Research survey and prospect on comprehensive processing of rapeseed

WU Moucheng, LI Xiaoding, YAN Fengwei, WANG Chengming, CHEN Maobing

College of Food Science and Technology, Huazhong Agricultural University Wuhan,430070, China
Email: wumch98@mail.hzau.edu.cn

Abstract

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million tons in 2005, ranked first in the world. Besides oil, rapeseed contains many other components, such as protein, glucosinolate, polyphenol, phytic acid, polysaccharide, cellulose, sterol, vitamine E and so on. Most of them have great utilized values, which reveal a fine prospect for the comprehensive utilization of rapeseed. Therefore, the research and development of comprehensive processing techniques of rapeseed is all along a hotspot around the world. In this paper, we introduced the research on rapeseed comprehensive processing in our laboratory, and display the vision of the developing direction in the field.

Key words:rapeseed,comprehensive processing, Research Survey,prospect

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million tons in 2005, ranked first in the world. Besides oil, rapeseed contains many other components, such as protein, glucosinolate, polyphenol, phytic acid, polysaccharide, cellulose, sterol, vitamine E and so on. Most of them have great utilized values, which reveal a fine prospect for the comprehensive utilization of rapeseed. Therefore, the research and development of comprehensive processing techniques of rapeseed is all along a hotspot around the world. In this paper, we introduced the research on rapeseed comprehensive processing in our laboratory, and display the vision of the developing direction in the field.

1 Basic research of rapeseed

1.1 Rapeseed protein

1.1.1 Classification of rapeseed protein isolate

Albumins and globulins were found to be the predominant proteins in Hua-Za 3 meal, comprising 36.8% and 31.6% of total proteins. While glutelins and prolamins were 29.1% and 2.5%,respectively. The molecular weight of fractionation was shown by SDS-PAGE and the structure of albumins is simple. Amino acid profile of the isolates indicated that the essential amino acid of each protein fraction nearly reach to 50%. Albumins have better function, including water absorption, oil absorption, emulsifying activity and emulsion stability. In addition, the albumin maintains their native capability because of low-temperature pressing, So albumin can be widely applied in the food and beverage industry.

1.1.2 Preparation of rapeseed peptides

Double-enzyme can not only increase the DH,but remove the bitter of RSP. In our experiment, Rapeseed Albumin was hydrolyzed with sequentially alcalase and flavourzyme. Degree (DH) were used as response values in analysis of response surface regression (RSREG), considering the mouth-sense, the optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows, pH :8.0, hydrolyzing temperature: 50.1°C,enzyme concentration: 0.38AU per gram of substrate, concentration of substrate:4.87%. Flavourzyme is used by step for 2h after reacted 1h with alcalase, the degree of hydrolysis of rapeseed albumin can go up to 28%.

Hydrolysates were clarified by filtration to remove insoluble substrate fragments. and the filtrate was lyophilized and freeze-dried for further use. The hydrolysates (RSP-R) was graded by Sephadex G-25 column with distilled water as eluant,fractions were pooled into three major groups(RSP-1, RSP-2 and RSP-3).Protein content in RSP-1,RSP-2 and RSP-3 were 71.76%, 79.23% and 86.14%;crude polysaccharides were 22.64%, 13.88% and 5.18%. Molecular weight of each grade were bigger than 5000,1052 and 563 Dal. Contents of amino acids determined by HPLC in three grades differed from each other.

1.1.3 Antioxidant activities of rapeseed peptides

After injecting 50, 100 mg/kg·d 15d for 15 d, MDA levels in serum in the RSP group were obviously lower than that of the control ($P < 0.05$) ;So RSP could improve the antioxidant capacity in vivo. The results in vitro showed that RSP had higher reductive activity and can scavenge hydroxyl radicals.

RSP had powerful inhibiting activity on active oxygen within certain concentration. As for malondialdehyde(MDA) formation and H_2O_2-induced of mice liver,RSP-R and RSP-1 had better inhibiting effects than RSP-2 and RSP-3,exhibiting dosage-depended. but little inhibiting effect on MDA induced by Fe^{2+}.There were not much influence of RSP on hemolysis of mice red blood cell. In a word, antioxidant activities of RSP-R and RSP-1 were better than others.

