Effect of Microwave-Assisted Aqueous Two-Phase Extraction of α -Solanine from *S. retroflexum* and Analysis on UHPLC-qTOF-MS

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Received: 23 July 2021 / Accepted: 27 December 2021 / Published online: 13 January 2022
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Abstract

A new, fast and efficient method, hyphenated microwave-assisted aqueous two-phase extraction (MA-ATPE) was applied in the extraction of α -solanine from *Solanum retroflexum*. This environmentally friendly extraction method applied water and ethanol as extraction solvents. Central composite design (CCD) was performed which included numerical parameters such as time, mass of plant powder and microwave power. The categorical factors included the chaotrope — NaCl or the kosmotrope — Na₂CO₃. Fitting the central composite design response surface model to the data generated a quadratic model with a good fit ($R^2=0.920$). The statistically significant ($p<0.05$) parameters such as time and mass of plant powder were influential in the extraction of α -solanine. Quantification of α -solanine was achieved using a robust and sensitive feature of the ultra-high performance quadrupole time of flight mass spectrometer (UHPLC-qTOF-MS), multiple reaction monitoring (MRM). The optimized condition for the extraction of α -solanine in the presence of NaCl and Na₂CO₃ was a period of 1 min at a mass of 1.2 g using a microwave power of 40%. Maximal extraction of α -solanine was 93.50 mg kg⁻¹ and 72.16 mg kg⁻¹ for Na₂CO₃ and NaCl, respectively. The synergistic effect of salting-out and microwave extraction was influential in extraction of α -solanine. Furthermore, the higher negative charge density of the kosmotrope (Na₂CO₃) was responsible for its greater extraction of α -solanine than chaotrope (NaCl). The shorter optimal extraction times of MA-ATPE make it a potential technique that could meet market demand as it is a quick, green and efficient method for removal of toxic metabolites in nutraceuticals.

Keywords α -Solanine · *S. retroflexum* · Response surface methodology · MA-ATPE · UHPL-qTOF-MS

Highlights

- Optimization of MA-ATPE resulted in good yields of α -solanine from *Solanum retroflexum*.
- The kosmotrope-Na₂CO₃ was a better extractor of α -solanine than the chaotrope-NaCl.
- The mass of plant powder and time had a significant ($p < 0.05$) effect on extraction.
- MA-ATPE is a green and competitive technique for reducing food toxicity.
- The enriched metabolites from MA-ATPE could potentially be applied in pharmacology.

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Introduction

Solanum retroflexum is one of many species within the Solanaceae family widely distributed and boasting over 3000 species of trees, shrubs and herbs. The consumption of *Solanum retroflexum* remains controversial in different cultural practices (Averbeke et al. 2007; Managa et al. 2020). In South Africa, *Solanum retroflexum* is an exclusively produced and consumed vegetable by African people, and due to its high nutrient composition in its leaves, its affordable means to alleviate malnutrition amongst poor rural based South Africans (Averbeke et al. 2007). On the contrary, *Solanum retroflexum* is an inedible plant and persistent weed in Europe and America, as it is perceived to be toxic (Karabegović et al. 2018).

The edibility or inedibility of *Solanum retroflexum* is due to its metabolic composition. Some of the classes of secondary metabolites derived from *Solanum* plants include polyphenols such as flavonoids, widely renowned for its

