STUDIES ON THE TECHNOLOGY OF EXTRACTING ESSENCE OIL FROM THE PURPLE PERILLA SEEDS BY SUPERCRITICAL FLUIDS

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Abstract: In order to obtain higher content of a -linonenic acid from purple perilla seeds, some extracting parameters affecting the extracting processes of SCE have been studied, such as pressure, temperature, density of CO_2 fluid, time of extracting and S/F (SC-CO_2/Fraction).The results of GC-MS analysis indicate that the content ofa -linonenic acid in extracts is the highest at the conditions of 20-25 MPa, 40? , and 9-10 L/min of CO_2 fluid, which reached 59.7%, the extraction ratio is 91.8%.

Keyword: a -linonenic acid (LNA); purple perilla seeds; essence oil; supercritical CO2(SC-CO_2); extracting parameters; extraction ratio

Introduction

The depth exploitation of ocean resources have a contribution to creating high quantity of living standard for human . It is an example that the health products produced by the extraction of ? -3 polyenic unsaturated fatty acid such as eicosapentaenoic acid(EPA) and docosahexenoic acid(DHA) from deep sea fish oil become popular all over the world. But they have the weakness of high cost, fishy smell, easily be oxidized and containing cholesterol etc. Comparing with this, **a**-linonenic acid(LNA) has a special physiological activity, which can be transformed into EPA and DHA by the action of desaturase and lenthened carbon chain enzyme after being absorbed by human body. Thus, we found that **a**-LNA has the same function to prepare EPA and DHA but it have not weakness of the products from deep sea fish oil.

EPA and DHA belong to fatty acid, which are the components constructing the brain cell , Nerve and the blood vessel of human body as well as various hormone, is named “ brain gold ” and are active factors for the source of human life . Once lacking of it ,some diseases will most probably touch you such as the disorder of organism metabolism, the variation of life mechanism, delay or stop of our physical development, decrease of intelligence or eyesight , bringing decrepitude forward， diseases of heart-brain blood vessel, cancer and senile dementia?

Purple perilla oil extracted from its seeds is an excellent health oil[1], which abounds in linolenic acid, 11,14-octadecadienoic acid and palmic acid etc, the content of unsaturated fatty acid reaches over 90%, among which the content of **a**-LNA reaches 50**~** 63%. Purple perilla oil has the following medical health functions: (1) increasing the functions of nerve system, benefiting mentality, improving both the ability of memory and the function of sight nerve and eyesight; (2) preventing decrepitude and senile dementia; (3)decreasing blood fat, preventing and curing artery porridgy cirrhosis ;(4)avoiding the condensation of blood platelets, diminishing the chance of forming the blood bolts, avoiding cardiac infarction and brain infraction; (5)improving the immunity function，functioning as defending the allergic diseases, controlling the growth of tumor and anti-cancer; (6) keeping the balance of the of fat, improving the situation of physical metabolism, functioning as the decrease of weight and body fat.

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Gansu Province, in western China, is a abundant area for generating a plant called purple perilla[2], especially in Qing Yang District. Despite its natural productivity of dozens of thousands of tons, for years it has been exporting with a humble as a result and not been shifting from its advantage in natural source to its potential advantage in high additional worth. Given that lacking of the ocean resources as an in-land province, they can use the technology of SCE to extract purple perilla oil from its seeds. The purple perilla oil can be regarded as quasi product of EPA and DHA, which equals to “plant brain gold”, besides its advantage of resources, the effect of purple perilla oil is same or better than that of products of deep sea fish oil and its price is lower, hence it has a wide market prospect. We studied the technology extracting essence oil from the purple perilla seeds by supercritical fluids, the emphasis is to look for the optimum technological conditions[3]. In this paper, we found that the content of**a** -LNA acid in extracts is the highest at the conditions of 20~ 25MPa，40？ ,and 9~ 10L/min of CO_2 fluid ,which reached 59.7%, the extraction ratio is 91.8%.

1. Experimental materials and apparatus

1-1. Experimental materials

The purple perilla seeds come from Qingyang of Gansu, which has faint scent, the color is brown, the skin has mesh beards. CO_2 is provided by the factory of Liujiaxia Chemical Fertilizer and the purity is 99.8%.

1-2. Experimental equipments

The extracting equipments are designed by ourselves. The volumes of the equipments are 0.3L and 5L respectively. The equipment of 0.3L is used to do experiment, which has a separating pot, the set parameters are extracting pressure and temperature, the flow rate of CO_2 , it has no cycle system of CO_2, the flow meter is vacated after being measured. The one of 5L is a small scaled equipment which has a extracting pot and two separating pot of 2L, the set parameters are extracting pressure and temperature, separating pressure and temperature, it has the cycle system of CO_2.

