Study on Proanthocyanidins-Rich Beer

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Report Outline

1. The purpose of the work

2. A brief review of the methods used

3. Conclusion

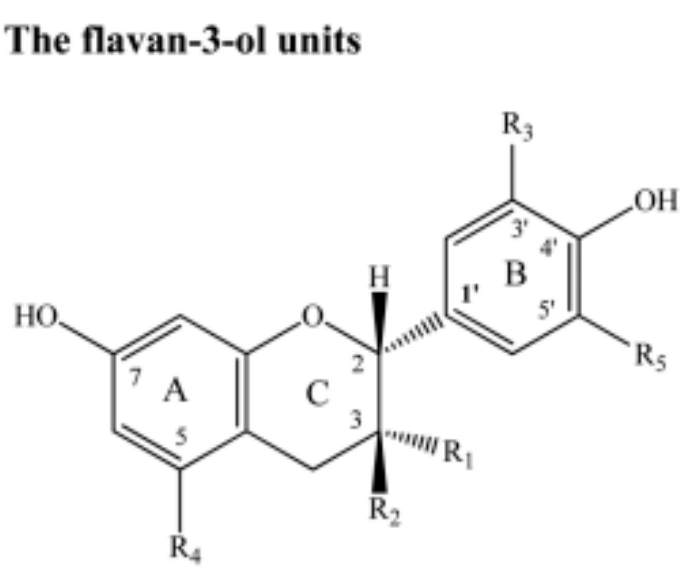
4. Suggestions for further work

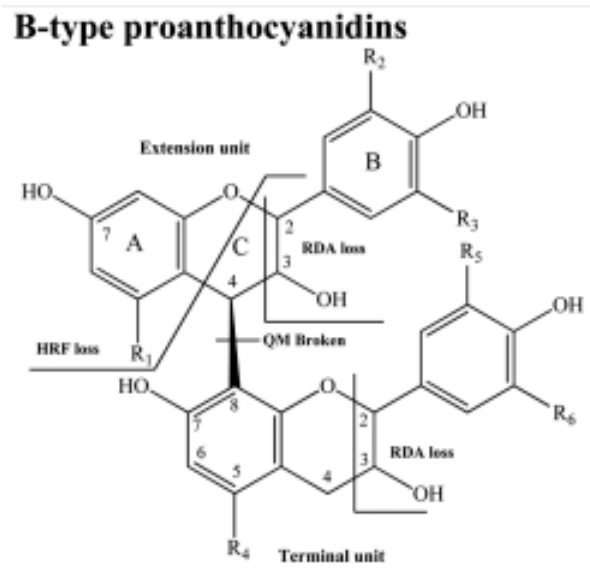
1. The purpose of the work

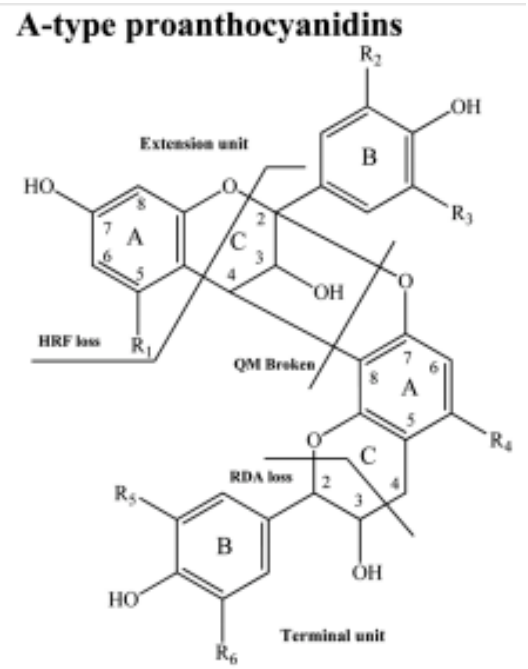
- To study the antioxidant effect of proanthocyanidins from Lycium Ruthenicum Murr (Gochi) in beer.
- To develop a beer rich in proanthocyanidins， using Gochi as a part of raw material to improve antioxidant capacity of beers and health- benefits.

2. A brief review of the methods used

Structures of Proanthocyanidins (PAs)







（1）About proanthocyanidins

- Proanthocyanidins (PAs) - condensed tannins.
- A class of colorless phenolics characterized by an oligomeric or polymeric structure based on flavan-3-ol units.
- PAs are antioxidative, anti-inflammatory, anti-hypertensive and hypocholesterolemic.
- May decrease the risk of cardiovascular diseases, cancer and neurodegenerative diseases.

PAs are the main polyphenolic components in many different plant-derived fruits, such as grains, berries, teas, and Lycium ruthenicum Murr(Gochi), and are reported to have a variety of health-promoting benefits.




（2）Lycium ruthenicumm Murr （Gochi)

- A shrub plants belonging to the Solanaceae family
- Growth in Tibet ,China; called Gochi
- Primarily comprised of flavonoids, essential oils and polysaccharides, and named as “The king of PAs”
- Being used for both traditional Chinese medicine and nutritional purposes in China and other Asian countries for centuries.

