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EFFECT OF SOAKING, HEATING AND AUTOCLAVING ON TANNIN AND PROTEIN CONTENTS OF CHICKPEA

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A study pertaining to the effect of various physico-chemical treatments such as soaking, heating and autoclaving on reduction in antinutritional factors such as tannins and trypsin inhibitors in chickpea grains and improvement *in vitro* protein digestibility was undertaken in 1997. The proximate composition of chickpea grain was: moisture 9.30%, crude protein 22.93%, crude fat 3.20%, crude fibre 5.40 and ash 3.30%. Average content of nitrogen free extract was 65.17%. In general, increasing soaking time from 4 hours to overnight significantly reduced tannin contents. Water soaking and 1% NaHC03 removed about 50% tannins, whereas 1% NaCl removed only 39%. The results suggest that soaking is better than heating and autoclaving in reducing the antinutritional factors in chickpea grains.

Key words: antinutritional substances in chickpea, autoclaving, heating, soaking

INTRODUCTION

Legumes are widely consumed throughout the world as a source of protein and other nutrients in human and animal diet. Supplementation of cereals with high protein legumes such as chickpea is potentially one of the appropriate solutions to protein-calorie malnutrition, especially in developing countries like Pakistan. Chickpea on average contains 21% protein and 61% carbohydrates (Gupta, 1988). It is used in cooked or boiled form, whole grain or in split form. However, its usefulness is limited by several factors including low protein digestibility and higher contents of ingredients that cause, flatulence. According to Liener and Kakade (1980), numerous toxic constituents are found in raw legumes and many of them may be destroyed by adequate soaking treatment.

Chickpea seeds, like many legumes, also contain a variety of chemical substances that cause digestive problems in human beings. Thus its nutritive value depends on its chemical composition, bioavailability of several essential nutrients and concentration of toxic and antinutritional substances occurring in food. Trypsin inhibitors (T1) are considered to interfere with protein digestion in biological systems (Liener and Kakade, 1980). High levels of trypsin inhibitory activity (TIA) cause pancreatic hypertrophy and growth inhibition. Sattar *et al.* (1990) reported significant loss (30.7%) of T1 with 12 hours. soaking and various radiation treatments. This paper reports the effect of soaking, heating and autoclaving on T1 and protein contents of chickpea.

MATERIALS AND METHODS

The study was conducted at the University of Agriculture, Faisalabad during 1997. The seeds of chickpea Cv. C-44 were obtained from the Directorate of Pulses, Ayub Agricultural Research Institute; Faisalabad. The cleaned seeds were soaked in tap water, sodium chloride (NaC1), sodium EDTA, sodium bicarbonate (NaHC03) solutions for 4 and 8 hours and overnight. After soaking, the water was drained off and the seeds were dried in an oven at 70°C to a constant weight. The individual samples were finely ground and passed through 100 mesh screen. As to the effect of heating, soaked and unsoaked samples were heated in an oven at 100°C for 10 hours and then ground finely to pass through 100 mesh screen. Pure seeds of chickpea were autoclaved at 121°C at 15 lb. pressure for 15, 20, 25 and 30 minutes. The autoclaved samples were finely ground. All the chemical analyses including proximate analysis of chickpea, T1 and protein digestibility were carried out in accordance with AOAC (1990).

RESULTS AND DISCUSSION

The proximate components of chickpea Cv. C-44 are shown in Table 1. These values are generally in accordance with those reported by Sattar *et al.* (1990) for CM-72.

Effect- of Soaking on ,Tannin Contents: Significant changes ($P < 0.05$) in tannin contents as affected by soaking duration and soaking solutions were observed (Table 2). Increased duration of

Table 1. Proximate composition of chickpea Cv. C-44

Moisture (%)	On dry matter basis (%)				
	Crude protein	Crude fat	Crude fibre	Ash	NFE
9.30	22.93	3.20	5.40	3.30	65.17

Each value is a mean of four samples.

Table 2. Effect of different soaking times and soaking solutions on tannin content (g/lOOg)in chickpea grains

Soaking solutions	Soaking time			Mean
	4 hours	8 hours	Overnight	
Control	1.32 a	1.32 a	1.32 a	1.32 a
NaCl (1%)	0.83 c	0.18 d	0.17 d	0.39 b
Sod. EDTA (1%)	0.94 be	0.24 d	0.17 d	0.45 b
NaHCOa (1%)	1.23 a	0.16 d	0.17 ab	0.52 b
Water	0.18 d	0.15 d	0.19 d	0.17 b
Mean	0.90	0.41	0.40	

Any two means not sharing a letter in common differ at 5% probability level.

Table 3. Effect of different soaking times and solutions on trypsin inhibitory activity in chickpea

Soaking solutions	Soaking time			Mean
	4 hours (TIU/ml)	8 hours (TIU/ml)	Overnight (TIU/ml)	
Control	152.63(4.02) "'	152.63(4.02) "'	152.63(4.02) *	152.63a(4.02)
NaCl (1%)	94.82(2.50)	98.05(2.58)	83.53(2.20)	92.13b(2.24)
Sod. EDTA (1%)	81,37(2.14)	93.21(2.46)	80.47(2.12)	85.01b(2.24)
NaHCOa (1%)	81,42(2.14)	65.20(1.98)	62.51(1.65)	69.71c(1.92)
Water	103.33(2.72)	95.22(2.51)	82.43(2.20)	93.66b(2.47)
Mean	102.71a(2.28)	100.86a(2.38)	92.31b(2.38)	

Any two means not sharing a letter in common differ at 5% probability level.
Values in parentheses indicate TIA (mg/g).

