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Effect of Organic Acid on Alcohol Resistance of Djulis (*Chenopodium formosanum* Koidz.) Pigment

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November, 2010

Introduction

Djulis (*Chenopodium formosanum* Koidz.)

- **Native cereal plant**
- **Colorful grains**
- **Insect repellant**
- **A rich source of nutrients**
- **A rich source of antioxidants: phenolics, and betalains**

(Chio and Yang, 2008; Tsai *et al*., 2010)

Table 1. Comparison of nutrition components in Djulis with other cereals

Composition	Djulis	Sweet potato	Wheat	Barley	Rice	Oats
Starch (%)®	55.7	28.6	68.4	62.6	77.2	66.2
Crude protein (%)	17.7	1.0	14.0	9.3	7.5	11.5
Dietary fiber (%)	17.5	2.4	11.3	15.3	0.3	5.1
Water (%)	10.1	69.0	13.0	11.6	14.3	9.4
Crude fat (%)	3.2	0.3	1.6	3.0	0.5	10.1
Calcium (ppm)	2523	340	290	340	50	390
Iron (ppm)	55.6	5.0	28.0	5.0	2.0	32.0
Zinc (ppm)	24.5	3.0	26.0	3.0	11.0	22.0
Sodium (ppm)	238.0	440.0	20.0	440.0	20.0	50.0

(Wu, 2008)

Table 2. Comparison of amino acids composition in Djulis and other cereals

Kind of amino acid (mg/100 gdry)	Djulis	Wheat	Barley	Rice	Corn
Threonine*	0.46	0.34	0.35	0.26	0.30
Valine*	0.60	0.43	0.52	0.45	0.42
Methionine*	0.10	0.25	0.35	0.20	0.35
Isoleuine*	0.51	0.39	0.44	0.31	0.45
Leucine*	0.85	0.69	0.80	0.56	1.44
Phenylalanine*	0.58	0.50	0.90	0.57	0.71
Histidine**	0.38	0.20	0.21	0.13	0.20
Lysine*	0.73	0.36	0.33	0.26	0.32
Arginine**	1.21	0.43	0.42	0.25	0.32
Glutamic acid	3.48	2.87	3.26	1.27	0.64

*Essential amino acid **Semi- Essential amino acid

(Wu, 2008)

Alcoholic beverages

- **Djulis should be regarded as a valuable product and has potential as an economic value-added in the alcoholic beverages product.**

- **However, the pigment of Djulis is quite sensitive to heat, pH, and alcohol due to the unstable resonance of the coloring structure (Herbach *et al.*, 2006a).**

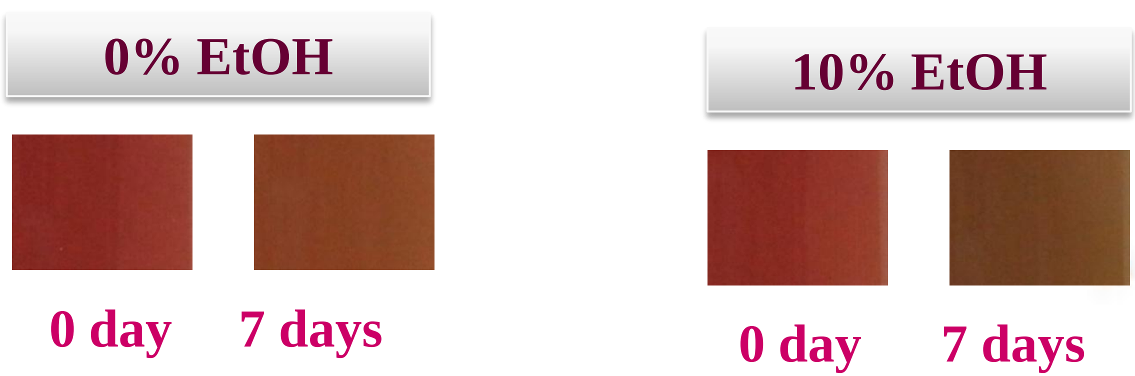
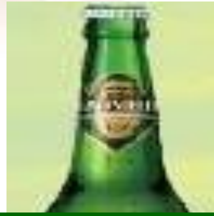


Figure 1. Comparison of color appearance in red Djulis model at 10% alcohol concentration after storage 7 days.

Alcoholic beverages

- **As long as the color of alcoholic beverages remains important to consumers, efforts by the industry to provide safe, suitable, stable and economical colorings will continue (Bell, 1989).**

- **Currently, there is limited information concerning the bleaching resistance of betacyanin in alcohol. There is a need to understand the degradation mechanism of betacyanin in alcohol, and how to improve the quality control of Djulis alcoholic beverage products.**



Beer
(2-6% alcohol)

Wine
(9-20% alcohol)

The color of alcoholic beverages is the first characteristic observed and decides its quality. If we can protect the pigment against bleaching from the alcohol , Djulis can be applied in all types of wine products with economic value.

(>40% alcohol)

(38-63% alcohol)

Figure 2. Types of alcoholic beverages

(http://www.google.co.th/image)

Djulis Pigment

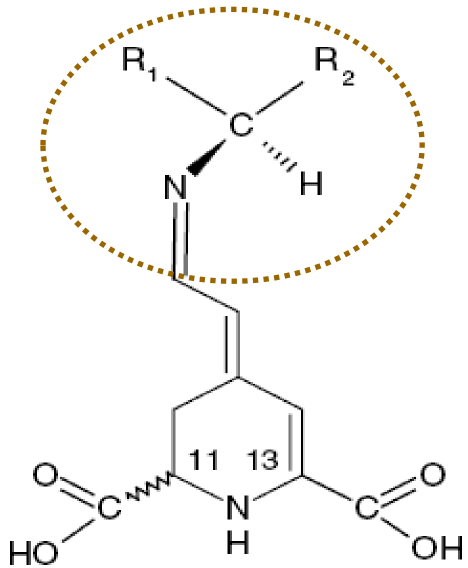
Betalains

Betaxanthins

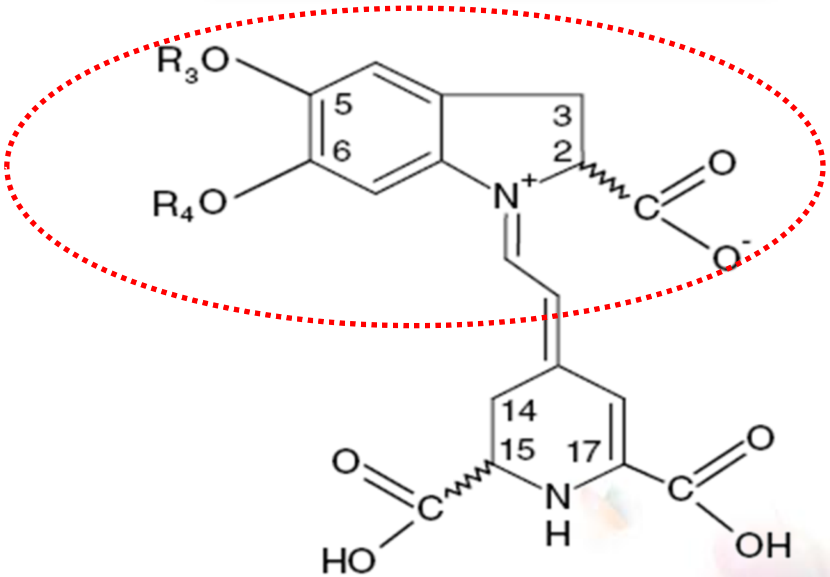
(475 to 485 nm)

Betacyanins

(530 to 545 nm)



(a)

