Kasetsart J. (Nat. Sci.) 37 : 353 - 367 (2003)

Reduction of Cholesterol in the Spray Dried Egg Yolk by Super Critical Carbon Dioxide Extraction

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ABSTRACT

The reduction of cholesterol in the spray dried egg yolk was studied by using supercritical carbondioxide extraction. Cholesterol contents were determined by gas chromatographic method (GC). The cholesterol content in dried egg yolk had mean value of 2321.40 mg cholesterol/100 g test sample. A statistical 3^3 factorial experiment in completely randomized design was performed to study the effect of factors in reduction of cholesterol in the spray dried egg yolk by supercritical carbondioxide extraction. These factors were 3 levels of CO_2 temperature : 40°C, 45°C and 50°C, 3 levels of CO_2 density : 0.70 g/ml, 0.80 g/ml and 0.90 g/ml and 3 levels of extraction time : 30 min, 45 min and 60 min respectively. The residual content of cholesterol in the spray dried egg, after extraction was determined by GC. Duncan's new multiple range test (DMRT) was used to differentiate treatment means. The results indicated that the factors effecting the extraction of cholesterol in the spray dried egg yolk were CO_2 density, CO_2 temperature and extraction time, respectively in order of importance. Response surface methodology (RSM) was used to determine optimum condition of cholesterol extraction. The processing conditions of 50°C temperature, 0.90 g/ml density, 350 bar pressure and 60 min extraction time gave the optimum condition of cholesterol extraction at 64.07 %. Supercritical carbondioxide extraction of the spray dried egg yolk had dramatic effect on proximate composition of the end product. The protein content increased 19.53 % and phospholipids content increased 18.74 %, but total lipid content reduced 17.39 %. The solubility value and emulsifying capacity value of the treated spray dried egg yolk were 16.31 % and 292.05 ml of oil/g protein, respectively. Consumer acceptability test indicated that the treated spray dried egg yolk can produce emulsifying product of good quality comparable to the fresh egg yolk.

Key words: super critical CO_2, cholesterol extraction, spray dried egg yolk

INTRODUCTION

Eggs are good protein food and rich in amino acids and essential fatty acids. The nutrients are found mostly in egg yolk. An egg of 50 grams contains about 213 mg. of Cholesterol or 0.43%. Consumers are concern about high level of cholesterol in egg yolk which may related to the blood clot in the veins leading to heart failure. There have been attempts to reduce the cholesterol in egg yolk by genetic selections, changing the feed formula with limited success because it may effect the egg production and survival of the embryo. Chemical and biochemical methods have been used such as hexane, ethanol and cyclodextrin to dissolve cholesterol but it may cause residual problems and changes in functional properties of egg yolk and protein denaturation. Reduction of

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Received date : 05/06/03 Accepted date : 09/09/03

cholesterol in egg yolk by super critical carbon dioxide (SC-CO_2) seems to be the best.

Cholesterol has chemical formula $C_{27}H_{46}O$ as shown in Figure 1 with melting point at 149-151°C (Kirschenbauer, 1960). It is a sterol which is white crystal, water insoluble, partly soluble in ethanol, benzene and petroleum ether, found mostly in animal tissue (Caret, 1995) Its functions to the body are as following; l. a component of plasma membrane which control the liquid state of the cell wall 2. a precursor of many hormones such as progesterone, androgen and estrogen 3. a transporter of fatty acid through the blood system 4. may change to vitamin D when exposed to UV light (Greenwald, 1991). The body obtains cholesterol from the food and it can be synthesized in the body as shown in Figure 2. The food with high cholesterol content are egg yolk, bacon, shrimp, crab, butter and cheese. Consumers should select low cholesterol food and polyunsaturated fat that help to reduce the cholesterol in the plasma and prevent the thrombosis. High level of cholesterol in the blood is resulted from low density lipo protein Cholesterol (LDL cholesterol) which may cause heart disease cholesterol is a co-carcinogen (Sakorn *et al.*, 1991. People with high level of cholesterol in the blood has 3 times more risk in getting heart disease. The high level of blood cholesterol is due to Low Density Lipid protein cholesterol (LDL cholesterol). It was found that 1% reduction in blood cholesterol would reduce the chance of getting heart disease by 2%. The factors causing high level of LDL cholesterol in blood are; 1. the body obtains too much cholesterol from the food 2. the liver synthesizes too much cholesterol 3. other tissues lack of LDL receptor, thus can not receive cholesterol from the blood.

Most of lipids in eggs are in egg yolk which is necessary for embryo development. Egg yolk is composed of 62.3% triglyceride, 32.8% of phospholipids and 4.9% sterol. Most of the sterol are cholesterol (USDA, 1989). The components of egg yolk and its lipids are shown in Figure 3a and b. Reduction of cholesterol in egg yolk is necessary because of consumers need to prevent heart disease. Extraction of cholesterol in spray dried egg yolk by Super Critical CO_2 was done by increasing temperature and pressure of SC-CO_2 to increase density and solvent power of CO_2. Cholesterol will dissolve in SC-CO_2 which will act as supercritical fluid (SCF) as shown in Figure 5. The molecules of solute will be surrounded by the molecules of SC-CO_2 and react with each other. The enthalpy is reduced and causing cholesterol to dissolve. CO_2 is selected because of its non-polar, non-flammable, non-toxic, non-stain and availability. It can be easily removed from the product at room temperature and normal pressure. SC-CO_2 will have lower viscosity but higher diffusivity to speed up the cholesterol extraction. SCF technology has been used in de-caffeinating of coffee, hop extraction, purification of vanillin, production of deflated soybean products. There are 3 related factors influencing the extraction by SC-CO_2. They are diffusion, solubility and matrix as shown in Figure 4. SC-CO_2 could be used to

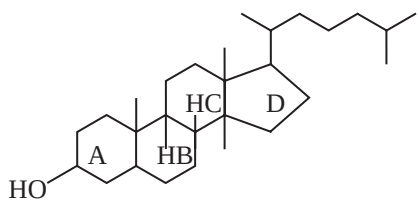

Figure 1 Structure of Cholesterol from Voet and Voet (1995).