1-3. Analytical apparatus

Gas chromatograph-mass spectrometer equipped with computer. The type is HPGC 6890/MS 5973.

2. The experimental method

2-1. Determination of content of water

The content of water is determined by the method of drying and constant weight. The temperature is 110？ . The results are obtained by the method of repeated constant weight.

2-2. Determination and calculation of material particle diameter

The materials are smashed and then the particle diameter is determined by screening, the specifications of mesh are 20m？ 40m？ 60m？ 80m？ 100m？ 120m？ 140m and the corresponding particle diameters are 0.065mm？ 0.088mm？ 0.097mm？ 0.149mm？ 0.25mm？ 0.40mm？ 2.0mm. The range of particle diameters can be determined and the corresponding content can be calculated after being screened. The following formula is used to calculate the particle diameter and the rate of extraction-intraparticle diffusion coefficient is calculated[4].

$$D=\frac{100}{\sum n[a/2(1/D_{k+1}+1/D_k)]}+\frac{3a_{\min}}{2D_{\min}} \qquad (1)$$

Where D is particle diameter, n is the number of granularity, a is the percent of each granularity, D_{k+1}

and D_k are the biggest and smallest diameter of each granularity, D_{min} is the smallest diameter, a_{min} is the percent of the smallest grade.

2-3. Study on the conditions of extracting technology

The extracting equipment of 0.3L is used to choose the optimum technological condition and the chosen optimum parameters then is used to do the extracting experiment of sample at the equipment of 5L, the technological condition is further optimized and the sample used to do experiments at small scaled equipment is obtained. In addition to the above, we also entrusted colleague to do the experiments of effect of pressure and temperature at the extracting equipment of 20L. The collected sample is analyzed by gas chromatography, liquid chromatography and gas chromatography-mass spectrometer (GC-MS) some extracting parameters affecting the extracting processes of SCE have been studied, such as pressure temperature, density of CO_2 fluid, time of extracting and S/F, the optimum technological parameters of extracting essence oil from the purple perilla seeds by supercritical fluids are obtained.

2-4 The calculation of the content of **a**-LAN and the extraction ratio

? Content of **a**-LNA

The chromatogram of total ion current is obtained by GC-MS. The existence of **a**-LNA can be known according to the standard mass spectrogram; the relative percent of **a**-LAN can be calculated according to the normalization method of peak area.

? The calculation of extraction ratio

$$R=(W-Ws) \times 100\%/Ws \qquad (2)$$

Where R is the extraction ratio(%), W is the content of **a**-LAN in extracts, Ws is the theoretical content of **a**-LAN in purple perilla seeds.

2-5 The conditions of chromatographic- mass spectrometric analysis

The chromatographic column: Hp-SMS(30m × 0.25mm × 0.25μm)

The temperature of column: the original temperature is 60? , then the temperature is reached 250? at the rate of 4? per minute.

Carrier gas: He, the flow is 1.2ml per minute, the split ratio is 40:1, the temperature of sample entrance is 300? .

The conditions of mass spectra: the ion source is 230? , ionization voltage is 70ev; the collected mass range is 33~ 550a.m.u

3 . Results and discussion

3-1 The effects of technological condition on the extraction ratio of essence oil from purple perilla seeds and the calculation of extraction rateWe studied the various factors affecting the extraction ratio. The extracting equipment of 0.3L is used to do the experiment under different pressure, the entrusted experiment is also done simultaneously at the extracting equipment of 20L . The effects of extracting pressure and temperature, density of CO_2 fluid, extracting time and S/F on the extraction ratio are shown in Fig 1-6. The reault of calculation of extraction rate is $2.7 \times 10^{-12} m^2/sec$