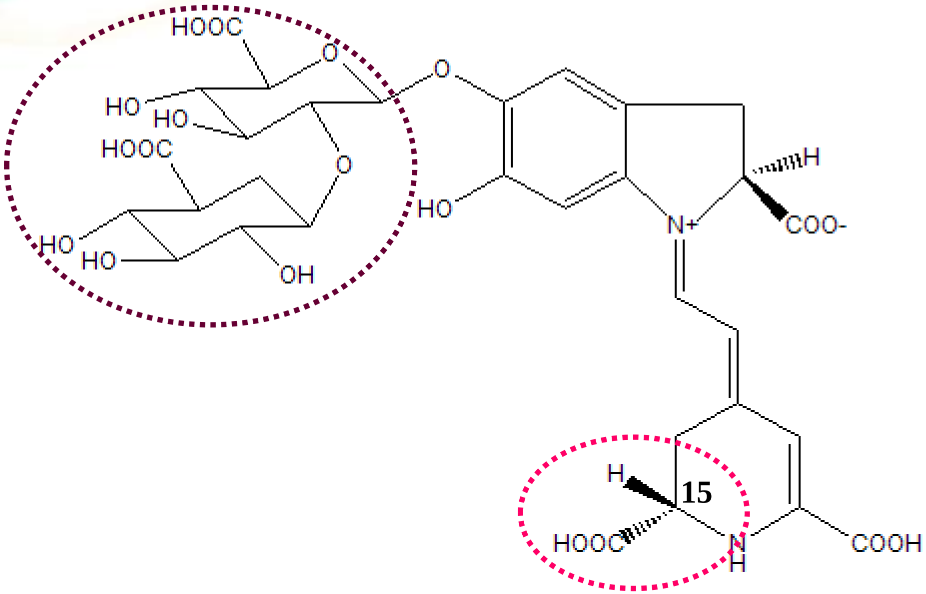


(b)

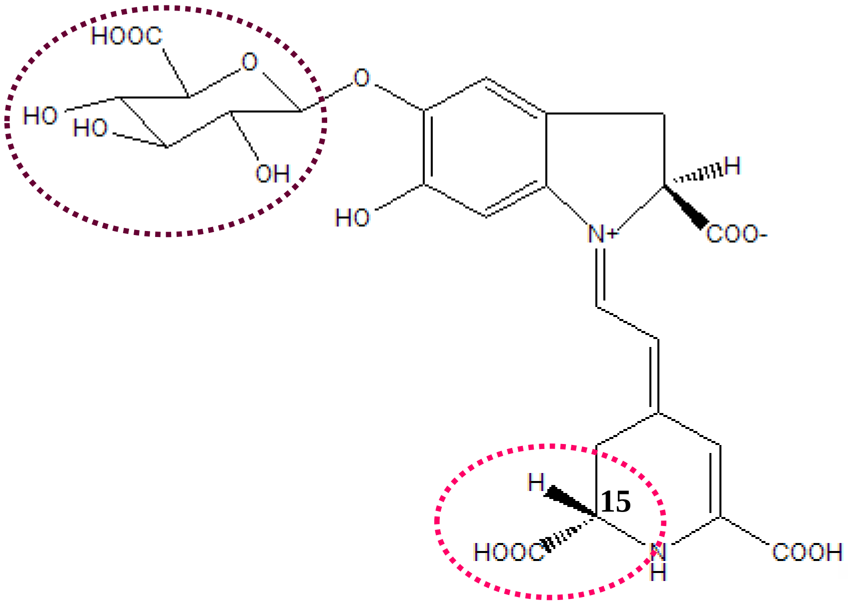
Figure 3. Chemical structure of betaxanthins and betacyanins

(Stintzing *et al*., 2007)

Betacyanins



Amaranthin



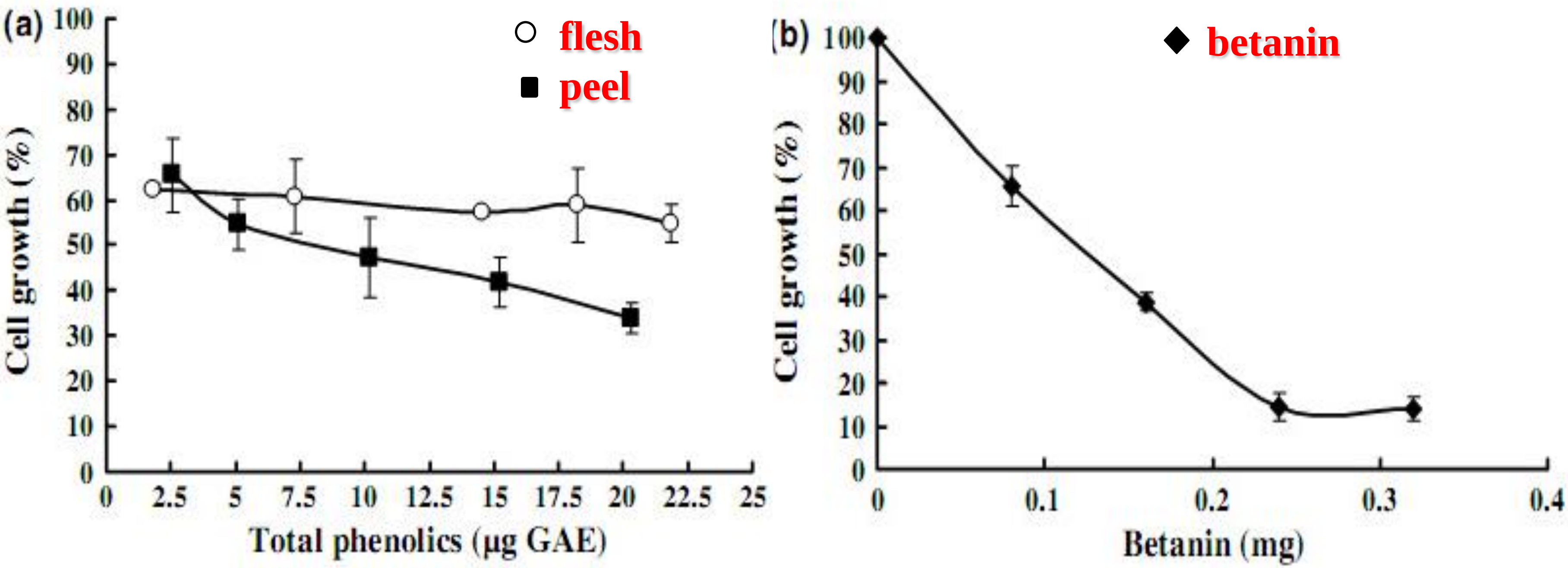
Betanin

Figure 4. Chemical structure of amaranthin and betanin in Djulis

(Herbach *et al.*, 2006)

Bioactivity of Betanin

Total phenolic content & Antiproliferative activity



(a) (b)

Figure 5. Inhibition of B16F10 melanoma cell proliferation by phyto-chemical extracts of *Hylocereus cacti*

(Wu *et al*., 2006)

Factors influencing betanin stability

- pH
- Temperature
- Alcohol
- Pigment content
- Light
- O_2

(Herbach *et al.*, 2006a)

