Cellular antioxidant activity of rice bran oil at different stages of refining

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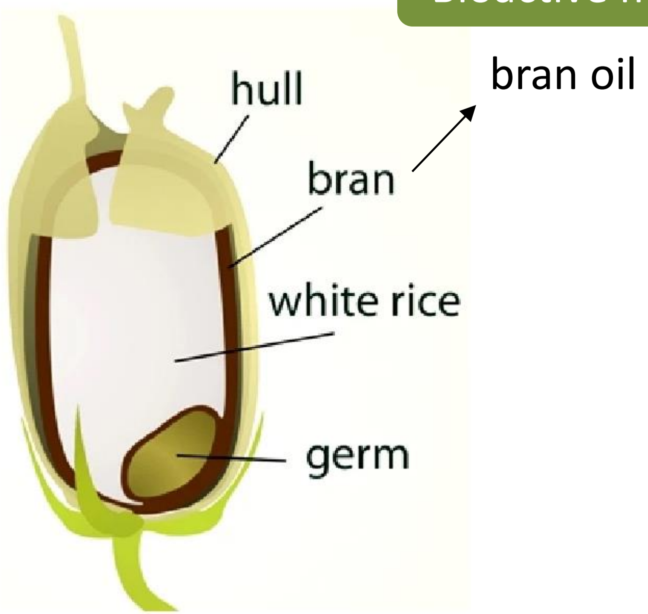
Structure of the presentation

- Introduction
- Cellular antioxidant activity (CAA) assay
- Results and discussions
- Summary and future perspectives

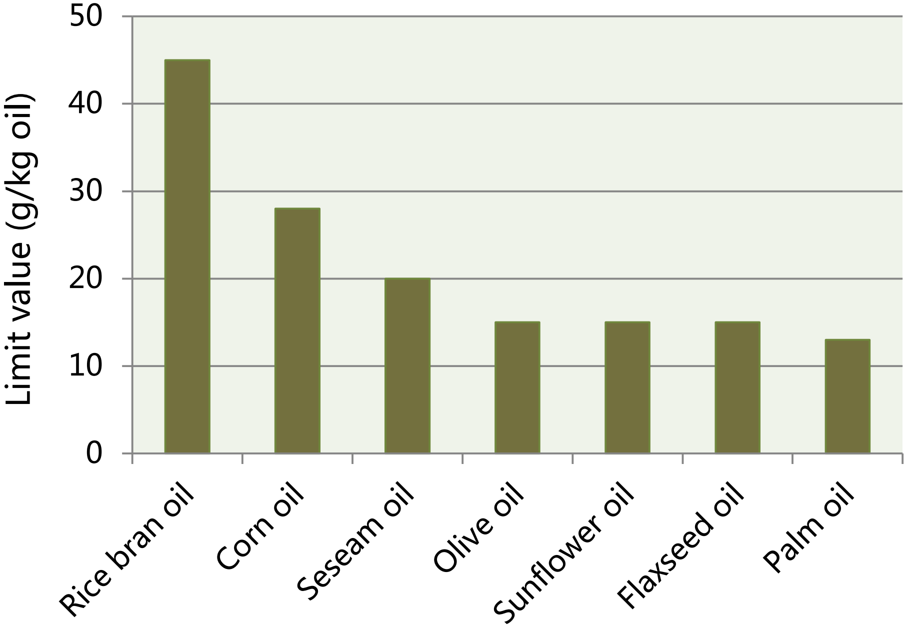
Rice bran oil & unsaponifiable matter

Bioactive molecules



Rice

Limit value of unsaponifiable matter of vegetable oil National Standard of China



Bioactive compound existed in RBO is in the form of unsaponifiable matter

Minor components in rice bran oil

Table 1
Minor components present in vegetable oils.

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Minor components	Oils					
	PO	OLO	SNO	RBO	SESO	LSO
Tocopherols (T) (mg/100 g of oil)						
α	22.9 ± 1.2[c]	13.3 ± 1.0[b]	36.9 ± 2.7[d]	13.2 ± 0.4[b]	1.1 ± 0.2[a]	23.4 ± 2.3[c]
β + γ	nd	1.1 ± 0.2[a]	2.93 ± 0.3[b]	23.3 ± 1.7[d]	65.7 ± 4.2[e]	12.1 ± 0.8[c]
δ	nd	0.8 ± 0.1[a]	0.67 ± 0.1[a]	2.1 ± 0.2[b]	3.8 ± 0.3[c]	0.6 ± 0.1[a]
Tocotrienols (T_3)(mg/100 g of oil)						
α	27.5 ± 2.1[b]	nd	nd	16.5 ± 0.9[a]	nd	nd
β + γ	1.1 ± 0.2[a]	nd	nd	54.2 ± 3.7[b]	nd	nd
δ	12.2 ± 0.6[b]	nd	nd	0.91 ± 0.1[a]	nd	nd
Total (T + T_3) (mg/100 g of oil)	63.7 ± 4.1[d]	15.2 ± 1.3[a]	40.5 ± 3.1[c]	110.2 ± 2.3[f]	70.6 ± 4.7[e]	36.1 ± 3.2[b]
Oryzanol (mg/100 g of oil)						
Methyl ferulate	nd	nd	nd	228 ± 10.6	nd	nd
Cycloartenyl ferulate	nd	nd	nd	105 ± 9.8	nd	nd
24-Methylene cycloartenyl ferulate	nd	nd	nd	492 ± 18.6	nd	nd
Campesteryl ferulate	nd	nd	nd	375 ± 16.3	nd	nd
β-Sitosteryl ferulate	nd	nd	nd	186 ± 11.8	nd	nd
Polyphenols (mg/100 g of oil)	nd	38.2 ± 3.6[b]	nd	nd	nd	32 ± 0.8[a]
β-Carotene (mg/100 g of oil)	41.8 ± 4.9[d]	2.4 ± 0.3[c]	0.3 ± 0.1[a]	0.7 ± 0.1[b]	0.6 ± 0.1[b]	0.8 ± 0.2[b]

Minor components in rice bran oil

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Table 2
Total antioxidant activity and lipid peroxides in serum of rats fed native (N) or minor constituents removed (MCR) oils.