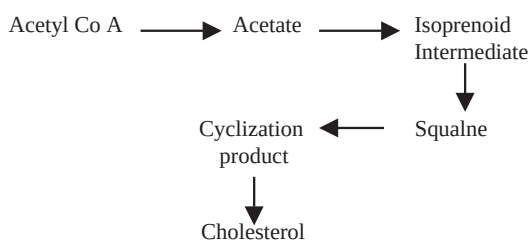

Figure 2 Cholesterol synthesis diagram.
Source : Voet and Voet (1995).

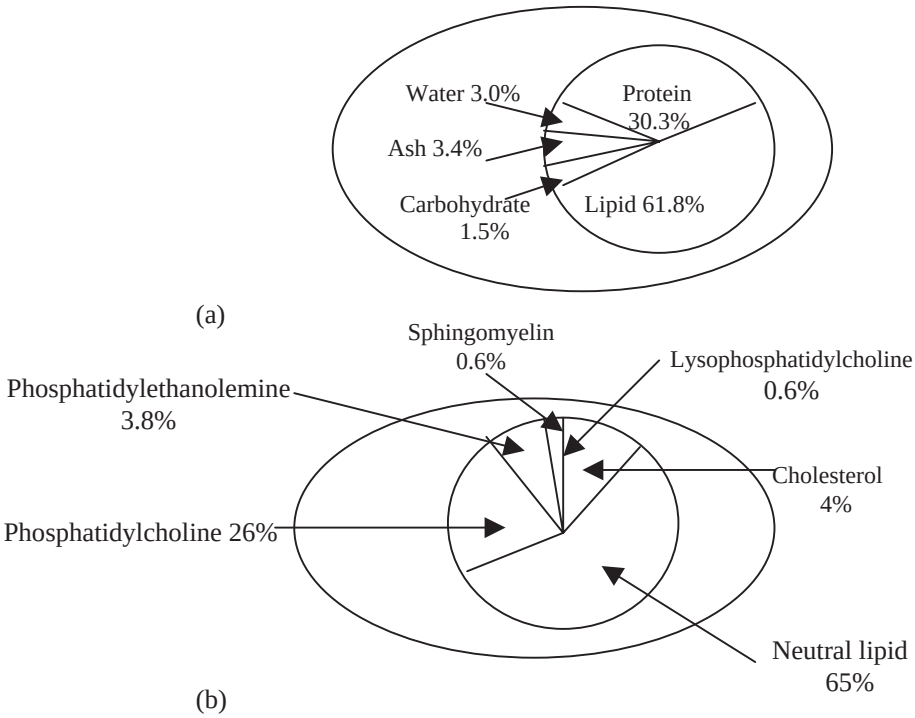

Figure 3 a. Components of egg yolk powder b. Components of lipids in egg yolk.

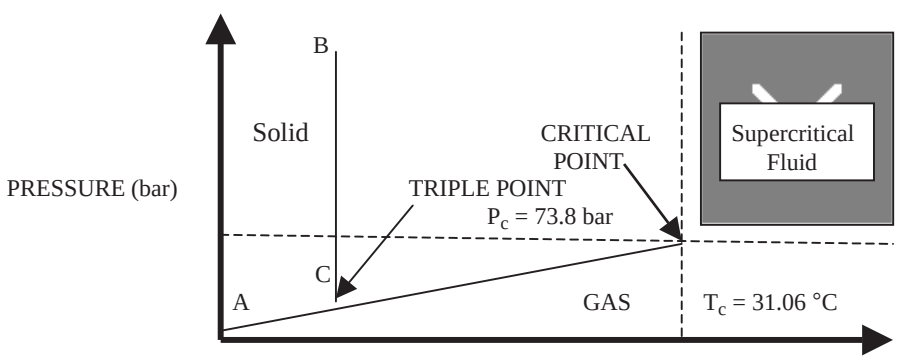

Figure 4 Diagram of CO_2 cycle modified from Clifford (1993) and Rizvi *et. al.* (1986).

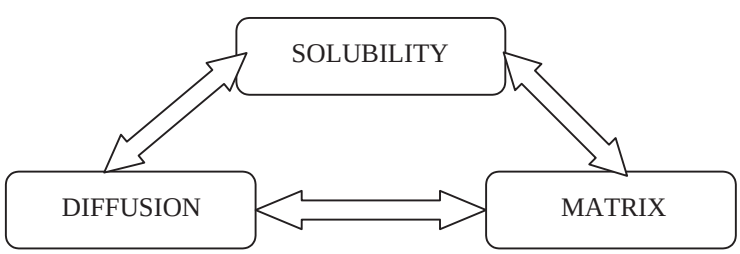

Figure 5 Relationship of factors effecting extraction by SC-CO_2 from Clifford (1993).

extract cholesterol from egg yolk powder without any effect on its functional properties (Froning *et al*., 1990; Zeidler *et al.,* 1994).

