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Thermal processing of 5-methyltetrahydrofolic acid in the UHT region in the presence of oxygen

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The thermal degradation of 5-methyltetrahydrofolic acid (5-MTHFA) in the presence of oxygen was investigated in the UHT region at 110°C, 120°C, 140°C and 150°C. The folate was processed in the presence of two different oxygen levels (0.3 and 6.8 ppm) in a phosphate buffer at pH 7. The degradation was found to be first-order with respect to the folate.

The Arrhenius activation energy for the aerobic and the anaerobic degradation was determined to be 106 kJ mol^{-1} and 62 kJ mol^{-1}, respectively.

The investigation shows that a low oxygen concentration during processing at UHT temperatures will decrease the folate degradation, but will not completely prevent it.

INTRODUCTION

Thermal processing affects many properties of foods. Of special interest are changes in the nutritional value of the processed foods. In particular, folate is one vitamin of concern. Deficiency in folate may cause megaloblastic anaemia (Hawkes & Villota, 1989), and it has recently been established that folic acid has a specific role in the prevention of neural tube defects (Wald *et al.*, 1991). The minimum requirement of folate is 50 µg per day (NRC, 1989). In the Scandinavian countries, milk is an important source of folate. Milk contains 50 µg litre^{-1} (Walstra & Jennes, 1984; Renner *et al.*, 1989), of which the dominant form (90–95%) is 5-methyltetrahydrofolic acid (5-MTHFA) (Shin *et al.*, 1975).

The most expansive thermal process being used for liquid foods today is the UHT (ultra high temperature) process, which utilizes temperatures in the range 135–150°C for a few seconds, for milk sterilization (Burton, 1988). This gives a product which does not require refrigeration for a long shelf-life (Ramsey & Swartzel, 1984). Losses of folate in milk during UHT processing are generally less than 20%, but losses of up to 43% have been observed (Andersson, 1993).

The degradation of 5-MTHFA in the presence of different oxygen levels and in the absence of oxygen in buffer systems has been examined by O'Broin *et al.* (1975), Chen and Cooper (1979), Paine-Wilson and Chen (1979), Ruddick *et al.* (1980), Mnkeni and Beveridge (1983) and Barrett and Lund (1989), and in food model systems by Day and Gregory (1983). Activation energies for the degradation of 5-MTHFA in the presence of oxygen in buffer systems were reported from 29.7 kJ mol^{-1} (Ruddick *et al.*, 1980) to 82.8 kJ mol^{-1} (Mnkeni & Beveridge, 1983). The activation energy for the anaerobic degradation has been reported by Barrett and Lund (1989) to be 97.4 kJ mol^{-1}.

These investigations, except those performed by Mnkeni and Beveridge (1983) and Day and Gregory (1983), have been carried out at temperatures below 100°C or at 100°C.

Considering the great difference between the activation energies reported for the degradation of 5-MTHFA in the presence of oxygen, there is a need for further research in order to gain a better understanding of the effect oxygen has upon the degradation of folate during thermal processing, and consequently whether in any way it is possible to increase the retention of folate during processing. Equipment has recently been developed for the purpose of obtaining kinetic data on rapid thermal reactions in the UHT region, providing the possibility of easily changing the experimental conditions (Viberg & Öste, 1995).

The aim of this investigation was to examine the effects of different oxygen levels on the thermal degradation of 5-MTHFA in the UHT region, as oxygen is a factor known to enhance the degradation of 5-MTHFA. The advantages of investigating the reaction in a buffer

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system, instead of a real food system, is that a greater control of different process parameters can be achieved and that it may be expected that the results can thus be explained in a more general way.

MATERIALS AND METHODS

Sample preparation

A fresh stock solution of 0.026 g of (6*R*,*S*)-5-methyl-5,6,7,8-tetrahydrofolic acid (calcium salt; Dr B. Schircks Laboratories, Jona, Switzerland) dissolved in 100 ml of 0.1 M Sörensen's phosphate buffer, pH 7.0, was made daily. This stock solution was kept cool and dark until needed. Prior to heat exposure, 1 ml of the stock solution was diluted with 100 ml of buffer with the same oxygen level as the buffer used in the continuous phase. Care was taken at all stages to protect the 5-MTHFA from light exposure.

Exposure to heat

The selected temperatures were 110°C, 120°C, 140°C, 150°C, to cover the whole temperature range normally used in UHT processing.

