Degradation of Oat Saponins during Heat Processing—Effect of pH, Stainless Steel, and Iron at Different Temperatures

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To understand the fate of oat saponins during processing, isolated avenacosides A and B were heated at 100 and 140 °C at different pH. The catalytic effect of soluble iron complexes and stainless steel was also examined. The avenacosides were stable when heated up to 100 °C for 3 h at pH 4–7. Heating at 140 °C, especially at pH 4, led to partial destruction of the oat saponins. A degradation product was detected and identified by mass spectrometry as desrhamnoavenacosides A and B. Addition of catalytic amounts of iron and stainless steel dramatically increased the rate of saponin breakdown at pH 4–6. This could in part explain the reduction of the saponin content in canned and roller-dried products.

Keywords: *Oats; saponins; isolation; heat processing*

INTRODUCTION

The interest in using oats in foods has increased in recent years, mainly due to its serum cholesterol lowering properties. These have been related mainly to the β-glucan content (Ripsin and Keenan, 1992), but minor components such as saponins and tocotrienol could contribute (Price et al., 1987; Querishi et al., 1986).

Saponins consist of a steroid or triterpene group linked to one or more sugar residues. Some saponins give a bitter taste to foods. Another property of saponins is their membranolytic activity. They probably combine reversibly with plasma membranes and thereby increase their permeability (Price et al., 1987). This property leads to inhibition of growth and sporulation of a wide range of fungal plant pathogens. Saponins with one sugar residue (monodesmosidic) have a higher membranolytic activity than saponins with two (bisdesmosidic) sugar residues (Price et al., 1987).

Two steroid saponins, avenacosides A and B, have been isolated from oat kernel and their structures elucidated (Tschesche et al., 1969; Tschesche and Lauren, 1971). They are both bisdesmosidic and have nuatigenin as aglycon. A glucose residue is bound at position C-26 and the other sugar residue, containing two glucose and one rhamnose residues, is bound at position C-3. In avenacoside B, the last mentioned carbohydrate contains one extra glucose moiety compared with avenacoside A.

Oat leaves also contain avenacosides, and studies have shown that a specific β-glucosidase, occurring naturally in oat leaves, is able to remove the C-26 bound glucose moiety (Grünweller and Kesselmeier, 1985). The desglucoform has less bitterness but a higher membranolytic activity than the avenacosides (Tschesche and Wiemann, 1977). The 26-desglucoavenacosides could also be detected in suspensions of unheated oatmeal in water (Önning and Asp, 1993). The avenacosides are probably broken down enzymatically since no desglucoavenacosides were found in suspensions of heat-treated oatmeal.

Almost all oats for human consumption are heat treated to inactivate lipases, before being milled and used in different food products. Oats are often also heated further, for example, by drum-drying of industrially made gruels. Because of this heating, oats also get their special roasted flavor.

Processing could affect the amount of saponins in oat products, but knowledge about the extent and nature of saponin degradation is incomplete. Some process methods have been shown to reduce the saponin content. Thus, cooking of legumes reduced the amount of saponins by 7–53% (Jood et al., 1986; Kataria et al., 1988; Gahlawat and Sehgal, 1992; Sharma and Sehgal, 1992), and autoclaving of legumes gave a reduction of 40% in one study (Sharma and Sehgal, 1992). In a heated and spray-dried oatmeal the avenacoside A and B content was reduced by 30% compared with the raw material (Önning, 1991).

The problem with most of the studies made so far is that we do not know for sure if the reduction is due to heat-induced degradation or if it is caused by formation of poorly extractable saponin complexes (Price et al., 1987). To understand this problem, we have chosen to heat isolated saponin fractions, i.e. avenacosides A and B. In this way it is also possible to detect degradation products.

MATERIALS AND METHODS

Materials. Saponins were isolated from oat kernels of the variety Vital (Svalöf AB, Svalöv). The oat kernels were milled to a particle size less than 0.5 mm (Retsch, Schieritz & Haurenstein AG, Arlesheim) prior to the extraction.

Extraction of Oat Saponins. The oatmeal (1 kg) was first defatted twice with petroleum ether (3 L, bp 60–80 °C) at room temperature. The saponins were then extracted twice from the defatted meal with methanol (3 and 2 L, respectively) at room temperature. A partition of the methanol extract between water and butanol (1:1 v/v) was thereafter made to remove more polar substances. TLC of the aqueous phase showed no avenacosides. The butanol extract was evaporated, and the residue weight was 10 g.

