Non-Wood Forest Products:
Health and Well-Being
Helsinki, Finland,
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Extraction, Isolation and Processing Technology on Berries

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- **General situation of berries in China**
- **Extraction, isolation methods for active ingredients from berries**
  - I. anthocyanins
  - II. polysaccharide
- **Processing technology on berries**
- **Technology extension Cases**

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1.General situation of berries in China

Vaccinium uliginosum

Lycium ruthenicum

Hippophae rhamnoides

Ilex purpurea

Ribes mandschuricum

Rubus idaeus

I. Berries' Distribution Regions

Wild regions

Cultivated regions

Blueberry planted in Wallen, one of our partners in Shandong

wallén™
沃林

The total area is more than 10^7 m^2

II. Varieties & Annual Output

About 39 species of berries from 11 families are recorded for edible fruits.

Typical Berries

- **Annual Output: 150,000 tons**

Chinese wolfberry

- **Annual Output: 355,000 tons**

Seabuck-thorn

- **Annual Output: 3,000 tons**

Blueberry

- **Annual Output: 150,000 tons**

Black Currant

- **Annual Output: 400,000 tons**

Mulberry

- **Annual Output: 130,000 tons**

Raspberry

Ⅲ. Utilization

Eaten Fresh

cosm-etics

Juice

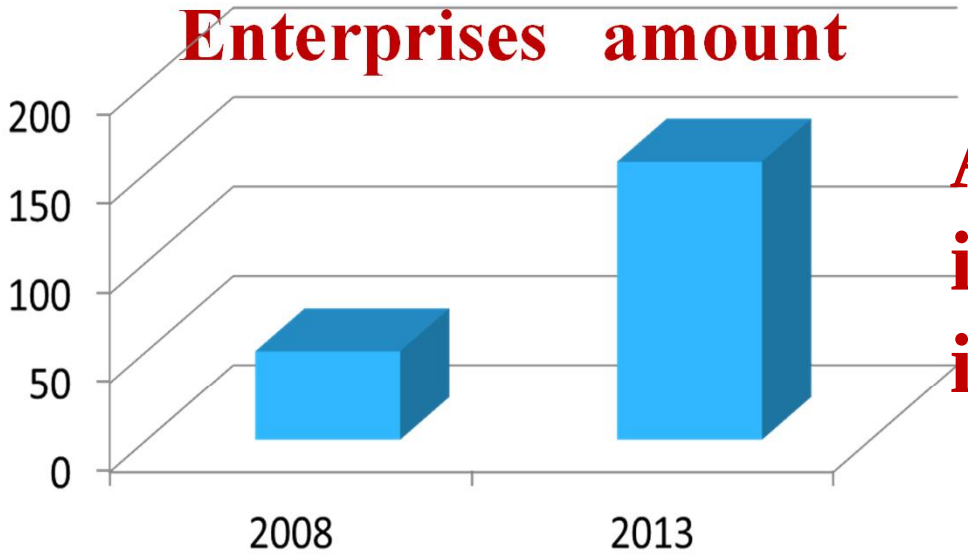
Berries

Medi-cine

Jam

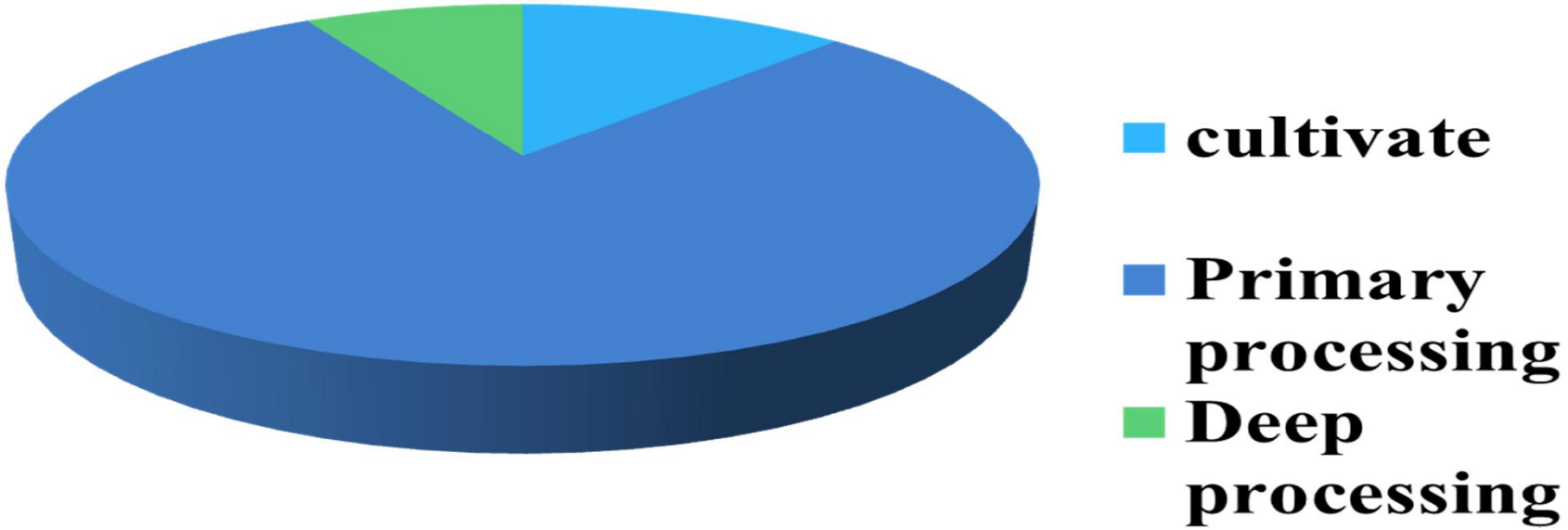
wine

IV/Enterprises/industry



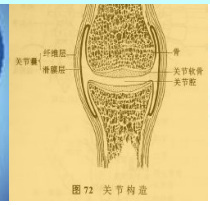
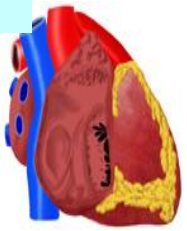
A threefold increase in related enterprises in recent 5 years.

Enterprise compositions



Q：Why do we focus on berries ?



①Healthcare values

Berry anthocyanins have good effect on eyes, which could alleviate eye fatigue and enhance the night vision.

Protect capillary and possess antioxidant properties.

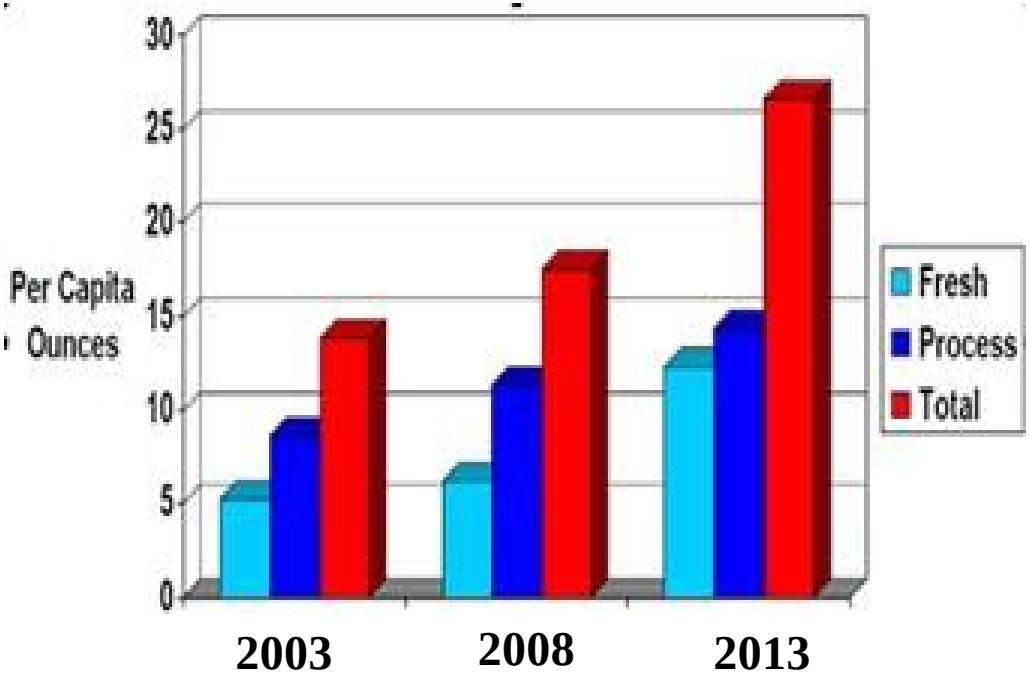
Delay aging process and improve memory.

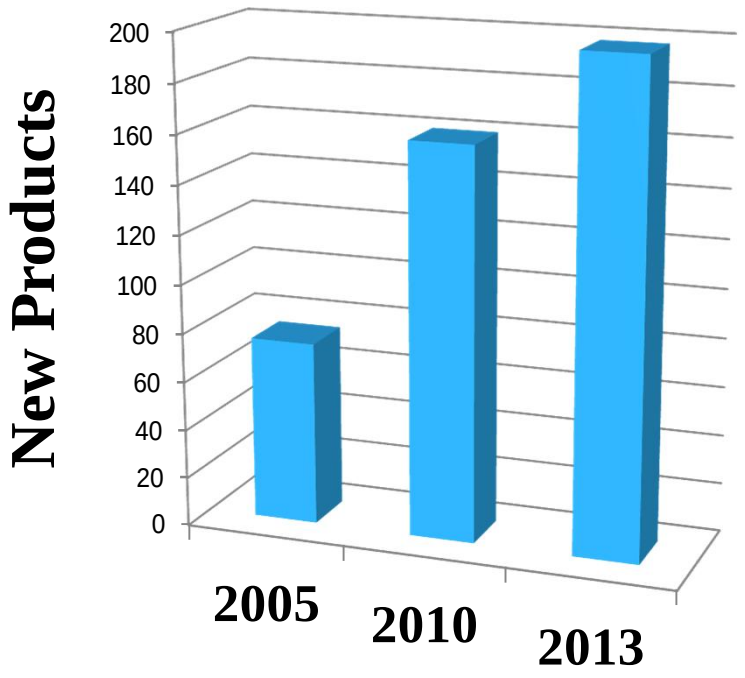
Remove free radicals and prevent cancer.

Have strong inhibition on enzyme activity related to occurring of cardiovascular diseases.

②Berries consumption is successive incremental

③ An increase in new product development





A： High value, Large market, Great demand

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2.Extraction, isolation methods for active ingredients from berries

Active Ingredients of Berries

Flavonoids hot !!!

Vitamin

Proanthocyanidin hot !!!

Resveratrol

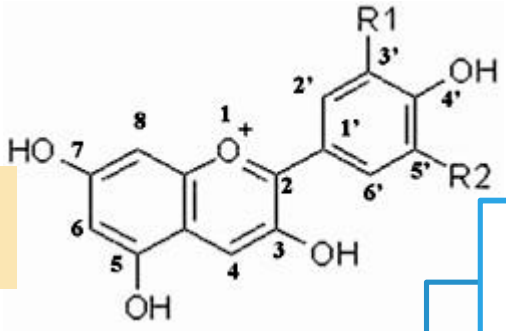
Protein

Organic Acid

Polysaccharides

Minerals

I/Anthocyanins



1. Extraction Methods

①Solvent Extraction

②Assisted Extraction

Organic Solvent

Additional Method

- Enzymatic hydrolysis
- Ultrasound
- Microwave
- Hydrostatic high-pressure
- Supercritical fluid

