Determination of natural vitamin A in fish liver by liquid chromatography

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Abstract: In order to accurately determine the content of vitamin A (vitamin A_1, vitamin A_2) in fish liver, sample pretreatment methods (water bath saponification, room temperature saponification, direct extraction) and detection methods (normal phase chromatography, reversed phase chromatography) were screened and applied to the determination of vitamin A in the liver of nine economic fish species. The results showed that vitamin A_1 and vitamin A_2 were separated effectively by reversed phase chromatography and showed good linear relationship within their respective linear ranges(R^2 > 0.99); the content of vitamin A extracted by water bath saponification was significantly higher than that by room temperature saponification and direct extraction (p <0. 05), and its average recoveries of vitamin A_1 and vitamin A_2 were 104. 52% and 90. 94%, respectively. Except for the freshwater snakehead and big mouth bass, the total content of vitamin A in the livers of other freshwater fishes and marine fishes was more than 200μg/100 g, and the total content of vitamin A in the liver of marine giant grouper was the highest, reaching 14 413.78μg/100 g. The water bath saponification method combined with reversed phase chromatography has good precision and is suitable for the determination of vitamin A in fish liver.

Keywords: Fish; Liver; Vitamin A; Extraction method; Detection method

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Vitamin A (V_A) is an essential fat soluble vitamin for human body, which can maintain vision. Epithelial tissue. Immunity and other normal physiological functions will lead to night blindness when the human body lacks V_A. Xerophthalmia. Growth retardation. Immune decline, etc [1-2]. V_A deficiency is a global health problem. About 250million preschool children lack V_A[3]. V_A is divided into natural V_A and synthetic V_A, and natural V_A mainly exists in the liver. Dairy. In egg yolk and red yellow plants, synthetic V_A is mostly used to apply ointment. V_{AD} oil and other products, but synthetic V_A has some potential safety hazards, which is easy to lead to hyperlipidemia. Pseudotumor. Eyelid conjunctivitis, etc [4-5]. Natural V_A includes retinol (V_{A1}) and dehydrogenated retinol (V_{A2}). Their structures are similar, and the only difference is that V_{A2} has a conjugated double bond on the 3, 4 carbon bond. V_{A1} is commonly found in mammals and marine fish, while V_{A2} is mostly found in freshwater fish [6]. The V_A content in fish liver is rich and economically available, which is an excellent raw material for obtaining natural V_A[7].

V_A itself is a trace element and its nature is unstable, so it is easy to be exposed to light. Hot. Oxygen. Acid. Alkali damage [8-9], so it is particularly important to choose an extraction method that can accurately reflect the real content of the sample. At present, V_A extraction methods mainly include saponification method and direct extraction method. Saponification method can be divided into room temperature saponification method and water bath saponification method. Water bath saponification method has a wide range of applications, but the operation is cumbersome and the temperature is high, which may cause certain losses to V_A. Room temperature saponification method has a mild effect, but it takes a long time; the direct extraction method has shorter extraction time and simpler operation.

The detection method of V_A includes ultraviolet method. Fluorescence method and liquid chromatography, in which UV method and fluorescence method have low sensitivity and cumbersome operation, and liquid chromatography is the main method to detect V_A at present. Liquid chromatography is also divided into normal phase chromatography and reverse phase chromatography. Normal phase chromatography allows more fat in the sample, and its mobile phase can also have a large span of polarity range [10]. It is stipulated in the Pharmacopoeia 2010 that normal phase chromatography is used to determine CIS and trans V_A in drugs [11], and the research results of Rebeca et al. [12] show that normal phase chromatography can well separate V_A isomers with good resolution. Reversed phase chromatography has higher stability [13], which is often used for the analysis and determination of V_{A1} content. At present, there are few studies on V_{A2} [14-16]. Due to the unstable nature of V_{A2}, now most research methods only take V_{A1} content as the total V_A content, which may cause the test results to be inconsistent with the actual results and cannot truly reflect the V_A content of the sample. In order to correctly evaluate the V_A content in fish liver, this study compared normal phase chromatography with reverse phase chromatography, and water bath saponification method with grouper liver as raw material. Room temperature saponification method. Three sample pretreatment methods of direct extraction were compared, and the best sample pretreatment was selected. Detection methods, and compared the types and total content of V_A in the liver of 9 economic fish, in order to provide preliminary basic data for the development and utilization of new natural V_A

resources.