Oils fed to rats	Total antioxidant activity	
	N (μg of Trolox equiv./dL)	MCR (μg of Trolox equiv./dL)
PO	22.8 ± 0.9^{b}	3.5 ± 0.4^{a}
OLO	26.3 ± 1.5^{b}	4.5 ± 0.3^{a}
SNO	9.2 ± 0.4^{b}	2.8 ± 0.2^{a}
RBO	35.2 ± 1.4^{b}	4.7 ± 0.2^{a}
SESO	29.7 ± 1.7^{b}	3.6 ± 0.5^{a}
LSO	20.6 ± 1.1^{b}	3.9 ± 0.2^{a}

	GNO		*RBO*	
Liver	*N*	*MCR*	*N*	*MCR*
Total antioxidant activity (μg of trolox equivalent/mg lipid)	7.3 ± 0.6^{c}	1.3 ± 0.2^{a}	21.6 ± 1.3^{e}	2.7 ± 0.3^{b}
Protein carbonyls (nmol/mg protein)	28.2 ± 1.3^{d}	33.6 ± 2.1^{e}	15.6 ± 1.4^{a}	22.7 ± 1.7^{c}
8-OHdG (nmol/mg protein)	4.8 ± 0.3^{d}	5.4 ± 0.4^{d}	1.9 ± 0.2^{a}	3.3 ± 0.4^{c}
Lipid peroxidation (nmol of MDA/mg protein)	12.3 ± 0.6^{d}	14.4 ± 1.0^{e}	6.2 ± 0.3^{a}	8.9 ± 0.4^{c}

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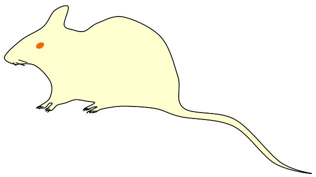
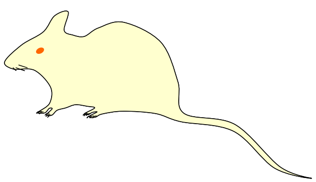


Group A: Minor components removed (MCR)oil



Group B: Native (N) oil

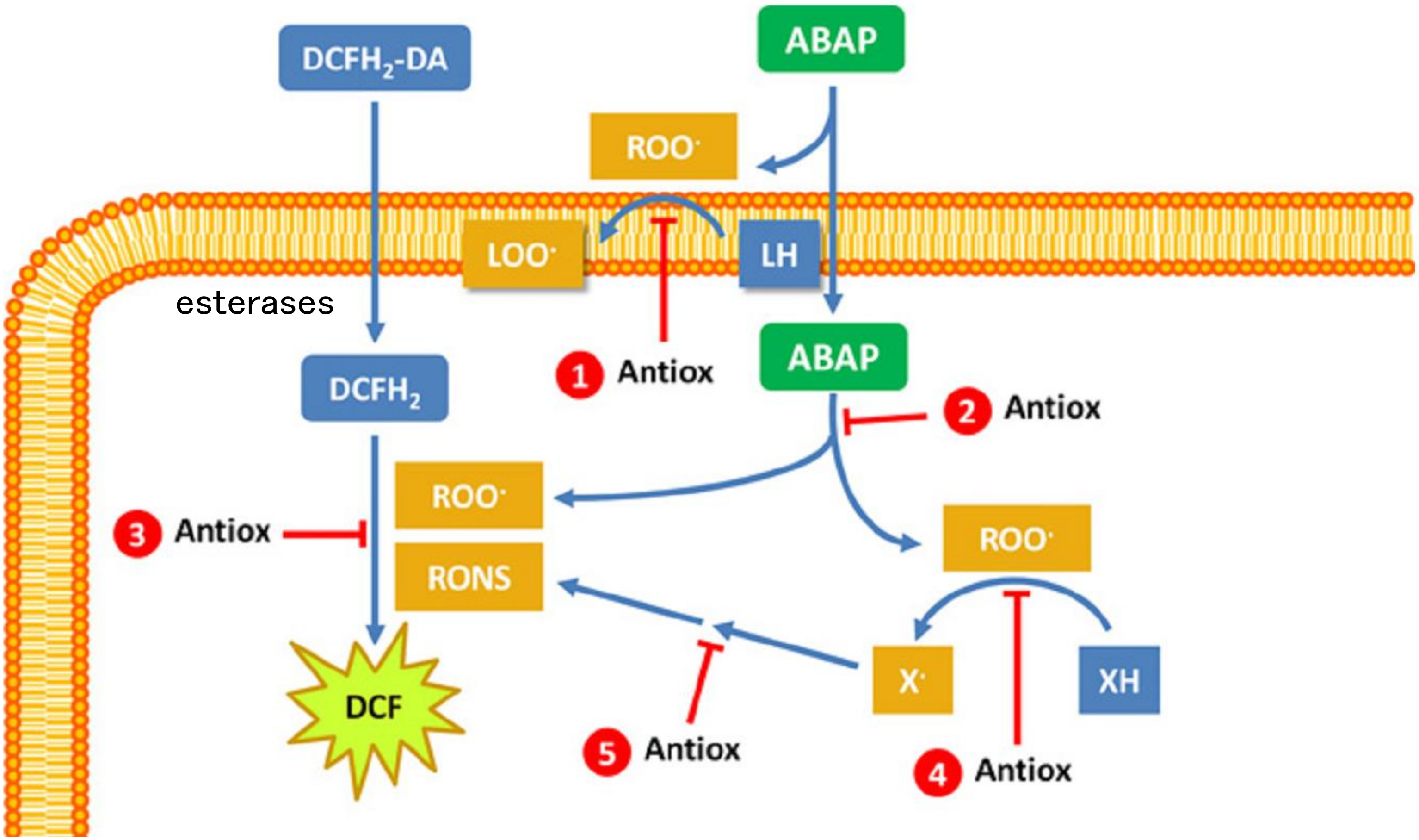
Minor components in rice bran oil

Therefore, while studying the effect of rice bran oil on antioxidant status in experimental systems, we should consider the contributions of minor compounds present in unsaponifiable fractions.