1.1.4 RSP can inhibit the growth of S_{180} tumor,the mechanism may have something to do with its antioxidant activities and increasing organize immune capability,RSP may induce the apoptosis of Hela cells as well.

RSP-R 100,150 mg/kg·d could inhibit the growth of S_{180} and increase thymus weight of S_{180}-bearing mice. Compared with cyclophosphamid, it did not decrease the immune viscera weight and interference the growth of S_{180}-bearing mice. RSP-R had little influence on macrophage phagocytosis and delayed-type hypersensitivity except high dose.But RSP-R could effectively improve the content of serum hemolysin IgM evidently.In addition, MDA levels in serum in the RSP group were obviously lower than that of the control, SOD activities in serum were significantly increased.The results showed that antioxidant activities and increasing organize immune capability of RSP were the most mechanism of its inhibiting tumor effect.

This dissertation demonstrate that the three grades of RSP can induce the apoptosis of Hela cells.RSP display evidently growth inhibitory effect in a dose-and time-dependant manner against Hela cells by MTT assay.

Fluorescence and electron microscopy assay illuminated that RSP can induce apopt- osis of Hela cells, which is dosage-dependent. So the inducing effect on the apoptosis of Hela cells may be one of anti-tumor effect mechanisms of RSP.

The attainting effect of RSP on Hela DNA can be assayed by single cell gel electrophoresis(SCGE),gel electrophoresis and TUNEL assay. In the distributions figure of cell cycle, S stage cells apparently increased through flow cytometry fluorescence. So RSP-2 can arrest Hela cell growth in S phrase. In addition, the results of RT-PCR showed that RSP-2 can decrease the expression of Bcl-2 gene.

1.1.5 This dissertation demonstrated the inhibiting effect of RSP on angiotension I-converting enzyme(ACE) activity of SHR. (*in Vitro*)

Rapeseed peptide with inhibiting effect on ACE of SHR can be gotten by hydrolysis rapeseed albumin. The peptide have the strongest inhibiting effect after hydrolyzed for 1h, it can reaches 42.68%. Hydrolysis with double-enzyme can improve effectively the inhibiting effect of RSP on ACE activity. The inhibiting activity was 69.13% when hydrolysis for 2hrs with alcalase and flavourzyme.

RSP-R and its three grades (isolated through Sephadex G-25) exhibited ACE inhibitory activity. Among which, RSP-3 showed the highest inhibition. When the RSP-3 concentration was augmented to 1mg/ml,the inhibition of ACE activity could nearly achieve to 96%.While RSP-2,RSP and RSP-1 were 78.43%, 69.13% and 60.92%, respectively, which illuminated RSP can be as a higher inhibition factor of ACE activity, All the results will extend the utilizing fields of RSP.

1.2 The rapeseed polyphenol

1.2.1 Structure of rapeseed polyphenol

The rapeseed polyphenol was purified by macroreticular resin, and separated by Sephadex LH-20, fractions were collected and marked as RSPP-0, RSPP-1, RSPP-2, RSPP-3, RSPP-4, RSPP-5, RSPP-6, RSPP-7. There were peaks near 330nm and 280nm in UV-vis spectra of fractions mentioned above. Peaks near 330nm denoted phenolic acids and peaks in 270—282.6nm denoted flavones, flavonoles or tannin, or hydroxylcinnamic acid. The IR spectra of fractions mentioned above were similar to the spectra of catechin and contained the structure information of phenyl and hydroxyl. RSPP could be separate well by gradient elution.

There were pseudomolecular ions (negative ion mode) which were equal to the cyaniding, and gallate + Na^+, and dicaffeoylquinic, and procyanidin dimmer, 1,2-disinapoly-2-feruloyldiglucoside, procyanidin trimer, 1, 2, 2’ — trisinapoldiglucoside in m/z.

There were 5 substance in HPLC-DAD-ESI-MS/MS spectra(negative ion mode) of RSPP. Substance 2 was correspond to 1,2-disinapoly-2-feruloyldiglucoside in pseudomolecular and fragment ions, substance 5 was correspond to kaempferol 3-sinapoyldiglucose. Substance 3 was the isomeric compound of substance 2 and 4 was 5. The structure of substance 1 could not be identified and its m/z of pseudomolecular and fragment ions were 494.2 and 462.0, it was abundant in RSPP. Substance 2 and 5 were reported from rapeseed for the first time.