pH

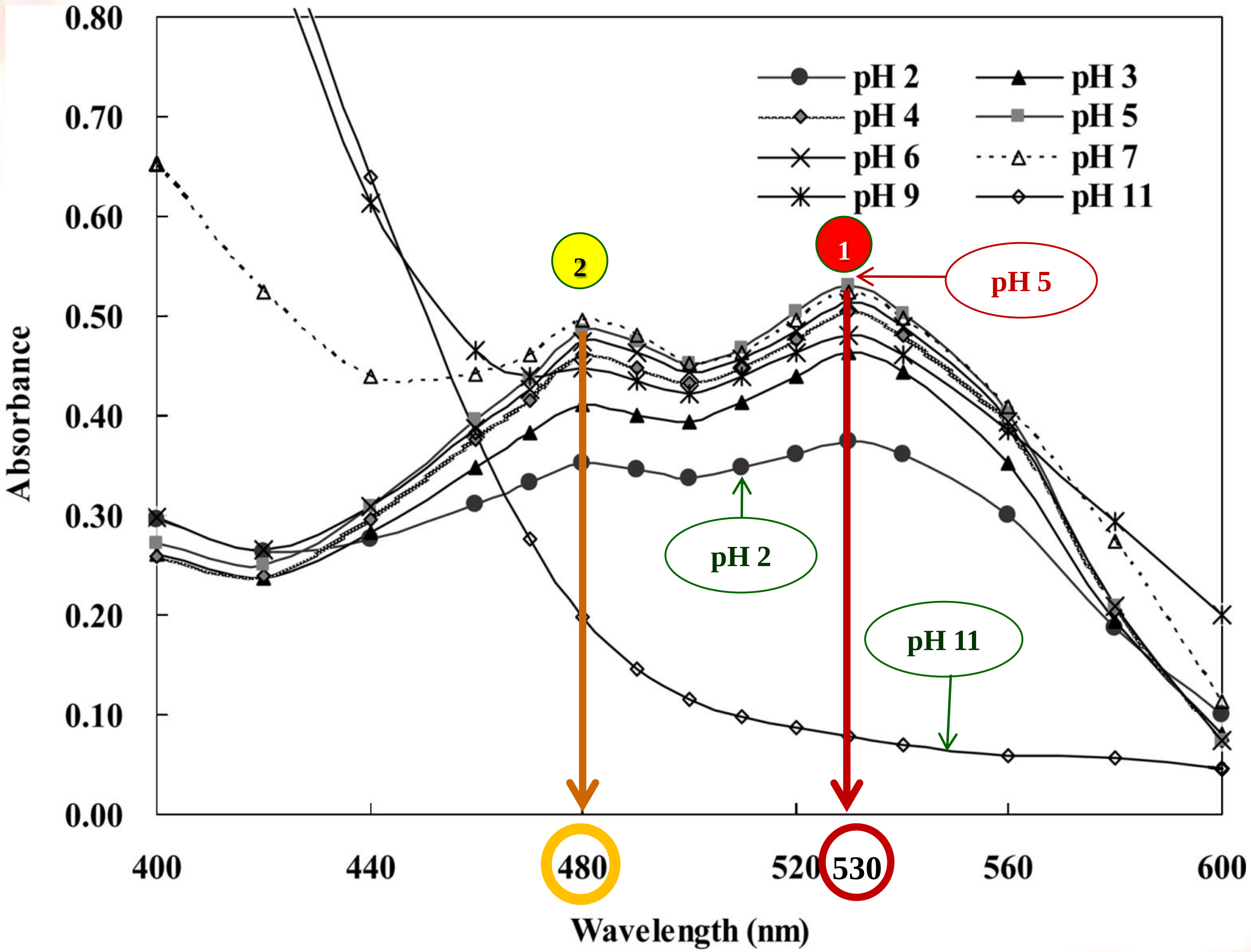


Figure 6. Absorbance spectrum of red Djulis grain pigments extracted with water and adjusted with buffer at pH 2-11.

(Tsai *et al*., 2010)

Thermal degradation

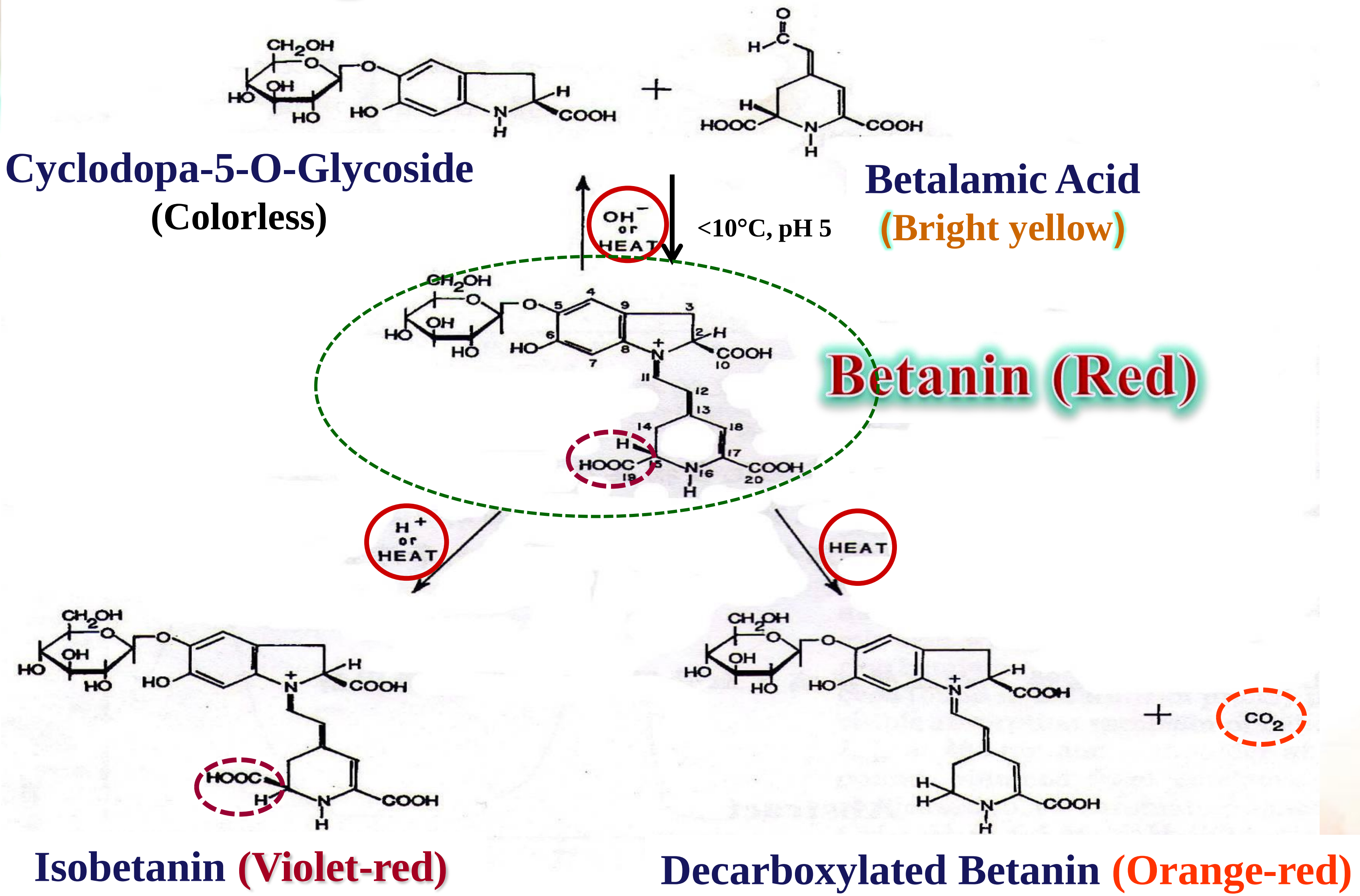


Figure 7. Proposed decomposition products and reactions of betanin

(Stintzing *et al*., 2004)

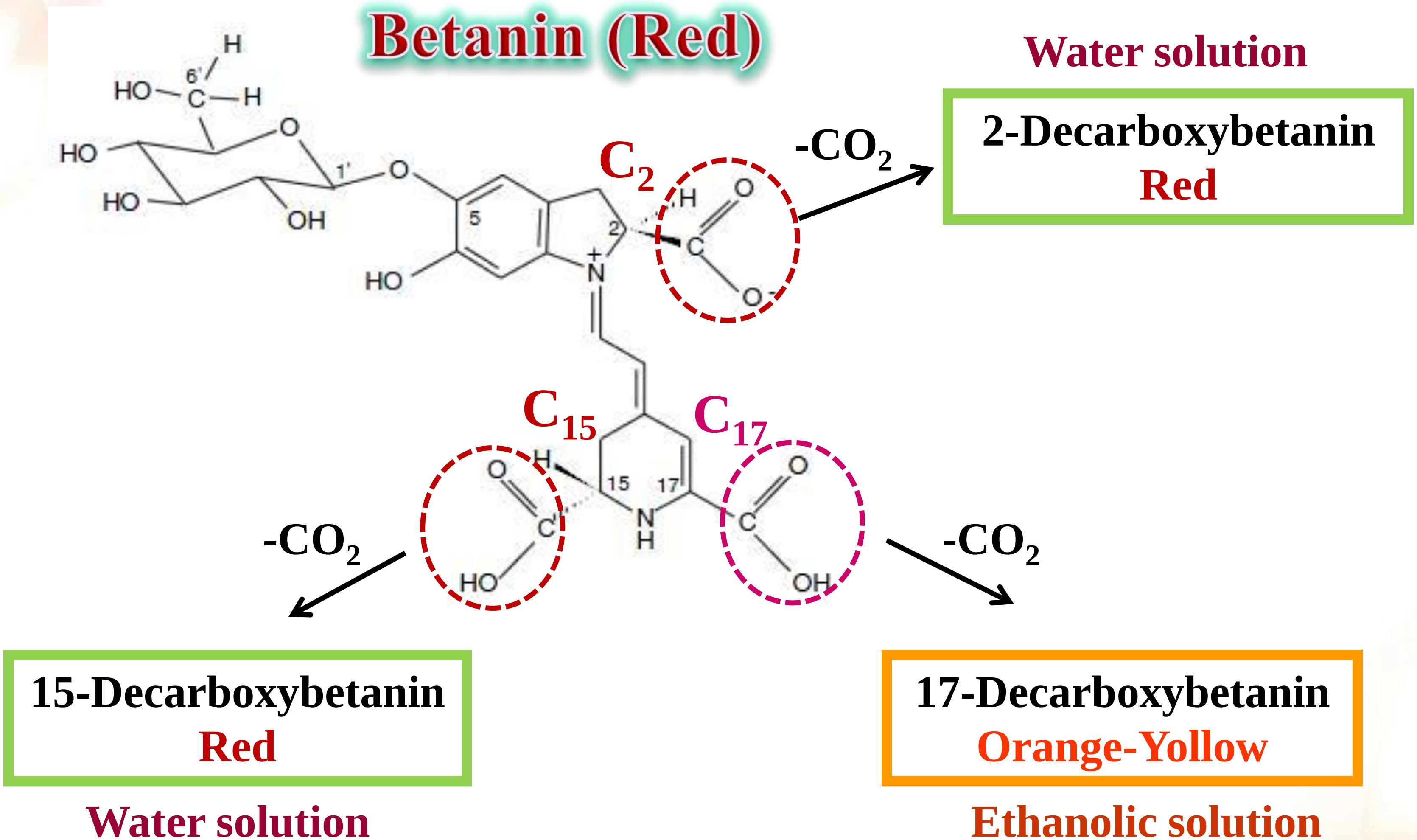


Figure 8. Betanin decarboxylation

(Herbach *et al.*, 2006a)

