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The effects of grinding, soaking and cooking on the degradation of amygdalin of bitter apricot seeds

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More than 650 metric tonnes of bitter apricot seeds are produced in Turkey per year as a by-product from the fruit canning industry. The seeds contain the toxic cyanogenic glycoside amygdalin in amounts up to around 150 μmol/g fresh weight. The effect of grinding, soaking and cooking on the degradation of amygdalin to prunasin, benzaldehyde cyanohydrin and HCN, has been studied, as has the release of these cyanides into the soaking water. Analysis for total cyanogenic potential (TCP), cyanogenic glycosides and non-glycosidic cyanogens were thus made on a number of differently processed seed batches. The parameters were: particle size, soaking time and temperature, the presence of a natural microflora, and the duration of cooking. Great reductions were obtained for all three values measured, i.e. from the initial TCP of 85 μmol/g and down to around 2–4 μmol/g. However, none of the products obtained were considered safe for human consumption, i.e. a further microbiological detoxification must be added.

INTRODUCTION

Apricot (*Prunus armaniaca*) is the most delicious stone fruit consumed during the summer season in Turkey. It is used fresh, or processed as apricot juice, nectar, jam or dried fruit. The amount of apricot seeds remaining after processing is quite large. Thus, over 600 metric tonnes of bitter apricot seeds were exported by Turkey during 1992–1993 season (Eagen Export Association, pers. comm.). These are mainly used in the cosmetic industry with a minor fraction going to the food and condiments industry for the production of, for example, marzipan. However, depending on origin (variety, growth conditions, etc.) bitter apricot seeds contain approximately 50–150 μmol per gram of dry weight of the toxic cyanogenic glycoside amygdalin, accompanied by minor amounts of prunasin (Abd El-Aal *et al.*, 1986; Tunçel *et al.*, 1990). This character puts constraints to their wider use for human or animal nutrition, i.e. they require adequate detoxification. Thus cyanogenic glycosides and their products of hydrolysis (Fig. 1) may give rise to both acute intoxications and to chronic human CNS syndromes such as Konzo (Tylleskaer *et al.*, 1992).

Recent investigations by the authors showed that the detoxification resulting from processing involving a tempe fermentation (*Rhizopus oligosporus*) enabled a removal of around 70% of total cyanide potential. However, additional improvement of the detoxification process is required to obtain a completely safe product (Tunçel *et al.*, 1990). The present paper describes the effects of grinding (particle size) and soaking (time and temperature) on the degradation of glycosides and release of cyanides into soaking water. In addition the effect of cooking (100°C) on the removal of non-glycosidic cyanogens was evaluated.

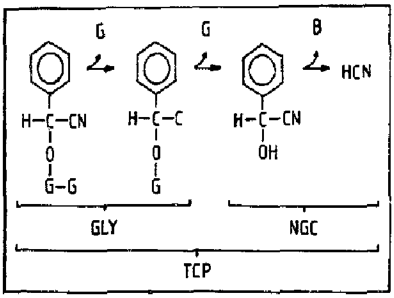

Fig. 1. The enzymatic breakdown of amygdalin in apricots (Tunçel *et al.*, 1990). TCP, total cyanogens measured as 'total cyanogenic potential'; GLY, glycosides; NGC, non-glycosidic cyanogens; G, glucose; B, benzaldehyde.

MATERIALS AND METHODS

Materials

Bitter apricot seeds were obtained from Izmir, Turkey. D-Amygdalin (A 6005) and pectinase (EC 3.2.1.15) from *Rhizopus* sp. (P 2401) — a source of hydrolytic enzymes for degradation of amygdalin standards (Brimer & Rosling, 1993) — were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of p.a. quality from Merck (Darmstadt, Germany). Picrate reagent sheets for the detection of released cyanide were prepared according to Brimer *et al.* (1983) from pre-coated ion-exchange sheets (Polygram ionex 25-SB-Ac, Machery-Nagel, Duren, Germany). Sheets were cut to size (dimensions of microtitre plate).

