Glycoalkaloid, phytosterol and fatty acid contents of raw and blanched leaves of the gboma eggplant (*Solanum macrocarpon* L.)

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Summary

The glycoalkaloid, sterol and fatty acid contents were determined in raw and blanched leaves of the gboma eggplant (*Solanum macrocarpon* L.). The results of the study revealed that 5 min blanching was sufficient to obtain a 35–56% decrease in the glycoalkaloid content in leaves to values below 200 mg·kg^{-1} of fresh weight, which is regarded as a safe level in foods. Processing also significantly decreased the free fatty acid content and increased the free sterol content. In raw and blanched leaves, however, the contents of total fatty acids and sterols were comparable. The total sterol content in *S. macrocarpon* leaves was as high as 3.2–4.0 g·kg^{-1} of dry weight, while the total fatty acid content was 23.0–32.1 g·kg^{-1} of dry weight. The cholesterol contribution to total sterols did not exceed 10%. While saturated compounds were most abundant in the free fatty acid fraction, total fatty acids were dominated by C_{18} polyunsaturated compounds. Additionally, isomerization of unsaturated fatty acids during hydrolysis and silylation of lipids from dried plant material was demonstrated. The presence of artefacts did not affect the results of quantitative analysis.

Keywords

Solanum macrocarpon; green leafy vegetables; glycoalkaloid; phytosterol, fatty acid; food processing

Indigenous solanaceous vegetable crops are grown and consumed in Africa as valuable sources of nutrients for the diversification of the diet [1]. Some of the species also provide a source of income for small-scale local farmers [2]. The gboma eggplant (*Solanum macrocarpon* L.) is one of the most important solanaceous crops, grown in the humid parts of Africa [3]. Both the leaves and fruits of *S. macrocarpon* are edible when cooked and are eaten as ingredients of soups and sauces, mixed with meat or other vegetables [4, 5]. The leaves are eaten shortly after the harvest, they can also be sun-dried and stored for future use [6, 7]. As the leaves of *S. macrocarpon* are consumed when cooked, the impact of the processing method on nutritional properties of the leaves is of the highest importance. Several processing techniques, including blanching and abrasion with or without salt, resulted in a significant decrease in protein, fat and mineral content, as well as in the level of some antinutrients (cyanide and phytate) [8].

Little is known about the glycoalkaloid (GA) content of *S. macrocarpon* leaves. It is widely accepted, that many *Solanum* species produce a number of GA of different toxicity to mammals [9]. Two solasodine-based GA (α-solamargine and α-solasonine) were reported in *S. macrocarpon* fruits [10]. Their contents were relatively high when compared to the common eggplant (*S. melongena* L.) and scarlett eggplant (*S. aethiopicum* L.), reaching values of 1.4–2.2 g·kg^{-1} fresh weight, which is considered potentially unsafe. As far as we are aware, there are no reports on the GA content of *S. macrocarpon* leaves. The toxicity of a diet containing unprocessed leaves to rats was relatively low when compared to other green leafy vegetables, with no potential to cause liver damage [11]. It was also reported that leaves should be boiled for 5–10 min, and the cooking water discarded, in order to reduce the level of toxic substances [11].

The lipid composition of *S. macrocarpon* leaves remains largely unknown, although the overall

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fatty acid profile was studied by GLEW et al. [7]. The total fatty acid content was 9.5 g·kg^{-1} dry weight, with α-linolenic acid being the most abundant (ALA, 18:3 omega-3): it accounted for 44.6% of the fraction. The production of essential long-chain omega-3 fatty acids in mammals, particularly eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), is based on the transformation of dietary ALA [12]. A deficit of dietary EPA and DHA, which are present mainly in the lipids of marine organisms, can thus be partially reduced by ingesting ALA of vegetable origin. The phytosterol content of *S. macrocarpon* leaves is also unknown. Recently, we reported an unusually high content of sterols in leaf surface waxes of the gboma eggplant [13], but there was no evidence that this reflected the overall high quantity of sterols in the whole leaves. The ingestion of phytosterols reduces the total cholesterol and the low-density lipoprotein (LDL) cholesterol level in blood, thus lowering the risk of cardiovascular diseases [14]. Hence, data on free and conjugated phytosterols in food are of a great interest.

In order to fully evaluate the nutritional value of *S. macrocarpon*, knowledge of the lipid content of fresh and processed leaves of this important crop species is necessary. Also, the risk associated with the ingestion of GA should be assessed. Therefore, the objective of the study was to determine the fatty acid, phytosterol and glycoalkaloid content in raw and blanched leaves of *S. macrocarpon*.

MATERIALS AND METHODS

Chemicals and apparatus

All solvents (petroleum ether, dichloromethane, chloroform, acetone, methanol, ethanol, *n*-butanol), as well as acetic acid, ammonia, HCl, KOH and Na_2SO_4, were of analytical grade and were obtained from POCh (Gliwice, Poland). Petroleum ether and dichloromethane were distilled before use. Silica gel for flash chromatography (particle size 40–63 μm), α-solanine and the standards used in lipid analysis (ethyl nonadecanoate, 19-methylarachidic acid, conjugated linoleic acid and methyl linoleate) were obtained from Sigma-Aldrich Poland (Poznań, Poland). Solasodine was obtained from MP Biomedicals (Santa Ana, California, USA). The MALDI matrix (2,4,6-trihydroxyacetophenone, THAP) and derivatizing agents – *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS), *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and 10% BF_3 in methanol were purchased from Sigma-Aldrich Poland.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a Biflex III spectrometer (Bruker Daltonics, Bremen, Germany) with an N_2 laser (λ = 337 nm). Electrospray ionization mass spectrometry (ESI-MS/MS) was carried out on a HCT Ultra ion trap mass spectrometer (Bruker Daltonics).