③ Our Work

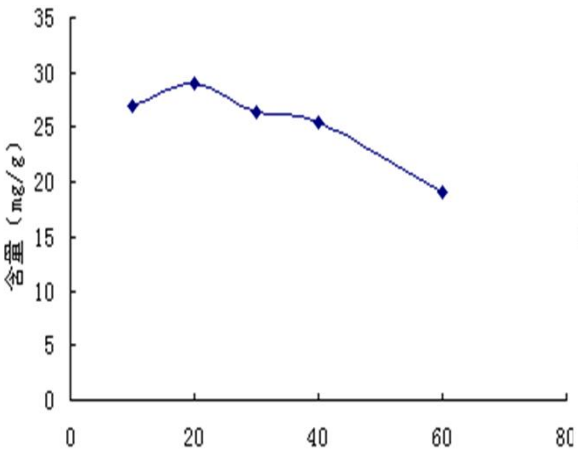
Blue-crop

Experiment Material：Blueberry

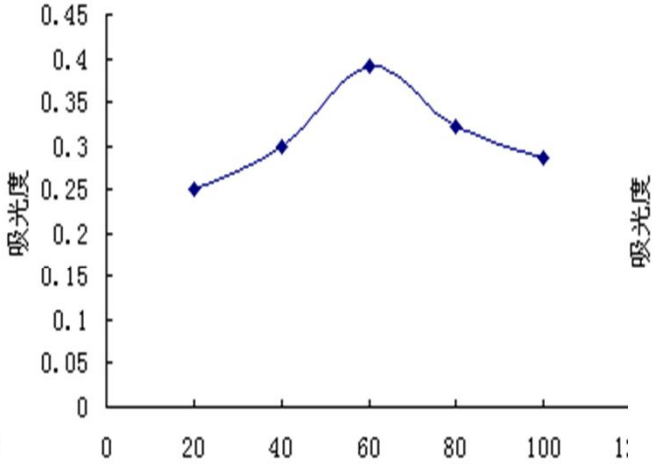
A: Solvent Extraction

Extractant： 2%Hcl-Methanol

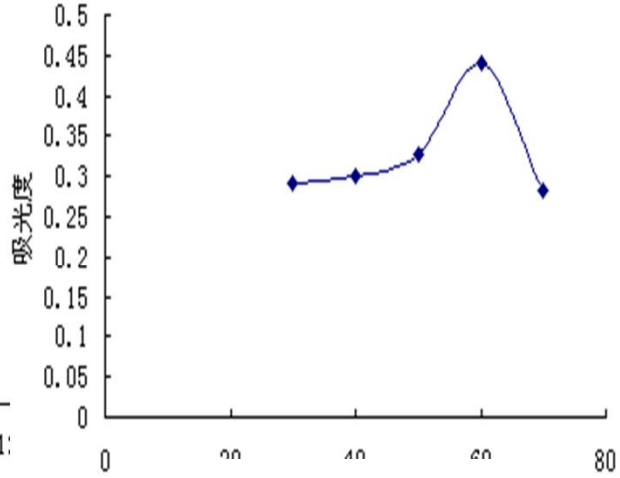
Extraction Rate



Ratio of Solid to Liquid



Time



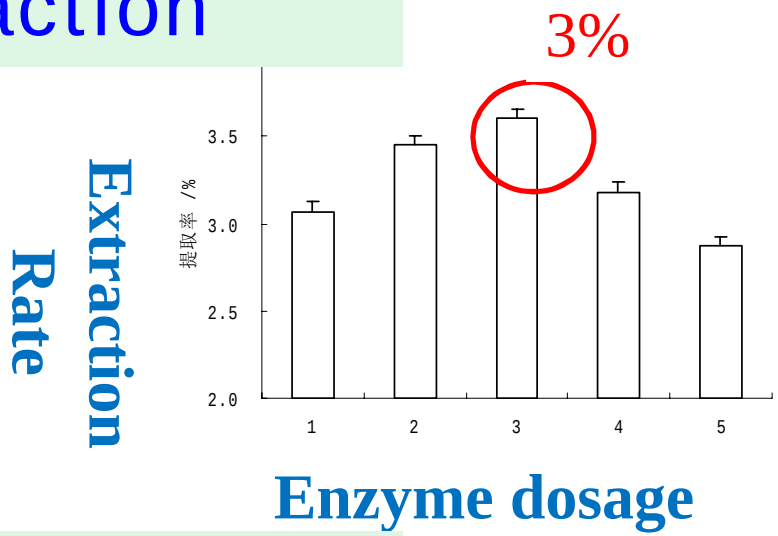
Temperature

Optimal Conditions：

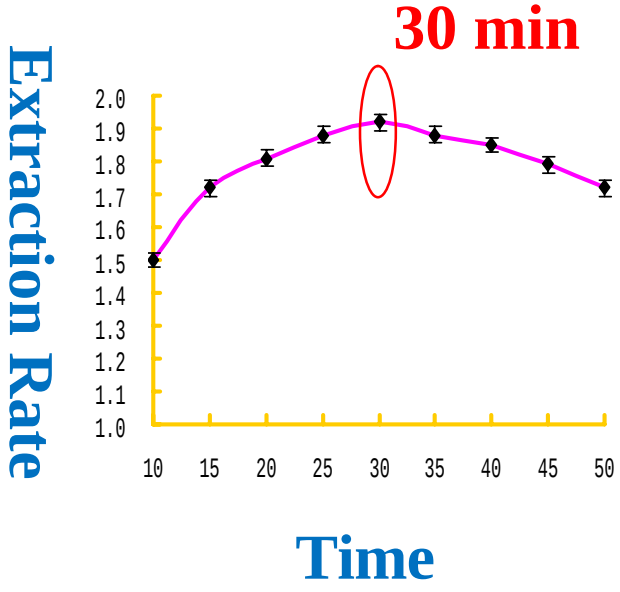
Ratio of Solid to Liquid: 1 to 10
Time: 60min
Temperature: 60°C

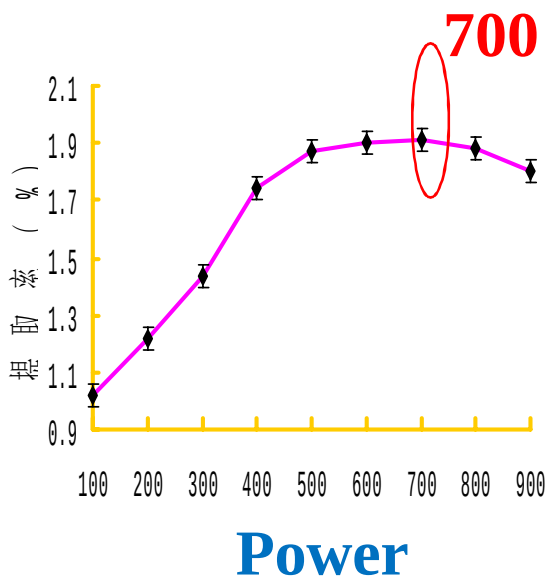
B: Enzyme-assisted Extraction

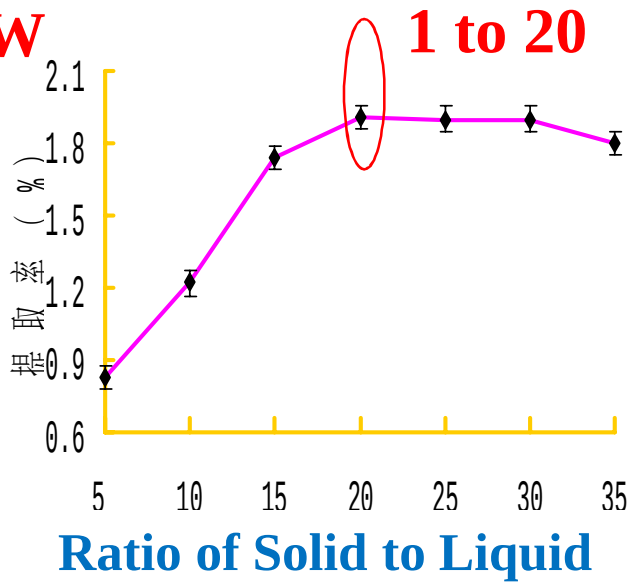
Cellulose Enzyme Hydrolysis → **Solvent Extraction** →



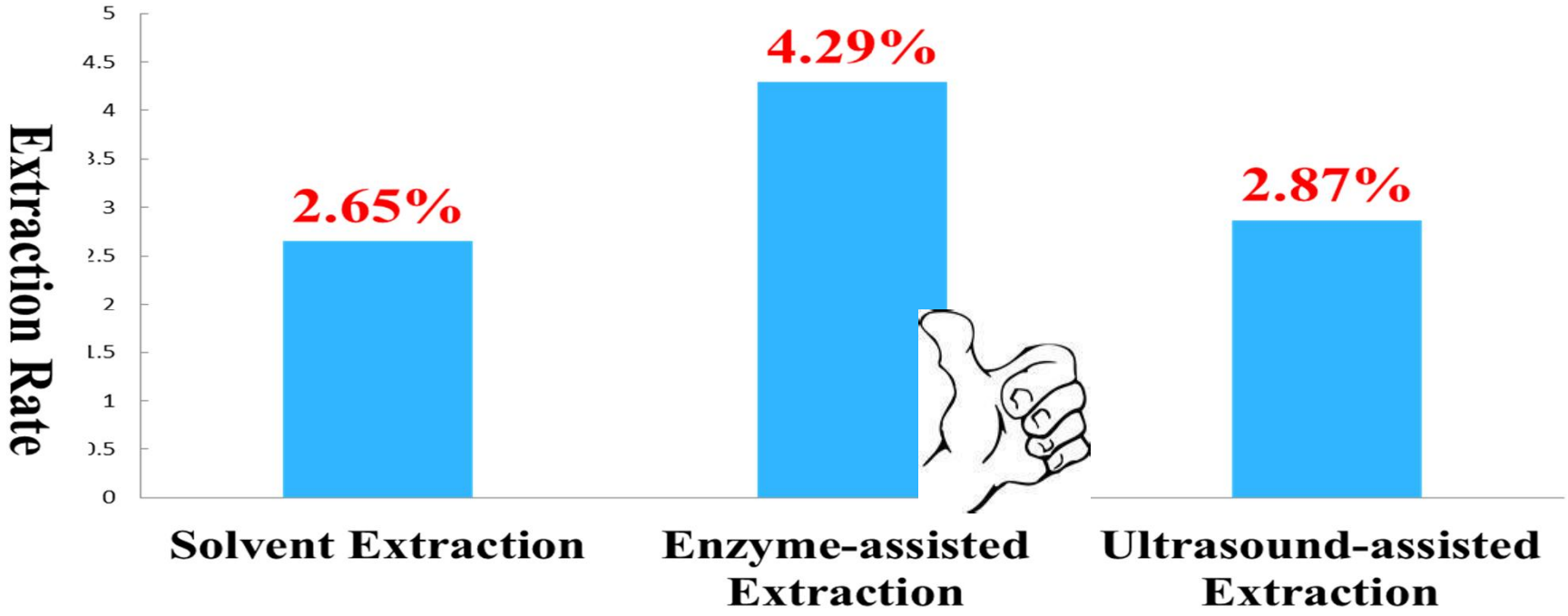
C: Ultrasound-assisted Extraction







The Best Extraction Method



Optimal Conditions of Enzyme-assisted Extraction:

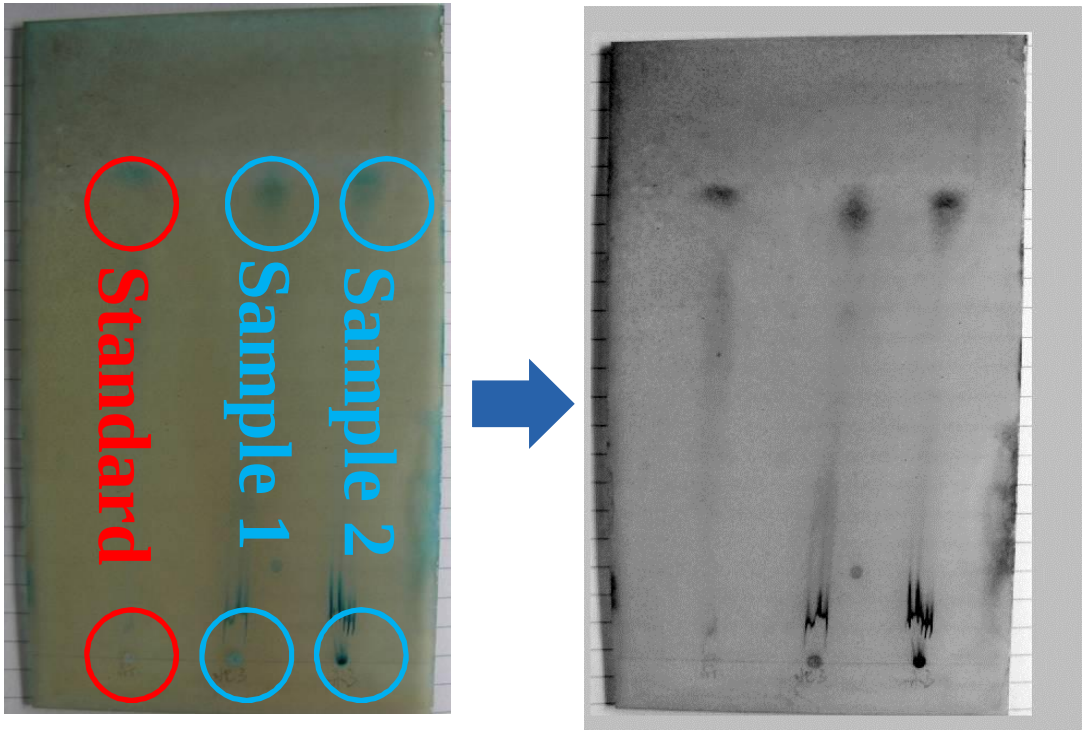
Ratio of Solid to Liquid: 1 to 10; **Time: 60min**