Purification on a Silica Gel Column. The crude extract (8 g) was applied to a silica gel SI-60 column (45 × 7 cm, Merck) and eluted with chloroform/methanol/water solution, first 70:32.5:5 (107 90-mL fractions) and then 70:30:5.5 (126 90-mL fractions) (v/v, flow rate 6 mL/min). TLC detection of

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saponins (Lütz, 1980) was made on every second fraction. Fractions containing avenacosides A and B were pooled, the solvent was removed with evaporation, and the residue was dissolved in water and thereafter freeze-dried.

LPLC Purification. The yellow freeze-dried samples from the silica gel chromatography were dissolved in 20% acetonitrile and injected onto a C_{18} Nucleosil column (250 × 20 mm, 10 μm, Macherey-Nagel) via a Rheodyne injection valve. A Kontron AC instrument (Model 110 A pump, Uvikon 735 detector, WxW recorder) was used, the flow rate was 3.4 mL/min, and detection was made at 200 nm. Elution of the saponins was performed with 20% acetonitrile in water for 45 min, then with a linear gradient up to 25% acetonitrile for 60 min, and finally with a second linear gradient up to 28% for 90 min. The fractions containing the saponins (monitored with HPLC) were collected, the solvent was evaporated, and the residue was dissolved in water and thereafter freeze-dried.

Processing of Oat Saponins. *Heat Treatment up to 100 °C.* Approximately 0.2 mg of avenacoside A or B was dissolved in 600 μL of 0.005 M of the following buffer solutions: sodium citrate buffers were used at pH 4–6, and a citrate/sodium phosphate buffer was used at pH 7. Seventy-five-microliter portions of the solutions were incubated in small glass vials (100 μL, crimp-top vial, Hewlett-Packard) at 22 °C for 30 min and 1, 3, and 24 h and at 60 and 100 °C for 3 h in a water bath.

Heat Treatment at 100 and 140 °C with and without Added Metal. Buffers and saponin concentrations were as before. The solutions were portioned (50 μL) in 5–6 mm diameter glass bulbs (R and L Slaughter Ltd., Romford, Essex, England). The bulbs were sealed with a flame and thereafter heated in a thermostatically controlled oil bath (±0.2 °C, Haake D8) at 100 and 140 °C for 10, 20, and 30 min and 1, 2, and 3 h, respectively.

To test if metal has any effect, 2 mg of fine powdered stainless steel 316 L was added to each bulb. This time the bulbs were heated at 100 °C for 10 and 30 min and 1, 1.5, and 2 h and at 140 °C for 5, 10, 20, and 30 min and 1, and 2 h.

Tests were also made by adding $FeCl_3$ [iron(III) chloride hexahydrate, Merck] in different amounts to the bulbs. The saponin and buffer concentrations were doubled in these experiments, and 25 μL of the saponin solution plus 25 μL of the $FeCl_3$ solution was added to the bulb just before the heat treatment. The experiments were carried out at pH 5 and 6. At pH 5 $FeCl_3$ was added to a concentration of 0.6, 1.2, and 2.4 mM, and at pH 6 the concentration of $FeCl_3$ was 2.4 mM. The bulbs were heated at 140 °C for 10 and 30 min and 1 and 2 h.

It takes 16 s for 50 μL of solution in the glass bulbs to reach 100 °C and 36 s to reach a temperature of 140 °C. After the heat treatment, the bulbs were immediately cooled in an ice/water bath.

Controls at room temperature were run at the same time.

TLC Analysis. Ten microliters of the different fractions was spotted onto a silica gel TLC plate (60F, Merck) alongside standard spots of avenacosides A and B. The TLC plate was developed in chloroform/methanol/water (70:30:5.5 v/v) (Lütz, 1980) for a distance of 7 cm. The spots were visualized by spraying the plate first with 1% vanillin solution in ethanol/3% perchloric acid in water (1:1 v/v, Godin reagent) and second with 10% sulfuric acid in ethanol and thereafter heating at 120 °C.

