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Food Chemistry **51** (1994) 79–81

0308-8146/94/$7.00

α-Amylase inhibitors in cowpea (*Vigna unguiculata*): Effects of soaking and cooking methods

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(Received 27 October 1993; revised version received and accepted 11 January 1994)

The effects of certain cooking and soaking methods on cowpea (*Vigna unguiculata* (L.) Walp.) α-amylase inhibitors were studied. The following ways of cooking were investigated: boiling in distilled water, autoclaving and cooking in a microwave oven. Soaking was done in water and alkaline solutions. Cooking was found to be ineffective in reducing amylase inhibitor activity except for autoclaving. Soaking in alkaline solutions under different conditions achieved 10–47% reductions of these anti-nutrients. The most pronounced decrease of amylase inhibitor (55%) was observed when soaked seeds were boiled. A marked increase of anti-amylasic activity was observed when seeds were submitted to extensive cooking. No such increase was shown in samples cooked in a microwave oven.

INTRODUCTION

It is a well-established fact that food legumes can synthesize and store certain biologically active substances named anti-nutritional factors (i.e. digestive enzyme inhibitors, lectins, tannins, phytic acid, etc.) in their seeds. These compounds are known to reduce the availability of nutrients to animals and humans (Rackis *et al.*, 1986; Gupta, 1987). However, bcause certain anti-nutronal factors are heat-labile, they can be denatured before eating by using appropriate cooking methods (Liener, 1980). The content of heat-resistant anti-nutrients can be partially reduced by processing the seeds with appropriate procedures such as soaking, dehulling, sprouting, etc. before cooking (Nowacki, 1980).

Cowpea (*Vigna unguiculata* (L) Walp.) is a widely consumed grain legume in developing countries of West Africa. In those regions it provides a cheap source of dietary proteins for rural and urban families. Like other legumes, cowpea seeds contain several anti-nutritional factors (Bressani, 1985). Previous studies have investigated the effects of domestic methods of processing and cooking on the main cowpea anti-nutrients (trypsin inhibitors, tannins, lectins, phytic acid) (Ologhobo *et al.*, 1984*a*,*b*; Laurena *et al.*, 1986; Ogun *et al.*, 1989; Uzogara *et al.*, 1990), but little information is available about the effects of processing on cowpea α-amylase inhibitors (Shekib *et al.*, 1988). The purpose of this study was to investigate the efficacy of soaking and cooking in reducing amylase inhibitor activity.

MATERIALS AND METHODS

Materials

Dry seeds of an Italian cowpea variety (*Vigna unguiculata* (L.) Walp.) were used in the present study. Seeds were thoroughly cleaned and freed from broken seeds, dust and other foreign materials.

Porcine pancreatic α-amylase (type IA, 1260 U/mg protein at 20°C) was obtained from Sigma Chemical Company (St. Louis, MO, USA); soluble starch was from Connaught Laboratories, Ltd (Willowdale, Canada). All common chemicals were of analytical grade.

Soaking

Seeds were soaked for 3 or 6 h at room temperature in distilled water or in 1 and 5% (w/v) aqueous solutions

of $NaHCO_3$. A 1 : 10 (w/v) seed:water ratio was used. The soak water was discarded and the seeds were dehulled since coats interfere with measurements (Piergiovanni, 1992), freeze-dried and ground with a Cyclotec 1093 mill, Tecator (Hogänas, Sweden)

Cooking

Soaked and unsoaked seeds were cooked in distilled water (1 : 20 w/v) as described below:

(a) in boiling water in a beaker for 1–2 h;
(b) autoclaved at 1 atm and 120°C for 10–30 min;
(c) in a microwave oven for 10–40 min keeping the water level constant inside the beaker by adding hot distilled water.

After each cooking test, seeds were drained, dehulled, freeze-dried and ground.

AI assay

Cowpea α-amylase inhibitors were extracted in 0·9% NaCl at 4°C or 2 h. The measurements were carried out by evaluating the iodine staining power spectrophotometrically at 565 nm, on partially digested starch in the presence of α-amylase and seed extract (Piergiovanni, 1992). The inhibitor content relative to each tested sample is the mean value of three replicate analyses.

RESULTS

The results of the soaking tests are summarized in Table 1. No remarkable reduction of amylase inhibitor activity was obtained by this procedure. As seen in the table, if soaking in water was limited to a short time, only 3 h, the inhibiting activity was actually increased. Longer soaking time (6 h) did not significantly change the initial amylase inhibitor activity.

The trend observed when alkaline solutions were used was different. Among the tested conditions, the heaviest reduction (around 40%) of anti-amylasic activity was observed on seeds soaked in $NaHCO_3$ (5%). Longer soaking times and more concentrated alkaline solutions were not considered because they caused breakage and loss of coat on a large fraction of seeds.

Table 1. Effect of soaking on amylase inhibitor activity

Soaking medium	Soaking time (h)	Amylase inhibitor (U/g dry sample)[a]
Dry seeds	—	586 ± 30
Water	3	1 114 ± 56
	6	510 ± 26
$NaHCO_3$ 1%	3	473 ± 24
	6	525 ± 26
$NaHCO_3$ 5%	3	311 ± 20
	6	340 ± 22

[a] One unit of inhibitor is that quantity which reduces the activity of two units of amylase by 50%.