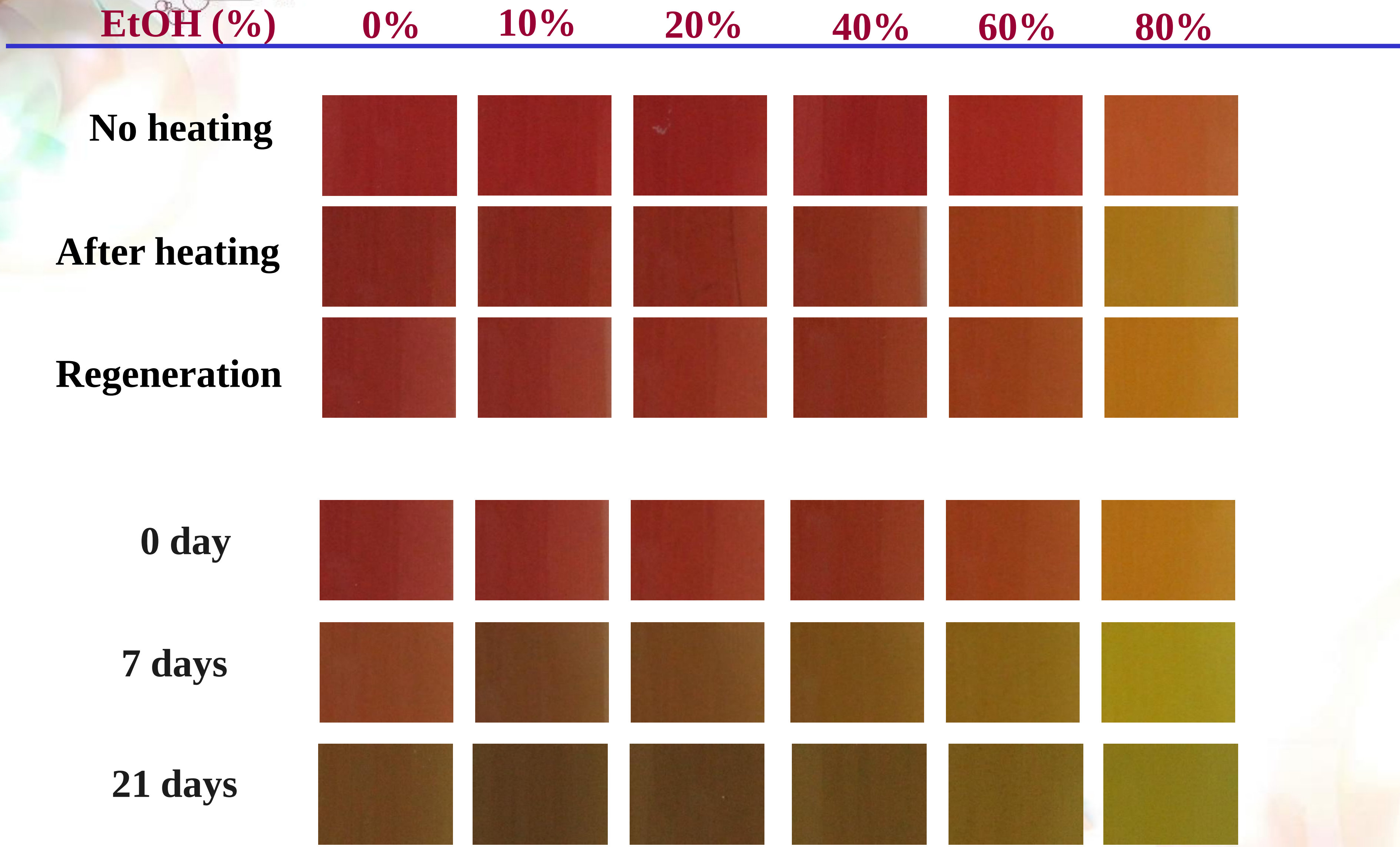
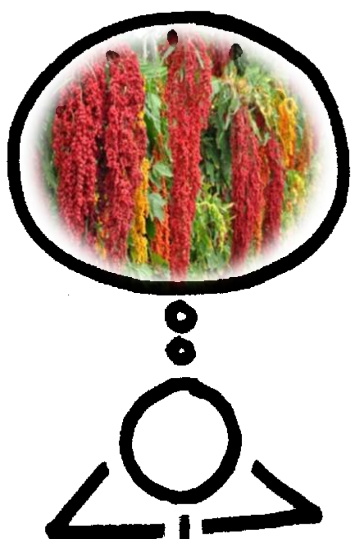


Figure 9. Comparison of color appearance in red Djulis model at pH 5.5 with different alcohol concentration during heating and storage at 25°C.

Objectives

- **To investigate the effect of organic acid (ascorbic, acetic and citric acid) on alcohol (10, 20, 40, 60% v/v) resistance of Djulis pigment model.**



Study on betanin stability after organic acid addition

Herbach *et al*. (2006b) selected additives (ascorbic, isoascorbic, and citric acid) to stabilize betacyanins during thermal treatment of purple pitaya juice at pH 4 and 6.

(http://www.google.co.th/image)

Table 3. Betacyanin retention (%) after heating (H) and cool storage (CS) of purple pitaya juices at 85°C for 1 hour

Additives	Additive concentration (%)	Juice heated at pH 4		Juice heated at pH 6	
		Betacyanin retention (%) [a]		Betacyanin retention (%) [a]	
		After H	After CS	After H	After CS
Without additive	–	36.9 ± 0.5e	36.4 ± 7.0e	23.2 ± 3.8f	44.3 ± 7.6d
Ascorbic acid	0.1	73.5 ± 2.4b	80.8 ± 4.4b	44.0 ± 4.8b	77.1 ± 5.3a
Ascorbic acid	1.0	82.9 ± 5.3a	91.4 ± 4.2a	53.4 ± 2.0a	80.7 ± 4.4a
Isoascorbic acid	0.1	73.6 ± 3.3b	78.4 ± 2.9b	35.3 ± 3.6d	64.0 ± 3.4b
Isoascorbic acid	1.0	75.3 ± 4.2b	80.0 ± 4.9b	39.5 ± 0.8c	66.2 ± 3.0b
Citric acid	0.1	43.3 ± 6.5d	44.8 ± 8.1d	27.6 ± 0.5e	51.0 ± 2.7c
Citric acid	1.0	55.1 ± 2.4d	55.5 ± 1.5c	21.1 ± 0.7f	47.4 ± 2.5e

Significant differences within values in the same column are indicated with different letters ($P < 0.05$).
[a] Calculated as betanin equivalents.

