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Study on the Preparation and Functional Properties of Rapeseed Protein

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Part1: Introduction



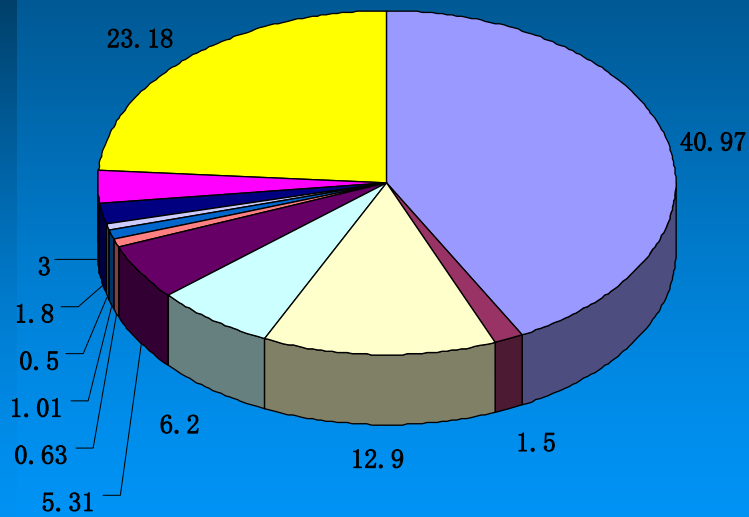


- ❖ Rapeseed is one of the most important source of edible plant oil and animal feed protein in China, also an important bio-energy crop.
- ❖ China have got remarkable achievement in rapeseed production, and the planting acreage and total output of rape are ranked the first in the world. In Hubei, Sichuan, Hunan, and other Yangtze River region, rape is the second largest exceeded only by rice crop , which plays an important role in chinese agriculture and rural economy .
- ❖ At present the production of rapeseed meal has more than 700 million tons. With the further development of rapeseed industry, especially the bio-diesel industry , we can get more rapeseed meal.





Composition of Rapeseed



- | | | |
|----------------------|----------------------------|-----------------|
| crude protein (%) | crude fat (%) | crude fiber (%) |
| crude ash (%) | water | calcium (%) |
| total phosphorus (%) | non-phytate phosphorus (%) | sinapine (%) |
| tannin (%) | others | |

- 
- ❖ Rape seed is increasingly becoming a major crop worldwide. Rapeseed meal contains 35%~45% protein after extracting the oil, the amino acid composition of which is well-balanced in regard to FAO requirements and is rich in **sulfur-containing amino acids** and **lysine**, generally the limiting constituent in both legumes and cereals.
 - ❖ Rapeseed protein is a very good complete protein and worth of development ,which has great value of utilization.