1.2.2 Bioactivities of rapeseed polyphenol

1.2.2.1 The anti-oxidative effects and mechanism of RSPPs

The effects of RSPPs (including RSPP and RSPP-1,RSPP-2, RSPP-3,RSPP-7)on anti-oxidative in some modified chemical systems, mice liver mitochondria, rat and mice red blood cell(RBC), mice serum and liver homogenate and mice in vivo were measured. The results showed that RSPPs were good deoxidant and could scavenge reactive oxygen species and inhibit lipoxygenase in some modified chemical systems. The capabilities of high deoxidating and inhibiting the enzymes linked with oxidation were revealed to be the mechanism of inhibiting oxidation of RSPPs. In vitro, RSPPs could inhibit the swelling of mice liver mitochondria and the auto-oxidation hemolysis of rat RBC and the hemolysis of mice RBC induced by H_2O_2 as well. RSPPs could also heighten the anti-oxidation capability of mice serum, and inhibit the formation of malondialdehyde(MDA) in mice liver mitochondria, rat RBC and mice liver homogenate. In vivo, there was a significant difference ($p<0.01$)between the controled group and the group *intra- peritoneal* injected with RSPP in MDA value of mice liver homogenate. All the above experiments indicated that RSPPs had evident anti-oxidation function in vivo.

1.2.2.2 Inhibiting Effects of RSPP on proliferation of Human Hepatocellular Carcinoma Cell Line SMMC-7221

RSPP at concentration of 25-400μg/mL displayed evidently growth inhibitory effects in a dose-and time-dependant manner against SMMC-7721 cells by MTT assay. Inhibiting rate of 400μg/mL RSPP for 3 days was 70.18%. The results of MTT assay could be authenticated by the results of proliferating cell nuclear antigen(PCNA) assay.

The results of morphological observation and alpha-fetoprotein(AFP) assay showed that RSPP could induce SMMC-7721 cells to differentiate into normal cells. According to the results of flow cytometer assay, RSPP could block SMMC-7721 cells in S-phase but could not induce SMMC-7721 cells apoptosis.

It was possible to infer 2 mechanisms for RSPP inhibiting SMMC-7721 cells from the results, one was that RSPP blocked SMMC-7721 cells in S-phase, another was that RSPP induced SMMC-7721 cells to differentiate into normal cells.

1.2.2.3 Effects of polyphenol from rapeseed on S_{180} tumor growth and organize immune capability in vivo

The tumor-bearing mice were gained by implanted S_{180} cells sustained in right front axillary. After *intra- peritoneal* injected samples 10d, the mice were executed. Tumor weight showed that RSPPs could inhibit tumor growth in vivo and the inhibiting rate of 50~200 mg/kg·d RSPP were between 30.23% and 44.19%, 50 mg/kg·d RSPP-1 and RSPP-3 were 39.53% and 32.56% respectively.

The tumor tissue were stained by HE, and pathology observation showed that the phenomena of condensation of the nucleus and phlegmonosis cells infiltration and degranulation of polymorphonuclear neutrophils appeared much more in RSPPs groups than in tumor control group, and the area of dead tumor cells in RSPP groups were bigger than the area in tumor control group. It was proved that RSPPs could inhibit tumor growth and increase organize immune capability.

The thymus index, spleen index, macrophage phagocytic rate and phagocytic index,delayed-type hypersensitivity,splenic antibody formation and serum hemolysin content of tumor-bearing mice could be increased significantly by RSPPs. RSPPs were beneficial to immunity of mice.

RSPPs could inhibit the activity of mice lactate dehydrogenase, heighten the activity of mice catalase, decrease the content of MDA in mice serum. The inhibiting tumor mechanism of RSPS may have something to do with its antioxidant activities and inhibition of lactate dehydrogenase

1.2.2.4 The anti-hyperglycemic activity of rapeseed polysacchrides in vivo

Diabetes mellitus mice were induced by alloxan and RSPP were administrated by *intra- peritoneal* injection for 12d. The serum glucose of mice showed that The serum level of blood glucose in diabetes mellitus mice decrease 17.99% and 24.14% in the 75 and 400mg/kg·d groups while remained no obvious change in the normal group injected 400mg/kg·d RSPP.

