APPENDIX

Appendix A: Meat products formula

Appendix B: Buffer solution preparation and analytical methods

Appendix C: Sensory evaluation

Appendix D: HPLC chromatogram trace of roselle anthocyanins

Appendix E: Statistical analysis

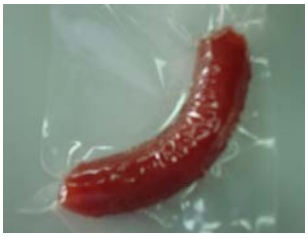
Appendix A

Meat products formula

Appendix A1 Chinese-style sausage formulation by sweeteners treatment (objective 4.2.1)

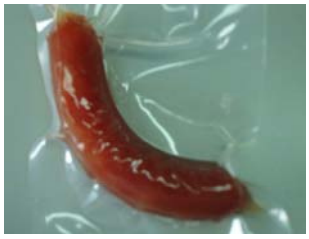
Ingredients	Formulations (Percentage)				
	Control*	**Sucrose**	**Lactitol**	**Maltitol**	**Xylitol**
Lean pork	65	65	65	65	65
Pork lard	16	16	16	16	16
Potassium nitrite	0.2	0.2	0.2	0.2	0.2
Chinese five spices	0.1	0.1	0.1	0.1	0.1
Monosodiumglutamate	0.3	0.3	0.3	0.3	0.3
Salt	1.8	1.8	1.8	1.8	1.8
Sucrose	16.6	16.6	-	-	-
Lactitol	-	-	16.6	-	-
Maltitol	-	-	-	16.6	-
Xylitol	-	-	-	-	16.6
Total (%)	**100**	**100**	**100**	**100**	**100**
Roselle	-	0.3	0.3	0.3	0.3

Control* formula adapted from Pinsirodom (2008)



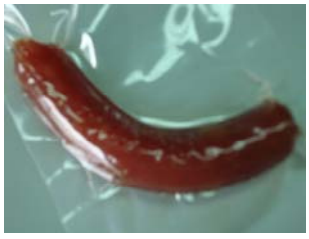
Control sample (Sucrose without RAE)

Sucrose added sample (with 0.3 % RAE)



Lactitol added sample (with 0.3 % RAE)

Maltitol added sample (with 0.3 % RAE)



Xylitol added sample (with 0.3 % RAE)

Figure Appendix A1 Chinese-style sausage formulation by different sweeteners treatment

Appendix A2 Chinese-style sausage formulation by xylitol concentration treatment (objective 4.2.2)

Ingredients	Formulations (Percentage)					
	Control 1	**Control 2**	**Control 3**	**Xylitol 11.6**	**Xylitol 16.6**	**Xylitol 21.6**
Lean pork	77.94	77.94	77.94	77.94	77.94	77.94
Pork lard	19.18	19.18	19.18	19.18	19.18	19.18
Potassium nitrite	0.24	0.24	0.24	0.24	0.24	0.24
Chinese five spices	0.12	0.12	0.12	0.12	0.12	0.12
Monosodiumglutamate	0.36	0.36	0.36	0.36	0.36	0.36
Salt	2.16	2.16	2.16	2.16	2.16	2.16
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**
Sucrose	16.6	16.6	-	-	-	-
Xylitol	-	-	16.6	11.6	16.6	21.6
Roselle	0.3	-	-	0.3	0.3	0.3

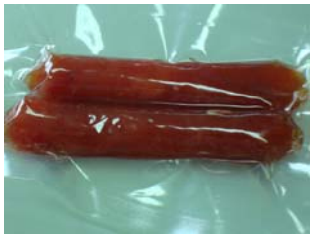
Control = Control samples, Xylitol = Xylitol treatment samples

Control 1 sample

(Sucrose with 0.3 % RAE)

Control 2 sample

(Sucrose without RAE)



Control 3 sample

(Xylitol without RAE)

Xylitol 11.6 %

(11.6 % Xylitol with 0.3 % RAE)

Xylitol 16.6 %

(16.6 % Xylitol with 0.3 % RAE)

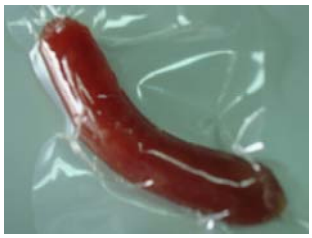
Xylitol 21.6 %

(21.6 % Xylitol with 0.3 % RAE)

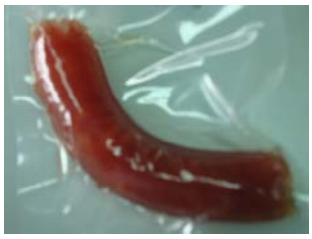
Figure Appendix A2 Chinese-style sausage formulation by xylitol concentration treatment

Appendix A3 Chinese-style sausage formulation by sucrose and xylitol treatment (objective 4.2.3)

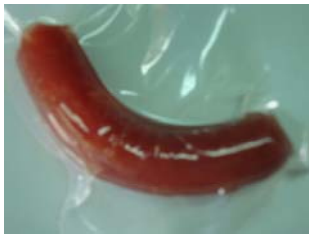
Ingredients (%)	Formulations (Percentage)		
	Control	**Sucrose 16.6**	**Xylitol 16.6**
Lean pork	77.94	77.94	77.94
Pork lard	19.18	19.18	19.18
Potassium nitrite	0.24	0.24	0.24
Chinese five spices	0.12	0.12	0.12
Monosodium glutamate	0.36	0.36	0.36
Salt	2.16	2.16	2.16
Total (%)	**1000**	**1000**	**1000**
Sucrose (%)	16.6	16.6	-
Xylitol (%)	-	-	16.6
Roselle extracts (%)	-	0.3	0.3



Control sample (without RAE)



Sucrose sample (with 0.3 % RAE)



Xylitol sample (with 0.3 % RAE)

Figure Appendix A3 Chinese-style sausage formulation by sucrose and xylitol treatment

Appendix A4 Vienna pork sausage formulation by sodium nitrite treatment (objective 4.3)

Ingredients (%)	Formulations (Percentage)					
	Cont 1*	**Cont 2***	**Ref 1**	**Ref 2**	**Treat 1**	**Treat 2**
Pork meat	54.58	54.58	54.58	54.58	54.58	54.58
Pork lard	21.44	21.44	21.44	21.44	21.44	21.44
Ice	21.44	21.44	21.44	21.44	21.44	21.44
Sodium tripolyphosphate	0.29	0.29	0.29	0.29	0.29	0.29
White pepper	0.29	0.29	0.29	0.29	0.29	0.29
Nutmeg	0.10	0.10	0.10	0.10	0.10	0.10
cardamom	0.05	0.05	0.05	0.05	0.05	0.05
Coriander	0.03	0.03	0.03	0.03	0.03	0.03
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Sugar	0.40	0.40	0.40	0.40	0.40	0.40
Monosodium glutamate	0.19	0.19	0.19	0.19	0.19	0.19
Smoke powder	0.19	0.19	0.19	0.19	0.19	0.19
Total	**100**	**100**	**100**	**100**	**100**	**100**
Sodium nitrite (ppm)	125	250	125	250	125	250
BHA (ppm)	-	-	200	200	-	-
Roselle extracts (%)	-	-	-	-	0.3	0.3

Control formula adapted from Heinz and Hautzinger (2007)

Appendix A5 Thai pork fermented formulation by sodium nitrite treatment (objective 4.3)

Ingredients (%)	Formulations (Percentage)					
	Cont 1*	**Cont 2***	**Ref 1**	**Ref 2**	**Treat 1**	**Treat 2**
Pork	56.86	56.86	56.86	56.86	56.86	56.86
Pork skin	30.62	30.62	30.62	30.62	30.62	30.62
Sticky rice	5.25	5.25	5.25	5.25	5.25	5.25
Garlic	4.37	4.37	4.37	4.37	4.37	4.37
Salt	2.19	2.19	2.19	2.19	2.19	2.19
Sugar	0.44	0.44	0.44	0.44	0.44	0.44
Sodium tripolyphosphate	0.26	0.26	0.26	0.26	0.26	0.26
Total	**100**	**100**	**100**	**100**	**100**	**100**
Sodium nitrite (ppm)	125	250	125	250	125	250
BHA (ppm)	-	-	200	200	-	-
Roselle extracts (%)	-	-	-	-	0.3	0.3

Control formulation adapted from Swetwiwathana et al., (2007)

Appendix A6 Thai pork fermented formulation by sodium nitrite treatment (objective 4.3)

Ingredients	Formulations*									
	%	**C 1**	**C 2**	**C 3**	**R 1**	**R 2**	**R 3**	**T 1**	**T 2**	**T 3**
Pork	56.86	✔	✔	✔	✔	✔	✔	✔	✔	✔
Pork skin	30.62	✔	✔	✔	✔	✔	✔	✔	✔	✔
Sticky rice	5.25	✔	✔	✔	✔	✔	✔	✔	✔	✔
Garlic	4.37	✔	✔	✔	✔	✔	✔	✔	✔	✔
Salt	2.19	✔	✔	✔	✔	✔	✔	✔	✔	✔
Sugar	0.44	✔	✔	✔	✔	✔	✔	✔	✔	✔
Sodium tripolyphosphate	0.26	✔	✔	✔	✔	✔	✔	✔	✔	✔
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Sodium nitrite (ppm)	125	✔	-	-	✔	-	-	✔	-	-
Sodium nitrite (ppm)	250	-	✔	-	-	✔	-	-	✔	
Sodium nitrite (ppm)	500	-	-	✔	-	-	✔	-	-	✔
BHA (ppm)	200	-	-	-	✔	✔	✔	-	-	-
Roselle extracts (%)	0.3	-	-	-	-	-	-	✔	✔	✔

Formulations*; C = Control test, R = Reference test, T = Treatment

Appendix B

Buffer solution preparation and analytical methods

Appendix B1 Mcllvaine's buffer system (pH 2.2-8.0)

Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$, M.W. 210.14); 0.1M-solution contains 21.01 g/l.