Comparison of chemical and CAA assays

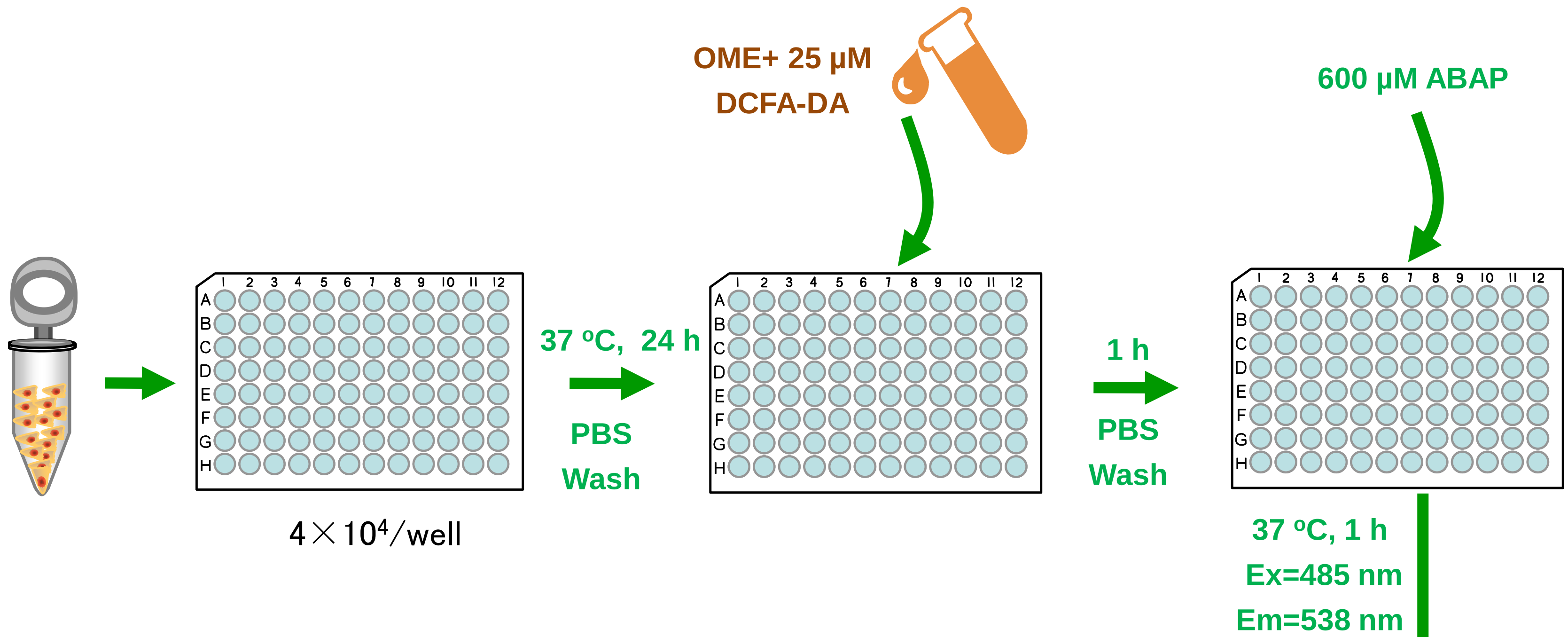
	Chemical assays	Cell-based assays
Assay Principles	Based on known chemical reactions between limited number of reagents	Based on interaction between added compounds and complex enzymatic reactions in biological system
Extraction of Active Ingredients	Some flexibility	Must take place in physiological saline solution; limited use of solvents
Use of Alcohol-based Solvents	Yes, if no interference with chemical reactions in assay	Yes, if properly diluted, tested for tolerance, and altered cellular behavior (depends on assay)
Dimethyl Sulfoxide (DMSO) as Solvent	Yes, with appropriate controls, if no interference with chemical reactions in assay; DMSO is a free radical scavenger	No, alters bioavailability, thereby defeating the purpose of testing bioavailability in vitro; DMSO is anti-inflammatory and can exaggerate mitochondrial ROS formation.
Data Analysis and Interpretation	Quantitative	Qualitative
Expectation of Linear Dose-responses	Yes	No
Applicability of Area-under-curve	Yes	No

Method and principle of CAA assay



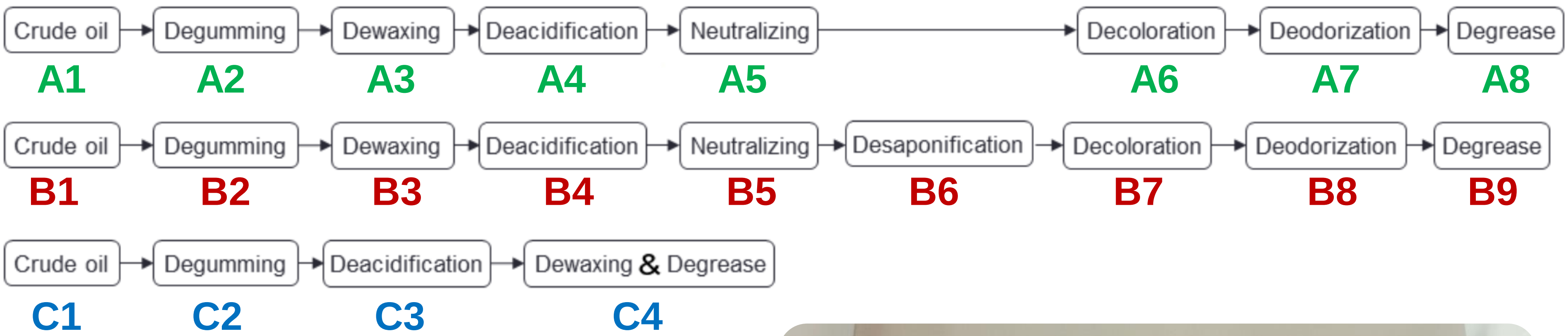
Antiox = Methanol extract of oil

Process flowchart of CAA assay



$$\mathrm{CAA}(\mathrm{unit}) = 100 - \frac{\int \mathrm{SA} - \int \mathrm{BA}}{\int \mathrm{CA} - \int \mathrm{BA}} \times 100$$