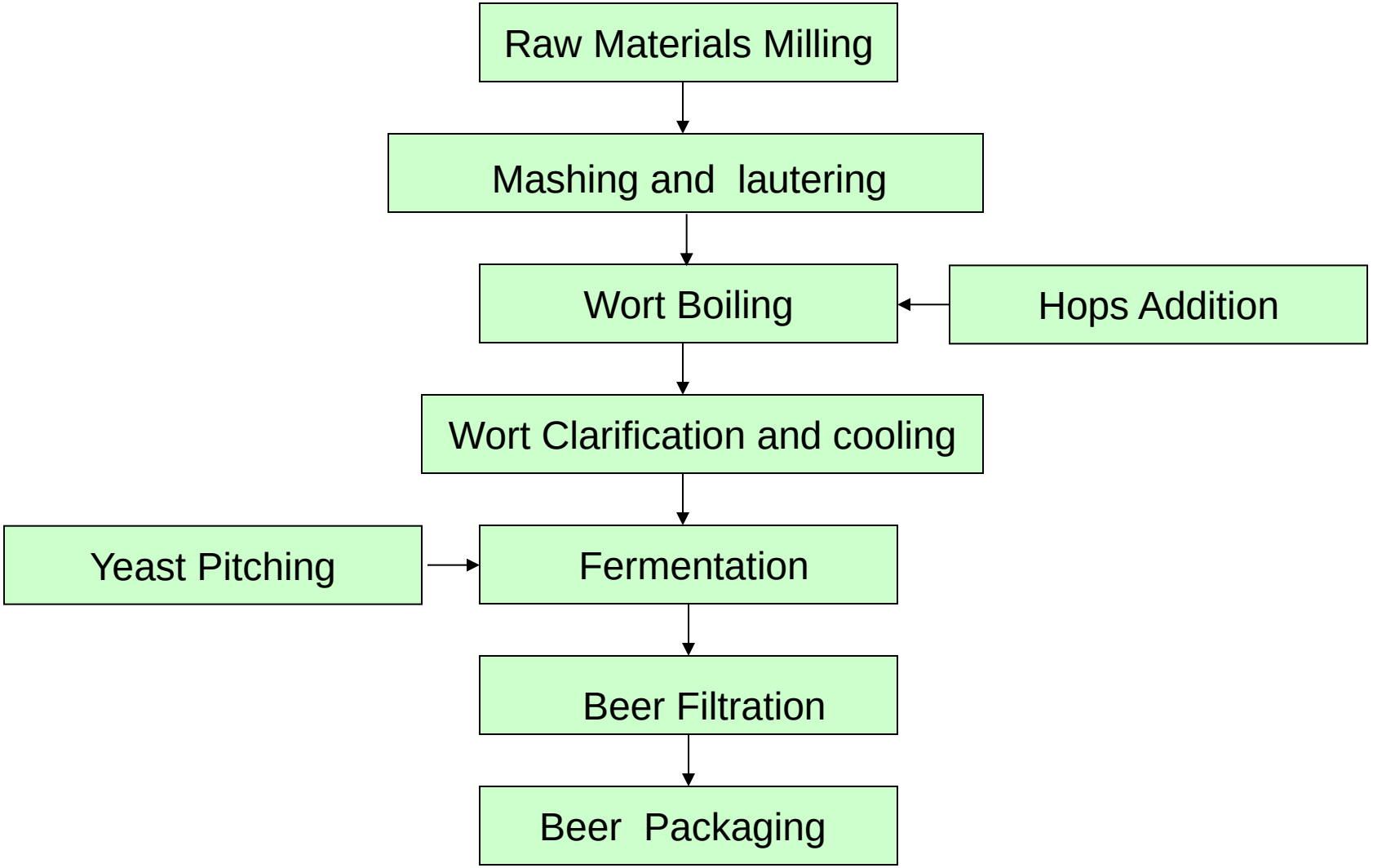
- Gochi contain higher water soluble PAs than hops and malt.
- Gochi could be used as a supplement for brewing to increase the amount of PAs in beer.