Disodium hydrogen phosphate (Na_2HPO_4, M.W. 141.98); 0.2M-solution contains 28.40 g/l, or Sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$, M.W. 178.05); 0.2M-solution contains 35.61 g/l.

To prepare 100 ml of the buffer, mix x ml 0.2 M disodium hydrogen phosphate and y ml 0.1 M citric acid as shown below.

pH required	0.2 M Na_2HPO_4 (ml)	0.1 M citric acid (ml)	pH required	0.2 M Na_2HPO_4 (ml)	0.1 M citric acid (ml)
2.2	2.00	98.00	5.2	53.60	46.40
2.4	6.20	93.80	5.4	55.75	44.25
2.6	10.90	89.10	5.6	58.00	42.00
2.8	15.85	84.15	5.8	60.45	39.55
3.0	20.55	79.45	6.0	63.15	36.85
3.2	24.70	75.30	6.2	66.10	33.90
3.4	28.50	71.50	6.4	69.25	30.75
3.6	32.20	67.80	6.6	72.75	27.25
3.8	35.50	64.50	6.8	77.25	22.75
4.0	38.55	61.45	7.0	82.35	17.65
4.2	41.40	58.60	7.2	86.95	13.05
4.4	44.10	55.90	7.4	90.85	9.15
4.6	46.75	53.25	7.6	93.65	6.35
4.8	49.30	50.70	7.8	95.75	4.25
5.0	51.50	48.50	8.0	97.25	2.75

Source: Dawson et al. (2003)

Appendix B2 Sodium carbonate-sodium bicarbonate buffer solutions (pH 8.8-10.6)

Sodium carbonate decahydrate ($Na_2CO_3 \cdot 10H_2O$, M.W. 286.2); 0.1M-solution contains 28.62 g/l.

Sodium carbonate ($NaHCO_3$, M.W. 84.0); 0.1M-solution contains 8.40 g/l.

To prepare 100 ml of the buffer, mix x ml 0.1 M Sodium carbonate and y ml 0.1 M Sodium carbonate as shown below.

pH required	0.1 M Na_2CO_3 (ml)	0.1 M $NaHCO_3$ (ml)
8.8	10	90
9.1	20	80
9.4	30	70
9.5	40	60
9.7	50	50
9.9	60	40
10.1	70	30
10.3	80	20
10.6	90	10

Source: Dawson et al. (2003)

Appendix B3 Ferrous ions chelating ability (FICA)

This method used to determine the ferrous ion chelating activities of roselle anthocyanin. Five-hundred microlitre solution containing extracts was mixed with 1.6 ml of distilled water and then the mixture was reacted with 50 µl of 2 mM $FeCl \cdot 4H_2O$ and 100 µl of 5 mM 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) for 10 min. The absorbance was read at 562 nm in a UV-Vis spectrophotometer. Five-hundred microlitre of distilled water, instead of roselle extracts solution, was used as control. Lower absorbance of the reaction mixture indicated higher chelating activity. The percentage of chelating activity on inhibition of $ferrozine - Fe^{2+}$ complex formation was calculated by the formula:

$$Ferrous\ ion\ chelating\ capacity\ (\%) = \left[1 - \frac{A_{562nm,sample}}{A_{562nm,control}}\right] x\ 100$$

Appendix B4 Trolox equivalent antioxidant capacity (TEAC) assay

The Trolox equivalent antioxidant capacity assay evaluates the capacity of a crude extract to scavenge $ABTS^{\bullet+}$ radicals. Briefly, a 7 mM solution of ABTS in water was prepared and $ABTS^{\bullet+}$ was formed after the addition of potassium persulfate to the solution at a final concentration of 2.45 mM. After 12–16 hr incubation in darkness at room temperature, the stock solution was diluted with ethanol until an absorbance of 0.7±0.02 at 734 nm was reached. After addition of 4.0 ml of diluted $ABTS^{\bullet+}$ solution to 40 µl of sample (or Trolox standard, 0.5-3 µM), the reaction mixture was incubated for 6 min in cuvett at 37°C. The decrease in absorbance at 734 nm using UV-Vis spectrophotometer (Hitachi U-2001, Japan) was determined at exactly 6 min after initial mixing for all samples. The absorbance of $ABTS^{\bullet+}$ without sample, *i.e.*, the control, also was measured. The TEAC value was calculated using the following formulae:

$$\% \, Inhibition = \left[\frac{Ac - As}{Ac} \right] x \, 100$$

$$TEAC \; value = \frac{\% \, Inhibition}{m}$$

where A_C is the absorbance of the control at t=6 min, A_S is the absorbance of the sample (or Trolox standard) at t=6 min, and *m* is the slope of the standard curve.

Trolox was used as standard and results were calculated based on standard curves such as the one presented here.

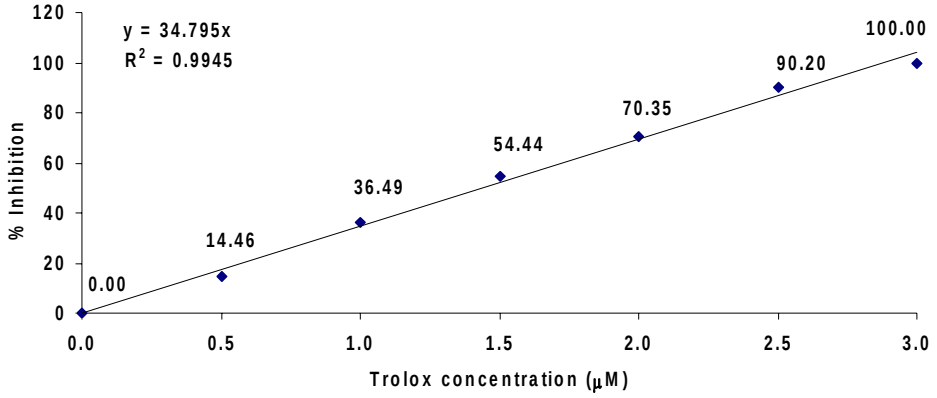


Figure appendix-B1 Trolox equivalent antioxidant capacity (TEAC) standard curve ranging from 0.5-3 micromolar (µM)

Appendix B5 Ferric thiocyanate antioxidant assay (FTC)

The FTC method was used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate. The substance is red, and denser color is indicative of higher absorbance. The TBA method measures free radicals present after peroxide oxidation.

Each sample solution (0.5 ml) was mixed with 0.5 ml of 2.51% linoleic acid in absolute ethanol, 1 ml of 0.05 M phosphate buffer (pH 7), and 0.5 ml of distilled water and placed in a screw capped tube. The reaction mixture was incubated in dark at 40C in an oven. Aliquots of 0.1 ml were taken at every 24 h during incubation and the degree of oxidation was measured by sequentially adding 75% ethanol (9.7 ml), 30% ammonium thiocyanate (0.1 ml) and 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.1 ml). After the mixture was rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The antioxidant activity was calculated as percentage of inhibition relative to the control.

$$OI\ (oxidative\ index) = \frac{Abs_{\ t-96hr}}{Abs_{\ t-0hr}}$$

$$AA\,(Antioxidant\ activity) = \frac{OI_{sample_{t=96hr}}}{OI_{control_{t-96hr}}}\ x\,100$$

$$AA\,(\%) = 100 - \left[\frac{Abs_{sample_{t-96hr}} - Abs_{sample_{t-0hr}}}{Abs_{control_{t-96hr}} - Abs_{control_{t-0hr}}}\right] x\,100$$

Appendix B6 Thiobarbituric acid reactive Ssbstances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were determined by the modified method of Min et al. (2009). Five-gram samples were weighed into a 50-mL test tube and homogenized with 15 ml of deionized distilled water using the homogenizer (Ultra-Turrax® T25Bbasic, Germany) for 10 s at the highest speed. One milliliter of sample homogenate was transferred to a disposable test tube (13x100 mm), and butylated hydroxyanisole (50 µl, 10%) and TBA/trichloroacetic acid (2 ml) were added. The mixture was vortex and then incubated in a boiling water bath for 15 min to develop color. The sample was cooled in cold water for 10 min, vortex again, and centrifuged for 10 min at 20,000xg. The absorbance of the resulting supernatant solution was determined at 532 nm against a blank containing 1 ml of deionized distilled water and 2 ml of TBA/trichloroacetic acid solution. The amounts of TBARS were expressed as milligrams of malondialdehyde per kilogram of sample. A standard curve was prepared using 1,1,3,3 tetramethoxypropane (TEP).