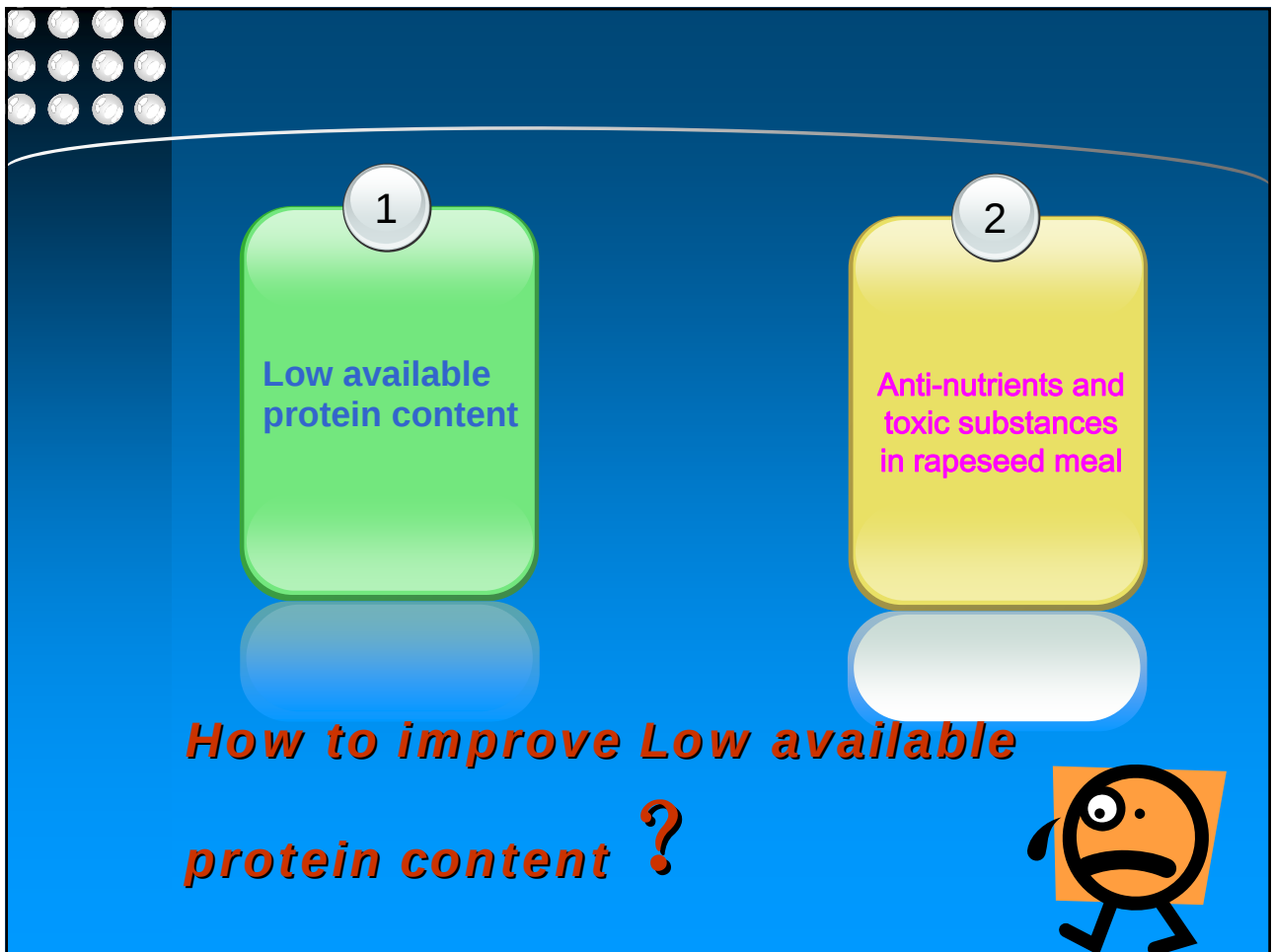


- Rapeseed after oil extraction, a protein-rich meal results and this is usually used for animal feeds or fertilizers.
- The crude protein content of soy protein isolate (SPI) in the industrial production can easily reach 90%. Compared with SPI, rapeseed protein had lower crude protein content and poorer color even using the similar preparation process.



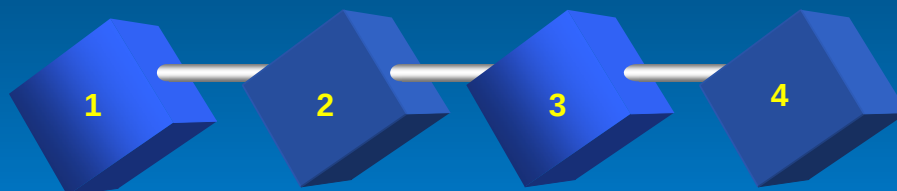
Reasons:

- 1) Compared with SPI, the components of rapeseed protein were more complex, their point and molecular weight had large distribution range. Part of the protein, its isoelectric point reach as high as 11, other protein's isoelectric point distributed at 4~8.
- 2) Cellulose content in rapeseed meal was obviously higher than soybean meal, and there were anti-nutrients and toxic substances in rapeseed meal which none in soybean meal.
- 3) There is big difference in Chemical composition, functional properties, and bioactivities between rapeseed protein and soybean protein.



Part2: Preparation of Rapeseed protein

Traditional extracting methods:



Aqueous phase method

Extract the protein in different aqueous phase and then precipitate it near the isoelectric point of rapeseed protein ,finally obtain the protein by separation and freeze-drying.