1 Materials and methods

1.1 Test materials

1.1.1 Raw materials and reagents

Grass Carp. Snakehead. Gold Pomfret. Order a basket of fish. Variegated carp. Pearl gentian grouper. Tilapia mossambica. Largemouth bass. Longpont grouper liver was purchased from Zhanjiang Xiashan products wholesale market. After the fish was killed, the liver was transported back to the laboratory with ice cubes to remove the surface fat and connective tissue. After being washed with precooled normal saline, the surface water was sucked dry with kitchen paper towels. After homogenization, it was immediately frozen in an ice box of $^{-8}$0℃.

Ascorbic acid. Anhydrous sodium sulfate. Potassium hydroxide. Petroleum ether. 2, 6 - Di tert butyl p-cresol (BHT), all analytically pure, Shantou Xilong company; anhydrous ethanol (analytical purity), Tianjin kemio company; methanol (chromatographically pure). V_{A1} standard (purity ≥ 95%), sigma company of the United States; V_{A2} standard (purity ≥ 93%), TRC Canada; isopropyl alcohol (chromatographic purity), Shanghai Aladdin company; N-hexane (chromatographic purity), thermofisher company, USA.

1.1.2 Instruments and equipment

$E_2$695 high performance liquid chromatograph (equipped with 2489 UV detector), waters, USA; $fa_2$004 analytical balance, Shanghai Sunny company; N^{-1}300 rotary evaporators, Shanghai ailang company; SHZ-B constant temperature water bath oscillator, Shanghai xunbo company; MGS^{-2}200h nitrogen blower, Shanghai Elang company; ultrasonic cleaning machine, Kunshan ultrasonic company; Z^{-1}6kl centrifuge, sigma company of the United States; D^{-2}4uv clear ultra pure water all-in-one machine, German Merck company; zls^{-3} vacuum centrifugal concentrator, Hunan Hexi company.

1.2 Test method

1.2.1 Preparation of V_A standard solution

25mg V_{A1} standard and 2.5mg V_{A2} standard were dissolved in 25mL and 5mL absolute ethanol respectively to make standard stock solution. Before use, the concentration of V_{A1} standard stock solution was corrected according to Appendix B of $gb_5$009.82-2016. After correction, the mass concentration of V_{A1} standard stock solution was 1.29mg/mL. V_{A2} did not correct its concentration because there was no reference method. Draw 0.25mLV_{A1} standard stock solution and 0.4mLV_{A2} standard stock solution respectively into the same 10mL volumetric flask, fix the volume with methanol, and prepare a mixed standard intermediate solution. Accurately absorb 0.1 respectively. 0.25. 0.5. 1.0. 2.0. Put 3.0mL mixed standard intermediate solution into 5mL Brown volumetric flask, fix the volume with methanol to the scale, and prepare V_{A1} and V_{A2} series mixed standard working solutions.

1.2.2 Sample pretreatment (V_A extraction)

Because V_A is unstable, use brown bottles and glass bottles wrapped with tin foil during operation, and try to avoid light exposure.

1.2.2.1 water bath saponification method

Referring to $gb_5$009.82-2016, accurately weigh 1.6g of grouper liver in a conical flask, add 20mL of water, mix well, add 1.0g of ascorbic acid and 0.1gbht as antioxidants, add 30mL of absolute ethanol, and then add a certain amount of potassium hydroxide solution with a mass fraction of 50% as saponification solution, mix well, put a cork on the back cover,

oscillate in a constant temperature water bath at 80℃, saponify for 30min, and immediately cool with ice water after saponification. Transfer the saponified matter into a 250mL Brown separating funnel with 30mL water, add petroleum ether to vibrate and extract for 5min, move the lower aqueous phase to another separating funnel for the second extraction, combine the ether phase and wash it to neutral, then filter it into a 1L rotary evaporation bottle through 3G anhydrous sodium sulfate, concentrate it on the rotary evaporator until it is nearly dry, dissolve the residue in fractions with methanol, dilute the V_A content extracted by different pretreatment methods and the liver of different fish to 10mL, and pass 0.22μm filter membrane to be tested.