This study is to find factors effecting cholesterol reduction in spray dried egg yolk by SC-CO_2 by using different conditions than those previous studies. Also to find optimum temperature density of CO_2 and extraction time and test the product quality.

MATERIALS AND METHODS

1. Measurement of cholesterol in spray dried egg yolk by Gas Chromatography according to Klantt *et al.* (1995). The samples were analyzed for the mean, standard deviation (S.D.) and coefficient of variation (C.V.)

2. Moisture content was measured according to AOAC (1990).

3. The color was obtained from Ultra Scan XE Hunter Colorimeter

4. Sensory evaluation was done by preparing mayonnaise samples using raw egg yolk, spray dried egg yolk and SC-CO_2 treated spray dried egg yolk as emulsifier and tested by 30 consumers using Hedonic scale (1-9).

5. SC-CO_2 equipment set up was shown in Figure 6. Liquid CO_2 in supply Tank (ST) would be pumped under high pressure by the compressor (C) which increased the pressure of CO_2 to 680 atm (10,000 psi). The CO_2 was filtered thru in line filter (5 micron, F) and sent to the compressor. Extraction vessel (EV) and separation vessel (SV) have 300 and 103 ml. capacity respectively. EV contains 115 g feed with fiber glass cover and thermocouple (T) inserted into the extraction tank. Heating was done by electrical element from outside and controlled by indicating temperature controller (ITC). Separation vessel was kept at ambient temperature. The pressure of both EV and SV were controlled by back pressure regulator (BPR) and read the value from the pressure gauge (P). CO_2 would act as solvent, it was changed to SC-CO_2 to dissolve cholesterol from spray dried egg yolk. The samples were measured for exact 2g weight, put into the thimbles, closed the caps and put into carrousels. The operations of SFE 768OT were controlled by the

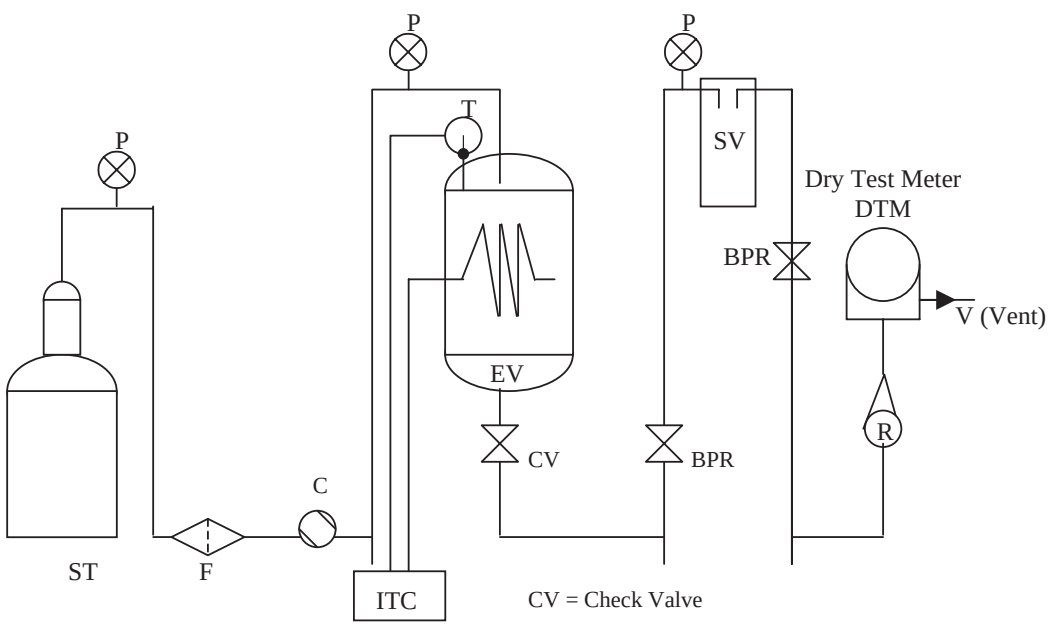

Figure 6 Super critical fluid extraction from Froning *et al.,* (1990)

computer. Before the operations, the extraction methods and sequences would be programmed into the computer. After starting the carrousels would rotate into the extraction chamber and cholesterol was extracted. The thimbles were then rotated to outside, samples were weighed and analyzed for cholesterol content and evaluated for the quality

6. **Experimental plan** Three factors were selected as follows : extraction time, temperature and density of CO_2 using 3^3 factorial experiment in completely randomized design with 2 replications, total of 54 runs. Factor A or the temperature had 3 levels; $a_1 = 40°C$, $a_2 = 45°C$ and $a_3 = 50°C$. Factor B or CO_2 density with 3 levels; $b_1 = 0.70$, $b_2 = 0.80$ and $b_3 = 0.90$ g/ml respectively. Factor C or extraction time had 3 levels; $c_1 = 30$, $c_2 = 45$ and $c_3 = 60$ min. respectively. Analysis of data was done using SAS program to get assumption underlying the analysis of the variation. Also to find factors that significantly effect the response of the variables and interaction between 2 and 3 factors. The data were analyzed for variation and compared means between treatments using Multiple Comparisons or Duncon's New Multiple Range Test (DMRT). Those significant factors were analyzed by response surface methodology (RSM) to find the trend of changes in the response and optimum level of the factors, extracted samples were analyzed for phosphorus phosphatide and lecithin using method of Pulliaien and Wallin (1994). Physical properties of the samples were studied; emulsifying by Caldironi and Ockerman, (1982); solubility by Suzuki and Shimizu (1982) and color using Hunter Color : meter.