Aqueous enzymatic extraction

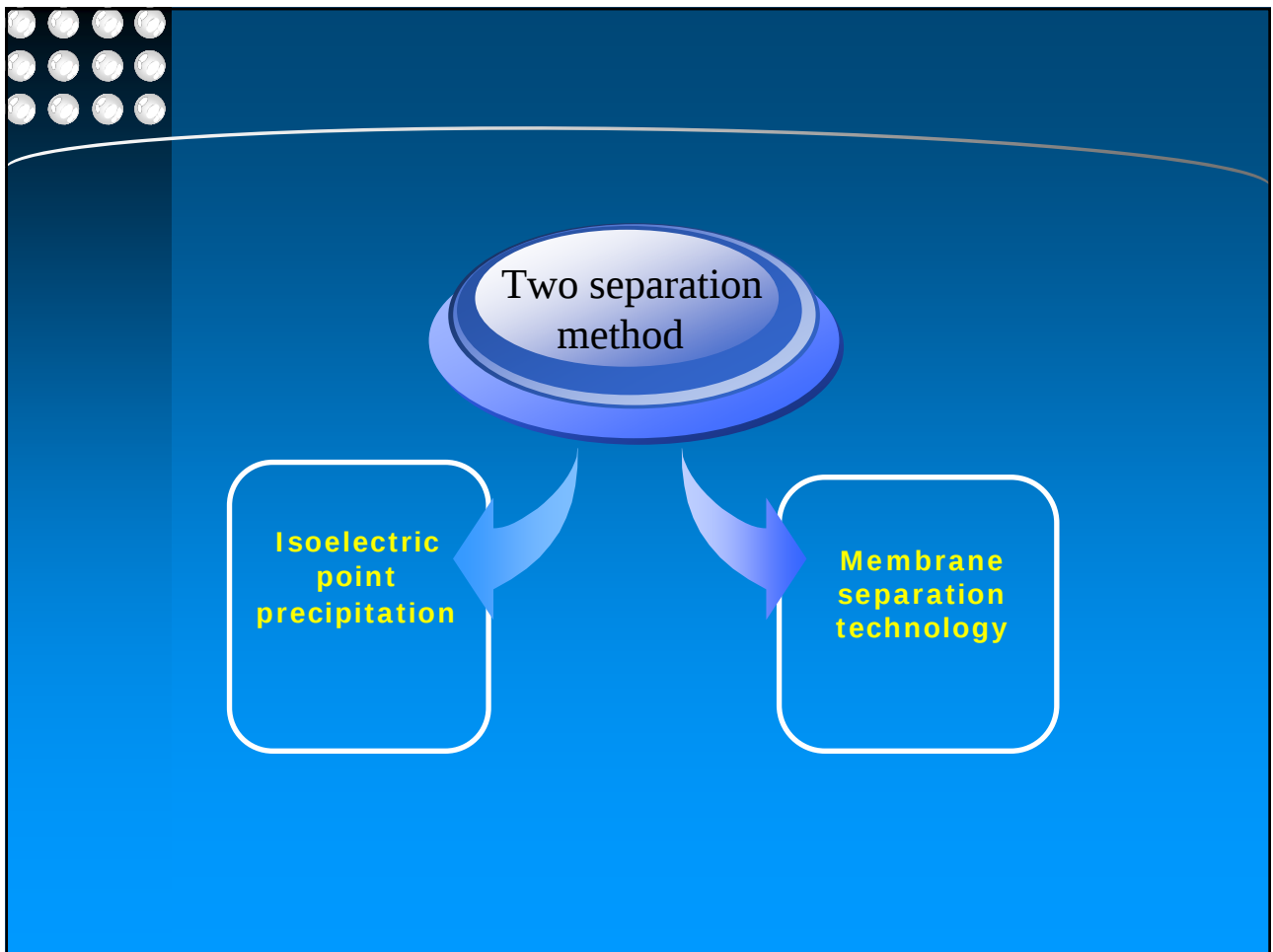
Protein is fully extracted using protase from rapeseed meal to improve the yield

Organic solvent method

rapeseed protein is extracted using acetone, ethanol, and other organic solvents

Two-phase extraction

Rapeseed meal is deal with CaCl_2 aqueous solution and dichloroethane then extracted protein