Compared with control group, the body increase and liver index and kidney index of the normal groups injected 400mg/kg·d RSPP remained no obvious change. RSPP at dose of 75 and 400mg/kg·d both could increase thymus index and spleen index significantly.

RSPP at dose of 400mg/kg·d could decrease MDA content in liver homogenate of normal mice, but have not effect on anti-ROS unit in serum. RSPP at dose of 75 and 400mg/kg·d both could decrease MDA content in liver homogenate and increase anti-ROS unit in serum of diabetes mellitus mice significantly.

The kidney and liver tissue were stained by HE, and pathology observation showed that PHF and RSPP could not prevent diabetes mellitus doing harm to kidney, but protect liver against diabetes mellitus.

1.3 Bioactivities of byproduct of extraction of rapeseed polysaccharides

1.3.1 The Anti-Oxidative Effect and Mechanism of rapeseed Polysaccharide (RSPS)

The anti-oxidative effect and mechanism of RSPS were studied. The deoxidation was measured by $K_3[Fe(CN)_6]$ and TCA system, the inhibition on lipoxygenase was detected by crude lipoxygenase**,**the content of malondialdehyde(MDA) and reactive oxygen species (ROS) were analysed by the reagent kits, the swelling of mice liver mitochondria were observed by the spectrophotometric method. The results showed that RSPS was a good reducer, the anti- ROS unit of 2.00 $mg \cdot ml^{-1}$ RSPS was 94.03,the inhibiting rate of 2.00 $mg \cdot ml^{-1}$ RSPS on lipoxygenase was 22.8% in some chemical modified systems**;** 2.00 $mg \cdot ml^{-1}$ RSPS made the radical-induced swelling of mice liver mitochondria be lower than the swelling of mice liver mitochondria without inducement; the inhibiting rate of 2.00 $mg \cdot ml^{-1}$ RSPS on radical-induced MDA formation are 30.3% in mice liver mitochondria, 54.7%(incubation) and 32.0%(incubation with Fe^{2+}) and 84.5%(incubation with H_2O_2) in liver homogenate respectively, the anti- ROS unit of mice serum added 10.00 $mg \cdot ml^{-1}$is 1340.13 *in vitro***;** *in vivo*, There was a significant difference(P<0.01) between the controlled group and the *intra- peritoneal* injected RSPP (400$mg \cdot kg^{-1}$ $bw \cdot d^{-1}$, 12d) group in MDA value in mice liver homogenate. All the results above showed that RSPS had anti-oxidation effect *in vitro* and *in vivo*, the capabilities of high deoxidation and inhibiting the enzymes associated with oxidation were revealed to be the possible mechanism of RSPS to inhibit oxidation.

1.3.2 Effects of rapeseed polysaccharides on S_{180} tumor growth and organize immune capability in vivo

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The tumor tissue were stained by HE, and pathology observation showed that the phenomena of condensation of the nucleus and phlegmonosis cells infiltration and degranulation of polymorphonuclear neutrophils appeared much more in RSPS groups than in tumor control group, and the area of dead tumor cells in RSPS groups were bigger than the area in tumor control group. RSPS could inhibit tumor growth and increase organize immune capability.

The thymus index, spleen index, macrophage phagocytic rate and phagocytic index,delayed-type hypersensitivity,splenic antibody formation and serum hemolysin content of tumor-bearing mice could be increased significantly by RSPS and RSPS were beneficial to immunity of mice.