RESULTS AND DISCUSSION

1. Factors effecting reduction of cholesterol in spray dried egg yolk by SC-CO_2 extraction

1.1 Checking certain properties of spray dried egg yolk

1.1.1 The average amount of cholesterol in initial spray dried egg yolk measured by GC. The average was 2,321.40 mg. cholesterol / 100 g as shown in table 1. The results were similar to those reported by campo (1994). The precision

Table 1 The amount of cholesterol in spray dried egg yolk analyzed by Gas Chromatograph.

Order	Weight of sample[1/] (g)	Gram sample/ml derivatized	mg/ml cholesterol in sample on standard curve	Mg cholesterol/100 g test sample
1	2.016	0.033	0.783	2330.703
2	2.013	0.033	0.773	2305.575
3	2.015	0.033	0.763	2274.526
4	2.016	0.033	0.765	2276.356
5	2.014	0.033	0.775	2311.833
6	2.016	0.033	0.800	23820839
7	2.017	0.033	0.793	2360.991
8	2.019	0.033	0.784	2331.148
9	2.016	0.033	0.779	2318.898
			Mean	2321.40
			Standard deviation, SD	35.46
			Coefficient of variation, CV	1.53

Note 1/ is the weight of spray dried egg yolk exclude the moisture; initial moisture is 3.27%

was good with low C.V. and S.D.

1.1.2 The total average moisture content of the sample was 3.27%.

1.1.3 The color values of the samples or L, a, b values were 81.54, 4.08 and 23.38 respectively, L indicates brightness, +a for redness and +b for yellowness.

1.2 Factors effecting cholesterol extraction from spray dried egg yolk by SC-CO_2 extraction

Figure 7 showed the cholesterol extracted spray dried egg yolk samples and extracted cholesterol which was further diluted in hexane. The sample was brighter, not caking and drier as compared to the control. Table 2 showed cholesterol

Table 2 Effect of temperature, density and pressure of CO_2 to cholesterol extraction in spray dried egg yolk by SC-CO_2.

Treatment	Code no. of sample	°C	Density (g/ml)	Time (min)	Pressure (Bar)	Cholesterol left in samples (mg/100 g)	% of extracted cholesterol
Control	-	-	-	-	-	2320.46	0.00
1	a1b1c1	40	0.70	30	115	2229.96	3.94
2	a1b1c2	40	0.70	45	115	2151.67	7.32
3	a1b1c3	40	0.70	60	115	2108.21	9.19
4	a1b2c1	40	0.80	30	164	2026.08	12.73
5	a1b2c2	40	0.80	45	164	1939.65	16.45
6	a1b2c3	40	0.80	60	164	1896.27	18.32
7	a1b3c1	40	0.90	30	281	1840.43	20.72
8	a1b3c2	40	0.90	45	281	1626.59	29.93
9	a1b3c3	40	0.90	60	281	1325.40	42.91
10	a2b1c1	45	0.70	30	133	2181.39	6.03
11	a2b1c2	45	0.70	45	133	2115.97	8.85
12	a2b1c3	45	0.70	60	133	2058.78	11.31
13	a2b2c1	45	0.80	30	189	1927.20	16.98
14	a2b2c2	45	0.80	45	189	1783.89	23.16
15	a2b2c3	45	0.80	60	189	1692.40	27.10
16	a2b3c1	45	0.90	30	316	1586.95	31.55
17	a2b3c2	45	0.90	45	316	1373.90	40.82
18	a2b3c3	45	0.90	60	316	1138.07	50.98
19	a3b1c1	45	0.70	30	151	2140.80	7.78
20	a3b1c2	50	0.70	45	151	2073.49	10.68
21	a3b1c3	50	0.70	60	151	2018.87	13.03
22	a3b2c1	50	0.80	30	214	1744.03	24.87
23	a3b2c2	50	0.80	45	214	1656.91	18.63
24	a3b2c3	50	0.80	60	214	1563.00	32.67
25	a3b3c1	50	0.90	30	350	1505.57	35.15
26	a3b3c2	50	0.90	45	350	1249.16	46.19
27	a3b3c3	50	0.90	60	350	833.99	64.07

reduction in SC-CO_2 treated samples obtained under different temperature, density and pressure of SC-CO_2 at different extraction times which indicated that the increase of the treated conditions resulted in the higher amount of cholesterol being extracted. This result agreed with the results of Froning *et al.* (1990) and Warren *et al.* (1991). The

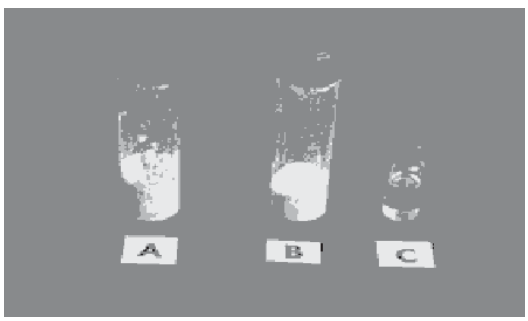

Figure 7 Sample of spray dried egg yolk.
A. Regular spray dried egg yolk
B. Spray dried egg yolk extracted by SC-CO_2
C. Extracted material diluted in hexane

data of extracted cholesterol were analyzed for variation and influence of various factors as shown in Table 3. The above factors had significantly effects ($p < 0.01$) on cholesterol extraction. There were also interaction between two factors, temperature and density of SC-CO_2 ; extraction time and SC-CO_2 density that had highly significant effects ($p < 0.01$) on cholesterol reduction. The above 3 factors has highly significant effects ($p < 0.01$) on the moisture content of the samples as shown in Figure 8. Increasing those conditions would reduce the moisture content. The above factors also had highly significant effects ($p < 0.01$) on color values or L, a and b as shown in Figures 9, 10 and 11 respectively. Increasing conditions of these factors resulted in more L value (brightness) lower "a" value (redness) and lower "b" value (yellowness). Froning *et al.*, and Unger (1995) reported that SC-CO_2 could extract pigment from the spray dried egg yolk

1.3 Effects of each factor on cholesterol reduction

Table 3 Analysis of variance to test influence of various factors effecting the amount of extracted cholesterol in spray dried egg yolk by SC-CO_2.