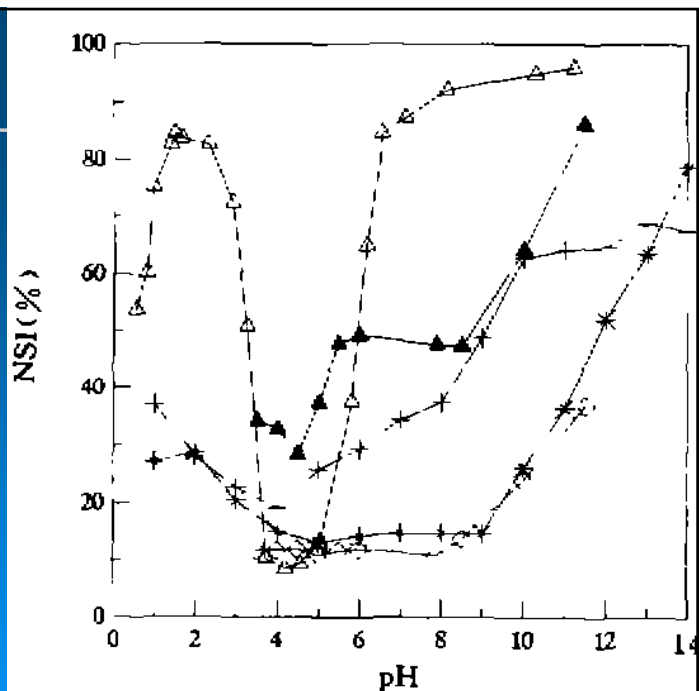




- ◆ Those toxic compounds have significantly lower molecular weights than rapeseed proteins.
- ◆ Precipitation at controlled pH or separation by ultrafiltration could potentially be used to separate the proteins from those toxic compounds.

◆ Compared with soybean protein, rapeseed protein's yield rate is generally low using the similar process.

◆ Compared with soybean protein, rapeseed protein shows a wider isoelectric point range on the solubility curve, two or more than two isoelectric point, so adopting the traditional extracting process can't precipitate the protein completely.





