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A Study on Vitamin A Content of Mare Milk using HPLC

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HPLC를 이용한 마유의 Vitamin A 함량 분석

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Abstract

Mare milk has a unique composition compared to other animal milks. This study was to determine the content of vitamin A in mare milk using HPLC and compared with cow milk. The RT(retention time) of vitamin A by HPLC was about 4.4 min in mare and cow milk. The results showed that vitamin A content of cow milk was higher than that of mare milk in each gram of milk sample. And the vitamin A content of mare cream was lower than that of cow cream in each gram of lipid. Consequently, vitamin A content of cow milk was higher than that of mare milk.

Key words : mare milk, vitamin A, HPLC

INTRODUCTION

Mare milk has interesting nutritional characteristics, especially with regard to diets for the elderly, convalescents and infants. Mare milk has a chemical composition similar to human milk such as a high level of polyunsaturated fatty acid, a low level of cholesterol and a protein distribution, which could permit its use in dietetics(Curadi et al., 2000). It contains less protein, oil and minerals but lactose content is high compared to other animal milks. Mare milk is also highly sensitive to preservation and transformation processes. Mare milk and its derivatives are widely used in the Euroasiatic region and for many years Mongolians used mare milk for making fermented airag. The fermented mare milk is not an exquisite beverage but mare milk is used as a treatment for several diseases as well as tuberculosis, gastro-intestinal disease and rheumatism.

Mineral content of mare milk has been reported to be lower than that of milk of other farm animals. Ash content of mare milk has been reported to be 0.3~0.5%, with extremes of 0.2 and 0.7%. Cow milk contained almost twice as much ash, potassium, phosphorus, magnesium and manganese, 50% more calcium, iron and copper and almost three times as much sodium and zinc as mare milk. The low sodium content of mare milk is a particularly desirable attribute for a dietary component for cardiovascular and hypertension patients(Csapo et al., 1995).

The study concerned with vitamin A in mare milk was no sufficiency. Therefore purpose of this study was to determine the content of vitamin A in mare milk and compare it with cow milk. Vitamin A content of mare and cow milk was determined by HPLC on the individual dairy product.

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MATERIALS AND METHODS

Materials

Mare milk and mare milk oil were obtained from Mogolia. The milk was stored frozen at -70℃. Cow milk and cream were purchased commercially. All trans retinol acetates were from Sigma. The high performance liquid chromatography (HPLC) was from Jasco.

Lipid Extraction of Sample

After sample weight measured, the sample put into the separation funnel. And put 10 mL ammonium solution and phenolphthalein solution of three drops. Add the 50 mL ethanol with 0.1% BHT shaked for 15 sec, the 100 mL ethyl ether shaked for 1 min and 100 mL petroleum ether. And then shake for 1 min. Transfer the supernatant liquid to 500 mL volumetric flask and the ether evaporated at 40℃(Fig. 1).

Preparation of Saponification

1 mL standard solution(retinol acetate diluted with methanol) and 5 mL absoluted ethanol(contain 0.1% ascorbic acid or 1% pyrogallol) put into the 50 mL centrifuge tube. After 2 mL-50% KOH solution put in the centrifuge tube, shake carefully. Then hydrolysis for 20 mins in the water bath at 80℃ and cooling. The ratio of diethyl ether and petroleum ether were 50 to 50. Vortexing for 1 min. After place for 1 min, vortexing for 1min. And put 15 mL water at 1℃ for cooling. After inverting this tube, centrifugation was at 1000 ×g for 10 min below 10℃. Transfer 10 mL of supernatant liquid to 250 mL volumetric flask. Then evaporating below 40℃. 2 mL methanol take out in flask and filtering with 0.2 μm syringe filter. Inject in the HPLC(Fig. 2).