Source	DF	Anova SS	Mean Square	F Value	Pr > F
A	2	1068.70	534.33	331.47	0.0001**
B	2	8788.87	4393.43	2725.41	0.0001**
C	2	1247.01	623.50	386.78	0.0001**
A*B	4	255.83	63.96	39.67	0.0001**
A*C	4	5.68	1.42	0.88	0.4883ns
B*C	4	509.87	127.47	79.07	0.0001**
A*B*C	8	24.24	3.03	1.88	0.1053ns

Note:- ** means statistically highly significant different ($P < 0.01$)
ns = no significant difference
A = temperature of CO_2 (°C)
B = density of CO_2 (g/ml)
C = time (min.)
A*B = interaction between A and B
A*C = interaction between A and C
B*C = interaction between B and C
A*B*C = combined interaction between A, B and C

1.3.1 Effect of temperature Table 4 and Figure 12 showed that the amount of extracted cholesterol increased significantly ($p < 0.05$) with increasing SC-CO_2 temperature at constant density.

1.3.2 Effects of SC-CO_2 density. Table 5 and Figure 13 showed that increasing CO_2 density would increase the amount of extracted cholesterol significantly ($p < 0.05$). The best conditions were at 50°C and 0.90 g/ml density of SC-CO_2. The pressure of SC-CO_2 would vary directly with density and temperature. But the density had more influence than the temperature in cholesterol

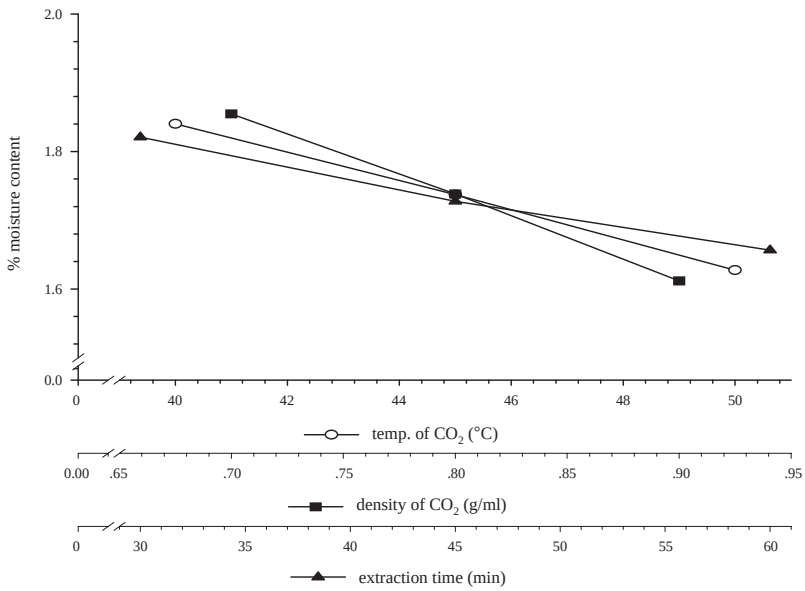

Figure 8 Influence of various factors on the moisture content of spray dried egg yolk extracted by SC-CO_2.

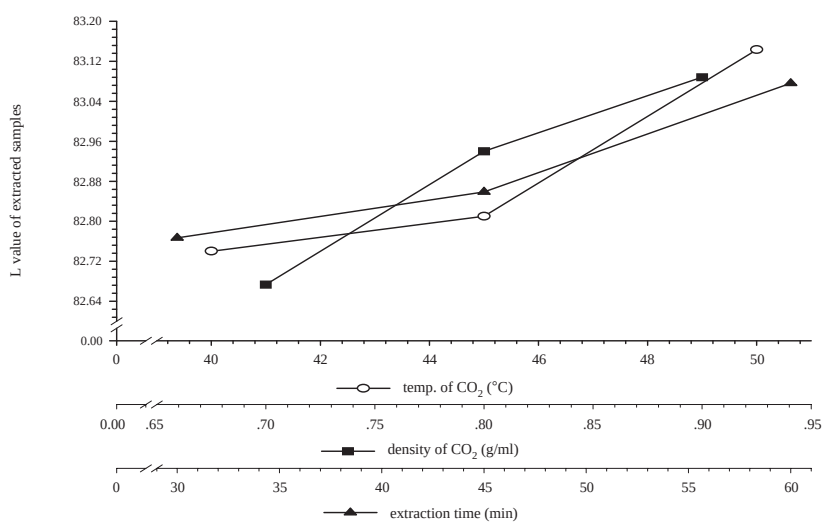

Figure 9 Influence of various factors on L value of spray dried egg yolk extracted by SC-CO_2.

reduction.

1.3.3 Effects of extraction time on cholesterol extraction were shown in Table 6 and Figure 14. The extracted cholesterol increased significantly ($p < 0.05$) with increase in extraction time. The best conditions were 60 minutes and 0.90 g/ml of SC-CO_2 at 50°C.