Different coloures at different pH values

acid

alkaline

（3）Brewing process



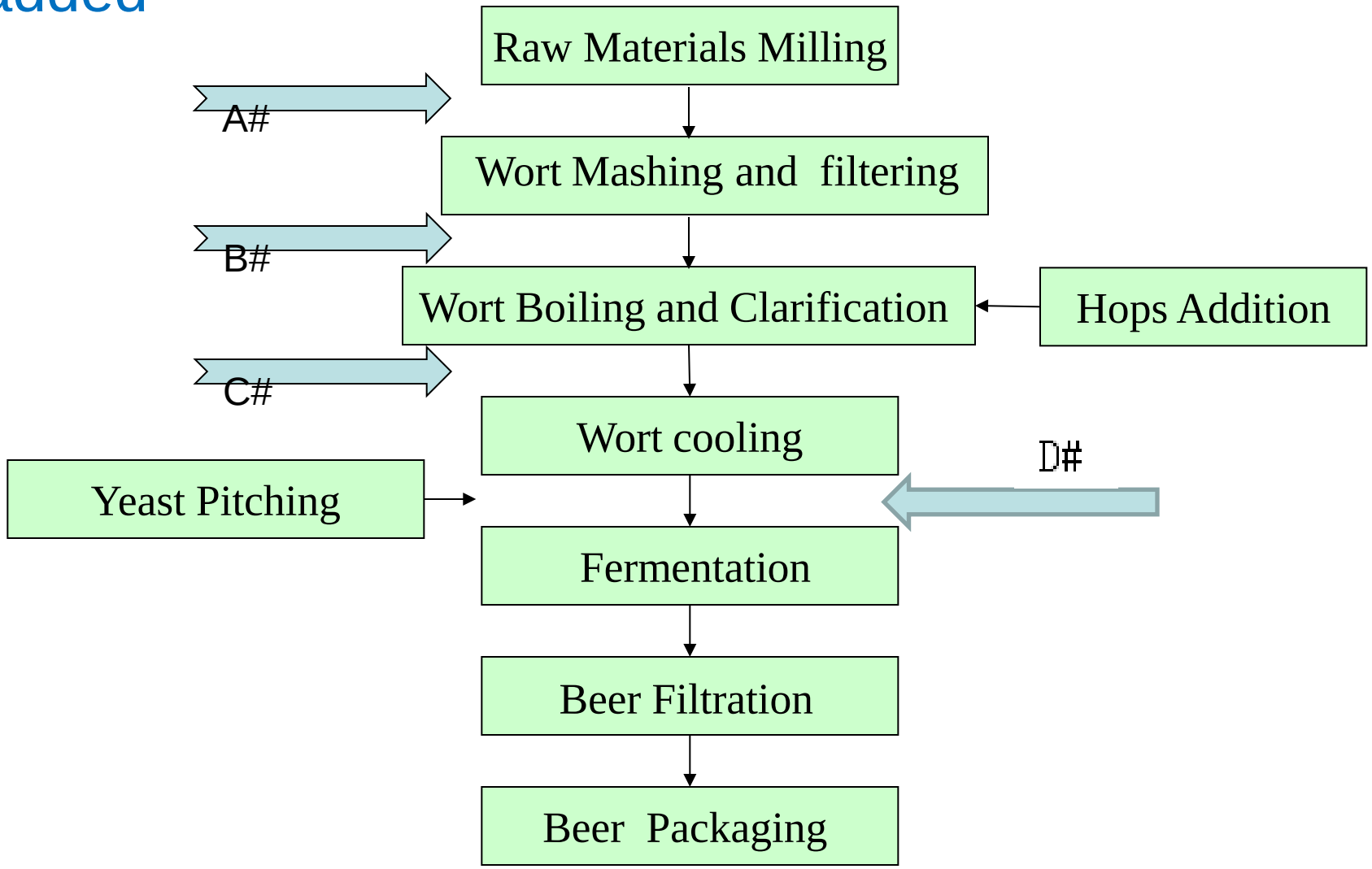
I. Brewing Material - Gochi

- Selection and preparation: full particles, no pests, no mildew and milling

- Experiment 1: The amount of Gochi added in end of boiling

  - 0#： control
  - 1#： 0.5g/L powders
  - 2#： 1.0g/L powders
  - 3#： 1.5g/L powders
  - 4#： 1.0g/L Dry whole Fruits

•Experiment 2: The amount of Gochi 0.5g/L powders added



Ⅱ. Brewing Conditions

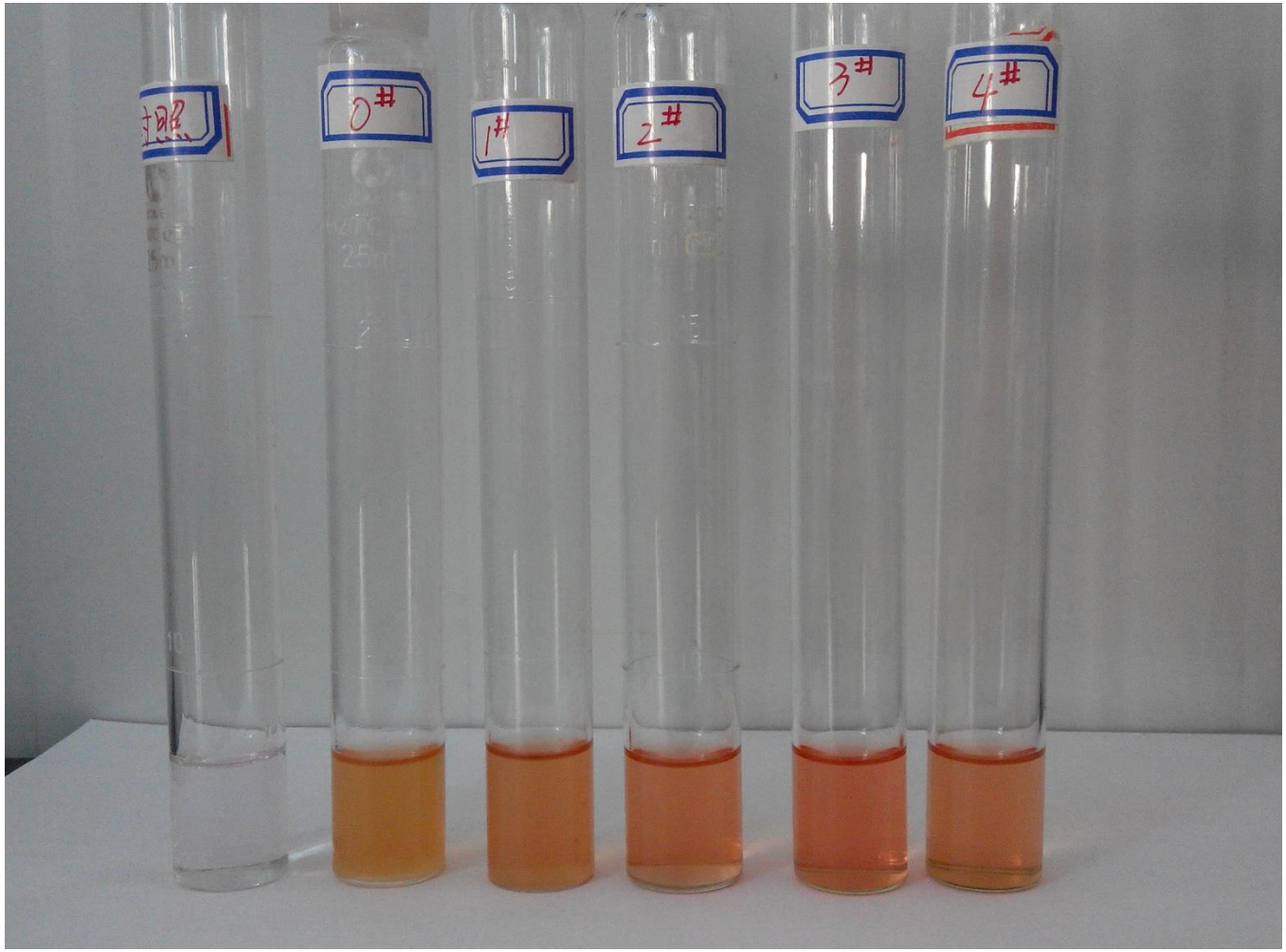
- Original wort concentration:12-13 ° P
- Pitching rate: 10^7 yeast cells/mL, Lager yeast.
- Fermentation: 10 days at 12° C.
- Addition of hops : 8 BU , at the 10min of wort boiling beginning; 4BU, at end of 30min before the end of wort boiling
- Lagering: in cask (5 days at 2° C).