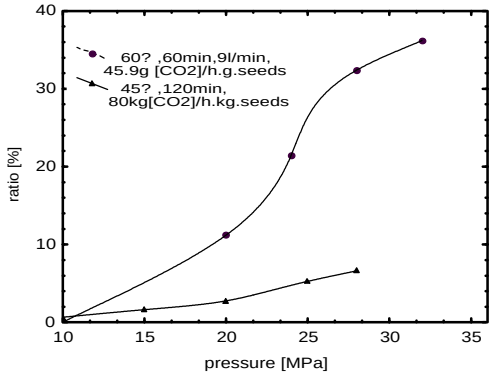

Fig.1 Effect of pressure on the ratio of perila seed oil

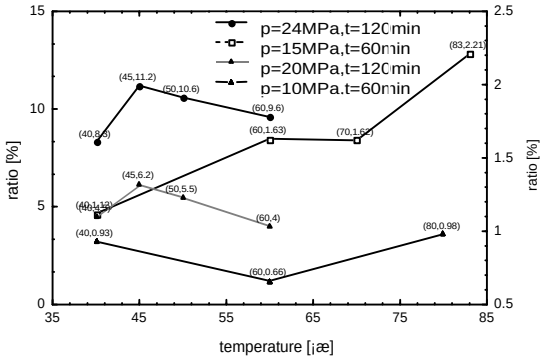

Fig.2 Effect of extract temperature on the ratio of perilla seed oil

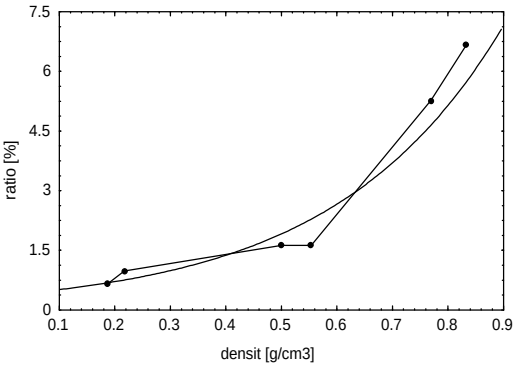

Fig.3 Effect of corbon dioxide density on the ratio of perilla seed oil

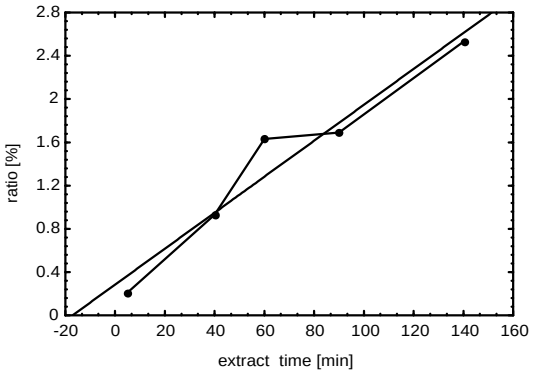

Fig.4 Effect of extract time on the ratio of perilla seed oil

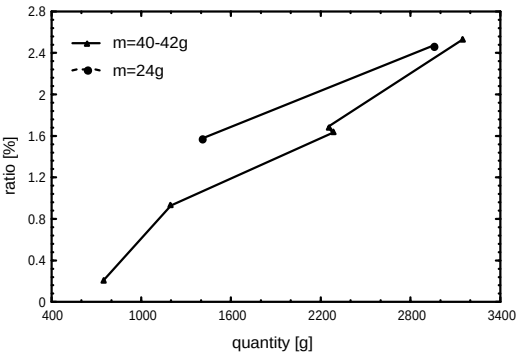

Fig.5 effect of cobon dioxide qantity on the ratio of perilla seed oil

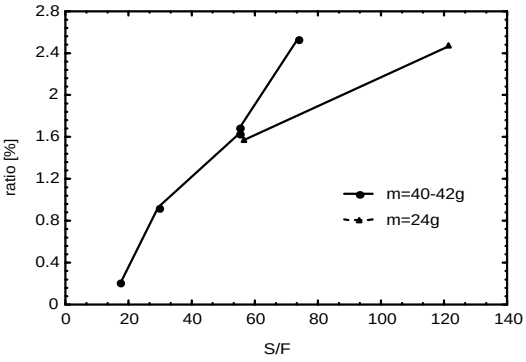

Fig.6 Effect of S/F on the rate of perilla seed oil

3-2 GC-MS analysis of essence oil from purple perilla seeds

In order to obtain the technological conditions of the highest content of **a**-LAN in essence oil, the extracting experiments of essence oil are done at the extracting equipment of 5L, the extracting pressure are 20? 25 and 30Mpa respectively. The temperature of extracting pot is 50? and the temperature of separate pot is 30? , the maximum supply of CO_2 is 44L/h, the extracting time is two hours. The obtained essence oil is analyzed by GC-MS.

3-2-1.Analysis of total ion current chromatograms of sample

The total ion current chromatograms are obtained under three different pressure (see fig 7). GC-MS is used to determine the higher content of substance under each pressure by the normalization method of peak area and the obtained contents are compared under three different pressure .The results of GC-MS analysis indicated that the pressure is higher, the numbers of extracts are more in the same condition(see table 1).