antioxidant activities (Uchida et al. 2017; Mahieddine et al. 2018; Fratianni et al. 2020) and steroidal alkaloids widely reported for its toxic effects. The surge in concentration levels of steroidal alkaloids (glycoalkaloids) is triggered by the exposure of *Solanum* plants to the sun's UV light or because of mechanical injury including peeling and slicing (Dao and Friedman 1994; Kasnak and Artik 2018). Some of the toxic effects of glycoalkaloids are due to anticholinesterase effects on the central nervous system (Caprioli et al. 2014; Lelario et al. 2019) and disruption of cell membranes (Blankemeyer et al. 1998; Nepal and Stine 2019). Symptoms of glycoalkaloid poisoning in humans include colic pain in the abdomen and stomach, diarrhoea, vomiting, burning sensation about the lips and mouth, fever, rapid pulse and headache (Uluwaduge et al. 2018; Deng et al. 2021). Other destructive effects of glycoalkaloids include craniofacial malformations in hamsters (Gaffield and Keeler 1996; Ni et al. 2018; Kumar et al. 2019) and a variety of organ malformations in frog embryos and mealworms (Friedman et al. 1991; Chen et al. 2021). Furthermore, the Centre for Food Safety (2015) reported on poisoning of patients after consumption of cooked potatoes, and subsequent investigations revealed that the poisoning was due to the glycoalkaloid α -solanine. As a result, regulatory bodies such as the Commission for Food and Agricultural Organization (FAO) and the World Health Organization (WHO) have established regulations for maximum permissible concentrations of glycoalkaloids, which currently stands at 200 mg kg⁻¹ for fresh potatoes (*Solanum lycopersicum*) sold in supermarkets.

In view of the toxic potential of glycoalkaloids contained in *Solanum* vegetables, food toxicology regulating bodies have come-up with maximum allowable limits of these metabolites. Besides these policy frameworks, more still need to be done to improve the nutritional value and safety of some plant foods, especially those consumed by humans. This could come in the form of environmentally friendly extraction techniques that target these poisonous glycoalkaloids in food. A variety of extraction methods have been studied for enrichment of glycoalkaloids from *Solanum tuberosum*. This includes bisolvent extraction using methanol and chloroform (2:1 v/v) and Soxhlet extraction involving 3% acetic acid in ethanol (Wang et al. 1972; Fried-niece et al. 2017; Abedini et al. 2020; Kaunda et al. 2021). However, these methods often apply toxic organic solvents and at time are used in large quantities, making the extraction method user-unfriendly. Furthermore, these techniques require multiple extraction steps from time to time making the process tedious and resulting in huge losses of the analytes of interest. Aqueous two-phase extraction (ATPE) has attracted interest amongst researchers for it's due to biphasic extraction capacity and selectivity, resulting in the achievement of target constituents that could be extracted either in the top or bottom phase (Xie et al. 2017, 2021). More

importantly, it is a green and efficient pre-treatment solution for separation and purification of compounds from natural products (Xie et al. 2017; Li et al. 2020).

In the view that ATPE has potential as a versatile technique, very little is known on the coupling of ATPE to other extraction methods, which could in turn attract interest amongst researchers. Hence, in this study, the one-pot extraction, microwave-assisted aqueous two phase extraction (MA-ATPE) of a toxic metabolite, α -solanine, from *Solanum retroflexum* was investigated. Additionally, information on multivariate approaches for extraction of toxic compounds from natural food sources is scarce. Therefore, this work also applied optimization based on chemometric tools such as central composite design (CCD) and response surface methodology (RSM) for α -solanine in *Solanum retroflexum*. The CCD and RSM approach are useful as it reduces the number of experiments, making it less laborious and time efficient (Silva et al. 2019). Application of MA-ATPE could be a fast, environmentally friendly and efficient method for extraction of α -solanine that could be vital by reducing toxicity of a popular vegetable, *Solanum retroflexum*, making it safe for consumption. Furthermore, this hyphenated environmentally friendly extraction technique could potentially be utilized on a commercial scale.

Materials and Methods

Chemicals and Reagents

The salts NaCl (anhydrous $\geq 99\%$ purity), Na₂CO₃ (anhydrous $\geq 99\%$ purity) and ethanol (99% CP) were purchased from Associated Chemical Enterprises (Johannesburg, South Africa) and Sigma-Aldrich (Johannesburg, South Africa). Ultra-pure water (0.005 μ S, 18 m Ω) using a Direct-Q 5UV distiller (MA, USA) was applied for the preparation of the salt solutions. A modified microwave oven (DM 350, Defy, Polokwane, South Africa) of 28 L capacity, working at a frequency of 2450 MHz, was used for microwave-assisted extraction. Chromatographic separation of the metabolites in the extracts was done using a reverse phase Shim-pack Velox C₁₈, 2.1 $\times$ 100 mm, 2.7 μ m with a serial number 227–32,009-03 (Columbia, USA). The UPLC was connected to a Shimadzu 9030 LC, qTOF-MS detector (Shimadzu, Kyoto). The solvents used for the chromatographic runs were methanol and formic acid, which were purchased from Romil Pure Chemistry (Cambridge, UK).

