

Rates of Cholesterol Ester Formation During Storage of Anchovy (*Engraulis capensis*) at Various Temperatures

Adrianus J. de Koning*

CSIR, Division of Food, Biological and Chemical Technologies,
FST Programme, Rosebank, Cape Town, South Africa

ABSTRACT

Anchovy were stored at 17, 8, 0, and -6°C . Samples were withdrawn periodically, the lipids extracted, purified, and analysed for free and esterified cholesterol. From the results of these tests the rates of the enzymatic esterification of cholesterol at the four temperatures were calculated. In the temperature range of -6 to 17°C the rate increased with temperature. Application of an Arrhenius plot afforded the esterification rate at any temperature within the range examined.

Key Words: Cholesterol; Anchovy oil; Rate of esterification; Arrhenius plot.

INTRODUCTION

Enzymatic formation of cholesterol ester from free cholesterol has been shown to occur during spoilage of anchovy (*Engraulis capensis*).^[1] As a result, the quality of the oil produced from the fish is reduced, as esterified cholesterol is much more difficult to remove from the oil than free cholesterol.^[2] This aspect becomes

*Correspondence: Professor Adrianus J. de Koning, CSIR, Division of Food, Biological and Chemical Technologies, FST Programme, 15 Lower Hope Road, Rosebank, Cape Town 7700, South Africa; Fax: +27 21 686 6116; E-mail: adkoning@csir.co.za.

particularly important in oils used for dietary supplements as these oils should have little or no cholesterol, whether free or esterified. Although this spoilage reaction has been known for many years,^[3] no data for the rates of esterification at different temperatures are available. The present study attempts to determine some of these by storing fresh anchovy in four different tests at 17, 8, 0, and -6°C . Samples were withdrawn at periodic intervals and the lipids extracted, purified, and analysed for free and esterified cholesterol. The results of these tests allow the calculation of the rates of esterification of cholesterol at the four storage temperatures and after employing an Arrhenius plot at any temperature within the range examined.

MATERIALS AND METHODS

General

Fresh anchovy were obtained from a local fish meal factory within 12 h of catching and transported to the laboratory in ice. The fish were weighed in batches of about 200 g and stored in polythene bags at 17, 8, 0, and -6°C . At day 0, and subsequently at known periodic intervals, the lipids were extracted, purified, and analyzed for free and esterified cholesterol. Four separate tests (A–D) were carried out; in only one test (A) were the anchovy stored at -6°C .

Extraction and Purification of the Lipids

The lipids were extracted from the anchovy with excess chloroform:methanol (2:1, by vol.) as described previously.^[4] The lipids were washed three times in a large volume of water according to the procedure of Folch et al.^[5] The chloroform solutions were dried over anhydrous sodium sulfate, filtered, and concentrated on a rotary evaporator in vacuo. The lipids were then taken up in a small volume of hexane, filtered over anhydrous sodium sulfate, concentrated on the rotary evaporator, and finally dried under high vacuum.

Total, Free, and Esterified Cholesterol

Total, free, and esterified cholesterol were determined in the various anchovy lipids by gas chromatography using stigmaterol as internal standard as described earlier.^[1]

RESULTS AND DISCUSSION

The lipid contents of the different batches of anchovy, together with the total and esterified cholesterol contents of these lipids, are recorded in Table 1. The total cholesterol content of the lipids showed a highly significant negative correlation with



Rates of Cholesterol Ester Formation

Table 1. Progressive esterification of cholesterol in anchovy (*Engraulis capensis*) stored at 17, 8, 0, and -6°C .