Detail information of rice bran oils



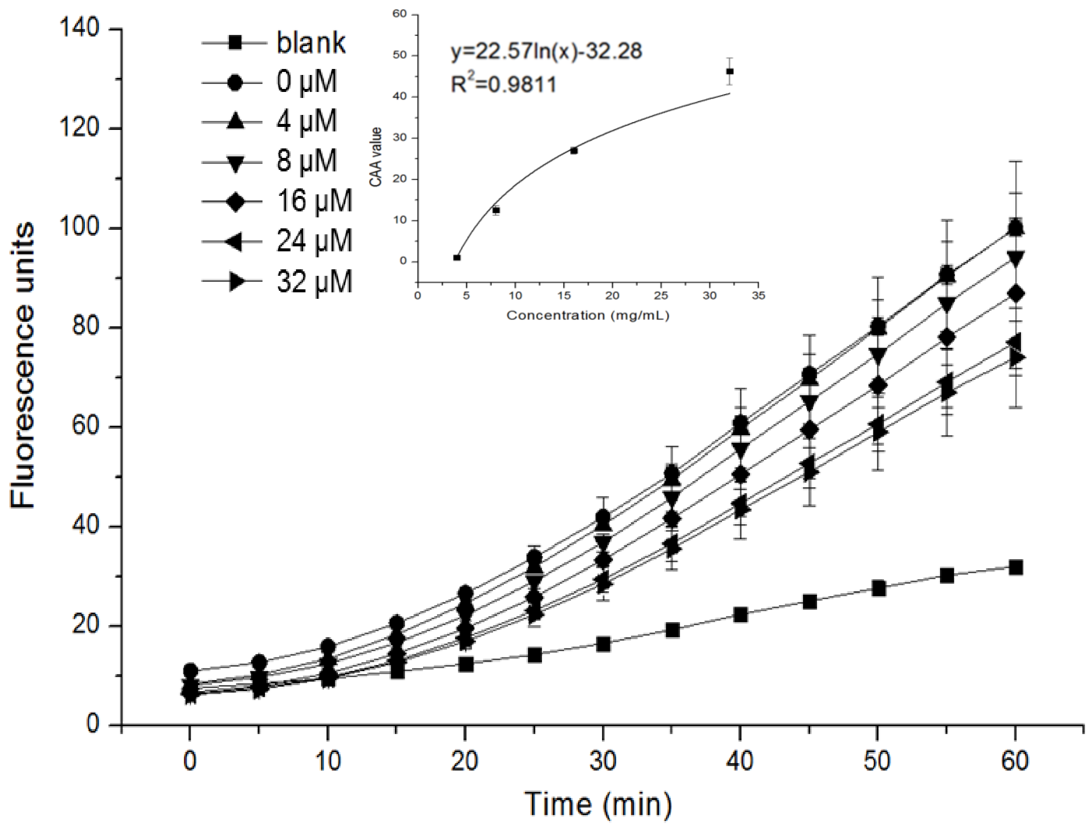
Group A: Qinhuangdao Factory (8 samples)

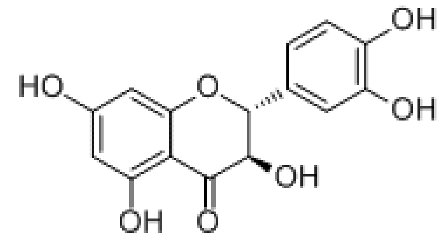
Group B: Taizhou Factory (9 samples)

Group C: Ha'erbin Factory (4 samples)

Samples of Group A from Qinhuangdao Factory

CAA assay model specification



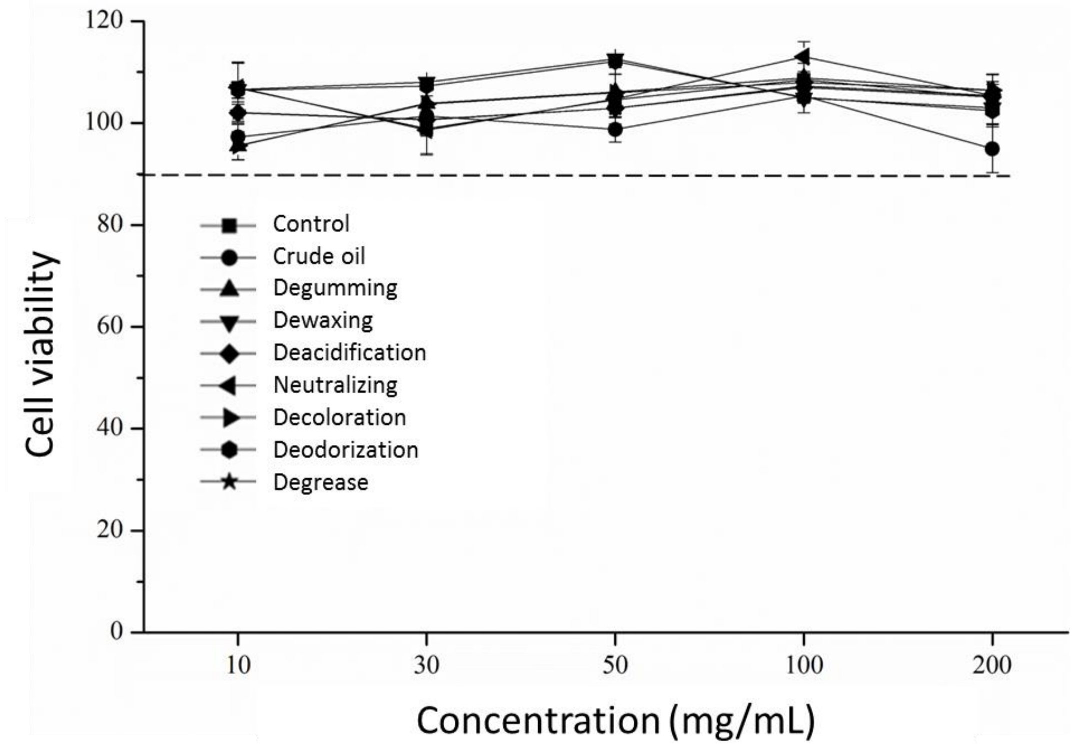


Quercetin

Fluorescence was inhibited by quercetin in a dose-dependent manner.

