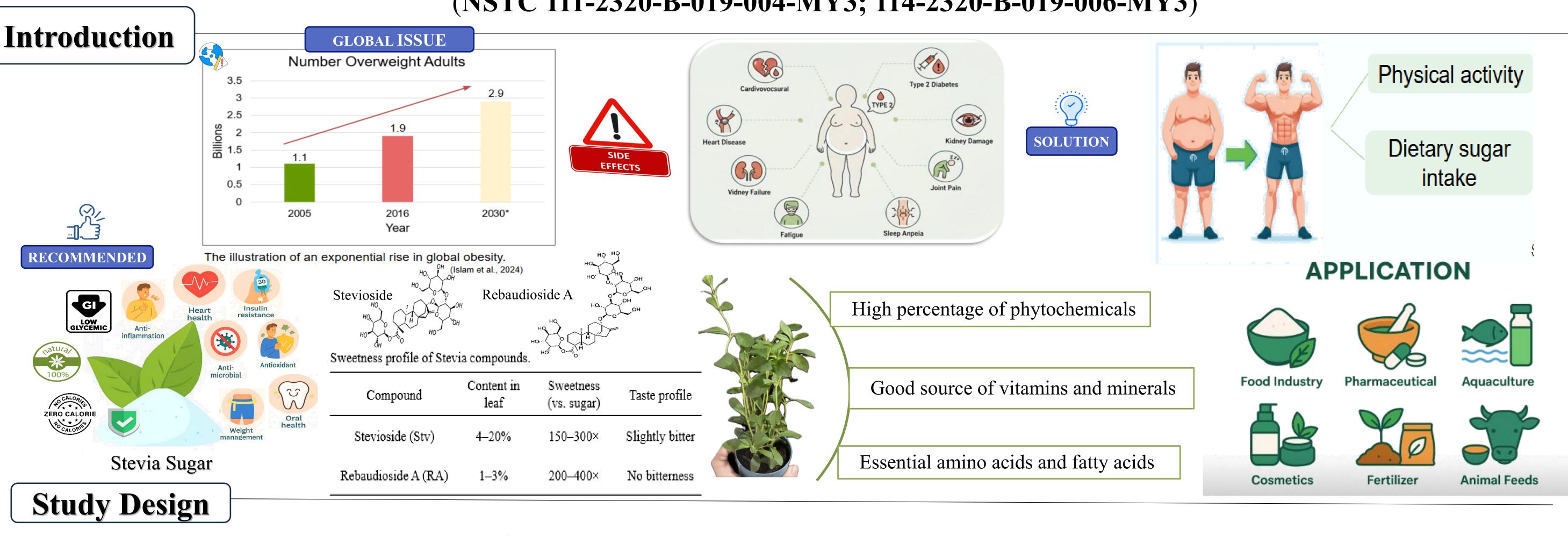


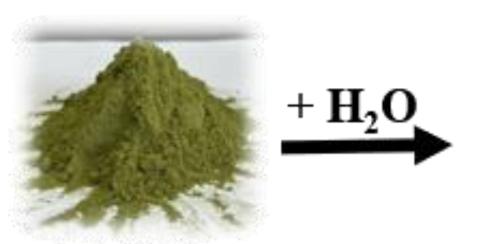
Optimizing Extraction of Stevioside and Rebaudioside A: Comparison of Thermal and Non-Thermal Processing Techniques

Mai Anh Le, Min Lang Tsai*

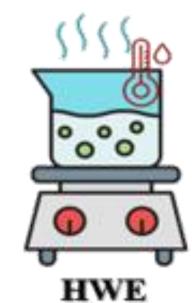
Department of Food Science, National Taiwan Ocean University, Taiwan

(NSTC 111-2320-B-019-004-MY3; 114-2320-B-019-006-MY3)









600000

500000

400000

援 300000

200000



Stevioside

y = 2E + 06x + 5227.4

0.15

0.2

Sample extracts Centrifuged Frozen Freeze-drying Filtered

Lyophilized sample



Yield (%) Amount (mg/g) Recovery rate (%)

Results

0.01

400

350

(g/gm) 250

 $(A)_{400}$

200

Table 1. Validation and monitoring test for steviol glycosides.