Sample Collection and Preparation

The leaves of *Solanum retroflexum* were obtained from a street vendor within the Thulamela District in Thohoyandou, South Africa. The plants were air dried until a constant

weight was obtained, and the leaves were ground into a fine powder with a blender at 2000 rpm and stored in glass containers. The containers were covered in paper bags to prevent light penetration. The MA-ATPE method consisted of powdered leaves placed in a 250-mL round bottomed flask (Fig. 1). Thereafter, saturated salt concentrations (5 mL) of 30% (w/v) involving Na_2CO_3 (kosmotrope) and NaCl (chaotrope) were prepared by weighing 15 g of salt in 50 mL of water and were added to the powdered leaves in the round bottomed flask. Thereafter, 99% ethanol extraction solvent (5 mL) was added resulting in ATPE (Fig. 1). The spontaneous formation of ATPE under the conditions stated above was also reported by Mokgehele et al. (2021). The ATPE mixture was then placed in a microwave oven (Fig. 1). This was conducted so that the microwaves could enhance extraction in ATPE, MA-ATPE. The volumes of the solvents after the extraction period remained unaltered. The setup of the MA-ATPE systems is shown in the appendix (Fig. 1). The MA-ATPE extracts, top-layer of ATPE system, obtained were then analysed on the UPLC-qTOF-MS for α -solanine.

Chromatographic and Mass Spectrometry Conditions

α -Solanine was separated using the column stated in the “Chemicals and Reagents” section. The column was maintained at 40 °C at a flow rate of 0.4 mL min⁻¹, and the injection volume was 5 μ L. Mobile phase A was 0.1% formic acid in ultrahigh purity water (v/v), and mobile phase B was 0.1% (v/v) formic acid in methanol.

The ultra-high performance quadrupole time of flight mass spectrometer (UHPLC-qTOF-MS) 9030 mass spectrometer was equipped with an electrospray interface (ESI) operating in positive mode. The ESI parameters were optimized for α -solanine by direct infusion of standard solutions into the mass spectrometer. The mass spectrometer was

operated in the multi reaction monitoring (MRM) mode to confirm the identity of α -solanine. High-purity nitrogen (N_2) was used as the nebulizing and drying gas. The optimum parameters were as follows: drying gas temperature, 250 °C; drying gas flow, 10 L min⁻¹; and collision energy, 50–80 V. Lab solutions software was used to run the LC-MS/MS instrument for data acquisition, and the mass range used was m/z 100–1000.

Preparation of Standards and Quantification of Samples

The stock α -solanine standard solution was prepared in methanol at a concentration of 1000 $\mu\text{g L}^{-1}$. The stock standard solution was stored at 4 °C in amber volumetric flasks. A series of nine working standard solutions at the concentration values of 15 to 1000 $\mu\text{g L}^{-1}$ were prepared from the stock standard solution by diluting with HPLC grade methanol. The α -solanine standards were quantified based on scheduled multiple reaction monitoring (MRM) where one m/z transition, from the precursor ion to the product ion, for α -solanine (868 $\rightarrow$ 722) was explored. The regression equation was $y = 52.1677x + 624.135$, the limit of detection (LOD) and limit of quantification (LOQ) were 0.3169 and 0.9509, respectively. The abovementioned transition was then applied for quantification of α -solanine from the ground leaves of *Solanum retroflexum* following MA-ATPE extraction. MRM quantification of α -solanine in the 36 samples studied was performed in duplicates (Table 1). The parameters evaluated for optimization of MA-ATPE of α -solanine were time, mass of plant powder, microwave power and the salt type (kosmotrope or chaotrope).