Cell viability of rice bran oil methanol extracts

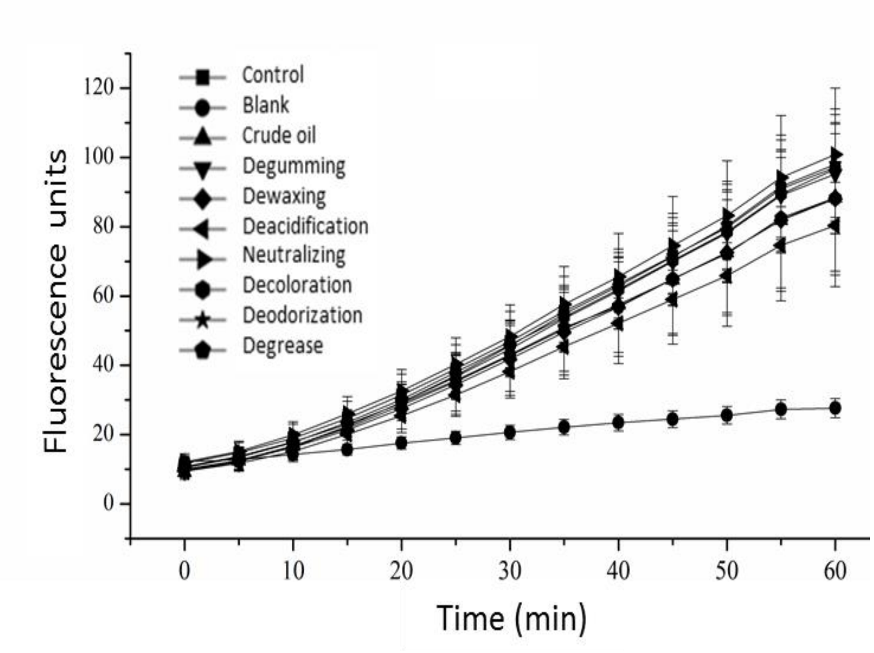
Qinhuangdao factory

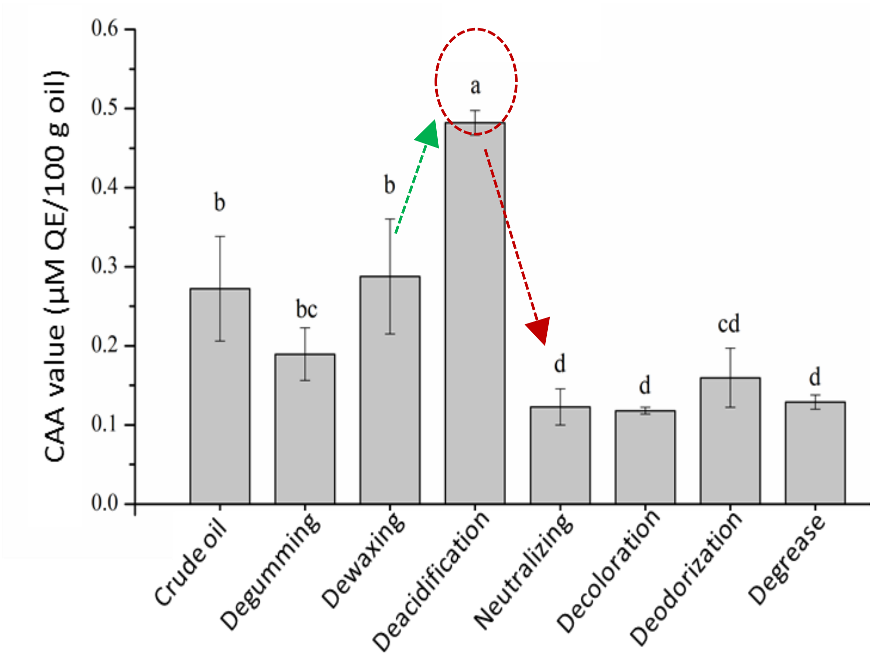


Cell viability was higher than 90%, cytotoxicity was not observed.

Effect of refining process on CAA value

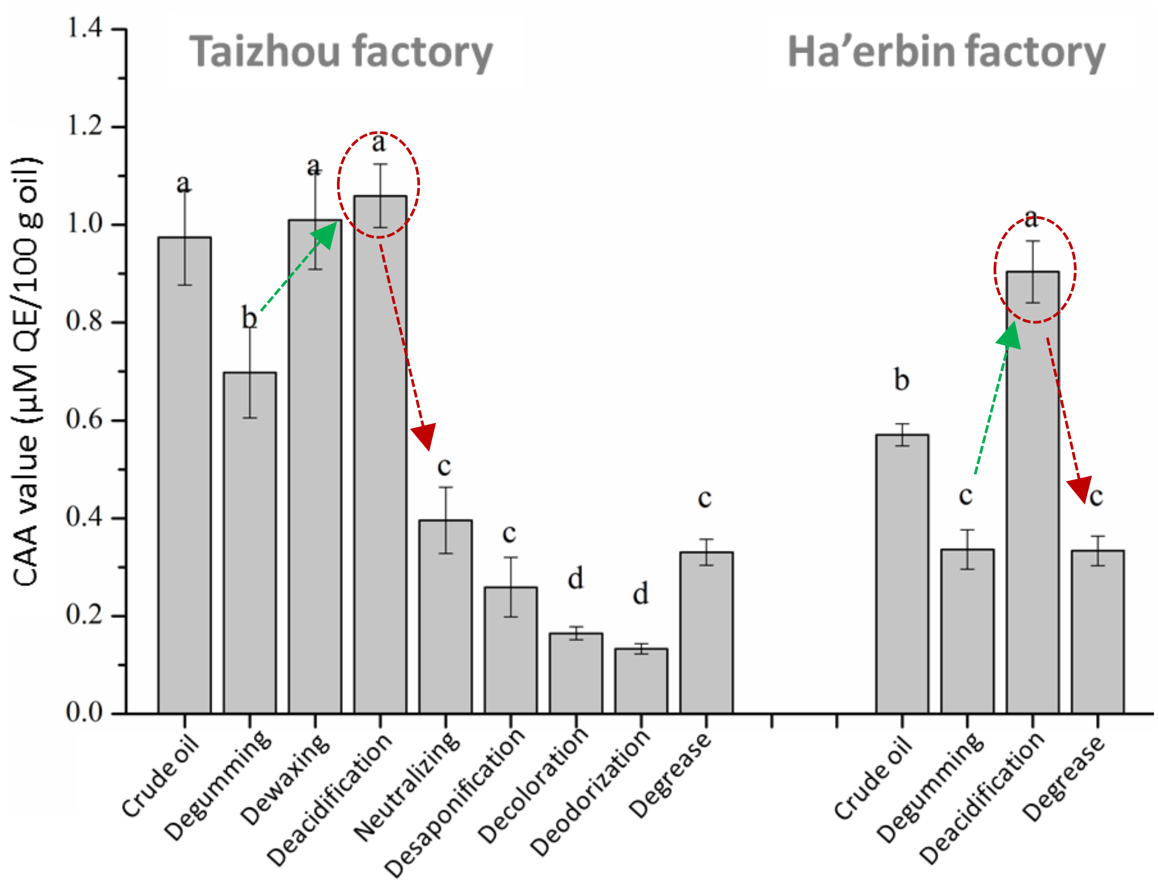
Qinhuangdao factory





Deacidification was a critical process that influenced the CAA value.

