Effect of Soaking and Cooking on the Saponin Content and Composition of Chickpeas (*Cicer arietinum*) and Lentils (*Lens culinaris*)

Raquel G. Ruiz,*,† Keith R. Price,† A. Eddie Arthur,‡ Malcolm E. Rose,§ Michael J. C. Rhodes,† and Roger G. Fenwick†

Food Molecular Biochemistry Department, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom, and The Open University, Walton Hall, Milton Keynes, MK7 6AA, United Kingdom

Changes in the saponin content and composition of both chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*) were investigated after the seeds were soaked in distilled water, citric acid, and sodium bicarbonate solutions. The effect of cooking for 30, 60, 90, and 120 min after the seeds were presoaked in distilled water was also studied. Soaking did not modify the saponin content or composition of chickpeas and lentils regardless of the pH of the soaking solution. The native saponin, soyasaponin VI, was partially degraded during cooking into soyasaponin I, and both of these saponins leached into the cooking solution, 2−5% and 6−14% for chickpea and lentil, respectively. An overall loss of saponin content was found for lentil (15−31% loss), but none was observed for chickpea.

Keywords: *Saponins; soaking; cooking; Cicer arietinum; Lens culinaris*

INTRODUCTION

Both chickpeas and lentils are leguminous crops that are used in various forms, mainly for human consumption, and that constitute excellent sources of protein, carbohydrates, fiber, minerals, and other nutrients. However, several antinutritional factors are found in biologically significant amounts in the raw seed (Chavan et al., 1986; Bhatty, 1988).

Saponins, which derive their name from their ability to form stable, soaplike foams in aqueous solutions, constitute a complex and chemically diverse group of compounds. In chemical terms, saponins contain a carbohydrate moiety attached to a triterpenoid or steroid aglycon. Among the naturally occurring compounds of grain legumes, saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial (Price et al., 1987; Fenwick et al., 1991). Fungitoxic (Gestetner et al., 1971), hemolytic (Khalil and El-Adawy, 1994), and membranolytic (Oleszek et al., 1994) activities have been ascribed to saponins. Conversely, a beneficial lowering of plasma cholesterol levels in humans has also been attributed to saponins (Sidhu and Oakenfull, 1986), while some have been reported to exhibit anticancer activity (Nishino et al., 1986; Konoshima et al., 1992) and an inhibitory effect on the infectivity of HIV *in vitro* (Nakashima et al., 1989). However, all of these behavioral properties are related to certain saponin structures rather than to all members of this family.

Group B saponins (Shiraiwa et al., 1991) had been thought to be monodesmoside saponins that contain only a sugar chain attached to the C-3 position of soyasapogenol B, but recently it has been found that they also contain a 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) moiety at the C-22 (Kudou et al., 1992). Soyasaponin I (Figure 1) (Price and Fenwick, 1984), also called soyasaponin Bb, belongs to the group B saponins. Soyasaponin VI (Figure 1) (Massiot et al., 1992), also known as soyasaponin βg or BeA (Kudou et al., 1992, 1993), contains the DDMP group at the C-22 position of soyasaponin I and might be the natural precursor of soyasaponin I. Yoshiki et al. (1994) found that group B saponins are widely distributed in legumes as DDMP-conjugated forms. DDMP saponins may have an important physiological activity in preventing lipid peroxidation or degeneration of DNA and proteins by free radical attack since Yoshiki and Okubo (1995) have reported an active oxygen scavenging activity of these saponins in soybean seed.

Legumes normally used in human nutrition need to be processed prior to consumption to reduce their levels of antinutritional factors. The traditional domestic methods of legume processing include milling, soaking, germination, fermentation, and cooking. Soaking of food legumes usually forms an integral part of bean processing methods such as cooking and germination. Cooking makes the beans edible by making them tender and also aids in flavor development. The degree of elimination of toxic constituents by soaking and cooking depends on several factors such as duration, type of solution, compound stability, and removal of soaking solution (Salunkhe and Kadam, 1989). The effects of soaking and cooking on saponins are poorly understood, and there is clearly a need to examine in detail the effects on both saponin composition and content of such processing since recent work has demonstrated a relationship between chemical structure and biological activity (Oleszek et al., 1994; Price et al., 1996).

Ruiz et al. (1995) developed a method for the quantitative determination of intact saponins in *Lupinus angustifolius* seed by high-performance liquid chromatography. This method has now been used to study the effect of soaking using different types of solution and cooking for different lengths of time on the saponin

* Author to whom correspondence should be addressed [fax 44(0)1603507723].
† Institute of Food Research.
‡ John Innes Centre.
§ The Open University.