III. Quantitation of proanthocyanidins

Detection of Proanthocyanidins with vanillin and HCl system

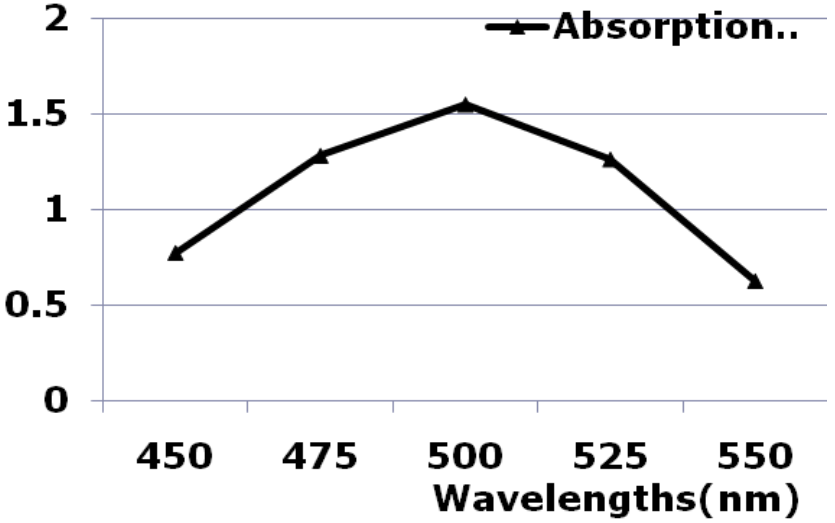
- Proanthocyanidins and monomers such as catechin, epicatechin has high chemical activity.
- The condensation of proanthocyanidins with vanillin produces red carbonium ions, under acidic conditions.
- Have specific absorption value at 500-530nm and the concentration of proanthocyanidins and its color are positive correlated.

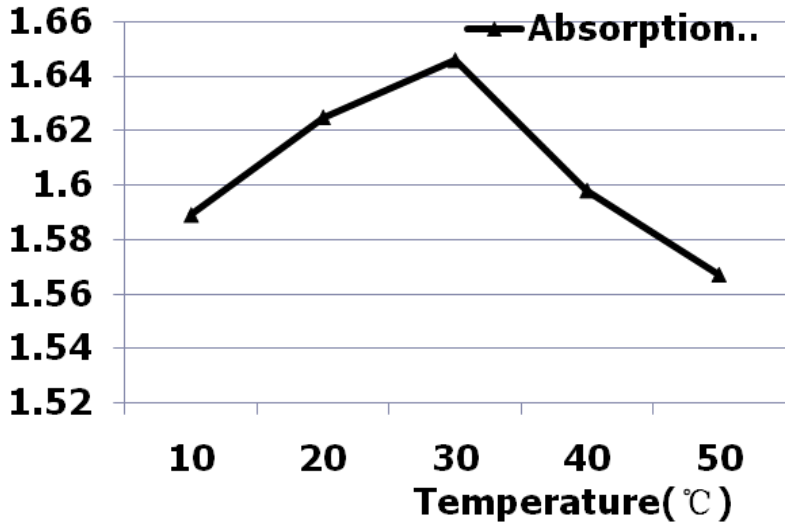
Chromogenic reaction of standard curve of catechin