2. Statistical analysis of data from cholesterol extraction were shown in Table 7 and 8. Response surface analysis was done with SC-CO_2 temperature (X_1), density (X_2) and extraction time (X_3) to the extracted cholesterol (Y). The regression analysis gave response highly significant ($p < 0.01$) to cholesterol extraction in linear, quadratic and cross product, Different levels of SC-CO_2 density ($X_2 * X_2$) had highly significant on cholesterol extraction ($p < 0.01$). Combination of both SC-CO_2 density and temperature ($X_2 * X_1$)

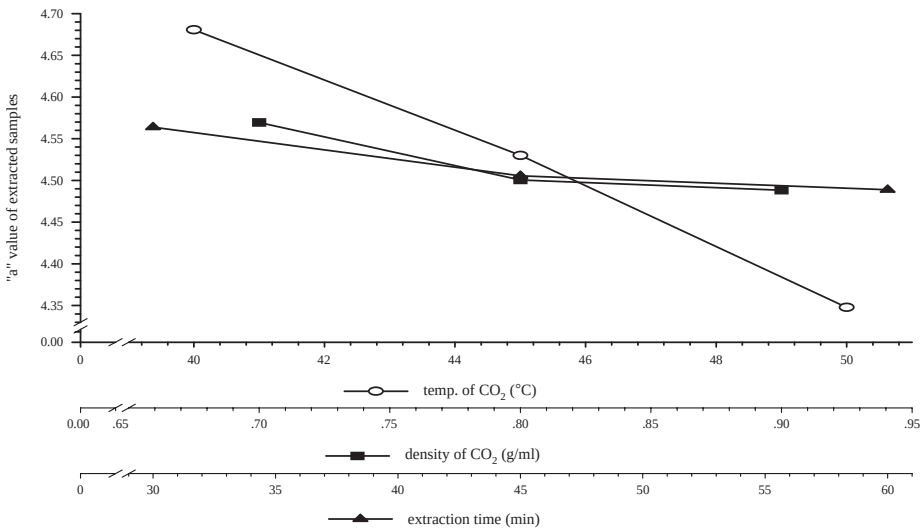

Figure 10 Influence of various factor on "a" value of Spray dried egg yolk extracted by SC-CO_2.

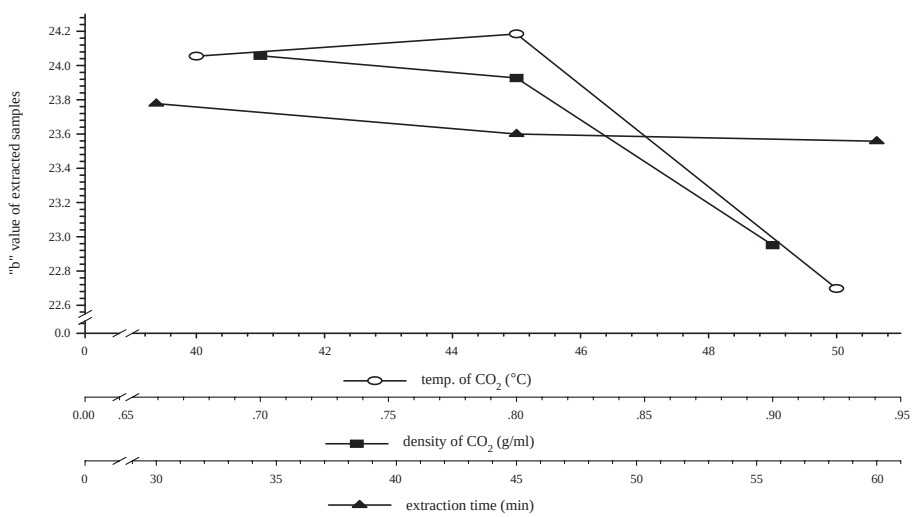

Figure 11 Influence of various factors on "b" value of spray dried egg yolk extracted by SC-CO_2.

as well as SC-CO_2 density and extraction time (X_2 * X_3) had highly significant effects ($p < 0.01$) on cholesterol extraction. From Table 9 the estimated parameters were used to write the equation to predict the response.

$$Y = 233.5670 - 1.5102X_1 - 559.55213X_2 - 2.1612X_3 - 0.0288X_1X_1 + 6.1675X_2X_1 + 194.1667X_2X_2 + 0.0057X_3X_1 + 2.8261X_3X_2 - 0.0003X_3X_3 \quad (1)$$

The best conditions for cholesterol extraction in spray dried egg yolk by SC-CO_2 were at 50°C, 0.9 g/ml density, 350 Bar pressure and 60 minutes extraction time which could reduce 64.07% of cholesterol in the samples. This was in agreement with Froning *et al.* (1990) that could get 65% cholesterol reduction in the spray dried egg yolk using SC-CO_2 at 55°C, 0.90 g/ml density at 380 Bar pressure for 60 minutes extraction time.

Table 4 Effects of CO_2 temperature on the extraction of cholesterol by SC-CO_2.

Extracting conditions			Mean or percent[1/] of extracted cholesterol
CO_2 density (mg/ml)	CO_2 temp (°C)	CO_2 pressure (Bar)	
	40	115	6.812f
0.7	45	133	8.730ef
	50	151	10.495ef
	40	164	15.828dc
0.8	45	189	22.410cd
	50	214	28.722bc
	40	281	31.185b
0.9	45	316	41.145a
	50	350	47.203a

Note [1/] the same alphabet in the same set of data indicated no statistically different ($P > 0.05$)

Table 5 Effects of CO_2 density on cholesterol extraction in spray dried egg yolk by SC-CO_2.