Statistical Analysis

The central composite design response surface model (CCD RSM) was fitted to experimental data to obtain the relationship between factors and optimize the response of Z (α -solanine yield) in relation to A (time), B (plant mass) using Design Expert 11 (Minneapolis, USA). By using CCD, a total of 36 experimental runs, done in duplicate, were designed which included 3 numerical factor levels for time (1 min, 5 min 30 s and 10 min), 3 factor numerical levels for mass of plant powder (0.2, 0.7, 1.2 g), 3 numerical factor levels for power (40, 70, 100%) and 2 categorical factor levels for salts which included the chaotrope (NaCl) and kosmotrope (Na_2CO_3).

The interaction between the various parameters studied and its resultant effected on the extraction of α -solanine (mg kg^{-1}) was fitted to experimental data by using a statistical multiple regression approach method of least square (MLS) and resulted in the lowest possible residual

Fig. 1 MA-ATPE setup containing the powdered *Solanum mauritianum* plant from which α -solanine was extracted

Table 1 Design layout from input variables in the central composite design and responses

Run	Factor 1:	Factor 2:	Factor 3:	Factor 4:	Responses		Mean $\pm$ SD Pre-dicted	
	Time (min)	Mass of plant powder(g)	Power (%)	Salt	Run 1	Run 2		
1	1	0.2	100	NaCl	32.07	36.19	34.13 $\pm$ 2.9	35.79
2	1	0.2	40	Na ₂ CO ₃	27.34	71.32	49.33 $\pm$ 31	45.89
3	1	1.2	40	Na ₂ CO ₃	72.00	115.0	93.00 $\pm$ 30	88.57
4	1	0.2	100	Na ₂ CO ₃	30.21	46.73	38.47 $\pm$ 11	34.3
5	1	0.2	40	NaCl	20.22	24.01	22.12 $\pm$ 2.7	23.27
6	1	1.2	40	NaCl	64.32	80.00	72.16 $\pm$ 11	73.36
7	1	1.2	100	NaCl	51.10	84.00	67.55 $\pm$ 23	67.39
8	1	0.7	70	NaCl	42.03	65.39	53.71 $\pm$ 16	58.46
9	1	1.2	100	Na ₂ CO ₃	45.28	71.37	58.32 $\pm$ 25	58.46
10	1	0.7	70	Na ₂ CO ₃	51.42	72.00	61.71 $\pm$ 15	65.31
11	5.5	0.7	70	Na ₂ CO ₃	15.16	100.0	57.58 $\pm$ 59	41.66
12	5.5	0.7	70	Na ₂ CO ₃	15.41	40.07	27.74 $\pm$ 17	41.66
13	5.5	0.7	40	Na ₂ CO ₃	32.55	62.03	47.29 $\pm$ 21	56.86
14	5.5	0.7	100	Na ₂ CO ₃	20.78	29.14	24.96 $\pm$ 6	28.72
15	5.5	0.7	70	NaCl	53.41	69.01	61.21 $\pm$ 11	57.23
16	5.5	0.7	100	NaCl	50.67	67.18	58.93 $\pm$ 12	56.35
17	5.5	1.2	70	Na ₂ CO ₃	34.28	53.55	43.92 $\pm$ 14	47.18
18	5.5	0.2	70	NaCl	22.69	32.57	27.63 $\pm$ 6.9	28.69
19	5.5	0.7	70	NaCl	62.90	65.14	64.02 $\pm$ 1.5	57.23
20	5.5	0.7	40	NaCl	50.73	73.00	61.86 $\pm$ 16	60.38
21	5.5	1.2	70	NaCl	59.35	73.00	66.17 $\pm$ 9.6	66.48
22	5.5	0.7	70	Na ₂ CO ₃	36.47	48.10	42.28 $\pm$ 8.2	-
23	5.5	0.2	70	Na ₂ CO ₃	10.82	13.63	12.22 $\pm$ 1.9	16.84
24	5.5	0.7	70	NaCl	51.12	70.92	61.02 $\pm$ 14	57.23
25	5.5	0.7	70	Na ₂ CO ₃	57.35	30.16	43.75 $\pm$ 19	41.66
26	5.5	0.7	70	NaCl	48.55	65.14	56.85 $\pm$ 12	-
27	10	1.2	40	Na ₂ CO ₃	34.45	42.71	38.58 $\pm$ 5.8	37.01
28	10	0.7	70	NaCl	30.09	49.86	39.97 $\pm$ 13	47.53
29	10	0.2	40	Na ₂ CO ₃	13.65	67.28	40.46 $\pm$ 37	-
30	10	0.2	100	Na ₂ CO ₃	-	-	-	-
31	10	0.2	100	NaCl	12.95	25.47	19.21 $\pm$ 8.8	20.61
32	10	1.2	40	NaCl	48.38	81.00	64.69 $\pm$ 23	66.66
33	10	0.7	70	Na ₂ CO ₃	-	33.25	33.24	9.54
34	10	1.2	100	NaCl	4.739	44.78	24.76 $\pm$ 28	-
35	10	1.2	100	Na ₂ CO ₃	16.65	40.01	28.33 $\pm$ 16	-
36	10	0.2	40	NaCl	23.26	26.59	24.93 $\pm$ 2.3	22.69

