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Adsorption of Cholesterol from Milk Fat in Supercritical Carbon Dioxide

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초임계이산화탄소를 이용한 유지방중의 콜레스테롤 흡착

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Summary

Reduction of cholesterol from milk fat by supercritical CO_2 extraction process in conjunction with an in-line adsorption system was systematically studied. Silicic acid and magnesium silicate were found to be the most suitable adsorbents of six adsorbents tested. Magnesium silicate adsorbed more cholesterol and less milk fat/g of adsorbent than silicic acid. Magnesium silicates of 30/60 mesh and 60/100 mesh show higher adsorption capacities than that of 100/200 mesh. The effective capacity of magnesium silicate was 1.07g of fat/g of adsorbent for 90% cholesterol reduction.

Introduction

Coronary heart diseases have been associated with diet high in cholesterol (Sweeney and Weihrauch, 1976). Because of the national interest in low cholesterol fats, it would be nutritionally and economically advantageous to remove cholesterol from animal fat.

Currently in the food processing industry, extraction techniques using supercritical fluids have been receiving wide interest because this techniques gives advantages over conventional separation methods. The supercritical fluid that had been receiving the largest amount of interest is CO_2 because it has the properties of non-toxicity, nonpolar nature, low cost, and fair solvent power (Rizvi et al., 1986a, b).

To date, studies have attempted cholesterol reduction of milk fat through supercritical fluid extraction (SFE) process. Using a simple, single pass system, Shishikura et al. (1986) were able to remove 92% of the cholesterol but at the expense of removing 75% of the triglycerides. The

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low yield of 25% meant that further research was required to enhance cholesterol selectivity. These researchers tried to overcome the problem of poor cholesterol reduction in simple extraction with supercritical carbon dioxide (SC-CO_2) by passing the solute-laden solvent through a silica gel column. Of the extracts that were passed through the column, 80% of the triglycerides were recovered with a cholesterol reduction of approximately 75%. Rizvi et al. (1989) used magnesium silicate as an adsorbent to remove the cholesterol from milk fat in a batch system and obtained 80% cholesterol reduction with a fat yield of 85%.

Unfortunately, there is no systematic study of reducing cholesterol from anhydrous milk fat (AMF) by SC-CO_2 extraction in conjunction with an in-line adsorption system.

There are several advantages using an in-line adsorption method to remove cholesterol from AMF. These include simple equipment, easy operation of the removal system, and possible high selectivity of cholesterol removal. Adsorption depends primarily on difference in the affinity of the adsorbing species for the adsorbent in a lipid system including cholesterol. Affinity is mainly determined by polar interactions, with nonpolar van der Waals forces playing a minor role. This means that polar grouping in the molecules to be separated exert a much greater effect than nonpolar hydrocarbon chains (Carroll, 1961; Cristie, 1982). Cholesterol in milk fat will be expected to bind to an adsorbent stronger than triglycerides because the polarity of cholesterol is higher than that of triglycerides (Shishikura et al., 1986).

The design of adsorption equipment requires the selection of an adsorbent. Several adsorbents were compared for their relative capacity to hold cholesterol from a hexane solution at room temperature and atmospheric pressure. The two most suitable adsorbents were tested at supercritical condition for their cholesterol adsorption characteristics.

Materials and Methods

Initial experiments were conducted to compare the ability of different adsorbents to adsorb the cholesterol from hexane solution at room temperature and atmospheric pressure. Six adsorbents were tested: Bentonite, Guar gum, carboxymethyl cellulose (CMC), alumina, silicic acid and magnesium silicate. The standard cholesterol solution (1mg/ml) was passed at a rate of 4.2ml/min through the column (0.9cm ×14cm) containing 500mg of different adsorbents. The eluents from each column were collected and their concentrations of the cholesterol were determined by GC (AOAC, 1984).

Commercial grade butter was purchased from the Cornell Dairy Store and converted into AMF by melting at 60℃, decanting the top layer and filtering through Whatman No. 1 filter paper under vacuum. AMF was subsequently stored at -20℃ for future use.

A conventional batch-type SC-CO_2 extraction equipment assembly as shown in Fig. 1 was utilized during this investigation.

The SC-CO_2 extraction was carried out at temperature of 40℃ and pressure of 241 bar as shown in Table 1. AMF was charged into the extraction vessel, then SC-CO_2 was admitted to the system. As the extraction vessel and preheater were heated to the desired temperature, the pressure was increased to the desired limit. The extraction was started by opening the pressure reduction valve, permitting SC-CO_2 to flow through AMF in the extraction vessel. The components of AMF that were sol-