(Herbach *et al*., 2006b)

Materials

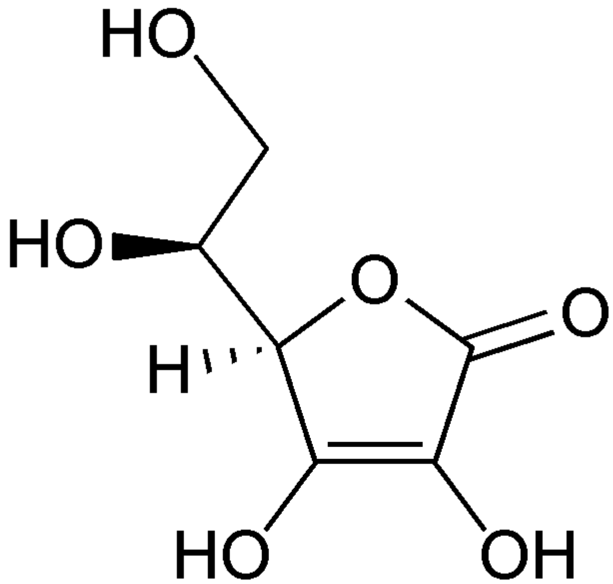
Djulis (*Chenopodium formosanum Koidz*)
was obtained locally from Taiwan,
and stored at 4℃

Fresh Djulis

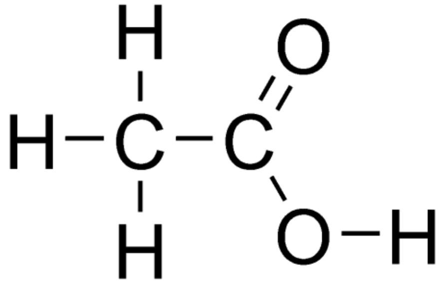
Djulis grains

Organic acids

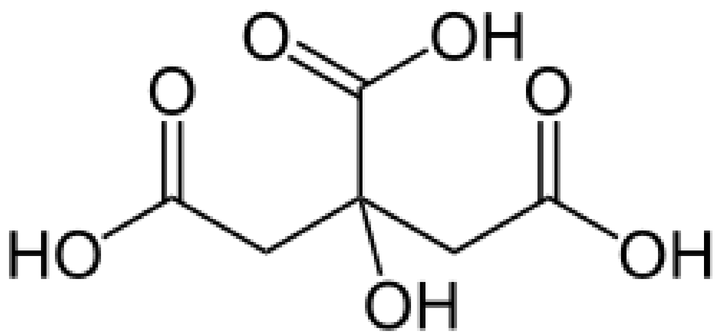
- ❑ Ascorbic acid
- ❑ Citric acid
- ❑ Acetic acid



Ascorbic acid



Acetic acid



Citric acid

Figure 10. The structure of organic acid used in this study.

Methods

Fresh Djulis

Pigment extraction

Alcohol concentration (10, 20, 40, 60%)

Control

Adjusted to pH 5.5

1% organic acid addition (ascorbic, acetic, citric acid)

Heating at 60°C, 30 min

Storage at 25 °C

Color qualities

Pigment retention, DI, Hunter L a b, Hue, Chroma, ΔE

Antioxidant capacities

DPPH, FRAP, TEAC

Statistical analysis

Figure 11. The flow chart to study the effect of organic acid on the color qualities and antioxidants capacity in Djulis pigment.

Results & Discussion

Table 4. Comparison of pigment retention of Djulis extract treated with different alcohol concentration and organic acid at pH 5.5 model system.

Organic acid	%EtOH	Pigment retention (%)	
		No heating	After heating
Control	0	$_{A}100.00^{a}$	$_{C}87.07^{b}$
	10	$_{BC}99.12^{a}$	$_{EF}84.19^{b}$
	20	$_{F}98.75^{a}$	$_{H}81.89^{b}$
	40	$_{H}97.53^{a}$	$_{L}74.95^{b}$
	60	$_{M}82.16^{a}$	$_{P}60.83^{b}$
1% Ascorbic acid	0	$_{A}100.00^{a}$	$_{A}89.73^{b}$
	10	$_{B}99.76^{a}$	$_{D}86.15^{b}$
	20	$_{D}99.52^{a}$	$_{F}84.00^{b}$
	40	$_{G}98.64^{a}$	$_{I}78.04^{b}$
	60	$_{J}89.84^{a}$	$_{M}67.33^{b}$
1% Citric acid	0	$_{A}100.00^{a}$	$_{B}88.52^{a}$
	10	$_{BD}99.66^{a}$	$_{E}84.47^{b}$
	20	$_{E}99.05^{a}$	$_{G}82.44^{b}$
	40	$_{H}97.67^{a}$	$_{K}76.10^{b}$
	60	$_{K}87.58^{a}$	$_{N}65.12^{b}$
1% Acetic acid	0	$_{A}100.00^{a}$	$_{C}87.2^{b}$
	10	$_{D}99.52^{a}$	$_{EF}84.33^{b}$
	20	$_{G}98.97^{a}$	$_{H}81.96^{b}$
	40	$_{I}97.60^{a}$	$_{J}77.40^{b}$
	60	$_{L}84.71^{a}$	$_{O}63.99^{b}$

$^{a-b}$: values with different letters within row are significantly different (P<0.05).

$_{A-O}$: values with different letters within column are significantly different (P<0.05).

Table 5. Betacyanin retention (%) after heating (H) and cool storage (CS) of purple pitaya juices at 85°C for 1 hour

Additives	Additive concentration (%)	Juice heated at pH 4		Juice heated at pH 6	
		Betacyanin retention (%) [a]		Betacyanin retention (%) [a]	
		After H	After CS	After H	After CS
Without additive	–	36.9 ± 0.5e	36.4 ± 7.0e	23.2 ± 3.8f	44.3 ± 7.6d
Ascorbic acid	0.1	73.5 ± 2.4b	80.8 ± 4.4b	44.0 ± 4.8b	77.1 ± 5.3a
Ascorbic acid	1.0	82.9 ± 5.3a	91.4 ± 4.2a	53.4 ± 2.0a	80.7 ± 4.4a
Isoascorbic acid	0.1	73.6 ± 3.3b	78.4 ± 2.9b	35.3 ± 3.6d	64.0 ± 3.4b
Isoascorbic acid	1.0	75.3 ± 4.2b	80.0 ± 4.9b	39.5 ± 0.8c	66.2 ± 3.0b
Citric acid	0.1	43.3 ± 6.5d	44.8 ± 8.1d	27.6 ± 0.5e	51.0 ± 2.7c
Citric acid	1.0	55.1 ± 2.4d	55.5 ± 1.5c	21.1 ± 0.7f	47.4 ± 2.5e

Significant differences within values in the same column are indicated with different letters ($P < 0.05$).

[a] Calculated as betanin equivalents.

(Herbach *et al*., 2006b)

