EDTA solution made them extremely soft, probably by removing Ca^{2+} from the middle lamellar region during the cooking process. The poor cooking condition persisted in dehulled lentil, suggesting its origin in the cotyledon where Phytic acid is located rather than in the seed coat. The cell wall pectin may play a complementary role in the poor-cooking condition of lentil.

Lack of cell separation and cell wall dissolution is typical of poor cooking lentils (Bhatty, 1990) that showed some evidence of a “lignification-like” mechanism at the cell junctions in the middle lamella. On the other hand, the cell wall was isolated in similar yields from good and poor cooking lentils, and showed a similar chemical composition. Means for hydration coefficient, solids lost, or water absorption were greater for the good-cooking lentil than for the poor-cooking lentil (Bhatty, 1995). The seed coat retarded water uptake and prevented solid loss in the poor-cooking lentils, thus increasing their shear force. However, when the seed coat composition was studied, no major differences were found in the chemical composition in terms of lignin, starch, protein or non-starch polysaccharides, although differences were found in the structure of the seed coat that would impede water uptake during cooking of the poor-cooking lentils. In apparent contrast to the previously described results, Ereifej and Shibli (1995) reported that physical and chemical properties and chemical composition were not related to differences in cookability of three newly developed lentil cultivars or a landrace cultivar (35 min to cook at 100 °C without previous soaking).

3.4. Dry-heat and Irradiation Treatments

With dry-heating processes lentil seeds have no direct contact with an external source of water. In these circumstances total nitrogen content was unaffected (Fasina et al., 2001; Urbano et al., 1995; Carbonaro et al., 1997; Porres et al., 2003), although protein solubility decreased due to thermal denaturation (Fasina et al., 2001; Sanz et al., 2001). Autoclaving significantly increases available carbohydrates like fructose or sucrose and decreases the total starch content (Table 12), not affecting the content of α -galactoside oligosaccharides or hydrosoluble vitamins thiamin, riboflavin and niacin (Urbano et al., 1995). Dry-heating caused a significant reduction in trypsin inhibitor activity in lentil seeds. Infrared heating of lentils did not cause any major effect on total or gelatinized starch or Trypsin inhibitor activity, but did significantly reduce the amount of α -galactoside oligosaccharides (Fasina et al., 2001).

Gamma irradiation is a food preservation technique that offers the potential to protect cereal grains and legumes from insect infestation and microbial contami-

nation during storage. Furthermore, irradiation can significantly alter the functional properties and increase the nutritional value of legume seeds due to inactivation of non nutritional components with antinutritional effect. Gamma irradiation of lentil seeds led to slight differences in the SDS-PAGE patterns of salt-insoluble proteins, causing a slight reduction in band density that could be indicative of minor radiolytic breakdown (Çelik et al., 2004). A general tendency to have higher hydration and swelling index values and lower cooking times were observed that were radiation dose-dependent.

3.5. Germination

Germination mobilizes reserve nutrients required for growth and contributes to the removal of some of the non-nutritive seed compounds such as phytic acid and α -galactosides. At the same time, the content of some other nutrients like minerals and vitamins such as B group and ascorbic acid as well as sugars (fructose, glucose and sucrose, Vidal-Valverde and Frias, 1992; Frias et al., 1996a) can be enhanced. Furthermore, seed sprouts present better organoleptic properties than the dry seeds. During germination, seed enzymatic systems activate and protease activity increases (Urbano et al., 2005a,b) and, consequently, protein nitrogen content decreases whilst non-protein nitrogen, peptides and free protein and non-protein amino acids increase (El-Mahdy et al., 1985; El-Adawy et al., 2003; Rozan et al., 2000; Kuo et al., 2004; Urbano et al., 2005a, b) (Table 17).

This protein pre-fractioning carried out in legume seed can be used into the preparation of dietetic products for population with gastro-intestinal dysfunctions. Hydrolysis and utilization of carbohydrates also takes place during germination and starch digestibility rises in lentil sprouts further increasing digestibility (Vidal-Valverde and Frias, 1992; Frias et al., 1998). Germination also alters the starch granule surface resulting in a higher resistance to temperature changes (Frias et al., 1998). Hemicellulose content is reduced during germination whilst cellulose and lignin increase (Vidal-Valverde and Frias, 1992). Loss of major constituents of the seed (protein and starch) during germination, gives an apparent increase on a dry weight basis of some seed constituents (Urbano et al., 1995; Kavas and Nehir, 1992; El-Adawy et al., 2003; El-Mahdy et al., 1985) (Tables 17 and 18).

Phytic acid decreases during lentil germination since naturally occurring phytases are activated and phytate is progressively degraded (Egli et al., 2002) leading to a decrease in its mineral-binding strength (Sandström and Sandberg, 1992). Vidal-Valverde et al. (2002a) have reported phytate degradation to lower inositol phosphate forms (penta- to triphosphates) using germination periods of 2, 4, and 6 days with or without light. These authors conclude that in order to maximize the beneficial effects of germination on the content of nutrients and non-nutritional components, the germination process should be carried out in the presence of light for a period of 6 days. Germination also seems to reduce hemagglutinating activity possibly due to proteolysis of lectins during germination (Chen et al., 1977).

Table 17. Effect of germination on the chemical composition of lentils

Reference	Klyen and McReady (1975)		El-Mahdy et al. (1985)		Kavas and Nehir (1992)		Danisová et al. (1994)		El-Adawy et al. (2003)	
	Raw	G72h	Raw	G96h	Raw	G96h	Raw	G48-96h	Raw	G72h G120h
(kJ per 100 g Dry Matter)										
Energy	1572	1592		1492	1647					
(Percent Dry Matter basis %)										
Total N			4.07-4.72	4.63-5.14	4.8	5.33	4.03	4.06	5.02	4.69 4.54
Protein N			3.58-4.09	3.16-3.55					0.54	0.93 1.37
Non Protein N			0.49-0.63	1.47-1.59			1.56	0.73	1.15	1.09 0.93
Fat	1.77	1.09							46.2	44.5 43.5
Starch									6.75	8.14 9.88
Fiber	5.1	4.0					4.33	6.24	4.16	5.87 6.97
Ash	2.87	2.93	2.62-3.18	2.99-3.84	2.62	2.85	3.73	3.30		
(mg mineral per 100 g Dry Matter)										
Na			4.30-6.90	4.60-7.10					11.0	11.6 11.7
K			143-157	180-200					240	280 300
Ca	36.5	44.0	40	45.0-50.0			78.6	73.1	76	86 94
Mg									48.5	49.2 49.7
Total-P			153-247	146-239			239	238	372	383 392
Phytate-P			120-195	60.4-96.8						
Inorganic-P			13.9-19.9	63.0-85.0						
Organic P; non phytate			13.1-38.1	22.6-57.0						
Fe	14.2	11.0	8.9-11.0	8.10-10.0	7.9	9.03	3.73	2.38	8.50	8.70 8.80
Mn									1.80	2.00 2.10
Zn	5.09	5.49	2.50-3.00	2.90-3.60	3.68	4.77				