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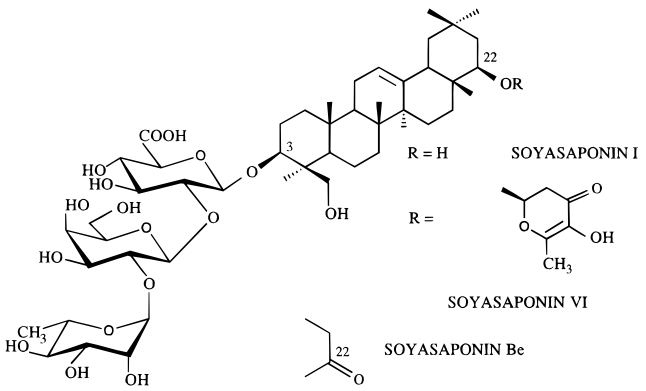

Figure 1. Chemical structures of soyasaponins I, VI, and Be.

content and composition of two cultivars of both chickpeas and lentils.

MATERIALS AND METHODS

Samples. Two cultivars of both chickpeas (*Cicer arietinum*), Fardon (type *Desi*) and Blanco Lechoso (type *Kabuli*), and lentils (*Lens culinaris*), Magda 20 (type *castellana*, ssp *macrosperma*) and Lyda (type *castellana*, ssp *macrosperma*), were obtained from crops grown in Córdoba and Albacete (Spain), respectively.

Pretreatment of the Seeds. Seeds of a similar size were selected and placed in a vacuum desiccator with silica gel for 92 h to achieve a relative constant weight with regard to the moisture content of the seeds.

The seeds were submitted to the following processes.

Soaking was performed in three types of solutions: distilled water, 0.1% citric acid (pH 2.77 ± 0.02), and 0.07% sodium bicarbonate (pH 8.79 ± 0.02). The proportion of seed to soaking medium was 1:3 w/v. The soaking period, 24 h at 25 °C, was chosen to obtain maximum seed weight and hydration. The soaking solution was drained and the soaked seeds were weighed. Both the soaking solutions and the soaked seeds were freeze-dried and the latter milled.

Cooking. Four portions of 100 g of seeds each were soaked in distilled water for 12 h at 25 °C. The soaking solution was drained, and the soaked seeds were weighed and boiled for 30, 60, 90, and 120 min in distilled water (seed:water ratio 1:3.2 w/v) using an iron saucepan. The cooking liquids and seeds were separated using a strainer and freeze-dried. The latter were then ground to a powder.

Saponin Analysis. The ground seeds were extracted with 70% aqueous ethanol containing 0.01% EDTA at room temperature. An internal standard of α-hederin was added to the sample prior to solvent extraction. The extracts were evaporated to dryness at less than 40 °C and dissolved in a mixture of 2 mL of water and 1-butanol (1:1). After centrifugation, the 1-butanol layers were collected and analyzed by high-performance liquid chromatography. Separations were performed on a column packed with Ultratechsphere 5 μ C_{18}, and chromatographic runs were carried out with an acetonitrile−water gradient elution system. The injection volume, flow rate, and run time were 10 μL, 0.9 mL/min, and 45 min, respectively, and detection was monitored by UV absorption at 205 nm as described in Ruiz et al. (1995).

Statistical Analysis. The data were subjected to the multiple comparison method of Tukey using Minitab 8.21 software (Macintosh version).

RESULTS AND DISCUSSION

The effect of soaking on the saponin content and composition of chickpeas and lentils is shown in Table 1. Soyasaponin VI (Figure 1) was the only saponin detected in both the unprocessed and soaked seeds of each of the two cultivars of chickpeas and lentils. No saponin was detected in any of the soaking solutions.