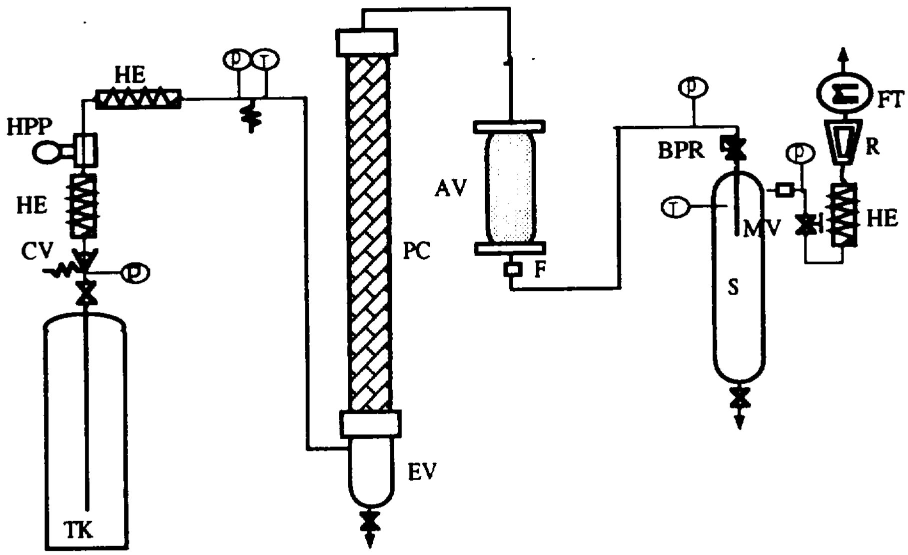

Fig. 1. Flow diagram of supercritical fluid extraction system.
(AV : adsorption vessel, BPR : back pressure regulator, CV : check valve, EV : extraction vessel, F : filter, FT : flow totalizer, HE : heat exchanger, HPP : high pressure pump, MV : metering valve, P : pressure gauge, PC : packed column, R : rotameter, S : separator, T : temperature indicator, TK : carbon dioxide tank)

Table 1. Experimental condition for SC-CO_2 extraction of AMF

Material : 50g anhydrous milk fat (AMF)
Solvent : Carbon dioxide, flow rate : 2.6kg/hr.

Extraction	First step	Second step
Temperature (℃)	40	40
Pressure (bar)	241	241
Separation		
Temperature (℃)	20	20
Pressure (bar)	atm.	atm
Magnesium silicate (g)	20	20

uble in SC-CO_2 passed out of the starting material into the adsorbent bed where cholesterol, phospholipids, cholesteryl ester and some glycerides are preferentially adsorbed and the rest of the triglycerides, still soluble in SC-CO_2, pass through the pressure reduction valve and into the separation vessel. After extracting with known quantities of carbon dioxide, the collected extracts in the separation vessel were removed and quantified. At the end of the process, the residual fraction was recovered from the extraction vessel. Cholesterol analysis of extracts were performed by the AOAC method (1984).

Results and Discussions

Adsorption capacities of several adsorbents at atmospheric pressure

In order to screen better adsorbents for cholesterol, several adsorbents were tested for their adsorption capacities for the cholesterol in hexane solutions at room temperature and atmospheric pressure as shown in Table 2. Silicic acid and magnesium silicate had better adsorption capacities as compared to the other adsorbents. This shows good agreement with the published literatures (Shishikura et al., 1986; Rizvi et al., 1989).

Table 2. Relative cholesterol concentration of eluents for various adsorbents

Adsorbents	GC peak area ratio (cholesterol/α-cholestane)					
	Amount of cholesterol solution passed through the column (ml)					
	10	25	35	45	55	65
Bentonite	2.08	-	-	-	-	-
Gua gum	4.68	-	-	-	-	-
CMC	3.29	-	-	-	-	-
Alumina	0.0	0.0	0.0	0.17	0.25	-
Silicic acid	0.0	0.0	0.0	0.0	0.02	0.24
Magnesium slicate	0.0	0.0	0.0	0.0	0.0	0.01

Adsorption capacities of silicic acid and magnesium silicate at supercritical condition

Two most suitable adsorbents, silicic acid and magnesium silicate, were tested at supercritical condition to compare their adsorption capacities for the cholesterol from milk fat in SC-CO_2 as shown in Table 3. Magnesium silicate had more adsorption capacity for cholesterol in AMF than silicic acid at supercritical conditions, and data taken over E1 to E4 indicated that the adsorbent retention capacity was gradually lost at higher extraction yield. King et al (1988) pointed out that the breakthrough volume asymptotically approaches a limiting volume defined by the void volume of the adsorbent bed as the adsorbent becomes saturated, hence there is a discrete adsorption range over which the adsorbent bed can be used for isolating components which are solubilized in the supercritical fluid phase.

Table 3. Percent cholesterol change of SC-CO_2 fractions

Fractions		Cholesterol change (%)	
		Silicic acid	Magnesium silicate
Extracts	E1	-87.2	-95.1
	E2	-96.9	-97.0
	E3	-93.6	-94.5
	E4	-82.0	-88.5

Table 4 shows the differences in the amount of adsorbate and color of extracts with silicic acid and magnesium silicate. Magnesium silicate adsorbed less milk fat/g of adsorbent at supercritical condition than silicic acid. This means that magnesium silicate had more adsorption capacity available for the cholesterol from AMF than silicic acid at supercritical conditions. Meanwhile, fat passed through magnesium silicate was lighter in color than that through silicic acid. In terms of fat yield efficiency, magnesium silicate was better than silicic acid to increase total yield of extract which is cholesterol-reduced.