HPLC Analysis. Avenacosides A and B were analyzed according to the method of Önning and Asp (1993). HPLC was performed on a Hewlett-Packard Series II 1090 liquid chromatograph fitted with a photodiode array detector. Twenty microliters of the fractions was injected onto a LiChrospher 100 RP8 column (125 × 4 mm, 5 μm, Merck). The flow rate was 2 mL/min, and UV spectroscopic data were collected from 190 to 400 nm. A linear gradient elution was performed with 25–40% acetonitrile in water during 15 min. Quantitation was done by injecting purified avenacoside A and B standards and comparing peak areas at 200 nm. The analyses were performed at least in duplicate. The coefficient of variation for the samples was 1.0% (12 injections).

MS Analysis. Fast atom bombardment (FAB) negative ion mass spectra were obtained using a Finnigan MAT 8430 mass spectrometer. Glycerol was used as matrix, and the samples were bombarded with an atom beam of xenon, with an energy of 7 keV. The source temperature was 60 °C.

Statistical Analysis. Analysis of variance (oneway) and mean separation (Duncan's multiple range test), subprograms of SPSS/PC, were used to determine the significance of differences in breakdown, with respect to pH and temperature.

RESULTS AND DISCUSSION

Isolation of Oat Saponins. The TLC solvent system separated the saponins efficiently: avenacoside A had an R_f value of 0.24, and avenacoside B had an R_f value of 0.17. This solvent system was also used in the first purification step, but the avenacosides were not fully separated. The total amount of avenacosides in the saponin-containing silica gel pools varied from 17% to 30% of the dry matter. The recoveries were 55% for avenacoside A and 60% for avenacoside B.

The two silica gel pools containing, respectively, 29% avenacoside A (total weight 310 mg) and 16% avenacoside B (total weight 440 mg) were further purified by reversed-phase chromatography. Totally, 51 mg of avenacoside A and 21 mg of avenacoside B were obtained after this step. The resulting freeze-dried samples were white, and the purity was determined to be at least 98% from TLC and HPLC analyses.

The identities of avenacosides A and B were confirmed with mass spectrometric analysis. The mass spectrum of avenacoside A showed characteristic ions at m/z 1062 (M^-) and 900 $(M - 162)^-$, and for avenacoside B, the spectrum showed ions at m/z 1224 (M^-), 1062 $(M - 162)^-$, and 900 $(M - 2 \times 162)^-$, corresponding to successive losses of glucose units.

In earlier investigations the avenacosides have been isolated from oat leaves. Tschesche et al. (1969) used silica gel and eluted the avenacosides with chloroform/methanol/water (65:70:35 v/v). Kesselmeier and Strack (1981) made first a purification with Sephadex LH-20 and used thereafter HPLC and a 250 × 9 mm column prepacked with Partisil 10 ODS.

Heat Treatment of Avenacosides. Degradation of nutrients and other food components on heating is dependent not only on time and temperature but also on other variables such as pH, chemical composition, the presence of catalytic factors, and water activity (Fennema, 1985). In the present study we have investigated the effect of time, temperature, pH, and catalytic factors on two oat saponins, avenacosides A and B.

Incubation of avenacosides A and B at pH 4–7 and at room temperature for up to 24 h gave no reduction in saponin content. There was also no change when the avenacosides were heated at 60 and 100 °C for 3 h. In earlier studies the reduction in saponin content after ordinary cooking has also been modest. Cooking of chickpea and black gram reduced the saponin content by 7–17% (Jood et al., 1986; Kataria et al., 1988). If the seeds were soaked before the heat treatment, the reduction was more extensive. Cooking of soaked faba bean seeds reduced the saponin content by 35% (Sharma and Sehgal, 1992).

Heating at 140 °C gave some reduction, especially at pH 4 (Table 1). The rate of breakdown was the same for avenacosides A and B, and after 3 h at pH 4, the reduction was about 50%. After 10 min of heating at pH 4, 4% of the avenacosides were broken down and a new peak was observed. In the HPLC separation these

Table 1. Avenacosides A and B Heated at 140 °C, pH 4 (Percent of Control Values)[a]

heating time	avenacoside A	avenacoside B	new peak A′ (aven A minus rhamnose)	new peak B′ (aven B minus rhamnose)
10 min	97	96	2	
20 min	95	94	4	3
30 min	92	90	5	4
1 h	84	84	8	8
2 h	66	68	14	13
3 h	55	52	18	13

[a] Means from duplicate determinations.