Table 1. Saponin Content[a] of Chickpeas and Lentils before and after Soaking Treatment

	saponin content (mg of soyasaponin VI/kg of dry wt)	
cultivar and treatment	seeds	soaking solution
C. arietinum Fardon		
unprocessed	752 ± 14[a]	
water soaking	744 ± 28[a]	ND[b]
citric acid soaking	737 ± 55[a]	ND
sodium bicarbonate soaking	758 ± 10[a]	ND
C. arietinum Blanco Lechoso		
unprocessed	711 ± 28[b]	
water soaking	670 ± 19[b]	ND
citric acid soaking	667 ± 21[b]	ND
sodium bicarbonate soaking	673 ± 25[b]	ND
L. culinaris Magda 20		
unprocessed	703 ± 14[b]	
water soaking	675 ± 18[b]	ND
citric acid soaking	684 ± 18[b]	ND
sodium bicarbonate soaking	670 ± 26[b]	ND
L. culinaris Lyda		
unprocessed	1139 ± 15[c]	
water soaking	1135 ± 36[c]	ND
citric acid soaking	1088 ± 52[c]	ND
sodium bicarbonate soaking	1097 ± 20[c]	ND

[a] Values are the mean of four determinations ± standard deviation. The same superscripts in the same column indicate no significant differences (family error rate = 0.05). [b] ND, not detectable.

Figure 2 shows the chromatograms corresponding to the unprocessed seeds of Fardon chickpeas, Blanco Lechoso chickpeas, Lyda lentils, and Magda 20 lentils. No significant differences were found between the unprocessed and the soaked seeds. Therefore, neither the saponin content nor the composition of either chickpeas or lentils was affected by soaking regardless of the pH of the soaking solution. Soyasaponin VI, within the seed matrix, was stable under the soaking conditions described above, although, in contrast, DDMP saponins have been reported to be stable at acidic pH and in the presence of hydrogen peroxide (K. Okubo, private communication) but unstable in basic pH (Tsurumi et al., 1992) and in the presence of ferric ion (Okubo et al., 1994).

The effect of cooking on the saponin content and composition of chickpeas and lentils is shown in Table 2. Both soyasaponins VI and I (Figure 1) were detected in all of the cooked seeds of both chickpeas and lentils. Soyasaponins VI and I were also detected in all of the cooking solutions from both cultivars of lentils. However, soyasaponin VI was detected in the cooking solutions from both cultivars of chickpeas only after 30 min of cooking but not after 60, 90 or 120 min of cooking.

Leaching of saponins into the cooking solutions was observed and ranged from 2 to 4%, from 3 to 5%, from 12 to 14%, and from 6 to 7% for Fardon chickpeas, Blanco Lechoso chickpeas, Magda 20 lentils, and Lyda lentils, respectively (Table 2). Therefore, leaching of saponins seemed to be more important in lentils, particularly for Magda 20 lentils, than in chickpeas, which can be attributed to a larger disruption of the seed structure in the case of lentils. The total saponin content of the cooking solutions from both cultivars of chickpeas showed a steady increase with cooking time, whereas leaching of saponins in the case of lentils seemed to cease after 30 min of cooking (Table 2).

Conversion of soyasaponin VI into soyasaponin I was also observed in all of the seeds studied (Table 2) and increased with cooking time; as soyasaponin VI decreased, soyasaponin I increased for both seeds and