The samples were thermally exposed in a miniaturized tubular heating system, which has been described in detail by Viberg and Öste (1995). The operation technique of the equipment is based on the thermal processing of small samples in a continuous flow of buffer solution. The sample is injected into the continuous flow of the system by an HPLC loop injector. The sample is then rapidly heated in a heating bath and caught in the holding bath by an immersed autoinjector. Holding times used in this present study ranged from 0.08 to 6.49 min. After a selected holding time, the autoinjector opens again and the sample continues with the flow throw a cooling bath. Finally, after processing, a fraction of the sample is withdrawn from the flow by a fraction collector. Three to four samples were removed for analysis at every utilized time–temperature combination.

The fraction withdrawn contained both sample and continuous phase. The proportions of sample and continuous phase in the withdrawn fraction is denoted the dilution factor. The dilution factor was determined by processing an inert standard. Sodium benzoate was chosen as such a standard, since it is easy to measure by absorption in the UV region. The samples of inert standard were processed in the same way as the folate samples. The sodium benzoate in the withdrawn fractions was measured spectrophotometrically at 223 nm (Varian DMG 100).

Oxygen measurements

The oxygen level was measured with an oxygen electrode (Model 97–08, Orion Research), connected to a pH-meter (PHM 63; Radiometer), after the cooling bath. According to the manual, the accuracy of the oxygen electrode was 0.05 ppm or 2%, whichever is larger.

Two oxygen levels were examined: 0.3 (0.25–0.35) and 6.8 (6.6–7.0) ppm (measured at room temperature). The highest oxygen level was attained by leaving bottles of buffer in a thermostatically controlled water bath so that the buffer could equilibrate with the oxygen in the environment. The lowest oxygen level was produced by flushing the buffer with nitrogen (AGA Plus; AGA Gas); finally, the middle oxygen level was attained by carefully mixing the two other buffers.

To maintain the oxygen levels in the bottles of unsaturated buffer, these were covered with a layer of paraffin oil. Hence the buffers could be used in the ambient atmosphere for a least 1 h without any measurable change in the oxygen levels.

Analysis of 5-MTHFA

The 5-MTHFA in the fraction withdrawn from the flow was stabilized with 2-mercaptoethanol (1.25% by volume) and was then immediately frozen to −40°C until analysis. The 5-MTHFA was analysed by HPLC according to Gregory *et al.* (1984), but, instead of the recommended column, a Zorbax® SB-C18 (Rockland Technologies) was used, since it was assumed that it could better resist the low pH of the mobile phase. The composition of the mobile phase was 7% acetonitrile and 93% phosphate buffer 0.033 M, pH 2.3. The pump used was a Varian 9010 connected to a Varian 9100 autosampler. The detection was made by a fluorescence detector (Varian 9070) with the excitation wavelength set at 296 nm and the emission wavelength set at 356 nm. The whole system was operated by a Varian Star Workstation.

The results of the analysis of 5-MTHFA in the fractions withdrawn were compensated for the dilution factor in order to obtain the true content of 5-MTHFA in the processed samples. Assumed losses of 5-MTHFA during heating and cooling were estimated mathematically.

First-order reaction

Many reactions concerning the deterioration of food components can be treated as simple monomolecular reactions with an order of one or zero (Labuza, 1979). In a first-order reaction the reaction rate constant (k) can be calculated from the original concentration or amount (C_0) and the amount remaining (C_t) at time t:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \qquad (1)$$

The Arrhenius equation, eqns 2 and 3, expresses the correlation between the specific reaction rate constant (k), the activation energy (E_a) and the absolute

temperature (T). In this way, the reaction rate at any temperature within the investigated temperature range can be calculated from the data collected at the selected temperatures.

$$k = A \exp^{(-E_a/RT)} \ (\text{min}^{-1}) \quad (2)$$

or

$$\ln k = \ln A - E_a/RT \quad (3)$$

RESULTS

Calculated specific rate constants with standard deviation determined at 110°C, 120°C, 140°C and 150°C at the different oxygen levels are presented in Table 1. At all combinations investigated more than 75% of the 5-MTHFA was degraded in the sample withdrawn at the longest holding time. The reaction could be described as a first-order reaction with respect to the 5-MTHFA, since linear regression of $\ln(C_t/C_0)$ versus time correlated well with a straight line on all occasions.

The apparent rate constant ($k_{\text{anaerobic}} + k_{\text{aerobic}}$) increased with increasing oxygen concentration. By extrapolation, the apparent rate constant could be determined in an oxygen-free system ($[O_2] = 0$, $k_{\text{aerobic}} = 0$). That the anaerobic rate constant found was greater than zero (Fig. 1), supports the theory that anaerobic degradation is a significant part of the total degradation of 5-MTHFA in the UHT region under the conditions of this investigation.