Table 1. the extracting situation of essence oil under different pressure

pressure / number / item	20MPa	25MPa	30MPa
the total numbers of chromatographic peak	38	42	49
the qualitative numbers of chromatographic peak	12	15	15

It is obvious that the extracting species are more under the pressure of 30MPa than those under the pressure of 20MPa. From the total ion current chromatogram we can see that the pressure is higher, the species and the numbers of light components are more.

In general, the extracted light components are more under the pressure of 20MPa, but the viscosity of extracted essence oil is smaller, the light components are easy volatile, the collection is difficult and the light components remained in sample is less. The heavy components in sample will increase with the pressure increasing, the viscosity of essence oil will increase and the species and mass of remained light components will increase. The experiments indicated that the light components in sample can be determined under the pressure of 30Mpa and can't be determined under the pressure of 20MPa.

3-2-2. Analysis of the content of a-LNA

Using the normalization method of peak area we calculated the content of a-LNA in essence oil (see table 2) from which we can see the content of LNA is the highest under the pressure of 20MPa.

Table 2. The contents of main components in essence oil

pressure / content(%) / name	20MPa	25MPa	30MPa
a -LNA	59.01	52.64	43.37
palmic acid	5.22	4.47	3.63
11,14,17-Eicosatrienoic acid	12.35	17.18	21.02
the total(%)	76.58	74.29	68.02

The main component of fatty oil extracted from purple perilla seeds by SEC is a-LNA (see fig 8), this is consistent with the result of paper[5]. Because esterification reaction is reversible, the action of acid and alcohol can from ester and water, the reaction can go on slowly even if no catalyst[6], the equation is

$$RCOOH + HOR' = RCOOR' + H_2O$$

As a-LNA in extracts can exist for above three kinds, we merge the contents of acid, alcohol and ester, then the content of a-LNA is calculated.

3-3. The confirmation of a - LNA by GC-HPLC

In order to prove further the existence and content of a-LNA, the methods of HPLC are used simultaneously to analysis the sample, the chromatograms are shown in fig 9. **?**-LNA is separated and determined by HPLC. The data of determination are shown in table 3.

Table 3. the contents of some components in essence oil by HPLC

number / content(%) / name	1# (extracted sample, 25MPa)	2# (the clarified components of extracted sample, 20MPa)	3#(the precipitated components of extracted sample, 20MPa)
a-LNA	55.9	59.7	59.2
?-LNA	0.4	0.5	0.5
palmic acid	6.6	6.6	6.4
oleic acid	23.2	19.2	19.2
arachidonic acid	<0.01	<0.01	<0.01
erucic acid	<0.01	<0.01	<0.01
fatty acid	99.0	99.8	99.5

The results of above table are consistent with the results by GC-MC, a-LNA is the main component in essence oil and its content reached 59.7%, therefore the condition of 20Mpa is confirmed.

4. Conclusion

The effects of technological parameters on the extraction ratio of essence oil extracted from purple perilla seeds have been studied, the optimum technological conditions have been obtained. Comparing with the theoretical content of 65%, the real content of a-LNA reached 59.7%, the extraction ratio is 91.8%.

The extracting pressure, temperature and amount of CO_2 fluid are very important factors affecting the efficiency of extraction. In general, the efficiency of extraction of essence oil increases with the increase of pressure, the extraction ratio under the pressure of 35~ 40Mpa can reach the maximum value, approaching to 40% [7], but we found that the content of a-LNA is the highest under the pressure of 20~ 25MPa. The higher temperature(80?) is of benefit to extract the essence oil from purple perilla seeds under the optimum pressure, but a-LNA will decompose over 50? , we selected 40~ 45? as the best temperature. As to extracting agent of CO_2, the density of CO_2 , amounts of CO_2 , flow rate of CO_2, extracting time and the value of S/F must be considered, which can be controlled by different way according to the difference of extracted equipments. In this study, the equipment of 0.3L is used and the flow rate is contoured at 9~ 10L/min. The longer extracting time and the major value of S/F will need large amounts of CO_2, which are of advantage to the increase of extraction ratio.

The optimum technological conditions in this study are obtained ,the pressure is 20~ 25MPa, the temperature is 40? , the flow rate of CO_2 is 9~ 10L/min, the extracting time is 120~ 180min.