Temperature: 45°C **Enzyme dosage: 3%**

The advantages of enzyme-assisted extraction

- **Simplicity & economy**
- **Mild treatment, product with high stability**

Identified by TLC



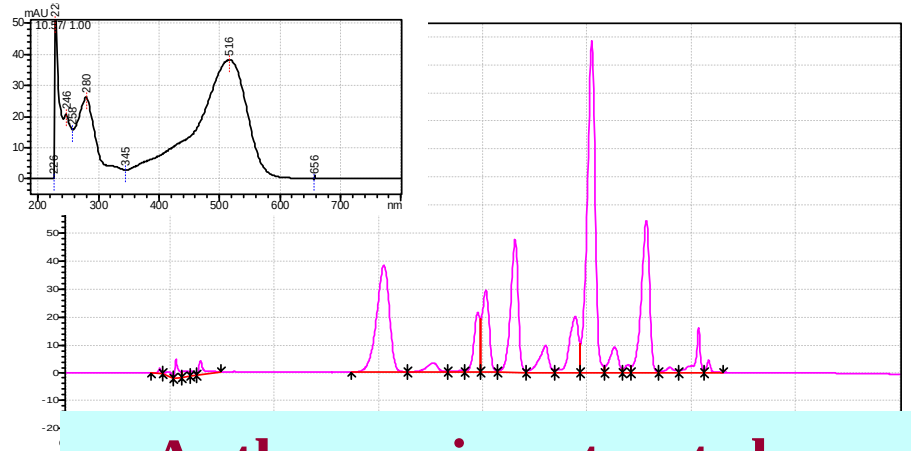
Standard:
cyaniding-3-glucoside

Sample 1:
Anthocyanin extracts by Solvent Extraction

Sample 2:
Anthocyanin extracts by Enzyme-assisted Extraction

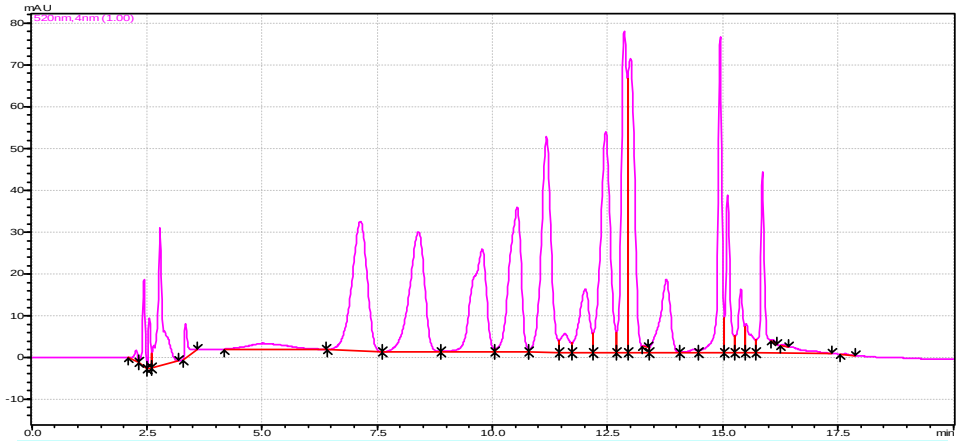
♦High extraction efficiency

Anthocyanin may be easily released, because the cell walls of berry were broken by cellulose enzyme.



Anthocyanin extracts by Solvent Extraction

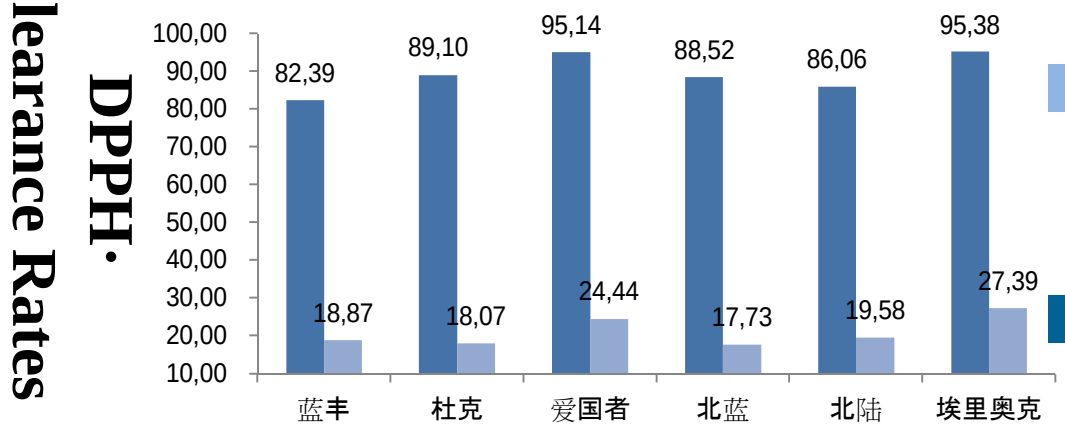
The extraction rate Anthocyanin of was increased by 61%, compared to solvent extraction.

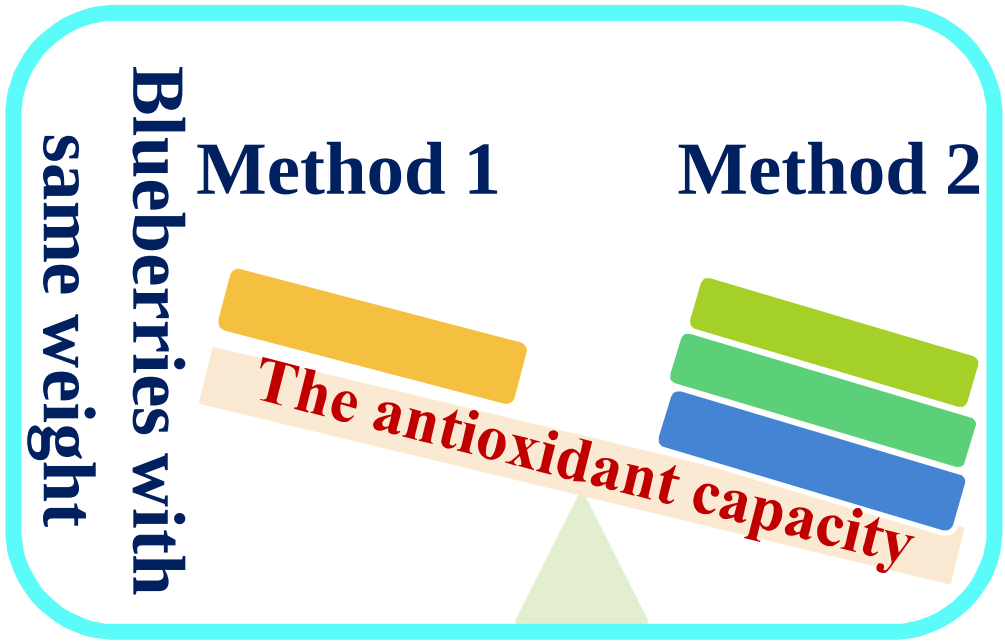


Anthocyanin extracts by Enzyme-assisted Extraction

♦Reduce the cost of products

The antioxidant capacity of anthocyanin extracts from 6 varieties blueberries





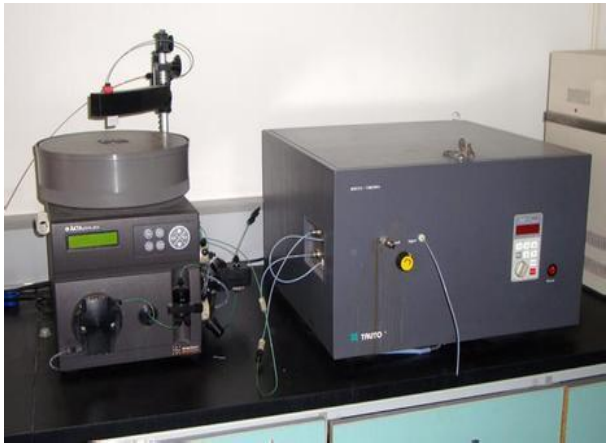
2. Isolation Methods

①Chromatography

②High-speed Countercurrent Chromatography

③Membrane Separation Technology

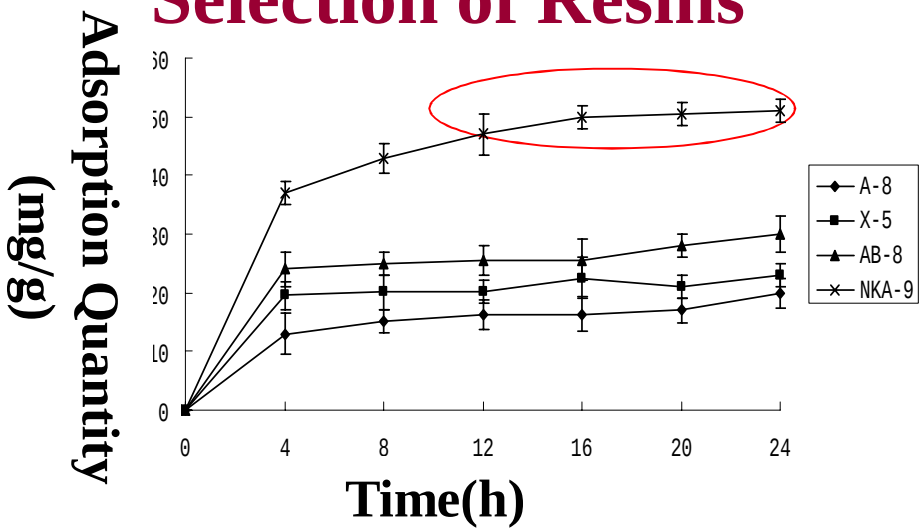
④High Performance Liquid Chromatography

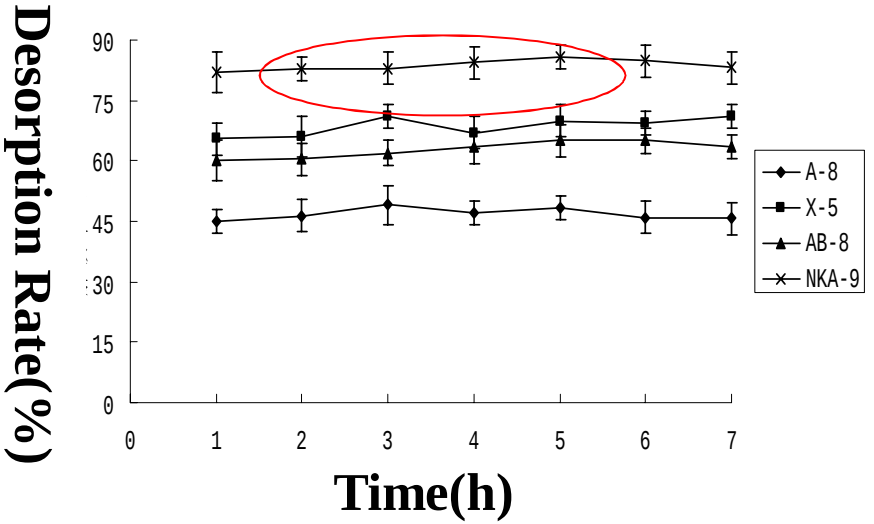


⑤ Our Work

A: Macroporous Resin Chromatography

Selection of Resins





The adsorptive properties of four macroporous resins to Anthocyanin:

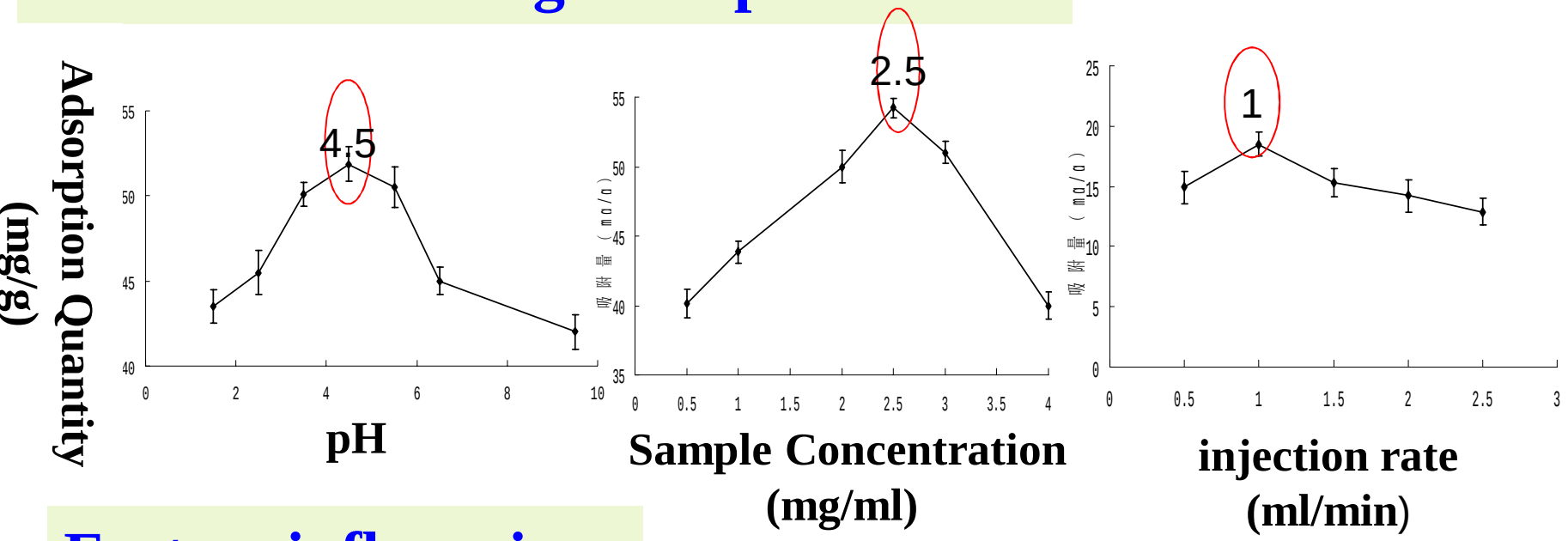
NKA-9 > AB-8 > X-5 > A-8

The adsorptive properties of four macroporous resins to Anthocyanin:

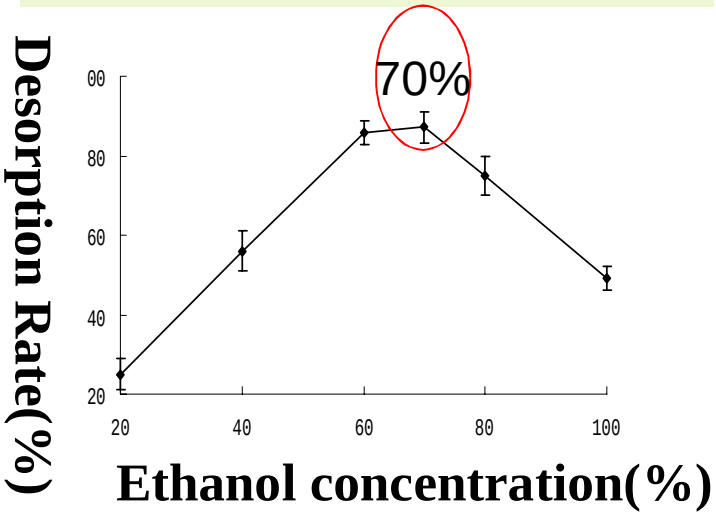
NKA-9 > X-5 > AB-8 > A-8

The optimal macroporous resin was NKA-9

Factors influencing adsorption



Factors influencing desorption



Optimal Conditions:

dynamic adsorption :

pH:4.5 injection rate: 1ml/min

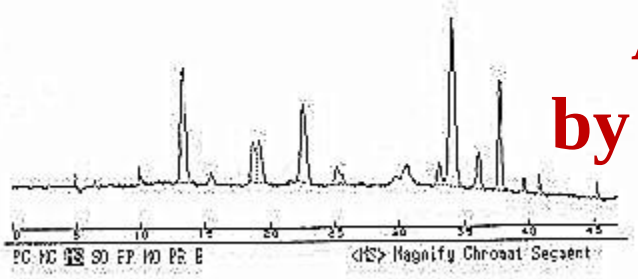
Sample Concentration: 2.5mg/ml

elution :

Ethanol concentration:70%

The purity of product reach 30%

B: Preparative high performance liquid chromatography



Anthocyanin monomer was obtained by further purification.

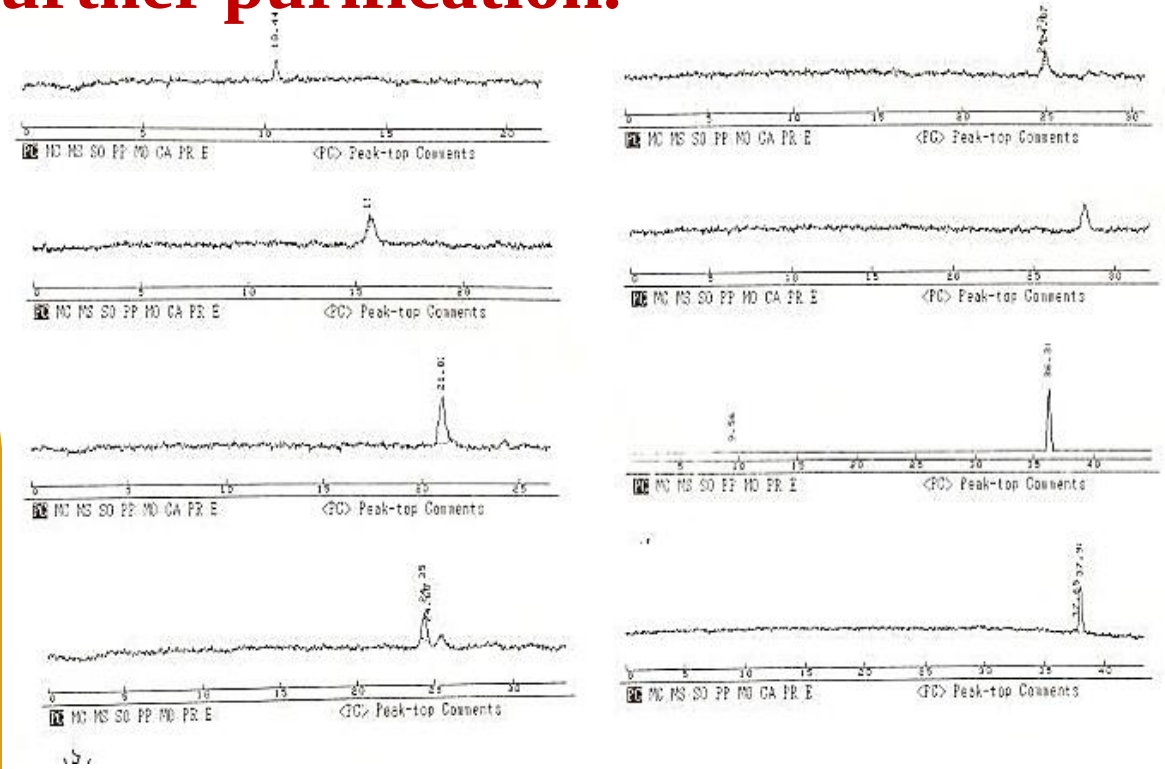
Conditions:

Mobile phase A:
Acetonitrile

Mobile phase B:
5% formic acid-water

Flow:1ml/min

Gradient elution

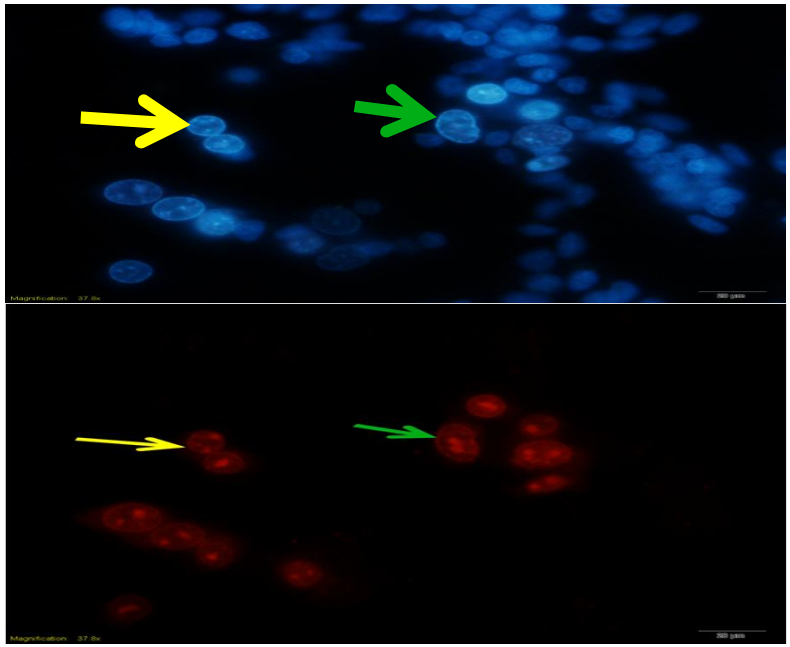


11 compounds were isolated !
Their purity＞ 90%

C: Identify the anthocyanin monomers by LC-MS

Peak No.	tR (min)	$[M+H]^-$			Formula	name
		Measured (m/z)	Predicted (m/z)	Error (ppm)		
1	5.269	465.1013	465.1028	-3.23	$C_{21}H_{20}O_{12}$	Delphinidin-3-O-gal
2	6.554	435.0911	435.0922	-2.53	$C_{20}H_{18}O_{11}$	Delphinidin-3-O-arab
3	7.228	479.1176	479.1184	-1.67	$C_{22}H_{22}O_{12}$	Petunidin-3-O-gal
4	8.703	449.1078	449.1078	86.16	$C_{21}H_{20}O_{11}$	Petunidin-3-O-arab
5	9.262	493.1324	493.1341	-3.45	$C_{23}H_{24}O_{12}$	Malvidin-3-O-gal
6	9.597	303.0486	303.0499	-4.29	$C_{15}H_{10}O_{7}$	Delphinidin
7	10.863	463.1222	463.1235	-2.81	$C_{22}H_{22}O_{11}$	Malvidin-3-O-arab
8	12.480	287.0547	287.0550	-1.05	$C_{15}H_{10}O_{6}$	Cyanidin
9	13.085	317.0649	317.0656	-2.21	$C_{16}H_{12}O_{7}$	Petunidin
10	17.841	301.0714	301.0707	2.33	$C_{16}H_{12}O_{6}$	Peonidin
11	18.462	331.0797	331.0812	-4.53	$C_{17}H_{14}O_{7}$	Malvidin

D: The Anti-cancer Activity of Anthocyanins



Hochest PI Hochest+PI

control

50

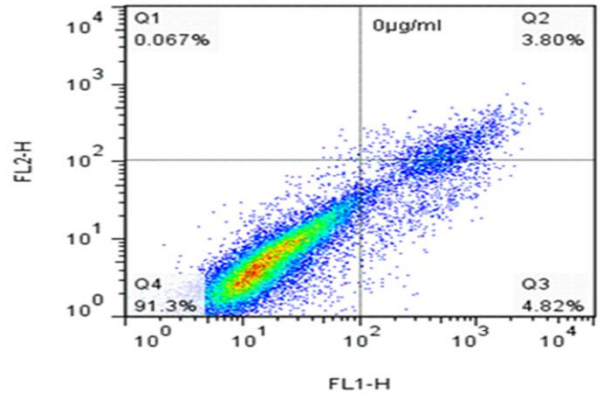
100

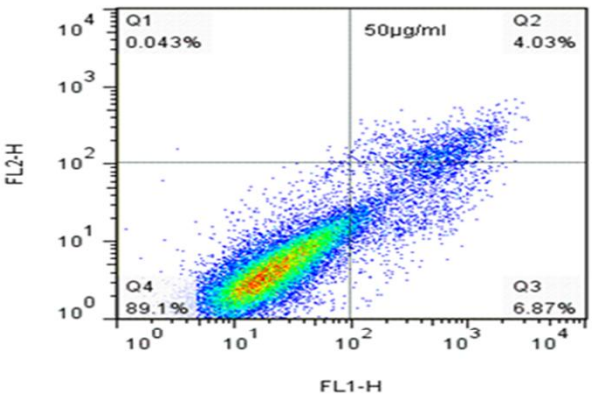
150

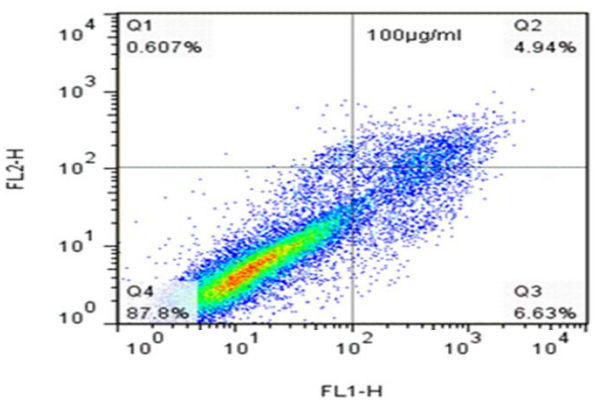
200

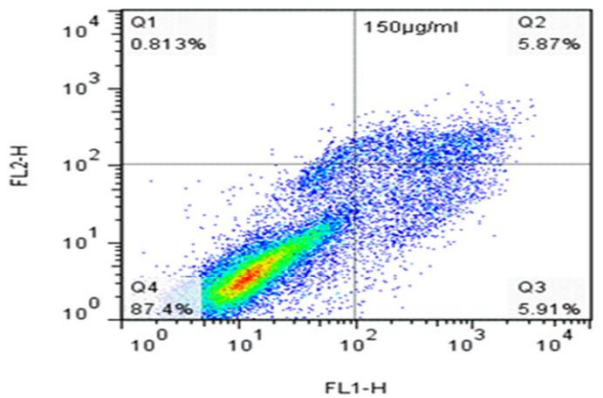
250

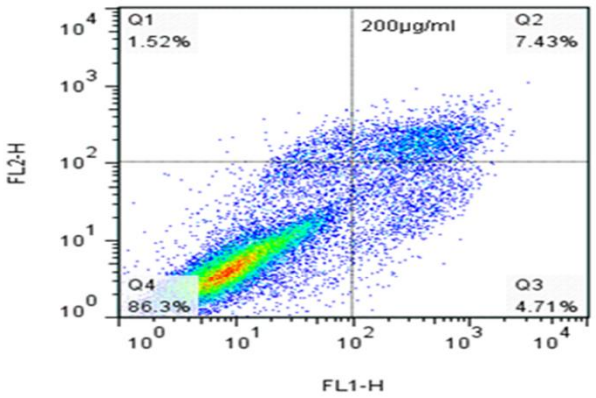
Different doses of anthocyanin caused the apoptosis and inhibited the proliferation of cell lines (Hep-G 2).