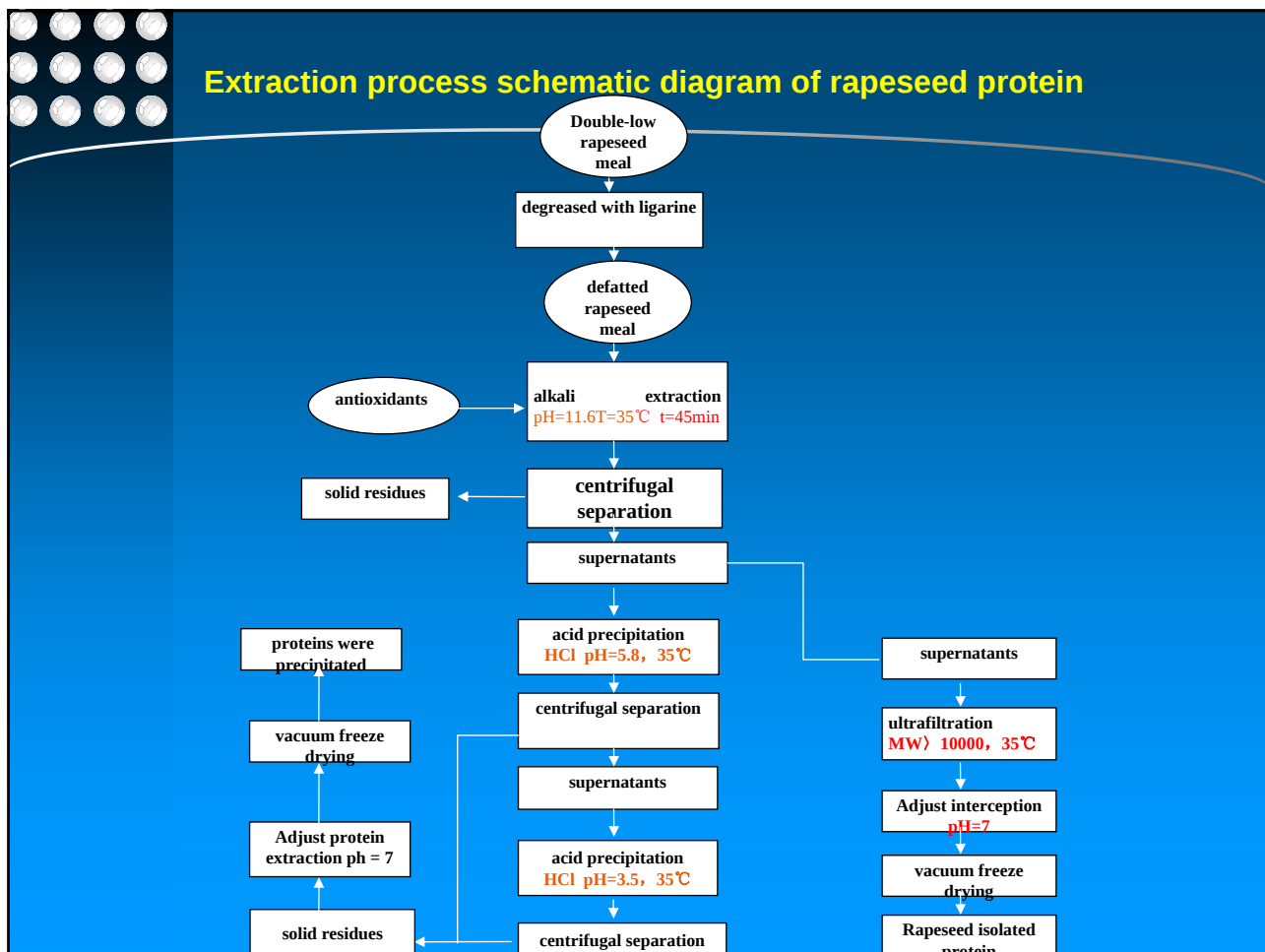
My experiment design and methods

The extraction of rapeseed proteins from double-low rapeseed meal with two methods :

- 1: alkali-dissolution and acid-deposition
 alkali-dissolution :pH=11.6
 acid-deposition : pH=5.8 and pH =3.6

- 2: membrane ultrafiltration:
 membrane pore size :MW=8K

Extraction process schematic diagram of rapeseed protein



The protein products

We obtained three types of rapeseed proteins by extraction :

Alkali-dissolution and pH=5.8 acid-deposition : precipitated protein (RP5.8)

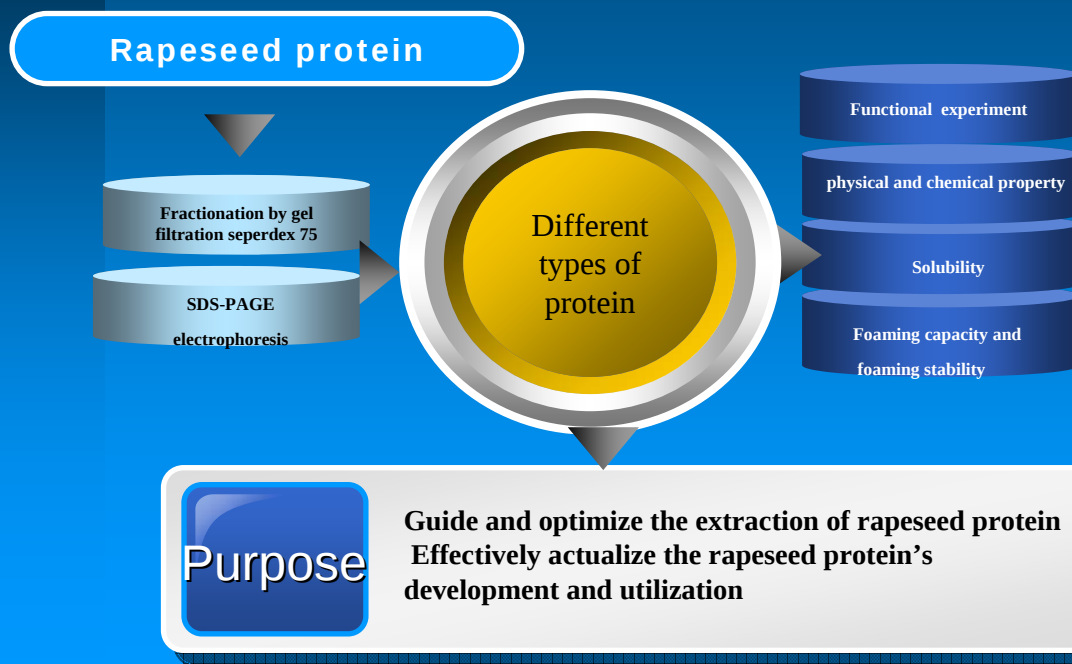
Alkali-dissolution and pH=3.6 acid-deposition : precipitated protein (RP3.6)

ultrafiltration protein : RPs

soybean protein: SP



Part3: Functional Properties of Rapeseed Protein





Protein Content of rapeseed and soybean protein

Protein sample	RPs	RP _{5.8}	RP _{3.6}	SP
Protein contents(%)	86.31	79.61	70.44	89.56