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Shimadzu QP-2010SE system (Shimadzu, Kyoto, Japan) equipped with a 30 m × 0.25 mm internal diameter (i.d.), film thickness 0.25 μm, RTX-5 capillary column (Restek, Bellefonte, Pennsylvania, USA). Helium was used as carrier gas at a flow rate of 1 ml·min^{-1}. All GC-MS analyses were carried out using electron-impact ionization (electron energy 70 eV, ion source temperature 200 °C). Gas chromatography with flame ionization detector (GC-FID) analyses were performed on a Trace GC 2000 Series gas chromatograph (Thermo Finnigan, Austin, Texas, USA). Argon was used as carrier gas at a flow rate of 1 ml·min^{-1}.

Plant material

The plants were grown from seed in semi-natural conditions in an unheated greenhouse. Two Tanzanian cultivars of *S. macrocarpon* were used: the Urafiki cultivar obtained from AVRDC – The World Vegetable Center, Tainan, Taiwan (accession number S00846), and the UVPP accession obtained from AVRDC – Regional Center for Africa (Arusha, Tanzania). Six-week-old plants were transferred to 8 dm^3 pots, which were kept well watered by irrigating 3 times a week (approx. 400–600 ml of water, depending on the ambient temperature). Leaves from three mature plants of each cultivar (approx. 16 weeks old) were then harvested. The three or four oldest and most damaged leaves of each plant were discarded, and the pooled leaves from one plant constituted a sample (30–53 g fresh weight). Thus, three independent samples of each cultivar were obtained. The leaves were chopped into small pieces, and half of each sample was blanched in 400 ml of boiling water for 5 min. The water was then discarded, and all the samples (both raw and blanched) were dried at 70 °C for 3 h. Prior to extraction, the samples were ground to a fine powder using a pestle and mortar.

Lettuce (*Lactuca sativa* L. cv. Królowa Majowych; PNOS Ożarów Mazowiecki, Ożarów Mazowiecki, Poland) and eggplant (*Solanum melongena* L. cv. Solara F_1; Royal Sluis, Enkhuizen, The

Netherlands) were grown in similar conditions. Their unprocessed leaves were dried in the same conditions as *S. macrocarpon* leaves and were used as reference materials during the study on the formation of artefacts in lipid hydrolysis and analysis.

Extraction and hydrolysis of glycoalkaloids

GA were extracted according to a modification of the method previously described by BROWN et al. [15]. About 1 g of powdered sample was suspended in 25 ml 5% acetic acid and placed in an ultrasonic bath for 15 min. The extract was then gently decanted and filtered, and the extraction procedure repeated. The pH of the pooled extracts was adjusted to 10 by adding 25% ammonia, and the solution was extracted with 100 ml *n*-butanol-water (4:1; v/v). The butanol layer was evaporated to dryness under reduced pressure and under a stream of nitrogen. The samples were then re-dissolved in 5 ml methanol.

An aliquot of each extract was hydrolysed in conditions proposed by NIKOLIC and STANKOVIC [16]. A sample with a known amount of internal standard (solasodine) was dissolved in 4 ml 10% (v/v) HCl solution in methanol-water (1:1; v/v). After the addition of 4 ml chloroform, the mixture was heated for 2 h at 70 °C. The chloroform layer was then evaporated to dryness under a stream of nitrogen. Trimethylsilyl derivatives of liberated aglycones were synthesized on the day of analysis by adding 0.1 ml MSTFA. The sample was then heated at 60 °C for 30 min.

Extraction, fractionation and hydrolysis of lipids

Lipids were extracted from the plant material according to a modification of the method previously described by FOLCH et al. [17]. Prior to extraction, known amounts of internal standards (ethyl nonadecanoate and 19-methylarachidic acid) were added to the plant material. About 0.1 g of the powdered sample was suspended in 8 ml chloroform-methanol (2:1; v/v) and placed in an ultrasonic bath for 15 min. The extract was then filtered, evaporated to dryness under reduced pressure and re-dissolved in 4 ml dichloromethane. Additionally, lipids from the fresh *S. macrocarpon* material were extracted by cutting the leaves into pieces and immediately immersing them in the above-mentioned solvent mixture. In this case, the extract was then dried using Na_2SO_4.

An aliquot of the extract was fractionated using flash-chromatography [18] on 8 g of silica gel in order to separate esters, triacylglycerols (TAG), free sterols and free fatty acids (FFA). The mobile phase flow rate was approx. 5–6 ml·min^{-1}. Petroleum ether (30 ml) was used to elute cuticular hydrocarbons. Then, compounds were eluted using petroleum ether (phase A) containing increasing amounts of an 85:15 (v/v) dichloromethane-acetone mixture (phase B). Wax esters, steryl esters and TAG were eluted together using the following mobile phases (A:B, v/v): 90:10 (25 ml), 80:20 (25 ml) and 70:30 (10 ml). The remaining free alcohols were removed using the last-mentioned mobile phase (15 ml). Finally, free sterols and FFA were co-eluted using the following phases: 40:60 (25 ml) and phase B (50 ml). The fractions were then evaporated to dryness under reduced pressure and under a stream of nitrogen.

The fractions containing esters and TAG, as well as aliquots of the whole extracts, were subjected to hydrolysis and trimethylsilylation according to the IUPAC procedure [19]. Briefly, 0.2 ml KOH solution in 99.8% ethanol (0.5 mol·dm^{-3}) was added to a sample and heated for 3 h at 70 °C. After the removal of ethanol under a stream of nitrogen, the hydrolysis products were silylated by adding 0.1 ml BSTFA + TMCS (99:1) and heating the mixture for 30 min at 90 °C. Alternatively, randomly selected extracts were subjected to hydrolysis in the same conditions, followed by acidification using 0.1 mol·dm^{-3} HCl solution and extraction of fatty acids with 2 ml chloroform. After removal of the solvent, 0.2 ml 10% BF_3 in methanol was added. The sample was kept at room temperature for 30 min, after which 1 ml distilled water was added and fatty acid methyl esters (FAME) were extracted twice with 2 ml petroleum ether. Free sterols and fatty acids were silylated on the day of analysis by adding 0.1 ml BSTFA + TMCS (99:1) and heating the mixture for 30 min at 90 °C.