Effect of refining process on CAA value

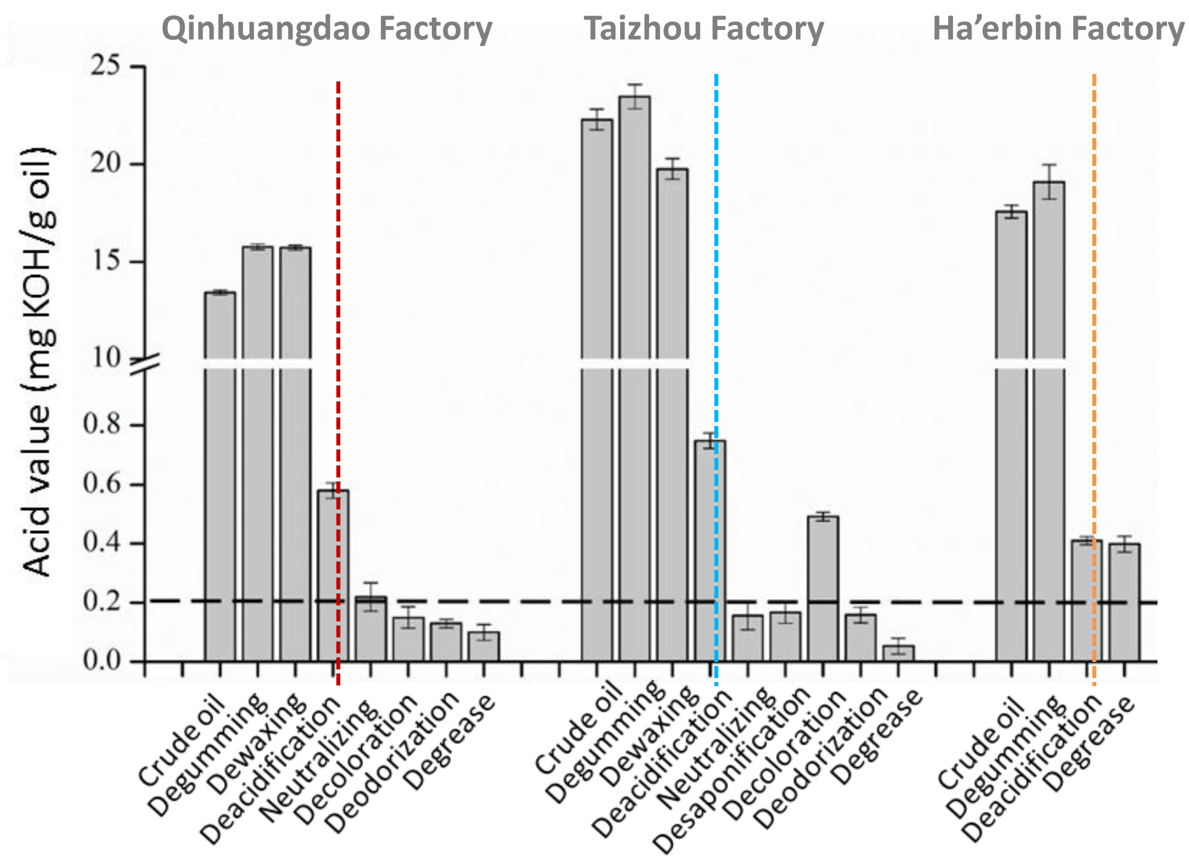


Why the CAA value changed significantly at the point of deacidification?

Results

Effect of refining process on acid value

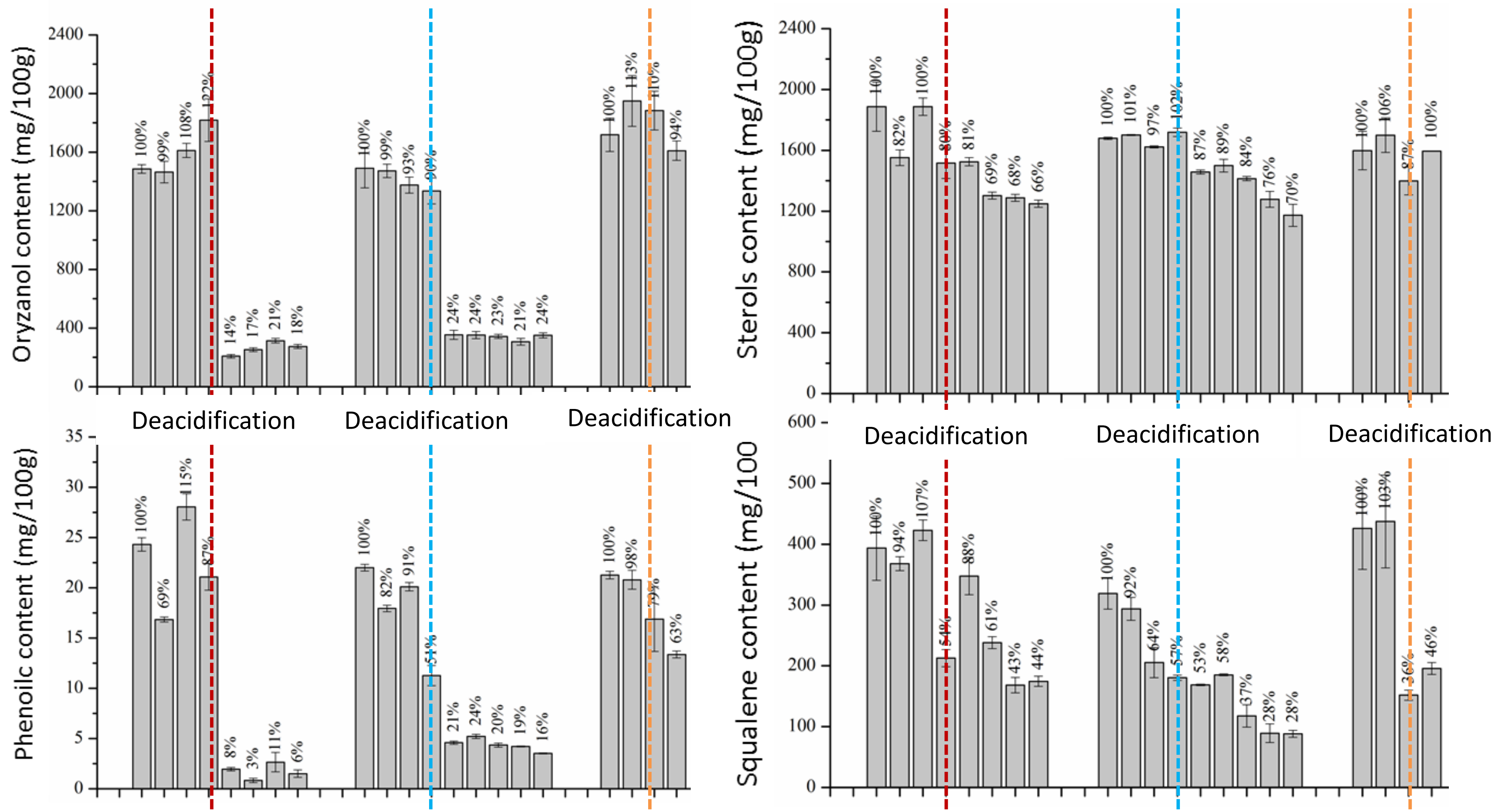
Deacidification



The deacidification process efficiently removed the free fatty acids in the oil.