1.2.2.2 room temperature saponification method

Refer to the method of Liu Bo et al. [17] and make some changes. Accurately weigh 1.6g of grouper liver of dragon pontoon into a conical flask, add 20mL of water, 1.0g of ascorbic acid and 0.1gbht, add 30mL of absolute ethanol, and then add a certain amount of potassium hydroxide solution with a mass fraction of 50% as saponification solution. After mixing, purge it under nitrogen flow for 5min, plug the bottle mouth with a rubber soft plug, and oscillate overnight. After being extracted with petroleum ether, wash it with water to neutral, concentrate it with rotary evaporator, and then fix the volume with methanol to 10mL, and pass 0.22μm filter membrane to be tested.

1.2.2.3 direct extraction method

Refer to the method of nimalaratne et al. [18] with slight changes. Accurately weigh 1.6g of grouper liver of dragon pontoon into a flat bottom flask, add 20mL of n-hexane, then add 1.0g of ascorbic acid and 0.1gbht, mix well, and then conduct ultrasonic treatment (ultrasonic power is 240W, ultrasonic frequency is 40khz) for a certain time. Then centrifuge at 5000r/min for 10min, take the supernatant, spin it on the vacuum centrifugal concentrator at 25℃ to dry, fix the volume with methanol to 10mL, and pass 0.22μm filter membrane to be tested.

1.2.3 Detection conditions of V_A by liquid chromatography

V_A was detected by reversed phase chromatography and normal phase chromatography respectively. Reversed phase chromatography condition:watersbehc$_1$8 column (250mm × 4.6mm, 5μm); the mobile phase is methanol water (volume ratio 96:4); flow rate 0.8mL/min; injection volume 10μL. Normal phase chromatography conditions:phenomenexlunasilica chromatographic column (250mm × 4.6mm, 5μm); the mobile phase is n-hexane isopropanol (volume ratio 99.7:0.3); flow rate 1mL/min; injection volume 10μL.

1.2.4 Standard curve drawing and sample detection

Put the mixed standard working solution of V_{A1} and V_{A2} series into liquid chromatography for detection, and draw the standard curve with the mass concentration (x) of V_{A1} and V_{A2} as the abscissa and the corresponding peak area (y) as the ordinate. Inject the sample solution into the liquid chromatography for detection, get the peak area, and then get the mass concentration of V_{A1} and V_{A2} according to the standard curve, and calculate the V_A content according to the following formula.

$$X_1 = \rho \times V \times f \times 100 / m \quad (1)$$

$$X_2 = (\rho \times V \times f \times 100 / m) \times 0.4 \quad (2)$$

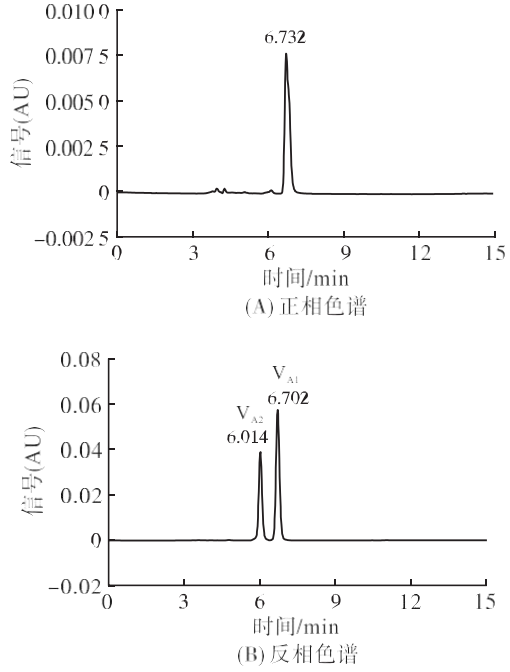
$$X_3 = X_1 + X_2 \quad (3)$$

Where:X_1, X_2, X_3 is V_{A1} respectively. V_{A2}

content and total V_A content, μg/100g; ρ is the mass concentration of V_A in the sample calculated according to the standard curve,μg/mL; *V* is the constant volume, mL; *f* is the conversion factor (the conversion factor of V_A is 1); *m* is the mass of the sample, g; 0.4 is the V_{A2} conversion coefficient, and the V_{A2} activity is 40% of V_{A1}, which is converted during calculation [19].

1.2.5 Data processing

V_A content is expressed by "mean±standard deviation" (*n*=3). The V_A content extracted by different pretreatment methods and the V_A content in the liver of different fish were analyzed by one-way ANOV$_A$. If the difference was significant, Duncan method was used for multiple comparisons, and *P*<0.05 was used as the judgment standard for the significance of the difference.