Table 18. Effect of germination on the essential and non essential amino acid content of lentils (g/16 g N)

Amino Acids	Kavas and Nehir (1992)	Urbano et al. (1995)	Danisová et al. (1994)
Non Essential	Raw G96h	Raw G144h	
Ala	3.47 ± 0.163.67 ± 0.54	2.37 3.55	
Arg	8.68 ± 1.757.64 ± 0.98	3.90 3.82	
Asp	11.56 ± 0.5417.47 ± 1.86	9.94 11.97	
Glu	14.58 ± 0.0012.73 ± 1.86	16.30 16.40	
Gly	3.36 ± 0.003.06 ± 0.52	4.43 4.56	
His	–	1.33 1.53	
Pro	1.19 ± 0.592.29 ± 0.03	2.58 2.72	
Ser	4.38 ± 0.165.01 ± 0.67	2.86 2.75	
Essential	Raw G96h	Raw G144h	Raw G48–96h
Cys	1.12 ± 0.001.28 ± 0.02	1.46 1.41	–
Ile	3.44 ± 2.704.09 ± 0.43	4.04 4.41	3.07 3.02
Leu	6.13 ± 0.747.30 ± 1.06	7.24 8.39	6.13 6.25
Lys	6.35 ± 0.527.15 ± 1.20	4.27 5.20	6.91 6.93
Met	0.22 ± 0.000.86 ± 0.03	1.10 1.30	0.10 0.35
Phe	4.34 ± 0.294.90 ± 0.50	5.52 6.34	4.61 4.65
Thr	2.69 ± 0.643.60 ± 0.57	2.49 2.46	3.68 3.71
Tyr	2.24 ± 0.003.25 ± 0.25	1.13 1.11	–
Val	4.19 ± 0.204.39 ± 0.64	3.04 3.39	4.62 4.38

The reduction of enzyme inhibitor activities during seed germination have been widely reported. Frías et al. (1995) found a progressive but slow decrease on trypsin inhibitor activity (TIA) after 6 days of lentil germination (12% reduction) followed by a faster decrease (up to 45% of control seed value) after 10 days of germination. Similar results were obtained by El-Mahdy et al. (1985). A considerable reduction in chymotrypsin and α -amylase inhibitor activity has been reported after 5-days germination by Sathe et al. (1983).

Vitamin content is also modified as consequence of germination process and while thiamine seems to decrease slightly, riboflavin and available niacin increases sharply as a consequence of germination (Kavas and Nehir, 1992; Urbano et al., 1995; Prodanov et al., 1997; Vidal-Valverde et al., 2002a). Similarly, it has been reported that lentil sprouts are a good source of vitamin C and E (Frias et al., 2002)

3.6. Fermentation

Fermentation is one of the oldest and most economical methods of food production and preservation known. Fermentation can be spontaneously initiated with the microbiota naturally present in the legume or controlled by the use of specific cultures or starters from a batch of previously fermented product. Legumes are fermented to improve their sensory characteristics such as flavor and taste, and to enhance their nutritive value by improving the density and availability of nutrients (Vidal-Valverde et al., 1993b; Svanberg and Lorri, 1997). This can be achieved by

degradation of antinutritional factors, by the pre-digestion of certain food components and by the synthesis of promoters for absorption (Frias et al., 1996b). In addition, by increasing the titratable acidity and reducing the pH of the food to levels below 4.5, fermentation precludes the proliferation of contaminating acid-intolerant species of bacteria and fungi.

Changes occurring during the fermentation process are mainly due to endogenous enzymes of the seed and the enzymatic activity of the microflora present in the legume. Differences in the final nutritional value of the fermented product depend on whether the whole seed or flour suspensions with different ratios of flour to water (Kozłowska et al., 1996) are used. Fermentation may be combined with other culinary (soaking and cooking) and technological (germination prior to the fermentation process) treatments to further improve the nutritional value of the fermented product.

Tabera et al. (1995) have studied the effect of natural fermentation for periods of 0–4 days at 42 °C and 79–221 g/L on the nutrient and antinutritional factor content of lentils. The authors reported a slight increase in the total nitrogen content of fermented lentils (Table 19) and a considerable reduction in the trypsin inhibitor activity (62.5%) and tannin/catechin ratio. Changes that were reflected in only a slight increase or no effect on the *in vitro* protein digestibility of the fermented product. Using similar fermentation conditions, Cuadrado et al. (1996, 2002b) obtained a considerable decrease in the levels of lectins and inositol phosphates present in lentils, thus increasing their nutritive potential.

After natural fermentation of lentils for 4 days at 30 °C, Vidal-Valverde et al. (1993b) found a reduction in pH, and in the content of trypsin inhibitor

Table 19. Effect of fermentation on the chemical composition of lentils

Reference	Vidal-Valverde et al. (1993b)		Tabera et al. (1995)		Vidal-Valverde et al. (1997)	
	Raw	4days	Raw	4days	Raw	4days
pH	6.0	3.8				
Titratable acidity	1.08	7.38				
Total Nitrogen (%)			4.12	4.78		
Total starch (g/100 g)	52.3	31.5				
Available starch (g/100 g)	44.8	28.6				
Fiber (%)	19.2	14.1				
Thiamin					0.387	0.370
Riboflavin	0.03	0.09			0.086	0.151
Available niacin					1.30	2.36
TIA (TIU/mg DM)	2.66	1.57	5.09	3.63		
Tannins (mg/g DM)			3.16	6.26		
Catechins (mg/g DM)			0.12	6.67		
α-galactosides (sum)	3.16	ND				

ND = Not detectable; TIA = Trypsin inhibitor activity

activity, sucrose, raffinose, ciceritol, stachyose, total and available starch, NDF, cellulose, and hemicellulose, whereas a considerable increase was found in the titratable acidity and the content of riboflavin, fructose, lignin, and the ratio of available to total starch. The effect of natural fermentation of lentils on the content of fructose and α -galactoside oligosaccharides was further corroborated by Frias et al. (1996b) using different temperatures and flour/water ratios. These authors also found a considerable increase in glucose content of fermented lentils. Using the same fermented samples, Kozłowska et al. (1996) obtained up to 70–75% reduction in the inositol phosphate content. Sotomayor et al. (1999) have reported a considerable decrease in starch content at 0 hours that was further reduced after 96 hours of natural fermentation. Endocorrosion seemed to be the main reaction pattern observed in the lentil starch granule as a consequence of the fermentation process.

