Supplementary material

Comparison of Methods for the Extraction of Saponins from *Sechium* spp. Genotypes and Their Spectrophotometric Quantification

Fátima Azucena Rasgado-Bonilla [1], Ramón Marcos Soto-Hernández [1,*], Luis Francisco Salomé-Abarca [2], Jorge Cadena-Íñiguez [3], Víctor A. González-Hernández [1], Lucero del Mar Ruiz-Posadas [1] and Sara Elisa Herrera-Rodríguez [4]

[1] Posgrado en Recursos Genéticos y Productividad-Fisiología Vegetal, Colegio de Postgraduados, Campus Montecillo, Texcoco, 56264 Estado de México, México; fatima.rasgado@colpos.mx (F.A.R.-B.); vagh@colpos.mx (V.A.G.-H.); lucpo@colpos.mx (L.d.M.R.-P.)
[2] Posgrado en Recursos Genéticos y Productividad-Fruticultura, Colegio de Postgraduados, Campus Montecillo, Texcoco, 56264 Estado de México, México; luis.salome@colpos.mx (L.F.S.-A)
[3] Posgrado en Innovación en Manejo de Recursos Naturales, Colegio de Postgraduados, Campus San Luis Potosí, Salinas de Hidalgo, 78600 San Luis Potosí, México; jocadena@gmail.com (J. C.-I.)
[4] Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., Subsede Yucatán, Tablaje Catastral 31264, Km 5.5, Carretera Sierra Papacal-Chuburná Puerto. Parque Científico Tecnológico de Yucatán, Mérida, 97302 Yucatán, Mexico; sherrera@ciatej.mx
* Correspondence: msoto@colpos.mx



Figure S1. Segregant genotype *Sechium* 633-M11. (a) Fruits at horticultural maturity (in planta). (b) Harvested fruits during thawing before processing.

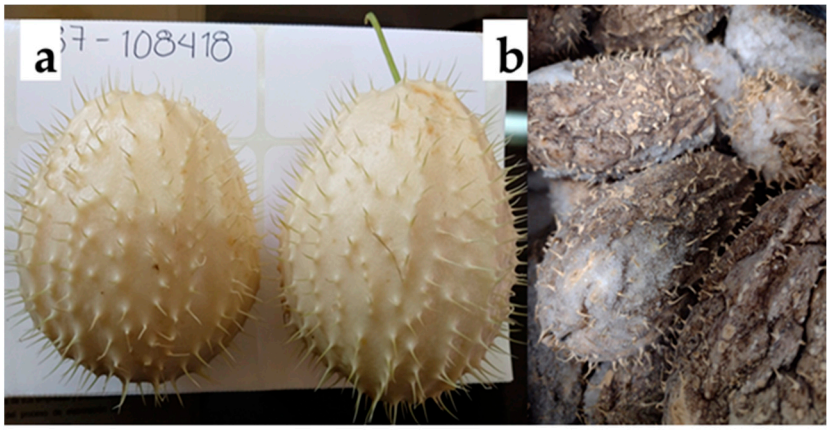


Figure S2. Segregant genotype *Sechium* 387-M16. (a) Fruits at horticultural maturity. (b) Harvested fruit during thawing before processing.