Table 4. Effect of different soaking times and solutions on *in vitro* protein digestibility of chickpea

Soaking solutions	Soaking time			Mean
	4 hours	8 hours	Overnight	
Control	98.59	98.59	98.59	98.59 NS
NaCl (1%)	78.30	78.06	80.92	79.09
Sod. EDTA (1%)	80.68	79.96	85.28	81.97
HaHC03 (1%)	79.24	81.87	87.37	82.83
Water	78.45	79.96	80.68	79.70
Mean	79.17 ±(1.09)	79.96 ±(1.56)	83.59 ±(3.30)	

Table 5. Effect of heating on tannins, trypsin inhibitors and *in vitro* protein digestibility of chickpea

Treatments	Tannins (g/100g)	Trypsin inhibitors		*In vitro* protein digestibility (%)
		TIU (J:IIP)	TIA (mg/g)	
Control	1.32 NS'	152.63 NS	4.02 NS	98.59
Heating without soaking	0.90	83.76	2.20	77.34
Heating with soaking	0.85	72.77	1,91	80,44
Mean	1,01 ± 0.38	93.28 ±59.96	2,46 ±1,58	81,35

Table 6. Effect of different time intervals of autoclaving on tannins, trypsin inhibitors and *in vitro* protein digestibility in chickpea

Treatments	Tannins (g/100g)	Trypsin inhibitors		*In vitro* protein digestibility (%)
		TIU (mP)	TIA(mg/g)	
Control	1.32 a	152.63 NS	4.02 NS	98.59
15 minutes	1.26 a	85.73	2.25	79,45
20 minutes	1.22 a	83.90	2.21	79.70
25 minutes	1.09 a	71:09	1,87	83.05
30 minutes	0.54 b	63:48	1,67	85.69
Mean	1,09 ±0.44	91.37 ±57.79	2,40 ±1,52	

Any two means not sharing a letter in common differ at 5% probability level:

and sodium EDTA removed about 70% and 66% tannin contents, respectively, which were statistically at par with each other. Soaking in 1% NaHC03 solution could not give satisfactory results and could remove only 36% tannins. Soaking in water versus all other treatments proved significantly better and removed 87% tannins. Overall, the mean tannin contents with 4 hours, 8 hours and overnight soaking of chickpea grains were 0.90, 0,41 and 0,40 g/100g, respectively (Table 2). The data in Table 2 also showed that soaking in different salt solutions had a significant effect on removal of tannins from the chickpea grains. Overnight soaking in water, 1% NaC1 and Sod. EDTA (1%) on average removed about 50% tannins, whereas 1% NaHC03 removed only 39%. These results corroborate the findings of Zia-ur-Rehman and Shah (1996) who reported 25- 50% removal of tannins from various legumes by overnight soaking in different solutions.

Effect of Soaking on Trypsin Inhibitors: Overnight soaking significantly decreased the T1 activity from the mean value of 102.71 T1U mP at 4 hours to 92.31 TIU mP (10.13% decrease) (Table 3). Soaking overnight in 1% NaHC03 also significantly reduced the trypsin inhibitory activity to 69.71 TIU ml-! as compared to soaking in 1% NaCl (92.31) or in 1% sodium EDTA (85.01) and water (93.66). The reduction in mean TIU was maximum with 1% NaHC03. A similar pattern of loss in trypsin inhibitory activity (TIA) was also observed as a result of heating (Table 5). These results are similar to those of Al-Bakir *et al.* (1982) and Sattar *et al.* (1990), who also reported a reduction in TIA in chickpeas, lentils and mung bean after soaking for 24 hours. Pawar and lngle (1987) also reported a significant decrease in TIA after soaking in NaHC03 solution.

Effect of Soaking on In *Vitro* Protein Digestibility: Results of *in vitro* digestibility indicated that neither soaking duration nor soaking solution had any significant effect on *in vitro* protein digestibility of chickpea (Table 4). However, when individual mean of each treatment was compared against standard (control) casein values by t-test, apparent increase in protein digestibility was noted in all the treatments. *In vitro* protein digestibility varied from 79.17 to 83.56% against the control value of 98.59%. These values fall within the range of 62 to 88% *in vitro* protein digestibility values reported by Khokhar and Chauhan (1986).

Effect of Heating on Tannin Contents, Trypsin Inhibitors and In *vitro* Protein Digestibility: Data showing effect of heating on different antinutritional factors are presented in Table 5. The tannin contents as influenced by various experimental treatments varied from 0.85 to 1.32 g/100g, with an average value of 1.01 ± 59.96 ml! and 2.46 ± 1,58 mg/g, respectively. *In vitro* protein digestibility exhibited the same pattern, the mean value being 81,35% (Table 5). These results agree with those of Rossi *et al.* (1984) and Sattar *et al.* (1990).

Effect of Autoclaving on Tannins, Trypsin Inhibitors and In *vitro* Protein Digestibility: Tannin content reduced from 1.32 in control to 0.54 g/100 g when autoclaved for 30 minutes, whereas autoclaving for 15, 20 or 25 minutes did not cause significant reduction in tannin content in chickpea grain. Almost similar effect was observed concerning different autocalving times on trypsin inhibitors (Table 6).

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