Results

Effect of refining process on minor components



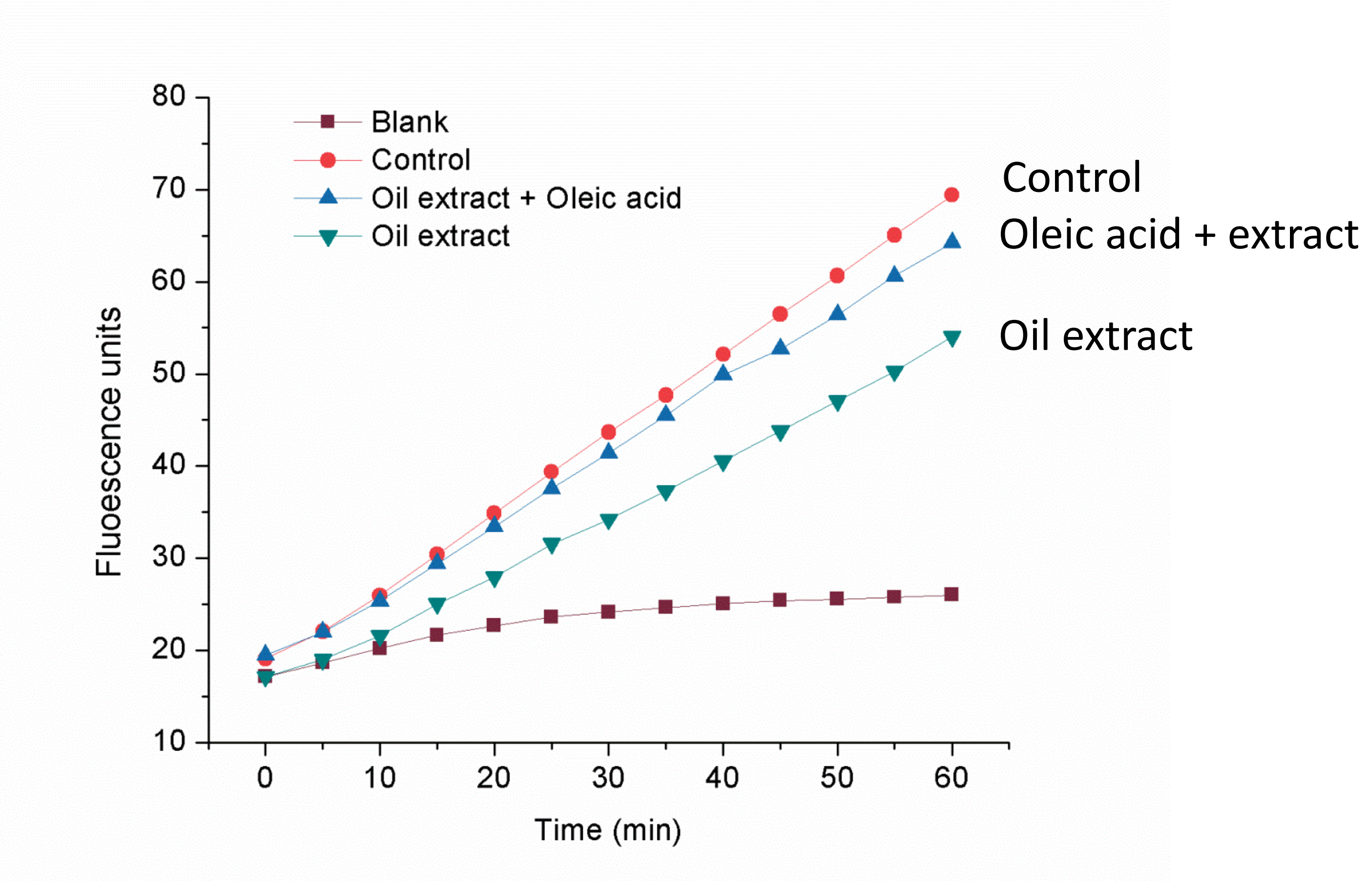
The dewaxing process efficiently removed the minor components in the oil.

Effect of refining process on CAA

- **Key points during the refining process:**
  - Acid value (free fatty acids)
  - Minor components.
  - Deacidification

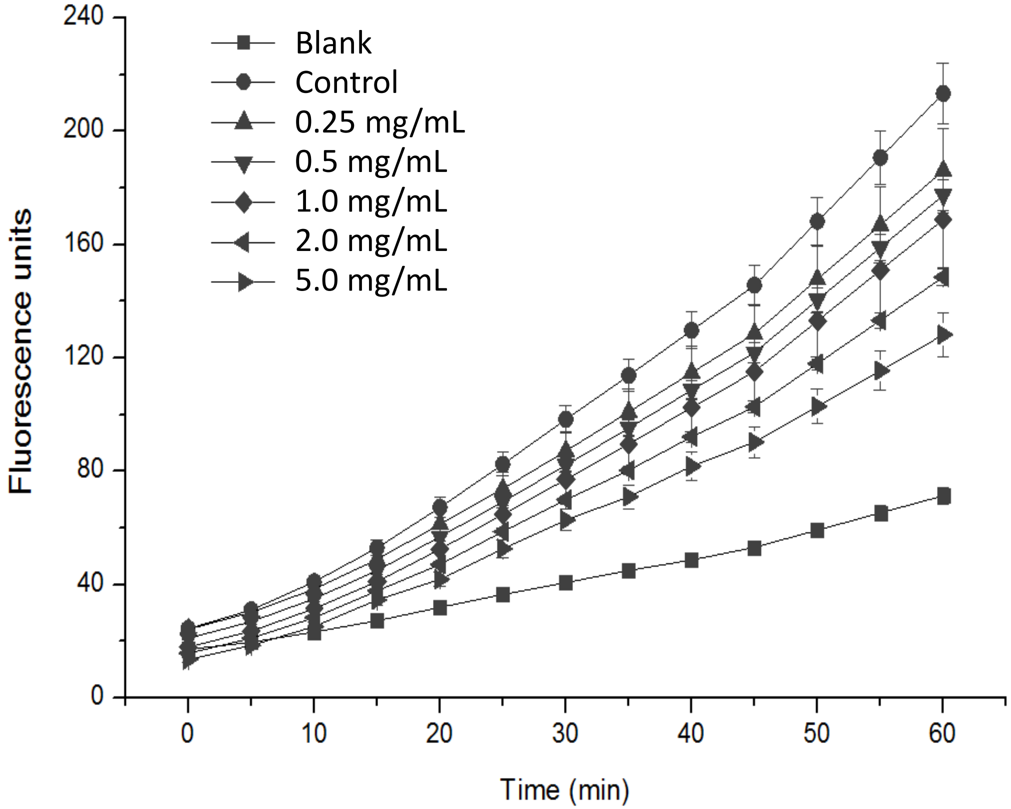
Whether the changes of CAA value are really related to free fatty acids and minor components?