RSPS could inhibit the activity of mice lactate dehydrogenase, heighten the activity of mice catalase, decrease the content

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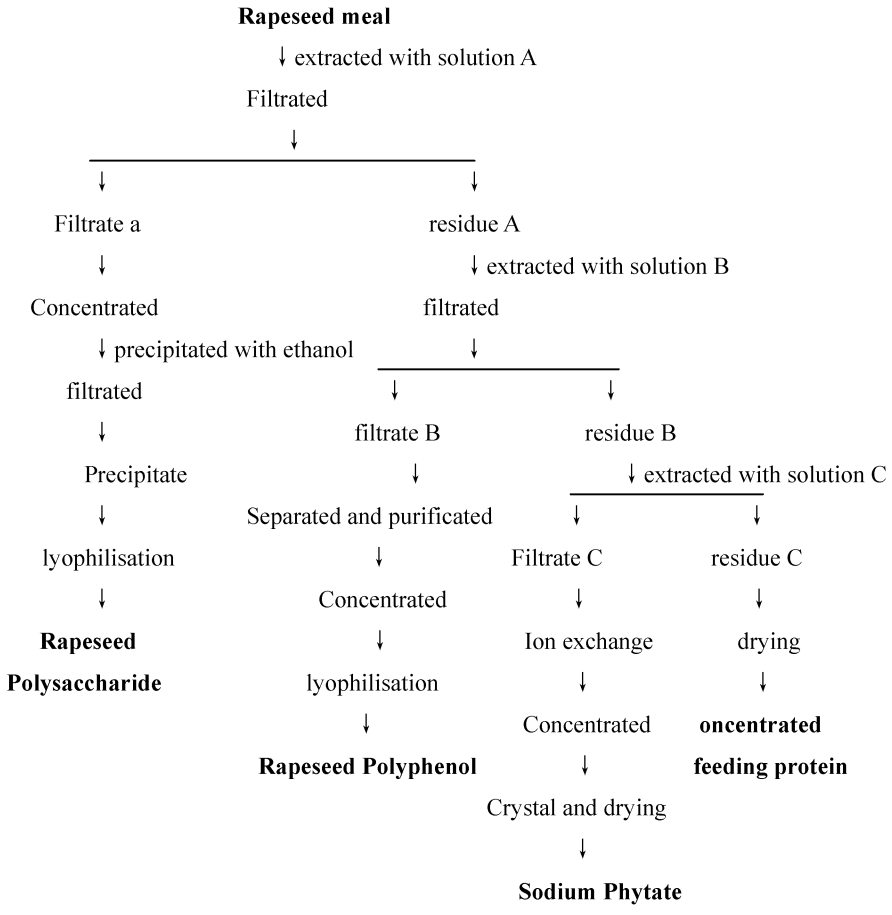
2. Studies on the process route of comprehensive utilization of rapeseed meal

2.1 Process route

We brought forward a process route of extracting polyphenol, polysaccharide and phytic acid from the rapeseed meal step by step as well as innoxious, high biological-performance concentrated protein, which was used to feed animals.



2.2 Preparation of rapeseeds polysaccharides (RSPS)

RSPS was extracted with water and then precipitated with ethanol, while the protein, the polyphenol and the phytic acid was also isolated respectively from the rapeseed cake and meal simultaneously. The optimized technical condition of isolating RSPS was studied through orthogonal test and the result was following: the rapeseeds cake and meal was marinated with 25 times of hot water at 100°C for 4 hours by solvent A. The yields of rapeseeds polysaccharides was 2.8% under this condition.

2.3 Extraction of polyphenol, phytic acid and protein in the rapeseed meal

The way of comprehensive utilization of polyphone, physic acid and protein in the rapeseed meal by extraction with solvents was studied; The extraction technology was optimized by fractional factorial design, central composite design and response surface analysis; mathematical models for predicting the extraction ratio of polyphone and physic acid were set up.

$$y_1=28.21260+0.29714x_1+0.03635x_3+0.06031x_4-0.39723x_1^2-0.43611x_3^2-0.21342x_4^2 -0.40250x_1x_3+0.01750x_1x_4+0.01500x_3x_4 \quad (1)$$

$$y_2=2.36842+0.15874x_1-0.11381x_3+0.12657x_4+0.11426x_1^2+0.38095x_3^2-0.13458x_4^2 +0.13463x_1x_3+0.19388x_1x_4+0.07588x_3x_4 \quad (2)$$

$$y_3=25.33261+0.20798x_1+0.61171x_3+0.16620x_4-0.05275x_1^2-0.80386x_3^2-0.31608x_4^2 +0.04000x_1x_3+0.60500x_1x_4+0.38000x_3x_4 \quad (3)$$

$$y_4=1.06082-0.05012x_1-0.01679x_3-0.00301x_4+0.13483x_1^2+0.01483x_3^2+0.02066x_4^2 +0.01538x_1x_3-0.04063x_1x_4-0.04313x_3x_4 \quad (4)$$

y_1, y_2 are the concentration of polyphenol and phytic acid in the extraction liquid of polyphenol respectively.y_3, y_4 are the concentration of phytic acid and polyphenol in the extraction liquid of phytic acid respectively. The four equations are anastomosed to the experimental data.