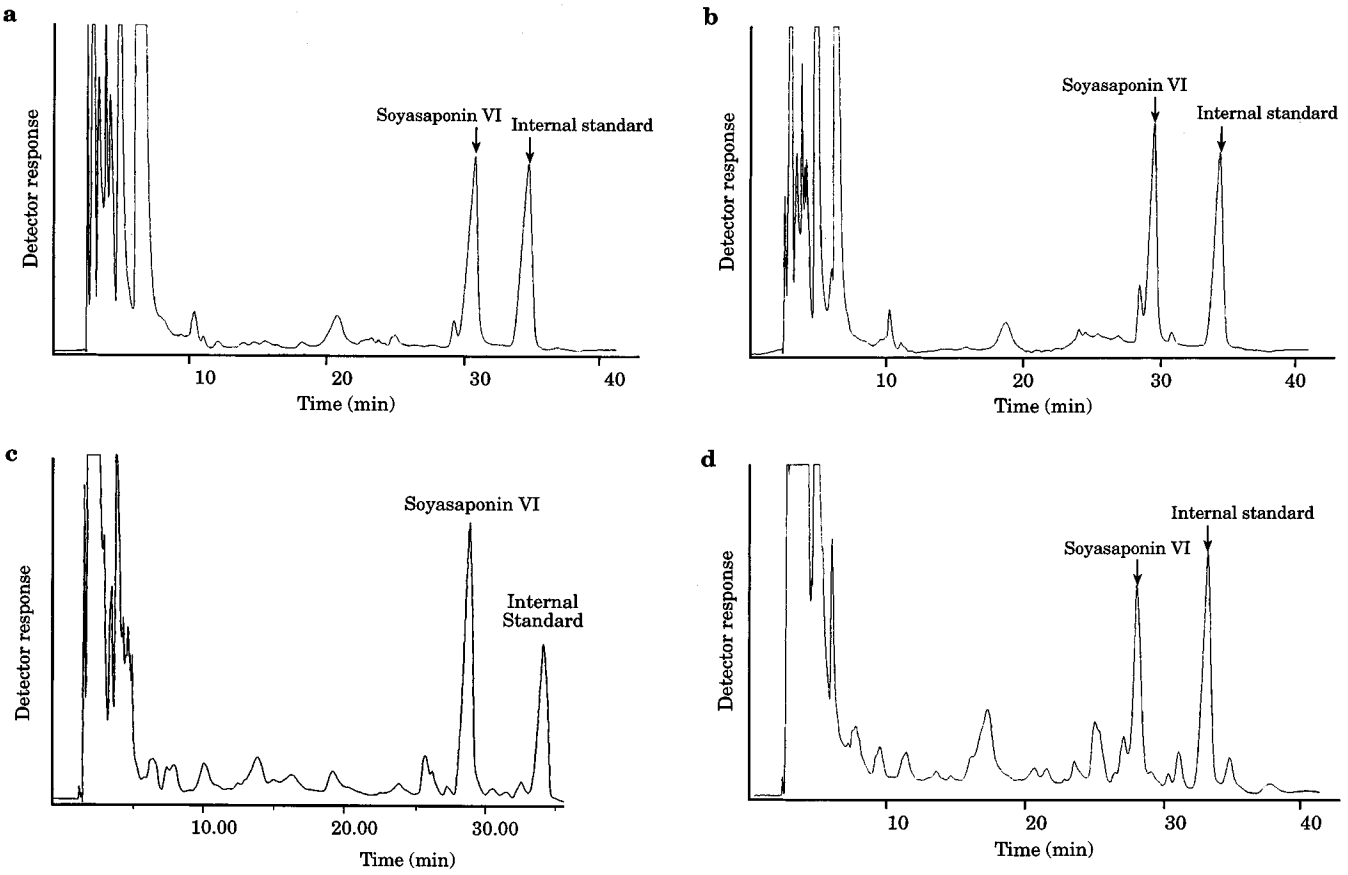

Figure 2. Chromatograms of saponins (soyasaponin VI) in unprocessed (a) Fardon chickpeas, (b) Blanco Lechoso chickpeas, (c) Magda 20 lentils, and (d) Lyda lentils with internal standard (α-hederin).

Table 2. Saponin Content[a] of Chickpeas and Lentils before and after Soaking plus Cooking Treatment

	saponin content (mg/kg of dry wt)							
	seeds			cooking solution				total saponin content of seeds and solution
cultivar and treatment	soyasaponin I	soyasaponin VI	total	soyasaponin I	soyasaponin VI	total	% saponin leaching	
C. arietinum Fardon								
unprocessed	ND[b]	752 ± 14	752 ± 14					752 ± 14[a]
30 min of cooking	407 ± 17	339 ± 13	746 ± 24	10 ± 1	5 ± 0	15 ± 1	2	761 ± 24[a]
60 min of cooking	513 ± 18	227 ± 21	740 ± 30	18 ± 2	ND	18 ± 2	2	758 ± 30[a]
90 min of cooking	585 ± 15	141 ± 4	726 ± 13	23 ± 2	ND	23 ± 2	3	749 ± 13[a]
120 min of cooking	640 ± 8	83 ± 7	723 ± 7	30 ± 2	ND	30 ± 2	4	753 ± 10[a]
C. arietinum Blanco Lechoso								
unprocessed	ND	711 ± 28	711 ± 28					711 ± 28[b]
30 min of cooking	408 ± 20	274 ± 6	682 ± 17	13 ± 1	5 ± 0	18 ± 1	3	700 ± 17[b]
60 min of cooking	519 ± 15	142 ± 15	661 ± 28	25 ± 2	ND	25 ± 2	4	686 ± 26[b]
90 min of cooking	543 ± 20	120 ± 7	663 ± 25	27 ± 3	ND	27 ± 3	4	690 ± 24[b]
120 min of cooking	588 ± 18	88 ± 2	676 ± 19	36 ± 6	ND	36 ± 6	5	712 ± 17[b]
L. culinaris Magda 20								
unprocessed	ND	703 ± 14	703 ± 14					703 ± 14[b]
30 min of cooking	105 ± 11	319 ± 9	424 ± 10	27 ± 0	40 ± 6	67 ± 4	14	491 ± 13[c]
60 min of cooking	176 ± 14	276 ± 7	452 ± 12	35 ± 1	29 ± 1	64 ± 0	12	516 ± 12[c]
90 min of cooking	191 ± 10	228 ± 19	419 ± 29	44 ± 1	25 ± 1	69 ± 0	14	488 ± 30[c]
120 min of cooking	233 ± 11	200 ± 13	433 ± 12	48 ± 0	22 ± 0	70 ± 0	14	503 ± 12[c]
L. culinaris Lyda								
unprocessed	ND	1139 ± 15	1139 ± 15					1139 ± 15[d]
30 min of cooking	381 ± 21	526 ± 36	907 ± 56	35 ± 1	20 ± 0	55 ± 1	6	962 ± 56[e]
60 min of cooking	510 ± 20	393 ± 43	903 ± 43	41 ± 4	12 ± 0	53 ± 3	6	956 ± 41[e]
90 min of cooking	519 ± 21	342 ± 11	861 ± 33	49 ± 1	10 ± 1	59 ± 1	6	920 ± 32[e]
120 min of cooking	543 ± 9	330 ± 26	873 ± 18	60 ± 0	6 ± 0	66 ± 0	7	939 ± 18[e]