The crude protein content of rapeseed ultrafiltration protein RPs was higher than precipitated proteins (RP_{5.8}、 RP_{3.6}) , by 6.71% and 15.87%.We used the assisted ultrasonic wave during extracted the rapeseed protein. , so the precipitated proteins contents was higher than that in the reported literature by 8.31%. (Guo 2001)

SDS-PAGE Electrophoresis

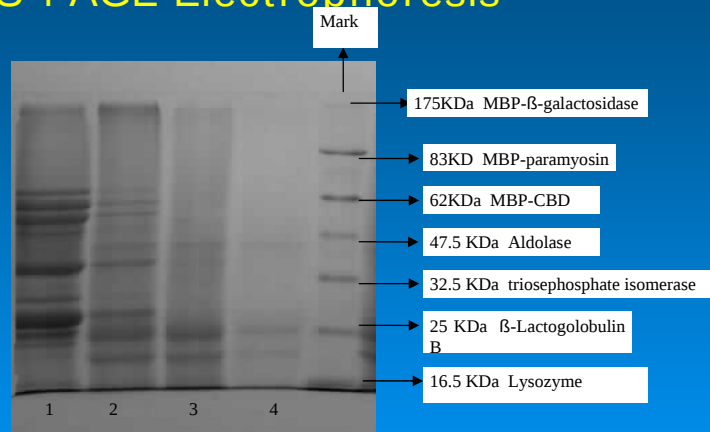


Fig2. Rapeseed protein electrophoresis (1) SP (2) RPs (3) RP_{5.8} (4) RP_{3.6}

The molecular weight distribution scope of ultrafiltration protein is wide , distributed mainly in 16~70KDa , the protein concentration in scope of 16~25 KDa and 65~100 KDa is higher than precipitated proteins RP_{5.8} and RP_{3.6}. RP_{5.8} mainly distributed at 18~25 KDa and 32~47.5 KDa , RP_{3.6} was at 16~25KDa. The soybean protein concentration was higher than rapeseed protein, mainly distributed at 16.5~83KDa.

Solubility

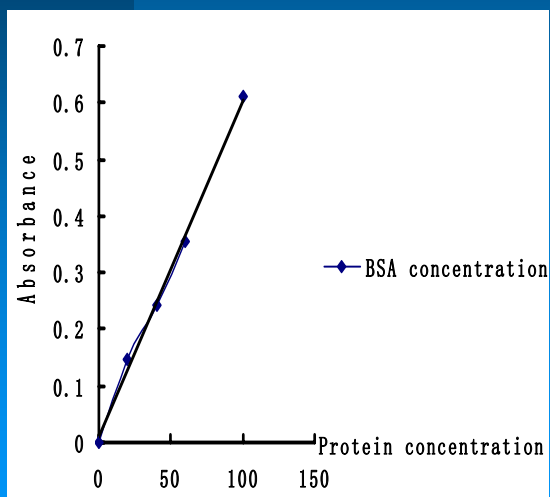


Fig.3 BSA Protein concentration standard curve

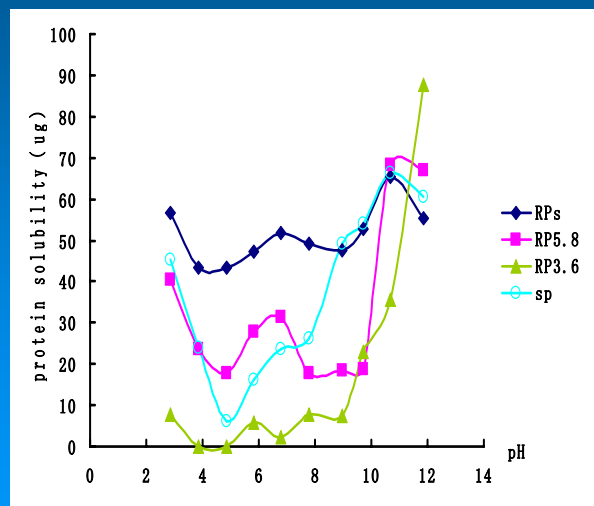


Fig4. Dissolution curve of rapeseed protein

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- The content of ultrafiltration rapeseed protein (RPs) was 86.31% and this protein dissolved completely when pH=10.8.
 - The solubility of precipitated proteins RP3.6 was worse when pH<10, almost less than 0.2mg/ml.
 - The solubility of ultrafiltration rapeseed protein RPs was better than soybean protein. The solubility of soybean protein was lowest at pH=4.5~5 while rapeseed protein had a wide isoelectric point interval.
 - This maybe the mainly reason for that the crude protein content of rapeseed ultrafiltration protein RPs was higher than precipitated proteins (RP5.8、RP3.6) by alkali-dissolution and acid-deposition extraction.

Foaming capacity and foaming stability

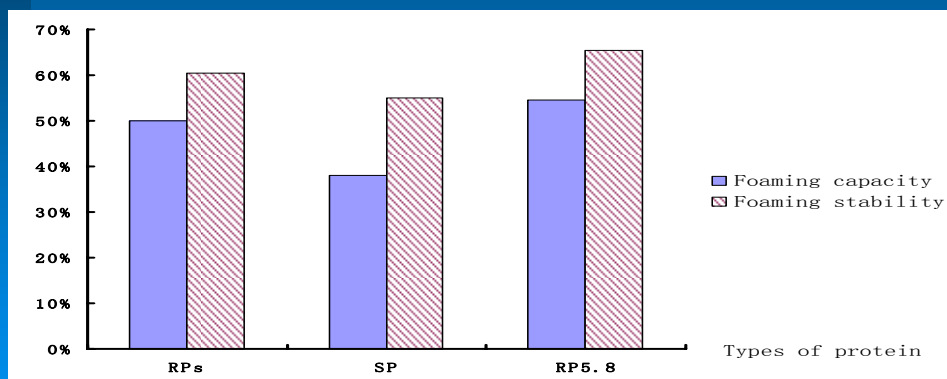


Fig.5. Foaming capacity and foaming stability comparison of rapeseed protein and soybean protein
1 Foaming capacity 2 Foaming stability



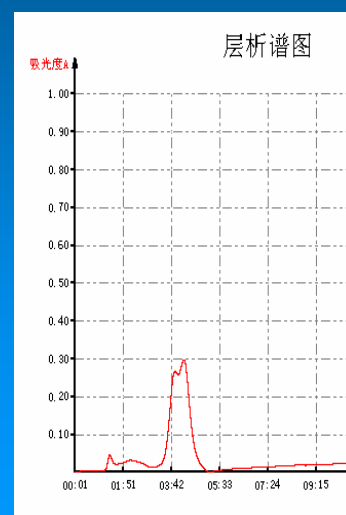
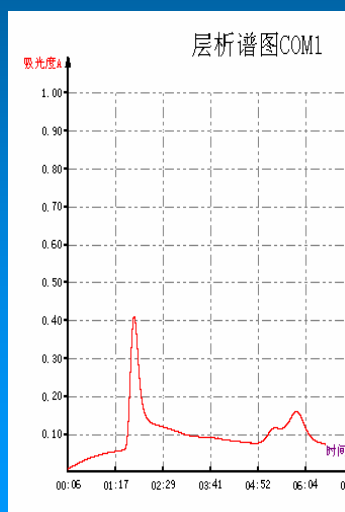
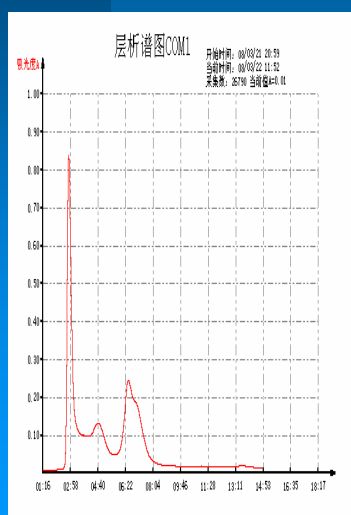
The foaming capacity and foaming stability of precipitated proteins RP5.8 were best, reaching 50% and 60.53%. Those were superior to soybean protein, maybe affected by surface viscosity. The lower surface viscosity of rapeseed protein was suitable for forming the foam and provide theoretical basis for rapeseed as food additive.