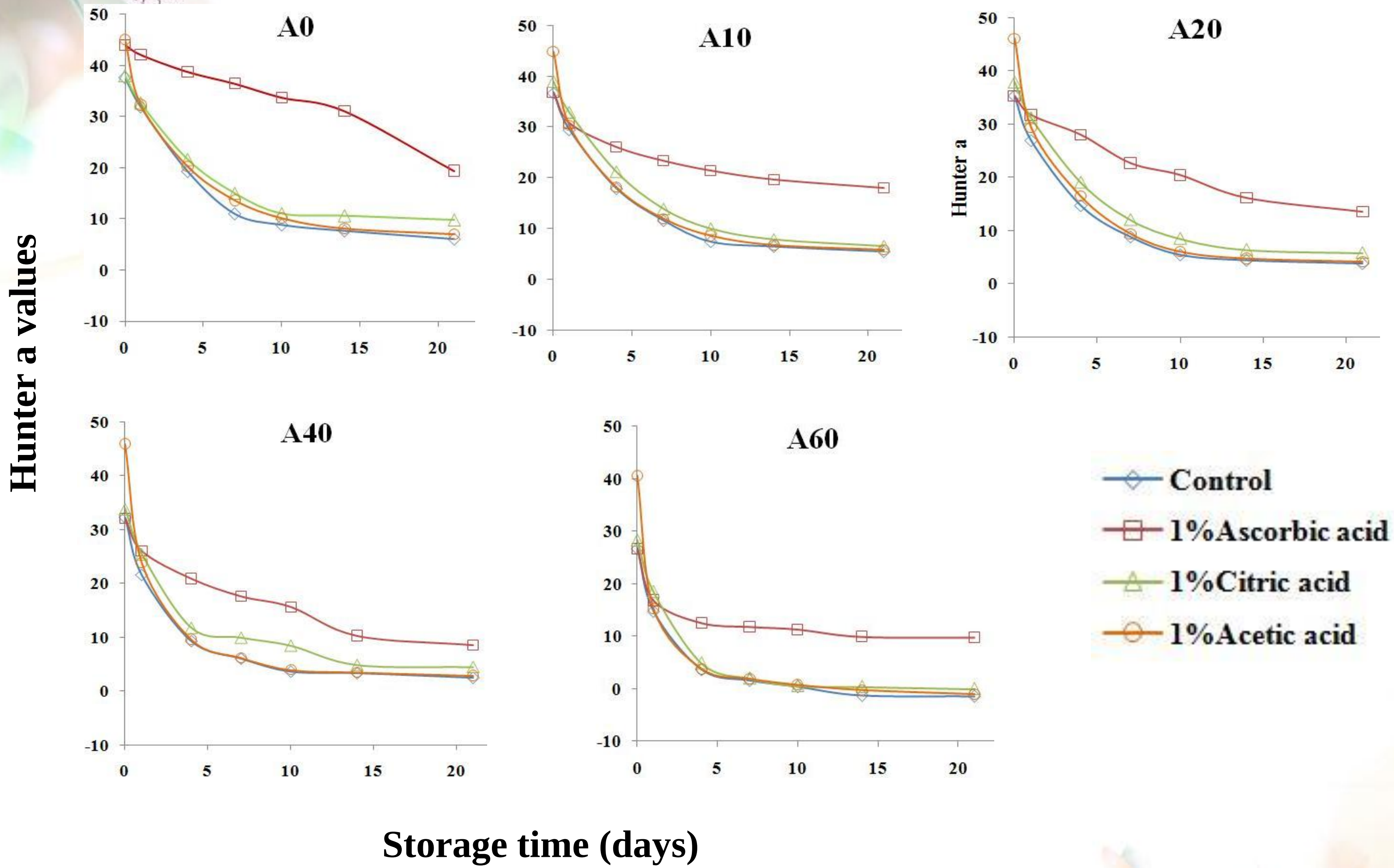


Figure 12. Changes in Hunter a value of red Djulis extract treated with (A0) 0%, (A10) 10%, (A20) 20%, (A40) 40%, (A60) 60% alcohol concentrations and organic acid addition during storage for 21 days.

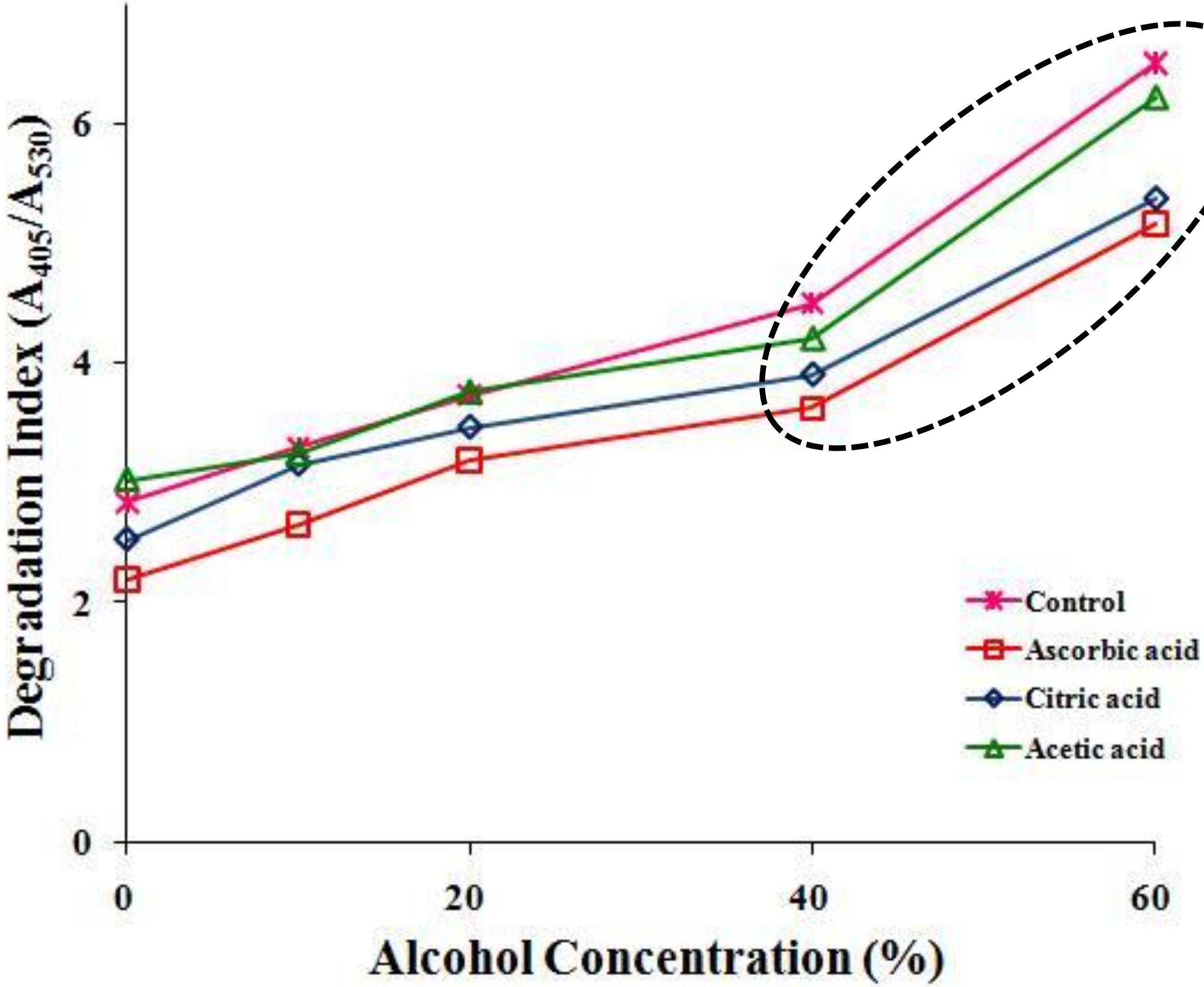


Figure 13. **Relationship between degradation indexes (A_{405}/A_{530}) of Djulis extract treated with alcohol (0, 10, 20, 40, and 60% EtOH) in control and 1% organic acid addition during storage for 21 days.**