MALDI-TOF MS and ESI-MS/MS analysis of intact glycoalkaloids

A solution of extracted GA in methanol was subjected to MALDI-TOF MS and ESI-MS/MS analyses. MALDI-TOF MS analysis was performed using 2,4,6-trihydroxyacetophenone (THAP) as matrix. The sample was mixed with the solution of the matrix in methanol, applied to the sample support plate and left to air-dry. Spectra were recorded in positive ion linear mode by averaging 160–220 scans.

The ESI-MS/MS analyses were carried out by injecting the extract directly to the ion source. Heater temperature of the drying gas (N_2) was 300 °C, flow of heated dry nitrogen gas was 7 l·min^{-1} and nebulizer gas (N_2) pressure was 69 kPa. Electrospray ionization was performed in positive ion mode at the scan range of *m/z* 50–1 200. The capillary voltage was 4 000 V and helium was used as the collision gas.

GC-MS and GC-FID analysis of GA aglycones

The products of GA hydrolysis were identified by GC-MS analyses of their trimethylsilyl derivatives. The injector temperature was 310 °C and the column temperature was programmed from 220 °C to 310 °C at 5 °C·min^{-1} and then held at 310 °C for 10 min. Quantification of aglycones was based on the results of GC-FID analyses, which were performed using a 30 m × 0.25 mm i.d., film thickness 0.25 μm, RTX-1 capillary column (Restek). The injector and detector temperatures were set at 320 °C, and the column temperature was programmed from 180 °C to 320 °C at 6 °C·min^{-1} and then held at 320 °C for 5 min. Solasodine was used as internal standard.

GC-MS and GC-FID analysis of fatty acids and sterols

Free sterols and fatty acids, as well as compounds liberated during the hydrolysis of esters, TAG and whole extracts, were analysed by GC-MS and GC-FID as their trimethylsilyl derivatives. GC-MS analyses were performed in the following conditions: the injector temperature was 310 °C, the column temperature was programmed from 100 °C to 310 °C at 4 °C·min^{-1} and then held at 310 °C for 10 min. GC-FID analyses were carried out using a 30 m × 0.25 mm i.d., film thickness 0.10 μm, Rxi-5HT capillary column (Restek). The injector and detector temperatures were set at 320 °C and the column temperature was programmed from 100 °C to 320 °C at 4 °C·min^{-1}. Nonadecanoic acid formed by the hydrolysis of ethyl nonadecanoate was used as internal standard in the quantification of hydrolysis products, while free sterols and FFA were quantified using 19-methylarachidic acid as internal standard. Response factors of the analytes were not determined, but were all assumed to be equal to the internal standard. Additional GC-MS and GC-FID analyses of FAME were carried out in the conditions specified above.

Statistical analysis

All quantitative results presented in this study are mean values obtained for three independent samples ± standard deviation. Aglycones liberated during GA hydrolysis, free sterols/fatty acids and sterols and fatty acids (FA) liberated from the ester/TAG fraction were analysed by GC-FID in triplicate, while products of the whole extract hydrolysis were analysed once. A one-way analysis of variance (ANOVA) followed by Fisher's protected least significance difference (LSD) test were carried out. Significant differences were accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Identification and quantification of glycoalkaloids

The extraction and hydrolysis of GA from *S. macrocarpon* leaves were performed in a two-step procedure, which yielded intact GA, as well as their aglycones liberated during the hydrolysis. The application of one-step hydrolytic extraction in a solid-liquid-liquid system [16] resulted in excessive contamination of the final sample, even when the preliminary extraction of the plant material with petroleum ether was applied, and thus was not used in the study. The results of MALDI-TOF MS analysis revealed the presence of two main GA with $[M+H]^+$ signals at *m/z* 866 and 884 (Fig. 1A). The exact composition of the

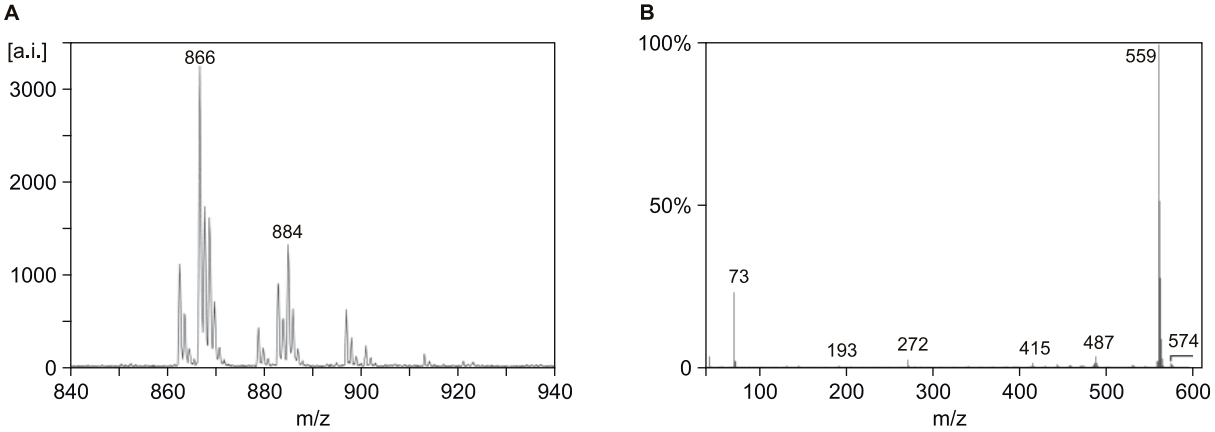

Fig. 1. Mass spectra of glycoalkaloids from *S. macrocarpon* leaves.