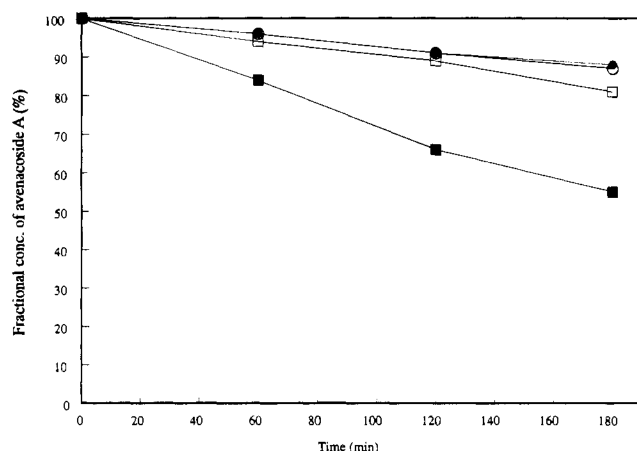

Figure 1. Effect of heat treatment at 140 °C on avenacoside A content, $n = 2$: (■) pH 4; (□) pH 5; (●) pH 6; (○) pH 7.

peaks eluted 0.7 min after avenacosides A and B, respectively.

The mass spectrum of the new peak detected when avenacoside A was heated gave a molecular ion at m/z 916, indicating that rhamnose was lost. Similarly, the breakdown product when avenacoside B was heated was found to be avenacoside B minus rhamnose.

Avenacoside A was also heated at pH 5, 6, and 7 at 140 °C and the breakdown after 3 h of heating was 19%, 12%, and 13%, respectively (Figure 1). At pH 5, desrhamnoavenacoside A could also be detected, but only after 3 h of heating. However, no new HPLC peaks appeared at pH 6 and 7, despite disappearance of the avenacoside peak.

For all pH values the reduction with time was almost linear. The regression coefficients were 0.996 for pH 4, 0.995 for pH 5, 0.994 for pH 6, and 0.999 for pH 7.

Very few studies have investigated the stability of saponins at higher temperatures. Autoclaving of soaked faba bean seeds for 15 min gave a reduction in saponin content of 40% in one study (Sharma and Sehgal, 1992).

The pH values used in these studies (4−7) are normal values found in food products. The avenacosides seem to be stable in this pH interval. Other studies have shown that saponins could be broken down to monodesmosides at low pH values (<3) (Oleszek et al., 1992).

Heat Treatment of Avenacoside A in the Presence of Stainless Steel Particles. Adding stainless steel to the glass bulbs containing avenacoside A solution had a great effect. Avenacoside A was already degraded at 100 °C (Figure 2). Heating for 10 min gave reductions of 59%, 51%, and 28% at pH 4, 5, and 6, respectively. The breakdown was significantly ($P <$ 0.05) lower at pH 7 than at the other pH tested. For example, heating for 1 h at pH 7 gave a reduction in

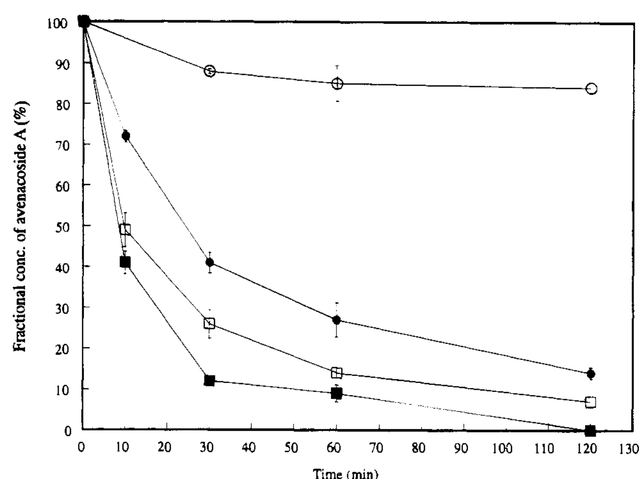

Figure 2. Effect of heat treatment (100 °C) with stainless steel particles at different pH on avenacoside A content, mean ± SD, $n = 2$–3: (■) pH 4; (□) pH 5; (●) pH 6; (○) pH 7.