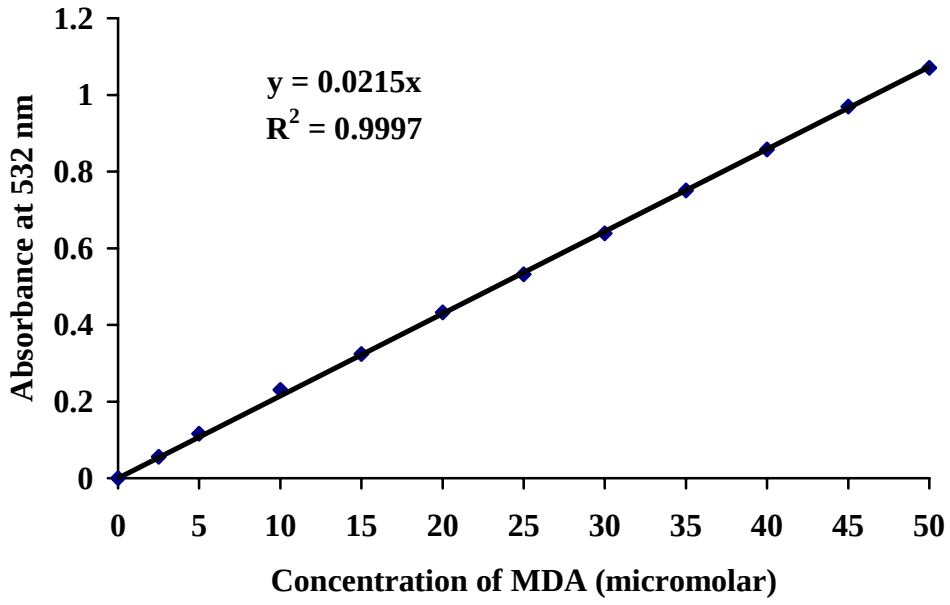


Figure appendix-B2 1,1,3,3 tetramethoxypropane (TEP) standard curve ranging from 0-50 micromolar (µM)

Appendix B7 Protein oxidation (total carbonyls) by 2,4-dinitrophenylhydrazones

Protein oxidation in sausage was followed by measuring the formation of protein carbonyls by converting them to 2,4-dinitrophenylhydrazones (DNPH) and the derivatives were measured spectrophotometrically. Two different measurements were made for protein oxidation: quantification of (a) carbonyls and (b) protein. Meat samples of 1 g were homogenized with 10 ml of 0.15 M potassium chloride (KCl) with the homogenizer (Ultra-Turrax® T25Bbasic, Germany) for 60 s. One ml of homogenate was transferred into a 2 ml Eppendorf vial, where 1 ml of 10% trichloroacetic acid was added. The sample was centrifuged for 5 min at 10,000xg, and the supernatant was removed. For sample (a) 1 ml of 2 M HCl with 0.2% DNPH and for sample (b) 1 ml of 2 M HCl was added. After an incubation of 1 h (shaken every 20 min), 1 ml of 10% trichloroacetic acid (TCA) was added. The sample was vortex and centrifuged for 5 min at 20,000xg. Supernatant was removed carefully without damaging the pellet with a Pasteur pipette. The pellet was washed with 1 ml of ethanol/ethyl acetate (1:1), shaken, and centrifuged for 5 min at 20,000xg; this procedure was repeated two to three times. After this, the pellet was completely dried with nitrogen. The pellet was dissolved in 1.5 ml of 20 mM sodium phosphate buffer with 6 M guanidine hydrochloride, final pH 6.5, shaken, and centrifuged for 2 min at 10,000xg. Carbonyls (sample a) and protein concentration (sample b) were measured at 370 nm and 280 nm, respectively. Concentration of carbonyls was calculated as:

$$Carbonyl\ concentration = \left[\frac{Abs_{370\ nm}}{21.0\ mM^{-1}cm^{-1}}\ x\,1000 \right]$$

Where: $21.0\ mM^{-1}cm^{-1}$ is the molar extinction coefficient of carbonyls. Protein quantification was determined using a standard curve made from BSA.

The inhibitions of roselle extracts against formation of protein carbonyls in Chinese-style sausage was calculated from the equation:

$$\text{Pr}\,otein\ carbonyl\ inhibition\ (\%) = \left(\frac{C_o - C_1}{C_0} \right) x\,100$$

Where: C_0 is the concentration (nM) of protein carbonyls per mg of protein in the control sample and C_1 is the concentration (nM) of protein carbonyls per mg of protein in the tested sample. The inhibitions were expressed as percentages.

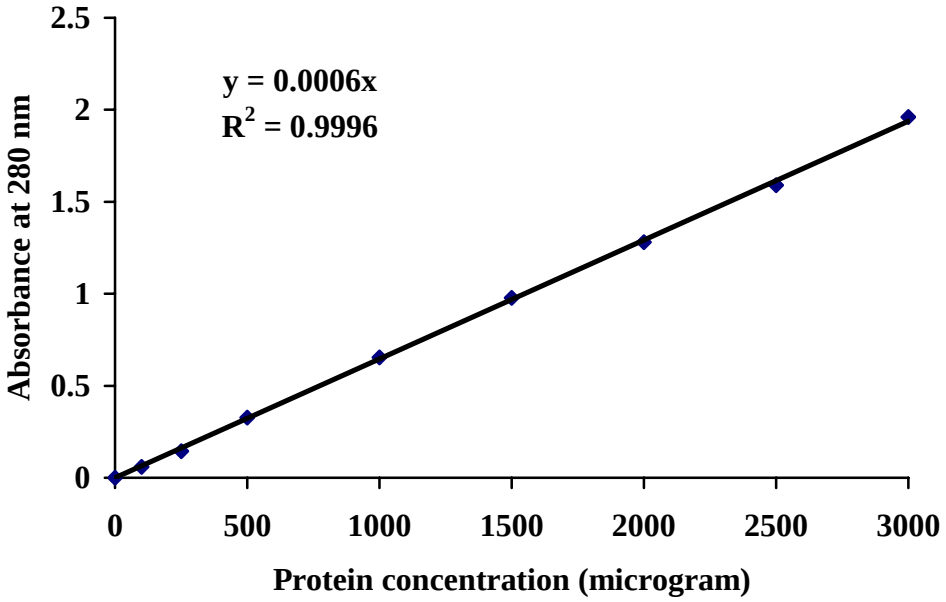


Figure appendix-B3 Bovine serum (BSA) standard curve ranging from 0-3000 microgram

Appendix B8 Nitrite scavenging activity

The anthocyanin extract and/or powder were diluted with the distilled water to a suitable concentration for analysis (up to 200 µg/ml). Three ml of anthocyanin samples were put in the tube (10 ml), then 2 ml of buffer pH 3.0*, 6.0* and 9.0**) and 0.1 ml of 200 µg/ml $NaNO_2$ were added, respectively. Finally, water was added up to 10 ml. The mixture was immediately incubated for 60 min in the water bath at 37°C. Then equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)-ethyline diamine hydrochloride, 2.5% Phosphoric acid) was added to the above mixture. The absorbance was measured, using a Spectrophotometer UV-Vis spectrophotometer (Shimadzu UV-1601, Japan) after 10 min at 538 nm. At the same time the control (without $NaNO_2$) and standard (without $NaNO_2$ and without pigment sample) were also measured. Ascorbic acid and butylated hydroxyanisole (BHA) were used as the positive control compounds. $NaNO_2$ scavenging activity was calculated using the following equation:

$$Sodium\ nitrite\ scavenging\ (\%\ Sa) = \frac{OD_s\left(OD_p - OD_c\right)}{OD_s}\ x\,100$$

Where: Sa is the $NaNO_2$ scavenging rate of tested sample (%), ODs is the OD value of standard, ODp is the OD value in the presence of tested sample and ODc is the OD value of control.

Remark: Buffer pH 3.0 and 6.0 = 0.1 M Citric acid buffer solution pH 3.0 and 6.0, respectively and Buffer pH 9.0 = 0.1 M Sodium carbonate-sodium bicarbonate buffer solutions pH 9.0

Appendix B9 Nitric oxide radical scavenging

Nitric oxide scavenging activity was determined according to Griess Illosvoy reaction. The reaction mixture contained: 10 mM sodium nitroprusside (SNP) in 0.5 M phosphate buffer, pH 7.4, and various doses (0-200 μg/ml) of the test solution (anthocyanin) in a final volume of 3 ml. After incubation for 60 min at 37°C, Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)-ethyline diamine hydrochloride, 2.5% Phosphoric acid) was added. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with α-napthyl-ethylenediamine was measured, using a Spectrophotometer UV-Vis spectrophotometer (Shimadzu UV-1601, Japan) at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated by using the formula:

$$Nitric\ oxide\ scavenging\ (\%) = \frac{A_{540\ nm\ of\ control} - A_{540\ nm\ of\ sample}}{A_{540\ nm\ of\ control}}\ x\ 100$$