A – MALDI-TOF MS spectrum of the whole glycoalkaloid fraction. **B** – EI-MS spectrum from the GC-MS analysis, obtained for the trimethylsilyl derivative of the aglycone.

fraction remains unknown, as the relative intensities of these two signals varied between analyses. Minor peaks were present only in single samples, and they were not attributed to any GA in the further ESI-MS/MS experiment. The formation of $[M+H]^+$ signals was confirmed by the analysis of commercially available α-solanine, which gave an intensive signal at m/z = 868. The presence of the common solanidine-based GA α-chaconine and α-solanine was then excluded, as these compounds would give signals at m/z 852 and 868, respectively. Among solasodine-based GA, which were already reported in *S. macrocarpon* fruits [10], α-solamargine would give a signal at m/z = 868 and α-solasonine at m/z = 884. However, the presence of the latter compound was also excluded, based on the preliminary GC-MS analysis of *S. macrocarpon* GA hydrolysis products. The analysis revealed the presence of only one aglycone, showing an intensive signal at m/z = 559 and minor signals at m/z 73, 487 and 574 (Fig. 1B). Such a spectrum is not characteristic of any common solanidine- or solasodine-type aglycones, which usually give main signals at m/z = 150 and m/z = 125, respectively [20]. In general, the lack of characteristic signals in the m/z region from 50 to 200 does not allow the aglycone structure to be clearly defined [21]. The possible molecular ion at m/z = 559, as well as the presence of a weak signal at m/z = 147, suggest the presence of two trimethylsilyl (TMSi) groups, the probable molecular weight (MW) of the aglycone was then 415 Da. Similar spectra were observed for tomatidine and soladulcidine, but the base peak in both spectra was at m/z = 125 [20]. Also, Van Gelder et al. described solanidine-type aglycones, bearing two hydroxyl groups and called solanidanediols [22]. However, their mass spectra would have a base peak at m/z = 150. On the other hand, the mass spectrum of the aglycone also showed a weak signal at m/z = 574, which may correspond to a molecular ion signal. In this case, the m/z = 559 signal would be a $[M-15]^+$ ion, which is quite common in TMSi derivatives. However, as the MW would be even (430 Da), the aglycone would contain two nitrogen atoms instead of one. Similar solanocapsine-type compounds were previously described, but the lack of characteristic signals at m/z = 112 and m/z = 130 in the mass spectrum excludes this group of aglycones [21].

Additional ESI-MS/MS analyses suggested the presence of two different aglycones attached to a chacotriose saccharide residue, which was supported by the neutral loss series of 146/146/162 (rhamnose/rhamnose/glucose) in the fragmentation pattern of compounds giving $[M+H]^+$ at m/z = 866 and m/z = 884 [23]. The latter compound, however, shows more complex fragmentation, with the additional loss of one or two molecules of water, suggesting the presence of at least two hydroxyl groups in the structure. Possible aglycone ions are then present in the spectra at m/z = 412 and m/z = 430, suggesting molecules of MW = 411 Da and MW = 429 Da, respectively. The $[M+H]^+$ signal at m/z = 884 may correspond to two different GA, as a minor signal at m/z = 414 occurs in the spectrum. The fragmentation pattern would then be consistent with the structure containing an aglycone of MW = 413 Da and a solatriose saccharide residue (neutral loss series of 146/162/162, i.e. rhamnose/glucose/galactose). Glycoalkaloids showing identical mass spectra were reported in tubers of *S. bulbocastanum* by Shakya and Navarre, and were tentatively identified as compounds containing solanidadienol, solanidenone and solanidenediol aglycones [24]. The possible fragmentation pattern of both GA is given in Tab. 1. However, these results are somehow inconsistent with the results of GC-MS analysis. We cannot exclude the possibility that the aglycone molecule underwent structural re-arrangement during the hydrolysis. This inconsistency, along with the atypical mass spectrum of the liberated aglycone, may point to the different

Tab. 1. The postulated fragmentation pattern of the main *S. macrocarpon* leaf glycoalkaloids, based on the results of ESI-MS/MS analysis.

$[M+H]^+$	Most prominent ions	Possible fragment structures
866	848 (1)	$[M+H-H_2O]^+$
	720 (100)	$[M+H-Rha]^+$
	702 (2)	$[M+H-Rha-H_2O]^+$
	574 (7)	$[M+H-Rha-Rha]^+$
	412* (22)	$[M+H-Rha-Rha-Glc]^+$
	394 (4)	$[M+H-Rha-Rha-Glc-H_2O]^+$
884	866 (100)	$[M+H-H_2O]^+$
	848 (11)	$[M+H-2H_2O]^+$
	738 (3)	$[M+H-Rha]^+$
	720/722 (3)	$[M+H-Rha-H_2O]^+/[M+H-Glc]^+$
	592 (1)	$[M+H-Rha-Rha]^+$
	-/576 (1)	$[M+H-Rha-Glc]^+$
	430* (2)	$[M+H-Rha-Rha-Glc]^+$
	412/414* (4/2)	$[M+H-Rha-Rha-Glc-H_2O]^+/$ $[M+H-Rha-Glc-Gal]^+$
	394 (3)	$[M+H-Rha-Rha-Glc-2H_2O]^+$

Relative intensities of the ions are given in parentheses.
* – signals attributed to aglycones. Rha – rhamnose, Glc – glucose, Gal – galactose.

Tab. 2. Glykoalkaloid aglycone contents in raw and blanched *S. macrocarpon* leaves.

Compound	Raw		Blanched	
	Urafiki	UVPP	Urafiki	UVPP
Aglycone [mg·kg^{-1}]	638 ± 109^{a}	577 ± 50^{ab}	415 ± 165^{bc}	254 ± 14^{c}
Total glykoalkaloids (dw) [mg·kg^{-1}]	1343	1214	873	535
Total glykoalkaloids (fw) [mg·kg^{-1}]	242	206	157	91

Values are the means from GC-FID analyses of three independent samples ± standard deviation, and the estimated total glycoalkaloid content expressed as equivalent of the compound of molecular weight 865 Da. Total glykoalkaloids are expressed per kilogram of dry weight (dw) and fresh weight (fw), respectively.
Values not sharing the same letters are significantly different from one another ($p \leq 0.05$).

chemical character of *S. macrocarpon* GA, when compared with commonly reported compounds. Also, these compounds may be more similar to the frequently reported saponins, for which certain structural re-arrangements during hydrolysis have already been described [25]. When α-solanine was analysed using ESI-MS/MS in the same conditions, the mass spectrum obtained was identical with that reported by Väänänen et al. [23] and Shakya and Navarre [24]. Also, the GC-MS analysis of the hydrolysis products of α-solanine revealed the presence of solanidine, as expected. At this point of the study, we cannot fully resolve the structures of *S. macrocarpon* GA. We could not isolate pure substances for structure elucidation because of the very limited availability of the plant material. Further experiments, involving the isolation of pure substances and NMR studies, are necessary in order to fully characterize these compounds.

The quantitative analysis was based on the results of GC-FID experiments. Solasodine was not detected in any of the preliminarily hydrolysed and analysed GA samples, and was thus used as an internal standard. The results are presented in Tab. 2, together with the estimated GA content expressed as the equivalent of the compound with MW = 865 Da. The average precision, expressed as the relative standard deviation (RSD) obtained for the mean value from three GC-FID analyses of the same sample, was approx. 3% for the samples of both raw and blanched leaves. The differences in GA contents between single plants were relatively high, particularly in the Urafiki cultivar, which was expressed by the high standard deviations given in Tab. 2. The average decrease in total GA content observed during 5 min blanching was approx. 35% for Urafiki cultivar and approx. 56% for UVPP cultivar. In both cases, the processing significantly ($p \leq 0.05$) reduced the GA content to the values considered to be safe in food [10]. It is, however, worth noting that the safety level of 200 mg GA per kilogram fresh weight was calculated for potato GA, which are considered relatively toxic to mammals [26]. Also, the results suggest that the reduction in GA content may be cultivar-dependent and, in some cases, the decrease in GA may be lower than expected.

Fatty acid and sterol analyses

The composition and quantitative analysis of *S. macrocarpon* fatty acids were already reported by Glew et al. [7]. Hence, although fatty acids were analysed in this study, the lipid analysis focused on the phytosterol content. In general, the GC analysis of native sterols offers a poorer resolution when compared to the analysis of their TMSi derivatives [27]. These authors suggested using an only slightly polar stationary phase of high thermal stability, e.g. 5% diphenyl – 95% dimethylpolysiloxane. The analysis of unsaturated FA using such a column does not permit unequivocal determination of the position and configuration of double bonds. Thus, identification of FA in this study was restricted to determination of chain length and the number of double bonds. The total content of FA and sterols was determined directly in the hydrolysed lipid extracts. The composition of FFA, free sterols and steryl esters was specified by the analysis of hydrolysed fractions obtained using flash-chromatography on silica gel.

A total of 11 FFA was identified on the basis of GC-MS analyses. Saturated fatty acids (SFA) accounted for more than 50% of total FFA in raw leaves, followed by polyunsaturated FA (PUFA) represented exclusively by C_{18} compounds, and minor quantities of monounsaturated FA (MUFA). The PUFA content in the FFA fraction was substantially lower than the value reported by Glew et al. in the total lipids of *S. macrocarpon* leaves [7]. As expected, during thermal processing of the leaves, the amount of FFA decreased significantly (by 65–80%) in both cultivars. In addition, the SFA/PUFA ratio increased from 1.14 to 3.36 in Urafiki cultivar, and from 1.67 to 3.51 in UVPP cultivar. Hence, the changes in FFA

content induced by blanching of leaves were unfavourable if the nutritional value is concerned. On the other hand, the free sterol fraction showed the opposite trend – we observed a significant increase in free sterols in processed samples, which suggests that an additional pool of sterols becomes available during blanching. At this stage of experimentation, however, we were unable to clearly identify the source of these sterols. Significant differences were detected between the studied cultivars. The free sterol fraction consisted of four commonly reported plant 4-desmethylsterols (campesterol, stigmasterol, β-sitosterol and isofucosterol). Small quantities of cholesterol, which is commonly reported in solanaceous plants [28], were also detected. This was the only component of the fraction that was unaffected by processing of leaves, and its contribution to the total free sterols decreased from 16–18% in raw leaves to 9–10% in blanched leaves. The identification of all sterols was based on mass spectra and retention parameters of their TMSi derivatives in GC-MS and GC-FID analyses [29]. Detailed results of the quantitative analysis of FFA and free sterols are given in Tab. 3.

The amounts of sterols liberated from steryl esters were 3–6 times higher than the values reported for free compounds. In addition to the previously identified 4-desmethylsterols, two 4,4-dimethylsterols (cycloartenol and 24-methylenecycloartanol) and one 4-methylsterol (citrostadienol) were detected. These 4,4-dimethylsterols accounted for 41–43% of esterified sterols. The total quantity of steryl esters, as well as the amounts of single sterols, were not affected by blanching, and raw and processed leaves contained comparable levels of these compounds. The cholesterol content did not exceed 6–7% of total sterols in all samples. The FA profile was different from that in FFA, the most abundant compounds being C_{18} PUFA, which accounted for more than 70% in all samples. As a consequence,

Tab. 3. Composition of free fatty acids and free sterols from raw and blanched *S. macrocarpon* leaves.