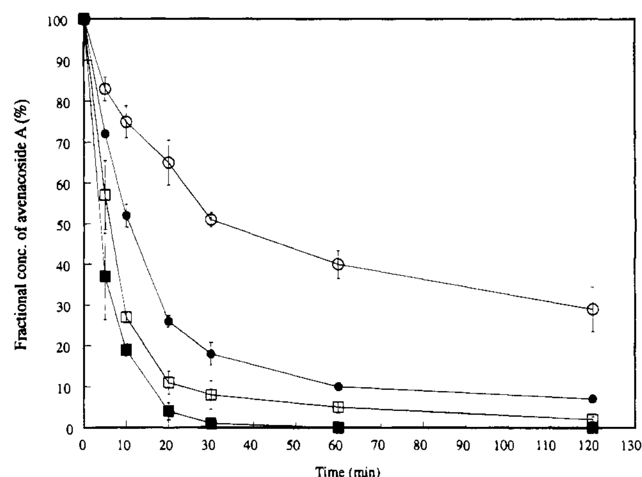

Figure 3. Effect of heat treatment (140 °C) with stainless steel particles at different pH on avenacoside A content, mean ± SD, $n = 2$–4: (■) pH 4; (□) pH 5; (●) pH 6; (○) pH 7.

avenacoside content of 15%, while heating at pH 4, 5, and 6 gave reductions of 91%, 86%, and 73%, respectively.

The breakdown was greater at 140 °C than at 100 °C (Figure 3). After 10 min of heating, the reductions were 81%, 73%, 48%, and 25% at pH 4, 5, 6, and 7, respectively. At this temperature the breakdown was also significantly lower at pH 7 ($P < 0.05$) than at pH 4, 5, and 6. In samples heated for 1 h at pH 4, 5, and 6 minimal amounts of avenacoside A could be detected (≤10% of original content), while in a sample heated at pH 7 about 40% remained.

In an earlier study different saponins (for example, diosgenin and saponins from alfalfa) were mixed with zinc and iron at room temperature (West et al., 1978). Saponins from alfalfa formed insoluble complexes with the minerals while digitonin remained in solution. In the present study, no precipitate could be seen when avenacoside A was mixed with stainless steel, and no reduction in saponin content was observed in the control samples, stored at room temperature, when stainless steel particles were added.

A plot of $\log_{10}$(concentration of avenacoside A) versus time ≤30 min generated a straight line, thus indicating first-order or pseudo-first-order kinetics for degradation of avenacoside A. The highest determined apparent first-order rate constants were observed, as expected,

Table 2. Apparent First-Order Rate Constants ($\times 10^{-3}$ s^{-1}) of Stainless Steel Catalyzed Breakdown of Avenacoside A

pH	100 °C	regression coefficient	140 °C	regression coefficient
4	1.1	−0.993	2.5	−0.999
5	0.72	−0.976	1.4	−0.972
6	0.49	−0.999	0.98	−0.993
7			0.36	−0.989

at pH 4 and increased almost twice when the temperature was raised from 100 to 140 °C (Table 2).

The rate of reaction is generally proportional to the catalyst concentration (Green, 1984). Thus, the determined apparent rate constants for the degradation of avenacoside A in the presence of stainless steel are only valid for the particular catalyst concentration used, i.e., 2 mg of steel particles in 50 μL of solution.

Despite the extensive breakdown, no new peaks appeared in the HPLC chromatograms. The same result was obtained even if the gradient was increased up to 100% acetonitrile. One explanation for this could be that the aglycon that contained the only UV-absorbing unit had been split up by the treatment. We cannot rule out a first step involving a fast absorption of the avenacosides to the steel particles, but we were unable to extract saponins or their degradation products from the stainless steel particles with chloroform/methanol (1:1).

Catalyst poisoning could be responsible for slowing down the reaction rate more than expected from the first-order rate constants when the solutions were heated for more than 30 min. A catalytic reaction could be poisoned in different ways (Hughes, 1984). If the above proposed reaction is catalyzed by solids, i.e., the reaction proceeds on the catalyst surface by means of adsorption and desorption processes, interfering substances could be adsorbed on the surface and hinder the reaction. Sensitive minerals are, for example, iron, nickel, cobalt, and copper, and examples of poisons are water, sulfur, nitrogen, phosphorus, oxygen, and CO_2.