References

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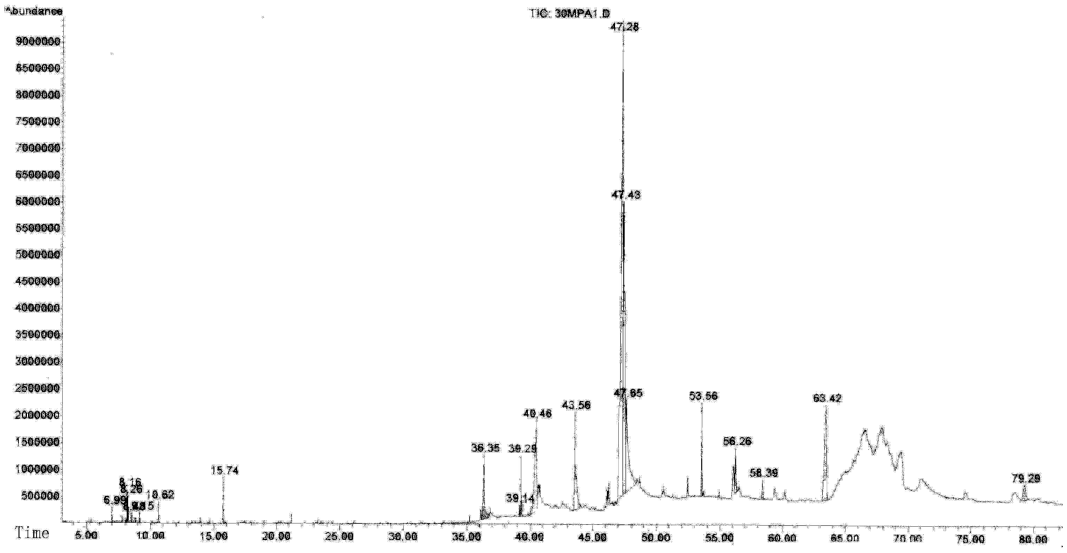

Fig.7 Ion chromatogram of perilla seed oil under 30 MPa pressure

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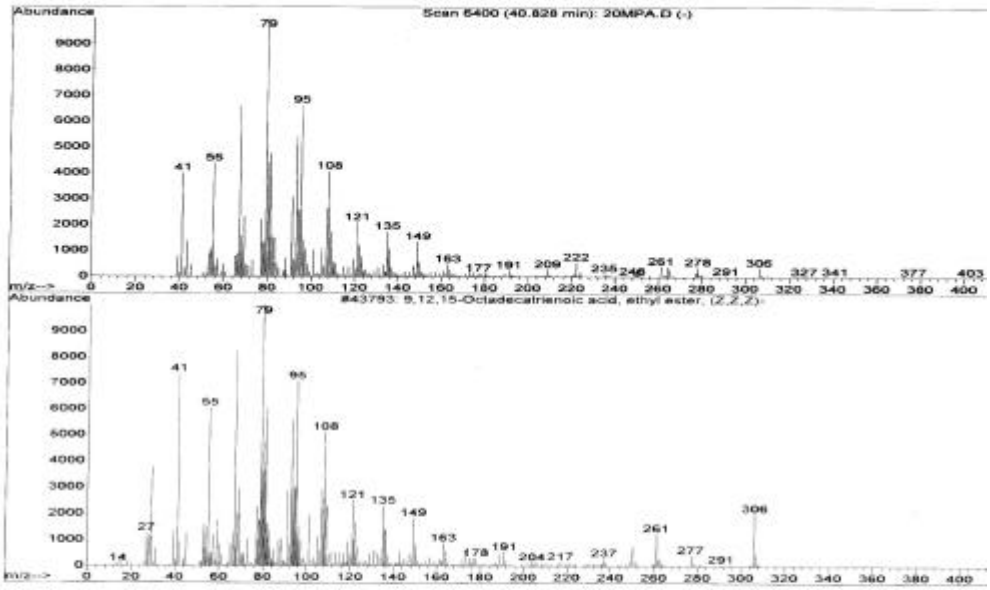
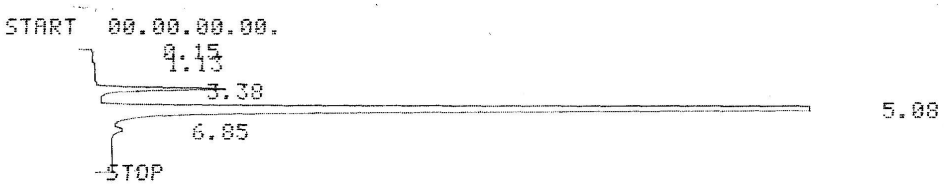


Fig.8 MS-chromatogram of 18:3 linolenic acid ethyl ester



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METHOD 41

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0		3.38	5.1256		205691
0		5.08	94.2238		3781179
0		6.85	0.6067	T	24349
	TOTAL		99.9999		4012974

Flg.9 HPLC chromatogram of perilla seed oil