Parameter	Stevioside	Rebaudioside A	
Linear Regression	y=2E+06x+5227.4	y=2E+06x+29797	
Coefficient of Determination (R ²)	0.9993	0.9999	
Limit of detection	0.005	0.002	
Limit of quantitation	0.016	0.005	

LOD: Limit of detection. Signal-to-noise (S/N)=3

LOQ: Limit of quantitation. Signal-to-noise (S/N)=10 Table 2. The precision validation of stevioside and rebaudioside A.

1.06

Data represent the results of three independent experiments (n = 3).

Concentration	Relative standard deviation (RSD, %)			
(mg/mL)	Intra-day precision		Inter-day precision	
_	Stevioside	Rebaudioside A	Stevioside	Rebaudioside A
0.25	1.57	1.04	1.14	1.00
0.125	1.17	1.75	1.58	1.44

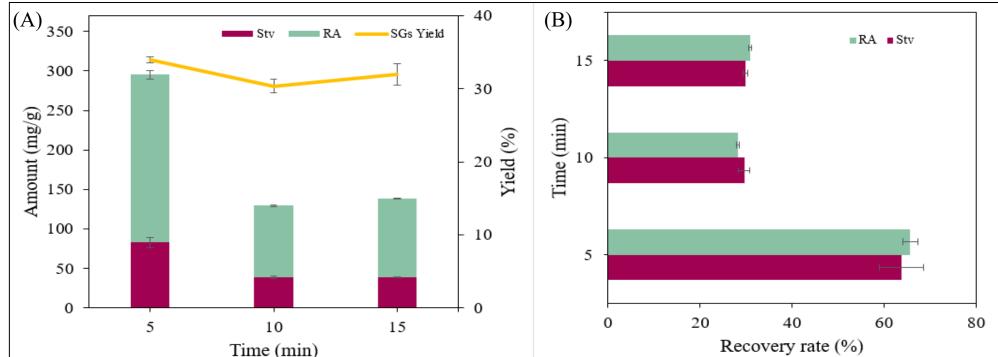
2.10

Concentration (mg/mL) Rebaudiosdie A y = 2E + 06x + 29797 $R^2 = 0.9999$ \$ 500000 يَّ 400000 ₹ 300000 ള് 200000 100000 0.25

0.1

Figure 1. Calibration curves of stevioside (A) and rebaudioside A (B) standards at various concentrations.

Figure 2. Yield of steviol (A)₃₅₀ glycosides (SGs) and amount in dried leaves (A), and recovery rate of stevioside (Stv) and rebaudioside (RA) (B) A obtained high-pressureby assisted extraction (HPAE) at different extraction times.