Compound	Raw		Blanched	
	Urafiki	UVPP	Urafiki	UVPP
Composition of the fatty acid fraction [%]				
12:0	2.0	4.0	9.1	10.0
14:0	2.4	3.9	8.6	10.7
16:1	1.2	1.2	2.9	2.7
16:0	31.9	36.2	33.1	35.3
18:3	26.0	20.6	13.5	13.7
18:2	19.9	16.4	8.3	7.6
18:1	0.8	0.8	1.6	1.2
18:0	11.9	13.8	14.8	15.4
20:0	1.4	1.1	2.1	1.4
22:0	1.5	1.1	3.7	1.3
24:0	1.1	0.9	2.4	0.7
Total content [g·kg^{-1}]				
Σ SFA	1.44 ± 0.14^{a}	1.22 ± 0.22^{a}	0.42 ± 0.03^{b}	0.53 ± 0.07^{b}
Σ MUFA	0.06 ± 0.01^{a}	0.04 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.01^{c}
Σ PUFA	1.26 ± 0.08^{a}	0.73 ± 0.07^{b}	0.13 ± 0.02^{c}	0.15 ± 0.02^{c}
Total free fatty acids	2.76 ± 0.21^{a}	1.99 ± 0.30^{b}	0.58 ± 0.06^{c}	0.70 ± 0.09^{c}
Cholesterol	0.06 ± 0.01^{a}	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.09 ± 0.03^{a}
Campesterol	0.02 ± 0.01^{a}	0.04 ± 0.01^{b}	0.06 ± 0.01^{c}	0.07 ± 0.01^{d}
Stigmasterol	0.08 ± 0.01^{a}	0.14 ± 0.02^{b}	0.19 ± 0.01^{c}	0.23 ± 0.04^{c}
β-Sitosterol	0.14 ± 0.02^{a}	0.24 ± 0.02^{b}	0.40 ± 0.04^{c}	0.53 ± 0.09^{d}
Isofucosterol	0.02 ± 0.01^{a}	0.04 ± 0.01^{a}	0.07 ± 0.01^{b}	0.08 ± 0.02^{b}
Cycloartenol	nd	nd	nd	nd
24-Methylenecycloartanol	nd	nd	nd	nd
Citrostadienol	nd	nd	nd	nd
Total free sterols	0.32 ± 0.04^{a}	0.54 ± 0.05^{b}	0.79 ± 0.06^{c}	1.00 ± 0.17^{d}

The content of single free fatty acids is given as a percentage of the fraction, quantitative values are given as mean values from GC-FID analyses of three independent samples ± standard deviation in grams per kilogram of dry weight.
Values in the same row not sharing the same letters are significantly different from one another ($p \leq 0.05$).
SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, nd – not detected.

Tab. 4. Composition of fatty acids and sterols in the ester/triacylglycerol fraction from raw and blanched *S. macrocarpon* leaves.

Compound	Raw		Blanched	
	Urafiki	UVPP	Urafiki	UVPP
Composition of the fatty acid fraction [%]				
12:0	0.4	0.4	0.2	0.6
14:0	1.0	1.2	1.2	1.4
16:1	1.1	1.1	1.2	1.2
16:0	16.4	16.6	14.8	15.6
18:3	36.1	36.2	33.7	33.4
18:2	37.0	36.3	38.0	38.0
18:1	0.5	0.4	0.5	0.4
18:0	4.7	5.1	5.4	5.2
20:0	1.4	1.3	2.2	2.1
22:0	1.1	1.0	2.0	1.6
24:0	0.4	0.3	0.8	0.5
Total content [g·kg^{-1}]				
Σ SFA	1.59 ± 0.09^{a}	1.41 ± 0.10^{a}	1.36 ± 0.30^{a}	1.21 ± 0.19^{a}
Σ MUFA	0.10 ± 0.01^{a}	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	0.07 ± 0.02^{a}
Σ PUFA	4.57 ± 0.19^{a}	3.92 ± 0.20^{ab}	3.65 ± 0.53^{b}	3.21 ± 0.47^{b}
Total fatty acids	6.26 ± 0.10^{a}	5.42 ± 0.30^{ab}	5.10 ± 0.83^{b}	4.50 ± 0.55^{b}
Cholesterol	0.12 ± 0.04^{a}	0.11 ± 0.04^{a}	0.11 ± 0.01^{a}	0.11 ± 0.04^{a}
Campesterol	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}
Stigmasterol	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}
β-Sitosterol	0.45 ± 0.05^{a}	0.43 ± 0.04^{a}	0.43 ± 0.03^{a}	0.49 ± 0.07^{a}
Isofucosterol	0.23 ± 0.04^{a}	0.22 ± 0.04^{a}	0.23 ± 0.02^{a}	0.24 ± 0.07^{a}
Cycloartenol	0.66 ± 0.23^{a}	0.55 ± 0.10^{a}	0.62 ± 0.08^{a}	0.70 ± 0.30^{a}
24-Methylenecycloartanol	0.15 ± 0.03^{a}	0.13 ± 0.02^{a}	0.15 ± 0.03^{a}	0.13 ± 0.03^{a}
Citrostadienol	0.13 ± 0.04^{a}	0.10 ± 0.03^{a}	0.13 ± 0.01^{a}	0.12 ± 0.04^{a}
Total sterols	1.88 ± 0.40^{a}	1.67 ± 0.26^{a}	1.80 ± 0.13^{a}	1.92 ± 0.52^{a}