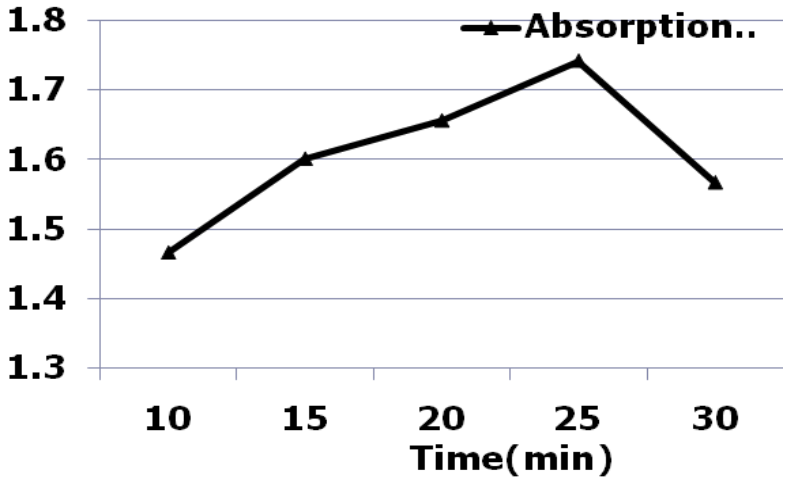


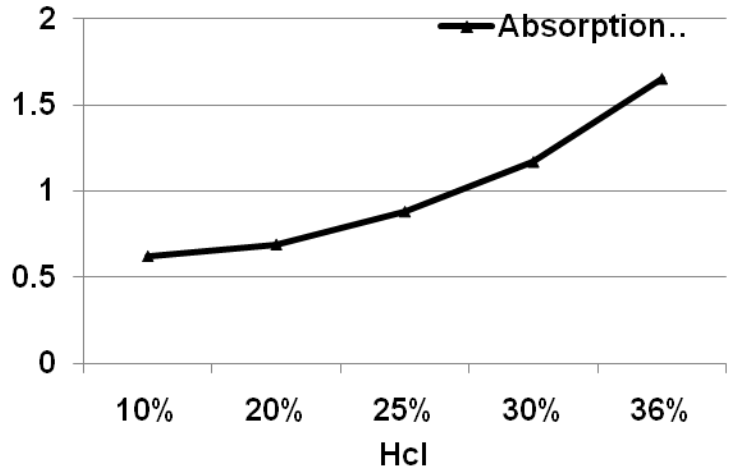
- To determine the optimim conditions:

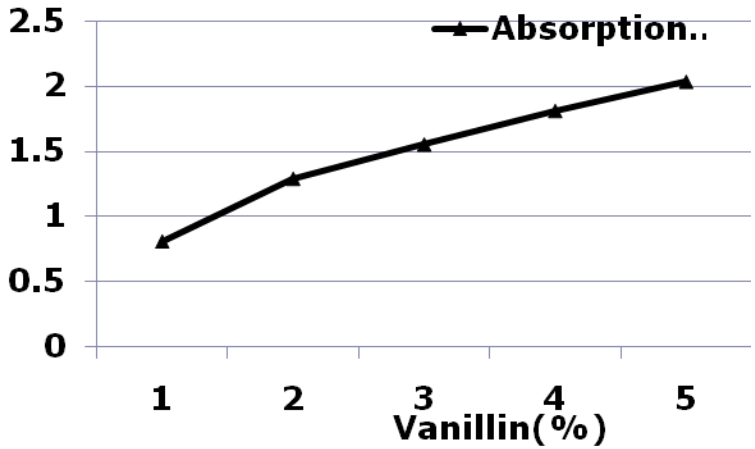
Reaction temperature and time, detection wavelength