Normal phase chromatography
Reversed phase chromatography
Signal
Time

Figure 1 Liquid chromatogram of mixed standard solution in normal phase and reverse phase chromatography

2 Results and analysis

2.1 Establishment of V_A detection method

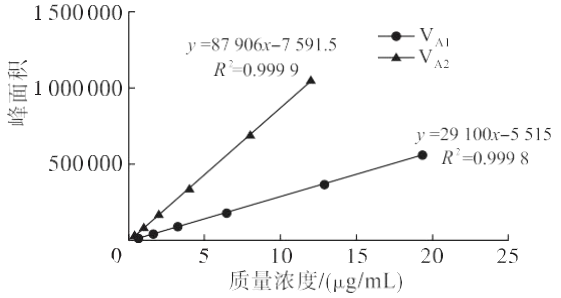
V_{A2} plays the same important role in visual regulation as V_{A1}. The mutual transformation of V_{A1} and V_{A2} is an adaptive response of animals to light changes. V_{A1} has the maximum absorption peak at 325nm, V_{A2} has the maximum absorption peak at 350nm, and V_{A2} is unstable. The change is fast, so 350nm is taken as the common measurement wavelength of both [14]. In order to more accurately refer to the method of nimalaratne et al. [18], with slight changes. Accurately weigh 1.6g of grouper liver of dragon pontoon into a flat bottom flask, add 20mL of n-hexane, then add 1.0g of ascorbic acid and 0.1gbht, mix well, and then conduct ultrasonic treatment (ultrasonic power is 240W, ultrasonic frequency is 40kHz) for a certain time. Then the V_A content in the sample was determined by centrifugation at 5000r/min. In this study, normal phase chromatography and reverse phase chromatography were compared.

Mix V_{A1} and V_{A2} with standard working solution (V_{A1} mass concentration is 12.9μg/mL, V_{A2} mass concentration is 8μg/mL) were detected by normal phase chromatography and reverse phase chromatography, and the obtained liquid chromatogram is shown in Figure 1.

It can be seen from Figure 1 that only one single peak can be detected by normal phase chromatography, and V_{A1} cannot be separated. Separate V_{A2} and replace the mobile phase. The mobile phase ratio and flow rate cannot be separated; and reverse phase chromatography, V_{A1}, V_{A2} separation is in good condition.

Add 19.35μg/mL V_{A1} and 12 μg/mL V_{A2} mixed standard solution was continuously

injected for 6 needles, and the stability of the test method was expressed by relative standard deviation (RSD). The results showed that the average RSD of reverse phase chromatography was 0.38%, which was less than 4.48% of normal phase chromatography, indicating that the stability of reverse phase chromatography was higher. Therefore, reversed phase chromatography was selected as the method to detect V_{A1} and V_{A2} in this study. Using reversed-phase chromatography, draw the standard curve according to 1.2.4, and the results are shown in Figure 2. It can be seen from Figure 2 that the linear equations of V_{A1} and V_{A2} are y=29100x-5515 and y=87906x-7591.5 respectively, and the correlation coefficients (R^2) are 0.9998 and 0.9999 respectively, which both have a good linear relationship in the range of 0. 645 ~ 19. 35 μg/mL and 0. 4 ~ 12 μg/mL, respectively.



Mass concentration

Peak area

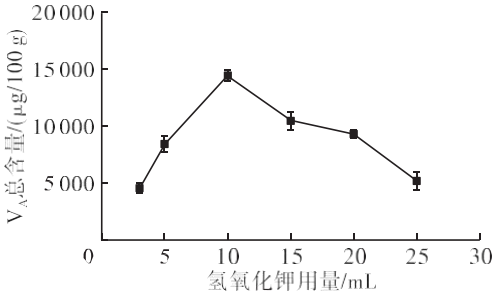
Figure 2 Standard curve of V_{A1}, V_{A2}

2.2 Determination of sample pretreatment method

2.2.1 Determination of extraction conditions for three sample pretreatment methods

2.2.1.1 Water bath saponification method

Saponification method is to remove the coexisting lipid compounds in the sample by saponification reaction between alkali and oil, convert V_A esters into free V_A, and separate the free V_A wrapped by oil for determination. Potassium hydroxide solution with a mass fraction of 50% is suitable for the saponification needs of most samples [20]. The increase of the amount of alkali is conducive to complete saponification. If the amount of alkali added is insufficient, the saponification reaction is not complete, which will cause the determination result to be low. However, if too much alkali is added, a large amount of water is required to wash it to neutral in the water washing process, resulting in waste. Moreover, V_A is a sensitive component, and too much alkali will cause certain oxidative damage to V_A. The V_A in the liver of grouper was extracted by water bath saponification with different dosage of potassium hydroxide (mass fraction 50%), and the total V_A content was detected by reversed-phase chromatography. The results are shown in Figure 3.