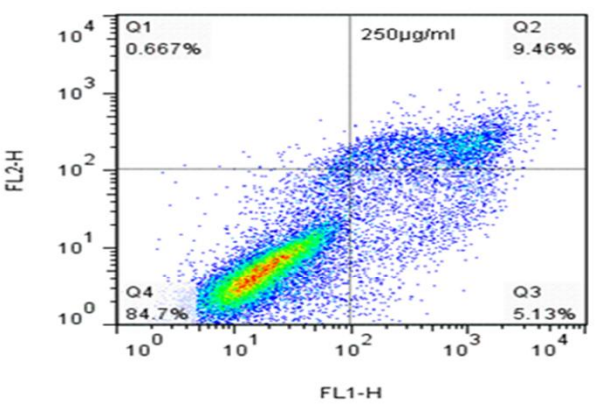


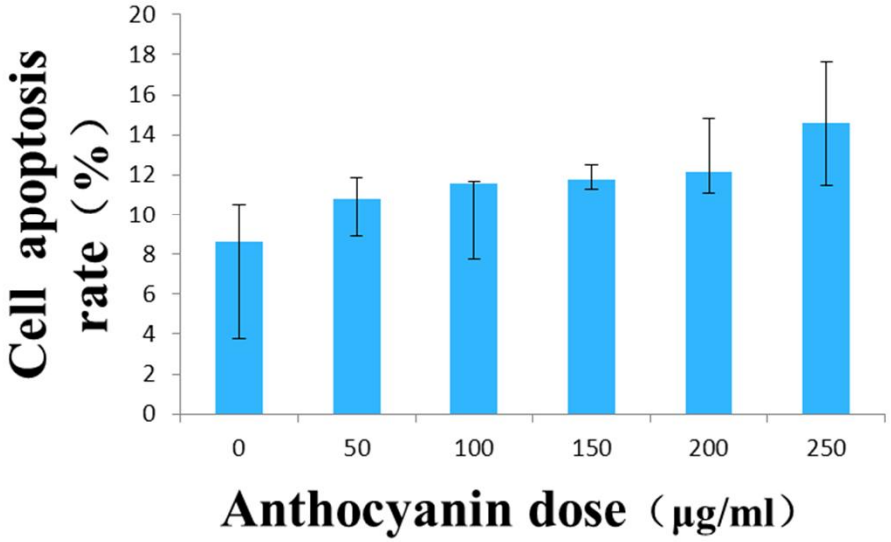










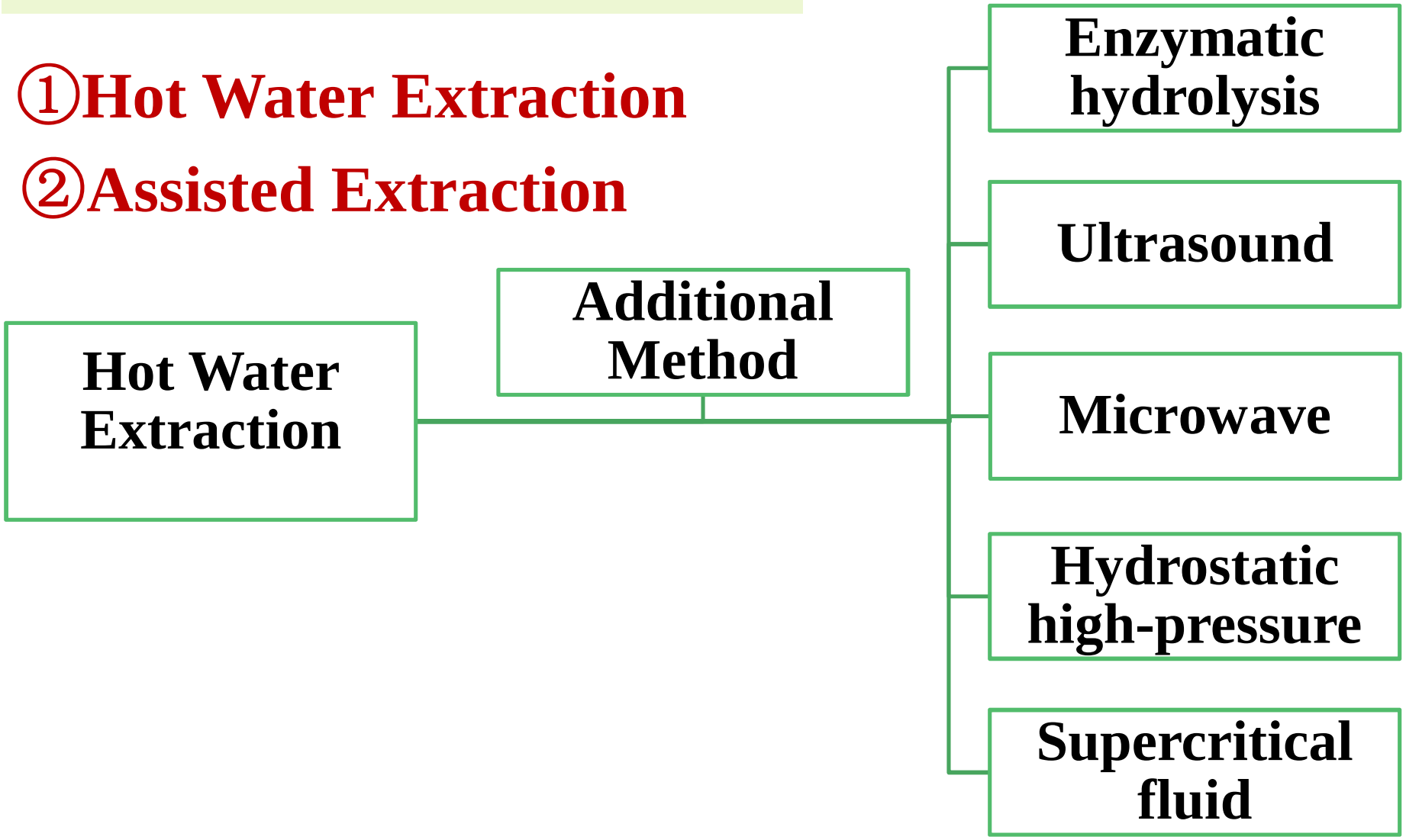


Increasing anthocyanin concentrations lead to the increase of apoptosis rate

1. Extraction Methods

①Hot Water Extraction

②Assisted Extraction

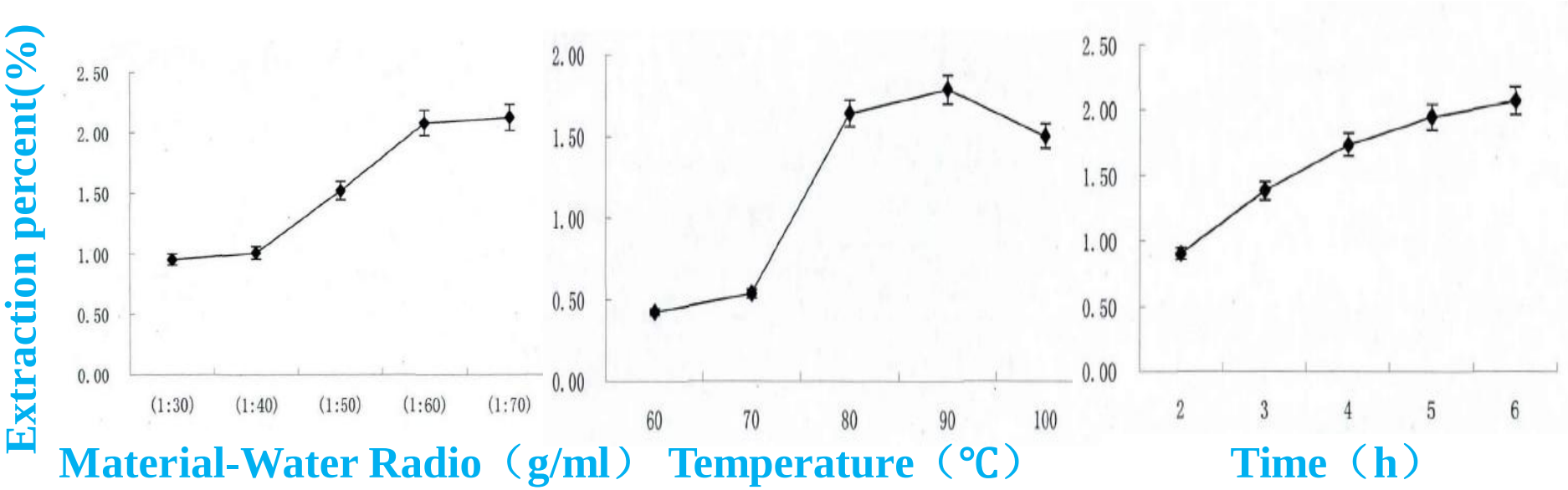


③ Our Work

Experiment Material：Dry Blueberry

Blue-crop

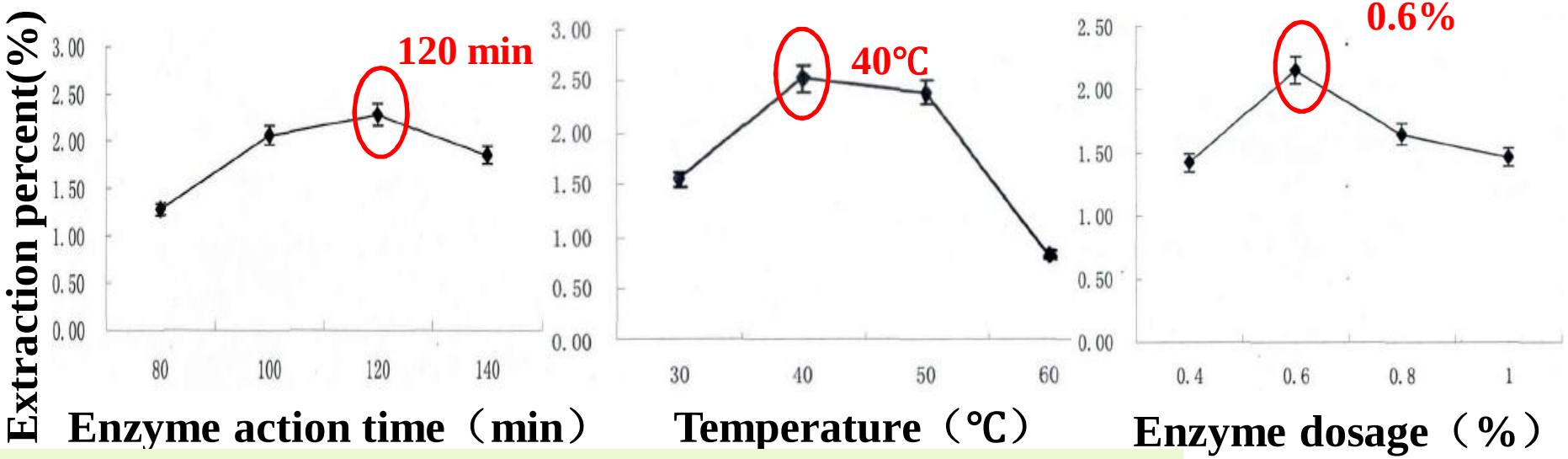
A:Hot Water Extraction