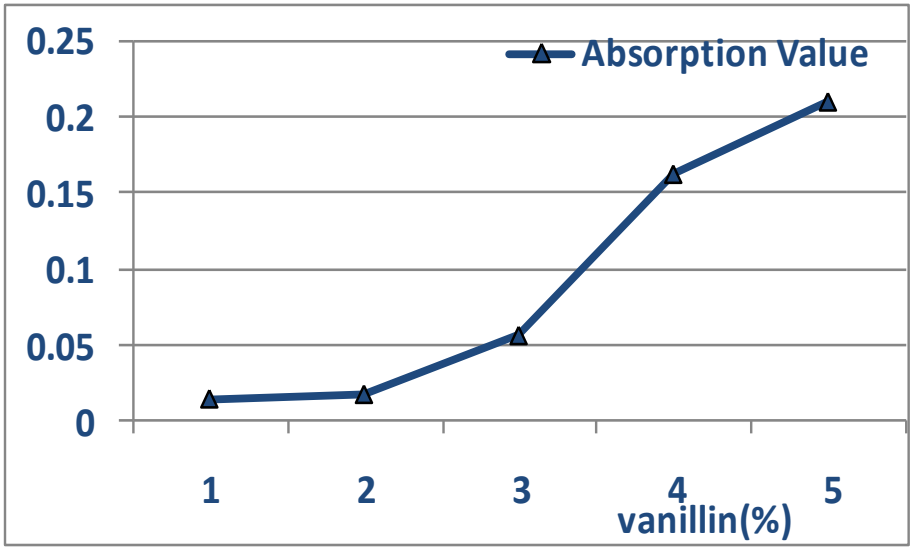








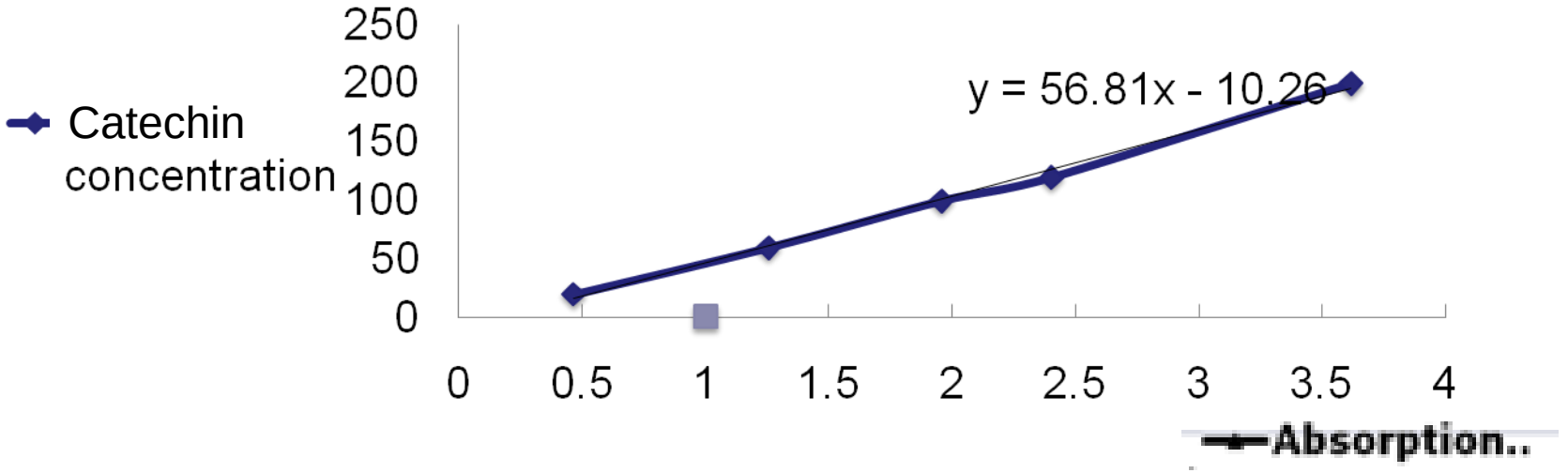
- To determine the optimum conditions: concentration of HCl and vanillin



Optimum conditions and standard curve of Catechin：

2% vanillin，1.5ml （36%）HCl，25℃，25min；
500nm to detect.