Dosage of potassium hydroxide

Total V_A content

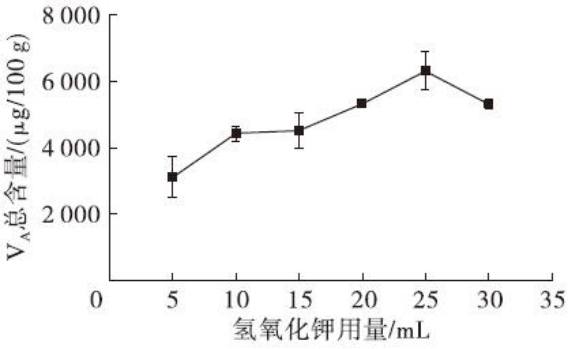
Figure 3 Effect of potassium hydroxide dosage on water bath saponification method

It can be seen from Figure 3 that with the increase of the dosage of 50% potassium hydroxide, the total V_A content first increased and then decreased. When the dosage of 50% potassium hydroxide was 10mL, the total V_A content reached the maximum. Then, with the increase of the dosage of 50% potassium hydroxide, the total V_A content began to decrease, which may be because too much alkali was added, which accelerated the oxidative damage of free V_A. Therefore, in the water bath saponification method, the dosage of 50% potassium hydroxide is 10mL.

2.2.1.2 Room temperature saponification method

Using room temperature saponification method, V_A in the liver of grouper was extracted with different amounts of potassium hydroxide (mass fraction 50%), and the total content of V_A was detected by reverse phase chromatography. The results are shown in Figure 4.

It can be seen from Figure 4 that with the increase of the dosage of 50% potassium hydroxide, the total V_A content showed a trend of gently increasing at first and then decreasing. When the dosage of 50% potassium hydroxide was 25mL, the total V_A content reached the maximum value, and then increased the dosage of potassium hydroxide, and the total V_A content decreased. Therefore, in the room temperature saponification method, the dosage of 50% potassium hydroxide is 25mL.



Dosage of potassium hydroxide

Figure 4 Effect of potassium hydroxide dosage on room temperature saponification method

2.2.1.3 Direct extraction method

The direct extraction method uses the cavitation effect of ultrasound to assist the extraction of organic solvents, so as to accelerate the release of substances in cells [21]. If the ultrasonic time is too short, the V_A extraction effect is not good, and if the ultrasonic time is too long, it will cause a waste of energy. Using direct extraction method, V_A in the liver of grouper was extracted under different ultrasonic time, and the total content of V_A was detected by reversed-phase chromatography. The results are shown in Figure 5.

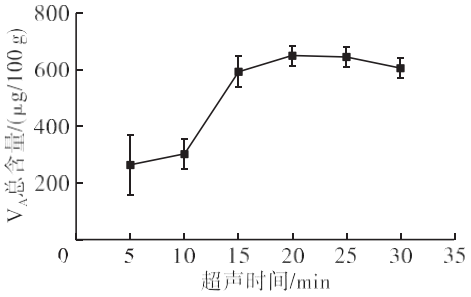


Figure 5 Effect of ultrasonic time on direct extraction method

It can be seen from Figure 5 that with the extension of ultrasonic time, the total V_A content method showed a trend of rising first and then stable. The total V_A content was the highest at 20min, but there was no significant difference with the total V_A content at 15min. Therefore, the ultrasonic time was selected as 15min.

2.2.2 Comparison of precision of different extraction methods

According to the conditions determined in 2.2.1, three sample pretreatment methods were used to extract V_A from the liver of grouper grouper, and the total content of V_A was detected by reversed-phase chromatography. The samples were injected four times in a day, and the precision was measured for three days. The relative standard deviation (RSD) was used to express the precision (the greater the RSD, the smaller the precision). The intra day RSD and intra day RSD of different extraction methods were calculated, and the results are shown in Table 1.