Sephadex chromatography of rapeseed protein

Gel chromatography is a separation and purification technology, it can separate the sample into few fractions based on different molecular weight. The composition of rapeseed proteins were complex, for example ultrafiltration rapeseed protein can obtain three fractions by purification and separation on Sephadex G-75 chromatogram.

Fig6. Gel chromatogram of three types of proteins,
(A) the RPs; (B) RP5.8; (C) RP3.6.





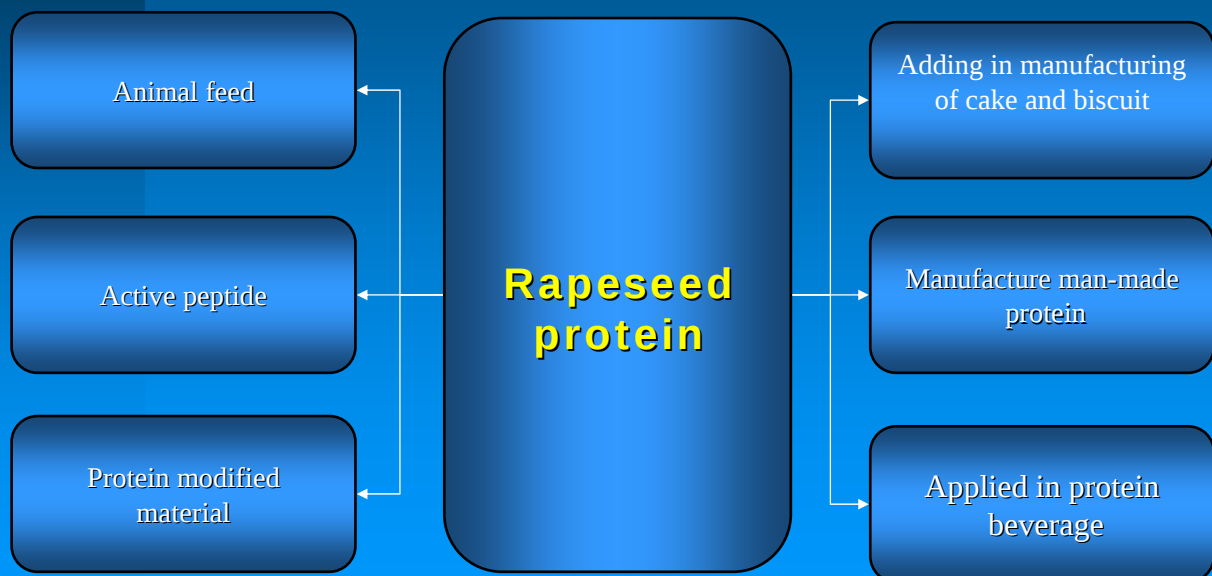
Part 4: Conclusion

we used assisted ultrasonic wave alkali-dissolution and acid-deposition method and ultrafiltration method to extract the rapeseed protein, and obtained three types of rapeseed protein RPs, RP5.8、 RP3.6.

- 1) The ultrafiltration rapeseed protein RPs had higher crude protein contents and better solubility for scavenging low molecular toxic substance.

- 
- 2) The contents of precipitated proteins RP5.8 and RP3.6 extracted by alkali-dissolution and acid-deposition were lower than ultrafiltration rapeseed protein RPs and soybean protein, but its parts of functional properties, such as foaming capacity and foaming stability were superior to soybean protein, so it can be considered as common food additive to use.
 - 3) We will further study the fractions of rapeseed protein in the future work to master more complete properties. Compared with the properties of edible soybean protein, these theories lay a theoretical foundation for effective development and utilization of rapeseed protein.

Patr5: The developing and utilization of rapeseed protein



Research on rapeseed is also a sustainable science!



Acknowledgements

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