Testing conditions			Mean of extracted cholesterol[1/]
Temperature of CO_2 (°C)	Density of CO_2 (mg/ml)	Pressure of CO_2 (Bar)	
	0.70	155	6.812f
40	0.80	164	15.828dc
	0.90	281	31.185b
	0.70	133	8.730ef
45	0.80	189	22.410cd
	0.90	316	41.145a
	0.70	151	10.495cf
50	0.80	214	28.722bc
	0.90	350	47.203a

Note [1/] the same alphabet in the same set of data indicated no statistically different ($P > 0.05$)

Noticed that his method used higher pressure than our method.

3. Testing the quality of spray dried egg yolk extracted by SC-CO_3

3.1 The SC-CO_2 extracted samples were analyzed as shown in Table 10. The moisture content, % cholesterol, % protein, % lipid and % phosphatide in SC-CO_2 extracted spray dried egg yolk were significantly different ($p<0.05$) from the control. There were reductions of 17.39% lipid but increased in protein and phosphatide 19.53% and 18.74% respectively.

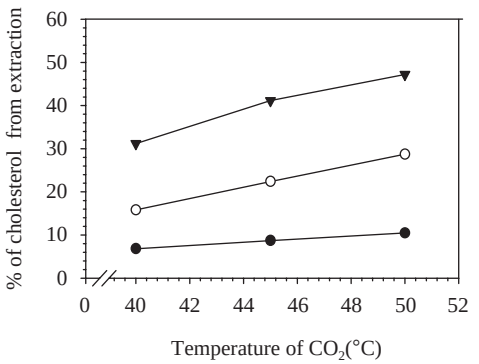

Figure 12 Relationship between the mean of extracted cholesterol to CO_2 temperature at constant CO_2 density.
—●— CO_2 density 0.7 g/ml
—○— CO_2 density 0.8 g/ml
—▼— CO_2 density 0.9 g/ml

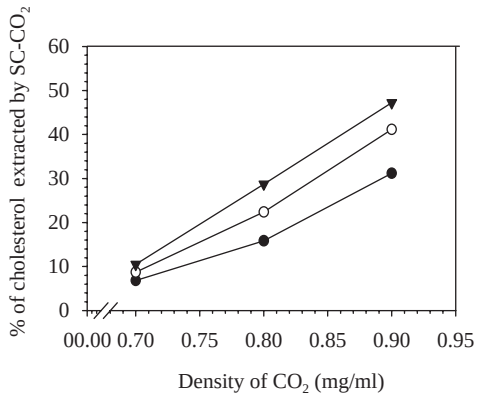

Figure 13 Relationship between the mean of extracted cholesterol to density of CO_2 at constant temperature of CO_2.
—●— CO_2 temperature 40°C
—○— CO_2 temperature 45°C
—▼— CO_2 temperature 50°C

Table 6 Relationship between CO_2 density and extraction time on the amount of extracted cholesterol by SC-CO_2.

Test conditions		% of extracted cholesterol
Density of CO_2 (mg/ml)	Time (min)	
	30	5.915c
0.7	45	8.917c
	60	11.175c
	30	18.192d
0.8	45	22.742ed
	60	26.027c
	30	29.168c
0.9	45	38.980b
	60	51.385a

Note [1/] the same alphabet in the same set of data indicated no statistically different ($P < 0.05$)

Table 7 Experimental plan and results of the tests.

Treatment	Free variables[1/]			Response[2/]	
	X1	X2	X3	Y1[3/]	Y2[3/]
1	40	0.70	30	4.06	3.81
2	40	0.70	45	7.79	6.84
3	40	0.70	60	8.21	10.16
4	40	0.80	30	12.36	13.09
5	40	0.80	45	17.00	15.89
6	40	0.80	60	18.58	18.05
7	40	0.90	30	18.80	22.64
8	40	0.90	45	29.57	30.29
9	40	0.90	60	42.18	43.63
10	45	0.70	30	6.64	5.42
11	45	0.70	45	8.53	9.17
12	45	0.70	60	12.13	10.49
13	45	0.80	30	17.43	16.53
14	45	0.80	45	23.58	22.73
15	45	0.80	60	27.52	26.67
16	45	0.90	30	31.97	31.13
17	45	0.90	45	41.88	39.76
18	45	0.90	60	51.86	50.09
19	45	0.70	30	7.42	8.14
20	50	0.70	45	11.20	10.15
21	50	0.70	60	14.05	12.01
22	50	0.80	30	25.72	24.02
23	50	0.80	45	29.56	27.69
24	50	0.80	60	33.56	31.78
25	50	0.90	30	34.10	36.19
26	50	0.90	45	45.36	47.02
27	50	0.90	60	65.34	62.80

Note [1/] X1 = CO_2 temp. (°C), X2 = CO2 density (g/ml)
X3 = extraction time (min)
2′ = results of the test
3′ = Y1 (% extracted cholesterol from first replication)
Y2 (% extracted cholesterol from second replication)

Table 8 Regression analysis of the results.

Regression	Degree of Freedom	Sum of Squares	R-Square	F-Ratio	Prob > F
Linear	3	11051.000	0.925	925.200	0.000**
Quadratic	3	51.567	0.004	4.317	0.009**
Cross product	3	663.950	0.055	55.585	0.000**
Total regress	9	11766.000	0.985	328.400	0.000

Note ** = statistically highly significant different

3.2 A study of certain physical properties the SC-CO_2 extracted samples had significantly higher percent solubility ($p< 0.05$) and more emulsifying capacity ($p < 0.05$) than the control as shown in Table 11. The treated samples showed more brightness but less redness and less yellowness than control.