Using the Arrhenius correlation, eqn 2, the activation energy (E_a) of the anaerobic degradation was found to be 62 kJ mol^{-1} ($r^2 = 0.9889$).

The aerobic reaction rate constants were determined by subtracting the previously determined anaerobic rate constant from the apparent rate constant (Table 1). The activation energy for the aerobic degradation of 5-MTHFA was then found to be 106 kJ mol^{-1} (106.6 kJ mol^{-1} at 6.8 ppm, $r^2 = 0.9884$, and 106.3 kJ mol^{-1} at 0.3 ppm, $r^2 = 0.9882$). The Arrhenius plots are presented in Figs 2 and 3.

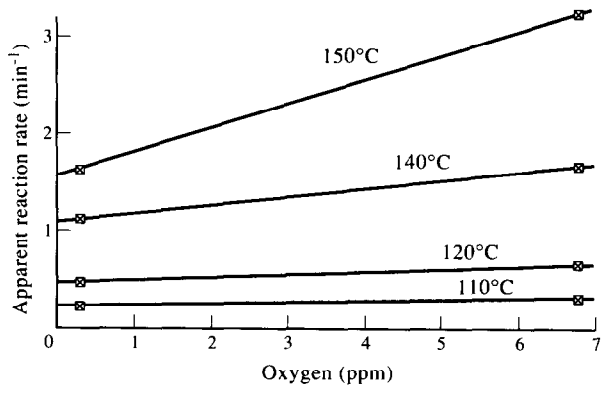

Fig. 1. Apparent reaction rate constants at 110°C, 120°C, 140°C and 150°C plotted versus oxygen concentration (ppm). The anaerobic reaction rate constants were determined by extrapolating the results obtained at 6.8 ppm and 0.3 ppm down to anaerobic conditions.

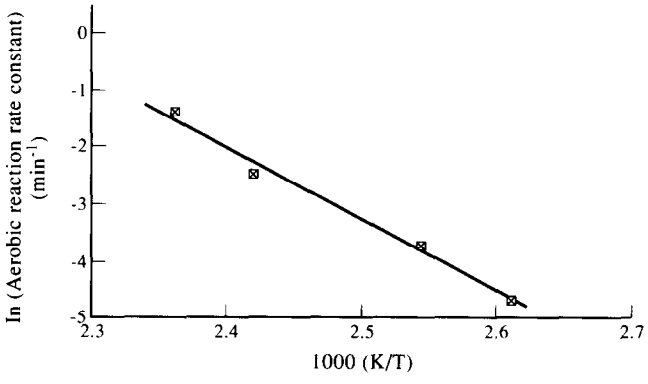

Fig. 2. Arrhenius plot of the aerobic degradation of 5-MTHFA. The activation energy was determined to be 106 kJ mol^{-1}.

DISCUSSION

Choice of buffer solution

The degradation rate of folate may depend on various factors. Paine-Wilson and Chen (1979) showed that the choice of buffer solution could affect the results. They performed their experiments on the degradation of several forms of folates at 100°C and noted that the degradation rate of 5-MTHFA (at pH 3) increased by a factor of approximately five between the buffer that gave the lowest degradation rate and the buffer that gave the highest degradation rate, and that the choice of buffer would probably have a greater influence on the degradation rate at higher temperatures than at lower temperatures.

The stability of 5-MTHFA in a buffer solution depends on the pH. The greatest thermal stability at

Table 1. Thermal degradation of 5-MTHFA in the presence of oxygen

	Temperature			
	110°C	120°C	140°C	150°C
Apparent reaction rate (min^{-1}) at $[O_2]$ = 0.3 ppm	0.242 ± 0.027	0.488 ± 0.125	1.12 ± 0.52	1.63 ± 0.25
Apparent reaction rate (min^{-1}) at $[O_2]$ = 6.8 ppm	0.300 ± 0.048	0.638 ± 0.085	1.65 ± 0.29	3.18 ± 0.36111

Calculated specific rate constants with standard deviation determined at 110°C, 120°C, 140°C and 150°C at the different oxygen levels.

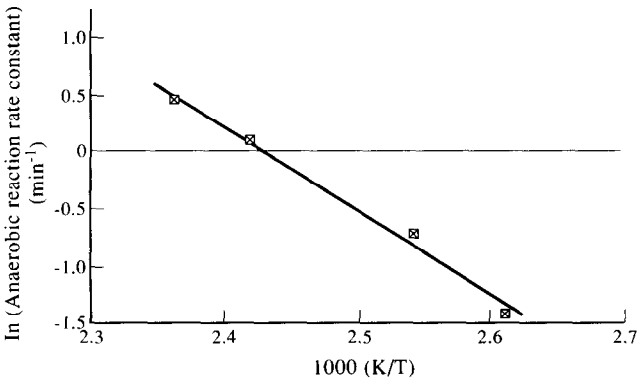

Fig. 3. Arrhenius plot of the anaerobic degradation of 5-MTHFA. The activation energy was determined to be 62 kJ mol^{-1}.