| Temp ($^{\circ}\text{C}$) | Test no. | Anchovy | | Lipids | | |
|--------------------------------|-------------|---------|------------------------------|-----------------------------|-------|----------------|
| | | Days | Lipids (g/100 g wet fish) | Cholesterol (g/100 g lipid) | | |
| | | | | Total | Ester | Δ Ester |
| 17 | A1 | 0 | 5.02 | 2.92 | 0.04 | |
| | | 1 | 4.77 | 2.97 | 0.46 | 0.42 |
| | | 2 | 4.85 | 2.80 | 0.60 | 0.56 |
| | | 3 | 4.79 | 2.60 | 0.87 | 0.83 |
| | | 4 | 4.04 | 3.26 | 1.57 | 1.53 |
| 8 | A2 | 0 | 5.02 | 2.92 | 0.04 | |
| | | 3 | 4.75 | 2.63 | 0.54 | 0.50 |
| | | 7 | 4.75 | 2.09 | 0.82 | 0.78 |
| | | 14 | 4.70 | 2.59 | 1.08 | 1.04 |
| | | 28 | 4.28 | 3.12 | 1.37 | 1.33 |
| 0 | A3 | 0 | 5.02 | 2.92 | 0.04 | |
| | | 8 | 5.00 | 2.90 | 0.21 | 0.17 |
| | | 15 | 4.70 | 2.70 | 0.28 | 0.24 |
| | | 28 | 4.60 | 2.79 | 0.67 | 0.63 |
| | | 49 | 4.02 | 3.36 | 0.91 | 0.87 |
| -6 | A4 | 0 | 5.02 | 2.92 | 0.04 | |
| | | 17 | 4.27 | 3.13 | 0.11 | 0.07 |
| | | 30 | 4.56 | 2.78 | 0.25 | 0.21 |
| | | 60 | 4.50 | 2.64 | 0.29 | 0.25 |
| | | 17 | B1 | 0 | 4.96 | 3.05 |
| 8 | B2 | 1 | 5.18 | 2.74 | 0.81 | 0.44 |
| | | 2 | 4.67 | 2.72 | 1.28 | 0.91 |
| | | 3 | 4.47 | 2.84 | 1.44 | 1.07 |
| | | 0 | 4.96 | 3.05 | 0.37 | |
| | | 4 | 4.55 | 3.21 | 0.96 | 0.59 |
| 0 | B3 | 8 | 4.53 | 3.17 | 1.29 | 0.92 |
| | | 15 | 4.37 | 3.33 | 1.37 | 1.00 |
| | | 0 | 4.96 | 3.05 | 0.37 | |
| | | 8 | 3.92 | 3.39 | 0.43 | 0.06 |
| | | 15 | 4.26 | 3.00 | 0.51 | 0.14 |
| 17 | C1 | 28 | 4.55 | 3.14 | 0.99 | 0.62 |
| | | 0 | 3.18 | 4.36 | 0.27 | |
| | | 1 | 4.13 | 3.65 | 0.96 | 0.69 |
| | | 2 | 3.64 | 4.28 | 1.66 | 1.39 |
| | | 3 | 3.71 | 3.97 | 1.94 | 1.67 |
| 8 | C2 | 4 | 3.38 | 4.39 | 1.88 | 1.61 |
| | | 0 | 3.18 | 4.36 | 0.27 | |
| | | 4 | 3.66 | 3.67 | 0.90 | 0.63 |
| | | 8 | 3.10 | 4.78 | 1.98 | 1.71 |
| | | 15 | 3.50 | 3.69 | 1.96 | 1.69 |
| | | 29 | 3.54 | 4.07 | 2.27 | 2.00 |

(continued)



Table 1. Continued.

| Temp (°C) | Test no. | Anchovy | | Lipids | | |
|--------------|-------------|---------|------------------------------|-----------------------------|-------|---------|
| | | Days | Lipids (g/100 g wet fish) | Cholesterol (g/100 g lipid) | | |
| | | | | Total | Ester | Δ Ester |
| 0 | C3 | 0 | 3.18 | 4.36 | 0.27 | |
| | | 8 | 3.62 | 3.98 | 0.60 | 0.33 |
| | | 15 | 3.54 | 3.00 | 1.12 | 0.85 |
| | | 40 | 3.28 | 4.11 | 1.60 | 1.39 |
| 17 | D1 | 0 | 3.95 | 3.89 | 0.11 | |
| | | 1 | 4.12 | 3.94 | 0.66 | 0.55 |
| | | 2 | 4.06 | 3.67 | 1.14 | 1.03 |
| | | 3 | 4.75 | 3.38 | 1.17 | 1.06 |
| | | 4 | 4.10 | 3.50 | 1.44 | 1.33 |
| 8 | D2 | 0 | 3.95 | 3.89 | 0.11 | |
| | | 4 | 4.09 | 3.30 | 0.30 | 0.19 |
| | | 7 | 3.93 | 3.57 | 1.13 | 1.02 |
| | | 15 | 3.66 | 4.02 | 1.58 | 1.47 |
| | | 28 | 3.86 | 3.23 | 1.33 | 1.22 |
| 0 | D3 | 0 | 3.95 | 3.89 | 0.11 | |
| | | 7 | 4.67 | 3.30 | 0.45 | 0.34 |
| | | 15 | 4.87 | 2.97 | 0.33 | 0.22 |
| | | 28 | 4.35 | 3.18 | 0.72 | 0.61 |
| | | 41 | 3.62 | 4.02 | 0.96 | 0.85 |

the lipid content of the anchovy: i.e., high cholesterol levels corresponding to low lipid levels and vice versa.