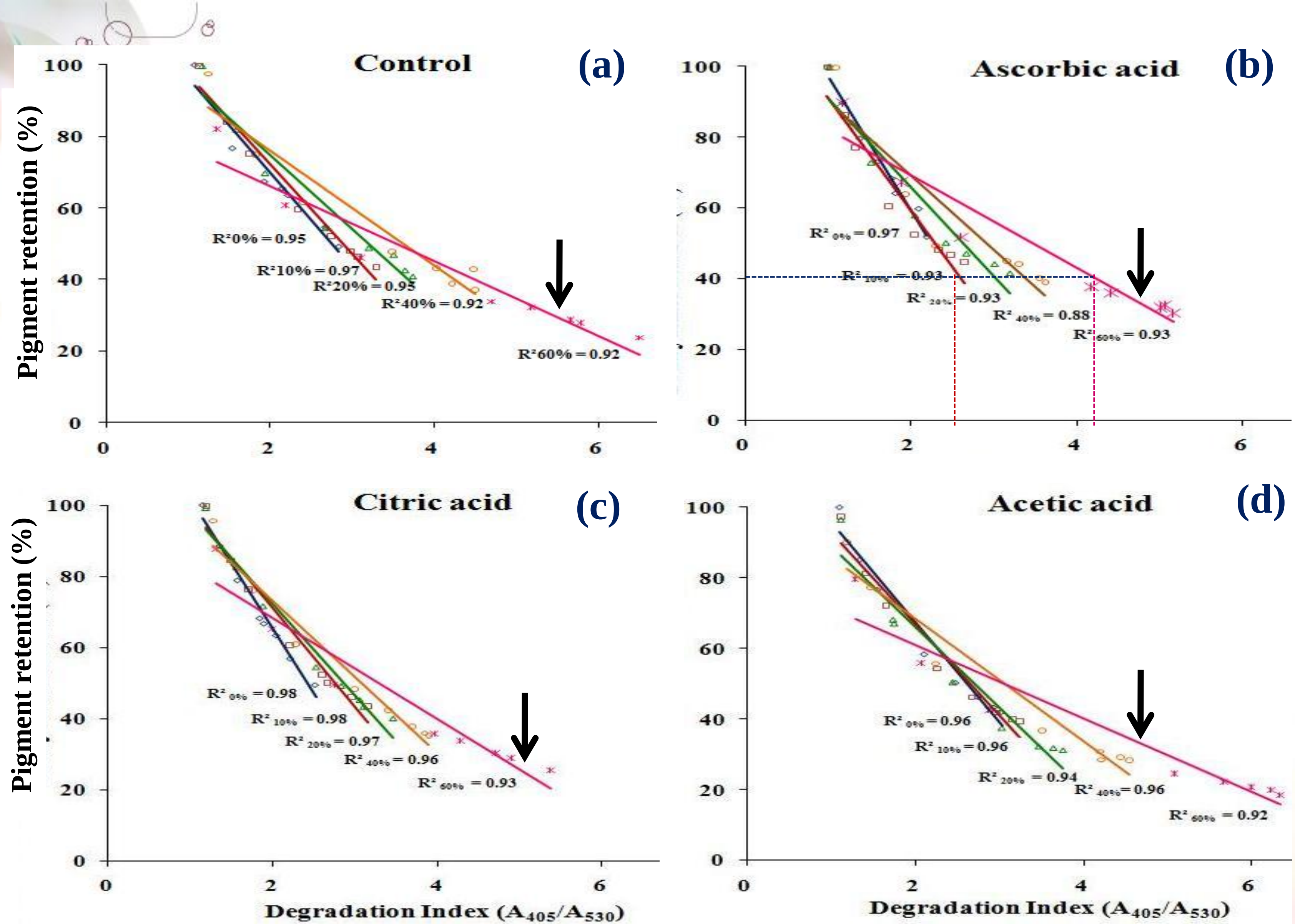


Figure 14. Relationship between pigment retention and DI of Djulis extract treated with alcohol (0, 10, 20, 40, and 60%) in (a) control (b) ascorbic acid, (c) citric acid and (d) acetic acid addition system during storage for 21 days.

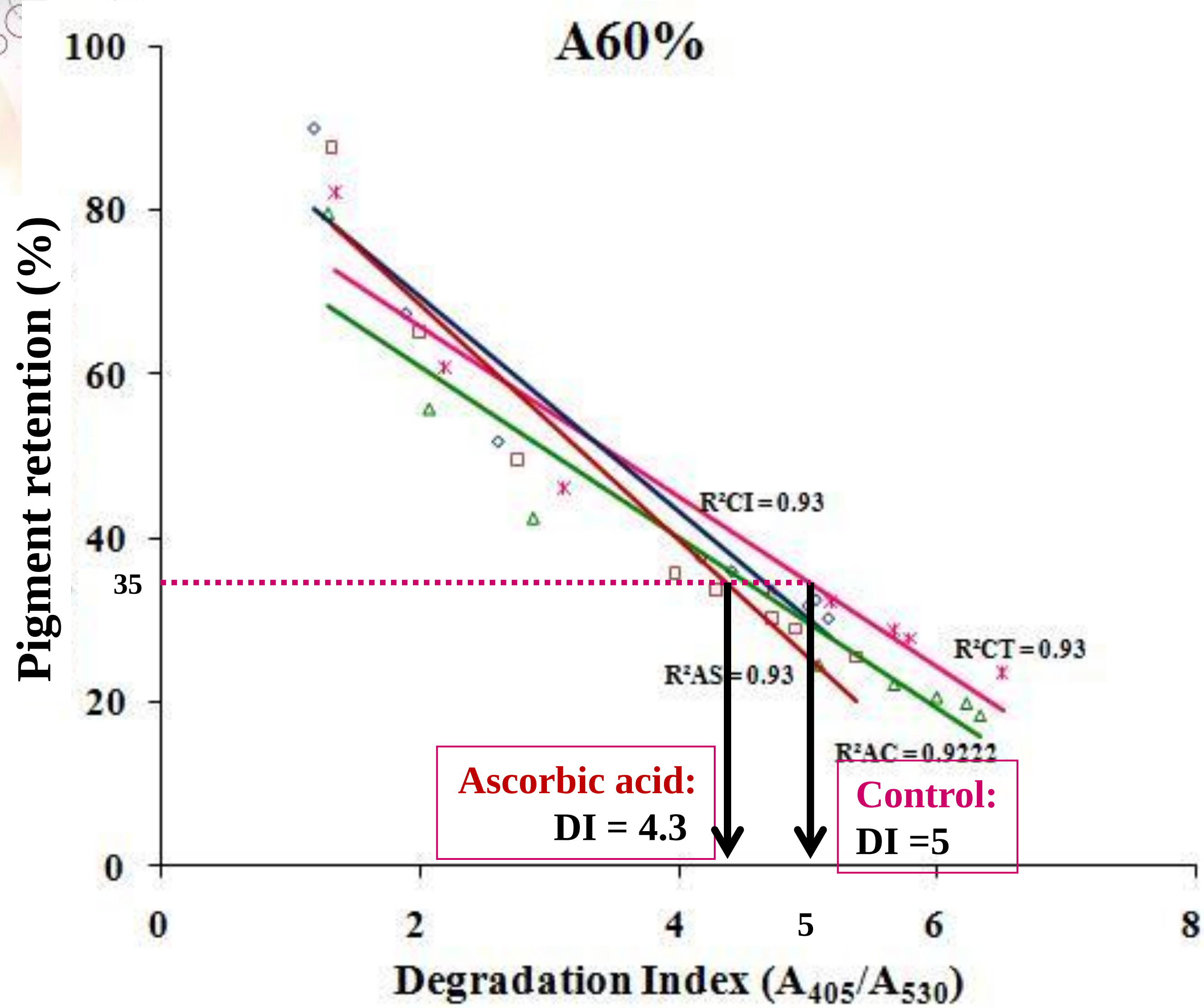


Figure 15.Relationship between pigment retentison and DI of Djulis extract treated with 60% EtOH in control (CT), and after 1% ascorbic acid (AS), 1% citric acid (CI) and acetic acid (AC) addition during storage for 21 days.

EtOH (%)

Hunter a values		0%	10%	20%	40%	60%
Control	0%: 37.64 60%: 5.98					
Ascorbic acid	0%: 43.94 60%: 19.40					
Citric acid	0%: 37.99 60%: 9.80					
Acetic acid	0%: 37.85 60%: 6.96					

