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Analytical Methods Section

A TLC method for the analysis of quinoa (*Chenopodium quinoa*) saponins

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A method is described which enables both the total saponin content and composition to be assayed in quinoa plant tissue. The saponin composition has been determined according to the three main groups of saponins found in quinoa which contain oleanolic acid, hederagenin and phytolaccagenic acid as the aglycone in each group. The method has been used to measure the saponin content of 15 ecotypes being used in a breeding programme in the UK and to compare the saponins present in sweet and bitter varieties.

INTRODUCTION

Quinoa is a crop that has been grown in South America for centuries (Sanchez-Marroqin, 1983) and has many potentially useful properties, such as resistance to drought and frost and can be grown in poor soil and at high altitude. The seeds have high nutritional value and are rich in essential amino acids which result in a protein quality comparable to that of whole dry milk (Mahoney *et al.*, 1975). For these and other reasons, quinoa is currently attracting considerable interest in Europe as both a human food and an animal feeding stuff (Risi & Galwey; 1984 Galwey *et al.*, 1990). The main problem with using the seed for human food is the bitterness found in the seed coat and which is thought to be caused by the presence of saponins (Price *et al.*, 1987) which are present as a natural protection against insects and birds.

There is a need for a range of methods for the analysis of saponins in such crops as quinoa. Plant breeders require simple and rapid methods to monitor breeding programmes, while food chemists require more information such as changes in saponin composition rather than just changes in total saponin content due to such factors as processing. In addition, saponins have a wide range of biological activities; for example, they have been shown to lower plasma cholesterol levels and to have adverse effects on the lining of the small intestine by increasing its permeability to other antigens (Gee *et al.*, 1989). Once again the important factor may well be the biological activity of an individual saponin rather than the level of total saponins.

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Methods have been described using chromatographic techniques such as GC (Ridout *et al.*, 1991), TLC (Curl *et al.*, 1985), HPLC (Kitagawa *et al.*, 1984), haemolytic methods (Elkowicz & Solsulski, 1982) and simpler techniques such as colorimetric assays (Honerlagen & Trelter, 1979). This paper reports a TLC method which goes some way to bridging the gap between simple methods and the more complex GC and HPLC methods and gives information on both total saponin content and saponin composition.

MATERIALS AND METHODS

Materials

Fifteen South American Lowland cultivars grown in the UK were supplied by John K. Kings and Sons Limited of Coggeshall, Essex, UK; sweet varieties (of S. American origin) were supplied by Dr N. Galwey of Cambridge University.

All reagents used were of AnalaR grade and solvents were redistilled before use. Solid phase extraction was carried out using C_{18} BakerElut columns which were purchased from J. T. Baker, UK. TLC plates [20 × 20 × 0·025 cm, coated with octadecylsilanised silica (RP18) and silica gel] were purchased from Whatman LabSales, Maidstone, Kent.

Extraction

Seed samples were cleaned by hand to remove other plant parts before being milled in a coffee grinder.

A 1 g flour sample was extracted with methanol (3 × 90 ml) in a round-bottomed flask under reflux (1 h). The combined methanolic extracts were pooled and

evaporated to dryness under reduced pressure. The dried saponin extract was redissolved in water (5 ml). A 1-ml aliquot was defatted by shaking with an equal volume of hexane. A portion of the defatted extract (200 μl) was loaded onto a C_{18} BakerElut column (3 g) preconditioned with methanol (6 ml) and water (6 ml) and then eluted sequentially with water (3 ml) and methanol (3 ml). The methanol eluate containing the saponins was then evaporated to dryness using nitrogen gas and redissolved in a mixture of methanol and water (3 : 1, v/v, 200 μl).

Thin layer chromatography

Aliquots of each extract (5 μl) were applied to the plate and eluted with methanol/water (2 : 1, v/v) for 12 cm. The plate was allowed to air-dry and the saponins were visualised by spraying with a mixture of *p*-anisaldehyde/sulphuric acid/glacial acetic acid (1 : 2 : 100 v/v/v) and heating uniformly with a stream of hot air until the background was uniformly light pink in colour. The plate was scanned with a TLC scanner which was set to monitor at 575 nm and the spot densities were measured.