Table 2. Effect of cooking on amylase inhibitor activity

Cooking mode	Cooking time (min)	Amylase inhibitor (U/g dry sample)[b]
Boiling in water	60	908 ± 45
	90	887 ± 44
	120	2 010 ± 98
Autoclaving	10	340 ± 22
	20	605 ± 30
	30	1 810 ± 89
Microwave oven	10	881 ± 44
	20	841 ± 40
	40	755 ± 38
Presoaked seeds Boiled in water[a]	30 60	265 ± 18 1 905 ± 93

[a] Soaking was carried out for 6 h in $NaHCO_3$ (5%).
[b] One unit of inhibitor is that quantity which reduces the activity of two units of amylase by 50%.

Table 2 shows the changes of amylase inhibitor activity in samples subjected to different cooking methods. The inhibitor activity was measured at different times for each procedure: at optimal cooking time and after extensive cooking. Cooking unsoaked seeds in boiling water for 1 h (the optimal time under these conditions), caused a 55% rise of inhibitor activity. Prolonged cooking under the same conditions not only brought about no reduction of the inhibitors, but even produced a further increase of anti-amylasic activity. After boiling for 2 h amylase inhibitor levels were in fact found to be three times as high as when measured in raw seeds and twice as high as after optimal cooking time.

The efficacy of autoclaving unsoaked seeds proved to be scarcely effective in eliminating amylase inhibitor. A 40% reduction of the inhibiting activity was observed in samples treated for 10 min, i.e. the optimal cooking time under these conditions. As for boiling, extensive cooking in an autoclave also produced a very strong increase of anti-amylasic activity (Table 2).

A different trend was observed in unsoaked seeds cooked in a microwave oven. While this cooking mode also did not cause the amylase inhibitor level to be reduced (at optimal cooking time (10 min) a 50% increase was observed), extensive cooking under these conditions did not cause an increased activity similar to that observed with the other cooking modes considered in this study.

Finally, the soaking of seeds in alkaline solutions before boiling gave the highest reduction (55%) of amylase inhibitor activity and halved the cooking time. Like the unsoaked samples, extensive cooking of soaked seeds caused a strong increase in inhibitor activity.

DISCUSSION

West African households commonly soak cowpea seeds with the addition of some *Kanwa*—a local alka-

line rock (Uzogara *et al.*, 1988)—prior to cooking. This habit is known to help to reduce the level of some anti-nutrients in grains (Laurena *et al.*, 1986; Uzogara *et al.*, 1988). Data collected in this study show that seed-soaking in alkaline solutions is scarcely effective in reducing amylase inhibitor activity. The lowering of inhibitor level seems to be related more to salt concentration than to soaking time. In fact, the increase of $NaHCO_3$ concentration, corresponding to higher ionic strength of the soaking solutions, produced greater reductions of amylase inhibitor activity. This loss of activity could be due to solubilization of the protein inhibitor or, more probably, to the extraction of ions essential for the inhibitor activity.

Very little information is available on the nature—whether heat-resistant or heat-labile—of α-amylase inhibitor in legume seeds. Recently Singh (1988) described the amylase inhibitors of chickpea and pigeonpea as heat-labile. Irshad & Sharma (1981) reported the purification of heat-resistant amylase inhibitor from peanut. The presence of amylase inhibitors with different heat-stabilities has been demonstrated in bean seeds (Iguti & Lajolo *et al.*, 1991). The results of this investigation suggest that the nature of cowpea amylase inhibitor is heat-resistant. In fact, cooking, was found to be a useless procedure when trying to make the anti-amylasic activity disappear. An exception was cooking in an autoclave which resulted in an appreciable lowering, though not complete removal, of amylase inhibitor. The highest reduction of the inhibitor activity was obtained by coupling soaking in alkaline solutions and cooking. However, the lost amylase inhibitor activity in this case can be mainly ascribed to soaking since a very similar extent of inhibitor reduction was observed in soaked-only samples.

Further investigations would, of course, be appropriate in order to evaluate the effect of other seed-processing modes on amylase inhibitor and, consequently, to identify more effective methods of destroying such anti-nutrients in cowpea seeds.

Of great interest, from a nutritional point of view, is the strong increase in amylase inhibitor activity observed when samples are submitted to extensive cooking. The use of a microwave oven seems to be useful to avoid this undesirable enhancement of inhibiting activity. Though the data collected are still inadequate for a full understanding of the nature of such increased activity, they can nevertheless provide a basis for making some observations. α-Amylase inhibitor enhancement cannot be related solely to the exposure of seeds to high temperatures for many hours. In fact, the heating of dry seeds (overnight at 105°C) did not produce any significant change in amylase inhibitor levels. Consequently, water plays a fundamental role in the changes undergone. This topic has been extensively studied in other anti-nutrients. For example, it is well-established that water is essential to reduce trypsin inhibitor activity in cowpea seeds (Phillips *et al.*, 1983). The present data, showing an increase in the inhibitor activity, seem to suggest that water has an opposite effect on amylase inhibitor.

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