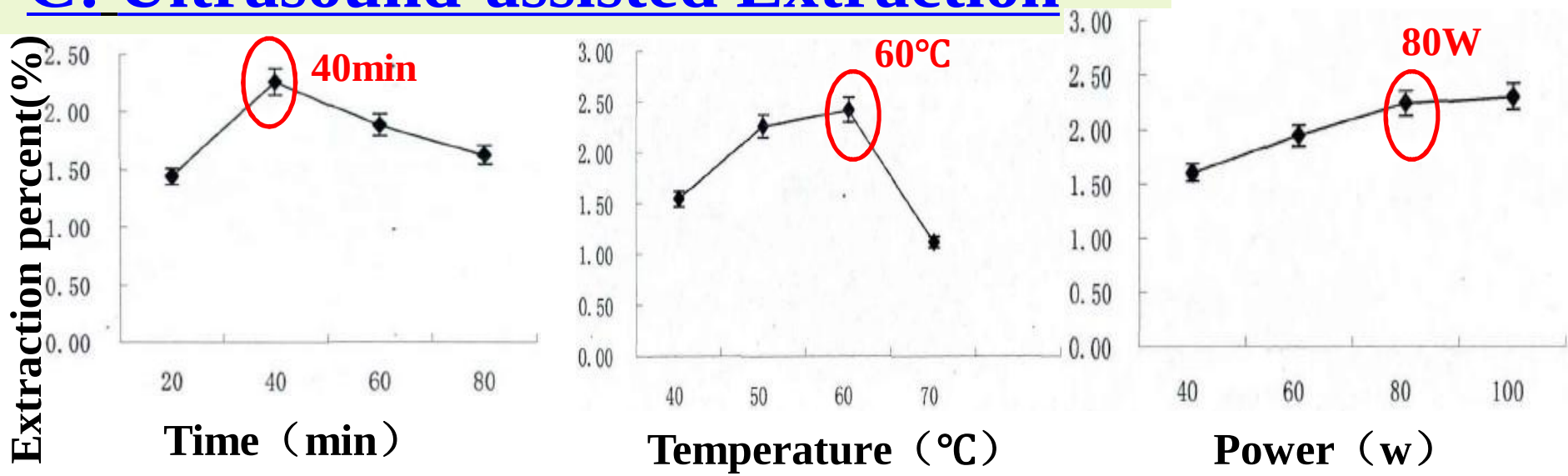
Optimal Conditions：

Material-Water Radio： 1 to 70
Time： 5h
Temperature： 70℃

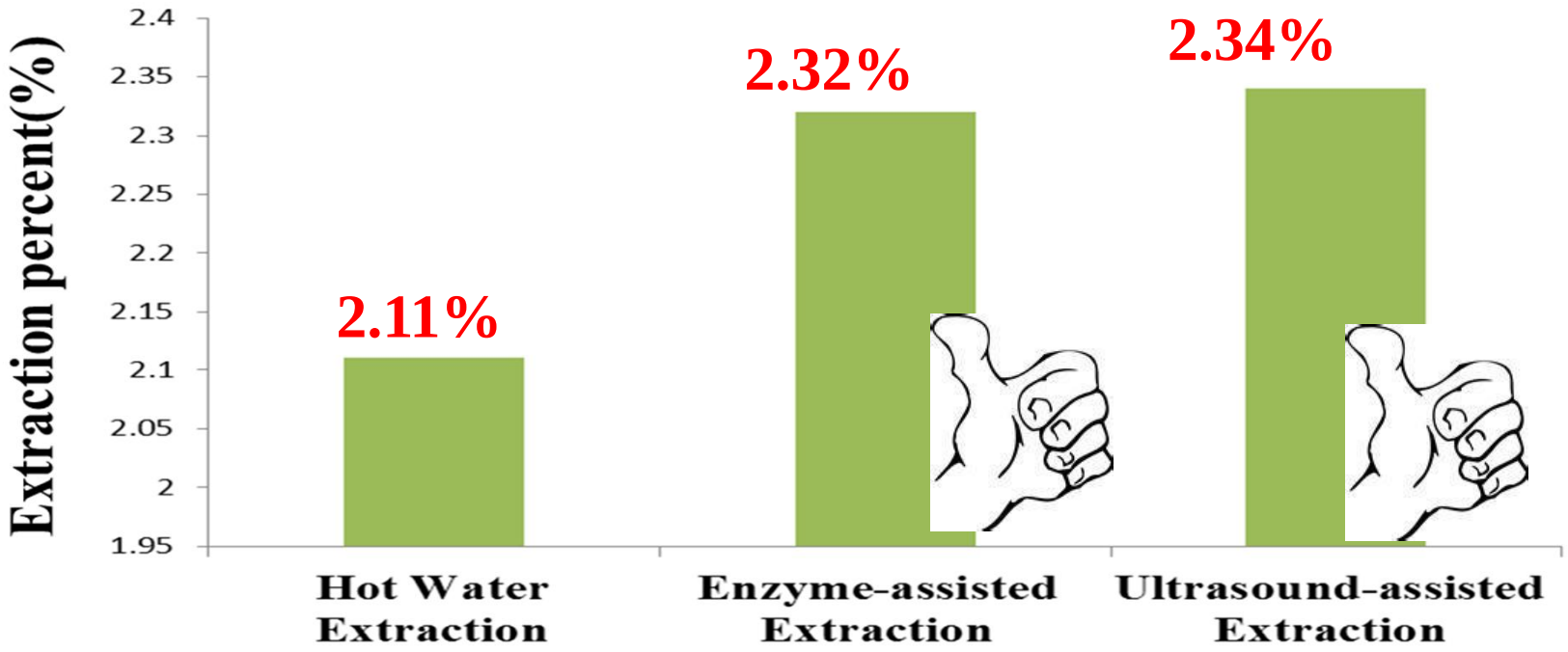
B: Enzyme-assisted Extraction



C: Ultrasound-assisted Extraction



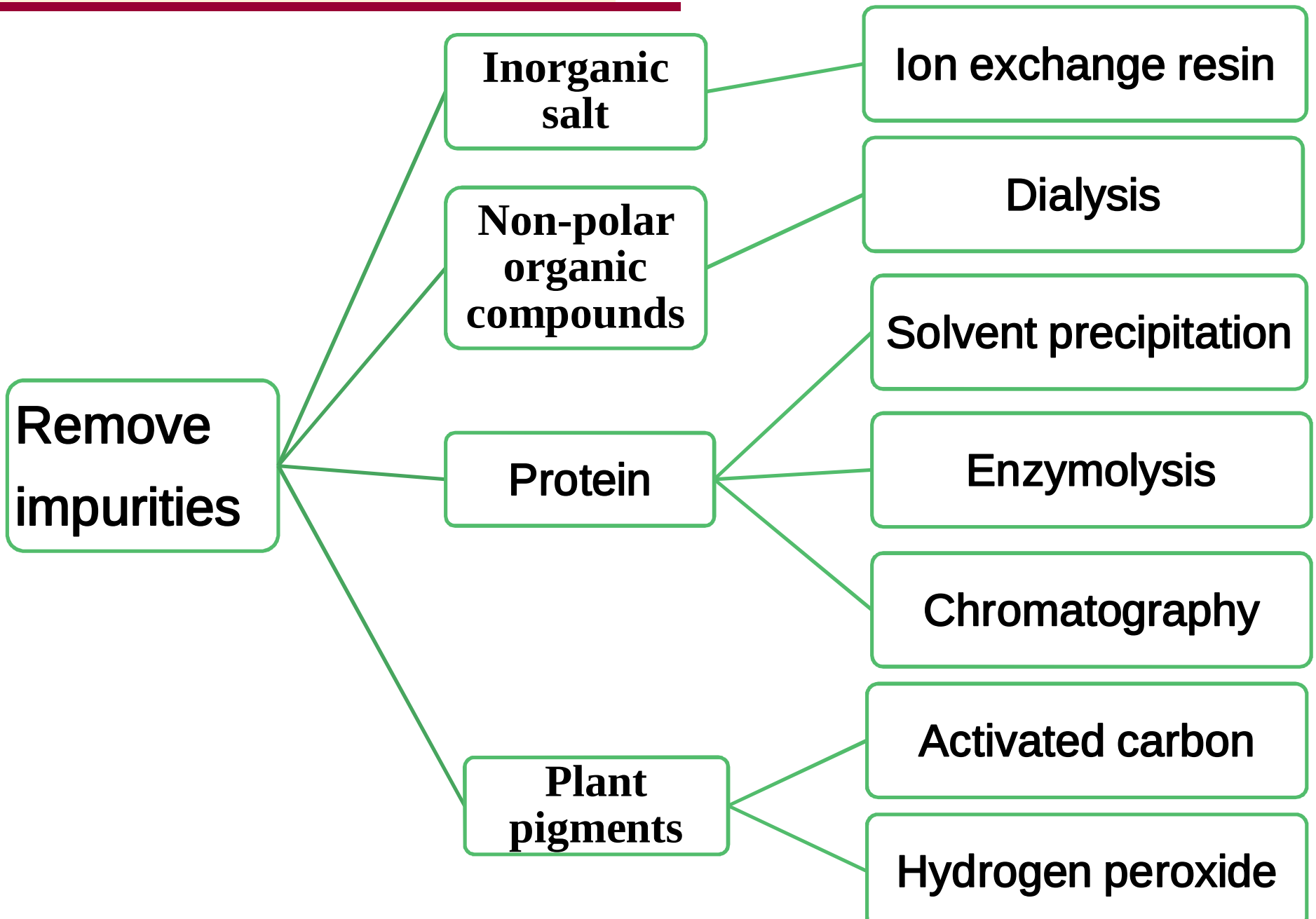
Ideal Extraction Methods



Enzyme-assisted Extraction & Ultrasound-assisted Extraction

- High extraction efficiency
- Mild treatment, product with high stability.