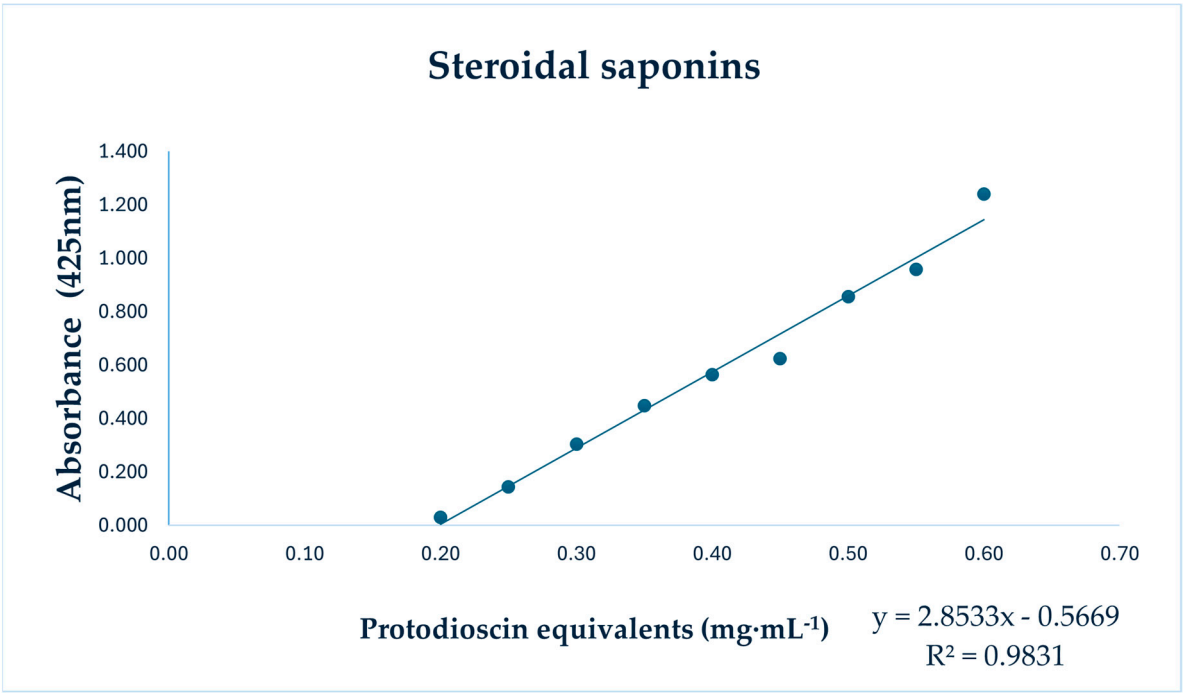


Figure S3. Calibration curve for Steroidal saponins quantified by the p-anisaldehyde-sulfuric acid assay at 425 nm, expressed as protodioscin equivalents (mg mL-1).

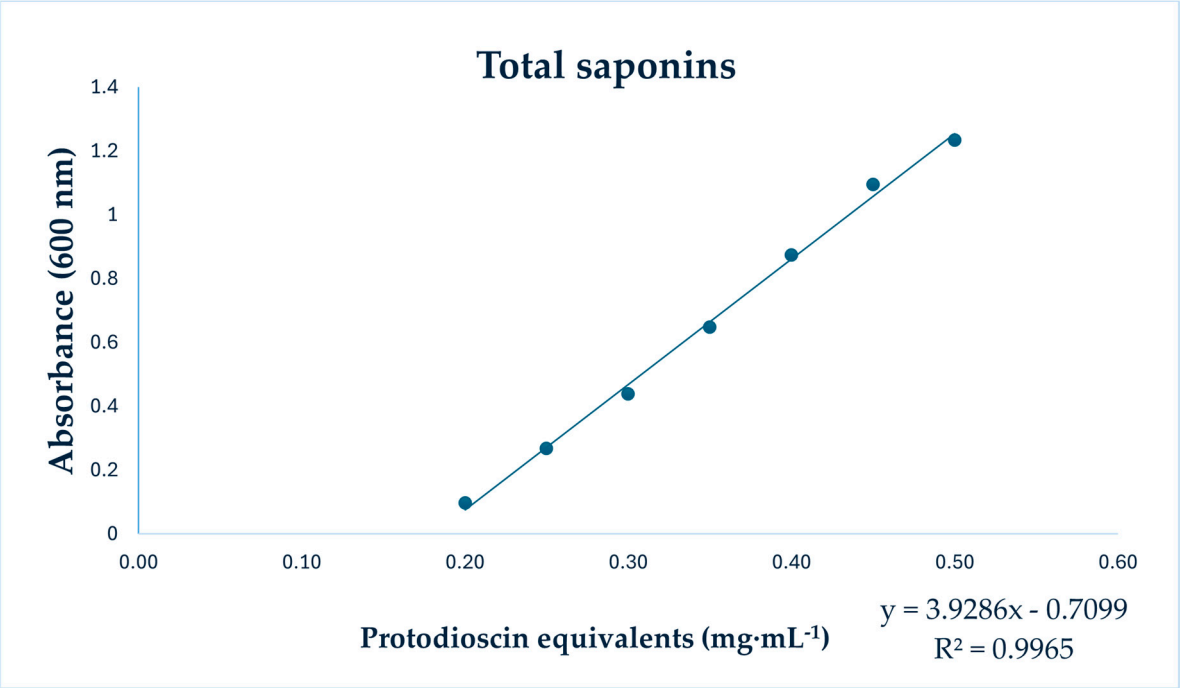


Figure S4. Calibration curve for total saponins quantified by the p-anisaldehyde-sulfuric acid assay at 600 nm, expressed as protodioscin equivalents (mg mL-1).