1mL sample + 3mL vaillin-methanol + 1.5mL HCl



Standard curve

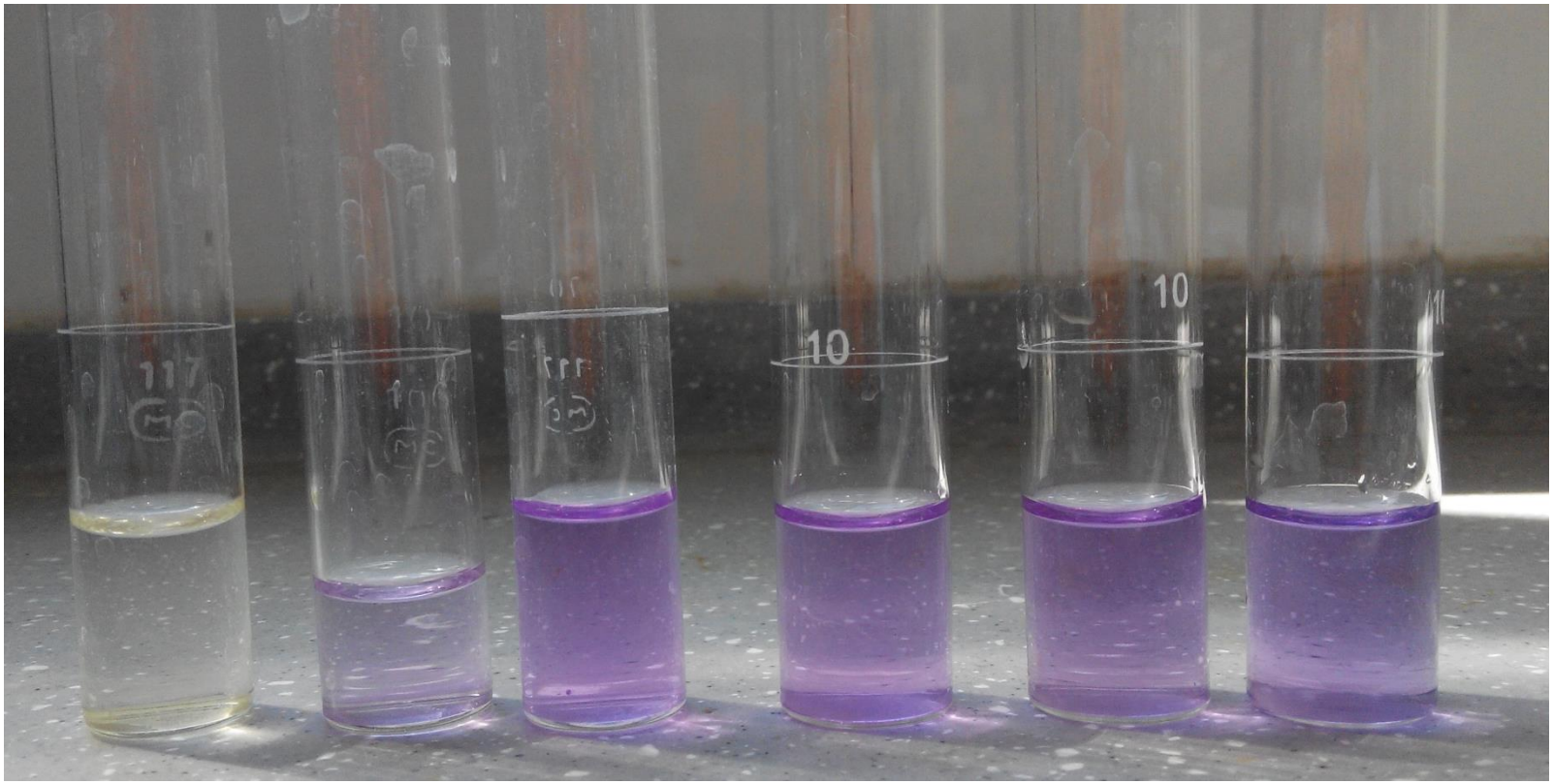
IV. Detection of antioxidant activities

Methods used to detect the total Antioxidant Activities of beer :

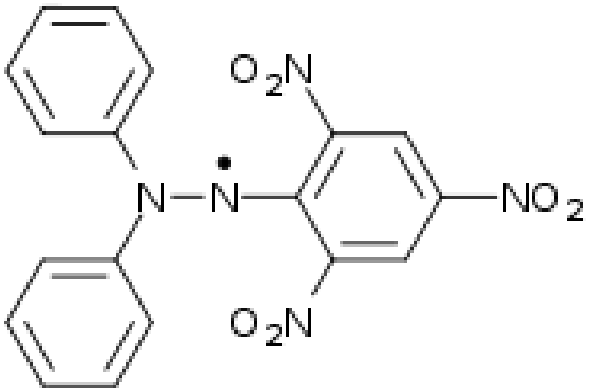
- Phosphomolybdenum coordination compound method
- Hydroxy radical-scavenging activities
- Anti–2,2-diphenyl-1-picrylhydrazyl (DPPH) radical capacity
- TBA (Thiobarbituric Acid)value

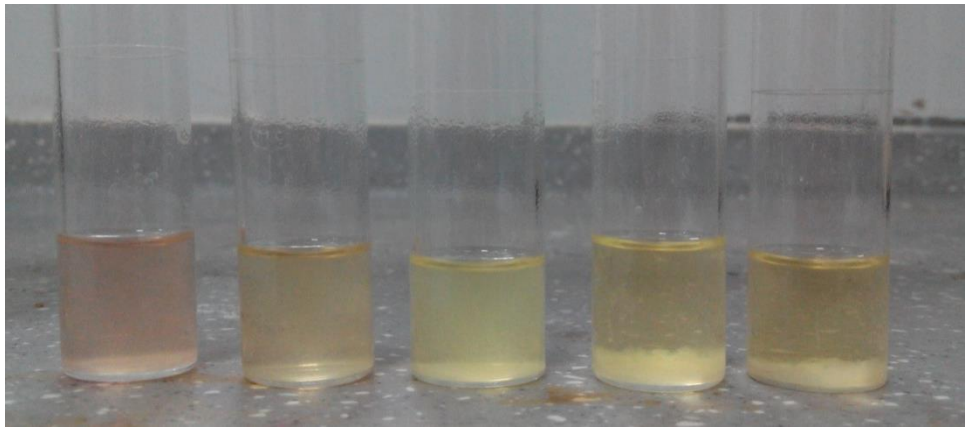
- Phosphomolybdenum coordination compound method

- Hydroxy radical-scavenging activities



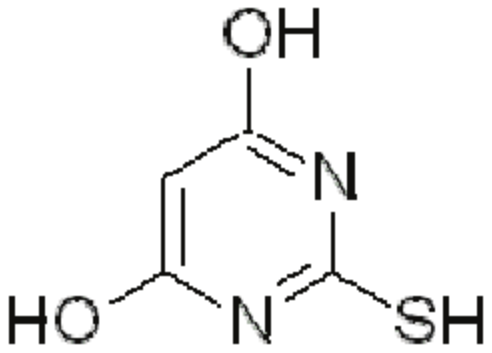
- **Anti–2,2-diphenyl-1-picrylhydrazyl (DPPH) radical capacity**