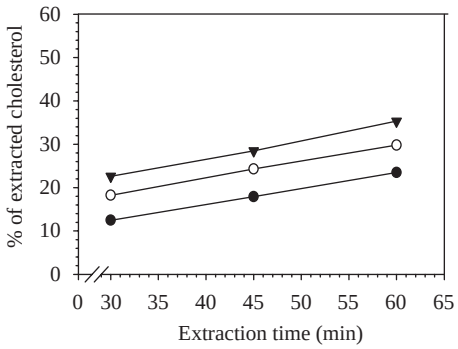

Figure 14 Relationship between the mean of extracted cholesterol extraction time at constant CO_2 temperature.

3.3 Sensory test; the SC-CO_2 extracted sample was used to make mayonnaise and compared with those made from raw egg yolk and regular spray dried egg yolk as shown in Table 12. Their color appearance, flavor, mouth feel and overall acceptability scores were no different ($p < 0.05$) than those made from raw egg yolk. Thus the spray dried egg yolk by SC-CO_2 was well accepted by the consumers.

CONCLUSION

1. There was 64.07% cholesterol reduction in spray dried egg yolk samples extracted by SC-CO_2.

2. Factors effecting cholesterol extraction by SC-CO_2 in order of importance were density, temperature of SC-CO_2 and extraction time. There were interactions between density of SC-CO_2 and

Table 9 Estimation and test of regression coefficient of the equation representing cholesterol extraction in spray dried egg yolk by SC-CO_2.

Parameter	Degrees of freedom	Parameter estimate	Standard error	T for HO : Parameter = 0	Prob > |T|
INTERCEPT	1	233.567	67.740	3.448	0.001
X1	1	-1.510	2.188	-0.690	0.494^{ns}
X2	1	-599.551	99.991	-5.596	0.000**
X3	1	-2.161	0.400	-5.395	0.000**
X1*X1	1	-0.029	0.023	-1.251	0.217^{ns}
X2*X1	1	6.167	0.814	7.571	0.000**
X2*X2	1	194.166	57.602	3.371	0.001**
X3*X1	1	0.005	0.005	1.055	0.297^{ns}
X3*X2	1	2.826	0.217	10.408	0.000**
X3*X3	1	0.000	0.002	0.152	0.880ns

Note : - ^{ns} No statistical significant different ($P > 0.05$)
** statistically highly significant different ($P < 0.01$)
X1*X1 = different temp. levels
X2*X2 = different density levels
X3*X3 = different extraction time
X2*X1 = interaction between density and temp. of CO_2
X3*X1 = interaction between time and CO_2 temp.
X3*X2 = interaction between time and CO_2 density

Table 10 Comparison of different important components in raw egg yolk, spray dried egg yolk and SC-CO_2 extracted yolk.

Sample	Amount of important component[1/]				
	% moisture[2/] content	Cholesterol[2/] (mg/100 g)	% protein[2/]	% lipid[2/]	% phosphatide[2/]
Raw egg yolk	51.29a	1153.47b	15.35c	31.68c	17.68c
Spray dried egg yolk	3.27b	2321.40a	32.07b	63.21a	27.81b
SC-CO_2 extracted	1.37c	833.99c	51.60a	45.82b	46.55a

Note [1/] = mean from analysis
[2/] = sample with some alphabet were no statistically different (P > 0.05)

Table 11 Physical properties of spray dried and SC-CO_2 extracted and raw egg yolk.

Sample	Physical properties[1/]				
	% solubility[2/]	Emulsification[2/] capacity (ml. Oil/ 1 g protein	Hunter color values[2/]		
			L	a	b
Raw egg yolk	10.42c	153.24c	64.28d	6.35a	31.56c
Spray dried egg yolk	14.35b	184.61b	81.54b	4.06b	23.38a
SC-CO_2 extracted	16.13a	292.05a	84.94a	3.93c	16.44b

Note [1/] = the mean from analysis samples
[2/] = the same alphabet indicated no statistically significant different (P > 0.05)

Table 12 Average likeness score of consumer to the sensory test of the mayon naise prepared by using 3 different emulsifier

Sensory quality	Average likeness score[1/]		
	Formula 1	Formula 2	Formula 3
1. Color of mayonnaise[2/]	6.67^a	5.43^b	6.23^a
2. Appearomce[2/]	6.63^a	5.30^b	6.66^a
3. Falvor[2/]	5.60^a	5.30^b	5.67^a
4. Viscosity in mouty[2/]	6.0^a	5.17^b	6.10^a
5. Total score	6.30^a	5.27^b	6.27^a

Note [1/] is the average score from the consumer test which formula 1. Is the emulsifier using liquid egg yolk. Formula 2 used spray dried egg yolk and. Formula 3 used sc-co_2 spray dried egg yolk

extraction time and temperature of SC-CO_2 and extraction time.

3. The equation to represent cholesterol extraction in the spray dried egg yolk by SC-CO_2 was obtained by response surface methodology.

4. SC-CO_2 extracted spray dried egg yolk had more brightness, less redness and less yellowness than the control.

5. SC-CO_2 extracted spray dried egg yolk showed increase in % protein, % phosphatide, % solubility and emulsification capacity but reduced in 1 % lipid.

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