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Interaction of oxalic acid with divalent metals—calcium, magnesium and zinc—during the cooking of cowpea (*Vigna unguiculata*)

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Two varieties of cowpea (*Vigna unguiculata*)—'Ife brown' and 'Kano white'—were examined for their oxalate contents. At least 80% of the oxalate, 35% of the calcium and 64% of the magnesium were found in the soluble form. Zinc was, however, not detected in the soluble form. The 'Ife brown' variety showed higher levels of the oxalate and minerals. Heating the cowpeas in water showed increases in both the insoluble oxalate and the soluble mineral levels, the changes being noticeable after only 20 min of heating at 140°C. Cooking the seeds in salt solutions led to increases in the levels of insoluble oxalate, implying in-seed interaction between the oxalate and the metal ions. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Cowpeas are an important source of vegetable proteins and also an important part of the diets of most Nigerians (Oyenuga, 1968). In the western part of Nigeria, cowpeas contribute up to 80% of the total dietary protein intake (Luse & Okwuraiwe, 1975). The dry seeds are usually boiled until soft, mixed with some condiments and then consumed as porridge. In another preparation, the seeds are soaked in water and the seed coats removed. The softened seeds are then ground into a paste, portions of which are either fried in oil to form a cake ('akara') or boiled in containers (leaves or small tins), after the addition of some condiments, and eaten as 'moinmoin'.

Cowpeas have been found to be a good source of calcium, magnesium and zinc (Etakakpan *et al.*, 1983; Oke, 1967), and also to contain some oxalic acid (Etakakpan *et al.*, 1983). Where it occurs, oxalic acid has been implicated in the non-availability of calcium, magnesium, iron and some trace metals on ingestion of the plant products (Hodgkinson, 1981). The levels of these minerals in some varieties of cowpeas have been found to be affected by cooking (Longe, 1983), as have the oxalate levels in some leafy vegetables (Pingle & Ramasastrio, 1978). The pattern of interaction between oxalic acid and some of these metals in aqueous solution has been studied (Faboya, 1990). In the present work, the in-seed interaction of oxalic acid with calcium, magnesium and zinc during the cooking of the cowpea seeds is examined.

MATERIALS AND METHODS

Sampling and sample preparation

The two varieties of cowpea employed in this work—'Ife brown' and 'Kano white'—were supplied by the National Cereals Research Institute, Moor Plantation, Ibadan, Nigeria.

About 500 g of hand-picked healthy seeds were ground into a fine powder using a micro-harmer miller and transferred into a clean air-tight polythene bag. Another 500 g of healthy seeds were placed in another air-tight polythene bag. Both bags were kept in a desiccator at room temperature until analysed.

Sample treatment

Raw samples

Five grammes of the powdered cowpeas were accurately weighed into a 100-ml glass-stoppered conical flask. Thirty millilitres of distilled water were added, and the flask and the contents were shaken mechanically for 15 min. Ten grammes of ammonium sulphate were added to precipitate heat-coagulable proteins (Hodgkinson & Zarembski, 1961), shaken for another 15 min and then set aside for 30 min at room temperature. The contents of the flask were carefully transferred into a centrifuge tube, centrifuged at 2000 rpm for 5 min, and the supernatant liquid decanted and filtered through a medium-porosity sintered funnel into a 100-ml volumetric flask. The residue was further stirred with three 10-ml portions of distilled water, centrifuged and the supernatant filtered into the volumetric flask. The pooled filtrate was made up to the mark with more distilled water.

Aliquots of this solution were used for the determination of water-soluble oxalate and the mineral determinations were carried out on the residue in the centrifuge tube.

Cooked samples, using distilled water
A number of healthy seeds weighing about 5 g were transferred into a 100-ml beaker and accurately weighed. Fifty millilitres of distilled water were added to the beaker, covered with a watchglass and heated on a hot-plate set at 140°C for the required length of time. The beaker was cooled to room temperature and the contents homogenized. The homogenate was allowed to stand for 30 min, and then centrifuged at 2000 rpm for 5 min. The supernatant was decanted and filtered through a medium-porosity sintered funnel into a 100-ml volumetric flask. The residue was further stirred with three 10-ml portions of distilled water, centrifuged and the supernatant filtered into the volumetric flask. The pooled filtrate was made up to the mark with more distilled water. The solution was used for the determination of the water-soluble parameters while the residue was used for the water-insoluble ones.

Cooked samples, using salt solutions
Fifty millilitres of the required salt solution were used in cooking about 5 g of accurately weighed healthy seeds for the required period. The seeds were then drained and homogenized with 50 ml of distilled water and the homogenate treated as for seeds cooked in distilled water.