Appendix B10 Peroxynitrite scavenging activity

Peroxynitrite ($ONOO^-$) was synthesized by the method described by Beckman et al. (1994). An acidic solution 2.11 M H_2O_2 in 1.85 M HNO_3 was mixed with 5 ml 2 M $NaNO_2$ on an ice bath for 1 s and 5 ml of ice-cold 4.2 M NaOH was added. Excess H_2O_2 was removed by treatment with granular manganese dioxide (MnO_2) pre-washed with 1.2 M NaOH and the reaction mixture were left overnight at -20°C. Collect the sample of $ONOO^-$, dilute ~20 times in 0.1 M NaOH and measure the UV/visible absorption spectrum in a quartz cuvette (previously blanked with the NaOH solution alone) between 240 and 400 nm. An absorption band at 302 nm should be evident. Calculate the concentration of $ONOO^-$ was calculated by using the formula:

$$(\varepsilon_{302}\ ONOO^- = 1670\ M^{-1}\ cm^{-1})$$

$$Concentration\ of\ \ peroxynitrite = \frac{\varepsilon_{302nm}}{1670\ M^{-1}cm^{-1}}$$

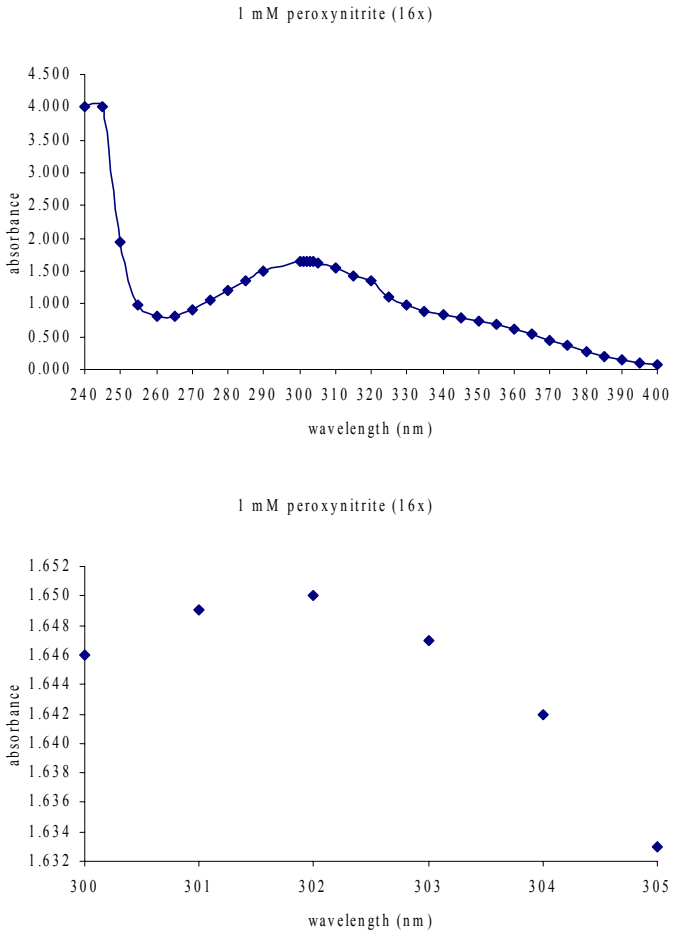


Figure appendix-B4 Absorbance of 1 mM peroxynitrite (16x) [$\lambda_{max\ @\ 301.8} = 1.651$]

An Evans Blue bleaching assay was used to measure peroxynitrite scavenging activity. The assay was performed by a standard method with a slight modification. The reaction mixture contained 50 mM phosphate buffer (pH 7.4) (435 µl), 0.1 mM DTPA (10 µl), 90 mM NaCl (10 µl), 5 mM KCl (10 µl), 12.5 µM Evans Blue (500 µl), various doses of plant extract (0–200 µg/ml) (30 µl) and 1 mM peroxynitrite (5 µl) in a final volume of 1 ml. After incubation at 25°C for 30 min the absorbance was measured, using a Spectrophotometer UV-Vis spectrophotometer (Shimadzu UV-1601, Japan) at 611 nm. The percentage scavenging of $ONOO^-$ was calculated by comparing the results of the test and blank samples. All tests were performed six times. Gallic acid was used as the reference compound.

$$Peroxynitrite\ scavenger\ (\%) = \left[\frac{A_0 - A_1}{A_0}\right] x\,100$$

Where: A_0 is the Absorbance of the control, and A_1 is Absorbance in the presence of the sample of extracts and standard.

Appendix B11 Nitrite residue assay

Ground sausage samples (2-5 g) were homogenised with 100 ml of 80°C distilled water in a 250 ml flask, using a homogenizer (Ultra-Turrax® T25Bbasic, Germany) for 60 s at high speed. The homogenate was washed with distilled water to 150 ml totally, sealed with an aluminium foil cap, and heated for 30 min in an 80°C shaking water bath (50 rpm). After cooling with ice water to room temperature, and filtering (Whatman No. 1) immediately, 10 ml of filtrate were transferred into a tube; 2 ml of Griess solution (1% sulfanilamide, 0.1% N-(1-naphthyl)-ethyline diamine hydrochloride, 2.5% Phosphoric acid) were placed in the tube, covered with aluminium-foil, and kept for 30 min. absorbance was measured, using a Spectrophotometer UV-Vis spectrophotometer (Shimadzu UV-1601, Japan) at 540 nm wave-length. The calibration curve was constructed by plotting the absorbance vs. the concentration.

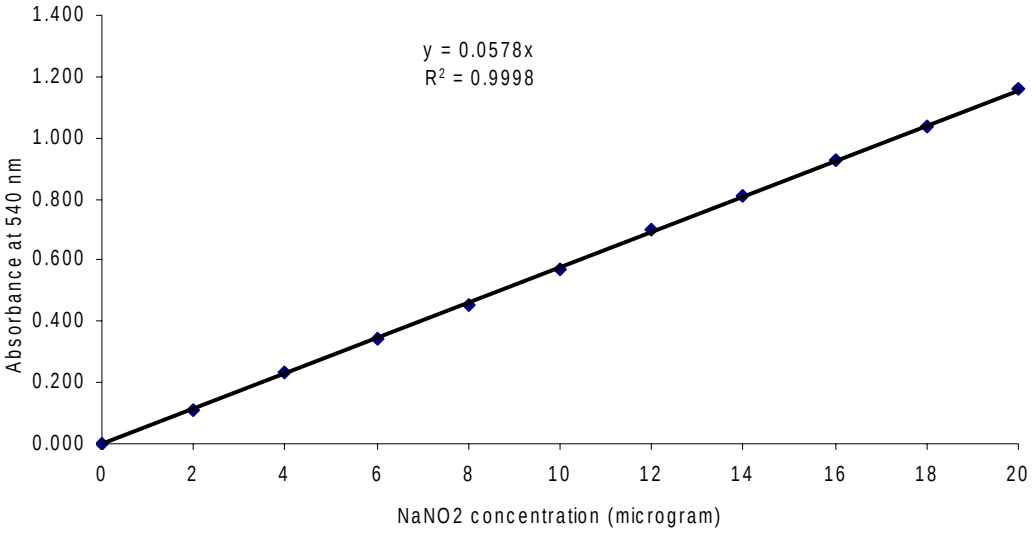


Figure appendix-B5 Sodium nitrite ($NaNO_2$) standard curve ranging from 0-20 microgram

Appendix C

Sensory evaluation

Appendix C1 Questionnaire for sensory evaluation (QDA)

A sensory evaluation test

(Quantitative Descriptive Analysis:QDA)

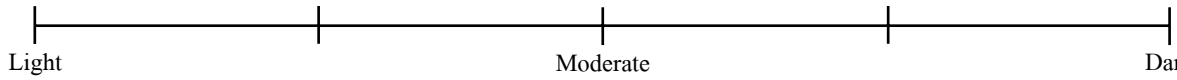
Product: Chinese-style sausage

Name.....Date.....

Instruction: Taste the sausage, one at a time and mark scale of each attribute on the scale line

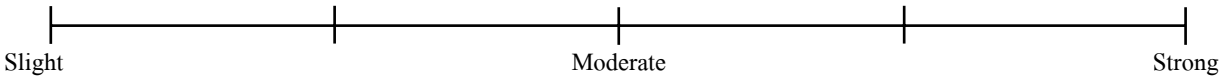
1. Appearance

1.1 Inner color

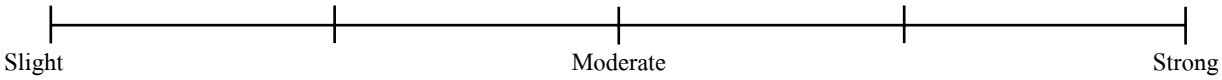


2. Odor

2.1 Normally oder

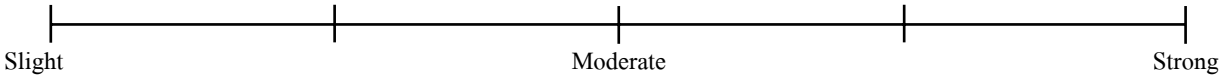


2.2 Rancid oder



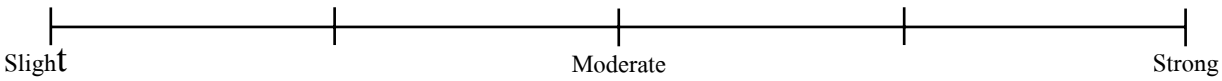
3. Taste

3.1 Sweet

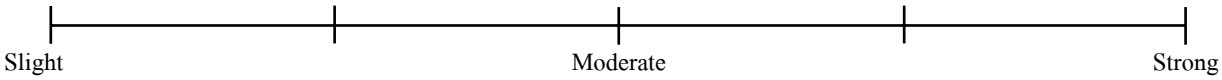


4. Mouthfeel texture

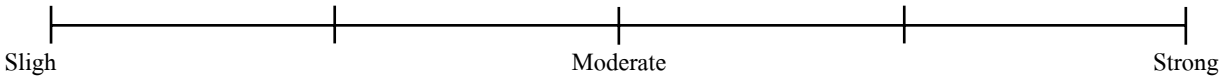
4.1 Hardness



4.2 Gumminess

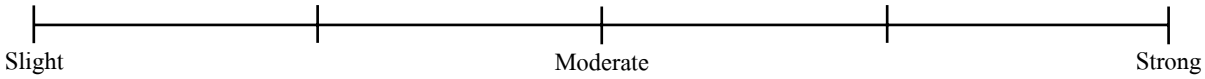


4.3 Chewiness



5. Overall acceptability

5.1 Overall acceptability



Appendix C2 Questionnaire for sensory evaluation (7-point hedonic scale)

A sensory evaluation test

(7-point hedonic scale)

Product: Chinese-style sausage

Name.....Date.....