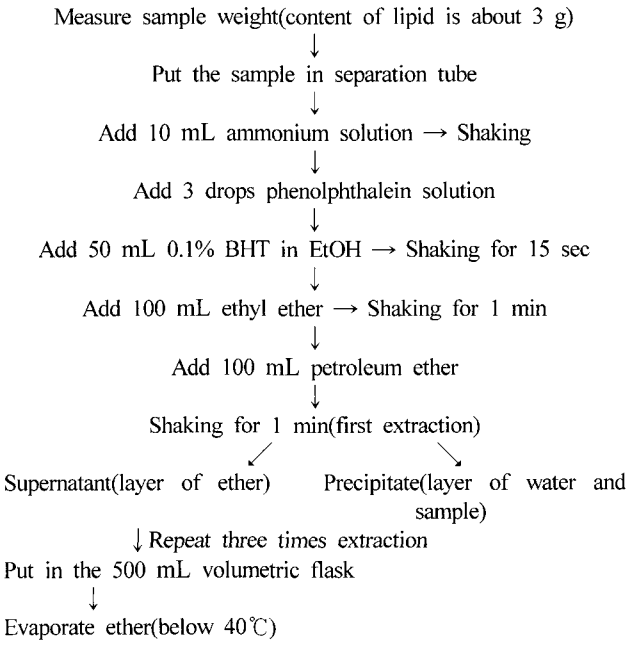

Fig. 1. Diagram of lipid extraction of sample for the vitamin A analysis.

Operation Condition of HPLC

Stationary phase was Crest Pak C18S, stainless steel, 3.9 mm×15 cm from Jasco(Japan). The ratio of acetonitrile, methanol and H_2O was 50 to 42 to 8 for the preparation of mobile phase. The Detector was UV detector from Jasco (Japan). Flow rate was 0.8 mL/min. Injection volume was 20 μL(Table 1).

RESULTS AND DISCUSSION

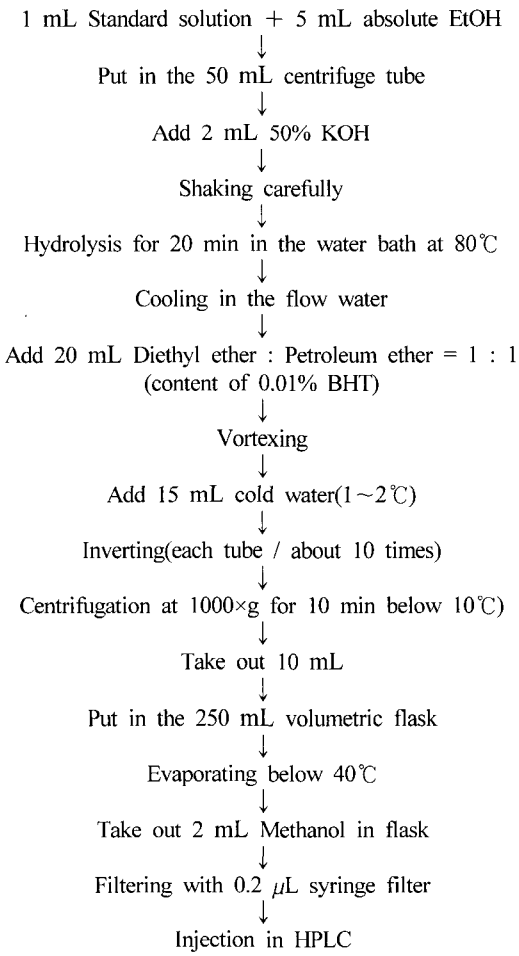

Fig. 2. Diagram of saponification for the vitamin A analysis.

Table 1. Operation condition of HPLC for the analysis of vitamin A

	Information of HPLC
Stationary phase	Crest Pak C18S, stainless steel, 3.9 mm×15 cm(Jasco, Japan)
Mobile phase	Acetonitrile : Methanol : H_2O = 50 : 42 : 8
Detector	UV detector(Jasco, Japan)
Flow rate	0.8 mL/min
Injection vol.	20 μL

Table 2. Lipid content of dairy products*

Sample	Lipid content(%)
Mare milk	1.50
Cow milk	3.69
Mare cream	69.95
Cow cream	36.26

* Mean value of samples analysed in triplicate.

The result of lipid content of mare, cow milk and other dairy products was shown in Table 2. The lipid content of mare cream was 69.95%. It was the highest in other dairy products. As the lipid content of mare and cow milk were 1.50 and 3.69%, respectively, cow milk contains more lipid than mare milk. As the lipid content of processed cow cream was 36.26%, it was lower than that of mare cream.