2. Isolation Methods



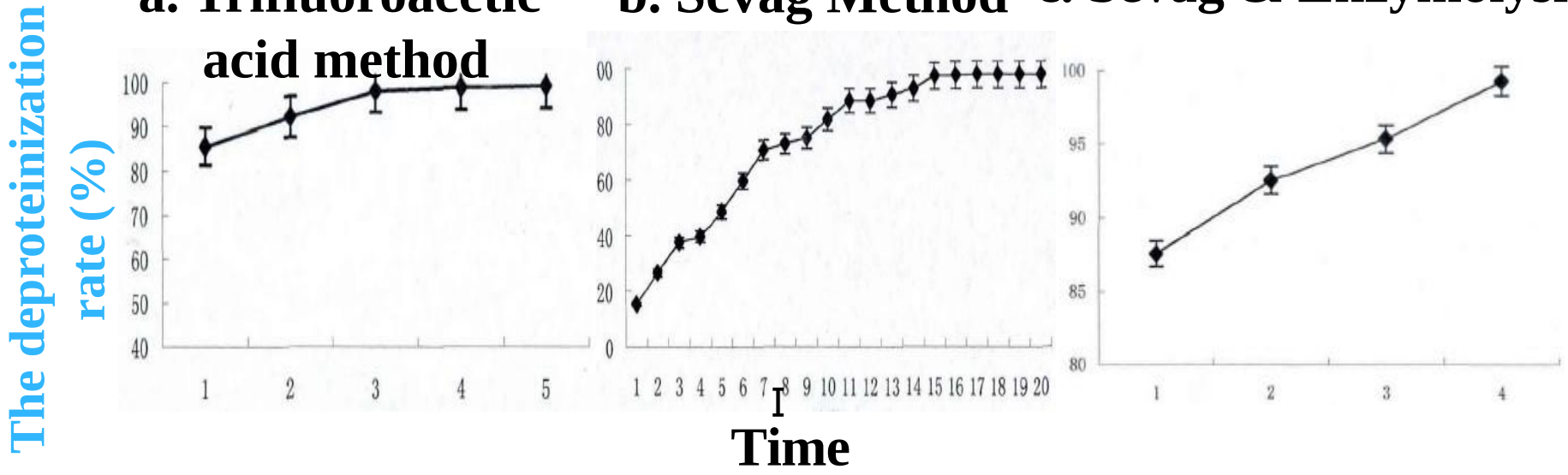
Our Work

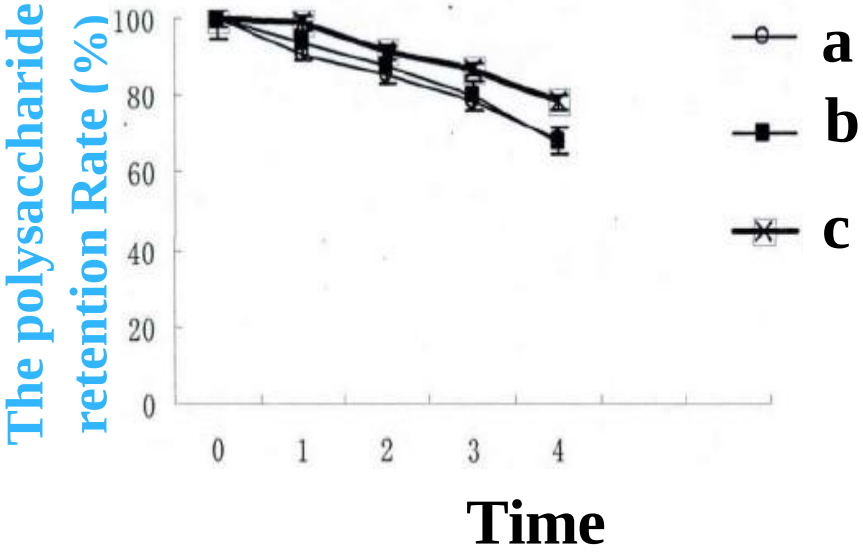
①Deproteinization

a. Trifluoroacetic acid method

b. Sevag Method

c. Sevag & Enzymolysis



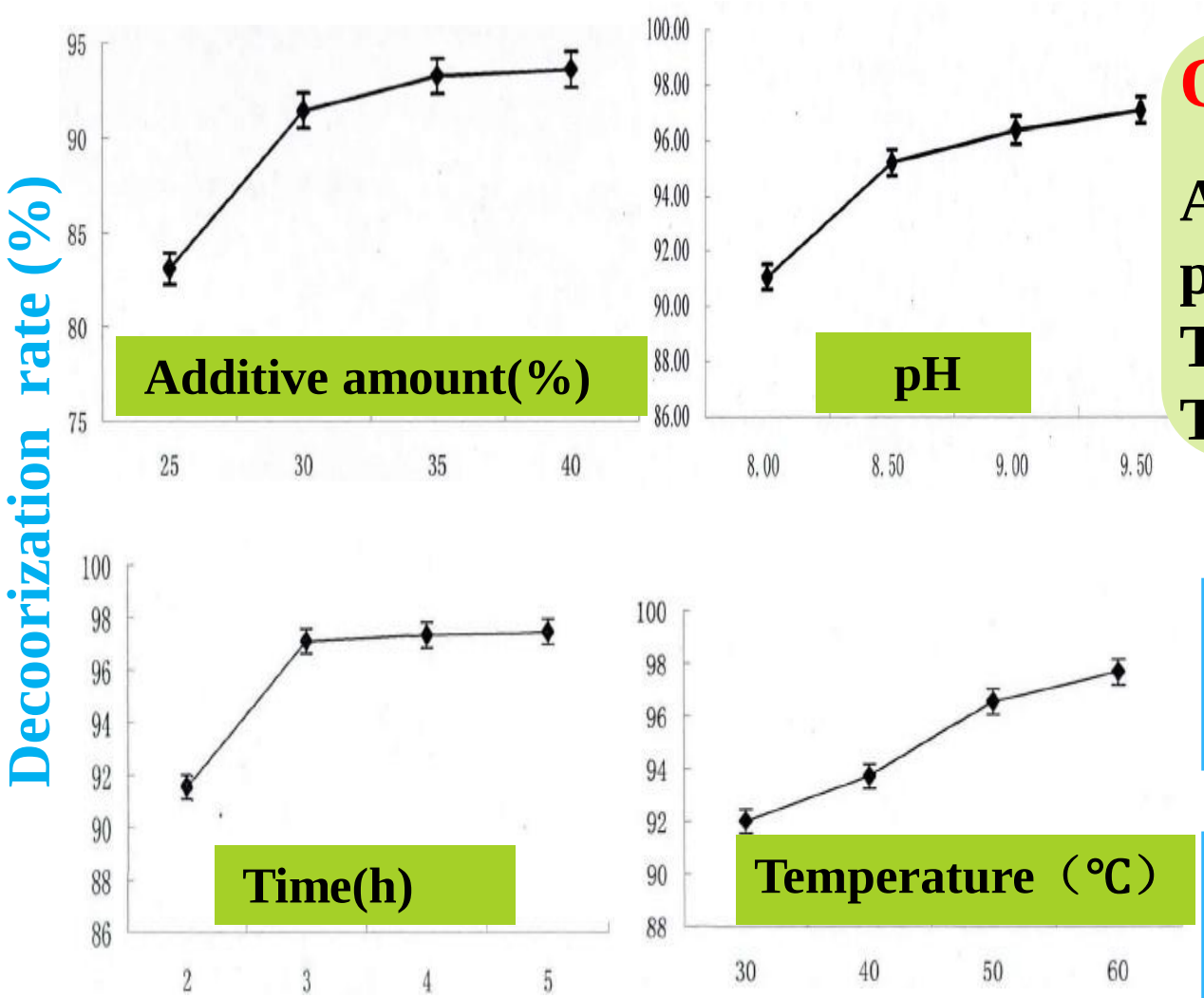


The Best Method

Sevag & Enzymolysis Method

- **High efficiency: 4 times**
- **High polysaccharide retention rate: 80%**

② Decoloration (Hydrogen Peroxide Method)



Optimal Conditions:

Additive amount:40%
pH: 8.5
Time: 4h
Temperature: 50°C

Decolorization rate: 93.11%

polysaccharide retention Rate: 79.24%

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3. Processing technology on berries

(1) Processing technology of berry jam

Berry → Wash → Blanch → Crush → Boil with sugar → Mix → Fill → Step cool

(2) Processing technology of berry juice

Berries → pre-process → Inactivate enzyme → Heat extract → Beat → Filter → Clarify → Filter → Mix → Sterilize → Fill

(3) Processing technology of berry wine

pre-process → enzymatic hydrolysis → Filter → Mix → Main fermentation → Isolation → Post-fermentation → aging → Mix → Clarify → Filter → Fill → Sterilize

$K_2S_2O_5$ (enzymatic hydrolysis)

$K_2S_2O_5$ (Main fermentation)

Add yeast (Main fermentation)

(4) Processing technology of berry vinegar

Berries → Choose & Wash → Crush → Adjust the sugar-acid ratio → alcoholic fermentation → acetic fermentation → aging → Clarify → Filter → Fill & Sterilize

(3) Our Work

A：Preparation of berry dry powder

Berries → Choose & Wash → Crush → Mix → Dry → Product

- Direct consumption

- yogurt

- icecream
- baked food

- ……

Selection of Drying Methods & Carriers

Experimental Material: Blueberry

Drum wind drying（DWD）

Freeze-drying（FD）

Time DWD:6h FD：6h

CMC

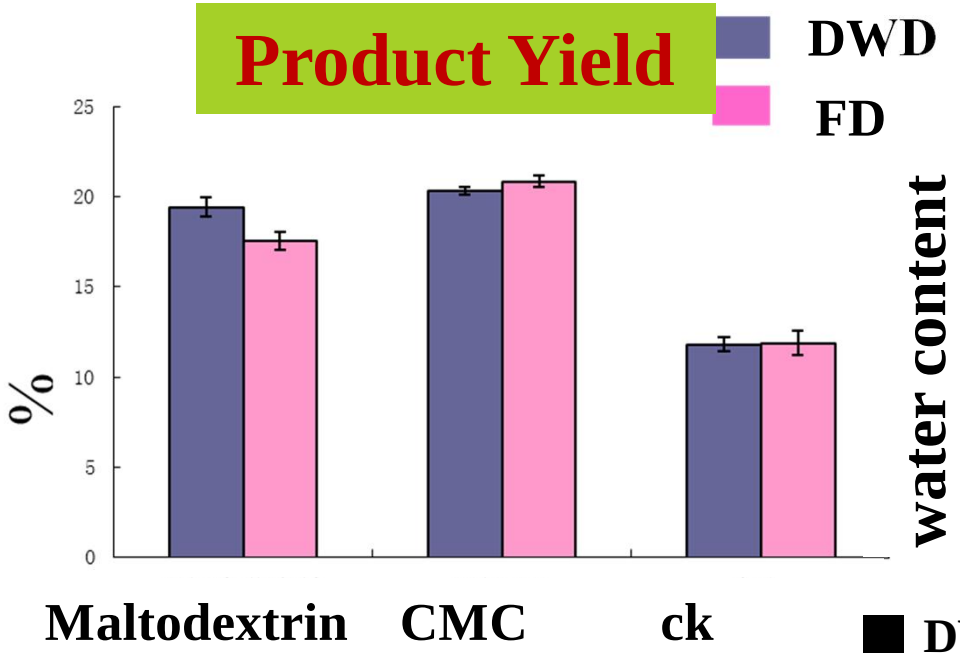
Maltodextrin

ck

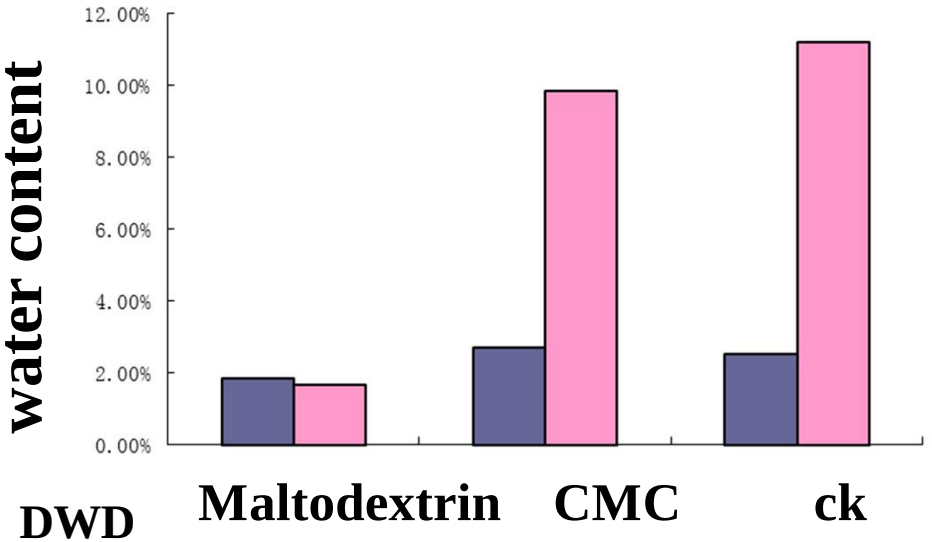
Groups		
	DWD	FD
Maltodextrin	A - 1	A - 2
CMC	B - 1	B - 2
ck	C - 1	C - 2