Instruction: Taste the sample from left to right and put the score

1 = Dislike Extremely

2 = Dislike Moderately

3 = Dislike Slightly

4 = Neither Like nor Dislike

5 = Like Slightly

6 = Like Moderately

7 = Like Extremely

	.....	.....	.....
Color	_____	_____	_____
Odor	_____	_____	_____
Taste	_____	_____	_____
Texture	_____	_____	_____
Overall liking	_____	_____	_____

Suggestion

_____

_____

_____

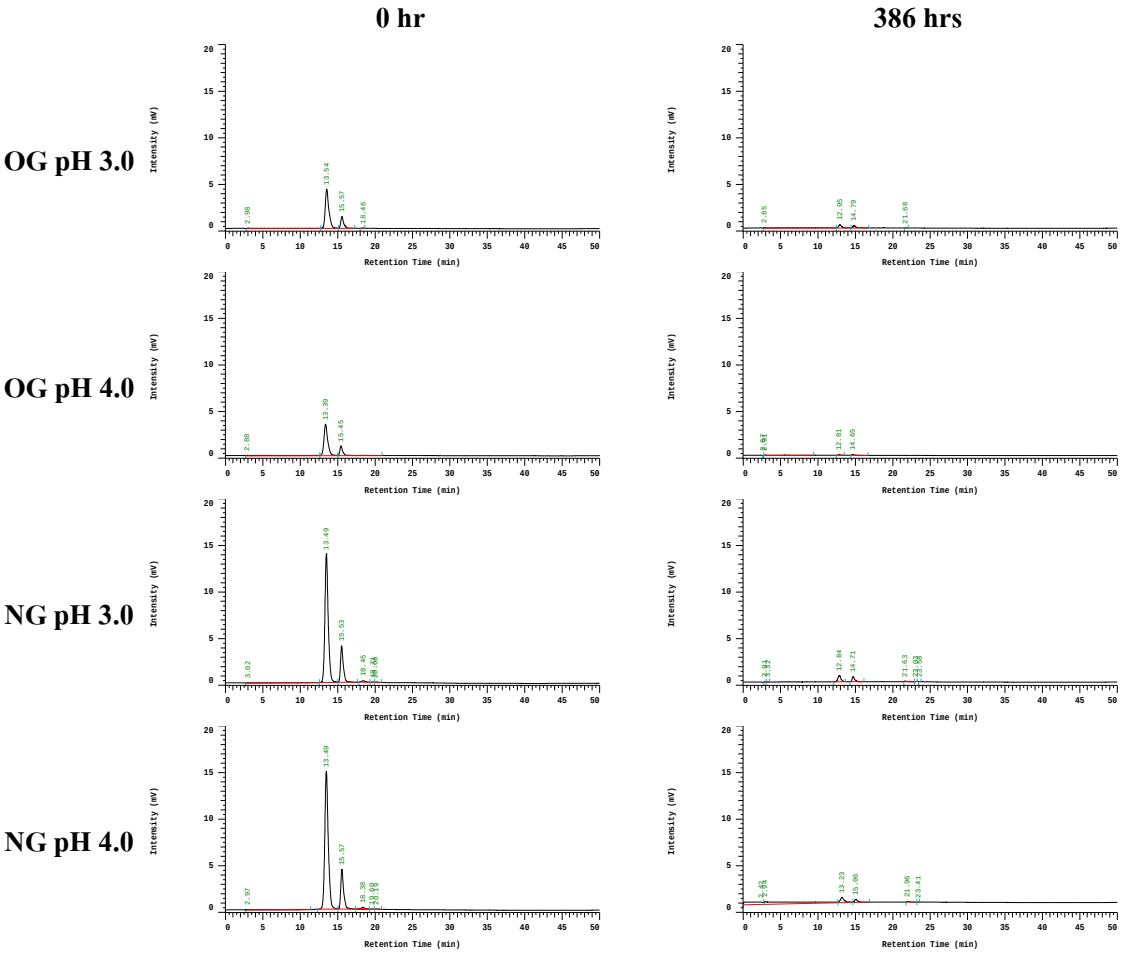
_____

_____

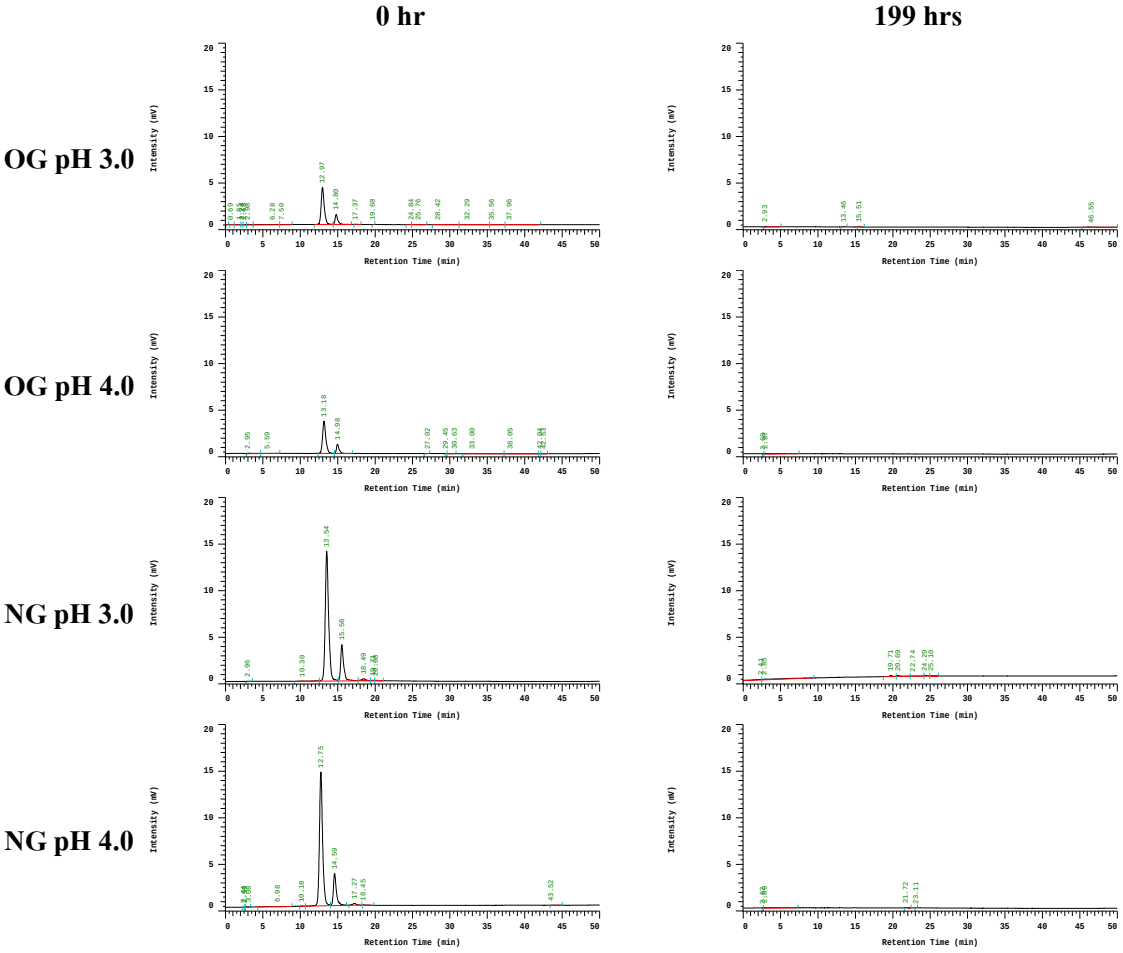
Appendix D

HPLC chromatogram trace of roselle anthocyanins

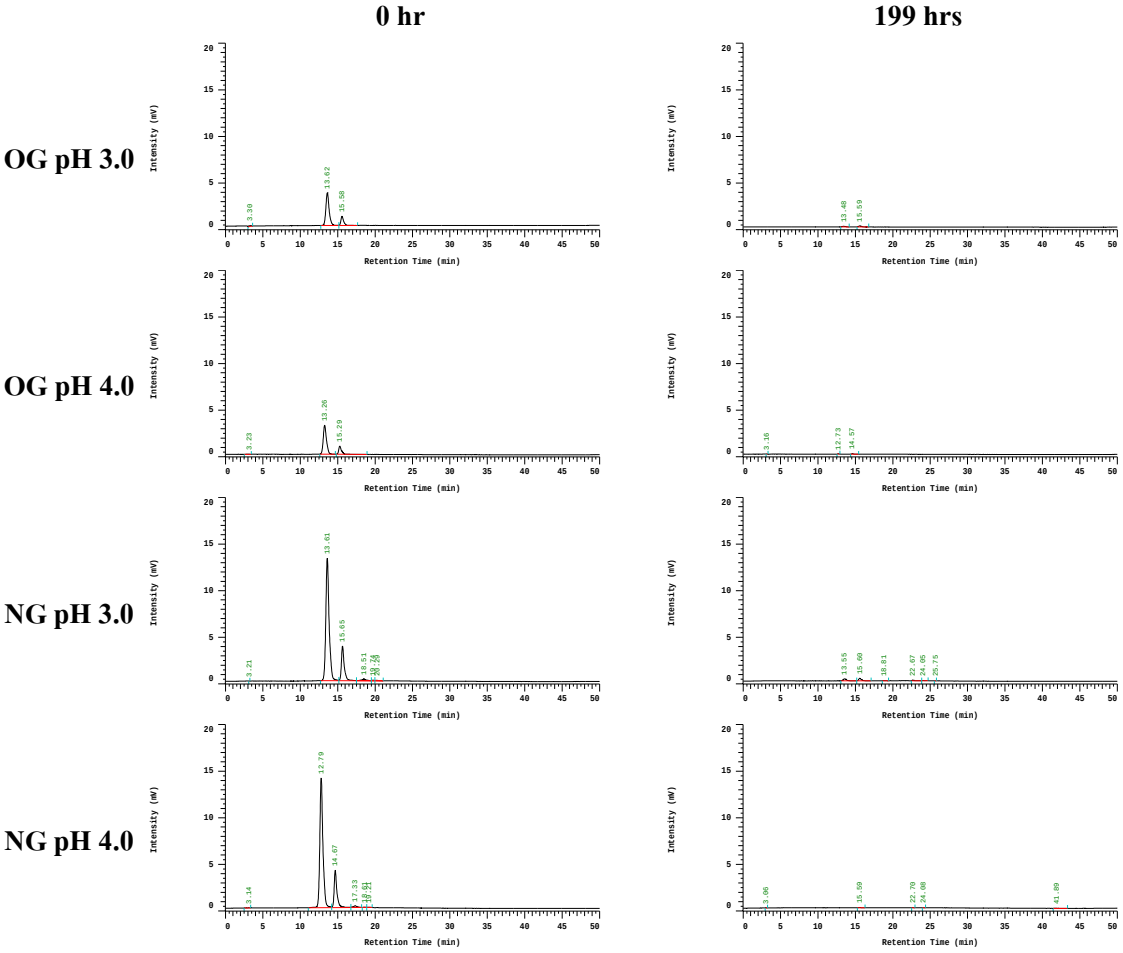
Appendix D1 HPLC chromatogram from roselle anthocyanin prepared in phosphate buffer with sucrose and heat at 50 °C for 0 and 386 hr



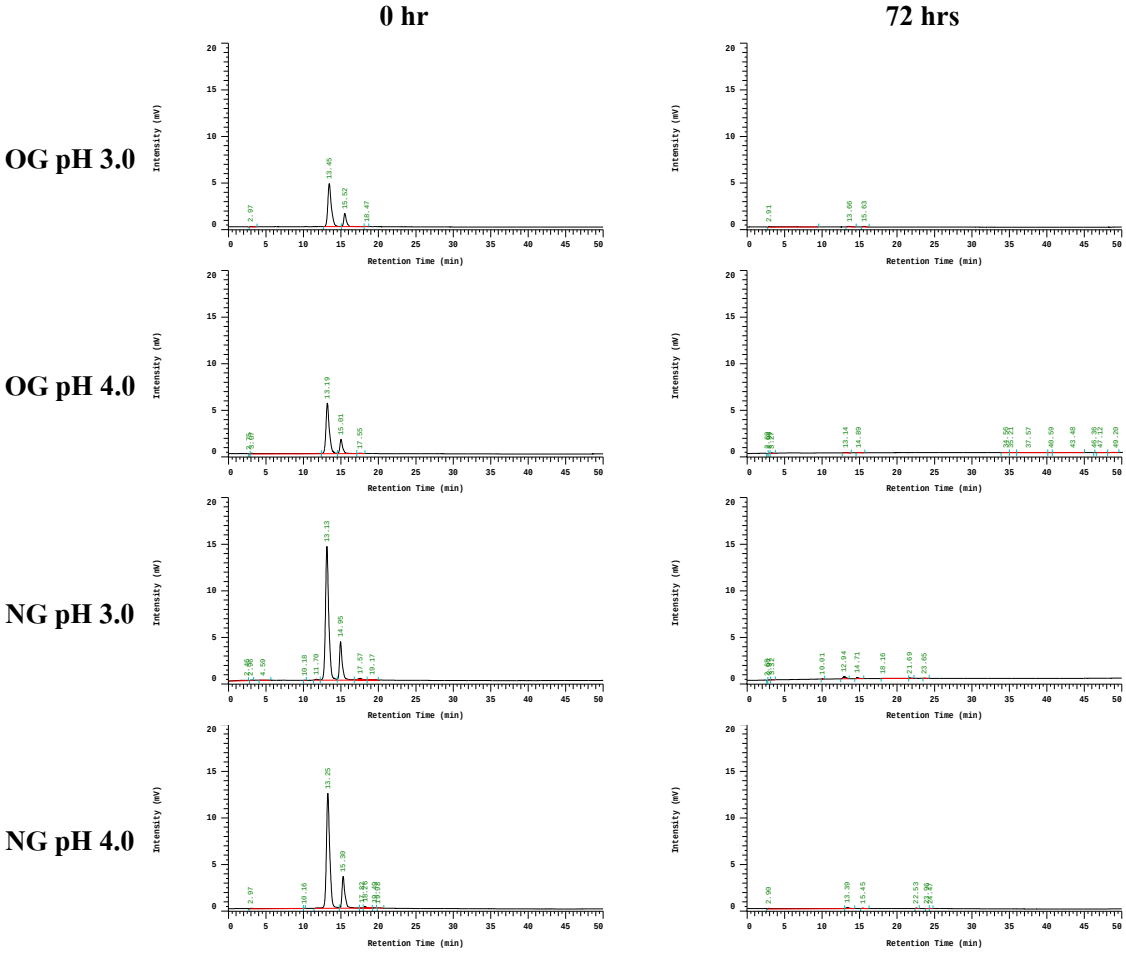
Appendix D2 HPLC chromatogram from roselle anthocyanin prepared in phosphate buffer with sucrose and heat at 60 °C for 0 and 199 hr



Appendix D3 HPLC chromatogram from roselle anthocyanin prepared in phosphate buffer without sucrose and heat at 60 °C for 0 and 199 hr



Appendix D4 HPLC chromatogram from roselle anthocyanin prepared in phosphate buffer with sucrose and heat at 70 °C for 0 and 72 hr



Appendix E

Statistical analysis

Appendix E1 Analysis of variance for Hunter L-value in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	36165.728	207	174.714	713957.594	.000
ACNS	942.373	1	942.373	3850953.080	.000
PH	5139.299	1	5139.299	21001459.451	.000
SUCROSE	1.102	1	1.102	4501.934	.000
TEMP	.000	0	.	.	.
TIME	22160.279	23	963.490	3937249.532	.000
ACNS * PH	9.796	1	9.796	40029.630	.000
ACNS * SUCROSE	10.518	1	10.518	42982.064	.000