Seed processing

Apricot seeds were rinsed three times with tap water and ground in a Simon mill (Henry Simon Ltd, Stockport, UK). The particle size was determined using Karlb Kolb (Germany) test sieves and was grouped as follows: (a) coarse 4–5 mm, (b) medium 2–3 mm, (c) fine < 1 mm for the effect of particle size. For the influence of soaking time/temperature, particle size 2–4 mm was used. Ground seeds were treated with different soaking and cooking conditions following the experimental design outlined below.

The influence of particle size and soaking time
Ground and sieved seeds were divided into subportions of 10 g. Seeds were mixed with 30 ml of water in beakers and incubated at approx. 30°C. At each of the times (start = 0 min, 30 min, 1 h, 3 h, 6 h and 22 h) two beakers were treated as follows:

(a) the total content of the beaker was poured into a coned paperfilter (Whatman ashless 41), the outlet going into a measuring cylinder;
(b) the filtrate (volume noted) was poured into 90 ml of 0·1 M *ortho*-phosphoric acid;
(c) the filter cake was poured into 90 ml of *ortho*-phosphoric acid.

All such prepared samples and whole washed seeds were analysed for total cyanogenic potential and for non-glycosidic cyanogens, as described below.

The influence of soaking time/temperature, natural flora and cooking
Portions of seeds (10 g) were soaked in 30 ml of tap water with or without the addition of a preservative (0·2% w/v of thymol), at two different controlled temperatures (25 and 35°C) for 4 and 22 h, respectively, to investigate the influence of the natural flora during soaking. To investigate the influence of cooking on the removal of non-glycosidic cyanogens, cooking was done after various times of soaking. Beakers were heated up on a hot plate (5 min) to boiling and placed in a open water bath at 100°C for 5, 15 and 30 min. Samples were then treated as outlined above, and analysed for cyanogens.

Chemical analysis

Extraction of glycosides and degradation products
Samples (filtrates or filter cakes plus 90 ml of 0·1 M *ortho*-phosphoric acid) were homogenised in a glass blender jar (Braun Multimix MX 32 type 4207, Braun, Germany) as follows: 15 s speed 1, 1 min speed 3, 1 min rest, 1 min speed 3 (Cooke, 1978). This results in stable acidic homogenates (designated A).

Analysis
Just prior to analysis, unstable working solutions (B) were prepared from 5 ml of the stable acidic homogenates (A), adding phosphate buffer (0·1 M Na_2PO_4/H_3PO_4, pH 7) to give B with a resulting pH of 6·5 (dilution factor was noted). Analysis was done using solid state detection of released HCN (Brimer & Mølgaard, 1986), measurements for total cyanogenic potential and non-glycosidic cyanogens, respectively, being performed as described by Brimer (1994). Standard graphs were produced by hydrolysis of aliquotes of a 1 mM aqueous solution of amygdalin, a 0·2% w/v solution of pectinase being used as source of hydrolytic enzymes for both standards and samples to be analysed for total cyanogenic potential (Brimer & Rosling, 1993). Sample hydrolysis was performed in microtitre plates (Brimer *et al.*, 1993), using the following set-up (Nout *et al.*, 1995). In a microtitre plate, the following wells were prepared in duplicate: (a) *amygdalin standards*: 10, 20 and 30 μl amygdalin + 100 μl pectinase + distilled water totalling 200 μl; (b) *total cyanogenic potential* (TCP = intact glycosides + degradation products): concentrated sample — 100 μl (B) + 100 μl pectinase; diluted sample — 10 μl (B) + 90 μl distilled water + 100 μl pectinase; (c) *non-glycosidic cyanogens* (NGC = HCN + cyanohydrins): concentrated sample — 100 μl (B) + 100 μl distilled water; diluted sample — 10 μl (B) + 190 μl distilled water. Plates were covered with picrate sheets and incubated at 25°C overnight. In this way, one microtitre plate

accommodated a three-level amygdalin calibration and three different samples (Nout *et al.*, 1995).