Vidal-Valverde et al. (1997) studied the influence of natural fermentation of lentils on the kinetics of thiamin, riboflavin and niacin. Natural fermentation significantly enhanced the content of riboflavin, total, and available niacin content of lentils, but had no effect or caused a slight reduction in thiamin content depending on the different fermentation conditions employed.

3.7. Hydroalcoholic Extraction of Non-nutritional Components

Sanz et al. (2001) developed an extraction procedure to reduce non nutritional components of lentil. They used 80% ethanol at room temperature or 50 °C for different periods of time (1–3 hours). Non-protein nitrogen content (12.9% of total nitrogen) was significantly affected by the different extraction temperatures (increasing at room temperature but falling at 50 °C Total nitrogen increased under all processing conditions. However, nitrogen solubility at pH 3, 5 or 7 was decreased as a result of the extraction process; a reduction that was further aggravated by extraction at 50 °C when compared to extraction at room temperature. The extraction process did not significantly affect the content of amino acids Lys, Hys or Tyr.

3.8. Aqueous Extraction of Protein for the Preparation of Isolates and Films

Monsoor and Yusuf (2002) have described the preparation of protein isolates from lentils, chickpeas or lathyrus beans using an alkaline (pH 11) extraction process followed by isoelectric precipitation of protein at pH 5.4. The authors found a 79.3% protein yield (extractability) for lentil, the highest of the three legumes studied, and a 90.7% protein content in the isolate with an *in vitro* protein digestibility of 95.2% that was very similar to a highly-digestible reference protein like casein and higher than the values found in the protein isolates from chickpeas or lathyrus beans. The thermal treatment of lentil protein isolate at 100 °C for 5 minutes led to a slight improvement in the *in vitro* protein digestibility of the final product.

Lombardi-Boccia et al. (2003) have established processing conditions intended for isolation of legume storage proteins (globulins G1 and G2); using this protocol, the authors obtained a lentil protein isolate with a protein, Fe, and Zn content of 89%, 9.0 and 9.4 mg/100g DM, respectively, in comparison to the 22.6%, 6.8, and 3.4 mg/100g DM originally present in the raw lentil flour. In addition, Fe dialyzability experienced a considerable improvement in the protein isolate when compared to the raw lentil flour in contrast to Zn dialyzability, which suffered a significant decrease as a result of the protein isolation process.

Bamdad et al. (2006) have applied a lentil protein isolate to the preparation of proteinous films oriented to packaging food products with the aim of ensuring a longer preservation process. The authors mixed an aqueous solution of lentil protein (5%) with glycerin to obtain a protein film in which they measured several physicochemical properties like color, tensile strength and percentage elongation, thickness, puncture strength, water vapor permeability, water content or soluble matter. The proteinous film shared similar properties with other soy, pea or whey proteinous films, and could be a feasible way to enhance the industrial use of lentil-based protein isolates.

3.9. Functional Properties

Lentil protein solubility is susceptible to a high degree of variation depending on the pH of the extraction solution used. In a similar way to other legumes, lentil protein exhibits a relatively wide isoelectric pH range of 4.0–6.0 in which the lowest solubility is observed. Protein solubility is sharply increased at both sides of the isoelectric pH range due to its high proportion of acidic and basic amino acids, reaching values that are close to 100% when the pH of the extraction solution is in the range of 10–12 (Carbonaro et al., 1997; Fasina et al., 2001; Sanz et al., 2001). Protein solubility from lentils and other pulses can be negatively affected by several technological treatments like thermal treatments or organic solvent extraction (Carbonaro et al., 1997; Fasina et al., 2001; Sanz et al., 2001), or positively by dehulling or germination processes (Ghavidel and Prakash, 2006). Sadowska et al. (1999) have reported the enhancing effect of fermentation process on protein solubility of lentils in the acidic pH range (3–5), whereas protein solubility was decreased in the neutral to basic pH range when compared to the raw lentil without processing. The fermentation process also produced modifications in other functional properties of lentil flour like the emulsifying capacity and the water or oil absorption capacity, and the functional properties of lentil-wheat flour mixes, affecting their bread-making properties.

Due to the influence of protein solubility on other important functional properties like the emulsifying capacity or the foam or gel forming capacity, the solubility profile of lentil protein is of utmost importance for lentil inclusion in the preparation of different food products. Succinylation of lentil globulins has been shown to modify the isoelectric pH from 4.5 in the native globulins to 3.5 in succinylated

proteins due to a higher negative charge of the later proteins (Bora, 2002); succinylation also improved protein solubility in the pH range of 4.5–8, but decreased it below pH 4. The functional properties of lentil globulins were affected by the succinylation process that improved the water absorption capacity, viscosity, emulsion activity and emulsion stability, decreasing the oil absorption capacity and not exerting any significant effect on foaming capacity or foaming stability. No effects on the functional properties of lentil globulins were observed as a result of different extents of succinylation.

Fernandez-Orozco et al. (2002, 2003) have studied the Superoxide Dismutase like-activity, peroxy radical-trapping capacity, trolox-equivalent antioxidant capacity and the content of soluble protein and several low molecular weight antioxidants like carotenoids, ascorbic acid, polyphenols and reduced glutathione in different lentil varieties, as well as the effect on these parameters of two processing technologies like cooking and germination. In general, SOD-like activity varied within different lentil cultivars and was not affected by the germination process, although it was considerably reduced by the cooking process. The SOD-like activity corresponded mainly to enzymatic SOD, and only minor proportions of the total activity corresponded to the total phenol contents, albumin protein, ascorbic acid or reduced glutathione. With regard to the other lentil components studied, the amount of soluble protein increased slightly with the germination process and was significantly reduced by cooking. The content of phenolic compounds was slightly decreased by both processing conditions, whereas considerable losses were observed in tocopherol and reduced glutathione. Ascorbic acid was not present in raw or cooked lentils, whereas significant amounts of this component were detected in germinated lentils. The antioxidant capacity varied among the different lentil varieties tested, and decreased significantly as a result of germination, with only slight changes being found in cooked lentils when compared to raw lentils. The authors observed a markedly higher molar percentage contribution of phenolic compounds to the antioxidant capacity in raw or cooked lentils when compared to the other low molecular weight antioxidants or soluble proteins. In contrast, the contribution of tocopherols, ascorbic acid and reduced glutathione in germinated lentils was considerably higher.