[a] Values are the mean of four determinations ± standard deviation. The same superscripts in the same column indicate no significant differences (family error rate = 0.05). [b] ND, not detectable.

cooking solutions. Figure 3 shows the chromatograms corresponding to saponins present in Fardon chickpeas cooked for 30−120 min.

While the total saponin contents of both Fardon and Blanco Lechoso chickpeas cooked for 30−120 min (Table 2) plus those of their corresponding cooking solutions do not differ statistically from the total saponin content of the unprocessed seeds, the total saponin contents of both cultivars of lentils are significantly lower than those of the raw seeds; the saponin losses were 27−31%

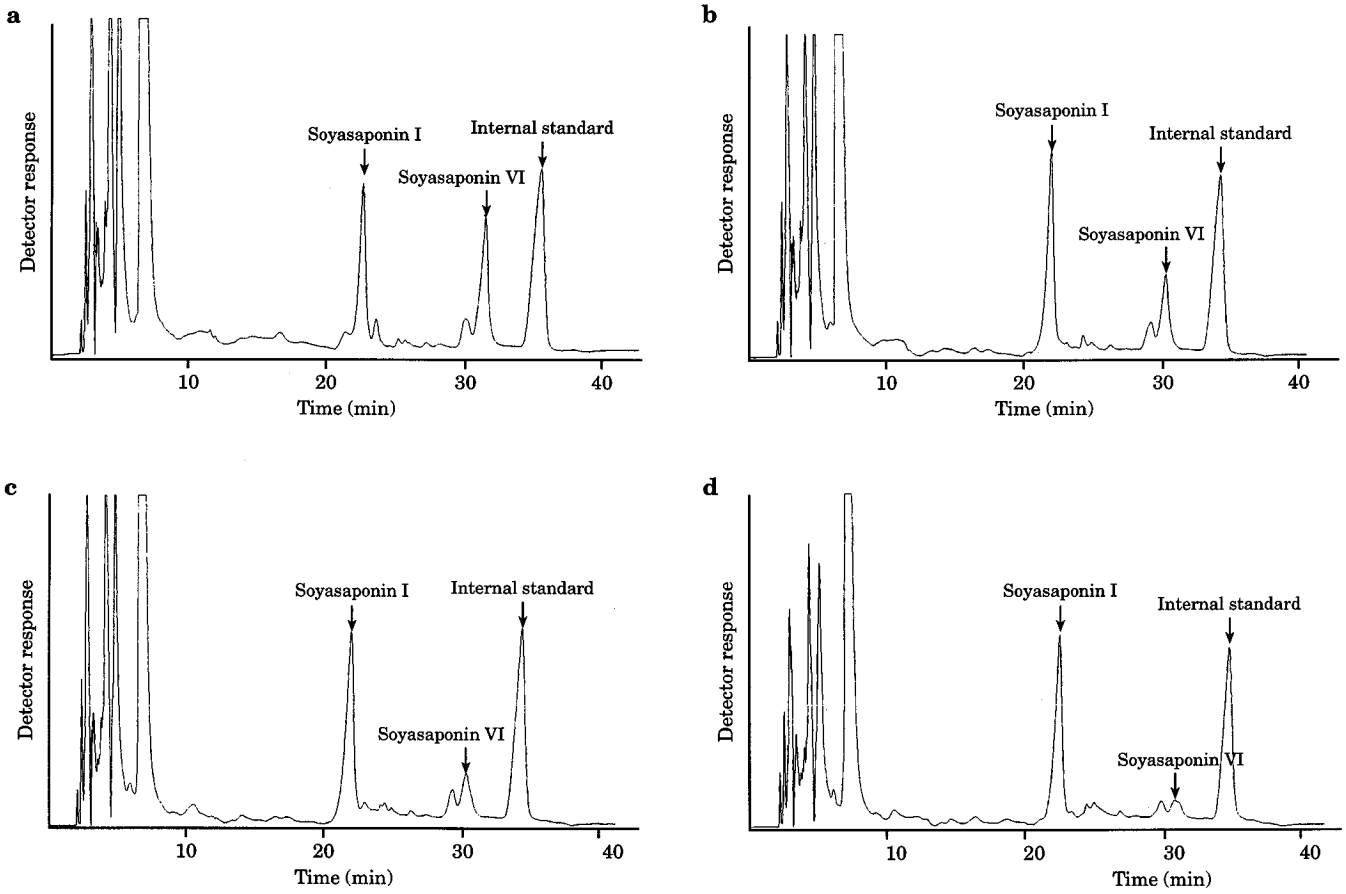

Figure 3. Chromatograms of saponins (soyasaponins VI and I) in Fardon chickpeas after cooking for (a) 30, (b) 60, (c) 90, and (d) 120 min with internal standard (α-hederin).

for the former and 15−19% for the latter. The saponin losses in the case of lentils might be due to a possible conversion of soyaponin VI into soyasaponin I plus soyasaponin Be (Figure 1), which possesses soyasapogenol E (Price et al., 1986) as aglycon and the same sugar chain as soyasaponin I, as reported by Kudou et al. (1992). Okubo et al. (1994) also found that soyasaponin VI easily changed into soyasaponins I and Be in the presence of Fe^{3+}. However, apart from conversion of soyasaponin VI into soyasaponin I, the latter might suffer further degradation as a consequence of heating, losing the different sugars of its glycosidic chain, which could also explain the reduction in saponin level reported in the case of lentils.

Studies on the effect of soaking and cooking on saponin content and composition are very few and contradictory. While Fenwick and Oakenfull (1983) found that cooking had little effect on broad beans, Khokhar and Chauan (1986) reported that soaking in a solution of mixed mineral salt removed much more of the saponins in moth beans than did soaking in water (30−36% and 9−18%, respectively) and ordinary cooking of the seeds presoaked in water reduced the saponin level by 12−15%. Jood et al. (1986) also found that common domestic processing and cooking treatments