(Baş and Boyacı 2007). Model parameters and model significance were determined at $p < 0.05$. The fitness of the model was determined by evaluating the coefficient of regression (R^2) obtained from the analysis of variance (ANOVA). The model fit generated the response surface that defined the behaviour of the response variable. By means of these plots, the optimized ranges for each factor that led to the highest response (i.e. concentration of α -solanine) that can be extracted (Baş and Boyacı 2007; Arteaga-Crespo et al. 2020).

Results and Discussion

MRM Quantification of α -Solanine Based on the 868 $\rightarrow$ 722 Transition

In this study, the extraction of α -solanine was performed of which was reported to be contained in *Solanum retroflexum*, using a hyphenated MA-ATPE approach (Daji et al. 2018; Mokgehele et al. 2021). The MA-ATPE was modified using different factors as shown in Table 1, on the recovery of target metabolite α -solanine. The presence

of α -solanine has been reported in *Solanum retroflexum* and other species within the *Solanum* genus (Daji et al. 2018; Mokgehele et al. 2021). Using a sensitive and robust tandem MS approach (UHPLC-qTOF-MS) with settings presented elsewhere (Gbashi et al. 2016; Mokgehele et al. 2021), it was possible to efficiently fingerprint these α -solanine as shown in Fig. 2 based on m/z 722 product ion. Thereafter, based on the $868 \rightarrow 722$ transition within the MRM method, α -solanine was quantified as a function of the various factors shown in Table 1.

Fit Statistics of Experimental and Predicted Data

The model fitted to the data was observed to have a quadratic fit; p values less than 0.001 indicate model terms are significant. The following terms, mass of plant powder, time and power, were found to be significant ($p < 0.05$), while power² was found to be insignificant when both the chaotrope and kosmotrope salts were applied during extraction (Fig. 3a and b). This indicated that the linear terms were adequate predictors of the experimental values obtained. The quadratic effect of the terms studied was found to be insignificant for Na_2CO_3 in particular (Fig. 3b). The linear effect of a variable indicates that the variable correlates in a directly proportional manner to the response variable (α -solanine), whereas the quadratic effect of a variable implies that the response variable is correlated with the square of that variable (Gbashi et al. 2016). In the same work, the authors highlighted that a significant linear effect of a variable ($p < 0.05$) means that the optimal level of the response falls out of the range of the experimental values for that variable; similarly, this was observed for Na_2CO_3 (Fig. 3b). The F value was observed to be 0.37 which indicated that it was not significant relative to the absolute error. The non-significant lack of fit was desirable. The goodness of fit between the experimental and the predicted values was $R^2 = 0.920$. Furthermore, the predicted R^2 of 0.7936 was in reasonable agreement

with the adjusted R^2 of 0.8594, i.e. the difference was less than 0.2.