Cari et al. (2002) prepared protein curds from 6 different legumes and studied the textural properties of the products obtained in relationship to their protein constituents. According to these authors, curds produced from soybeans, chickpeas, and faba beans exhibited a better texture and higher texture scores for hardness, springiness and cohesiveness than curds produced from lentils, smooth peas or mung beans due to the higher amount of 11S globulins with superior levels of sulfur-containing amino acids in the former three legumes. Zhao et al. (2005) prepared spaghetti with semolina containing 5–30% milled flours of green pea, yellow pea or lentil and evaluated physical-chemical properties, descriptive sensory and consumer acceptance characteristics. Lentil fortification decreased the lightness, shiny appearance, elasticity and overall quality of spaghetti products and there was a trend for the optimal cooking time to increase and significant increments in the

cooking loss and firmness of the product. With regard to consumer acceptance, panelists liked the control spaghetti (100% wheat semolina) the most; spaghetti containing lentil flour had a lower color score than the products containing other 3 legume flours, but had higher flavor acceptability than spaghetti containing chickpea and yellow pea flours. General comments of the panelists indicated that legume spaghetti had a beany off flavor that could be related to the activity of lipoxygenase activity prior to drying and cooking of pasta product.

Banerjee et al. (2003) have designed different lentil-based extruded expanded products aimed at the preparation of snack foods, and studied the effect of temperature and moisture on process optimization. Incorporation of lentil to the snack food significantly enhanced the protein content of the functional product, and the extrudate properties could be significantly modified by different temperature and moisture conditions used in the preparation process designed by response surface methodology.

Lentil has been pin-milled and air classified into starch and protein fractions for incorporation into food and feed products and for industrial utilization (Gonzalez and Perez, 2002). The isolated lentil starch was microwave-irradiated and extruded in order to improve its functional and rheological characteristics. Both treatments caused internal granule compaction that was reflected in higher density and lower water absorption and swelling power of the modified starches. The restricted swelling and solubilization of processed starches could be responsible for the reduction of their amylographic viscosity. Moisture, crude protein and fiber contents were also reduced by the thermal treatments, which also reduced the retrogradation tendency of starch; that could be interesting from an industrial point of view, since starch retrogradation has been one of the limiting factors for a wider industrial use of legume-derived starches.

Dalgetty and Baik (2006) have prepared breads with wheat flour fortified with 3, 5, and 7% legume hulls or insoluble fiber, or with 1, 3, and 5% soluble cotyledon fibers isolated from pea, lentil, or chickpea flour. Inclusion of legume hulls or insoluble fiber led to increases in dough water absorption, mixing time and loaf weight, but decreased loaf volume when compared to control bread without legume hulls or insoluble fiber. Color and crumb uniformity was better in breads containing soluble fiber when compared to breads containing either hulls or insoluble fiber. In general, legume fiber or hull fortification of bread led to higher moisture content compared to the control bread.

4. BIOLOGICAL EVALUATION OF RAW AND PROCESSED *LENS CULINARIS*

4.1. Effect of Lentil Composition on Daily Food Intake

Legumes are seldom eaten raw, but are usually processed as previously described. Daily food intake of a raw lentil diet with a protein content in the range of 23–24.5% by growing rats was inferior to a casein-methionine control diet (Urbano

et al., 1999; Porres et al., 2003). Lower food intake can be attributed to a range of factors (Mercer et al., 1989) as experimental animals respond with a voluntary suppression of food intake to a nutrient imbalanced diet. A range of possible causes are discussed below but the cause or causes are not yet confirmed.

Carbohydrates.- The content of palatable soluble sugars in lentils is usually low (Frias et al., 1996a,b) and could lower intake. However, the increase in soluble sugars in response to germination and fermentation processes could improve palatability and thus increase food intake. Excessive germination time (5–6 days or more), may hamper intake due to changes in food texture or food chemistry (Rozaan et al., 2000). The high content of dietary fiber present in lentil may have contributed to the lower daily food intake obtained, due to its satiating effect (Slavin, 2005).

Protein level and protein quality of the diet.- Increased amounts of dietary protein can reduce food intake (Johnson and Anderson, 1982). However, the reduced food intake derived from lentil consumption appears to be related to protein quality rather than to the levels of protein in the diet. Dietary amino acid imbalances can decrease food ingestion (Harper et al., 1970), and specific relationships between amino acid ratios and daily food intake indicate these food constituents are active participants in the regulation of food consumption.

Minerals and vitamins.- Mineral and vitamin deficiency can lead to an important reduction in the nutritive utilization of food, and experimental animals show a depression in food intake as one of the initial symptoms of mineral-vitamin deficiency (O'Dell and Reeves, 1989). Lentils are generally deficient in certain essential minerals like calcium or iodine, and in liposoluble vitamins, choline, and niacine. Moreover, a sufficient amount of minerals and vitamins to meet the nutrient requirements does not ensure the availability of these nutrients for the animal. Supplementation of a mineral-vitamin premix to a raw lentil diet led to a significant increase in daily food intake by growing rats (Porres et al., 2003).

Fat.- The quantity and nutritional quality of fat present in the diet is known to have a significant influence on daily food intake (Le Magnen, 1983). Lipid peroxidation processes may cause a reduction in the organoleptic properties of food (Villaume et al., 1993). Unsaturated fatty acids are by nature more prone to become rancid by the action of lipoxygenase enzyme present in lentil (Maccarrone et al., 1997). However, the low levels of fat present in lentils suggest they are an unlikely cause of suppressed palatability

Non-nutritional components.- The presence of α -galactoside oligosaccharides in legumes has a negative effect on daily food intake due to the flatus-producing potential of these compounds. However, thermal treatment reducing α -galactoside of lentils has no effect on dietary intake (Urbano et al., 1995). Possibly these compounds are not present in sufficient quantities in lentils to play a major role in food intake. Dietary levels of α -galactoside oligosaccharides up to 3% do not exhibit any measurable flatulence effect, but do show important prebiotic

benefits. Moreover, Maillard products formed during the thermal treatment can negatively affect the palatability of heat-treated foods (Sarriá et al. 2001) and might have affected the palatability of lentils, thus hindering the beneficial effects of removing α -galactoside oligosaccharides. While the astringent sensation produced by tannins can lead to a significant reduction of daily food intake levels found in lentils do not appear to be high enough to exert any significant effect.