reduced the saponin level of chickpeas and black grams significantly. However, since the assay used in both cases probably did not distinguish between saponins and oligosaccharides, and as the conditions of soaking have been found to have a significant effect on the oligosaccharide contents of legumes (Vidal-Valverde et al., 1993), these results should be treated with caution.

The differences in saponin recovery between chickpeas and lentils found in the present study could be attributed to the fact that in the case of lentils, especially the cultivar Magda 20, the seed structure was highly disrupted, releasing more saponins into the cooking solution. The saponins might be then more liable to suffer damage by heat or the possible presence of Fe^{3+} in the saucepan, whereas for the chickpeas, the structure of which was modified to a much smaller extent by heating, soyasaponins VI and I would be more protected and suffer less degradation in the protective environment supplied by the seed.

The work described here provides quantitative results using intact saponins rather than their products from acid hydrolysis, made for the first time on the effect of soaking and cooking, not only on the total saponin content but also on the true saponin composition. These data have established the fate of soyasaponin VI in the seed matrix, which has been shown to be stable after soaking but unstable when chickpeas and lentils were cooked. As a result of cooking, conversion of soyasaponin VI into soyasaponin I, leaching of both saponins, and reduction of saponin level in the case of lentils were recorded. Thus, this study proves that the important physiological properties ascribed to DDMP saponins (Yoshiki and Okubo, 1995) might be lost as a consequence of certain types of legume processing. Since some of the biological properties reported for saponins are now recognized to be dependent on their particular chemical structures, this type of information will allow further understanding of not only the relationship between chemical structure and bioactivity but also the impact of processing on that bioactivity in relation to both human and animal health.

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