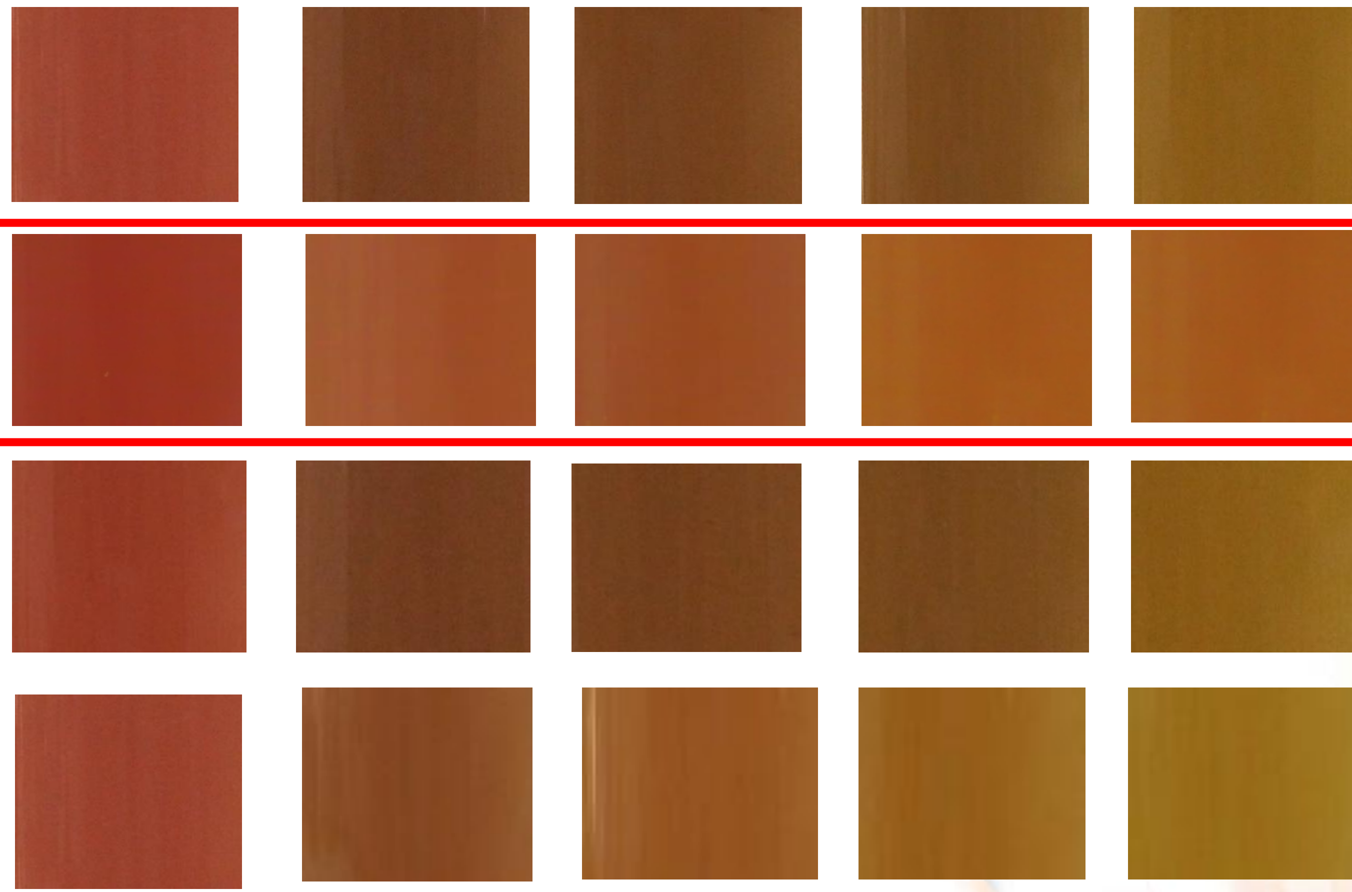


Figure 16. Comparison of color appearance in red Djulis model (Control, 1% organic acid) with different alcohol concentration after storage for 21 days

Table 6. Changes of the antioxidant capacity of Djulis extract treated with 40% alcohol concentration after organic acid addition during storage 21 days.

Organic acid	Storage (days)	DPPH (%)	FRAP (mmole/l)	TEAC (mM)
Control	0	$_{I}84.25^{a}$	$_{E}1.09^{a}$	$_{D}1.21^{a}$
	7	$_{L}70.35^{c}$	$_{E}0.90^{b}$	$_{D}1.03^{b}$
	14	$_{K}73.34^{b}$	$_{E}0.65^{c}$	$_{D}0.95^{c}$
	21	$_{M}68.52^{d}$	$_{E}0.64^{c}$	$_{D}0.94^{c}$
1%Ascorbic acid	0	$_{F}88.14^{b}$	$_{A}69.17^{a}$	$_{A}34.50^{a}$
	7	$_{G}87.29^{c}$	$_{B}56.22^{b}$	$_{B}33.85^{a}$
	14	$_{H}86.43^{a}$	$_{C}50.98^{c}$	$_{C}31.01^{b}$
	21	$_{J}82.71^{b}$	$_{D}45.18^{d}$	$_{C}30.97^{b}$
1%Citric acid	0	$_{E}88.85^{b}$	$_{E}1.29^{a}$	$_{D}1.66^{a}$
	7	$_{E}88.96^{b}$	$_{E}1.02^{b}$	$_{D}1.55^{b}$
	14	$_{C}90.95^{a}$	$_{E}0.94^{c}$	$_{D}1.47^{c}$
	21	$_{F}G87.81^{c}$	$_{E}0.90^{c}$	$_{D}1.51^{bc}$
1%Acetic acid	0	$_{B}91.99^{b}$	$_{E}1.10^{a}$	$_{D}1.41^{a}$
	7	$_{D}89.96^{c}$	$_{E}0.95^{b}$	$_{D}1.34^{b}$
	14	$_{A}93.92^{a}$	$_{E}0.89^{bc}$	$_{D}1.25^{c}$
	21	$_{C}90.41^{c}$	$_{E}0.84^{c}$	$_{D}1.22^{c}$

$^{a\text{-}g}$: values with different letters within column are significantly different (P<0.05).

$_{A\text{-}0}$: values with different letters within column are significantly different (P<0.05).

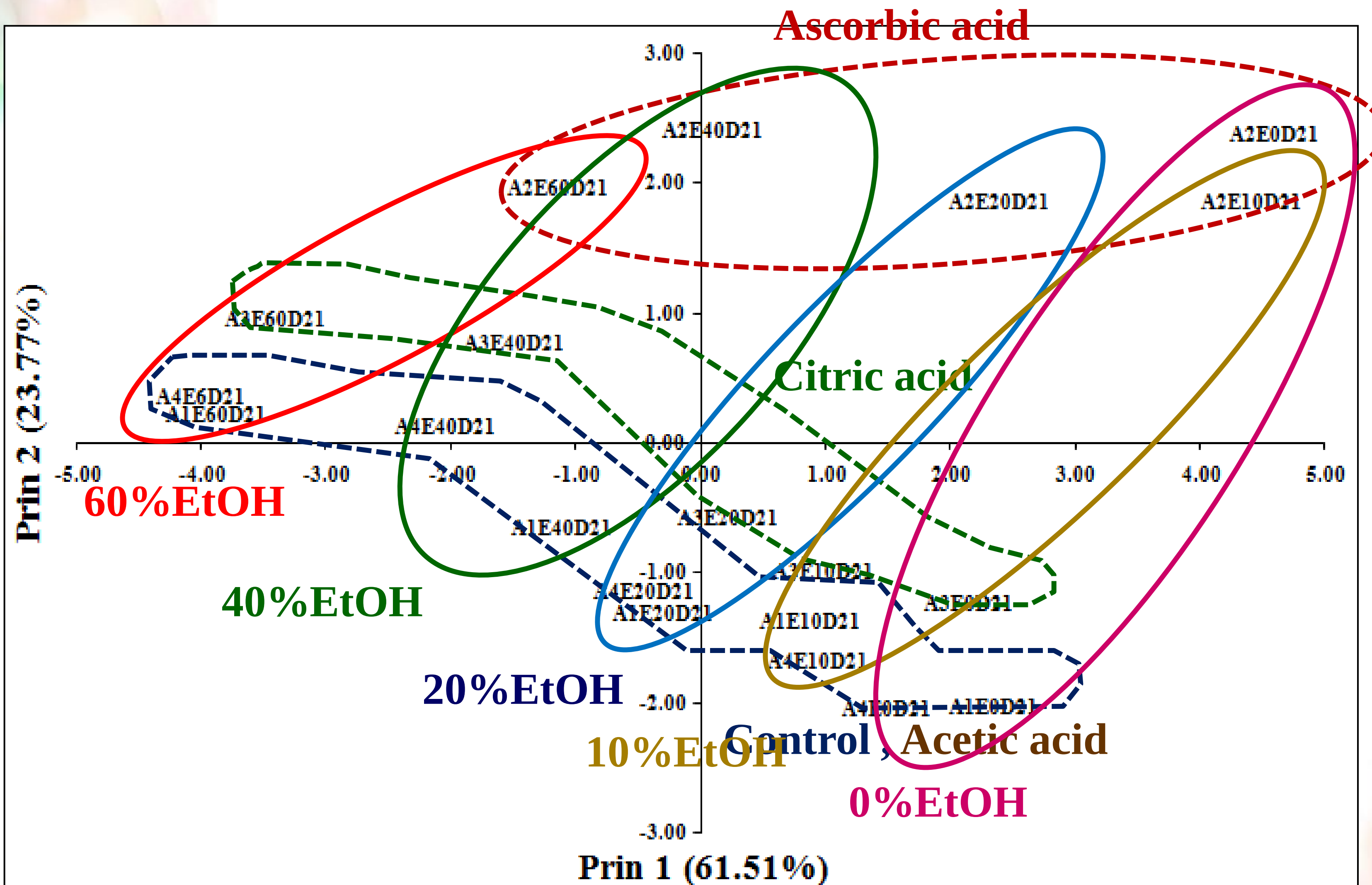


Figure 17.Principle component analysis of Djulis extract treated with different alcohol concentration and organic acid addition (A1: control, A2: ascorbic acid, A3: citric acid, A4: acetic acid) during storage for 21 days

Conclusions

- The color degradation of Djulis betacyanin occurred in alcohol solution and increased when alcohol concentration increased during heating or storage.
- Addition of 1% ascorbic acid in Djulis pigment model system will exhibit the highest pigment retention and hunter a value compared to the other treatments.
- Addition of organic acid will also enhance the stability of antioxidant capacities, especially ascorbic acid in FRAP reducing power and TEAC.
- PCA confirmed that the concentrations of alcohol as well as the kind of organic acid were important factors in classifying the samples.

Acknowlagements

國立屏東科技大學

- **NPUST Scholarship**
- **Pi-Jen Tsai, Ph.D**
- **Department of Tropical Agriculture and International Cooperation (DTAIC), NPUST**
- **Department of Food Science, NPUST**
- **Kasetsart University, Thailand**


THANK YOU …
FOR YOUR ATTENTION !!