4.2. Nutritive Utilization of Protein from Raw and Processed Lentils

4.2.1. Digestive Utilization

Digestive utilization of lentil protein determined *in vivo* using the growing rat as experimental model ranges from 62.3–92.0% (Table 20), using *in vitro* methodologies it varies from 37.6–49.6% and from 82.3–92.5% depending on the different *in vitro* experimental conditions used. The *in vivo* digestive utilization of lentil protein is higher than that found for the common bean (Nestares et al., 2001), similar to faba beans and chickpeas (Fernandez et al., 1996; Nestares et al., 1996), and lower than what has been reported for peas and lupin (Urbano et al., 2003; Porres et al., 2006).

Table 20. Digestive utilization of protein (%) from lentils and effect of technological processing methods

	Raw	Heated/ Cooked	Germination	Fermentation	Ext. EtOH
<i>In vivo</i>	75 ¹ ;75.6 ⁷ ; 79.0 ⁸ ; 62.3 ¹⁰ ; 79.0 ¹¹ ; 65.7 ¹³ ; 76.1 ¹⁵ ; 80.7–84.2 ¹⁶	71.5 ⁷ ; 79.0 ⁸ ; 89.5 ¹³ ; 72.1–78.7 ¹⁵ 81.7–85.1 ¹⁶	72.1 ⁷		
<i>In vitro</i>					
pH-stat	87.7 ² ; 82.5 ⁵ ; 92.4 ⁶	81.66 ⁵		77.4–92.6 ²	91.4–92.8 ⁶
pH-drop	84.20 ¹² ; 82.3–84.8 ¹⁷		85.15–87.53 ¹²		
Pepsin digestion	37.6 ³ 41.5 ⁴	67.8–72.7 ³ , 47.3 ⁴			
Pancreatin digestion	25.0 ⁴	38.3 ⁴			
Pepsin + Pancreatin digestion	49.6 ⁴ ; 95.2 ⁹ 74.8 ¹⁴	63.9 ⁴ ; 97.9 ⁹	80.5 ¹⁴ 92.5 ¹⁴		
	69.78 ¹⁴				

1. Combe et al. (1991); 2. Tabera et al. (1995); 3. Rehman and Shah (2005); 4. Shekib et al. (1986); 5. Carbonaro et al. (1997); 6. Sanz et al. (2001); 7. Urbano et al. (1995); 8. Porres et al. (2003); 9. Monsoor and Yusuf (2002); 10. Cuadrado et al. (2002a); 11. Sarwar and Peace (1986); 12. El-Adawy et al. (2003); 13. Manan et al. (1987); 14. El-Mahdy et al. (1985); 15. Hernández-Infante et al. (1998); 16. Savage and Scott (1989); 17. Carbonaro et al. (1996)

The poorer digestive utilization of plant proteins when compared to animal proteins can be attributed to the specific structure of plant proteins that makes them more resistant to the attack by digestive proteinases, and to the presence in legumes of certain non-nutritional components that interfere with the activity of digestive enzymes and induce a higher secretion of endogenous nitrogen to the digestive tract. One of the main factors responsible for the higher losses of endogenous nitrogen derived from the consumption of legume-based diets appears to be the presence of lectins in legumes. Lectins bind to glycoproteins of the brush border membrane and increase the turnover of intestinal mucosal cells, thus inducing a higher nitrogen loss in feces as well as other metabolic effects. However, lectins present in lentils have been described to be of the mannose/glucose specific type (Rubio et al., 1995), and did not exert any particular effect on the digestive utilization of protein due to their low level in lentils and their low degree of reactivity (Grant et al., 1983; Hernández-Infante et al., 1998; Cuadrado et al., 2002a). Instead, the low digestibility of lentil proteins could be attributed to the reduced digestibility of lentil globulins and/or the inhibitory effect of the lentil seed coat (Cuadrado et al., 2002a).

Protease inhibitors (trypsin and chymotrypsin) present in lentils can interfere with the digestive utilization of protein by binding and blocking the activity of these digestive proteinases. This interfering action will lead to an incomplete digestion of dietary protein, and to a higher fecal excretion of endogenous nitrogen. In addition, the experimental animals have a higher requirement for sulfur containing amino acids in order to synthesize new digestive proteinases; amino acids that are limiting in legume seeds (Green and Lyman, 1972).

Tannins also exist in lentils which may interact with dietary protein and decrease its susceptibility to the attack by digestive proteinases. Furthermore, tannins themselves can interact with digestive enzymes interfering with their activity. Higher tannin/catechin ratios imply a higher degree of polymerization of tannins and, therefore, a lower digestive utilization of protein (Yoneda and Nakatsubo, 1998).

The role of phytic acid on digestive utilization of protein is not clear in view of the differences reported by several authors that either point out to a reduction in the activity of trypsin and chymotrypsin caused by phytic acid, or else do not find any relationship between a reduction in phytic acid content and improvements in the digestive utilization of protein *in vitro* or *in vivo* (Singh and Krikorian, 1982; Manan et al., 1987; Knuckles et al., 1989; Porres et al., 2005, 2006).

4.2.1.1. *Effect of processing on the digestive utilization of protein*

Thermal treatments.- Dry heating or autoclave treatment of non-soaked lentil seeds caused a reduction in the content of TIA, tannins and phytic acid, and increased the tannin/catechin ratio, without significantly affecting the digestive utilization of protein assessed by *in vitro* (Monsoor and Yusuf, 2002) or *in vivo* (Urbano et al., 1995; Porres et al., 2003) methodologies. This lack of improvement in digestive utilization of protein can be attributed to the higher degree of tannin polymerization and protein denaturation that results from the thermal treatment and is able to counteract the benefits of eliminating heat-labile non-nutritional

components like TIA. On the other hand, soaking of lentil seeds followed by a traditional or autoclave cooking process, caused a significant decrease in phytic acid and tannin content (Rehman and Shah, 2005), and led to a significant increase in the *in vitro* and *in vivo* protein digestibility (Savage and Scott, 1989; Rehman and Shah, 2005), and the *in vitro* starch digestibility (Rehman and Shah, 2005). In contrast, other authors have found no appreciable differences or even a reduction in the digestive utilization of lentil protein as a result of different soaking and cooking processes. Carbonaro et al. (1997) did not find a significant difference in protein digestibility of lentils measured by an *in vitro* ph-stat methodology between raw and cooked lentils, Aparna et al. (2000) found a reduction in the *in vitro* protein digestibility of lentils after cooking in a salt, bicarbonate, tartaric or citric acid solution, and Hernández-Infante et al. (1998) reported that a reduction in the content of TIA and hemagglutinins caused by microwave treatment of raw or soaked lentils or by cooking previously soaked lentils was not reflected in any improvement of protein digestibility.