Oxalate determination

Water insoluble
Oxalate extraction and estimation were carried out on the samples by employing the modified Andrews & Viser (1951) method as earlier described by Faboya *et al.* (1983).

Water soluble
Aliquots of the water extracts were pipetted directly into the inner tube of the Clausen extractor, and the oxalate extracted and estimated as described by Faboya *et al.* (1983).

Mineral determination

Solid samples
Five grammes of the powdered cowpea sample were dry-ashed in a muffle furnace set at 550°C. The ash was extracted with 3 M HCl and the calcium, magnesium and zinc contents determined using AAS.

Liquid samples
The aqueous extracts were aspirated directly as in the AAS determination of minerals.

RESULTS AND DISCUSSION

Oxalate and mineral contents of the raw cowpeas

The varieties of cowpeas (*Vigna unguiculata*)—the 'Kano white' and 'Ife brown'—were examined raw for their oxalate and mineral contents. Table 1 shows that healthy, mature cowpeas contained oxalates, both in the soluble and insoluble forms, the soluble portion accounting for at least 80% of the total oxalate.

This result agreed with the findings by earlier workers (Hodgkinson, 1981; Vityakon & Standal, 1989) that over 5% of the oxalate in plants was in the soluble form, mainly sodium and potassium oxalate. The levels of soluble oxalate found in both varieties of cowpeas were, however, much higher than earlier reported by Etakakpan *et al.* (1983) for uninvested cowpeas. The raw samples also contained calcium and magnesium in both the soluble and insoluble forms. Zinc was, however, only found in the insoluble form. In both varieties, a greater proportion (about 60%) of the calcium was found in the insoluble form, while a greater proportion (>60%) of magnesium was in the soluble form. This

Table 1. The oxalate and mineral contents of raw cowpea (*Vigna unguiculata*)

Variety	'Ife brown' Water soluble (%)	'Ife brown' Water insoluble (%)	'Ife brown' Total (%)	'Kano white' Water soluble (%)	'Kano white' Water insoluble (%)	'Kano white' Total (%)
Oxalate	0.240 ± 0.005 (82.2)*	0.052 ± 0.003 (17.8)*	0.292 ± 0.006	0.665 ± 0.002 (88.9)*	0.083 ± 0.004 (11.1)*	0.748 ± 0.004
Calcium	0.0024 ± 0.0003 (40.6)	0.0035 ± 0.0001 (59.3)	0.0059 ± 0.0003	0.0034 ± 0.0003 (36.9)	0.0058 ± 0.0003 (63.1)	0.0092 ± 0.0004
Magnesium	0.0720 ± 0.0001 (64.4)	0.0398 ± 0.0003 (35.6)	0.1118 ± 0.0002	0.0761 ± 0.0002 (67.3)	0.0369 ± 0.0001 (32.7)	0.1130 ± 0.0002
Zinc	N.D.	0.0045 ± 0.0001	0.0045 ± 0.0001	N.D.	0.0052 ± 0.0001	0.0052 ± 0.0001

N.D., not detectable.
*Percentage of total.

Table 2. Changes in the water-insoluble oxalate and the water-soluble minerals of cowpeas during cooking in water

	Cooking time (min)	'Ife brown'	'Kano white'
Insoluble oxalate (%)	20	0.057 ± 0.003 (9.6)*	0.085 ± 0.006 (2.4)*
	40	0.061 ± 0.002 (17.3)	0.091 ± 0.003 (9.6)
	60	0.076 ± 0.006 (46.2)	0.102 ± 0.003 (22.9)
Soluble magnesium (%)	20	0.074 ± 0.004 (2.8)	0.078 ± 0.0001 (2.6)
	40	0.085 ± 0.002 (17.8)	0.089 ± 0.0002 (17.1)
	60	0.084 ± 0.003 (16.5)	0.086 ± 0.0001 (13.2)
Soluble zinc (%)	20	0.0017 ± 0.0001	0.0018 ± 0.0002
	40	0.0021 ± 0.0001	0.0030 ± 0.0003
	60	0.0024 ± 0.0002	0.0035 ± 0.0003
Soluble calcium (%)	20	0.0028 ± 0.0002 (16.7)	0.0038 ± 0.0003 (11.8)
	40	0.0035 ± 0.0001 (45.8)	0.0043 ± 0.0001 (26.5)
	60	0.0044 ± 0.0003 (83.3)	0.0045 ± 0.0001 (32.4)

*Percentage increase, based on results on Table 1.

could be due to the greater solubility of magnesium salts compared with both calcium and zinc, as earlier partly demonstrated by Faboya (1990). In both varieties, however, the soluble forms of calcium and magnesium accounted for at least 35 and 64%, respectively, of their total concentrations.