100°C was in HCl/KCl buffer at pH 7.0 (Paine-Wilson & Chen, 1979), while O'Broin *et al.* (1975) reported the greatest stability at pH 9.0 in (Tris/HCl buffer, 0.1 M) at room temperature. However, the activation energies for the degradation of 5-MTHFA were found to be of the same magnitude in the pH range 3–6 examined by Mnkeni and Beveridge (1983), indicating that the degradation mechanism is independent of pH.

Despite the difficulties encountered by previous examiners in the measurement of oxygen levels in phosphate buffers (Ruddick *et al.*, 1980), this buffer was chosen here since phosphates are the most common salts in bovine milk.

Special advantages of the apparatus used

When a solution is heated, its capacity to dissolve gases decreases. On the other hand, its ability to dissolve gases will increase with increasing pressure.

By using an HPLC pump placed before the heating bath, and pumping a buffer at high velocity through a small capillary, the pressure will increase so that the dissolved oxygen will remain dissolved in the buffer during the whole thermal process. This implies that errors due to diffusion resistance between separate phases, a problem pointed out by Barrett and Lund (1989), will be decreased.

Kinetics of 5-MTHFA degradation

The determination of the order of a reaction is an important part in the construction of a kinetic model, although many deteriorative reactions in food can be approximated as zero-order or first-order (Labuza, 1979).

The degradation kinetics of 5-MTHFA is fairly well understood and the same conclusions can be drawn from most investigations. Chen and Cooper (1979), Paine-Wilson and Chen (1979), Mnkeni and Beveridge (1983) and Barrett and Lund (1989) came to the conclusion that the reaction follows pseudo-first-order kinetics of 5-MTHFA in the presence of oxygen. Day and Gregory (1983) found, however, that the degradation of 5-MTHFA could be described by a second-order reaction, when oxygen was the limiting factor in the reaction, but they did not have enough data to present a kinetic model of the degradation. Barrett and Lund (1989) suggested that the degradation of 5-MTHFA should be divided into an anaerobic and an aerobic pathway.

In the present investigation, the degradation was found to be first-order with respect to 5-MTHFA and the conclusions of Barrett and Lund (1989), that the degradation of 5-MTHFA ought to be separated into an aerobic and an anaerobic pathway, could be confirmed.

Losses of oxygen during the reaction have, in previous investigations, been found to be first-order (Barrett & Lund, 1989). Unfortunately, it would be impossible to measure the losses of oxygen during the reaction inside the apparatus used in this investigation. In our study, the degradation rate of 5-MTHFA increased proportionally when the 5-MTHFA was processed at three different oxygen levels, indicating a unimolecular reaction of oxygen with 5-MTHFA. Thus, despite the fact that no direct measurements of oxygen losses could be made during the reaction, we can establish that the reaction is first-order with respect to oxygen. The degradation of 5-MTHFA in the presence of oxygen could then be described as an overall second-order reaction that is first-order with respect to 5-MTHFA and to oxygen.

The aerobic part of the total reaction, eqn 4, can be reduced to a pseudo-first-order reaction, eqn 5, when oxygen is present in such excess that the changes in concentration of oxygen are very small compared to the concentration changes of 5-MTHFA.

$$\frac{d[5-MTHFA]}{dt} = k_{aerobic}[5-MTHFA][O_2] \quad (4)$$

$$\frac{d[5-MTHFA]}{dt} = k'_{aerobic}[5-MTHFA] \quad (5)$$

$$k'_{aerobic} = k_{aerobic}[O_2] \quad (6)$$

$$\frac{d[5-MTHFA]}{dt} = k_{aerobic}[5-MTHFA][O_2] + k_{anaerobic}[5-MTHFA] \quad (7)$$

The whole process, including both the aerobic and the anaerobic degradation, eqn 7, can be simplified in the same way, eqn 8.

$$\frac{d[5-MTHFA]}{dt} = (k'_{aerobic} + k_{anaerobic})[5-MTHFA] \quad (8)$$

All unknown parameters of the degradation, eqn 4, can then be determined by examining the reaction at at least two oxygen levels.