Box Plots Evaluating the Effect of Time, Mass of Plant Powder and Power on α -Solanine Extraction

In Figs. 4 and 5 are the box-and-whiskers plots of the effect of time, mass and power on the MA-ATPE extractability of α -solanine from leaves of *Solanum retroflexum*. From these plots, a proportional increase in α -solanine was observed with an increase in the mass of plant powder when NaCl and Na_2CO_3 were applied during extraction (Figs. 4a and 5a). This indicated that the mass of the plant powder played a key role in the recovery α -solanine. The increased enrichment of α -solanine was more notable when Na_2CO_3 was used compared to NaCl. For instance, the concentration of α -solanine extracted increased from approximately $14.286 \text{ mg kg}^{-1}$ (0.2 g) to 72.16 mg kg^{-1} (1.2 g) which equated to a fivefold increase (Fig. 5a). The observed enhancement in the yield of α -solanine with an increase in mass can be attributed to the increased mass transfer of metabolites from the plant matrix to the solvent when larger weights of the plant material were used (Doulabi et al. 2020). Additionally, the high α -solanine extractions was most likely due to the low solvent to mass ratio, i.e. 0.12 (m/v) when 1.2 g was used compared to 0.02 (m/v) with 0.2 g in a 10 mL mixture, which generally contributed to high microwave energy absorption of the plant material as the solvent absorbed most of the microwave energy (Doulabi et al. 2020). Conversely, increased solvent volumes have been reported to reduce the heating efficiency in microwave extraction, limit the breakage of solid cell walls and inhibit the driving force for the mass transfer of compounds (Doulabi et al. 2020). Similarly, Alara and Abdurahman (2019) reported that larger volumes of solvent required more energy and time to maximize extraction of analytes from the plant matrix.

It was also observed from Figs. 4b,c and 5b,c that an increase time and power led to a general decrease in the extraction of α -solanine. This highest extraction of α -solanine was observed at 40% microwave power,

Fig. 2 Molecular transition of α -solanine ($m/z = 868$) to β -solanine ($m/z = 722$)

Fig. 3 Pareto chart of standardized effects of time, mass of plant powder and power on the extraction α -solanine at 868 $\rightarrow$ 722 **a** NaCl and **b** Na_2CO_3

indicating that power had an influence on extraction. Microwave power was reported by Kuhnert and Danks (2002) and Khan et al. (2018) to cause superheated solvents. Additionally, an increase in microwave power resulted in the superheated extraction by water, resulting in quicker and easier penetration of solvent into the plant matrix. Furthermore, the thermal energy supplied by varying microwave power can overcome cohesive (solute–solute) and adhesive (solute–matrix) forces by reducing the activation energy needed for the desorption

process, as seen at 40% power (Vergara-Salinas et al. 2013; Gbashi et al. 2016). However, at microwave powers greater than 40%, a steady decrease was observed due to excessive microwave irradiation energy degrading the α -solanine analyte (Routray and Orsat 2012; Valdés et al. 2015). Therefore, the optimal extraction power was observed at 40%. Moreover, according to the box-and-whiskers plots, the best extraction was generally observed at shorter times; this could probably be due to the synergistic effect of extraction time and microwave energy. A

Fig. 4 Box-and-whiskers plots evaluating (a) time, (b) mass and (c) power on the enrichment of α -solanine when NaCl was used to aid extraction

Fig. 5 Box-and-whiskers plots evaluating (a) time, (b) mass and (c) power on the enrichment of α -solanine when Na₂CO₃ was used to aid extraction

similar observation was reported by Martino et al. (2006) and Kaderides et al. (2019).