PH * SUCROSE	148.092	1	148.092	605171.119	.000
ACNS * PH * SUCROSE	41.205	1	41.205	168382.331	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	424.883	23	18.473	75489.524	.000
PH * TIME	865.331	23	37.623	153744.563	.000
ACNS * PH * TIME	134.128	23	5.832	23830.669	.000
SUCROSE * TIME	454.949	23	19.780	80831.439	.000
ACNS * SUCROSE * TIME	67.157	23	2.920	11931.844	.000
PH * SUCROSE * TIME	220.070	23	9.568	39100.184	.000
ACNS * PH * SUCROSE * TIME	205.415	23	8.931	36496.417	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	.102	416	2.45×10^{-4}		
Corrected Total	36165.830	623			

Appendix E2 Analysis of variance for Hunter a-value in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	144887.793	207	699.941	4918504.522	.000
ACNS	209.892	1	209.892	1474920.157	.000
PH	22416.322	1	22416.322	157520098.850	.000
SUCROSE	3.023	1	3.023	21240.443	.000
TEMP	.000	0	.	.	.
TIME	93321.127	23	4057.440	28511742.587	.000
ACNS * PH	7.840E-02	1	7.840E-02	550.944	.000
ACNS * SUCROSE	29.176	1	29.176	205017.681	.000
PH * SUCROSE	559.859	1	559.859	3934144.637	.000
ACNS * PH * SUCROSE	237.338	1	237.338	1667782.397	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	1532.416	23	66.627	468188.237	.000
PH * TIME	3529.081	23	153.438	1078215.141	.000
ACNS * PH * TIME	412.905	23	17.952	126151.806	.000
SUCROSE * TIME	1146.321	23	49.840	350227.290	.000
ACNS * SUCROSE * TIME	165.649	23	7.202	50609.575	.000
PH * SUCROSE * TIME	702.238	23	30.532	214549.662	.000
ACNS * PH * SUCROSE * TIME	661.282	23	28.751	202036.795	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	5.92×10^{-2}	416	1.42×10^{-4}		
Corrected Total	144887.852	623			