The standard curve for the determination of vitamin A by HPLC was made with retinol acetate. The results were shown in Table 3. According to the change of dilution concentration, the value of retinol acetate was increased from 10.37 to 99.33 as straight line. Peak area was increased also. So standard equation of retinol acetate was as follows :

$$Y = 13664.36X + 32297.75 (r=0.93)$$

The comparison of vitamin A content in cow milk with mare milk was given in Table 4, Fig. 3 and Fig. 4. The RT of vitamin A by HPLC was about 4.4 min in mare and cow milk. The peak area of cow and mare milk was 423115 and 141345, respectively. Therefore, vitamin A content of cow milk was about three times as much as mare milk. This result was like lipid content of Table 2. The reason was supposed that vitamin A was due to fat soluble vitamin.

Table 3. The standard values of retinol acetate in the analysis by HPLC

Dilution	Con.(I.U/mL)	Peak area
270	10.37	157924
90	31.11	478849
30	93.33	1302279

Table 4. The comparison of vitamin A content in cow with mare milk

Sample	RT(min)	Area	Height
Cow milk	1.579	11546	1381
	2.567	443730	39110
	4.458	**423115**	**20606**
	5.533	26455	1808
Mare milk	2.633	12545	1899
	4.333	**141345**	**6271**
	5.492	654445	32239

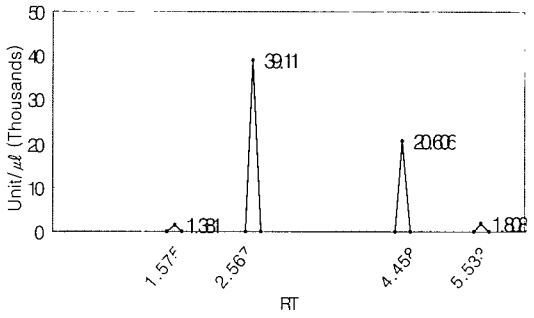

Fig. 3. HPLC chromatogram of vitamin A in cow milk.

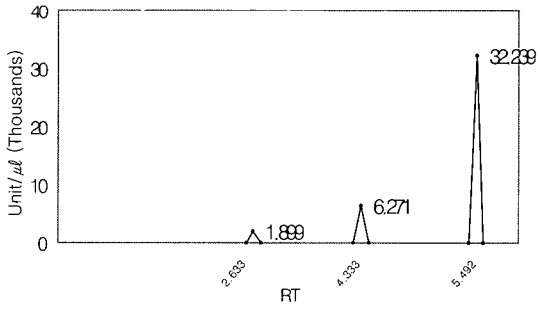

Fig. 4. HPLC chromatogram of vitamin A in mare milk.

The content of vitamin A of dairy products was shown in Table 5. Vitamin A contents of cow milk powder, cow cream and mare milk oil were 406, 562 and 2326 I.U/sample 100 g, respectively. And the content of vitamin A in each gram of lipid was 15.04, 15.49 and 23.26 I.U. Vitamin A content of

Table 5. Vitamin A content of different cow, mare milk and their products*

Sample	Content(I.U)/ Sample 100g	Content(I.U)/Lipid g
Cow milk powder	406	15.04
Cow cream	562	15.49
Cow milk	112	30.41
Mare milk	42	27.99
Mare milk oil	2326	23.26

* Mean value of samples analysed in triplicate.

cow milk was higher than that of mare milk in each gram of lipid. Also vitamin A content of cow milk was higher than that of mare milk in each gram of sample. Consequently, vitamin A content of cow milk was higher than that of mare milk. This result supports previous study that the content of vitamin A of cow milk powder was higher than that of mare milk powder(Marconi et al.,1998).

요 약

마유는 다른 동물의 젖과는 달리 매우 독특한 구성성분을 이루고 있다. 이 연구의 목적은 HPLC를 이용하여 마유의 비타민 A 함량을 결정하고, 우유의 비타민 A 함량과 비교분석하기 위한 것이다. HPLC를 이용하여 마유와 우유의 비타민 A 함량을 분석한 결과 RT는 약 4.4 분이었다. 그리고 시료 100g당 우유와 마유의 비타민 A 함량은 각각 112 I.U, 42 I.U로서 우유가 마유보다 약 2.7배 가량 높았으며, 지방 1g 당 비타민 A 함량은 우유가 30.41 I.U, 마유가 27.99 I.U로 마유가 우유보다 약간 적은 것을 알 수 있었다. 결과적으로 우유의 비타민 A 함량은 마유의 비타민 A 함량보다 높았다.

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