Table 1. Fast atom bombardment mass spectra of TLC fractions

Negative mode		Positive mode	
m/z	Assignment	m/z	Assignment
Ions from spot 1:			
963		1157	
925		995	
809		883	
793		499	PH-w$^+$
647		481	PH-2w$^+$
515	P-H$^-$	469	
497	P-H-w$^-$	453	PH-w-f$^+$
483		439	OH-w$^+$
481		421	OH-2w$^+$
467			
455	O-H$^-$		
453			
437	O-H-w$^-$		
Ions from spot 2:			
969	M-H$^-$	1009	MK$^+$
941		993	MNa$^+$
809		965	
647		833	
601		483	
499		455	HH-w$^+$
479		437	HH-2w$^+$
471	H-H$^-$	409	HH-w-f$^+$
453	H-H-w$^-$		
375			
Ions from spot 3:			
967		929	MH$^+$
955		767	MH-h$^+$
927	M-H$^-$	439	OH-w$^+$
809		421	OH-2w$^+$
765	M-H-h$^-$		
455	O-H$^-$		
437	O-H-w$^-$		
Ions from spot 4:			
1177	M-H$^-$	1201	MNA$^+$
1015		1009	MH-w$^+$
985		499	PH-w$^+$
853		481	PH-2w$^+$
739			
691			
515	P-H$^-$		
479			

Quantification

Five seed samples with saponin levels covering the range 1·3 to 13·8 g kg^{-1} saponin were analysed both by this method and by a gas chromatographic method (Ridout *et al.*, 1991) to give a calibration graph relating TLC spot intensity to saponin concentration. Each sample was analyzed in duplicate.

Each spot on the TLC plate was removed and the saponins, where present, identified by the use of fast atom bombardment mass spectrometry (fab). The aglycone identity was confirmed by acid hydrolysis of the extract and subsequent co-chromatography with standard aglycone samples using TLC on silica gel plates eluted with chloroform/methanol (96 : 4 v/v).

RESULTS

The extraction procedure is shown in Fig. 1 and quantification of the TLC plate in Fig. 2. The peak areas, derived from measurement of the individual TLC spots, were translated into saponin content by comparison with results obtained from the analysis of the same seed samples using a standard GC method described previously (see Fig. 3). Only spots which had previously been identified as containing saponins were quantified. All the saponins identified were contained in four spots, each of which contained saponins of a particular class. These were identified from the fragmentation patterns of their mass spectra using fast atom bombardment in both positive and negative modes (Table 1) and chromatography of the acid-released sapogenols.

As can be seen from these data, spot 1 (R_f = 0·10)

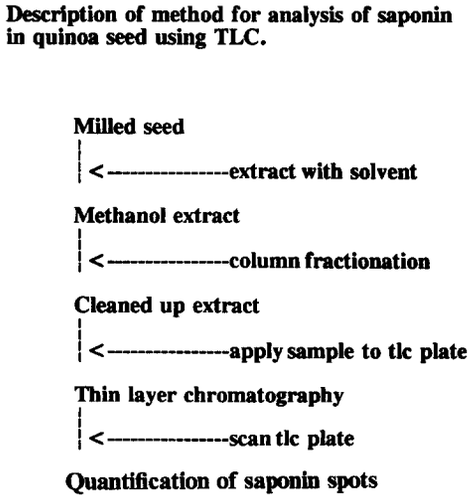


Fig. 1. Flow chart of the extraction procedure.

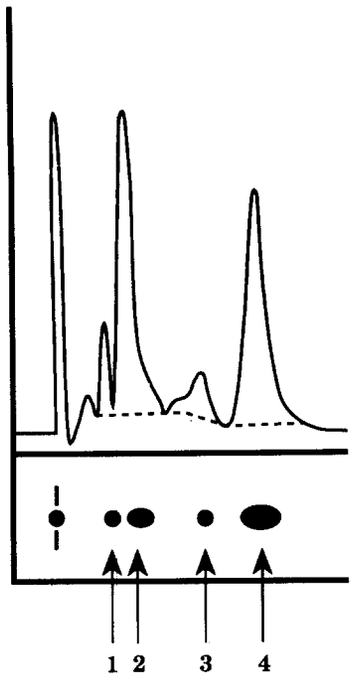


Fig. 2. TLC plate and quantification.