Appendix E3 Analysis of variance for Hunter b-value in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	7540.505	207	36.428	267421.163	.000
ACNS	1933.389	1	1933.389	14193353.250	.000
PH	2.392	1	2.392	17558.391	.000
SUCROSE	10.275	1	10.275	75430.712	.000
TEMP	.000	0	.	.	.
TIME	3316.198	23	144.183	1058469.397	.000
ACNS * PH	.136	1	.136	995.434	.000
ACNS * SUCROSE	4.198	1	4.198	30820.318	.000
PH * SUCROSE	7.986	1	7.986	58629.513	.000
ACNS * PH * SUCROSE	18.900	1	18.900	138745.789	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	161.847	23	7.037	51658.715	.000
PH * TIME	527.872	23	22.951	168487.014	.000
ACNS * PH * TIME	59.750	23	2.598	19071.220	.000
SUCROSE * TIME	85.174	23	3.703	27185.974	.000
ACNS * SUCROSE * TIME	19.492	23	.847	6221.506	.000
PH * SUCROSE * TIME	33.530	23	1.458	10702.184	.000
ACNS * PH * SUCROSE * TIME	31.599	23	1.374	10085.707	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	5.67×10^{-2}	416	1.36×10^{-4}		
Corrected Total	7540.562	623			

Appendix E4 Analysis of variance for chroma in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	91607.438	207	442.548	2209199.667	.000
ACNS	1685.885	1	1685.885	8415938.055	.000
PH	15351.057	1	15351.057	76632477.590	.000
SUCROSE	66.115	1	66.115	330047.311	.000
TEMP	.000	0	.	.	.
TIME	55219.541	23	2400.850	11985041.253	.000
ACNS * PH	30.975	1	30.975	154625.170	.000
ACNS * SUCROSE	39.541	1	39.541	197387.020	.000
PH * SUCROSE	397.541	1	397.541	1984523.174	.000
ACNS * PH * SUCROSE	176.421	1	176.421	880694.386	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	720.794	23	31.339	156443.722	.000
PH * TIME	5217.528	23	226.849	1132430.357	.000
ACNS * PH * TIME	514.536	23	22.371	111676.742	.000
SUCROSE * TIME	889.975	23	38.695	193163.234	.000
ACNS * SUCROSE * TIME	111.973	23	4.868	24302.920	.000
PH * SUCROSE * TIME	496.512	23	21.587	107764.740	.000
ACNS * PH * SUCROSE * TIME	459.526	23	19.979	99737.205	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	8.333E-02	416	2.003E-04		
Corrected Total	91607.521	623			

Appendix E5 Analysis of variance for hue value in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	569293.665	207	2750.211	4394703.269	.000
ACNS	6508.411	1	6508.411	10400123.425	.000
PH	39461.256	1	39461.256	63057167.503	.000
SUCROSE	842.998	1	842.998	1347069.325	.000
TEMP	.000	0	.	.	.
TIME	356345.330	23	15493.275	24757499.998	.000
ACNS * PH	12.404	1	12.404	19820.689	.000
ACNS * SUCROSE	66.108	1	66.108	105636.849	.000
PH * SUCROSE	817.447	1	817.447	1306241.191	.000
ACNS * PH * SUCROSE	128.201	1	128.201	204858.988	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	5652.797	23	245.774	392734.571	.000
PH * TIME	24865.102	23	1081.091	1727531.449	.000
ACNS * PH * TIME	2602.502	23	113.152	180811.842	.000
SUCROSE * TIME	3249.163	23	141.268	225739.303	.000
ACNS * SUCROSE * TIME	1284.371	23	55.842	89233.174	.000
PH * SUCROSE * TIME	2157.934	23	93.823	149924.905	.000
ACNS * PH * SUCROSE * TIME	1713.282	23	74.491	119032.223	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	.260	416	6.26×10^{-4}		
Corrected Total	569293.925	623			

Appendix E6 Analysis of variance for A_{420} in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	1.680	207	8.115E-03	4662.807	.000
ACNS	.935	1	.935	537230.482	.000
PH	.155	1	.155	89045.086	.000
SUCROSE	3.510E-02	1	3.510E-02	20165.171	.000
TEMP	.000	0	.	.	.
TIME	.236	23	1.025E-02	5889.363	.000
ACNS * PH	7.526E-04	1	7.526E-04	432.453	.000
ACNS * SUCROSE	2.957E-03	1	2.957E-03	1698.884	.000
PH * SUCROSE	2.080E-03	1	2.080E-03	1195.151	.000
ACNS * PH * SUCROSE	6.823E-05	1	6.823E-05	39.204	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	2.061E-02	23	8.961E-04	514.879	.000
PH * TIME	.111	23	4.828E-03	2773.842	.000
ACNS * PH * TIME	2.118E-02	23	9.208E-04	529.094	.000
SUCROSE * TIME	4.369E-02	23	1.899E-03	1091.368	.000
ACNS * SUCROSE * TIME	1.070E-02	23	4.653E-04	267.373	.000
PH * SUCROSE * TIME	5.844E-03	23	2.541E-04	146.003	.000
ACNS * PH * SUCROSE * TIME	7.410E-03	23	3.222E-04	185.105	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	7.240E-04	416	1.740E-06		
Corrected Total	1.681	623			

Appendix E7 Analysis of variance for A_{520} in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	28.514	207	.138	125116.587	.000
ACNS	.388	1	.388	352832.620	.000
PH	4.666	1	4.666	4237762.890	.000
SUCROSE	1.295E-03	1	1.295E-03	1175.939	.000
TEMP	.000	0	.	.	.
TIME	17.487	23	.760	690573.309	.000
ACNS * PH	7.202E-03	1	7.202E-03	6541.946	.000
ACNS * SUCROSE	1.600E-02	1	1.600E-02	14530.673	.000
PH * SUCROSE	.116	1	.116	105049.503	.000
ACNS * PH * SUCROSE	3.791E-02	1	3.791E-02	34433.492	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	.436	23	1.898E-02	17237.811	.000
PH * TIME	1.440	23	6.262E-02	56881.154	.000
ACNS * PH * TIME	.232	23	1.009E-02	9161.332	.000
SUCROSE * TIME	.291	23	1.263E-02	11474.604	.000
ACNS * SUCROSE * TIME	3.769E-02	23	1.639E-03	1488.524	.000
PH * SUCROSE * TIME	.151	23	6.571E-03	5968.603	.000
ACNS * PH * SUCROSE * TIME	.147	23	6.385E-03	5799.088	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	4.580E-04	416	1.101E-06		
Corrected Total	28.514	623			

Appendix E8 Analysis of variance for color density in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	38.081	207	.184	7971.938	.000
ACNS	2.378	1	2.378	103035.746	.000
PH	6.507	1	6.507	281974.518	.000
SUCROSE	4.435E-02	1	4.435E-02	1921.872	.000
TEMP	.000	0	.	.	.
TIME	20.983	23	.912	39532.402	.000
ACNS * PH	5.949E-03	1	5.949E-03	257.797	.000
ACNS * SUCROSE	1.473E-02	1	1.473E-02	638.232	.000
PH * SUCROSE	.138	1	.138	5976.544	.000
ACNS * PH * SUCROSE	3.958E-02	1	3.958E-02	1715.303	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	.511	23	2.223E-02	963.153	.000
PH * TIME	2.268	23	9.862E-02	4273.534	.000
ACNS * PH * TIME	.360	23	1.566E-02	678.605	.000
SUCROSE * TIME	.384	23	1.670E-02	723.660	.000
ACNS * SUCROSE * TIME	4.618E-02	23	2.008E-03	87.010	.000
PH * SUCROSE * TIME	.189	23	8.205E-03	355.537	.000
ACNS * PH * SUCROSE * TIME	.181	23	7.870E-03	341.021	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	9.600E-03	416	2.308E-05		
Corrected Total	38.091	623			

Appendix E9 Analysis of variance for degradation index in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	301.589	207	1.457	18366.444	.000
ACNS	4.109	1	4.109	51797.423	.000
PH	24.608	1	24.608	310211.488	.000
SUCROSE	5.059	1	5.059	63768.862	.000
TEMP	.000	0	.	.	.
TIME	189.940	23	8.258	104103.867	.000
ACNS * PH	3.570E-03	1	3.570E-03	45.003	.000
ACNS * SUCROSE	6.765E-03	1	6.765E-03	85.285	.000
PH * SUCROSE	.320	1	.320	4036.039	.000
ACNS * PH * SUCROSE	2.875E-02	1	2.875E-02	362.381	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	2.567	23	.112	1407.169	.000
PH * TIME	6.461	23	.281	3541.021	.000
ACNS * PH * TIME	1.033	23	4.493E-02	566.413	.000
SUCROSE * TIME	6.553	23	.285	3591.516	.000
ACNS * SUCROSE * TIME	.584	23	2.539E-02	320.073	.000
PH * SUCROSE * TIME	1.021	23	4.440E-02	559.757	.000
ACNS * PH * SUCROSE * TIME	.872	23	3.790E-02	477.713	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	3.300E-02	416	7.933E-05		
Corrected Total	301.622	623			