Figure S5. *Agave lechuguilla* (reference material for steroidal saponins). (a) Fresh leaves upon collection. (b) Leaf tissue after cutting, before drying/extraction.



Figure S6. Digital voucher and photographic record of *Agave lechuguilla* used as reference material for steroidal saponins: (a) field salbel/sample documentation; (b) representative leaf material photographed before processing.

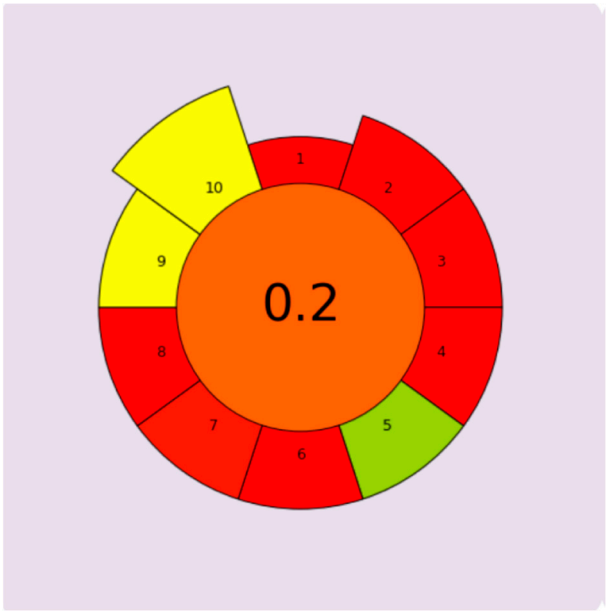


Figure S7. Analytical greenness report for the Gravimetric method of extraction of saponins in Sechium genotypes (M1) using the AGREEprep metric. The evaluation was performed using the default weights (value of 2) for all 10 criteria as described in the original tutorial.

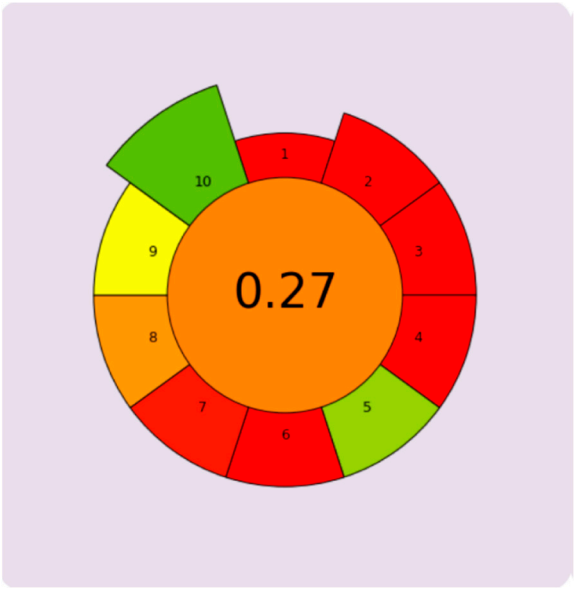


Figure S8. Analytical greenness report for the Gravimetric method of extraction of saponins in Sechium genotypes (M2) using the AGREEprep metric. The evaluation was performed using the default weights (value of 2) for all 10 criteria as described in the original tutorial.