Mathematically expressed the relationship is:

$$\text{Total cholesterol (g/100 g lipid)} = 7.11 - 0.89 \times \text{lipid (g/100 g wet fish)}$$

$$r = -0.84 \text{ and } n = 51$$

where r is the correlation coefficient and n the number of samples analyzed. This relationship is similar to that observed for the total cholesterol levels of press oils of different pelagic fish species.^[6,7] Esterified cholesterol increased on storage of the anchovy, these increases at 17, 8, 0, and -6°C can be expressed as a function of the time in days by 13 straight lines, indicating a zero order enzymatic reaction.^[8] The slopes of the lines correspond to the average daily increases in cholesterol ester contents of the anchovy lipids at the different temperatures. These rates for the four tests are recorded in Table 2.

Scrutiny of the rates at each temperature shows good agreement in the various tests, the rate at 17°C ranging from 0.32 to 0.42, at 8°C from 0.042 to 0.063, and at 0°C from 0.018 to 0.034 g cholesterol/(100 g lipid)/(day). In the single test at -6°C a value of 0.0043 g cholesterol/(100 g lipid)/(day) was obtained. The straight line



Table 2. Rates of cholesterol ester formation in anchovy (*Engraulis capensis*) lipids at 17, 8, 0, and -6°C .

| Temperature ($^{\circ}\text{C}$) | Test | Rate [g cholesterol/ (100 g lipid)/(day)] | r | n |
|---------------------------------------|------|---|------|-----|
| 17 | A1 | 0.35 | 0.97 | 5 |
| | B1 | 0.37 | 0.98 | 4 |
| | C1 | 0.42 | 0.93 | 5 |
| | D1 | 0.32 | 0.95 | 5 |
| Mean | | 0.37 | | |
| 8 | A2 | 0.042 | 0.91 | 5 |
| | B2 | 0.063 | 0.89 | 4 |
| | C2 | 0.062 | 0.83 | 5 |
| | D2 | 0.045 | 0.77 | 5 |
| Mean | | 0.053 | | |
| 0 | A3 | 0.018 | 0.99 | 5 |
| | B3 | 0.022 | 0.94 | 4 |
| | C3 | 0.034 | 0.97 | 4 |
| | D3 | 0.019 | 0.95 | 5 |
| Mean | | 0.023 | | |
| -6 | A4 | 0.0043 | 0.99 | 4 |

relationship between the logarithm of the reaction rate and the reciprocal of the absolute temperature ($1/T$) yields an Arrhenius plot, which is shown in Fig. 1.

The mathematical expression of this plot is:

$$\log k \text{ esterification} = 19.81 - 5892/T \quad r = -0.98 \quad n = 13$$

where k is the rate constant of the reaction, r is the correlation coefficient and n is the number of data points. The activation energy for this reaction, calculated from the slope of the line, is 112.77 kJ/mol.^[8] This is larger than the activation rates of 99.95 and 76.77 kJ/mol found for the enzymatic hydrolysis of anchovy phospholipids and neutral lipids respectively^[9] resulting in a relatively steep slope of the present Arrhenius plot. However, it should be realized that although a spoilage rate is measured, the current study is concerned with an esterification rather than a hydrolysis reaction of the anchovy lipids. The steep slope of the Arrhenius plot means that lowering the storage temperature of the anchovy in the delay between catching and processing will be extremely effective in minimizing the cholesterol ester content of the resulting anchovy oil. This is therefore the recommendation made to fish oil producers for improving the quality of the anchovy oil used in dietary supplements.