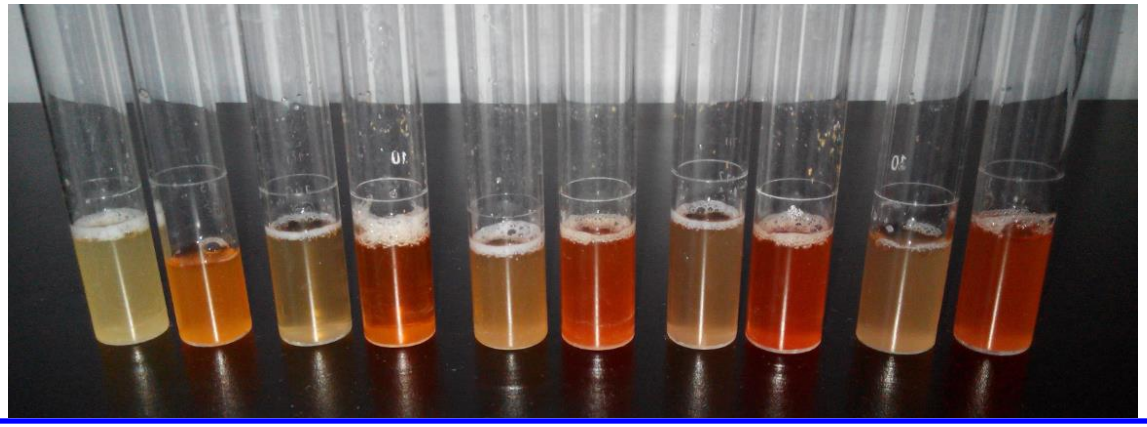
The radical scavenging capacities of beers were measured based on their ability to quench the DPPH radical , decrease the absorbance of DPPH radical at 515 nm.

- **Detection of TBA value**



Thiobarbituric acid

Thiobarbituric acid can react with carbonyl compounds in beer to generate a substance of yellow.At 530nm wavelength, they have specific absorption value.
The lower value is, the more fresh beer is.



Analytical data Experiment 1 of beer

Beer sample	0	0.5g/L RL powder	1.0g/LRL powder	1.5g/LRL powder	1.0g/LDry Fruits
	0#	1#	2#	3#	4#
pH value	4.67	4.67	4.65	4.65	4.6
Original gravity	12.87	12.18	12.97	13.06	12.67
Real degree of fermentation	68%	66%	68%	68%	67%
Diacetyl	0.06	0.05	0.06	0.05	0.07
Alcohol (v/v)	5.54	5.12	5.64	5.68	5.38
Residual sugar	4.36	4.278	4.316	4.354	4.135
Acidity	1.96	2.02	2.06	2.00	2.02

According to analysis method of GB/T4928-2008
Page24-30：See page 12 experiment program

Results of measurements of Experiment 1

Sample	Total polyphenol	PAs	TBA Value	Phosphomolybdenum coordination compound method	Hydroxy radical-scavenging activities	Anti–DPPH radical capacity
	Result mg/L	A500	A530	A695	A588	A515
0#	288.89	47.686	0.6691	0.319	0.144	3.50%
1#	295.36	59.048	0.4927	0.369	0.177	4.10%
2#	298.89	68.138	0.3392	0.519	0.200	10.69%
3#	326.93	68.706	0.2860	0.572	0.262	17.86%
4#	305.78	60.753	0.5334	0.522	0.199	14.28%

TBA : 2-Thiobarbituric acid

Sensory evaluation

0#: normal, typical lager beer (control)

1#: normal, taste clean, tiny fruit flavors than control

2#: normal, taste clean, tiny fruit flavors than control

3#: normal, taste clean and more fresh than others

4#: normal, taste clean, tiny fruit flavors than control

Analytical data Experiment 2 of beer

Beer Sample	Contral	Add when mashing	Before boiling	After boiling	After cooling
	0#	A#	B#	C#	D#
pH value	4.51	4.53	4.56	4.49	4.51
Real degree of fermentation	69.50%	66.90%	67.60%	68.20%	68.90%
Acidity	1.80	1.70	1.83	1.89	1.93
Total polyphenol A600:mg/L	264.04	333.74	455.1	457.56	433.78
TBA value A530	0.602	0.525	0.532	0.514	0.534
Abs value of PAs A500	0.53	0.69	0.844	0.955	0.834

Page31-34：See page 13 experiment scheme

Results of measurements of Experiment 2

Beer Sample	Phosphomolybdenum coordination compound method	Hydroxy radiad-scavenging activities	Anti–DPPH radical capacity
	A695 (value of diluted 5 times)	A588 (value of diluted 5 times)	(value of not diluted)
0#	0.686	0.133	32%
A#	0.676	0.103	52%
B#	0.797	0.102	29%
C#	0.662	0.09	39%
D#	0.67	0.114	52%

3. Conclusion of Experiment 1

- Using (Gochi) will enhance the PAs contents.
- Adding (Gochi) powder 0.5g/L in wort will enhance the PAs content about 23.8%.
- (Gochi) powder will provide more PAs than dry fruit under same condition.
- Using (Gochi) will enhance the antioxidant indexes significantly for final beer.

3. Conclusion of Experiment 2

- Adding Gochi at mash-in, the TBA value is lower (that means the beer is more fresh) while the total antioxidant ability and the PAs contents are lower.
- Loss PAs in wort boiling
- The better use of Gochi is added after boiling.

4. Suggestions for further work

- To develop the better extraction method of PAs from Lycium ruthenicumm Murr (Gochi)
- To determine the structures and degrees of polymerization

Thank you