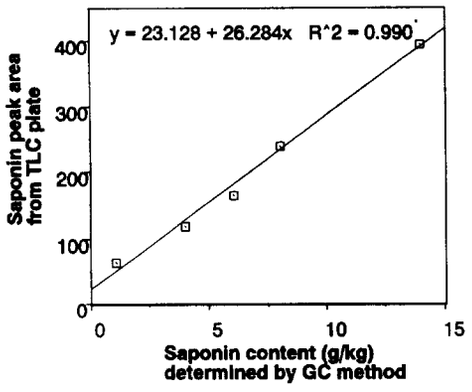
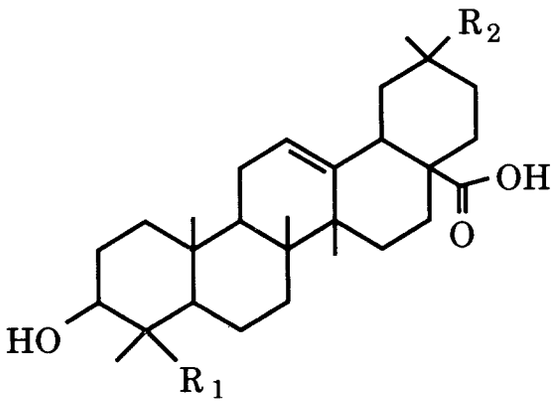


Fig. 3. Calibration curve for the TLC method.



Sapogenol	R_1	R_2
Oleanolic acid	$-CH_3$	$-CH_3$
Hederagenin	$-CH_2OH$	$-CH_3$
Phytolaccagenic acid	$-CH_2OH$	$-C(=O)-OCH_3$

Fig. 4. Structures of the three sapogenols present in quinoa saponins.

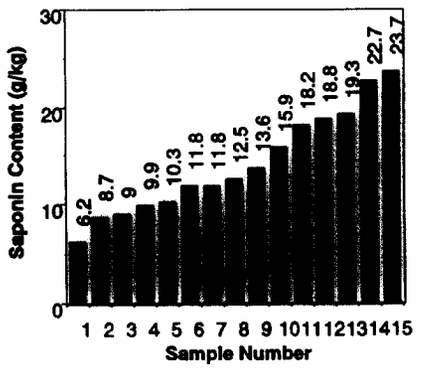


Fig. 5. Total saponin content of 15 cultivars.

contained saponins possessing both phytolaccagenic acid and oleanolic acid (see Fig. 4). Fab-mass spectra gave ion fragmentation patterns showing, as well as molecule ions, specific ions indicating the presence of both phytolaccagenic acid (m/z 515, Aglycone-H-) and oleanolic acid (m/z 455, Aglycone-H-) while acid hydrolysis yielded phytolaccagenic acid ($R_f = 0{\cdot}08$) and oleanolic acid ($R_f = 0{\cdot}35$). Spot 2 ($R_f = 0{\cdot}14$) contained saponins possessing only hederagenin, (m/z 471, Aglycone-H-) and acid hydrolysis yielded hederagenin ($R_f = 0{\cdot}10$). Spot 3 ($R_f = 0{\cdot}27$) contained saponins possessing oleanolic acid saponins (m/z 455, Aglycone-H-) and acid hydrolysate yielded oleanolic acid ($R_f = 0{\cdot}35$). Spot 4 ($R_f = 0{\cdot}36$) contained saponins possessing phytolaccagenic acid (m/z 515, Aglycone-H-) and acid hydrolysis yielded phytolaccagenic acid ($R_f = 0{\cdot}08$).

By combining the peak areas for each of the four spots a total saponin content for each sample was calculated; the saponin composition of each sample was calculated from the peak area of each spot and expressed as a mixture of oleanolic acid and phytoaccagenic acid monodesmosidic saponins (spot 1), hederagenin saponins (spot 2) and oleanolic acid (spot 3) and phytolaccagenic acid bisdesmosidic saponins (spot 4). Total saponin contents are given in Fig. 5 which show a range from 6·2 to 23·7 g kg^{-1}.

Saponin composition for the 15 cultivar samples, with increasing total saponin content, is shown in Fig. 6. There is a slight decrease in the proportion of

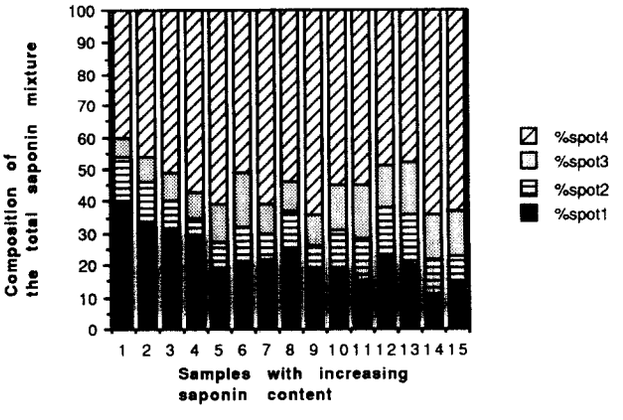


Fig. 6. Saponin composition of the 15 cultivars.

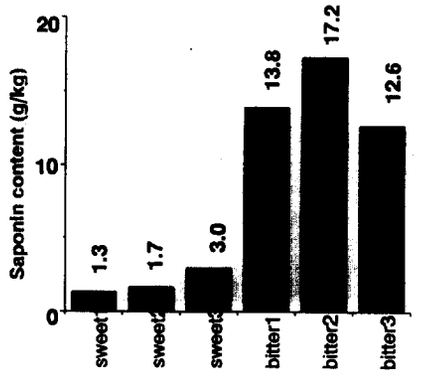


Fig. 7. Total saponin content of sweet and bitter varieties.

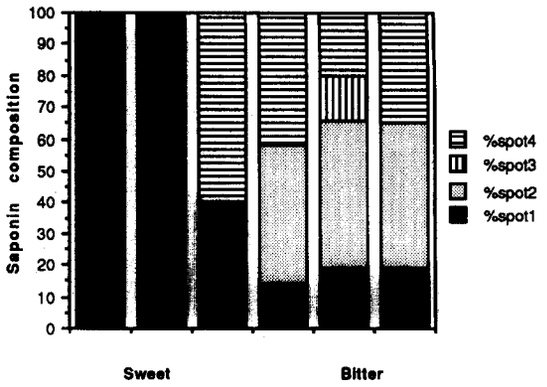


Fig. 8. Saponin composition of sweet and bitter varieties.

saponins residing in spot 1 (40%-14%) with increasing saponin content, while in all cases the predominant saponin species are present in spot 4 (42%-65%), the phytolaccagenic acid (type II) saponins. The hederagenin and oleanolic acid (type II) saponins associated with spots 2 and 3 range from 6% to 16% and 6% to 17%, respectively, but do not show any relationship to overall saponin content.

The total saponin content of three bitter and three sweet varieties (Fig. 7) was found to be 13 g kg^{-1}, 14 g kg^{-1} and 17 g kg^{-1} (bitter) and 1·3 g kg^{-1}, 1·7 g kg^{-1} and 3·0 g kg^{-1} (sweet). The saponin composition (Fig. 8) shows only the presence of spot 1 saponins in two of the sweet samples with the third having a mixture of these and spot 4 saponins comprising 40% and 60% of the total mixture, respectively. The bitter varieties showed a distribution similar to the 15 varieties previously discussed but with a lower proportion of spot 4 (20%-42%) and no spot 3 saponins detected in two of the three samples.

DISCUSSION

The 15 UK-grown cultivars all had relatively high levels of saponin when compared with the sweet varieties. The range of 6·2 g to 23·7 g of saponin in 1 kg of seed should be sufficient to give scope for genetic manipulation of the saponin content. This could conceivably be increased for improved pest resistance or decreased for improved sensory characteristics. Although most of the saponins can be removed by processing such as washing or abrasion of seed, the remaining saponins in the seed may be of a sufficient level to generate biological activity.

The presence of phytolaccagenic acid saponins is of interest because they are not normally present in plants used for human food and are associated with toxicity due to their presence in pokeweed, which is known to be toxic to animals. The saponin composition of the bitter cultivars studied here was predominantly phytolaccagenic acid saponins, while that of two of the sweet varieties has no detectable amounts of this class of saponin.

The method described here is relatively fast for this type of analysis. With a continuous programme up to 10 samples per day can be analyzed. It is therefore rapid and simple enough to monitor moderate numbers of cultivars from a breeding programme, while at the same time giving information on the saponin composition which may be of equal importance to the overall level of saponins when the biological significance of these compounds in food and feedingstuff is considered.

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