Product Quality

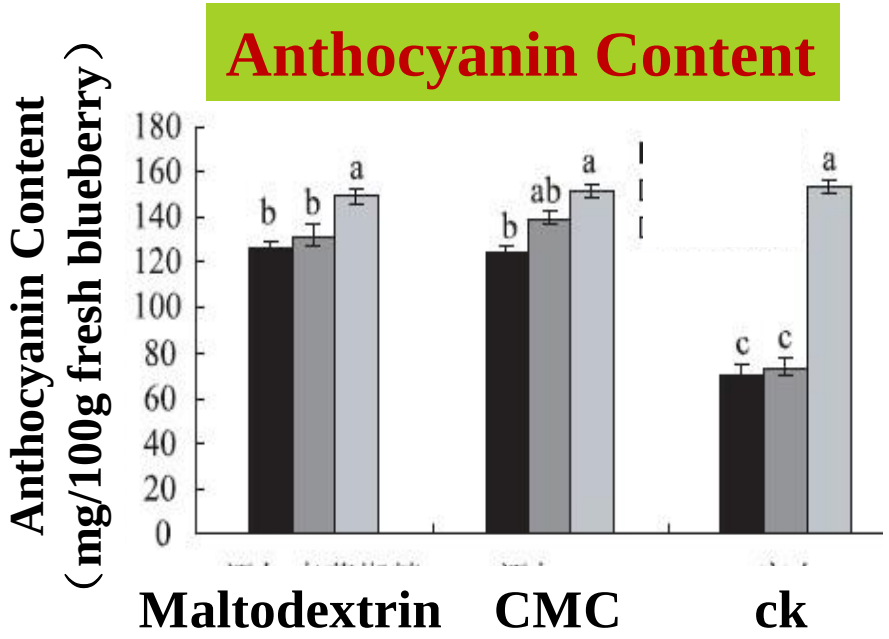
Product Yield



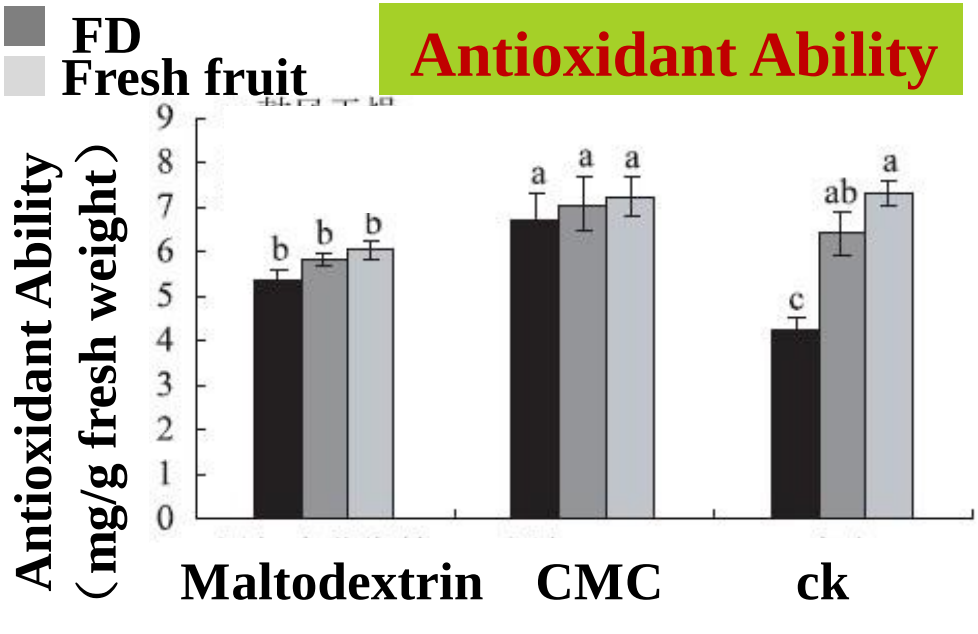
Water Content



Anthocyanin Content



Antioxidant Ability

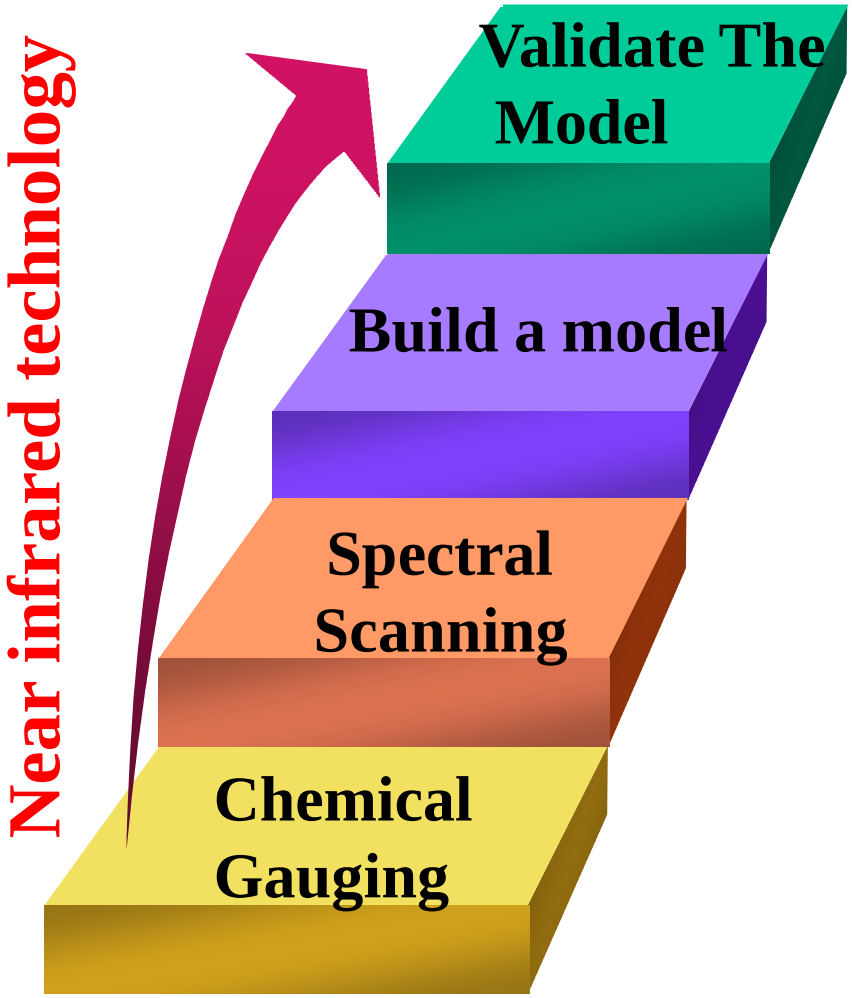


Product Appearance

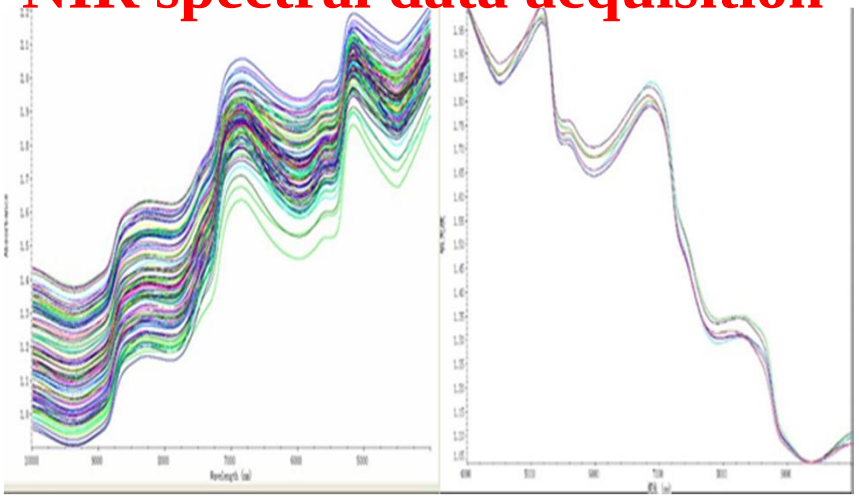
	DWD	FD
Maltodextrin	Purple powder Firm structure Difficult collection Got damp after 12h at RT	Purple powder Crisp structural Got damp after 12h at RT
CMC	Purple powder Low drying rate Firm structure Difficult collection Not easy to get damp	Purple powder Crisp structural Low drying rate Not easy to get damp
ck	Purple powder Got damp easily	Purple powder Got damp easily

Optimal Conditions：Add maltodextrin & Freeze-drying

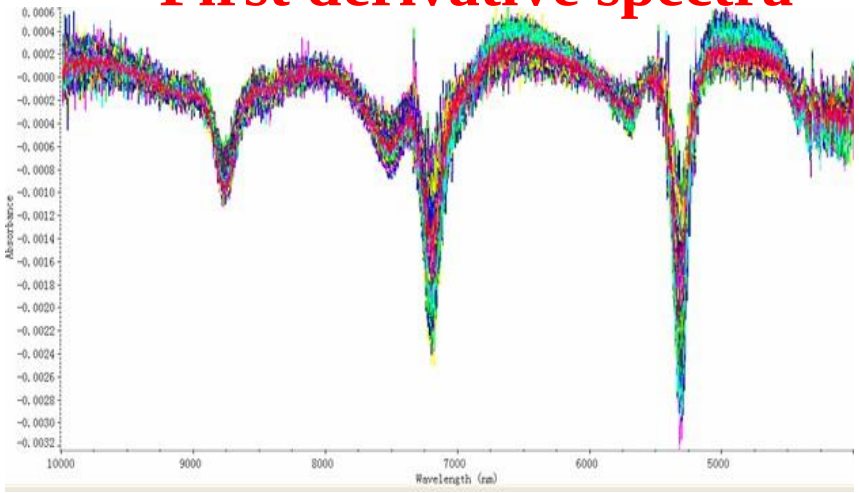
B: Non-destructive assessment technology



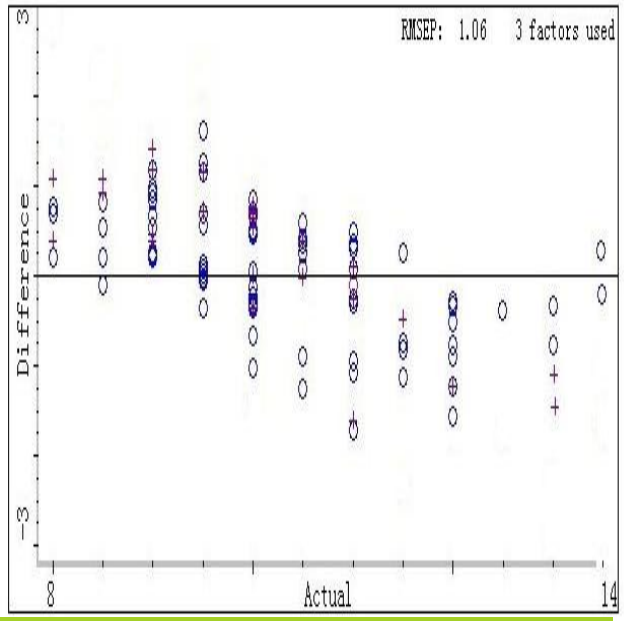
NIR spectral data acquisition



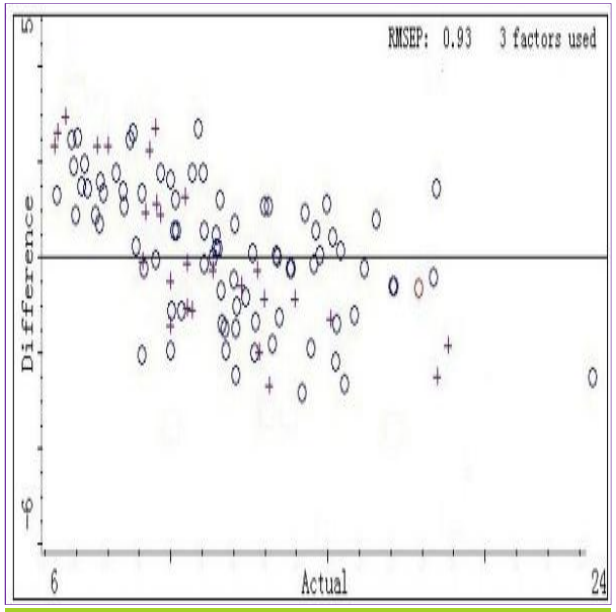
First derivative spectra



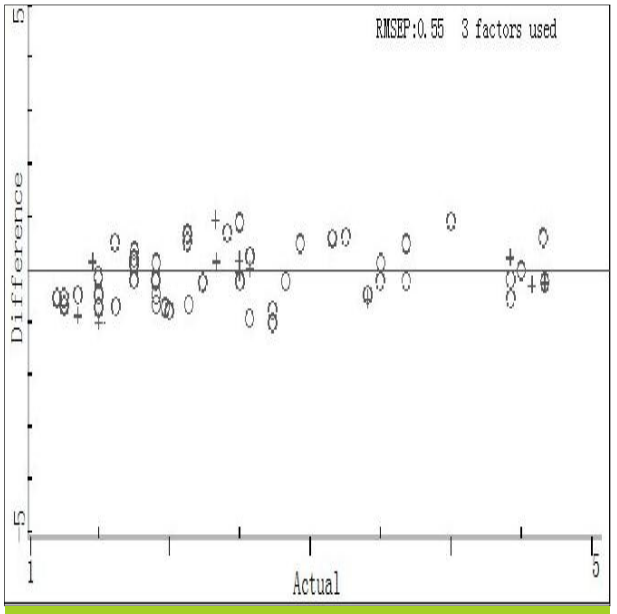
3 Models established



Soluble Solids



Total Phenol



Anthocyanin

The 3 models via NIR tool help our producers to realize the quality evaluation of blueberry without any damage to fruit.

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4. Technology Extension Cases

1/Haida biological technology co., LTD

Capsule production line




Blueberry anthocyanin capsules:

Preparation technology of berry dry powder

Production capacity: 100,000 / hour

Profit of the product

成果应用（效益）证明

项目名称	蓝莓花青素高通量检测及产业化研究
应用单位	南阳市海达生物技术有限公司
应用成果起止时间	2011 年 7 月至今
通讯地址	河南省南阳市龙升工业园区
经济效益	
年度	2011 年 7 月至 2012 年 7 月
新增产值	90 万
新增利税	9 万
节支金额	
其他收入	7 万
年增收金额	16 万

应用情况:

我公司自 2011 年 7 月至今,采用于北京林业大学生物科学与技术学院张柏林教授课题组（项目编号：200904014-3）合作研发的蓝莓冻干果粉作为原料,应用于蓝莓花青素胶囊制备,该产品最大程度保留了蓝莓中花青素的含量和活性,对增视明目和保护肝脏起到积极作用。运输、携带、服用方便，产品投放市场以来，收到广大消费者的欢迎。

应用单位（盖章）　应用单位财务部门（盖章）　法人签章

2012 年 10 月 20 日

newly added output value :900,000 RMB

Enterprise Standard

Q/NHS

南阳市海达生物技术有限公司企业标准

Q/NHS 022-2011

蓝莓花青素胶囊

Q/41BA/0716-2011

Blueberry anthocyanin capsules

2011-7-6 发布　2011-7-12 实施

南阳市海达生物技术有限公司　发布

2/Orogen native products co., LTD

Berry juice production line




Blueberry juice: High anthocyanin content

Enterprise Standard

14.0

Q/HXJS

河北喜奥保健食品有限责任公司企业标准

Q/HXJS 0001S—2012

果汁及果汁饮料

备案号：13****S-2012
备案日期：2012年1月13日
有效期至：2015年1月12日

2012年1月5日发布　　2012年1月5日实施

河北喜奥保健食品有限责任公司　发布

Juice and Juice Drinks

Conclusions

♦Establish the optimum extraction and purification process of anthocyanin and polysaccharides;

♦Develop technology related to drying and NIR for the processing of blueberry ;

♦Have a good cooperation with medium-sized companies, and help them to set up production lines.

ACKNOWLEDGMENT:

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Thank you for your attention

Welcome to BJFU!

Beijing Forestry University