Assessment

The density of the red-brown spots on yellow background typical of positive reactions, was assessed by measuring the absorption of transmitted light at 540 nm using a microtitre plate reader (Dynatech MR5000) (Brimer *et al.*, 1993; Brimer 1994). Using the amygdalin standards for calibration in each picrate sheet, extinctions of either dilute or concentrated sample which were closest to the standard, were used for calculations. Degradation products labelled as 'non-glycosidic cyanogens' were based upon well type (c), the difference of total cyanogenic potential (well type b) and degradation products being labelled as 'glycosides'. All were expressed as μmol/g fresh weight of seeds processed, i.e. dry weight was not determined on the different soaked qualities. The detection level was 1 μmol HCN/g.

Determination of dry weight

Seeds rinsed with tap water were weighed and dried to constant weight at 105°C.

RESULTS AND DISCUSSION

The effect of particle size and soaking time on the degradation of amygdalin and release of cyanides into soaking water is shown in Table 1. The data show a similar trend as observed earlier (Tunçel *et al.*, 1990), indicating that endogenous β-glycosidase activity causes significant degradation of amygdalin during grinding and subsequent soaking. As expected, finer particles in general result in faster degradation of glycosides. Thus, finely ground seeds contained no glycosides after 0·5 h of soaking, while soaking of medium and coarsely ground seeds left 3–5 μmol/g and 5–8 μmol/g of amygdalin respectively after 6–22 h (Table 1). In soaking water no glycosides were found, while up to around 13 μmol/ml of non-glycosidic cyanogens were accumulated from finely ground seeds. Accumulation was found slightly lower for soaking waters of medium and coarsely ground seeds (Table 1).

In Table 2, the effect of soaking time versus temperature is presented. To investigate the influence of the natural flora during soaking, soaking was further done

Table 2. The effect of soaking time and temperature on the degradation of amygdalin (μmol/g) in ground bitter apricot seed (2–4 mm particle size)[a]

Treatment, soaking	Fresh weight basis[b]		
	NGC	TCP	GLY
25°C/4 h$^+$	12·6	23·8	11·2
25°C/4 h$^-$	13·0	34·0	21·0
25°C/22 h$^+$	10·3	16·4	6·1
25°C/22 h$^-$	10·0	17·2	7·2
35°C/4 h$^+$	10·9	17·3	6·4
35°C/4 h	10·7	14·8	4·1
35°C/22 h$^+$	11·4	14·7	3·3
35°C/22 h$^-$	12·8	13·8	1·0

[a] Concentrations in soaked seeds (= filtercakes) based on the fresh weight of the seeds processed; $^+$ soaked with addition of thymol; $^-$ soaked without thymol.
[b] TCP, total cyanogenic potential; GLY, glycosides (amygdalin + prunasin); and NGC, non-glycosidic cyanogens (*cf.* Fig. 1).

Table 1. The effect of particle size and soaking time on the degradation of amygdalin and release of cyanides into soaking water (μmol/g FW)[a]

Seed treatment	NGC	TCP	GLY	Soaking water treatment	NGC[b] (=TCP)
Raw	2·1 (2·3)	83·9 (91·7)	81·8 (88·9)		
Ground fine	23·5 (27·0)	38·1 (43·8)	14·6 (16·8)		
Ground medium	8·6 (9·9)	27·6 (31·7)	19·0 (21·8)		
Ground coarse	7·8 (9·0)	21·3 (24·5)	13·5 (15·5)		
Soaked 0 h fine	10·6	19·4	8·8	0 h fine	12·9
Soaked 0 h medium	8·9	16·5	7·6	0 h medium	6·2
Soaked 0 h coarse	7·2	23·1	15·9	0 h coarse	1·3
Soaked 0·5 h fine	21·3	21·3	—[c]	0·5 h fine	11·1
Soaked 1 h fine	15·6	15·6	—	1 h fine	12·6
Soaked 1 h medium	10·9	15·1	4·2	1 h medium	7·4
Soaked 1 h coarse	10·6	18·1	7·5	1 h coarse	9·7
Soaked 3 h fine	20·6	20·6	—	3 h fine	13·3
Soaked 3 h medium	13·5	23·9	10·4	3 h medium	7·1
Soaked 3 h coarse	10·0	21·0	11·0	3 h coarse	4·8
Soaked 6 h fine	15·6	15·6	—	6 h fine	11·0
Soaked 6 h medium	14·6	18·1	3·5	6 h medium	5·0
Soaked 6 h coarse	11·4	16·4	5·0	6 h coarse	5·0
Soaked 22 h fine	14·7	14·7	—	22 h fine	7·0
Soaked 22 h medium	11·9	16·3	4·4	22 h medium	5·1
Soaked 22 h coarse	7·1	15·7	8·6	22 h coarse	4·3