Chromatographic Profile of MRM-Based Quantification of α -Solanine

Chromatograms depicting the highest and lowest concentrations of α -solanine (mg kg⁻¹) obtained when NaCl and Na₂CO₃ was applied is included in Fig. 6a–d. As seen in the chromatogram, the MRM transition of α -solanine 868 → 722 is observed at a retention time of 3.80 min (Fig. 6a,b,c and d). The fragmentation profiles showing

the product ions of α -solanine are also included in Fig. 6f. As seen in Fig. 2, α -solanine is composed of the solanidine aglycone unit glycosylated to solatriose. Solatriose is a trisaccharide composed of glucose, rhamnose and galactose monosaccharides. Of interest in this study was the 868 → 722 transition which was due to the loss of rhamnose at collision energy of 65 eV (Figs. 2 and 6f). Other product ions of α -solanine were also observed which included m/z 576, 445 and 414 which were due to losses of glucose, loss of the [Glu + H – H₂O – CO]⁺ adduct (m/z 131) and galactose, respectively (Kuuranne et al. 2000; Yuan et al. 2018).

Fig. 6 Chromatogram of the lowest **a** Na_2CO_3 , **b** NaCl and highest **c** Na_2CO_3 , **d** NaCl concentration of α -solanine (mg kg^{-1}) for run 2 and **f** mass spec of α -solanine

Response Surface Equations and Corresponding for NaCl and Na_2CO_3 and the Resultant Optima

Response equations, Eqs. 1 and 2, corresponding to NaCl and Na_2CO_3 , respectively, and the resultant response surfaces evaluating the multivariate interaction between the mass of plant powder, power and time are shown in Figs. 7 and 8. Equations 1–3 and 4–6 represent the response surface equations for NaCl (Fig. 7a–c) and Na_2CO_3 (Fig. 8a–c), respectively, where

A = time; B = mass of plant powder, C = power and Z = extraction yield (mg kg^{-1}).

$$Z = -10.5 + 197.7B + 0.31C - 77.9B^2 - 0.00063C^2 - 0.596BC \quad (1)$$

$$Z = 47.7 + 13.27A + 0.24C - 0.981A^2 - 0.0004C^2 - 0.0689AC \quad (2)$$

$$Z = 7.4 + 6.67A + 131.3B - 0.631A^2 - 48.6B^2 - 2.96AB \quad (3)$$

Fig. 7 Surface plots showing the interaction between the parameters A&B (a), A&C (b) and C&B (c) on the concentration of α -solanine extracted in the presence of NaCl

Fig. 8 Surface plots showing the interaction between the parameters A&B (a), A&C (b) and C&B (c) on the concentration of α -solanine extracted in the presence of Na_2CO_3

$$Z = 52.1 - 7.26A + 75.3B + 0.571A^2 - 14.9B^2 - 5.17AB \quad (4)$$

$$Z = 145.2 - 10.03A - 1.00C + 0.331A^2 + 0.0030C^2 + 0.0223AC \quad (5)$$

$$Z = 98.4 + 57.3B - 1.64C - 8.9B^2 + 0.0101C^2 - 0.269BC \quad (6)$$

As the mass of plant powder was increased, in the presence of NaCl and Na_2CO_3 , a proportional increase in the yield of α -solanine was obtained (Figs. 7a and 8a). The

observed increment in the concentration of α -solanine with an increase in mass can be attributed to the increased mass transfer of metabolites from the plant matrix to the extraction solvent (Doulabi et al. 2020). This concurs with observations from the Pareto chart, Fig. 3 a and b, which indicates the significant linear effect ($p < 0.05$) of mass of plant powder on the extraction of α -solanine. In Fig. 9 a and b, the predicted optimal extraction of α -solanine in the presence of Na_2CO_3 and NaCl was 88.57 mg kg^{-1} and 73.36 mg kg^{-1} , with a desirability score of 0.804 and 0.868, respectively. The high (> 0.8) desirability score of Na_2CO_3 and NaCl indicated its closeness to the target requirement of 1 and hence the greater reliability of this optimum for maximal enrichment of α -solanine. Additionally, comparisons of the concentrations of α -solanine obtained in Table 1 and (Figs. 7a-c and 8a-c) indicated that more of α -solanine was extracted for Na_2CO_3 compared to NaCl . This suggested that, generally, α -solanine extraction was probably favoured by the presence of multiply charged ions (kosmotropes), Na_2CO_3 in this case, rather than NaCl . The doubly charged carbonate ions from Na_2CO_3 , probably formed stronger hydrogen bonds with the solvation sphere surrounding α -solanine than singly charged chloride ions, enhancing the extent of its precipitation (salting-out) from the hydration sphere and its subsequent extraction by ethanol. This observation is correlated with the Hofmeister series as narrated by Kang et al. (2020), Dogra et al. (2020)