CONCLUSION

The enzymatic esterification of free cholesterol during spoilage of anchovy in the delay between catching and processing adversely affects the quality of the resulting



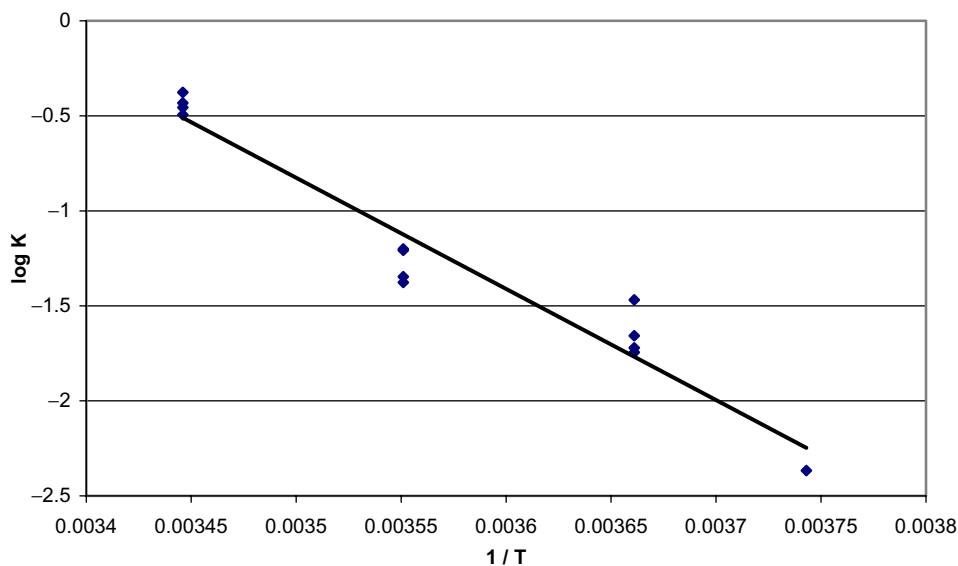


Figure 1. Arrhenius plot of the esterification of cholesterol in anchovy during storage at various temperatures. (View this art in color at www.dekker.com.)

anchovy oil since, in contrast to free cholesterol, esterified cholesterol is only removed with difficulty. This is a serious problem for anchovy oils, which are rich in omega-3 fatty acids and used as dietary supplements, since these oils should have little total cholesterol. The present investigation has shown that esterification is very temperature-dependent; for instance the rate at 17°C is 16 times that at 0°C. Icing the fish in the delay period is therefore very effective in reducing the progressive esterification of cholesterol.

REFERENCES

1. de Koning, A.J.; Hearshaw, K.D.; van der Merwe, G.H. Free and esterified cholesterol in a number of South African fish oils and their corresponding meals. *Fat Science Technology* **1993**, *95*, 27–31.
2. Marschner, S.S.; Fine, J.B. Physical Process for Simultaneous Deodorization and Cholesterol Reduction of Fats and Oils. United States of America Patent 4,804,555, 1989.
3. Lovern, J.A.; Olley, J.; Watson, H.A. Changes in the lipids of cod during storage in ice. *Journal of the Science of Food and Agriculture* **1959**, *10*, 327–336.
4. de Koning, A.J. Phospholipids of marine origin I. The hake (*Merluccius capensis*, *Castelnau*). *Journal of the Science of Food and Agriculture* **1966**, *17*, 112–117.
5. Folch, J.; Lees, M.; Sloane-Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **1957**, *226*, 497–509.



Rates of Cholesterol Ester Formation

327

6. de Koning, A.J.; Mol, T. The cholesterol content of South African fish oil and its seasonal variation. *Fat Science Technology* **1992**, *94*, 60–63.
7. de Koning, A.J. Properties of South African fish oils: a review. *International Journal of Food Properties* **1999**, *2*, 205–216.
8. Laidler, K.J.; Meiser, J.H. *Physical Chemistry*; The Benjamin/Cummings Publishing Company, Inc.: Menlo Park, 1982; 350–367.
9. de Koning, A.J. The free fatty acid content of fish oil Part IV. Rates of free fatty acid formation from phospholipids and neutral lipids in anchovy (*Engraulis capensis*) stored at various temperatures. *Fat Science Technology* **1995**, *97*, 341–346.

Received May 19, 2003

Accepted September 2, 2003



Copyright of International Journal of Food Properties is the property of Marcel Dekker Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.