The results additionally show that the oxalate and mineral levels also varied with the variety of cowpea analysed. The 'Kano white' consistently had higher results than the 'Ife brown'. These findings agreed with those of earlier workers (Kasidas & Rose, 1980), that a considerable variation in plant content could occur depending on the variety, among other things.

Effect of cooking the cowpeas in distilled water

Each variety of cowpea was cooked at 140°C for varying periods of time, between 20 and 60 min, to investigate the effect on the levels of soluble minerals and insoluble oxalates. The results are shown in Table 2.

There were general increases in the levels of soluble minerals, the increases being noticeable even only after 20 min of heating. The 'Ife brown' showed larger increases in soluble calcium than the 'Kano white'.

Both cowpea varieties were, however, comparable with respect to the changes in the levels of soluble magnesium and zinc. Although the changes did not follow any particular pattern, the increases within the first 40 min of heating were not as pronounced as those within the last 20 min. These increases in the levels of minerals, as the cowpeas were being heated, probably resulted from the softening of the cowpea seeds, leading to greater extractability of the soluble salts. These could also be partly due to the greater solubility of the salts with increasing temperature. The levels of insoluble oxalates increased with increasing periods of heating, with the 'Ife brown' showing as much as a 46% increase after 60 min of heating. The 'Kano white' generally exhibited lower increases. These increases could be due either to the additional formation of insoluble salts through the interaction of the oxalate and the divalent ions, or to greater extractability of the insoluble salts owing to softening during increased periods of heating. Similar changes in the insoluble oxalate had earlier been noticed by Pingle & Ramasastrio (1978) during the cooking of Amaranthus leaves.

Effect of cooking cowpeas in salt solution on the oxalate level

In order to check whether the increases in the levels of insoluble oxalate observed during the cooking of the cowpeas in water were due to greater extractability or increased interaction between the oxalate and the metal ions, the cowpeas were cooked in solutions containing Ca^{2+}, Mg^{2+} or Zn^{2+} ions. The results are presented on Tables 3 and 4.

Soluble oxalate

There were large decreases in the levels of soluble oxalate in both varieties as they were cooked in the salt solutions. The decreases became larger as the cooking time became longer. After 60 min of cooking in 0.01 M calcium acetate solution, decreases of about 67 and 87% had occurred in 'Ife brown' and 'Kano white' varieties, respectively. Similar degrees of decrease were obtained for cooking the cowpeas in 0.01 M magnesium acetate and 0.01 M zinc acetate solutions. Employing stronger salt solutions did not seem to significantly affect the levels of decreases in soluble oxalate as the cooking time increased. Cooking in 0.1 M calcium oxalate solution for 60 min resulted in 67 and 87% decreases in the 'Ife brown' and 'Kano white' varieties, respectively.

These decreases would be expected as a portion of the soluble oxalate would have leached into the cooking solution during the heating, leading to a direct interaction with the metal ions in the solution. This would be in addition to the in-seed interaction occurring as the cooking liquid penetrated the seeds.

Insoluble oxalate

Cooking in solutions containing either Ca^{2+} or Zn^{2+} ions resulted in increases in the insoluble oxalate level

Table 3. Changes in the oxalate contents of the 'Ife brown' variety of cowpea during cooking in salt solutions

Cooking solution	Cooking time (min)	Soluble oxalate (%)	Insoluble oxalate (%)
0.01 M Calcium acetate	20	0.207 ± 0.001 (13.8)*	0.064 ± 0.003 (23.1)**
	40	0.112 ± 0.001 (53.3)	0.074 ± 0.001 (42.3)
	60	0.080 ± 0.003 (66.7)	0.083 ± 0.002 (59.6)
0.01 M Magnesium acetate	20	0.227 ± 0.0004 (5.4)	0.040 ± 0.003 (−23.1)
	40	0.126 ± 0.001 (47.5)	0.050 ± 0.002 (3.8)
	60	0.101 ± 0.002 (57.9)	0.057 ± 0.002 (9.6)
0.01 M Zinc acetate	20	0.217 ± 0.001 (9.6)	0.059 ± 0.002 (13.5)
	40	0.019 ± 0.001 (50.4)	0.065 ± 0.003 (25.0)
	60	0.090 ± 0.003 (62.5)	0.080 ± 0.003 (53.8)
0.01 M Calcium acetate	20	0.202 ± 0.002 (15.8)	0.069 ± 0.001 (32.7)
	40	0.107 ± 0.002 (55.4)	0.078 ± 0.003 (50.0)
	60	0.080 ± 0.001 (66.7)	0.091 ± 0.003 (75.0)
0.01 M Magnesium acetate	20	0.214 ± 0.003 (10.8)	0.057 ± 0.002 (9.6)
	40	0.119 ± 0.003 (50.4)	0.064 ± 0.003 (23.1)
	60	0.090 ± 0.002 (62.5)	0.084 ± 0.005 (61.5)
0.01 M Zinc acetate	20	0.207 ± 0.001 (13.8)	0.062 ± 0.001 (19.2)
	40	0.114 ± 0.001 (52.5)	0.70 ± 0.003 (34.6)
	60	0.084 ± 0.001 (65.0)	0.090 ± 0.003 (73.1)