The results showed that 6 ml/g rapeseed cake, 65%(v/v) solvent B, 1.3g/100ml assistant substance, 51°C, 20min and extracting 3 time for obtaining polyphenol, 1%(v/v) solvent C, 20~30°C and extracting 2h for obtaining phytic acid were the optimized conditions. The extraction ratio of polyphenol about 2.82%, phytic acid 2.34% and protein content 70.2% respectively. The poisonous glucosinolate and anti-nutritious polyphenol and phytic acid in the protein were 0.76umol/g, 1.33mg/g, and 3.01% respectively, which declined 96.6%, 95.9%, and 38.5% accordingly.

2.3.1 Separation and purification rapeseed polyphenol

Experiments of 8 kinds of macroreticular resin adsorbing and desorbing rapeseed polyphenol showed that special 1 was the most appropriate resin in all for the preliminary purification of polyphenol, and the adsorbing capacities of resin could be utilized fully by arranging column in series. In column of special 1,impurity in rapeseed polyphenol could be removed well, and these condition was best for purifying polyphenol in mass production: 74%(v/v) acidic ethanol (containing 0.5%(v/v) 0.1mol/L HCl) was the desorbing solution, velocity of flow was 1BV/h (BV-column bed volume).

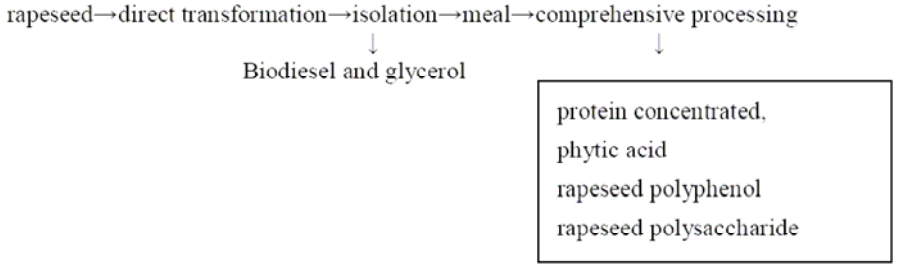
2.3.2 After primary purification as 1.3.1, we used chromatography column with Sephadex LH-20 to for further purification. Subfraction-1of polyphenol could be got with the decolourizer of methanol: H_2O=1:3 while subfraction-2 could be got with the decolourzer of methanol: H_2O=1:1.

3. Studies on the process route of comprehensive utilization of rapeseed

China has the second greatest consumption of petroleum. It reached 245.7 million tons in 2002, exceeding Japan and just behind America. In 2003, China consumed 83 million tons diesel oil and 40.16 million tons gasoline, 32% of the total consumption of petroleum was imported. In 2004, domestic consumption of energy sources increased at an average annual rate of 15% while the amount of domestic petroleum output only 1.67%. Therefore, the lack of energy sources will hinder the development of mankind.

Biodiesel, namely longchain fatty acid methyl ester from vegetable oils or animal fats, is a kind of renewable and environmental protection energy source, which can substitute for diesel. Biodiesel can not be produced cosmically in China mainly because its production cost is higher than the market price. So, we must explore the new thoughts and methods on the production of biodiesel to reduce its production cost.

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million ton in 2005, ranked first in the world. Therefore, we have found a unique technological route to produce biodiesel directly from rapeseed with the further comprehensive processing of rapeseed meal. The route was as follows:



The characteristics of route are following:

A. Abandon the conventional technological route with two steps at present which the oils are squeezed firstly from rapeseed and then transformed to biodiesel. The new route is to directly extract and transform the oils simultaneously, which change two steps into one step. So, the expenditure of pressing oil was decreased and then the cost of biodiesel product can be reduced greatly.

B. The highly active solid catalyst for ester transformation is used during the transformation of biodiesel. So, the disposal cost of KOH or NaOH alkalescent catalysis used in conventional production of biodiesel is reduced and the environmental pollution can be avoided too.

C. The continuum production of biodiesel and online exclusion of glycerol are implemented by means of the technology of constant methyl esterification at normal pressure, the products of biodiesel and glycerol with high quality will be obtained.

D. The quality of protein in rapeseed meal after rapeseed transformed directly to biodiesel was much better than that in the rapeseed meal squeezed conventionally from rapeseed because the former was obtained at the temperature being lower than 70°C. For example, the content of lysine in the former is doubled that of the latter. The feeding Bioavailability(biological

value) is further high and more benefit for the development of rapeseed meal.

E. The rapeseed meal byproduct after rapeseed transformed to biodiesel was used to extract and isolate the impurity in rapeseed meal such as glucosinolate, phytic acid, polyphenol, polysaccharide, cellulose etc by water solvent in divided step, the feeding protein concentrated was obtained rather than by the classical preparation method of protein concentrated from extracted protein. Simultaneously rapeseed polysaccharide, rapeseed polyphenol and phytic acid were recovered and prepared from the waste water. The four products including feeding protein concentrated, phytic acid, rapeseed polyphenol and rapeseed polysaccharide were obtained in the technological route. The output value and profit of these products were several ten times higher than that of rapeseed meal.

The development of rape biodiesel and comprehensive processing of rapeseed meal can lighten the lack of energy sources, and resolve the low profit of oil corporation as well which produces only oil and rapeseed meal such singleness variety of product. The increase of enterprise benefit may raise the purchase price of rapeseed so that the income of peasant and the enthusiasm of peasant planting rape will increase. It is significant to adjust the industry situation of countryside in China, promote the development of countryside economic, and walk out a way making the peasant affluent which transforms from agricultural products to industry products.

At present, the above comprehensive processing of rapeseed meal have successfully applied in industrialization production. The testing of biodiesel transformed directly from rapeseed has finished. The product line of biodiesel with 500 tons annual production had been built already. We are looking forward to researching and developing rapeseed together with our colleagues.

References

1. Wu Moucheng, Yuan Junhua, Shao Jinhua, Zhang Yan. “Studies of Comprehensive Processing and Utilization of Rapeseed”. Journal of Huazhong Agricultural University. 1999. 18(6).589-591
2. Wu Moucheng, Zeng Xiaobo et al. Research Progress on Processing and Comprehensive Utilization of Rapeseed. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P287-291.
3. Wu Moucheng Shao Jinhua, et al. Preparing Rapeseed Protein Isolate from Dehulled Rapeseed Meal which Oil was Extracted at Proper Temperature. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P306-312..
4. Zeng Xiaobo, Wu Moucheng et al. Preparation of Rapeseed Protain Concentrate by Washing with Acetone Solvent. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P299-305..
5. Wu Moucheng, Zeng Xiaobo et al. Study on Removal of Glucosinolates, Tannins and Phytates from Rapeseed Meal. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P292-298..
6. Wu Moucheng,Zeng Xiaobo. Studies on preparation and its functional properties of rapeseed protein concentrate. 11th International Rapeseed Congress, Copenhagen, Denmark. Abstracts, 450
7. Xue Zhaohui, Yin Jingzhang, Wu Moucheng, Yuan Junhua. Preparation and antihypertensive effect of rapeseed peptide (RSP). 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 689-693
8. Wu Moucheng, Zeng Xiaobo, Yuan Juan. Inhibition effect of rapeseed peptides (RSP) on tumor. 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 586-591
9. Xue Zhaohui, Yin Jingzhang, Wu Moucheng, The hydrolysis of rapeseed albumin with alcalase. 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 592-598
10. Xue Zhaohui, Wu Moucheng, Yin Jingzhang, Ruan Zheng, Isolation Characterization of Defatted Hua-Za 3 Meal protein. Food Science, 2004, 25(4):25-28
11. Zhou Quancheng, Wu Moucheng. Concentration of tocopherols by supercritical carbon dioxide with cosolvents. European Food Research and Technology. 2004, 219:398-402(SCI source)