Germination.- The process of germinating *Lens culinaris* seeds has been shown to decrease the levels of TIA, tannins and phytic acid (Vidal-Valverde et al., 1994; Urbano et al., 1995; El-Adawy et al., 2003) and improve the digestibility of lentil protein after short term germination period of up to 75 hours (El-Adawy et al., 2003). In contrast, Urbano et al. (1995) have reported a noticeable reduction in the digestive utilization of lentil protein after 6-days germination in spite of the above mentioned decrease in the content of non-nutritional components. Changes in the food matrix and seed chemical composition that take place during germination could be partially responsible for the enhancing or inhibitory effect of this technological process on protein digestibility.

Fermentation.- Shekib (1994) have studied the effect of natural fermentation of *Lens culinaris* for a period of 4 days on the content of different nitrogen fractions and the nutritive utilization of protein, finding a considerable increase in the levels of non-protein nitrogen and a slight increment in crude protein content, whereas no significant change was observed in the amount of protein nitrogen. With regard to the nutritive utilization of protein, fermentation process led to a significant improvement in the *in vitro* protein digestibility of the fermented product.

Tabera et al. (1995) have reported that natural fermentation of lentils using temperatures of 28–35 °C, and lentil flour concentrations of 79–150 g/L during 0–4 days decreased the levels of TIA by 17.1–62.5% and the tannin/catechin index, increasing the tannin content. The changes in tannin content and tannin/catechin index were associated to the soaking process of lentils carried out prior to fermentation. However, very slight changes in the *in vitro* protein digestibility of lentils were observed by these authors as a result of natural fermentation process.

Ethanol Extraction.- Lentil flour extraction with 70% ethanol during 1–3 hours at room temperature or 50 °C led to a reduction in protein solubility at different pH, but did not cause any major alteration in *in vitro* protein digestibility assessed by

a pH-stat methodology (Sanz et al., 2001). Similar results have been reported by Porres et al. (2005, 2006) for lupin in which the amount of insoluble nitrogen was increased as a consequence of α -galactoside-oligosaccharide extraction using 50% ethanol at 40°C without any major effect on protein digestibility assessed by *in vitro* or *in vivo* methodologies.

Protein amino acid digestibility.- The pattern of fecal amino acid composition in rats that consumed lentil diets was characterized by the excretion of endogenous protein that contains high amounts of methionine, cystine and lysine (Sarwar and Peace, 1986; Porres et al., 2002). This high excretion justifies the lower fecal digestibility of these amino acids when compared to the rest of dietary amino acids and crude protein. In addition, sulfur-containing amino acids of lower digestibility are deficient in legume-based diets, where the nutritional demands for these amino acids are increased for the *de novo* synthesis of pancreatic enzymes due to the effect of non-nutritional components like TIA or tannins, all that contributes to the lower nutritive utilization of lentil protein.

The chemical score of lentil protein is within 61–88%, whereas the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) ranges between 52–71%, and the chemical score corrected by the digestibility of the limiting amino acid ($AAS_{(ASAA)}$), usually the sulfur-containing amino acids in lentils, ranges between 41–57% (Sarwar and Peace, 1986; Porres et al., 2002; Iqbal et al., 2006). The thermal treatment in autoclave alters the nutritive utilization of lentil as indicated by the lower PDCAAS and $AAS_{(ASAA)}$ indices, and by the lower true digestibilities of methionine and tyrosine (Porres et al., 2002). However, chemical score and protein digestibility were not affected by the autoclave treatment.

In conclusion, it is difficult to assess the influence of any individual non-nutritional component on protein digestibility based on the effect of different technological treatments, given that multiple changes in chemical composition and food matrix are known to take place during the course of processing that may consistently affect the nutritive utilization of protein. The effects of processing on protein digestibility should be ascribed not only to changes in a single food component but rather to the combined effect of several food constituents.

4.2.2. Metabolic utilization

The nutritional quality of raw lentil protein assessed by nutritional indices like the percentage of retained to absorbed nitrogen, weight gain or Protein Efficiency Ratio

Table 21. Metabolic utilization of protein from lentils

PER	0.30 ¹ ; 0.64 ² ; 0.83 ³
Weight gain (g/day)	0.67 ¹ ; 1.44 ² ; 6.7 ⁴ ; 0–0.23 ⁵
Nitrogen retention (mg/day)	51.3 ¹ ; 74.9 ²
% Retained to Absorbed Nitrogen	19.1 ¹ ; 26.4 ² ; 71.8 ⁴ ; 29.9 ⁵ ; 41.0 ⁶

1. Urbano et al. (1995); 2. Porres et al. (2003); 3. Hernández-Infante et al. (1998);
4. Combe et al. (1991); 5. Cuadrado et al. (2002a); 6. Manan et al. (1987)

(PER) is usually low (Table 21) and inferior to other legumes like the pea, faba bean or lupin (Fernandez et al., 1996; Urbano et al., 2003; Porres et al., 2006), although similar to beans and chickpeas (Nestares et al., 1996; Nestares et al., 2001). The deficiency in sulfur-containing amino acids and certain essential minerals and vitamins together with the low availability of some of these nutrients from lentils are responsible, at least in part, for the low nutritional value of lentil protein. In fact, supplementation of raw lentil flour with methionine or a mineral-vitamin premix (Combe et al., 1991; Urbano et al., 1995; Porres et al., 2003) led to a significant improvement in the metabolic utilization of protein. The beneficial effects of methionine supplementation to a raw or cooked lentil diet have been also reported by Savage and Scott (1989), who, nevertheless, found a higher biological value in cooked lentils than in raw lentils even after methionine supplementation. With regard to the mineral-vitamin supplementation of raw lentil flour, the improvement in nutritive utilization of dietary carbohydrates may have contributed to the improved nutritive utilization of protein, given that protein can be then oriented to plastic rather than to energetic needs.

Miller McCurdy et al. (1978) and Davis et al. (1984) have studied the nutritive utilization of lentil protein using an experimental model based on the protozoan *Tetrahymena pyriformis*. This protozoan requires the same amino acids essential for weanling rats and can degrade intact proteins; the growth period requires only 4 days and maintenance costs are relatively inexpensive. The growth responses of *Tetrahymena* to lentil were significantly lower than those obtained from casein or yellow pea (Miller McCurdy et al., 1978; Davis et al., 1984). The albumin fraction of lentils was of superior quality to promote *Tetrahymena* growth than the whole lentil seed protein, whereas the globulin fraction was similar or slightly inferior. Methionine was the first limiting amino acid in lentils and growth responses of *Tetrahymena* improved significantly in response to supplementation of this amino acid to lentils. Nevertheless, even when lentil powders were supplemented with essential amino acids to simulate casein, they supported less than half as much growth of *Tetrahymena* than the intact casein standard. Lentil protein concentrate had a superior nutritional value when compared to the unprocessed legume flour and this nutritional value was improved by the individual supplementation of Met or Cys, but not by the combination of Met + Cys or Met + Cys + Lys.