Heat Treatment of Avenacoside A in the Presence of Iron. Most of the iron added to the solutions was bound to citrate in soluble complexes. Adding $FeCl_3$ to the glass bulbs increased the breakdown compared with samples heated with no addition (Figure 4). Heating at 140 °C at pH 5 for 10 min gave a reduction in avenacoside A content of 5−20%, depending on how much $FeCl_3$ was added. A higher rate of breakdown was seen with increasing $FeCl_3$ concentration, especially in the beginning of the heat treatment. When the highest amount of $FeCl_3$ was added (2.4 mM) and the solution was heated at pH 6 instead of pH 5, a similar reduction was seen; i.e., 62% of avenacoside A was broken down after 120 min of heating.

The experimental results indicate a first-order rate of reaction in the tested time range. The amount of variation not explained by this model was small (average $r^2 = 0.986$).

Calculated rate constants were 6.2×10^{-5}, 7.9×10^{-5}, and 1.0×10^{-4}/s when 0.6, 1.2, and 2.4 mM $FeCl_3$, respectively, were added. The rate constants were determined as the difference between the first-order rate constants in the presence and in the absence of the metal ion (Taqui Khan and Martell, 1967). The reaction rate was low compared with the rate when stainless steel was added; for example, rate constants at pH 5 were 1.0×10^{-4}/s when 2.4 mM $FeCl_3$ was added and 1.4×10^{-3}/s when 2 mg of stainless steel particles was

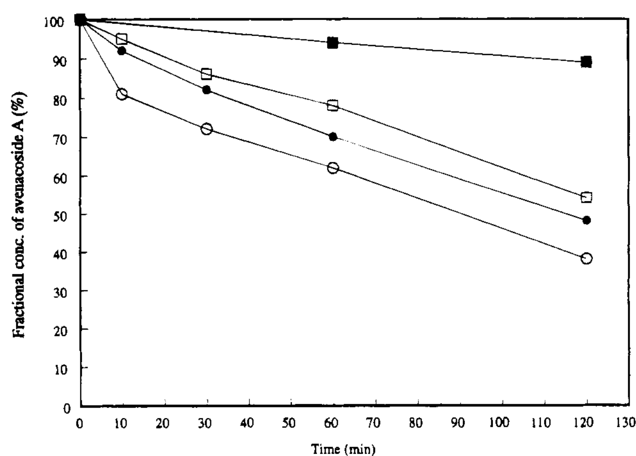

Figure 4. Effect of heat treatment (140 °C) with different concentrations of $FeCl_3$ on avenacoside A content, pH 5, n = 2: (■) 0 mM; (□) 0.3 mM; (●) 0.6 mM; (○) 1.2 mM.

added. This result was not surprising since the highest concentration of $FeCl_3$ added (2.4 mM) corresponds to 16 μg of iron, a quite low amount compared with the quantity of stainless steel. The result could also be due to differences in catalytic capacity between surface (solid metal) and solution (ions).

A linear regression of the rate constant versus $FeCl_3$ concentration gives a line with a regression coefficient of 0.908 and a y-intercept of 0.24. This indicates that iron had a catalytic effect, but the rate constant did not increase linearly with the iron concentration. A similar result was obtained in a study investigating the effect of copper on the degradation of vitamin A (Wilkinson et al., 1982).

As when stainless steel particles were added, no new peaks could be detected by HPLC, even if the gradient was increased to 100% acetonitrile. TLC analysis of the samples did not show any new spots.

Industrial food products are often in contact with stainless steel and iron, but it is difficult to predict from these studies what effect this could have on the saponins. There is probably some influence since the saponins were reduced by 60% when beans were canned in one study (Drumm et al., 1990), and in another study, steam injection (30 s at 140 °C) and thereafter roller-drying at 180 °C decreased the saponin content in sweet and bitter quinoa by 70% (Gee et al., 1993).

The most effective methods to reduce the saponin content so far have been sprouting, soaking, and abrasion. By sprouting, the losses were 37−51% in chickpea and black gram (Jood et al., 1986), 56−66% in moth bean (Khokhar and Chauhan, 1986), and 77% in faba bean (Sharma and Sehgal, 1992). Using a combination of abrasion and soaking, the amount of two quinoa saponins (A and B) was reduced by 56% and 100%, respectively, compared with the raw material (Ruales and Nair, 1993).

The physiological/nutritional importance of reducing the saponin content in foods remains an open question and further research is needed.

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