Table 1 RSD comparison of three sample pretreatment methods

%

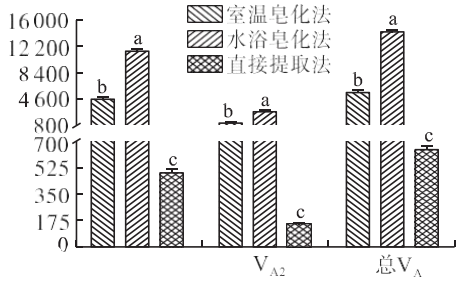
Pretreatment method	Average intraday RSD	Average daytime RSD

Water bath saponification	1.07	10.64
Room temperature saponification	1.39	2.77
Direct extraction method	6.91	10.44

It can be seen from Table 1 that the daily RSD of water bath saponification method and room temperature saponification method are 1.07% and 1.39% respectively, and the difference between these two extraction methods is small, while the difference with the daily RSD of direct extraction method (6.91%) is large; the daytime RSD of room temperature saponification method is the smallest, which is 2.77%, while the daytime RSD of water bath saponification method and direct extraction method is larger. This may be because the reaction temperature of room temperature saponification method is relatively mild, while the reaction temperature of water bath saponification method is higher, V_A is unstable and sensitive to temperature, and the longer the storage time is, the more V_A loss is caused, while the direct extraction method is prone to emulsification in the ultrasonic process, resulting in the instability of the whole system, which has a certain impact on the detection, thus making its RSD larger, which is consistent with the research results of Jiang Bo et al. [23]. The intraday RSD of the three sample pretreatment methods is less than 10%, and the intraday RSD is less than 15%, which meets the requirements of the analytical method [24].

2.2.3 Comparison of V_A content with different extraction methods

According to the conditions determined in 2.2.1, three sample pretreatment methods were used to extract V_A from the liver of grouper, and the content of V_A was detected by reversed-phase chromatography. The results are shown in Figure 6.



Note: different letters indicate that there are significant differences between different methods in the same category, $P<0.05$

Figure 6 Comparison of V_A content in the liver of longpont with three pretreatment methods

It can be seen from Figure 6 that among the three sample pretreatment methods, water bath saponification method V_{A1}. The content of V_{A2} and total V_A were significantly higher than that of saponification and direct extraction at room temperature ($p<0.05$), while that of saponification at room temperature V_{A1}. The content of V_{A2} and total V_A were significantly higher than that of direct extraction ($p<0.05$).

The results of this study showed that the direct extraction method had the lowest V_A content. Different extraction methods are applicable to different test objects, although the direct extraction method is effective on mink kidney [12]. The extraction effect of V_A from milk powder [18] and vegetable oil [23] is good, but according to the results of this study, this method is not suitable for the extraction of V_A from the liver of grouper. The room temperature saponification method has no heating treatment, the effect is more mild, and the reaction rate is also low. The fat content of this research object is high, so the

saponification reaction time is longer at room temperature. However, long-term exposure to alkaline environment has caused damage to V_A, which may be the reason why the V_A content of room temperature saponification method is lower than that of water bath saponification method. After comparative analysis, combined with the test results in Table 1, the water bath saponification method is selected to pretreat the samples.

2.2.4 Recovery rate of water bath saponification method

To homogenize the liver of grouper longpont, press low. V_{A1} is added at medium and high levels. V_{A2} standard is pretreated by water bath saponification method, and then the V_A content is detected by reversed-phase chromatography to calculate the spiked recovery. The results are shown in Table 2.

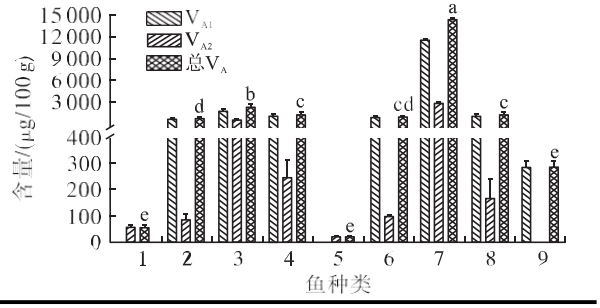
Table 2 Standard addition recovery rate

VA	Content in sample/μg	Scalar addition/μg	Measured value/μg	Recovery rate/%	Average recovery/%
V_{A_1}	11.73	4.69	16.62	1 4. 26	1 4. 52
	11.45	6.87	18.21	98.4	
	11.59	9. 27	21.87	11 .9	
V_{A_2}	2.82	1.16	3.79	83.62	90.94
	2.90	1.64	4. 37	89. 63	
	2.74	2. 26	4.99	99.56	

It can be seen from Table 2 that the recovery rate of V_{A1} standard addition is between 98.40% and 110.90%, and that of V_{A2} standard addition is between 83.62% and 99.56%. The recovery rate is good. Based on the above results, this study chose the water bath saponification method to extract V_A. Because the RSD of the water bath saponification method is relatively large during the day, it needs to be determined as soon as possible on the day of extraction.