Combe et al. (2004) have studied the differential effect of lentil feeding on proteosynthesis rate in different tissues when compared to a casein control diet. According to these authors, feeding of cooked lentils induced a partitioning of the flux for protein synthesis, preferentially to the benefit of intestinal tissues at the expense of liver and muscle tissue. The reduced amino acid flux to the liver in lentil-fed animals was not related to a change in real digestibility and could be attributed to a higher intestinal amino acid uptake. Thus, there would be an important effect of the alimentary tract on amino acid availability for the support of other physiologic processes like growth. The trophic effect of lentils in small and large intestine can be produced by products derived from gut fermentation of complex carbohydrates or proteins that are not digested in the small intestine.

4.2.2.1. *Effect of technological treatments on the metabolic utilization of lentil protein*

Thermal processing.- The dry heat or thermal treatment in autoclave of lentils did not improve the metabolic utilization of protein assessed by the percentage of retained to absorbed nitrogen or the Protein Efficiency Ratio when compared to the raw lentil (Urbano et al., 1995; Porres et al., 2003). Furthermore, Hernández-Infante et al. (1998) found a significant reduction in PER as a result of traditional cooking of lentils, although they did not find any significant effect caused by autoclave cooking. In contrast, Manan et al. (1987) have reported a significant improvement in the NET Protein Utilization (NPU) and Biological Value of lentils after soaking in water at room temperature for 4 hours and cooking for 40 minutes followed by drying at 105 °C. Finally, Miller McCurdy et al. (1978) have reported that lentils cooked for the shortest period of time retained a higher nutritional value of protein assessed by the *Tetrahymena pyriformis* experimental model.

Germination.- Germination of lentils for a 6-day period caused a significant reduction in the metabolic utilization of protein assessed by the percentage of retained to absorbed nitrogen, but did not affect the Protein Efficiency Ratio (Urbano et al., 1995). Similar undesirable effects of a long germination period have been reported by other authors in soyabean and pea (Bau et al., 2000; Urbano et al., 2005a,b). It appears that germination for longer periods has a detrimental effect on the organoleptic and nutritional properties of sprouted legume seeds, whereas germination for shorter periods of 48–96 hours have been shown to significantly improve the metabolic utilization of peas (Urbano et al., 2005a,b).

4.3. **Nutritive Utilization of Minerals**

4.3.1. *Effect of Processing Conditions*

Lentils are good dietary sources of essential minerals like P, Mg, Ca, K, Zn, Fe, and Mn. Nevertheless, mineral bioavailability from legumes is usually poor as a result of their high dietary fiber content or the presence in legumes of non-nutritional components like phytic acid, oxalate, or polyphenols that may interfere with mineral absorption. Dietary fiber is capable of causing significant losses of Mg and calcium by means of a solvent drag mechanism or ionic interactions with calcium and other food components in case of magnesium, or by adsorption of calcium to cellulose and interaction with other dietary fiber components like lignin (Hardwick et al., 1991; Luccia and Kunkel, 2002). Massey et al. (2001) have found appreciable quantities of oxalate in cooked lentils (1.18 mg/g or 100 mg/serving). Even though these values are lower than those reported for the soyabean and some of its products, the common bean or peanut butter, they are still over the recommendations made for patients with kidney stone (10 mg per serving or 50–60 mg/day).

Lentils have a substantial proportion of phosphorus in the form of phytic acid and low contents of free inorganic phosphorus (El-Mahdy et al., 1985; Porres et al., 2004). Phytic acid phosphorus has been described as being poorly available

for monogastrics (Reddy et al., 1982). Furthermore, at the basic pH found in the small intestine of monogastrics, phytic acid may form an insoluble complex with divalent and trivalent cations, which renders them unavailable for absorption (Cheryan, 1980). Nevertheless, when low levels of Ca are present in the diet and the dietary source of P is mainly phytate, it has been shown that part of phytate-phosphorus from lentils and faba beans was available for absorption by growing rats in an attempt to maximize the absorption of this mineral and thus meet the nutritional requirements of the animal. The degree of phytic acid degradation can be modulated by endogenous phytase present in the seed (although phytase activity is usually negligible in non-germinated pulses), by the presence in the brush border membrane of a phosphatase with phytase activity, or by the ability of phytase to absorb the phytic acid molecule. In addition, phytic acid that escapes degradation in the small intestine will be efficiently hydrolyzed by the microbiota present in the large intestine of the animals where the absorption of phosphorus could take a especial relevance under conditions of decreased dietary P supply or unpaired absorption in the small intestine.

Calcium digestibility from lentils has been assessed by an *in vitro* methodology by Sahuquillo et al. (2003). These authors found that digestibility (46.6%) was higher in lentils when compared to other legumes like the bean or the chickpea. Using an *in vivo* experimental model, the digestive utilization of calcium assessed by the Apparent Digestibility Coefficient ranged between 54.3–59.8% (Urbano et al., 1999; Porres et al., 2003). These values are lower than the digestive utilization of calcium from a casein-methionine control diet with similar protein content tested by Urbano et al. (1999). A finding that may be attributed to dietary factors like the lower protein quality of lentil protein, the presence of low levels of vitamin D, or the influence of dietary fiber and non-nutritional components like phytic acid or oxalate.

Due to the low amount of calcium provided by lentil diets, experimental animals made an extremely efficient metabolic use of this mineral, which was reflected in the high percentages of retained to absorbed calcium found in the animals that ingested raw or processed lentils. The low amount of calcium provided by lentils caused a reduction in the amount of this mineral accumulated in the *Longissimus dorsi* muscle, but not in the femur, probably due to the short experimental period of 10 days that was used.

Dahl et al. (1995) found no significant effect of dietary lentils on the final calcium balance of nine human volunteers, although they did report a reduction in the urinary calcium and sodium excretion and increments in the amount of potassium excreted. Homeostasis of P is closely associated with that of calcium. Therefore, in view of the low levels of calcium provided by lentil diets, metabolic utilization of phosphorus was low in spite of a reasonable net absorption, and a large amount of this mineral was excreted in the urine by the experimental animals (Urbano et al., 1999; Porres et al., 2003, 2004).