*Percentage decrease based on the level in the raw sample.
**Percentage increase based on the level in the raw sample.

Table 4. Changes in the oxalate contents of the 'Kano white' variety of cowpeas during cooking in salt solutions

Cooking solutions	Cooking time (min)	Soluble oxalate (%)	Insoluble oxalate (%)
0.01 M Calcium acetate	20	0.203 ± 0.002 (80.5)*	0.087 ± 0.003 (4.8)**
	40	0.111 ± 0.002 (83.3)	0.101 ± 0.001 (21.7)
	60	0.082 ± 0.001 (87.7)	0.128 ± 0.003 (54.2)
0.01 M Magnesium acetate	20	0.214 ± 0.003 (67.8)	0.069 ± 0.002 (−16.7)
	40	0.130 ± 0.001 (80.5)	0.071 ± 0.003 (−14.5)
	60	0.095 ± 0.001 (85.7)	0.099 ± 0.004 (19.3)
0.01 M Zinc acetate	20	0.206 ± 0.004 (69.0)	0.080 ± 0.001 (−3.6)
	40	0.112 ± 0.002 (83.2)	0.097 ± 0.002 (16.7)
	60	0.084 ± 0.003 (87.4)	0.122 ± 0.001 (47.6)
0.1 M Calcium acetate	20	0.196 ± 0.002 (70.5)	0.094 ± 0.006 (13.3)
	40	0.113 ± 0.003 (83.0)	0.111 ± 0.006 (33.7)
	60	0.077 ± 0.003 (88.4)	0.135 ± 0.003 (62.7)
0.1 M Magnesium acetate	20	0.205 ± 0.003 (69.2)	0.084 ± 0.001 (1.2)
	40	0.129 ± 0.003 (80.6)	0.105 ± 0.001 (26.5)
	60	0.088 ± 0.003 (86.8)	0.127 ± 0.004 (53.0)
0.1 M Zinc acetate	20	0.198 ± 0.004 (70.2)	0.088 ± 0.003 (6.0)
	40	0.112 ± 0.003 (83.2)	0.114 ± 0.001 (37.3)
	60	0.81 ± 0.004 (87.8)	0.130 ± 0.003 (56.6)

*Percentage decrease based on the level in the raw cowpea.
**Percentage increase based on the level in the raw cowpea.

with time. With both species, about a 50% increase was recorded after heating for 60 min in a 0.01 M solution of either metal ion. Cooking in the 0.1 M solutions resulted in higher increases, with the 'Ife brown' exhibiting higher values than the 'Kano white' in all cases. When compared with the results obtained from heating the cowpeas in water, the increases observed in Tables 3 and 4 were most probably the result of an increased in-seed interaction between the oxalate and the metal ions as the seeds got softer. The decreases, rather than increases, in the insoluble oxalate observed during the heating of the cowpeas in solutions containing Mg^{2+} ions were consistent with earlier observations by Faboya (1990) that Mg^{2+} partially inhibited the precipitation of calcium and zinc oxalates.

CONCLUSION

At least 80% of the oxalate, 35% of calcium and 64% of magnesium found in cowpeas were in the soluble form. Both the oxalate and the mineral levels in the seeds varied with the variety. Cooking the cowpeas in distilled water resulted in increases in the levels of insoluble oxalate and soluble minerals. The fact that larger increases in the level of insoluble oxalate resulted from cooking the seeds in solutions containing Ca^{2+} and Zn^{2+} showed that in-seed interaction took place between the oxalate and the metal ions during the cooking. This, therefore, confirms that, during normal processing of cowpeas into food, a portion of its Ca^{2+} and Zn^{2+} content is made unavailable either as the insoluble form or owing to in-seed interaction between the soluble oxalate and the metals in their soluble form. Decreases in the insoluble oxalate level were obtained during the cooking of the cowpeas in solutions containing Mg^{2+}. This further confirmed that Mg^{2+} in solution inhibited the precipitation of calcium and zinc oxalates (Faboya, 1990).

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