The content of single fatty acids is given as a percentage of the fraction, quantitative values are given as mean values from GC-FID analyses of three independent samples ± standard deviation in grams per kilogram of dry weight.
Values in the same row not sharing the same letters are significantly different from one another ($p \leq 0.05$).
SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

the SFA/PUFA ratio was approx. 0.33–0.37. A statistically significant decrease in PUFA and total FA was, however, observed as an effect of blanching of Urafiki cultivar. The molar ratio of sterols and FA in the ester fraction was approx. 0.22–0.24. Consequently, most of FA detected in the fraction were liberated from triacylglycerols (TAG) and wax esters, which in flash-chromatography were eluted together with steryl esters. Wax esters, however, were present in trace amounts, as we detected only small quantities of primary alcohols, mainly C_{28}. The results of the GC-FID analyses of the ester/TAG fraction are given in Tab. 4 and those of FA and sterols liberated from total lipids of *S. macrocarpon* leaves are given in Tab. 5. The sum of FFA and FA liberated from the ester/TAG fraction accounted for only 38% and 23% of total FA in raw leaves of Urafiki and UVPP cultivars, respectively. The corresponding values obtained for processed leaves were 25% and 17%, respectively. Free sterols and sterols liberated from steryl esters accounted for 64–68% of total sterols in raw leaves, and 73% of total sterols in blanched leaves of both cultivars. The FA profile was similar to that previously reported by Glew et al. [7]. The most abundant compounds were C_{18} PUFA, and the SFA/PUFA ratio was 0.48–0.50 in UVPP cultivar and 0.42–0.44 in Urafiki cultivar. However, the absolute amount of FA was approx. 3 times higher than previously described [7]. The increase in sterol content, in comparison to the content of free and esterified sterols, was associated with elevated 4-desmethylsterols, probably originating from the hydrolysis of glycosylated forms. The composition of the fraction varied broadly from sample to sample, particularly where the cycloartenol and citrostadienol content was concerned. Cholesterol accounted for 7–9% of total sterols. Both total FA and sterols were unaffected by the processing of the leaves and no significant differ-

Tab. 5. The total fatty acid and sterol contents in lipids from raw and blanched *S. macrocarpon* leaves.

Compound	Raw		Blanched	
	Urafiki	UVPP	Urafiki	UVPP
Composition of the fatty acid fraction [%]				
12:0	0.3	0.2	0.2	0.2
14:0	0.6	0.6	0.6	0.6
16:1	1.7	2.4	1.4	1.6
16:0	21.2	24.2	21.0	23.3
18:3	43.3	44.5	42.5	43.5
18:2	25.4	20.0	25.7	22.4
18:1	0.6	0.8	0.7	0.9
18:0	6.0	6.3	6.3	6.3
20:0	1.0	1.0	0.8	1.1
22:0	nd	nd	0.55	nd
24:0	nd	nd	0.24	nd
Total content [g·kg^{-1}]				
Σ SFA	6.92 ± 0.83^{a}	10.38 ± 3.79^{a}	6.82 ± 0.74^{a}	9.41 ± 3.14^{a}
Σ MUFA	0.54 ± 0.11^{a}	1.02 ± 0.42^{a}	0.48 ± 0.16^{a}	0.76 ± 0.36^{a}
Σ PUFA	16.33 ± 1.51^{a}	20.70 ± 2.89^{a}	15.67 ± 2.22^{a}	19.62 ± 3.62^{a}
Total fatty acids	23.79 ± 2.08^{a}	32.10 ± 6.98^{a}	22.96 ± 3.11^{a}	29.78 ± 5.92^{a}
Cholesterol	0.25 ± 0.08^{a}	0.28 ± 0.14^{a}	0.25 ± 0.04^{a}	0.29 ± 0.09^{a}
Campesterol	0.26 ± 0.04^{a}	0.27 ± 0.08^{a}	0.22 ± 0.02^{a}	0.31 ± 0.02^{a}
Stigmasterol	0.34 ± 0.05^{a}	0.36 ± 0.11^{a}	0.33 ± 0.04^{a}	0.41 ± 0.28^{a}
β-Sitosterol	1.24 ± 0.48^{a}	1.12 ± 0.46^{a}	1.36 ± 0.16^{a}	1.50 ± 0.37^{a}
Isofucosterol	0.36 ± 0.05^{a}	0.34 ± 0.10^{a}	0.39 ± 0.02^{a}	0.40 ± 0.12^{a}
Cycloartenol	0.59 ± 0.23^{a}	0.49 ± 0.13^{a}	0.67 ± 0.09^{a}	0.72 ± 0.46^{a}
24-Methylenecycloartanol	0.22 ± 0.05^{a}	0.22 ± 0.12^{a}	0.21 ± 0.05^{a}	0.23 ± 0.02^{a}
Citrostadienol	0.17 ± 0.13^{a}	0.16 ± 0.09^{a}	0.16 ± 0.05^{a}	0.17 ± 0.11^{a}
Total sterols	3.44 ± 0.23^{a}	3.24 ± 0.87^{a}	3.55 ± 0.38^{a}	4.03 ± 0.42^{a}

The content of single fatty acids is given as a percentage of the fraction, quantitative values are given as mean values from GC-FID analyses of three independent samples ± standard deviation in grams per kilogram of dry weight.
Values in the same row not sharing the same letters are significantly different from one another ($p \leq 0.05$).
SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, nd – not detected.

ences were detected among all samples.

During the analysis of TMSi derivatives of FA, liberated from the ester fraction or the total lipids, we observed random formation of artefacts, which were not formed when the FFA fraction was analysed. In general, unsaturated FA with 18 carbon atoms elute before the saturated C_{18} compound on slightly polar and non-polar stationary phases. However, in addition to the signals identified during the FFA analysis, two more signals appeared, eluting just after the 18:0 compound (Fig. 2A, 2B). Their mass spectra confirmed that they are 18:3 (signal A) and 18:2 (signal B) FA. There are no common methylene-interrupted C_{18} unsaturated FA with such an elution order [30]. Analysis of the standard conjugated linoleic acid (CLA) suggested that signal B corresponded to CLA. The other, however, eluted too early to be a conjugated 18:3 FA [31]. Also, the analysis of hydrolysed lipids from lettuce and common eggplant dried leaves revealed the presence of the same signals. In contrast, when the lipids from fresh tissue of *S. macrocarpon* were analysed, there were hardly any artefacts (Fig. 2C). Moreover, the analysis of FAME, which were synthesized after the hydrolysis of lipids from dried *S. macrocarpon* leaves, revealed the presence of only minor quantities of these compounds (Fig. 2D). It was reported that the hydrolysis of linoleic acid esters alone did not result in the formation of CLA [32]. We confirmed these findings by hydrolysing methyl linoleate in the same conditions in which the lipid samples were processed. The formation of artefacts was not detected in this experiment. Summing up, the results suggest that the formation of artefacts during the hydrolysis and silylation of FA is a complex process and is observed mainly in lipid samples from dry plant material. On the other hand, the presence of minor quantities of artefacts in methylated samples indicates that their