2.3 Comparison of V_A content in liver of 9 Economic fish species

Choose Grasscarp. Snakehead. Gold Pomfret. Order a basket of fish. Variegated carp. Pearl gentian grouper. Grouper with dragon pontoon. Tilapia mossambica. Big mouth bass, nine common economic fish, were studied, including Grasscarp. Snakehead. Variegated carp. Tilapia mossambica. Big mouth bass is freshwater fish, and the rest are sea fish. The liver of these fish was pretreated by water bath saponification method, and then the V_A content was detected by reversed-phase chromatography. The results are shown in Figure 7.



Fish species

Content

Note:1. Snakehead; 2. Grass Carp; 3. Variegated carp; 4. Tilapia mossambica; 5. Largemouth Bass; 6. Gold Pomfret; 7. Grouper with dragon pontoon; 8. Pearl gentian grouper; 9. Order a basket of fish. The total V_A content was analyzed, and different letters showed significant differences, $P<0.05$

Figure 7 Comparison of V_A content in liver of 9 Economic fishes

It can be seen from Figure 7 that among the nine kinds of fish, the total content of V_A in the liver of grouper is 14413.78μg/100g, significantly higher than other fish ($p<0.05$). The total content of V_A in silver carp liver was 2337.36μg/100g, lower than grouper, but significantly higher than other fish ($p<0.05$).

The total content of V_A in the liver of Pearl gentian grouper and tilapia was 1265.13 respectively. One thousand two hundred and eighty-seven point eight fiveμg/100g, significantly higher than that of snakehead. Grass Carp. The total content of V_A in the liver of golden pomfret was 1019.71μg/100g, the total V_A content in Grasscarp liver is 765.03μg/100g, both of which are significantly higher than that of snakehead. Big mouth bass and point basket fish (P<0.05), snakehead. Compared with other fish, the total content of V_A in the liver of largemouth bass and point basket fish was 57.01, respectively. 23.02. Two hundred and eighty-seven point five sixμg/100g. The liver of snakehead and Largemouth Bass only contains V_{A2}, that of basket fish only contains V_{A1}, and that of the other six species of fish all contain V_{A1}. V_{A2} two types of V_A.

In this study, the liver of freshwater fish contains V_{A2}. Generally speaking, V_{A2} mostly exists in freshwater fish, but with the deepening of research, it is found that V_{A2} also exists in many seawater fish. In this study, four species of marine fish were determined, including Golden Pomfret. Grouper with dragon pontoon. V_{A2} exists in the liver of three kinds of marine fish, pearl gentian grouper. Kondrashev et al. [25] also found V_{A2} with a high proportion in the bodies of two kinds of marine fish, hexagramma bifida and sardines. Studies have shown that fat soluble vitamins cannot be excreted from the body through body fluids and are easy to accumulate in the body. The content of V_A in the liver increases with the increase of V_A level in the diet, and the two are significantly linearly and positively correlated [26]. The nine kinds of fish selected in this study are all artificially raised. Among these kinds of fish, the grouper is the largest individual, the longest breeding time, and the V_A content accumulated in the body is also the highest. The types and contents of V_A in the liver of different fish are different from those of fish. Individual size. Their own diet and feeding management level are related to many factors [27].

3 Conclusion

In this paper, the content of V_A in fish liver was determined by screening the sample pretreatment methods and detection methods of V_A extraction in fish liver. It was determined that water bath saponification method could extract V_A from fish liver at most, and reverse phase chromatography was used to detect and separate V_{A1}, V_{A2} is in good condition. With high stability, water bath saponification combined with reversed-phase chromatography is suitable for the analysis and determination of V_A in fish liver. V_A is abundant in the liver of fish. Among the nine fish species tested, the total content of V_A in the liver of other fish species, except snakehead and largemouth bass, reached 200μg/100g, of which the total content of V_A in the liver of sea water fish Dragon Grouper is the highest, reaching 14413.78μg/100g. The research results provide preliminary basic data for the development of new natural V_A resources.

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