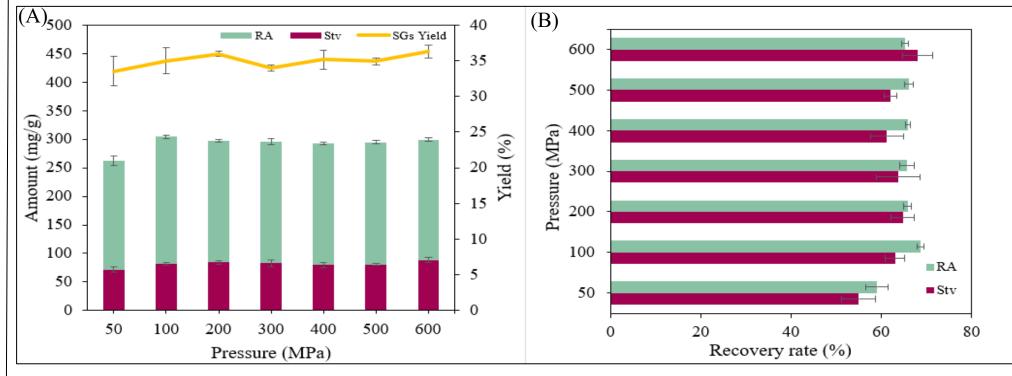
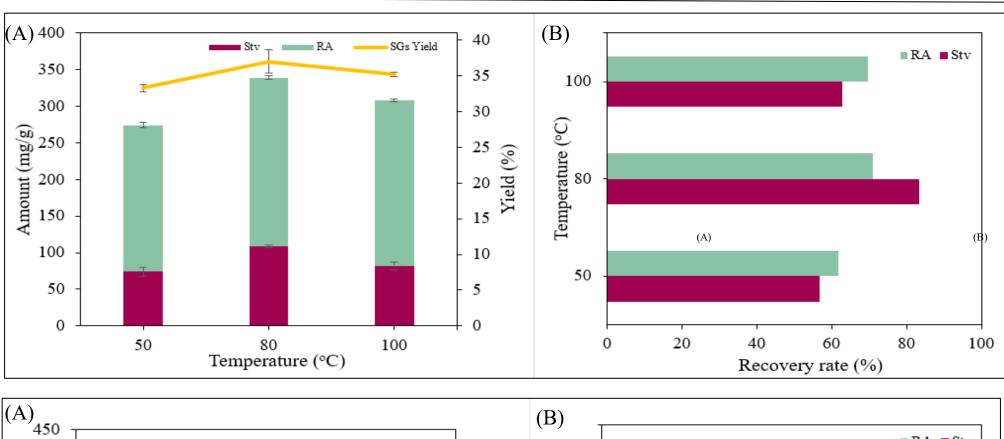


Figure 3. Yield of steviol glycosides (SGs) and amount in dried leaves (A), and recovery rate of stevioside (Stv) and rebaudioside (RA) high-pressureobtained assisted extraction (HPAE) at different pressure ranges.



o₅ Yield (%)

1.77

1.13

Recovery rate (%)

Figure 4. Yield of steviol glycosides (SGs) and amount in dried leaves (A), and recovery rate of stevioside (Stv) and rebaudioside A (RA) (B) obtained by microwave-assisted extraction (MAE) at varying temperatures.

Figure 5. Yield of steviol glycosides (SGs) and amount in dried leaves (A), and recovery rate of stevioside (Stv) and rebaudioside A (RA) (B)high-pressureobtained by assisted extraction (HPAE) at

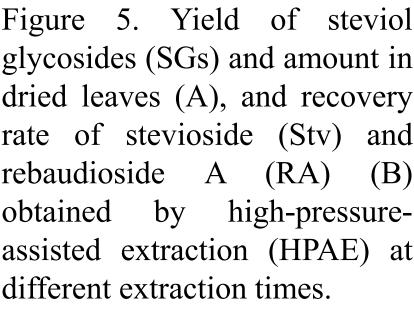
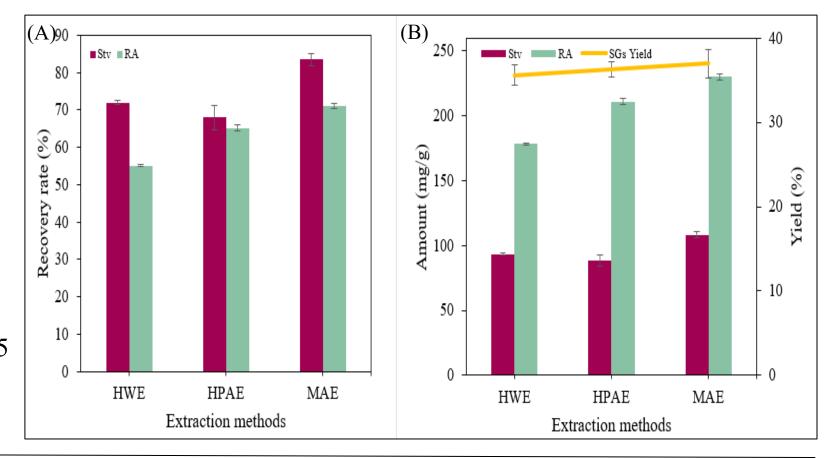