Table 4. Quantity of adsorbate and color of extracts

Adsorbents	Quantity of adsorbate (wt%)	Color of extracts
Silicic acid	14.4	yellow
Magnesium silicate	11.5	white

Table 5 showed % cholesterol change in the extract and the residue as a function of the quantity of the residue. As the amount of the residual oil was small, even though % cholesterol reduction in the residue was low, % cholesterol reduction in the extract was high, and the total fat yield was also high. Therefore it is recommended to take more extract, and then leave less residue to increase total cholesterol reduction in the extract.

Based on those results, magnesium silicate turns out to be the best adsorbent in terms of cholesterol reduction and fat yield than silicic acid. Therefore, magnesium silicate was chosen as an adsorbent thereafter.

Table 5. Relationship between amount of residue and % cholesterol change

Trial #	Residue		Total extracts	
	fat yield (wt%)	chol. change (%)	fat yield (wt%)	chol. change (%)
2M	24.1	-37.6	83.3	-80.1
11M	10.9	-31.4	85.6	-88.5
10M	2.4	-18.7	88.5	-92.6

Adsorption capacity of different size of magnesium silicate

Fig. 2. shows the adsorption capacity of different size of magnesium silicate. Magnesium silicates of 30/60 mesh and 60/100 mesh show almost the same adsorption capacity. However, magnesium silicate of 100/200 mesh becomes saturated with the cholesterol after 24g of fat was extracted with 20g of adsorbent. This means that the finer the particles of adsorbent, the greater the surface area available for adsorption and the better the separations that can be obatined. If the particles are too small, however,

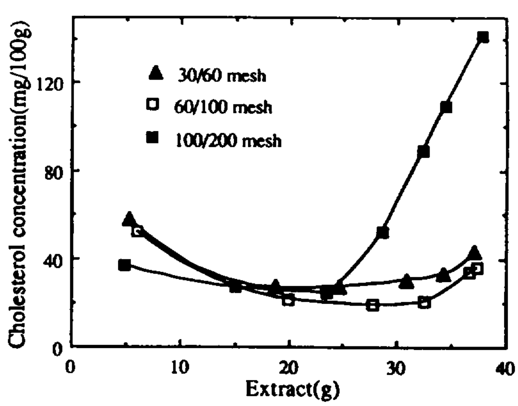

Fig. 2. Adsorption capacity of different size of magnesium silicate.

the flow rate through the column may be rather slow and, in practice, a compromise must be sought which allows reasonable flow rates without unduly prejudicing the quality of the separations (Carroll, 1961).

Breakthrough quantity of magnesium silicate

Fig. 3. shows breakthrough quantity of magnesium silicate for cholesterol adsorption from AMF. It shows that in order to get more than 90% cholesterol reduced-milk fat, 20g of fresh magnesium silicate had to be repaked in the adsorption column after about 43% of the milk fat was extracted. Therefore, the effective capacity of magnesium silicate was 1.07g of fat/g of adsorbent for 90% cholesterol reduction.

Based upon those results presented here, supercritical SC-CO_2 extraction in conjunction with an in-line adsorption of cholesterol in AMF by magnesium silicate was found to provide a promising procedure which was commercially viable for lowering the cholesterol level in AMF.

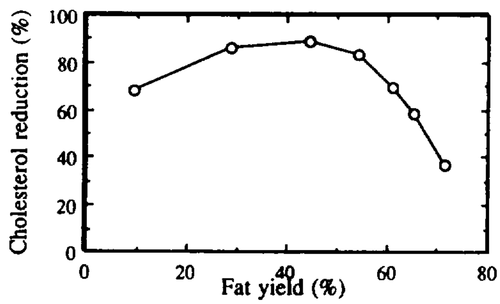

Fig. 3. Breakthrough curve of magnesium silicate for cholesterol adsorption from AMF.

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〈국문초록〉

초임계이산화탄소를 이용한 유지방중의 콜레스테롤 흡착

초임계 이산화탄소에 의한 추출장치에 흡착장치를 부착하여 유지방으로부터 콜레스테롤을 제거하는 연구가 수행되었다. 실험한 여섯종의 흡착제중 실리실산과 마그네시움 실리케이트가 가장 좋은 콜레스테롤 흡착성을 보였다. 마그네시움 실리케이트는 실리실산보다 흡착제 g당 더 많은 콜레스테롤을, 그리고 더적은 유지방을 흡착하였다. 30/60 메쉬와 60/100 메쉬의 마그네시움 실리케이는 100/200 메쉬의 마그네시움 실리케이트보다 높은 콜레스테롤 흡착성을 보였다. 마그네시움 실리케이트의 유효용량은 90% 콜레스테롤 제거에 대해서 흡착제 g당 1.07g의 지방이었다.