Digestive utilization of Mg from lentils (45.9%, Porres et al., 2004) was inferior to a casein-methionine control diet (Urbano et al., 2006), possibly as a consequence of the low vitamin D content of lentils or the high amount of phytic acid and

magnesium provided by lentil diets. Nevertheless, net Mg absorption was still sufficient to meet the nutrient requirements of experimental animals. Metabolic utilization of magnesium from lentils was low due to the high intake and net absorption of this mineral, although it was higher than what has been reported for other legumes like the chickpea or the common bean by Nestares et al. (1997, 2003) who obtained null balances of this mineral.

Regarding the digestive utilization of zinc, Porres et al. (1996) have reported null absorption values of this mineral from lentils using the growing rat as experimental model, and associated this low absorption to the phytic acid content of lentil flour studied. Due to the low absorption of zinc from lentils by the experimental animals, the balances of this mineral in growing rats that ingested lentil diets were very reduced or null in spite of a low urinary excretion (Porres et al., 1996). In contrast, Sebastián et al. (2001) found a considerable amount of dialyzable zinc in lentils and other raw legumes like chickpeas and common beans. Similar results have been reported by Sahuquillo et al. (2003) using an *in vitro* model based on the amount of soluble mineral after simulated gastric and intestinal digestions, and by Lombardi-Boccia et al. (2003), who described a percentage of dialyzable zinc close to 25% in raw lentil flour, similar to chickpeas and beans and higher than lupins. Zinc dialyzability decreased slightly in isolated globulins when compared to the raw lentil flour.

Iron bioavailability from legumes, and specifically lentils, determined in humans by an extrinsic tag methodology was found to be low (Gillooly et al., 1983; Lynch et al., 1984). These low bioavailability values are in agreement with the low *in vitro* availability of this mineral from lentils reported by Hazell and Johnson (1987), Sebastián et al. (2001), Sahuquillo et al. (2003), and Lombardi-Boccia et al. (1991, 2003), although the latter authors found a significant improvement in Fe dialyzability after isolating the globulin protein fraction of lentils (up to 10% dialyzable Fe), which was higher than Fe dialyzability from globulins isolated from other legumes like lupin, common bean or chickpea. The low iron absorption found using *in vitro* or *in vivo* models can be attributed to several factors e.g. 1) the presence of seed components that act as inhibitors of iron absorption (inositol phosphates, tannins, oxalate or structural components), 2) the different Fe storage in lentil and/or differing behaviour of lentil ferritin with regard to iron absorption, compared to iron associated with soybean ferritin which is reasonably absorbed (Davila-Hicks et al., 2004), 3) the low levels of seed components with an enhancing effect on iron absorption, such as organic acids (Teucher et al., 2004) and sulfur-containing amino acids (Layrisse et al., 1984), and 4) the possible endogenous iron losses in the gastrointestinal tract that would be increased by lentil flour experimental diets.

4.3.1.1. *Effect of processing on mineral availability*

Mineral and vitamin supplementation.- Supplementation of raw lentil flour with a mineral-vitamin premix gave rise to a significant increase in the nutritive utilization of calcium, magnesium and zinc that matched a significant improvement of different

parameters related to growth of the animals and nutritive utilization of the lentil meal. Nevertheless, due to the greater amount of these minerals provided by the mineral-supplemented when compared to the unsupplemented lentil diet, a reduction was observed in the digestive utilization of total phosphorus and the percentage of phytate-phosphorus absorbed by the animals (Porres et al., 1996, 2003, 2004).

Thermal treatment.- Dry heating of lentils led to a significant increase in the digestive utilization of Ca and P by growing rats (Urbano et al., 1999). A finding that the authors related to the reduction in the levels of phytate and hemicellulose present in heated lentils. In contrast, no significant effect on Ca, P or Mg digestibility by growing rats was found in response to autoclaving of lentils seeds when compared to the raw lentil flour (Porres et al., 2003, 2004). Traditional or autoclave cooking of lentils resulted in a significant reduction in calcium and zinc dialyzability, but no changes in Fe dialyzability when compared to raw lentil (Sebastiá et al., 2001). Nevertheless, dialyzability of the three minerals studied was significantly improved in a commercial ready-to-eat lentil product as a result of the addition of preserving agents like EDTA or citric acid to the commercial product that are well known enhancers of mineral solubility and dialyzability (Porres et al., 2001; Yeung et al., 2002). Similar results have been reported by Viadel et al. (2006) using a Caco-2 cell line *in vitro* model. Quinteros et al. (2001) have studied the effect of the same technological processes described by Sebastiá et al. (2001) on the amount of soluble Fe from lentils and its speciation, reporting a decrease in the content of total and soluble Fe as a result of traditional or autoclave cooking process, whereas an increase in the amount of soluble Fe was found in the ready-to-eat commercial lentil product. In all the legume samples studied by these authors, the amount of soluble Fe (III) present in legume seeds was significantly higher than the amount of soluble Fe (II) with a potentially higher bioavailability.

Lombardi-Boccia et al. (1991) have reported a considerable reduction in Fe dialyzability as a result of the extrusion process, and a considerable improvement in the dialyzability of this mineral after enzymatic dephytinization of lentils. Similar results have been reported in pea by Urbano et al. (2006), who found a significant improvement in iron absorption after treatment of the pea flour with exogenous microbial phytase which led to inositol hexaphosphate levels that were below those reported by Sandberg and Svanberg (1991) to be detrimental for Fe availability (above $0.5 \mu\text{mol/g}$), and in lupins or whole wheat bread by Porres et al. (2001, 2005), who observed a higher dialyzability of Fe and other minerals like P, Zn or Mn after phytase enzyme treatment. On the other hand, Hazell and Johnson (1987) have observed a significant decrease in the percentage of diffusible ^{59}Fe after addition of phytic acid to different plant foods.

Germination.- Germination of *Lens culinaris* for a period of 6 days led to a significant increase in the digestive utilization of Ca, P, and Zn by growing rats assessed by the Apparent Digestibility Coefficient (Porres et al., 1996; Urbano et al., 1999).

This improvement in mineral availability can be attributed to the reduction in the levels of non-nutritional components like phytic acid and tannins that are able to interfere with the availability of the above mentioned cations, to changes in the content of the different dietary fiber fractions (Vidal-Valverde and Frias, 1992; Urbano et al., 1999; Urbano et al., 2006), and to the synthesis of different ligands like free amino acids or organic acids with an enhancing effect on mineral absorption (Sripriya et al., 1997).

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