Figure 7. Comparison of steviol glycosides (SGs) yield, amount (A), and recovery rates (B) of stevioside (Stv) and rebaudioside A (RA) obtained by hot water extraction (HWE), microwave-assisted extraction (MAE), and high-pressure-assisted extraction (HPAE) under optimal conditions.

*HWE operated at 60 min, 100 °C; HPAE operated at 5 min, 600 MPa; MAE operated at 3 min, 80 °C, 1000 W.



Disscusion

This study compared the efficiency of three extraction methods recovering Stv and RA from Stevia leaves, including MAE, HPAE, and HWE.

MAE performed optimally at 3 minutes, 80 °C, 1000 W, maximising yield and sweetener recovery while minimising thermal degradation. Rapid internal heating and cell wall rupture enhanced solvent penetration and mass transfer, especially for stevioside, which showed superior recovery in dried leaf extracts.

HPAE conducted at 600 MPa for 5 minutes was identified as optimal, enhancing steviol glycoside yield and stevioside content while maintaining sweetener stability. The applied pressure promoted molecular diffusion and solvent penetration, helping prevent degradation and solubility limitations associated with longer extraction times.

Both MAE (36.96%) and HPAE (36.28%) achieved slightly higher overall yield than HWE (35.59%). MAE (1000 W, 3 min, 80 °C) resulted in the highest recovery rate for Stv (83.48%) and RA (71.12%), while HPAE (600 MPa, 5 min) demonstrated balanced recovery of both compounds (67.96% for Stv and 65.22% for RA). Although HWE required a longer extraction time (60 min at 100 °C), it achieved considerable yields.

In conclusion, MAE showed the most efficient in terms of recovery, whereas HPAE emerged as the most promising non-thermal technique, combining high efficiency with preservation of sweetening compounds. These findings highlight the potential of novel extraction strategies to produce sustainable, health-promoting natural sweeteners for food applications.

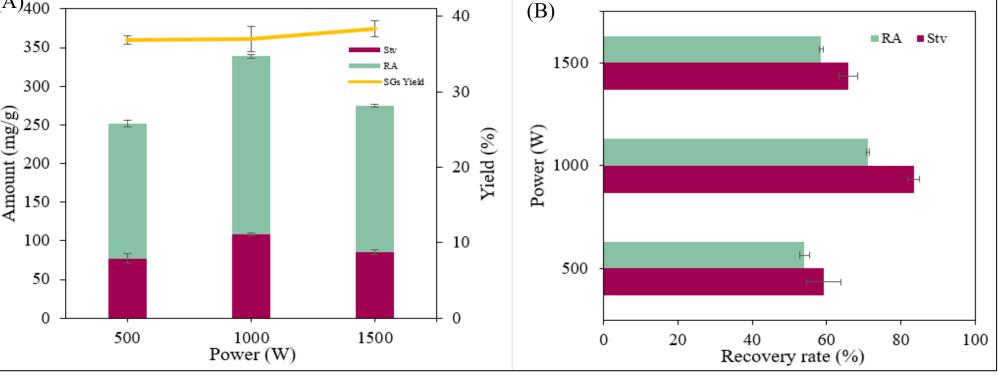


Figure 6. Yield of steviol glycosides (SGs) and amount in dried leaves (A), and recovery rate of stevioside (Stv) and rebaudioside A (RA) obtained by microwave-assisted extraction (MAE) at different power ranges.

Acknowlegdement

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