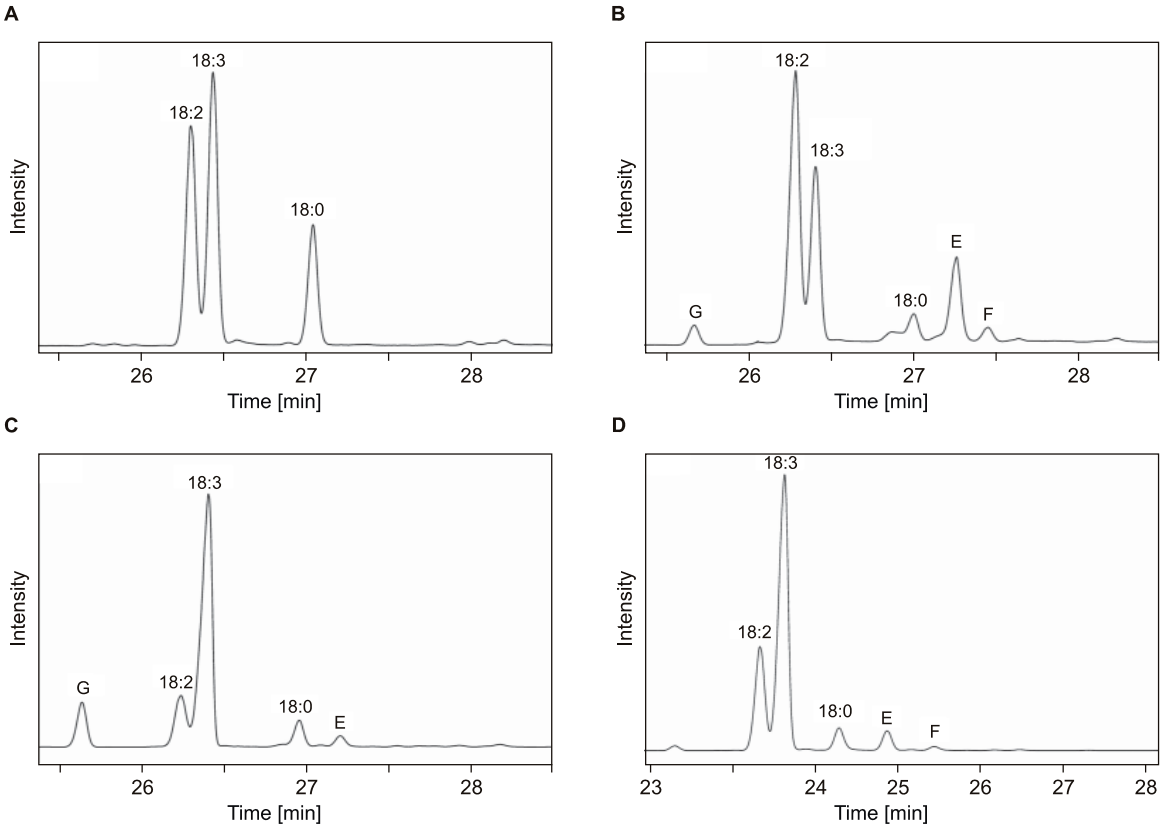

Fig. 2. Chromatograms from GC-FID analyses showing the C18 fatty acid region of *S. macrocarpon* lipid fractions.

A – TMSi derivatives of free fatty acids extracted from the dried material, B – TMSi derivatives of FA liberated during the hydrolysis of total lipids from the dried material, C – TMSi derivatives of FA liberated during the hydrolysis of total lipids from the fresh material, D – methyl esters of FA liberated during the hydrolysis of total lipids from the dried material.
E – unidentified 18:3 fatty acid, F – possible conjugated 18:2 fatty acid, G – phytol.

formation is somehow associated with the sample processing method, and not just with the hydrolysis and silylation of FA. As the concentration of CLA in plant material is usually very low, it may be only concluded that CLA detected in samples was somehow formed during sample preparation and was not present in the original sample. The mechanism of its formation during analytical process will be studied in the future. At this point, we were unable to fully characterize the structure of the second compound, which was identified as an unknown 18:3 FA. It was present almost exclusively in samples after hydrolysis/silylation, and there were no procedures available, which would allow preparative separation of single TMSi derivatives of FA, as these are strongly susceptible to chemical degradation. When interpreting the analytical results obtained for TMSi derivatives of FA, these observations should be taken into account. However, while the intensities of the artefact signals varied broadly from one analysis to another, the total area of all the signals attributed to 18:2 and 18:3 FA remained constant for one sample. The average RSD value for the GC-FID analyses of C_{18} PUFA in the ester/TAG fraction was less than 2%, thus making the quantitative analysis repeatable and highly reliable.

CONCLUSIONS

The impact of blanching on the composition of glycoalkaloids, fatty acids and sterols from *Solanum macrocarpon* leaves was studied. The GA content was significantly reduced during this process and reached values regarded as safe in foods. The amounts of FA and sterols detected in *S. macrocarpon* leaves were relatively high when compared with other vegetables [7, 14]. The elevated contents of PUFA, as well as phytosterols, makes *S. macrocarpon* a valuable secondary source of these compounds. Leaf blanching re-

duced the amounts of FFA, but did not affect the overall sterol and FA contents. Summing up, leaf processing reduces the potential toxicity of *S. macrocarpon*, without affecting its nutritional values as far as the lipid composition is concerned. Additional study on identification of GA and assessment of their toxicity is needed.

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