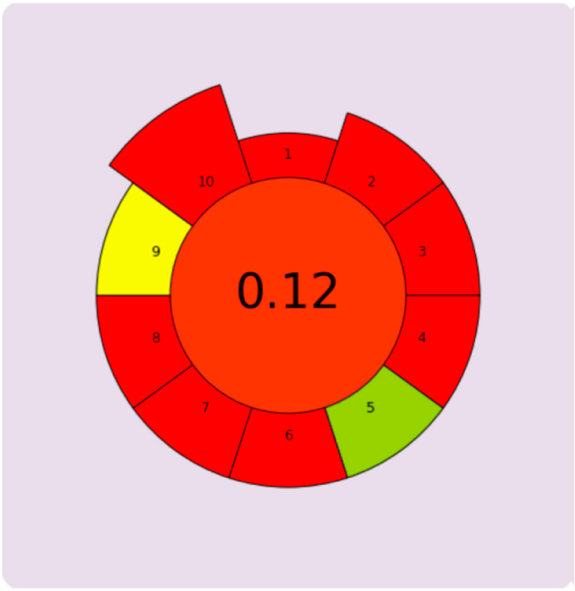


Figure S9. Analytical greenness report for the Gravimetric method of extraction of saponins in Sechium genotypes (M3) using the AGREEprep metric. The evaluation was performed using the default weights (value of 2) for all 10 criteria as described in the original tutorial.

Table S1. AGREEprep evaluation of the analytical greenness of the gravimetric saponin extraction methods (M1-M3) applied to *Sechium* genotypes, based on the 10 principles of green sample preparation.

AGREEprep Criterion	Description	Method 1 (M1)	Method 2 (M2)	Method 3 (M3)
C1	Sample preparation placement	Off-line (Ex situ)	Off-line (Ex situ)	Off-line (Ex situ)
C2	Hazardous substances (g or mL)	High	Moderate	Extreme
C3	Sustainability/Renewability	<25% sustainable/renewable; single-use	<25% sustainable/renewable; single-use	<25% sustainable/renewable; single-use
C4	Waste (g or mL)	220	387.5	425
C5	Size economy of the sample (g or mL)	1	1	1
C6	Sample throughput (samples/h)	Low	Moderate	Very Low
C7	Integration/automation	5-steps; manual	5-steps; manual	6 + -steps; manual
C8	Energy comsuption	500	150	800
C9	Post-sample preparation	Non-MS analytical platforms	Non-MS analytical platforms	Non-MS analytical platforms
C10	Operatorꞌs safety	Low (Neurotoxic) 2	Moderate 1	Very Low (Carcinogenic) 4+
Final Score	AGREEprep score	0.2	0.27	0.12

Table S2. Spectrophotometric saponin content (mg g-1, DW, protodioscin equivalents) in juice and bagasse of *Sechium* 633-M11 and 387-M16 across *Agave lechuguilla*/*Sechium* mixing ratios. Results at 600 nm (Totals), 425 nm (steroidal), and the triterpenic component (600 – 425 nm). .

Sechium **genotypes**	**Matrix**	***Agave*: *Sechium***	**Saponins fraction (mg g^{-1})**		
			Totals 600 nm	**Steroidal 425 nm**	**Triterpenic (600– 425 nm)**
633-M11	Juice	20:80	70.20 ± 3.78	52.96 ± 2.83	17.24 ± 0.96
		50:50	62.55 ± 1.10	92.23 ± 3.53	ND
		80:20	53.77 ± 1.91	128.01 ± 0.78	ND
	Bagasse	20:80	73.17 ± 2.33	31.221 ± 0.45	41.95 ± 1.89
		50:50	62.83 ± 2.85	94.62 ± 1.50	ND
		80:20	47.00± 0.95	110.02 ± 1.01	ND
387-M16	Juice	20:80	82.24 ± 1.28	45.33 ± 0.47	36.91 ± 1.74
		50:50	56.29 ± 0.76	91.98 ± 1.38	ND
		80:20	52.90 ± 1.57	91.98 ± 1.38	ND
	Bagasse	20:80	86.92 ± 0.88	41.45 ± 2.55	45.48 ± 1.74
		50:50	78.13 ± 5.13	96.35 ± 2.21	ND
		80:20	67.12 ± 0.72	132.15 ± 10.51	ND

Values are mean ± SE (n = 3 independent extractions per genotype x matrix x mixture). Calibration used protodioscin; results are reported as protodioscin equivalents (PE). The triterpenic component was computed per replicate as Δ= Total 600 nm – Steroidal 425 nm (mg g-1). ND = not detected (Δ ≤ 0 or absorbance ≤ blank + 3 SD per predefined criterion). Mixing ratios denote mass fractions (m/m) of *Agave lechuguilla* leaf extract: *Sechium* extract in the assay.