The effect of temperature and oxygen

The advantage of processing at higher temperatures for a short time compared to processing at lower temperature and longer holding times is that the destruction of spores and microorganisms becomes relatively greater than the losses of nutrients (Burton *et al.*, 1965). Nevertheless, it is preferable to have some knowledge about what effects a thermal process will have upon different food components and whether the retention of nutrients can in any way be optimized. Apart from time and temperature, the chemical environment may affect the degradation rate. In the present investigation, the effect of oxygen has been studied since it is supposed to enhance the degradation of 5-MTHFA during thermal processing.

In previous investigations, the determined activation energy varied between 29.7 and 82.8 kJ mol^{-1}. These investigations seem to be divided into two groups, one in which an activation energy of about 30 kJ mol^{-1} was found (Chen & Cooper, 1979; Ruddick *et al.*, 1980) and another in which the activation energy was determined to be higher than 80 kJ mol^{-1} (Barrett & Lund, 1989; Mnkeni & Beveridge, 1983). The difference in activation energy found could mean that several degradation mechanisms are possible. It is suggested that this could be due to chemical (Mnkeni & Beveridge, 1983) or physical causes (Barrett & Lund, 1989). The two investigations (Mnkeni & Beveridge, 1983; Barrett & Lund, 1989) in which a higher activation energy was found, as demonstrated in this study, were performed with different processing techniques; thus the higher activation energy is not dependent on the design of the heating conditions. The activation energy reported by Mnkeni and Beveridge (1983) would probably be even higher since they did not consider the anaerobic pathway. The apparent activation energy in our study was 77 kJ mol^{-1} (6.8 ppm) when the anaerobic pathway was disregarded. This is of the same magnitude as the activation energy (79.4–82.8 kJ mol^{-1}) determined by Mnkeni & Beveridge (1983).

Barrett and Lund (1989), who separated the degradation of 5-MTHFA into an anaerobic and an aerobic pathway, reported that the activation energy of the anaerobic degradation was higher than that of the aerobic pathway. By extrapolating their result into the UHT region, the anaerobic degradation should be the dominant degradation pathway at temperatures over $\approx$120°C ($k_{anaerobic} > k_{aerobic}$). This could not be confirmed in our study since the activation energy for the aerobic pathway was found to be higher than the activation energy for the anaerobic pathway. In addition, the effect of oxygen became more pronounced when the higher temperatures were investigated. The observation of Barrett and Lund (1989) that the anaerobic degradation of 5-MTHFA can not be neglected is, however, confirmed by the present study.

Because, during continuous thermal processing, the pasteurization or UHT sterilization of liquid foodstuffs generally takes less than 1 min, one can conclude that the effect of the presence of oxygen becomes an important factor in the degradation of 5-MTHFA only at 140°C and 150°C. Applying the information found using the buffer system should, however, be done with caution. That the activation energy could be significantly lowered in food systems by food components (apple juice, 32.8 kJ mol^{-1}; tomato juice, 44.3 kJ mol^{-1}) was suggested by Mnkeni and Beveridge (1983).

The minor effect of oxygen was surprising, since an investigation of indirect UHT processing of milk at 140°C for 4 s at different oxygen levels performed by Andersson and Öste (1992*a*,*b*) showed a retention of 96% of folate with 0.6 ppm oxygen, 67% with 2.3 ppm oxygen and only 20% with 5.6 ppm oxygen. According to these results, anaerobic degradation can be disregarded in milk processing.

These examples indicate a general weakness of investigations performed in model systems with purposes other than to determine relative effects. Numerical values should be used as a reference for other investigations and not to predict results in other matrices, or in conditions other than the one under examination.

Nevertheless, our study shows that the aerobic degradation of 5-MTHFA is an important reaction pathway in the UHT region, and one can conclude that degassing of milk, or any liquid foodstuff, before thermal treatment will always have a positive effect on the retention of folate. As well as a low oxygen level, the natural content of ascorbic acid in milk ought to have a considerable protective effect on the folate. It would be unwise, however, to ignore the anaerobic degradation without a proper examination under the conditions of interest.

CONCLUSIONS

Reported kinetic data on the degradation of 5-MTHFA in the presence of oxygen vary. This study shows that the overall reaction is second-order in the presence of oxygen. The Arrhenius activation energy for aerobic and anaerobic degradation was found to be 106 kJ mol^{-1} and 62 kJ mol^{-1}, respectively. The results of this investigation are similar to the results reported by Barrett and Lund (1989) and Mnkeni and Beveridge (1983). These experiments confirm the importance of degassing liquid foods before thermal processing to reduce the oxygen content, in order to maximize the amount of 5-MTHFA retained during thermal processing.

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