[a] Concentrations in soaked seeds (= filtercakes) based on the fresh weight of the seeds processed, figures in brackets μmol/g DW.
[b] No glycosides found in soaking waters.
[c] — (= below limit of detection).
TCP, total cyanogenic potential; GLY, glycosides (amygdalin + prunasin); and NGC, non-glycosidic cyanogens (*cf.* Fig. 1).

with and without the addition of the preservative thymol. Results for 25°C correlate well with those presented in Table 1, the effect of the natural flora being insignificant at this temperature. The degradation of glycosides proved to be significantly ($P<0{\cdot}01$) more efficient at 35°C, as seen already at 4 h, an influence of the natural flora being indicated as a trend when looking at the time course of the hydrolysis. In agreement with the results from Table 1, the total cyanogenic potential decreases very slowly under the conditions used, independent of particle size, temperature and the existence of a natural microflora. Thus, the non-glycosidic cyanogens tend to be quite stable under the aqueous conditions of natural soaking, as also reported from studies on the breakdown of the cyanogenic glucoside linamarin in cassava (Mlingi *et al.*, 1993). Removal of the accumulated non-glycosic cyanogens (cyanohydrins + HCN) might possibly be accomplished by filtering followed by drying of the seed cake, as results indicate from studies on cassava. In the present study we wanted to investigate the influence of open pan cooking in the soaking medium. As seen from the results in Table 3, a great reduction of non-glycosidic cyanogens is achieved after only 5 min of cooking. Complete removal seems to be difficult to achieve even with cooking times of 30 min, however, in general, the glycosidic fraction is rather stable to even prolonged cooking (Table 3).

The results presented show, that both grinding and soaking cause a considerable reduction in the total cyanogenic potential, resulting in accumulation of non-glycosidic cyanogens in the wet seed material as well as in the soaking water. In spite of the great reductions, none of the wet seed products reach levels of total cyanogenic potential as low as those set for cassava flour (CAC, 1988) and gari from cassava (FAO, 1989), i.e. 10 and 2 mg HCN/kg (equalling 0·4 and 0·07 mmol HCN/kg), not even after cooking. For samples of medium and coarse particle size, remaining concentrations of glycosides (amygdalin + prunasin) are also well above these levels, pointing to the necessity of using microbiological processing in order to obtain acceptable food or feed products from a surplus of bitter apricot seeds.

Table 3. The effect of cooking on the degradation of amygdalin (μmol/g) in ground and soaked bitter apricot seed (2–4 mm particle size)[a]

Sample	Treatment (min of cooking)	Fresh weight basis[b]		
		NGC	TCP	GLY
Soaked 25°C/4 h'	0	12·6	23·8	11·2
	5	4·9	8·4	3·5
	15	—[c]	2·0	2·0
	30	—	—	—
Soaked 25°C/22 h'	0	10·3	16·4	6·1
	5	2·7	9·7	7·0
	15	—	7·2	6·5
	30		3·8	3·8
Soaked 25°C/22 h	0	10·0	17·2	7·2
	5	2·3	11·8	9·5
	15	1·0	8·3	7·3
	30	—	6·5	6·4
Soaked 35°C/22 h'	0	11·4	14·7	3·3
	5	3·9	7·3	3·4
	15	4·0	7·8	3·8
	30	3·0	6·6	3·6
Soaked 35°C/22 h'	0	13·0	13·8	0·8
	5	2·2	7·1	4·9
	15	1·0	7·1	6·1
	30	—	4·6	4·6

[a] Concentrations in soaked seeds (= filtercakes) based on the fresh weight of the seeds processed; ' soaked with addition of thymol; soaked without thymol.

[b] TCP, total cyanogenic potential; GLY, glycosides (amygdalin + prunasin); and NGC, non-glycosidic cyanogens (*cf.* Fig. 1).

[c] —, below limit of detection.

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