and Wang et al. (2021). Similarly, the salting-out effect was also reported by Sazali et al. (2019) and Mokgehle et al. (2021). Hence, α -solanine extraction is better achieved with the divalent Na_2CO_3 rather than monovalent NaCl .

Comparison of MA-ATPE to MAE and ATPE

Studies evaluating the extraction of glycoalkaloids involving either MAE or ATPE have been reported. For instance, Kondamudi et al. (2017) examined MAE for obtaining glycoalkaloids from *Solanum tuberosum*. In that study, the concentrations of α -solanine extracted ranged from 15.40 to 28.12 mg kg^{-1} at an optimal extraction time of 10 min. In another study, Maldonado et al. (2014) examined ATPE systems for extraction of α -solanine from *Solanum tuberosum* peels and obtained concentrations of 71 mg kg^{-1} . However, this study has shown that hyphenation of microwave extraction and salting out through MA-ATPE, in the presence of kosmotrope- Na_2CO_3 , can significantly reduce the extraction period (1 min) for α -solanine while simultaneously obtaining greater concentrations (93.50 mg kg^{-1}) than what was reported by Kondamudi et al. (2017) and Maldonado et al. (2014). In this view, MA-ATPE is an economical and efficient extraction method for α -solanine.

Fig. 9 Optimal conditions for extraction of α -solanine in the presence of **a** NaCl and **b** Na_2CO_3

Conclusions

The application of MA-ATPE, a synergy of microwaves and salting-out, has demonstrated to be an energy efficient and time-saving method for enrichment of α -solanine from *Solanum retroflexum*. This is evident in the lower times (1 min) and microwave power (40%) required by MA-ATPE for maximal extraction of α -solanine. Furthermore, MA-ATPE is environmentally friendly method due to its non-requirement for toxic organic solvents for extraction. This technique only utilizes water and ethanol, which is a green solvent, for extraction. The maximal amount of α -solanine extracted was 93.50 mg kg⁻¹ and 72.16 mg kg⁻¹ for Na₂CO₃ and NaCl, respectively. Fitting the central composite design response surface model to the data generated a quadratic model with a good fit ($R^2 = 0.92$). It was statistically deduced that time and mass of plant powder had a significant effect ($p < 0.05$) on the extraction of α -solanine in MA-ATPE. The effect of microwave power was determined to be insignificant. The application of multiply charged salts such as the kosmotrope-Na₂CO₃ was shown to be a comparably better extractant of α -solanine than the chaotrope-NaCl and is in agreement with the Hofmeister effect. Therefore, this cost-cutting technique, MA-ATPE, can potentially be escalated to be applied as a reliable means to minimize the concentrations of toxic compounds in other food sources. The MA-ATPE method has some limitations and extraction can be improved by potentially coupling MA-ATPE with miniaturization techniques such as hollow fibre miniaturized extraction (HFME) or the exploration of natural deep eutectic solvents (NADES) as extraction solvents.

Funding This study is financially supported by the University of Venda, Sasol Inzalo and the National Research Foundation.

Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent not applicable.

Consent to Participate Informed consent not applicable.

Conflict of Interest Tebogo Mphatlalala Mokgehele declares that he has no conflict of interest. Ntakadzeni Madala declares that he has no conflict of interest. Wilson Mugeru Gitari declares that he has no conflict of interest. Nikita Tawanda Tavengwa declares that he has no conflict of interest.

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