Effect of free fatty acid on CAA value



Answer 1: Oil extracts with free fatty acids exhibited lower CAA value.

Effect of methanol extract on CAA value



Answer 2: Higher concentration of oil extracts exhibited higher CAA value.

Jiangnan University

Results

Effect of refining process on antioxidase

	T-AOC	SOD	CAT
Control	0.66 ± 0.02	5.87 ± 0.24	12.66 ±1.50
H_2O_2	0.40 ± 0.01	1.79 ± 0.06	4.39 ± 0.20
Quercetin + H_2O_2	**0.26±0.03**	**3.15±0.03**	**9.33±0.59**
Crude oil + H_2O_2	0.23±0.00	4.15±0.10	7.48±0.37
Deacidification oil + H_2O_2	**0.71±0.03**	**3.24±0.07**	**10.47±0.20**
Refined oil product + H_2O_2	0.20±0.02	5.31±0.25	5.65±0.57

- **Crude oil**
  - Higher free fatty acids
  - Higher minor components
- **Deacidification oil**
  - Higher minor components
  - Less free fatty acid
- **Refined oil**
  - Less free fatty acid
  - Less minor components

- The rice bran oil with abundant minor components and less free fatty acids exerted higher activity of antioxidase.

Pearson correlation coefficient analysis

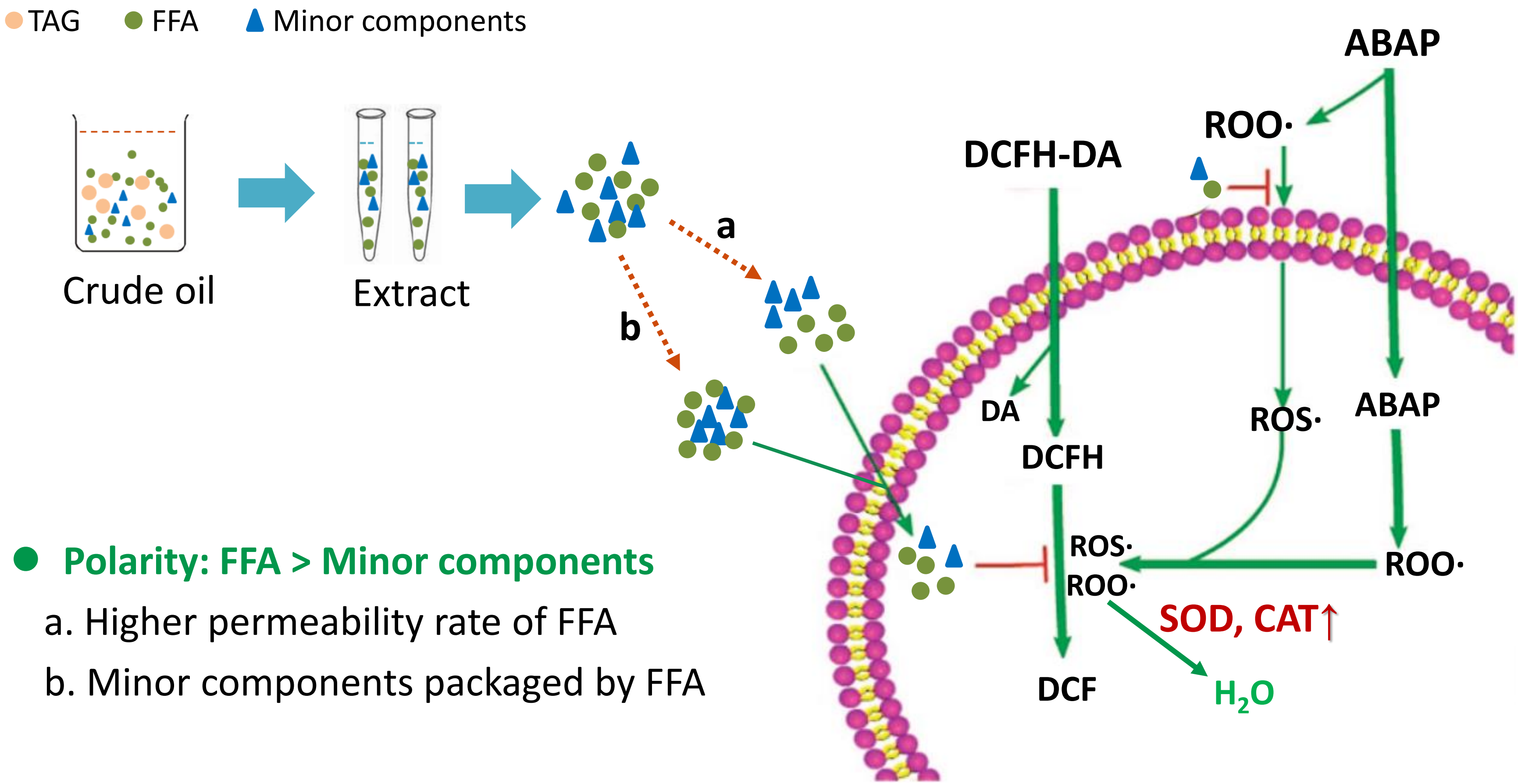
Tab. Pearson correlation coefficient among minor components and CAA value

	Tocopherol	Squalene	Campesterol	Stigmasterol	Oryzanol	Polyphenol
CAA value	0.644	0.585	**0.909******	**0.838******	**0.725***	0.661

(*p<0.05, **p<0.01)

- CAA value was significantly correlated with sterol and oryzanol.

Proposed mechanisms



- **Polarity: FFA > Minor components**
  a. Higher permeability rate of FFA
  b. Minor components packaged by FFA

Summary & Perspectives

Edible oil needs precise and appropriate processing

- **Attention should be given to minor constituents**
  - RBO contains sufficient amounts of endogenous minor constituents .
  - Minor constituents have a vital role in providing anti-oxidant properties.
- **Refining methods for oils should be optimized**
  - Edible oil needs precise and appropriate processing.
  - Refining methods should be optimized to retain oryzanol and sterol.

Acknowledgement

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