Appendix E10 Analysis of variance for monomeric anthocyanins in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	2219.914	207	10.724	8910.411	.000
ACNS	54.137	1	54.137	44980.436	.000
PH	174.917	1	174.917	145332.840	.000
SUCROSE	4.839	1	4.839	4020.564	.000
TEMP	.000	0	.	.	.
TIME	1451.517	23	63.109	52435.609	.000
ACNS * PH	.416	1	.416	345.400	.000
ACNS * SUCROSE	.384	1	.384	319.125	.000
PH * SUCROSE	2.100	1	2.100	1745.097	.000
ACNS * PH * SUCROSE	1.073	1	1.073	891.468	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	37.238	23	1.619	1345.196	.000
PH * TIME	66.549	23	2.893	2404.074	.000
ACNS * PH * TIME	9.422	23	.410	340.356	.000
SUCROSE * TIME	11.195	23	.487	404.406	.000
ACNS * SUCROSE * TIME	7.362	23	.320	265.950	.000
PH * SUCROSE * TIME	8.675	23	.377	313.380	.000
ACNS * PH * SUCROSE * TIME	7.142	23	.311	258.008	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	.501	416	1.204E-03		
Corrected Total	2220.415	623			

Appendix E11 Analysis of variance for polymeric anthocyanins in roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	2610.047	207	12.609	4785.793	.000
ACNS	53.039	1	53.039	20131.245	.000
PH	195.770	1	195.770	74305.644	.000
SUCROSE	6.926	1	6.926	2628.630	.000
TEMP	.000	0	.	.	.
TIME	1728.251	23	75.141	28520.347	.000
ACNS * PH	.162	1	.162	61.436	.000
ACNS * SUCROSE	.319	1	.319	120.895	.000
PH * SUCROSE	4.390	1	4.390	1666.117	.000
ACNS * PH * SUCROSE	.650	1	.650	246.735	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	35.822	23	1.557	591.154	.000
PH * TIME	74.091	23	3.221	1222.682	.000
ACNS * PH * TIME	11.189	23	.486	184.650	.000
SUCROSE * TIME	14.093	23	.613	232.566	.000
ACNS * SUCROSE * TIME	8.364	23	.364	138.030	.000
PH * SUCROSE * TIME	14.960	23	.650	246.872	.000
ACNS * PH * SUCROSE * TIME	7.671	23	.334	126.593	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	1.096	416	2.635E-03		
Corrected Total	2611.143	623			

Appendix E12 Analysis of variance for ferric ions chelating ability of roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	6791.000	71	95.648	141.622	.000
ACNS	650.694	1	650.694	963.454	.000
PH	201.376	1	201.376	298.169	.000
SUCROSE	4.770	1	4.770	7.063	.009
TEMP	.000	0	.	.	.
TIME	1995.220	6	332.537	492.372	.000
ACNS * PH	1304.195	1	1304.195	1931.063	.000
ACNS * SUCROSE	738.298	1	738.298	1093.165	.000
PH * SUCROSE	429.317	1	429.317	635.670	.000
ACNS * PH * SUCROSE	22.042	1	22.042	32.636	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	165.812	6	27.635	40.918	.000
PH * TIME	36.179	6	6.030	8.928	.000
ACNS * PH * TIME	52.010	6	8.668	12.835	.000
SUCROSE * TIME	95.227	6	15.871	23.500	.000
ACNS * SUCROSE * TIME	263.075	6	43.846	64.921	.000
PH * SUCROSE * TIME	38.505	6	6.418	9.502	.000
ACNS * PH * SUCROSE * TIME	63.889	6	10.648	15.766	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	97.254	144	.675		
Corrected Total	6888.255	215			

Appendix E13 Analysis of variance for Trolox equivalence antioxidant capacity of roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	64.289	71	.905	1287.587	.000
ACNS	52.688	1	52.688	74921.806	.000
PH	.874	1	.874	1242.841	.000
SUCROSE	7.180	1	7.180	10209.246	.000
TEMP	.000	0	.	.	.
TIME	1.440	6	.240	341.366	.000
ACNS * PH	.235	1	.235	333.735	.000
ACNS * SUCROSE	.259	1	.259	368.337	.000
PH * SUCROSE	.714	1	.714	1015.513	.000
ACNS * PH * SUCROSE	.378	1	.378	537.996	.000
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	1.168E-02	6	1.947E-03	2.768	.014
PH * TIME	1.601E-02	6	2.668E-03	3.794	.002
ACNS * PH * TIME	1.706E-02	6	2.843E-03	4.043	.001
SUCROSE * TIME	5.928E-02	6	9.880E-03	14.049	.000
ACNS * SUCROSE * TIME	2.126E-02	6	3.544E-03	5.039	.000
PH * SUCROSE * TIME	1.225E-02	6	2.041E-03	2.903	.011
ACNS * PH * SUCROSE * TIME	2.553E-02	6	4.255E-03	6.051	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	.101	144	7.032E-04		
Corrected Total	64.391	215			

Appendix E14 Analysis of variance for ferrous thiocyanate of roselle extracts prepared in different pH with and without sucrose during heating 50 to 70 °C

Source	SS	df	MS	F	Sig.
Corrected Model	768.523	71	10.824	20.685	.000
ACNS	1.251	1	1.251	2.391	.124
PH	10.560	1	10.560	20.181	.000
SUCROSE	30.150	1	30.150	57.618	.000
TEMP	.000	0	.	.	.
TIME	339.828	6	56.638	108.236	.000
ACNS * PH	.768	1	.768	1.468	.228
ACNS * SUCROSE	.600	1	.600	1.146	.286
PH * SUCROSE	3.481	1	3.481	6.652	.011
ACNS * PH * SUCROSE	2.880	1	2.880	5.503	.020
ACNS * TEMP	.000	0	.	.	.
PH * TEMP	.000	0	.	.	.
ACNS * PH * TEMP	.000	0	.	.	.
SUCROSE * TEMP	.000	0	.	.	.
ACNS * SUCROSE * TEMP	.000	0	.	.	.
PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP	.000	0	.	.	.
ACNS * TIME	9.362	6	1.560	2.982	.009
PH * TIME	6.463	6	1.077	2.059	.062
ACNS * PH * TIME	25.232	6	4.205	8.036	.000
SUCROSE * TIME	27.773	6	4.629	8.846	.000
ACNS * SUCROSE * TIME	11.507	6	1.918	3.665	.002
PH * SUCROSE * TIME	3.050	6	.508	.972	.447
ACNS * PH * SUCROSE * TIME	23.641	6	3.940	7.530	.000
TEMP * TIME	.000	0	.	.	.
ACNS * TEMP * TIME	.000	0	.	.	.
PH * TEMP * TIME	.000	0	.	.	.
ACNS * PH * TEMP * TIME	.000	0	.	.	.
SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * SUCROSE * TEMP * TIME	.000	0	.	.	.
PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
ACNS * PH * SUCROSE * TEMP * TIME	.000	0	.	.	.
Error	75.353	144	.523		
Corrected Total	843.875	215			

Appendix E15 Correlations among physicochemical properties of OG and NG roselle extracts

	L-value	a-value	Chroma	A420nm	A520nm	CD	DI	Mono-ACNs	Poly-ACNs
Roselle extract	-.649**	.430**	.586**	.944**	.521**	.677**	-.125**	-.117*	.080
pH	.163**	-.220**	-.188**	-.050	-.195**	-.164**	.211**	-.224**	.229**
Sucrose	-.155**	.173**	.162**	.032	-.127**	.032	.174**	.118**	-.126**
Temperature	.198**	-.186**	-.177**	-.007	-.039	-.136**	.245**	-.118**	.130**
Time	.399**	-.438**	-.386**	-.054	-.242**	-.307**	.456**	-.420**	.429**
L-value	1	-.952**	-.981**	-.723**	-.857**	-.971**	.708**	-.549**	.573**
a-value		1	.961**	.529**	.861**	.918**	-.779**	.704**	-.719**
Chroma			1	.675**	.882**	.971**	-.676**	.554**	-.574**
A420nm				1	.680**	.802**	-.222**	-.038	.003
A520nm					1	.928**	-.580**	.496**	-.506**
Color density (CD)						1	-.573**	.460**	-.481**
Degradation index (DI)							1	-.827**	.856**
Monomeric-ACNs								1	-.995**

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively.

Appendix E16 Correlation among antioxidative capacities of the OG and NG roselle extracts

	FICA[1]	TEAC[2]	FTC[3]
Roselle extract	.307**	.905**	.039
pH	.171*	.117	-.112
Sucrose	.026	.334**	-.189**
Temperature	-.237**	.002	-.293**
Time	-.382**	-.044	-.424**

FICA[1] = Ferrous Ions Chelating Ability (% inhibition). TEAC[2] = Trolox Equivalence Antioxidant Capacity, FTC[3] = Ferric Thiocyanate Method (Antioxidant activity in linoleic acid emulsion system).

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively.