KINETICS OF VITAMIN A LOSS IN A FOOD SYSTEM DURING HEAT PROCESSING

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ABSTRACT

Vitamin A is nutritionally significant and is used as an indicator for the performance of fat soluble vitamins in products and processes. There was a paucity of kinetic data relating to its loss in food systems during thermal processing and therefore it was important to develop kinetic models that described destruction rates, and their dependence on factors such as temperature and the microenvironment of the food.

A real food system, beef liver, was used to study the loss of vitamin A on thermal processing. Two different heating methods were employed to obtain kinetic data - a steady state and an unsteady state procedure. In the steady state method, liver puree was heated in glass capillary tubes. The order of reaction, rate constants and their dependence on temperature were determined. Using experimental design techniques, the effects of composition (fat, protein and moisture contents, pH and copper concentration) on vitamin A loss were determined. The ranges of compositional variables were those expected in manufactured meat products conforming to practical and New Zealand legal requirements. The unsteady state method was employed to see if the steady state reaction kinetic parameters were valid on scale-up, and if steady state results could be used to predict vitamin A losses in commercial thermal processing operations.

For natural beef liver puree, vitamin A loss on steady state heating (103 - 127^{o}C) could be described by first order kinetics. The reference rate constant, k_{122}, had the value 125.0 x 10^{-5} s^{-1} and the activation energy was 112 $\pm$ 9 $kJmol^{-1}$ at the 95% level of confidence.

Where fat, protein and moisture contents were studied at one level of copper and pH to determine their effect on vitamin A loss, it was found that either moisture content or fat content could explain most of the observed variation. However, moisture content was the preferred variable to model the effect of composition, as it explained a greater proportion of the variation. Moisture content increased the rate of vitamin A loss as it increased in value from 52 - 72%. The activation energies were very similar for the mixtures, and only when the copper level was changed did

E_a change. The change in rate moving from 102°C to 122°C was less in runs where copper was present at high concentrations than when it was at a low level. This indicates that changing copper concentration probably brough about changes in the mechanism of vitamin A loss.

Under similar compositional and heating conditions to those used in steady state, pilot plant canning trials gave greater vitamin A losses than predicted from the steady state data. Part of the discrepancies was due to destabilisation of the system which led to some heat transfer by convection and to fat migration to the outer parts of the can. Neither of these effects was taken into account by the prediction method. The remaining differences were probably due to a change in the kinetics between the steady state and unsteady state experiments.

Kinetic parameters were calculated from the unsteady state data for comparison with the steady state values. The activation energies were the same but the reference rate constants were different. The difference was only slight with a liver mixture of 56% moisture content but was significant at 69% moisture content. Therefore it was not possible to relate k_{122} in the steady and unsteady state and so the steady state data did not scale up completely.

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1. INTRODUCTION

Heat processing is a common method of food preservation. The basic function of a thermal process is to eliminate or reduce micro-organisms and/or enzymes that would, upon storage, result in deterioration of the food or could endanger the health of the consumer. However, undesirable changes to nutrients, particularly proteins and vitamins, occur concurrently with these desirable modifications. There is a need to design heat processes which give the required microbial and enzymic destruction but minimise the nutrient loss. Such a process design needs not only data on the thermal destruction of microorganisms but also data on the thermal destruction of nutrients. Unfortunately, at the present time, there are few such data available.

The determination of these data would assist in:

(1) Product improvement - to minimise the nutrient loss in an existing process.
(2) New product development - to identify opportunities via the design of new processing and/or packaging methods (e.g. aseptic processing, retortable pouch).
(3) Growing consumer awareness about nutrients and the effects of processing.
(4) Mandatory governmental requirements such as nutrient labelling. (Lenz and Lund, 1980; Suguy and Karel, 1980).

The most sensitive nutrients to changes caused by heat processing are proteins and vitamins. Heating, in general, affects all vitamins to some extent, but the heat sensitive vitamins A, B-1 (thiamin) and C (ascorbic acid) are sometimes taken as indicators for the performance of the other vitamins (Klaui, 1979; Lund, 1979). Ascorbic acid and thiamin are used as indices for retention of water soluble vitamins and vitamin A is used as the index for fat soluble vitamins. Most of the research effort has been concentrated on ascorbic acid and thiamin because they are considered to be heat labile and are relatively easy to assay (Chittaporn, 1977). With respect to the fat soluble vitamins, little information is available, especially with respect to

the rates of destruction as a function of temperature, water activity and oxygen (Labuza et al., 1978). Vitamin A has probably been selected as an index nutrient for fat soluble vitamins because it is easier to measure than the other fat soluble vitamins - D, E and K; and it has important nutritional significance.

Although no coenzyme role has been demonstrated, vitamin A has other functions:

(1) Maintenance of proper vision.
(2) Maintenance of spermatogenesis in the male.
(3) Maintenance of the placenta and prevention of resorption of the foetus in the female.
(4) Maintenance of bone development and growth.
(5) Maintenance of the mucus secreting cells of epithelia, the biosynthesis of glycoproteins, and the prevention of keritinization.
(6) Interaction with vitamin E in regulating stability of biological membranes.
(7) Interrelated with thyroid hormone function.
(8) Involved in production of corticosteriods and in glycogenesis.
(9) Influences synthesis of serum and muscle proteins.

Vitamin A deficiency and xerophthalmia are chronic public health problems in many developing nations (Paden et al., 1979). Even in developed countries, deficiencies exist. Pennington (1976) stated that vitamin A is one of the nutrients most commonly deficient in US diet surveys and it might serve as one of the indices of adequacy of diet.

Because of the nutritional significance of vitamin A and its use as an indicator for the performance of fat soluble vitamins in products and processes, the objective of this study was to determine the kinetics of vitamin A loss in a food system on heat processing. The little quantitative data that existed for vitamin A were for pharmaceutical preparations only (Garrett, 1956; Shah et al., 1976; Slater et al., 1979). Therefore it was very important that kinetic models be developed which describe destruction rates, and their dependence on factors such as temperature and the microenvironment of the food.

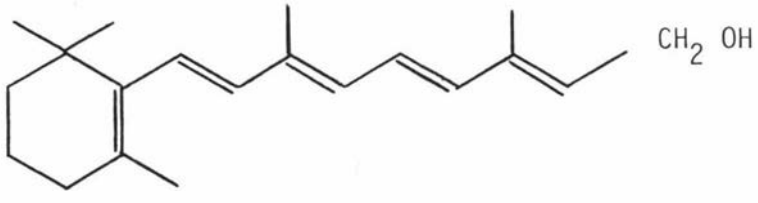
2. LITERATURE REVIEW

This chapter outlines factors affecting stability of vitamins, particularly vitamin A, during heat processing. Reported losses of this vitamin in various products, differing conditions and thermal processes are reviewed. Methods used for determining kinetic models, and their possible application to the study of vitamin A are discussed.

2.1 VITAMIN A

Vitamin A, retinol, occurs naturally only in animals. It is present in highest concentrations in the liver, with other important sources being egg yolk and dairy produce. The carotenoids are precursors and do occur more widely, being found in green and yellow pigmented plant foods. Approximately 50% of the vitamin A in the Western diet is derived from animal tissue as retinol, and the other 50% is derived from plant materials as provitamin A carotenoids (Hurt, 1979).

The substance first recognised as vitamin A (early in the 1900's) is now termed retinol or vitamin A_1. It is an unsaturated cyclic alcohol with 20 carbon atoms and 5 conjugated double bonds. This system of conjugated double bonds is an easy point of attack by oxygen.



The carbon skeleton is a trimethylcylohexenyl ring with an isoprenoid side chain. Vitamin A can exist in different isomeric forms with different biological activities, the all-trans (full bioactivity), the 2-mono-cis (neo) or 13-cis (3/4 activity), the 6-mono-cis or 9-cis, and the 2,6-di-cis or the 9,13-di-cis (¼ activity). In nature, it occurs predominantly in the form of fatty acid esters as the all-trans isomer.

In addition, a second molecular variation known as A_2 ($C_{20}H_{28}O$) exists in the tissue of freshwater fish but is biologically less active than A_1 ($C_{20}H_{30}O$).

Vitamin A is sensitive to air, oxidising agents, and ultraviolet light. Its decomposition is accelerated by increasing temperature and is catalyzed by mineral ions, in particular copper, and to a lesser extent iron. At pH 4.5 or lower, partial isomerisation of vitamin A from the all-trans form to cis forms will occur with a resultant decrease in vitamin potency due to the lower potencies of the cis isomers (de Ritter, 1976).

2.2 FACTORS AFFECTING STABILITY OF VITAMINS DURING HEAT PROCESSING

In optimising the conditions of processing a food, it is important to remember that certain chemical changes are inevitable if the safety of the food at the time of its consumption is to be ensured. However, it is important to minimise those changes which reduce the quality of the product. Thus the key variables which influence vitamin loss must be determined. Vitamin loss on heating is dependent not only on temperature and time but also on other variables such as pH, oxidation-reduction potential, medium composition, the presence of catalytic factors such as heavy metals, composition of the gaseous phase and water activity (Lund, 1975; Tannenbaum, 1976).

Temperature is perhaps the most important of these variables because of its broad influence on all types of chemical reactions. Generally, increasing the temperature increases the rate of reaction and this can be expressed by the Arrhenius equation.

The rates of reaction for the destruction of microorganisms and chemical reactions are very different. Therefore time is an important variable in determining the amount of change that occurs on heat processing. Time can also have an important influence on the relative importance of concurrent reactions.

Another variable, pH, influences the rate of many reactions. It has been shown to affect the rate of loss on heating of pantothenic acid (Hamm and Lund, 1978), folic acid (Paine-Wilson and Chen, 1979), thiamin (Farrer, 1955; Feliciotti and Esselen, 1957; Mulley et al., 1975b) and ascorbic acid (Lee et al., 1977).

Oxidation-reduction systems influence the destruction of thiamin (Dwivedi and Arnold, 1973) and folacin (Chen and Cooper, 1979).

The composition of the product is important since this determines the reactants available for chemical transformation. Thiamin destruction is affected by the type of food, for example in pureed meats and vegetables the destruction occurred at different rates (Feliciotti and Esselen, 1957). The blend of raw materials may cause unexpected interactions e.g. the rate of oxidation can be accelerated or inhibited depending on the amount of salt present. In fabricated foods, the composition can be controlled by adding approved chemicals, such as acidulants, chelating agents or antioxidants, or by removing undesirable reactants. The storage stability of ascorbic acid in tomato juice has been found to be influenced by the presence of copper ions (Lee et al., 1977).

The composition of the gaseous phase is important mainly with respect to the availability of oxygen as a reactant. In situations where it is desirable to limit oxygen, it is unfortunately almost impossible to achieve complete exclusion. The consequences of a small amount of residual oxygen sometimes becomes apparent during subsequent behaviour of the product. For example, the early formation of a small amount of dehydroascorbic acid can lead to browning during storage.

One of the most important variables controlling reaction rates in foods is water activity (a_w). Destruction of α-tocopherol was related to a_w by Widicus et al., (1980). Wanninger (1972) postulated that moisture content affected the rate constant but not the activation energy of the storage stability of ascorbic acid. Labuza (1980) postulated that if viscosity of the system increases with decreasing water activity, the overall rate constant decreases as well.

2.3 STABILITY OF VITAMIN A DURING PROCESSING

Two recent reviews stated that vitamin A has good stability during cooking and processing operations, but losses do occur when the foods are heated in the presence of oxygen (Lang, 1970; Barratt, 1973). Many authors have reported the percentage loss of vitamin A in a product that was given a particular treatment (Tables 2.1 and 2.2). For unsupplemented products such as meat and egg/egg products, cooking gave losses of 0-14%. Stability of vitamin A in fortified products was more varied under cooking conditions. Losses ranged from 0-90% depending on the product and heating time and conditions (Table 2.2). For example, reconstituting enriched nonfat milk powder by boiling for 2-30 min. resulted in 2-6% loss (Bauernfeind and Allen, 1963), whereas heating vitaminised margarine for 40 min. at 130-160^{o}C gave a vitamin A loss of 90% (Benterud, 1977).

For commercial thermal processing operations, retentions vary widely. Negligible losses of vitamin A were reported in the pasteurisation, sterilisation and spray-drying of milk; and in the spray-drying of whole egg (Table 2.1). Canning of composite foods has resulted in losses ranging from 0-100%. Processing, e.g. extrusion of fortified products,appears to give losses of up to 50% (de Muelenaere and Buzzard, 1969; Lee et al., 1978).

Obviously, the differences found in vitamin A retention were caused by different processing conditions and differences in the microenvironment of the food system. However, the data are usually not complete enough to determine the effect of vitamin concentration and temperature on the rate of thermal destruction of vitamin A in foods. Therefore, optimisation of the process for vitamin retention from existing data is not possible.

As indicated in Section 2.2, vitamin loss on heating is dependent not only on time and temperature but also on other variables. Table 2.3 gives details of additional variables which affect vitamin A stability. Generally, vitamin A is regarded as being more stable in basic than acidic pH (O'Brien, 1967; Harris and Karmas, 1975). However, Hannukainen and Niinivaara (1974b) showed the opposite result when the pH of pork liver was lowered from pH 6 to 5.

Table 2.1: Effects of Heat Processing on Unsupplemented Foodstuffs

Reference	Product Type	Process/Product	% Vitamin A Loss
Ford et al., 1969	MILK	UHT milk	0
Thompson, 1969		HTST and UHT milk	0
Cho and Cho, 1970		30 min /64°C 15 s/74°C	Substantial
Goerner and Oravcova, 1970		230-300 min < 20°C	3.9
		150 min < 30°C	7.3
		120 min < 50°C	11.2
Gorner and Koszacka-Setaffy, 1970		Pasteurisation 85°C	15
		Evaporation 46-57°C	5-6
		Contact drying 3s/ 150°C	2.4-2.8
Aristova and Bekhova, 1974		Sweetened condensed milk	10-17
Legge and Richards, 1978		Human breast milk; 8min/4°C-100°C	0
Uherova and Goerner, 1979		Steam injection .36MPa to 140°C/ 3-4s, cooled to 76°C	2.7
Goerner and Uherova, 1980		Prepasteurisation 85°C, then UHT 3-4s/140°C	2.8
Denton et al., 1944	EGG AND EGG PRODUCTS	Spray-drying whole egg	0
		Spray-drying egg yolk	33
Fey and Braun, 1974		Prodn: sponge pastry	14
		scrambled egg,	13
		boiled egg	3.4
Kizlaitis et al., 1964	MEAT	Braising liver to 77°C	0-10
Hannukainen and Niinivaara, 1974a		Mincing and cooking liver	12.4

CONTINUED...

Table 2.1 - continued

Reference	Product Type	Process/Product	% Vitamin A Loss
Hellendoorn et al. 1971	COMPOSITE FOODS	Canning meat and vegetables	0-100
de Ritter et al., 1974		Frozen dinners/pies	3
Labuza, 1974		Canning soups contg meat	15-20
Auffray et al., 1978		Canned baby foods	54 ± 8.5

Table 2.2: Effect of Heat Processing on Fortified Foodstuffs

Reference	Product Type	Process/Product	% Vitamin A Loss
Bauernfeind et al., 1953	CEREALS AND CEREAL BASED PRODUCTS	Cookies and cakes baked 190-205°C	0-20
Fukushi et al., 1967		Enriched flour in bread	34.8
de Muelenaere and Buzzard, 1969		Extrusion corn/soybean/peanut mixture	52.5
Cort et al., 1976		Enriched flour in bread	0
		Cooking fortified rice	1
Benterud, 1977		Commercial baking: bread	10-20
		biscuits and sweet rusks	20-30
Rubin et al., 1977		Bread baked 20 min/213°C	5-17
		Corn grits: boiling water 4-6 min	20-25
		10-30 min	25-30
		Corn meal cooked 5 min	13
Lee et al., 1978		White corn extrud. acetate; retinyl palmitate 130°C	10 10-50
Paden et al., 1979		Fortified sugar in cakes	24
Parrish et al., 1980		Flour in baked foods	0-20
Bauernfiend and Allen, 1963	BEVERAGES	Reconst. nonfat milk powder 2-30 min/100°C	2-6
Brooke and Cort, 1972		Tea, 5 and 60 min boiling	0-96
Head and Hansen, 1979		Fortified milks, 16s/74°C	0
Willich et al., 1954	MISCELLANEOUS	Peanut butter; heated 71°C	5
		82°C	7
Benterud, 1977		Water-free melt carbohydrates and retinyl palmitate	
		15 min/100°C	4
		15 min/110°C	10
		15 min/130°C	12

CONTINUED ...

Table 2.2 - continued

Reference	Product Type	Process/Product	% Vitamin A Loss
Benterud, 1977		Vitaminised Margarine	
		24 min/130-160°C	50
		40 min/130-160°C	90
		12 min/175-200°C	50
		20 min/175-200°C	90
Paden et al., 1979	MISCELLANEOUS (cont.)	Fortified sugar in coffee	
		15 min/100°C	10-11
		Fortified fat	
		30 min/160-180°C	20-40

As retinol is a highly conjugated structure, it is susceptible to oxidation, directly in the presence of oxygen and by the action of peroxides. Antioxidants enhance stability, e.g. ascorbic acid added to butter (Alifax, 1969). The addition of mineral salts tends to have a destabilising influence. Iron was shown to have no effect when pork livers were heated (Hannukainen and Niinivaara, 1974b) or in fortified rice, flour and corn meal (Cort et al., 1976). However, in model systems such as aqueous solutions, ferrous ions catalysed loss (Lucy, 1966; Fisher et al., 1972). Copper, was shown to be significantly correlated with vitamin A loss (Hannukainen and Niinivaara, 1974b).

Composition also appears to markedly influence the stability of vitamin A as shown in Table 2.3. The effects of protein and carbohydrates are conflicting, whereas with moisture content there is an obvious trend. In the case of enriched milk powder and margarine, vitamin A stability decreased with an increase in moisture content (Bauernfeind and Allen, 1963; Imamura et al., 1967a). With regards to fat, vitamin A loss is dependent on its peroxide value. Once oxidation of the fat commences, there is concomitant loss of vitamin A. There are also antivitamin factors in foods such as lipoxidase, thyroxine and citral. Their action is nullified by heat processing.

Though the factors in Table 2.3 are possibly important in vitamin A loss, not all would be encountered in every food processing operation. For example in canning, light, high levels of nitrite, mineral salts and antivitamin factors are unlikely to be important.

Table 2.3: Factors Affecting Vitamin A Stability

Reference	Factor Affecting Stability	Conditions	Effect(s) on Vitamin A
O'Brien, 1967	pH	50% EtOH soln vit A + linoleate hydroperoxide.	Rate of oxidation increased as pH decreased.
Hannukainen and Niinivaara, 1974b		Minced and cooked pork liver; pH reduced 6 to 5.	Better retn in lower pH samples.
Lucy, 1966	OXYGEN	Retinol in EtOH + dissolved air.	Autoxidn; rate influenced by phys. state of retinol.
Fisher et al., 1972		Aqueous colloidal dispersion of retinol plus O_2	Autoxidn; radical catalysed.
Goussault et al., 1977	UV RADIATION	Veg. and meat baby food packed in glass and stored in light (365-580 nm)/10 or 37-40^{o}C for 90 days.	Sensitive to light; destn inhibited by coloured glass at ≤ 20^{o} but not at 40^{o}C.
Hannukainen and Niinivaara, 1974b	ANTI- AND PRO-OXIDANTS	Pork livers boiled in water for 30 min.	Redox potential and copper correlated with loss, ascorbic acid and iron not correlated with loss, $NaNO_2$ helped stabilise.
Alifax, 1969		Ascorbic acid added to butter.	Increased stability.
Gupta and Rao, 1970		Oral liquid vit prepn plus antioxid. held at room temp and 45^{o}C.	Antioxidant enhanced stability.
Klaui, 1971		Vit A palmitate + antioxid./45^{o}C	Stability depended on type of antioxid.
Imamura et al., 1967a		Addn of cottonseed and coconut oils to margarine	Cottonseed oil increased peroxide value, worse affect on stability
O'Brien, 1967		50% EtOH soln vit A + linoleate hydroperoxide + antioxid.	Oxidn 1st order; protection from antioxid.

continued...

Table 2.3 - continued

Reference	Factor Affecting Stability	Conditions	Effect(s) on Vitamin A
Hayashi and Nishii, 1971		Vit A in oily soln	Destruction depended on peroxide values
Gronowska-Senger et al., 1978		Rats fed vit A and peroxide contg lard	Lard with highest peroxide value-poorest utilisation of vit A
Davies and Worden, 1954		Mixed ration feedstuff + mineral salts/ambient	Destructive action of mineral salts
Bauernfeind and Allen, 1963		Reconstn. of enriched nonfat milk powder contg added Cu and Fe	Accelerated losses in comparison with no added minerals
Lucy, 1966	ANTI- AND PRO-OXIDANTS (cont .)	Retinol in saline or water plus Fe^{2+}	Degradation catalysed by Fe^{2+}
Fisher et al., 1972		Retinol in aq. colloidal dispersions + Fe^{2+}	Autoxidation catalysed by Fe^{2+}
Cort et al., 1976		Fortified flour, rice, and corn meal + minerals	Stable in presence of Fe, Zn, Mg and Ca in flour, and corn meal; and in presence of Fe and Zn in rice
Rubin et al., 1977		Fortified flour in bread	Ca and Mg might worsen loss
Matthews and Workman, 1978		Canned veg. and meat dinners for babies	Vit A decreased with increases in Fe
Manz et al., 1980		Choline and minerals in livestock feeds; 4 months at ambient	Vit A loss of 7.75%

continued...

Table 2.3 - continued

Reference	Factor Affecting Stability	Conditions	Effect(s) on Vitamin A
Imamura et al., 1967b	PROTEIN	Addn. skim milk to margarine	Increased stability; suppressed increase of peroxide value
Bhattacharya, 1968		Proteins, after different treatments added to vit A	Effect depended on prepn. of protein; protein and fats offered better protection than proteins alone
Bhattacharya, 1969		Mixtures of vit A + diff. proteins + surfactants	Proteins retarded autoxidation
Imamura et al., 1967b	CARBOHYDRATE	Addn. skim milk to margarine	Increased stability; suppressed increase of peroxide value
Bhattacharya, 1969		Mixtures of vit A prepd.with carbo-hydrates and non-protein macro-molecules	Rapid oxidation
Bauernfeind and Allen, 1963	MOISTURE CONTENT	Stored, enriched nonfat milk powder	Increased moisture content resulted in increased loss
Imamura et al. 1967a		Vit A added to margarine	Increased moisture content resulted in increased loss
Imamura et al. 1967a	FAT	Addn of cottonseed and coconut oils to margarine	Cottonseed oil increased peroxide value; worse affect on stability
Alifax, 1969		Butter + added ascorbic acid	Disappearance coincided with autoxidation phase of fats
Gronowska-Senger et al., 1978		Rats fed lard + vit A	Lard with highest peroxide value - poorest utilisation
Lepkovsky, 1966	ANTIVITAMIN A FACTORS	Lipoxidase, thyroxine, citral	Oxidises fat and leads to loss; aggravates vit A deficiency; acts as an antagonist

2.4 REACTION KINETICS

To optimise nutrient retention, it is important that meaningful data for evaluating the kinetic parameters be available for the thermal destruction of nutrients under the variety of conditions found in foods. These parameters describe the effect of nutrient concentration and time-temperature regimes on loss.

Kinetic parameters can either be determined for the nutrient in a model system or in a food system. Model systems have the advantage in that they are better defined and controlled, and analysis is simpler and more accurate than those involving complex tissue samples. A major disadvantage of the approach is that it fails to consider that there may be factors present in the complex tissue samples which influence the stability of the vitamin. Consequently, observations made in the experiments with simple model solutions of vitamin A may not necessarily apply to complex tissue samples (Navankasattusas, 1978).

The first experimental step is to obtain data for the change in the concentration of reactant as a function of time. From this, the mathematical form of the rate expression can be determined. Generally, the data at each temperature are compared to possible models for the kinetics (often zero-, first-, or second-order models). For many food components, a first-order kinetic model adequately describes the destruction (Stumbo, 1973). This procedure provides a model which describes the kinetics of the reaction. It does not necessarily represent the actual mechanism of the reaction.

Once the concentration-dependence of the rate at fixed temperature has been determined, the temperature-dependence of the reaction rate constant is evaluated. This can be expressed by the Arrhenius equation:

$$k = Ae^{E_a/RT}$$

where k = rate constant
A = frequency factor
E_a = activation energy
R = gas constant
T = absolute temperature.

This equation is found to fit many of the available experimental kinetic data (Aiba et al., 1973). Data conforming to the Arrhenius equation yield a straight line when ln k is plotted against 1/T. Food reactions generally observe the Arrhenius relationship over a certain intermediate temperature range but deviations from this relationship can occur at high or low temperatures (McWeeny, 1968). Thus, it is important to remember that the Arrhenius relationship for food systems can be used only over a range of temperatures that has been experimentally tested. McWeeny lists four reasons for deviations from the Arrhenius relationship occurring,most of which are induced by either high or low temperatures:

(i) enzyme activity may be lost,
(ii) the reaction pathway may change or it may be influenced by a competing reaction(s),
(iii) the physical state of the system may change,
(iv) one or more of the reactants may become depleted.

His fourth reason can only arise if the apparent order of reaction changes.

The only comprehensive kinetic data for vitamin A have been obtained for pharmaceutical preparations, not for food systems. Garrett (1956) determined an activation energy for vitamin A in liquid multivitamin preparations (pH 3.2) of 61 $kJmol^{-1}$. In 1976, Shah et al. demonstrated that vitamin A in multivitamin drops decomposed in accordance with a first order reaction and the activation energy had a value of 95 $kJmol^{-1}$. Slater et al. (1979) measured the shelf-life stability of vitamin A in multivitamin tablets and determined that degradation was pseudo-first order. Analysis of Slater's data gave an activation energy of 118 $kJmol^{-1}$.

For food systems, some rate constants were calculated from the literature. Analysis by the author of some published data on the heating of enriched ghee (Maqsood et al., 1963) gave a first order reaction for vitamin A degradation at both 100 and 200°C. The rate constants were $k = 1.09 \times 10^{-3}\ s^{-1}$ (heating ghee in boiling water) and $k = 18.70 \times 10^{-3}\ s^{-1}$ (frying ghee at 200°C). For the reconstitution of a fortified whey-soy drink mix by boiling in water (Dellamonica et al., 1979), a first order rate constant of $k = 3.07 \times 10^{-3}\ s^{-1}$ was calculated. This

variability in the value of the rate constants for different products and the paucity of comprehensive kinetic data for vitamin A loss on thermal processing of foods indicates the importance of determining kinetic models for this vitamin.

2.5 DETERMINATION OF KINETIC MODELS

To determine the kinetic model for destruction of food components or characteristics during heat processing, two procedures can be used - these are known as steady state and unsteady state procedures. In the steady state method,small samples are used so that the time required to reach the processing temperature is small in comparison with the processing time at that temperature. Thus the heating process approximates a "square" process and all the sample is exposed to virtually the same processing conditions. In unsteady state processes, larger samples (e.g. a whole can) are used and both come-up times and variation in processing conditions with position are significant. In many such processes,steady state may never be achieved.

In the steady state procedure, the containers used to quickly heat the sample to the processing temperature are sometimes called thermal death time containers (Stumbo, 1973; Lenz and Lund, 1980). As all the food is at constant temperature (i.e. steady state) during processing, the final data analysis is simpler than for unsteady state, processing. After processing, the sample from each time/temperature combination is analysed to determine the concentration of the desired nutrient. In the unsteady state procedure, any container may be used for processing. The tin-plated mild steel can, which may or may not be lacquered internally and/or externally, is the container most commonly used in commercial thermal processing operations. Destruction occurring during a thermal lag (heat-up or cool-down) is part of the final data analysis. As with the steady state procedure, the raw data consist of concentration of the desired component versus processing conditions (temperature/time combination). However, in this case generally only the mass average concentration of the component in the can before and after processing is known. To determine the concentration in different parts of the can, a model is needed to predict the temperature distribution through the can and this in turn is used to predict the concentration. Thus the calculation of the kinetic model is more complex than with the steady state procedure (Taimmanenate, 1980).

2.6 CONCLUSIONS AND RESEARCH OBJECTIVES

The lack of kinetic data for food systems therefore justified research into the rate of vitamin A loss during thermal processing. If data existed for different food systems, the extent of loss of this vitamin during commercial heat processing operations could be predicted. Therefore, in this study it was decided to investigate a food rather than a model system. This was because data collected in model systems may not relate to vitamin A destruction in foods, where a number of factors can interact.

Approximately 50% of the vitamin A in the Western diet is derived from animal tissue. Beef liver, which has a naturally high retinol content (100-400 $\mu g g^{-1}$), is an important source. Further, it is often heat processed, is readily available, and is cheap. It was therefore chosen as the food system to be studied.

Both steady and unsteady state heat processing were investigated. The steady state procedure, because of its ease of final data analysis and the small amount of raw material required, was employed to characterise and quantify the kinetics of vitamin A loss in natural beef liver, and in beef liver and fat mixtures. Unsteady state heating of cans was used to see whether the steady state kinetic parameters were also valid in unsteady state heating, and whether steady state kinetic parameters could be used to predict vitamin A losses in commercial thermal processing operations. The temperature range studied was the typical canning range for meat products - 102 - 127^{o}C. Oxygen, pH, antioxidants, pro-antixodants, protein, carbohydrate, moisture content, fat, all have an effect on loss of vitamin A on heat processing. However in canning, many of these will not be important. Factors most likely to affect the rate of vitamin A loss on heat processing of liver were fat, protein and moisture contents, pH and the presence of heavy metals such as copper. These factors were therefore investigated to determine their effect on vitamin A loss on thermal processing. The effect of carbohydrate was not considered because of the low level in natural liver and formulated liver products.

The research objectives were therefore:

(1) To determine the kinetic parameters for the loss of vitamin A on heat processing of natural beef liver under steady state conditions.

(2) To find the effects on the kinetic parameters, of fat, protein, moisture content, pH and copper in liver mixtures.

(3) To establish whether kinetic parameters determined in steady state heat processing could be used to predict losses in unsteady state heat processing of cans.

3. METHODS

In order to determine the effects of heating on vitamin A loss in a food system, it is important to be able to accurately measure the vitamin A concentration. This chapter first reviews vitamin A determination in foods and describes in detail the assay used. Secondly, methods to analyze the proximate composition of liver are given. Finally the two heating methods (steady and unsteady state) used to investigate the thermal stability are described.

3.1 VITAMIN A DETERMINATION

3.1.1 Literature Review of Vitamin A Determination in Foods

In selecting a method to determine vitamin A, the nature of the food product, as well as forms and levels of vitamin A present, must be considered. The major part of vitamin A in unsupplemented foods, such as milk, butter, cheese, and liver is present as mixed esters of vitamin A alcohol (retinol). However, in eggs, the largest part of the vitamin A is the unesterified alcohol, retinol, with lesser amounts of retinaldehyde and retinyl esters.

Parrish (1977) gave an excellent review of the determination of vitamin A in foods. Physico-chemical methods have replaced the biological methods with laboratory animals, which were used during the early years of vitamin investigations. However, biological methods are still necessary to study availability of vitamin A in foods, to determine biopotency of different forms and isomers of vitamin A, and similar problems. Determinations by physico-chemical methods are more rapid, controllable, and precise than animal procedures.

Most of the common methods used to identify and measure vitamin A content are based on the properties of vitamin A, which include its absorbance at 320 nm, its fluorescence, and its ability to form coloured compounds with antimony trichloride and other reagents. A variety of methods have been developed (Table 3.1); except for possibly gas-liquid chromatography and some of the special purpose methods, they have been used with varying degrees of success for determining vitamin A in foods.

Table 3.1: Classification of Methods to Determine Vitamin A (Retinol) Proposed by Parrish (1977)

(i)	Spectrophotometric:	direct reading
		destructive irradiation
		convert to anhydroretinol
(ii)	Colorimetric:	blue colour - Carr-Price, or other blue-colour reagents
		dichlorohydrin reagent
		others such as phenols, ferrous compounds, phosphomolybdic acid
(iii)	Fluorometric:	direct reading
		destructive irradiation
(iv)	Chromatographic:	(used in conjuction with (i), (ii) or (iii))
		adsorption columns
		partition columns
		thin layer
		paper
		gas liquid
		liquid-liquid
		HPLC
(v)	Others:	electrochemical, infra-red, X-ray diffraction etc. for special identification of isomers, structures.

Since this review appeared in 1977, methods of determining vitamin A in foods have concentrated largely on high pressure liquid chromatographic (HPLC) techniques. Methods have been published by Cohen and Lapointe (1978); Frolik et al. (1978); Henderson and McLean (1979); Barnett et al. (1980); and Thompson et al. (1980). This interest in HPLC is based on claims of speed, versatility and reliability. However, these claims should be tempered by the realisation that in the overall vitamin A analysis, hydrolysis and extractions are by far the time-consuming steps, and unless foods are analyzed regularly for vitamin A content, it is difficult to justify the expense of an HPLC unit.

With foods, it is generally not possible to use one of the methods in Table 3.1 directly. This is because either the concentration of vitamin A is small in comparison to other components that may interfere, or vitamin A is bound so that it cannot be extracted without pretreatment. An alkaline digestion is generally used in the vitamin A determination for foods to free the vitamin from the stabilizing matrix, from the lipids in which it might be dissolved, or from substances in the food that might interfere with the extraction. Saponification does not materially destroy vitamin A in most natural or supplemented foods. The esterified vitamin A is converted to the alcoholic form, retinol; vitamin A is then determined as retinol, which yields results by colorimetric or spectrophotometric procedures similar to those obtained on the esters.

The Carr-Price antimony trichloride colorimetric procedure (Carr and Price, 1926) is by far the most widely accepted method to determine vitamin A in foods. It is the official AOAC method for feeds, premixes and foods. The Carr-Price, or similar colorimetric method, is better than the spectrophotometric method in analyzing sample extracts with relatively low quantities of vitamin A in the presence of steriods, vitamin E, etc., where spectrophotometric results are falsely high, even after chromatography.

Disadvantages and problems attributed to the Carr-Price method include: rapidly fading blue colour, sensitivity of the reagent to moisture, formation of water-insoluble precipitates on glassware from the reaction of reagent and moisture, interference from carotenoids, and cost of the reagent. However, there are solutions to these problems.

In this study a modification of the official AOAC method, employing the Carr-Price colorimetric procedures, was used to determine the vitamin A content of beef liver and beef liver mixtures. It was chosen because it is a well-recognised and widely applicable technique. HPLC looked promising, but was not considered because a unit was not available at the commencement of research and refinement of techniques had yet to be published. Thus the assay method used was based on that described by Haggett (1976). This procedure was a development of methods used in the Vitamin Assay Laboratories of Roche Products Pty Ltd, Australia. The principle is that the food homogenate is first saponified with ethanolic potassium hydroxide. After extracting and washing the unsaponifiable matter, the trans-retinol is separated by thin layer chromatography. It is quantitatively recovered in chloroform. Trans-retinol is colorimetrically measured according to the Carr-Price method at 610 nm (Carr and Price, 1926). The actual method is given in the following sections.

3.1.2 Reagents

Unless otherwise stated all aqueous solutions were prepared with glass distilled water.

3.1.2.1 Ethanol (95%)

Aldehyde-free by Schiff's test.

3.1.2.2 Potassium Hydroxide Solution (50%)

Laboratory grade potassium hydroxide (500g) was dissolved in water and made up to 1 litre of solution.

3.1.2.3 Diethyl Ether

Peroxide free.

3.1.2.4 Ether-Saturated Distilled Water

Distilled water (1 l) was vigorously shaken with petroleum ether (ca 100 cm^3) and peroxide-free diethyl ether (200 cm^3) for 2 min in a separating funnel. The bottom layer was used as required.

3.1.2.5 Cyclohexane

Analytical reagent.

3.1.2.6 Ethyl Acetate

Analytical reagent.

3.1.2.7 Chromatography Solvent

Cyclohexane (375 cm^3) and ethyl acetate (125 cm^3) were added to a glass stoppered cylinder and shaken. The tanks were filled to a depth of 1 cm. The solvent was completely renewed after 6-8 developments.

3.1.2.8 Spotting Solvent

Petroleum ether (20 cm^3), triethylamine (2 cm^3) and absolute ethanol (2 cm^3) were mixed together.

3.1.2.9 Thin Layer Chromatography Plates

Kieselgel G (Merck) was dried on a tray for 1 hr at 100°C. To the dry Kieselgel (50 g) was added water (100 cm^3) and was vigorously shaken for 1 min. At the same time, to Kieselguhr (17 g) (containing 10% calcium sulphate) was added water (32 cm^3) and was also shaken vigorously for 1 min. The plate spreader was divided into two unequal compartments (5:1), into which the two solutions were poured. Glass plates (20 x 20 cm) were spread to a depth of 0.6 mm. After spreading they were air dried. The plates were activated at 100°C for 2 hrs immediately prior to use.

3.1.2.10 Petroleum Ether

Reagent grade (40 - 60°C fraction).

3.1.2.11 Chloroform

Reagent grade. If not clear, was purified by distillation, the first and last 10% being discarded.

3.1.2.12 Reference Standard

Trans-retinol palmitate; crystalline, Sigma grade; Type I; synthetic 513000 $\mu g g^{-1}$; contained BHA and BHT to retard oxidation. Stored at 0 - 5°C.

3.1.2.13 Antimony Trichloride (Carr-Price) Reagent

Either:

(i) 100 cm^3 ampoule Carr-Price reagent (BDH) or

(ii) reagent grade antimony trichloride crystals from unopened, tightly stoppered bottle. Antimony trichloride crystals (100 g) were added to chloroform and diluted to 500 cm^3. It was warmed under reflux on a water bath, and shaken to dissolve the crystals. Cooled, and acetic anhydride (15 cm^3) added. If solution was not clear, it was filtered, centrifuged, or allowed to settle and then decanted.

3.1.3 Standardisation

The top of the ampoule containing the USP Reference Standard of retinol was knocked off and the oil expressed into a small tared beaker. It was weighed accurately. The oil was transferred to a volumetric flask and diluted to volume with chloroform. Dilutions of the Standard solution were made with chloroform so that aliquots treated as described later gave absorbances in the 0.1 - 0.7 range at 610 nm. Absorbance was plotted against μg retinol cm^{-3}. If this plot was a straight line, a factor was calculated for determining the retinol content in beef and beef liver mixtures (see Section 3.1.4.3).

3.1.4 Method for Determining Trans-Retinol in Beef Liver Puree

3.1.4.1 Saponification and Extraction of Unsaponifiable Matter

Sample was weighed into a 250 cm^3 round bottomed flask. Ethanol (50 cm^3) and potassium hydroxide solution (15 cm^3) were added and mixed. It was saponified for 30 min in a water bath at 65 - 70°C. After saponification, the flask was cooled to room temperature under running water.

The contents of the flask were transferred to a 500 cm^3 separating funnel by rinsing with ethanol (30 cm^3) followed by distilled water (70 cm^3). Diethyl ether (80 cm^3) was added and shaken vigorously for 2 min; petroleum ether (80 cm^3) was added and shaken gently (about 10 to and fro motions). It was left to stand until the layers separated. The bottom aqueous layer was removed. The extract was washed with ether-saturated water: (i) 50 cm^3, (ii) 70 cm^3, (iii) 100 cm^3. The water was merely poured through the extract for the first washing, gentle agitation (2-3 to and fro motions) for the second wash and more vigorous (8-10) for the final wash.

The organic extract was transferred to a 250 cm^3 stoppered cylinder and the volume, V_f, noted. The separatory funnel was rinsed with small quantities of petroleum ether and added to the extract in the stoppered cylinder. The extract was dried with anhydrous sodium sulphate for 1.5 - 2.0 hrs. The organic layer was evaporated at a temperature of 40^{o}C, along with a petroleum ether rinse (30 cm^3) of the desiccant, to 1 cm^3. Immediately spotting solvent (1 cm^3) was added.

3.1.4.2 Thin Layer Chromatography

The activated plates were scribed along all edges for ca 2 mm and then scored ca 2 cm from the top. The sample was loaded with a Pasteur pipette as a thin band just below the inert strip boundary. The flask was washed with spotting solvent (0.5 cm^3) and washings loaded onto plate immediately below sample band. It was run to inert boundary with acetone to concentrate band then placed in a tank and left to develop in the dark to the scored line.

The wet plate was rapidly inspected under ultraviolet light (366 nm) and the band corresponding to retinol (fluoresced greenish-yellow) marked; scraped off quantitatively and transfered to sintered glass crucible containing chloroform (10 cm^3). It was extracted with further chloroform (30 cm^3) into a 50 cm^3 stoppered cylinder, and made up to a specified volume (V), which was noted.

3.1.4.3 The Carr-Price Method

The spectrophotometer (Hitachi, model 101) was adjusted to 0% absorbance at 610 nm using chloroform (1 cm^3) and Carr-Price reagent (3 cm^3). The tube containing chloroform solution of vitamin A (1 cm^3) was placed in the instrument. Carr-Price reagent (3 cm^3) was added in 1-2 sec. The absorbance was read at first transitory pause point (absorbance in the range 0.20 - 0.50) ca 10 sec after commencement of addition. The solution should have been clear and blue. Other colours indicated isomerisation of retinol.

(i) Absorbance Correction for Sample Losses:

$$A_c = \frac{A_s \times V_i}{V_f}$$

where A_c = absorbance correction
A_s = sample absorbance
V_i = initial volume of diethyl ether and petroleum ether added (~160 cm^3)
V_f = final volume of organic solvents, cm^3

(ii) Concentration in Sample:

$$\text{Concn} = \left[\frac{A_c - \alpha}{\beta}\right] \times \frac{V}{w}, \quad \mu g g^{-1}$$

where α = intercept value from standard curve
β = constant from standard curve
w = mass of sample taken, g
V = volume of chloroform extract, cm^3

3.1.5 Accuracy of the Assay Procedure

3.1.5.1 Recovery

No significant losses occurred during the saponification and extraction stage of the assay procedure. This agrees with the findings of Haggett (1978). Recovery from thin layer chromatography plates was in the range 94-98%, and was determined by adding a known quantity of vitamin A to the plate and proceeding with the assay from Section 3.1.4.2 to 3.1.4.3.

3.1.5.2 Isomers

The Carr-Price assay as such does not distinguish between the vitamin A isomers and so only total vitamin A is determined. Thus nothing is known about the biological potency. By using thin layer chromatography it was relatively easy to detect trans-retinol by its characteristic yellowish-green fluorescence at 366 nm and characteristic R_f value of 0.460 to 0.548 when concentrated into a band by acetone and then developed in cyclohexane-ethyl acetate (3:1) solvent. It was noticed that heated liver samples gave additional fluorescent and absorption bands when observed under UV. However, only vitamin A, determined as trans-retinol, was measured as only this band was removed from the thin layer plate. An additional check that isomers were not measured was observation of the chloroform extract-Carr-Price solution, which was clear and blue. Other colours, e.g. red, pink/purple, indicate isomerisation of retinol.

3.1.5.3 Reproducibility

Reproducibility of the Carr-Price method is $\pm$ 3 - 10% depending upon the product (AVC, 1966). For beef liver puree and beef liver mixtures, replication of analysis was virtually always within 8 $\mu g\ g^{-1}$ of the mean which is a variability of up to 5% in the determined retinol concentration.

3.2 PROXIMATE ANALYSIS OF BEEF LIVER

To determine the effect of composition on the loss of vitamin A on heating, it was important to know the actual composition of the liver so that it could be adjusted to the desired fat, protein and moisture levels etc. Analyses were mainly based on those of the AOAC (1980) or modifications of them.

3.2.1 pH Determination

The pH was measured at 20°C on an expanded scale pH meter; model PHM26c (Radiometer), with a 2 buffer adjustment at pH 7.0 and 4.0.

3.2.2 Redox Potential (E_h) Determination

E_h was determined in the range 18 to 22°C, usually at 20°C. It was measured by a PHM26c pH meter (Radiometer) using a platinum electrode P101 and a calomel electrode (Leistner and Wirth, 1965).

$$E_{h_{20}} = E_{calomel} + 249 + 58.1\ (pH - 7)\ mV$$

3.2.3 Moisture Determination

Air oven method (AOAC, 24.003, 1980). Ca 5 g sample dried at 100°C for 16 hours.

3.2.4 Crude Fat Determination (Soxhlet)

Dried sample from 3.2.3 extracted with petroleum ether (boiling point range 40 - 60°C) for 5 hours (AOAC, 24.005, 1980).

3.2.5 Protein Determination (Kjeldahl)

AOAC, 24.027, 1980.

3.2.6 Copper Determination (Zinc Dibenzyldithiocarbamate Method)

Pearson, p 81, 1976.

3.3 REAGENTS FOR SAMPLE ADJUSTMENT

The proximate composition of the base raw material was altered by one or more of the following reagents. Unless otherwise stated, all aqueous solutions were prepared with glass distilled water.

3.3.1 pH Adjusters

(i) ca 1 M HCL: Dilute 86.2 cm^3 of 36.5% (11.6 M) HCL to 1 l with water.

(ii) ca 1 M NaOH: Dissolve 10 g NaOH pellets in water and dilute the solution to 250 cm^3 with water.

3.3.2 Concentrated Solution of Copper Ions (200 $\mu g\ cm^{-3}$)

Dissolve 0.157 g of $CuSO_4.5H_2O$ (BDH Analytical Reagent) with water and dilute to 200 cm^3.

3.3.3 Fat

Commercially produced lard.

3.3.4 Protein Source

Beef liver protein concentrate (88.5% protein, 8.3% moisture) was prepared according to Toledo's (1973) low temperature extraction process for animal protein concentrates. Preparation was a 3 stage solvent extraction at 45°C, using 85% (by weight) isopropanol. The solvent-to-solids ratio was 4.3:1. After each extraction stage, the solids and extract were separated by vacuum filtration. After the last extraction stage, the solids were spread evenly in a 3 mm layer on a pan and vacuum dried at 0.35 bar at 40°C for 2 hr.

3.3.5 Water

Glass distilled water.

3.4 HEATING METHODS

To determine the kinetic model for destruction of food components or characteristics during heat treatment, two procedures can be used - a steady state procedure where thermal death time cans or tubes are commonly used or an unsteady state procedure where food is heated in any container (Lenz and Lund, 1980). In the steady state procedure, heating and cooling to and from the desired temperature should be instantaneous so that no significant destruction occurs during the heat-up and cooling periods. In practice, this is difficult to obtain.

3.4.1 Steady State Heating Theory

The problem of thermal lags in steady state procedures has been tackled in different ways.

(i) Thermal lags can be ignored, should they be small and insignificant and result in only small errors to the processing effect.
(ii) Procedures can be found to correct for these lags.
(iii) The thermal lag periods can be reduced so that either they can be considered negligible or their correction needs not be so extensive.

Thermal lags can generally be ignored if the thermoresistor (Stumbo, 1948), capillary tube (Stern and Proctor, 1954) and flask methods (Levine et al., 1927) are employed. In the first two methods very small sample sizes are used (0.10 cm^3), and in the last one low temperatures (< 100^oC) are employed with the assumption that not much destruction occurs during the come-up time. Some workers who have used these methods, ignored thermal lags and obtained kinetic data, include: Lyster (1970) who investigated the denaturation of α-lactalbumin and β-lactoglobulin in heated milk in capillary tubes; Mulley et al., (1975a) who studied the kinetics of thiamin degradation by heat using a thermoresistor; and Eagerman and Rouse (1976) who used the flask method to determine heat inactivation-time relationships for pectinesterase inactivation of citrus juices. The flask method was also used by Chen and Cooper (1979).

Thermal lags have also been ignored when the holding time at a specified temperature is considerably greater than the come-up time e.g. Navankasattusas (1978) who investigated the stability of vitamin B_6 in the thermal processing of foods, and Saguy et al. (1978) who studied the thermal kinetic degradation of betanin and betalamic acid.

Procedures for correcting for thermal lags fall into three general categories - graphical methods, numerical methods and experimental manipulation. Graphical methods were used by Farkas et al. (1962), Saper et al. (1962), Gupte et al. (1964) and Burnette and Flick (1978).

Two numerical methods can be used to correct for thermal lags. Those that assume a "z" value and employ developed methods to obtain an equivalent time at a specified temperature (Sognefest and Benjamin (1944); Tan and Francis (1962), and Lin et al. (1970)); and those based on iterative procedures which ultimately determine "z" values and equivalent processing times (Resende et al., (1969) and Hayakawa et al., (1977)). The iterative procedures were developed to account for the thermal lags as z values could not be used when it is the z value which is to be determined. Experimental manipulation to correct for thermal lags involve determining the come-up time and then starting actual timing from this period. This method was used by Feliciotti and Esselen (1957) and Hamm and Lund (1978).

The third way of tackling this problem is to reduce the lag periods. This can be done by using very small samples but there may be a problem with regards to analysis because of the sample size. If the sample is to be heated in water or oil baths, then the container could either be preheated prior to addition of the sample, constructed from a material of high thermal conductivity such as metal and/or be thin walled, agitated to increase the rate of heat transfer, or a booster heater could be used to rapidly bring up the bath temperature.

3.4.2 Unsteady State Heating Theory

In the unsteady state, it is impossible to reduce the lag time and it varies with different positions in the container. The temperature of the food in a container is dependent not only on time but also on position. The temperature distribution in the container at any given process time must be determined,as the calculation of the kinetic parameters is based on the mass average retention concentration. Two methods to determine the temperature distribution are experimental measurement and theoretical prediction. These methods have been reviewed by Taimmanenate (1980). Experimental measurement of temperature has developed from the use of thermometers to thermocouples, where more points in the can may be followed by continuous recording potentiometric devices. However there is a limitation on the number of points that can be followed, and on the time interval of recorded temperature. Therefore, a theoretical determination is needed for calculating temperature distribution in the can.

Provided heating within a can is by conduction only, both analytical and numerical methods exist to predict temperature distribution as a function of time and position (Taimmanenate, 1980). These, and their use are described in Section 7.2.3.

3.4.3 Steady State Heating Method

To minimize the thermal lag during heating and cooling, containers had to be chosen which were readily available and relatively cheap, inert, heat-sealable, and have a wall thickness thin enough to avoid excessive thermal resistance to heat penetration and yet strong enough to withstand internal pressure created when heated to 127^{o}C. Glass vials made from capillary tubing were chosen. These tubes were heated in an oil bath for stated times and temperatures. The food product was at constant temperature (steady-state) during processing after the initial come-up period.

3.4.3.1 Preparation of Vials

Boro-silicate glass capillary tubing, with an internal diameter of 3 mm and an external diameter of 5 mm, was cut into 130 mm lengths. One end of the length was heat sealed with a natural gas flame. Approximately 0.5 g of beef liver puree or beef liver puree mixture, was put into each vial using a Pasteur pipette attached to a 10 cm^3 disposable syringe,and ensuring no smearing of the sample occurred at the top end. The vials were then heat sealed under vacuum (head-space 45 mm) and stored in the frozen state (-18^{o}C) until required for heat processing.

3.4.3.2 Heating Aparatus

A diagrammatic representation of the apparatus used is shown in Figure 3.1. The temperature in the insulated oil bath was thermo-statically controlled to $\pm$ 0.2^{o}C using a Variac or a "Simmerset" temperature controller. A Voss stirrer was used to ensure an even temperature distribution throughout the bath. The vials were placed in a holder made of 2 metal sheets with holes punched equidistantly in them. The sheets were connected with a metal rod at each corner. Each tube was separated from the other by 1.5 cm to ensure that the heating medium could easily circulate between them.

3.4.3.3 Determination of the Temperature-Time Profile in the Vials

A vial prepared in a similar fashion to the tubes containing beef liver puree (BLP) was used to follow the temperature-time profile of the BLP during heating and cooling. A 24 gauge copper-constantan thermocouple was inserted in the tube with the sensing junction immersed in the puree approximately at the goemetric centre of the sample. The vial was sealed with epoxy resin. The thermocouple was connected to a recording potentiometer (12 point Honeywell-Brown with a 72 second print cycle) which printed its temperature during heating and cooling.

The temperature-time profile of the vial during the come-up time was calculated using a finite difference program, the listing of which can be seen in Appendix A.2. The program evaluated the temperatures through the glass wall and the liver every 2 seconds and showed that the time for the slowest heating point in the sample to rise to within 0.1^{o}C of the heating medium was 60 seconds.

Components:
1. Thermally insulated container
2. Heating element, 450W
3. Voss stirrer, 200-250V, 0-33 s^{-1} (Voss Instruments, U.K.)
4. Capillary holder
5. Vial and thermocouple
6. Vial containing beef liver puree
7. Temperature recorder
8. Temperature controller
9. Oil heating medium, 10.3 litres

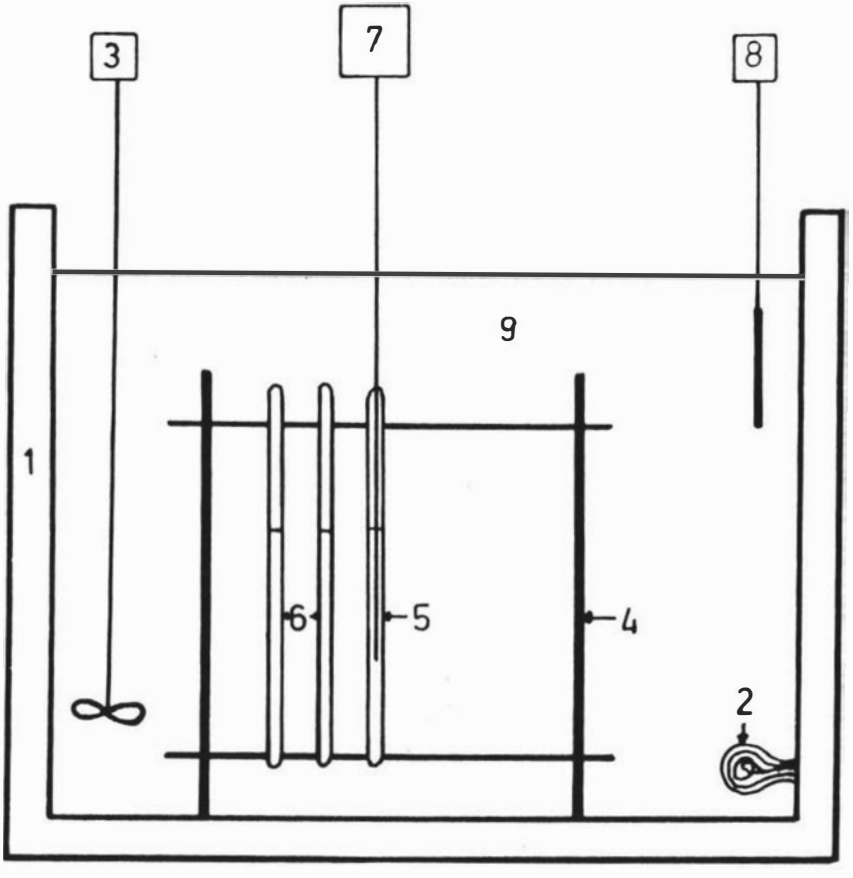


Figure 3.1 Heating apparatus for steady state heating

3.4.3.4 Conditions of Heating and Cooling

In all steady state heating experiments, the following general procedure was practised. The capillary holder was preheated in an air-oven to minimize temperature drops in the medium once this and the samples were placed in it. For each run, eighteen to twenty vials were heated at the specified temperature in the range 102-127^{o}C, typical meat canning temperatures. At pre-selected times 3 or 4 tubes, randomly chosen, were withdrawn from the holder and plunged into a crushed ice/water bath. After cooling, the tubes were washed and stored in the dark at -18^{o}C until analyzed for trans-retinol content. Immediately prior to analysis, the vials were opened and the BLP blown out under a stream of compressed air into 250 cm^{3} round bottom flask. Analysis for trans-retinol was then conducted according to the method described in 3.1.4. Control samples, which were not heated, were treated in a similar manner to the test ones.

3.4.4 Unsteady State Heating Method

Prior to heat processing in the autoclave, several pre-processing steps were necessary and these, along with the actual thermal processing conditions are described.

3.4.4.1 Pre-Processing Conditions

Thawed beef liver mixtures were put into code 3 anti-sulphur cans (74 x 112 mm). During filling, a net headspace of zero mm was sought to minimize can to can variations in the temperature responses of the samples. However, on processing, the headspace was no longer negligible. This was because the water holding capacity of the meat decreased on heating with the consequence that there was shrinkage of the meat, and fat and water release. After filling, but prior to sealing, the cans were covered with cling wrap followed by aluminium foil, and suspended in a 60^{o}C water bath to preheat the liver. On equilibration, the cans were vacuum sealed at a pressure of 0.5 bar. They were then put back into the water bath for the temperature to re-equilibrate to 60^{o}C.

3.4.4.2 Processing Conditions

The cans taken from the water bath were processed in a retort, 0.80 m long x 0.55 m internal diameter (laboratory-scale, horizontal autoclave, Berry Engineering Ltd, N.Z.). This involved putting four preheated cans (2 with thermocouples located in their geometric centre and 2 to be used for vitamin A analysis) into the retort and processing at 112 or 120°C to achieve an F value of 2 or 6 (Section 7.2). The retort was vented for 2 minutes and zero heating time taken as the time when the retort steam temperature reached 100°C. In cooling, the retort pressure was reduced to atmospheric and the retort filled to 80 - 85% of its volume with cooling water. This water level was maintained by a continuous flow of cold water. The total cooling time was 40 minutes.

To follow the temperature-time profile of the beef liver mixtures, 2 of the cans in each run had centrally located thermocouples. A tension method of thermocouple mounting was used (Board, 1965). The two wires passed through diametrically opposed holes in the can walls and were sealed with a heat resistant epoxy resin adhesive. Standard 24 gauge copper-constantan thermocouples were used. These were connected to a 12-point Honeywell-Brown recording potentiometer operating on a 72 second print cycle during the processing runs. The centre temperature of the liver was measured and recorded by the potentiometer.

Control samples (2 further cans) were subjected to exactly the same treatment as the four processed ones except they were not put into the retort. The seamed controls, after being held at 60°C for the same time as the test samples, were water cooled for 40 minutes. After cooling, the control and processed cans were frozen and stored at -18°C until analyzed for trans-retinol content. They were completely thawed and the can contents thoroughly mixed before duplicate samples were taken for analysis.

4. CHARACTERISATION OF THE KINETICS OF VITAMIN A LOSS ON STEADY STATE HEATING OF LIVER

4.1 INTRODUCTION

Published data (Tables 2.1 and 2.2) gave only total vitamin A losses in particular processes. In order for predictions to be made of losses in any process, it was necessary to characterise and quantify the temperature, time and concentration dependence of its loss.

Natural beef liver was the food system chosen to investigate vitamin A loss. Five heating times at each of five temperatures, in the range 103 -127^{o}C, were used to determine the order of reaction, calculate rate constants and determine the activation energy from the Arrhenius equation. Steady state heating using small glass vials was used. These containers heat rapidly to the processing temperature with virtually no heating lag. After equilibration all the food product is at constant temperature (i.e. steady state) during processing. This work has been published in J. Fd Sci., 46, 32,(1981), and a copy of the paper is given in Appendix A.1.

4.2 EXPERIMENTAL

4.2.1 Sample Preparation

Fresh beef liver had visible fat and connective tissue removed. The liver was cut into 1.0 cm cubes and pureed in a Waring Blendor for ca 90 seconds. The proximate composition of the puree was:

Moisture Content	=	69.6%
Crude Protein	=	19.6%
Extractable Fat	=	2.4%
Ash and Carbohydrate	=	8.4% (by difference)
pH	=	5.81 ± 0.01

The puree was then filled into vials as specified in section 3.4.2.1.

4.2.2 Thermal Processing Conditions

The vials were heated and cooled as described in Section 3.4.3.4. For each temperature, three vials were removed at five different times as shown in Table 4.1.

Table 4.1: Temperature-Time Combinations for Thermal Processing of Beef Liver Puree

Temperature (^{o}C)	Time (min.)					
102.9	0	15	30	60	90	120
111.0	0	15	30	45	60	75
118.3	0	15	25	35	45	55
122.1	0	10	18	25	32	40
126.7	0	10	16	22	28	34

4.3 RESULTS AND DISCUSSION

4.3.1 Temperature-Time Profiles of Heated Tubes

Experimental temperature-time profiles of heated beef liver puree in glass vials during heating and cooling are shown in Figure 4.1. These profiles were typical of the heating profiles of the system. Figure 4.2, also shows a temperature profile during the come-up time, but it was determined by modelling the heat transfer within the vial wall and the sample using a finite difference program (Appendix A.2). This was done because the come-up time was so short (ca 60 seconds) and hence difficult to measure.

The times taken to reach actual temperature, and 90% of the desired temperature (as calculated by the finite difference program) are shown in Table 4.2.

Table 4.2: Length of "Come-Up" Times in Heated Tubes

TEMPERATURE OF RUN (^{o}C)	TIME TO REACH			
	0.1^{o}C of Steady State Temperature at		90% of Steady State Temperature at	
	Surface (s)	Centre (s)	Surface (s)	Centre (s)
102.9	54	60	9	15
111.0	54	60	9	15
118.3	54	60	9	15
122.1	54	60	9	15
126.7	54	60	9	15

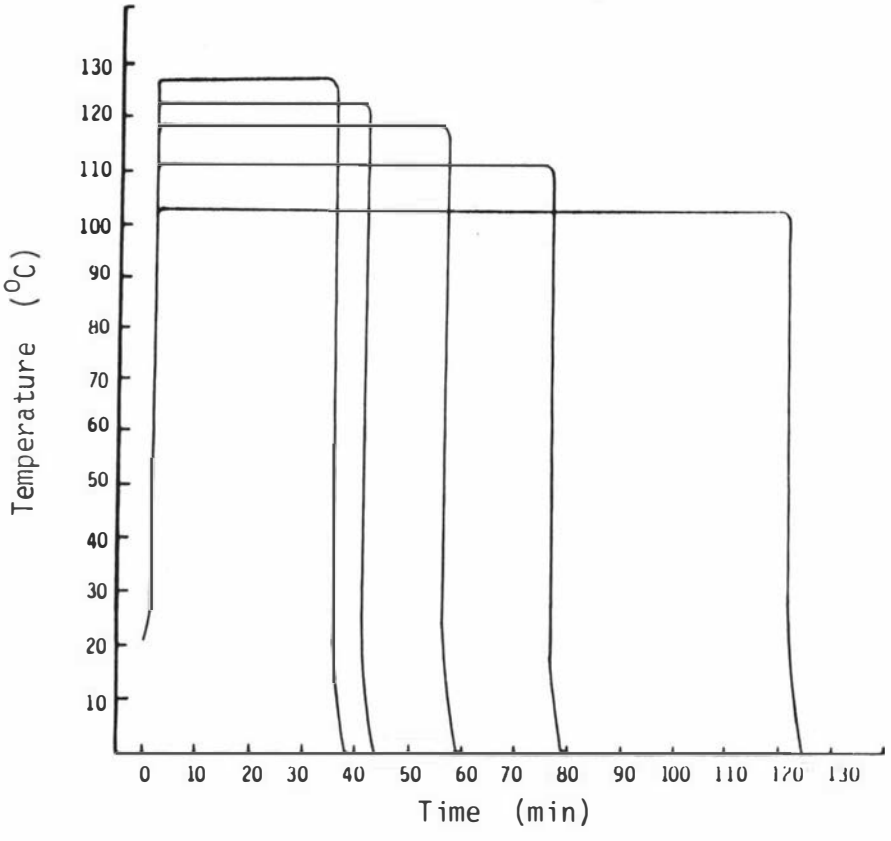


Figure 4.1 Temperature-time profiles for heating and cooling vials during steady state processing.

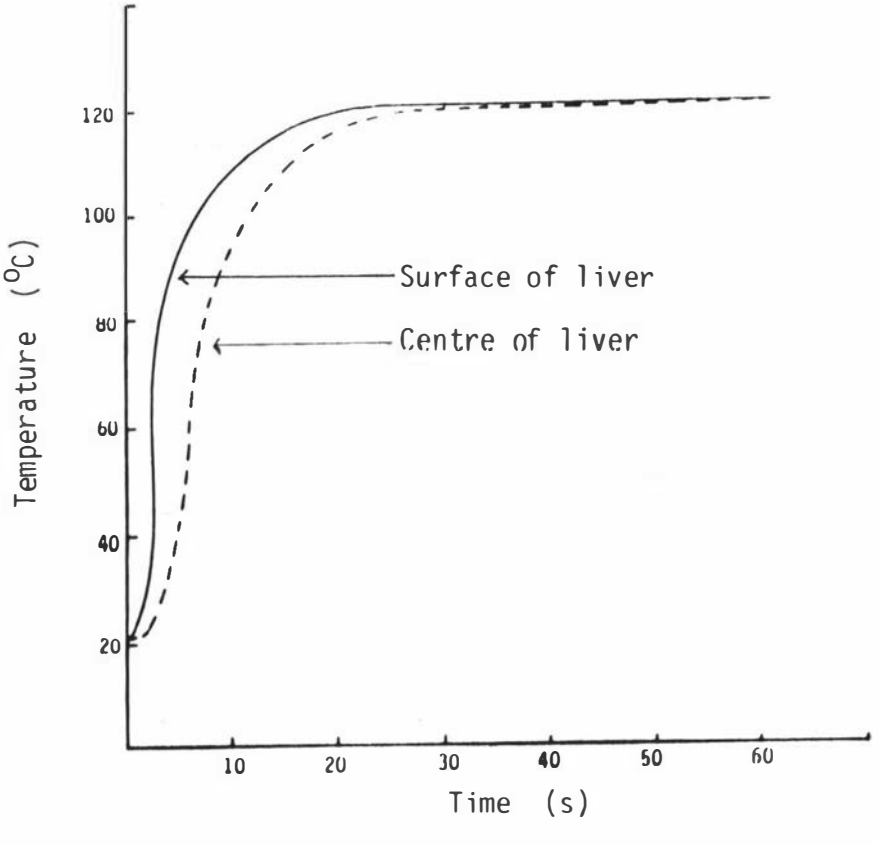


Figure 4.2 Calculated temperature-time profile during "come-up" time for 122.1°C heating process

Consideration of the heat transfer characteristics through the glass vial (Table 4.2 and Figure 4.2) indicated that the temperature difference between the centre of the liver and the glass-liver interface during the initial heating period was ca 20^{o}C after three seconds and ca 3^{o}C after thirty seconds. In fifteen seconds, 90% of the desired temperature was reached,and after 60 seconds the centre of the sample was at the steady state temperature. Thus, as the thermal lags were very small in comparison with the total processing times (minimum of 34 minutes), instantaneous heating and cooling were assumed.

4.3.2 Extent of Reaction During Come-Up Time

Incorporated into the finite difference program to calculate the temperature-time profile of the vial was a numerical integration to evaluate the extent of retinol loss that occurred during the come-up period. This could then be compared with the extent of loss which would have occurred in this time if heating had been instantaneous (Table 4.3). Kinetic parameters established in Section 4.3.3 were used in this calculation.

Table 4.3: Extent of Vitamin A Loss in Sixty Seconds"Come-Up"Time in Heated Tubes

TEMPERATURE (^{o}C)	EXTENT OF VITAMIN A LOSS (%) DURING COME-UP		
	Accounting for Actual Heat Transfer		Assuming Instantaneous Come-up
	Liver Surface	Liver Centre	
102.9	0.83	0.70	1.03
111.0	1.75	1.55	2.23
118.3	3.36	2.96	4.27
122.1	4.62	4.09	5.90
126.7	6.74	6.00	8.63

The difference in the extent of vitamin A loss from that predicted for instantaneous heating and that achieved experimentally, was not significant compared with the repeatibility of the vitamin A assay, where replication was within 8 $\mu g g^{-1}$ which would indicate a variability of up to 5% in the determined concentrations. A square process was therefore assumed.

4.3.3 Estimation of Parameters Characterising Thermal Loss of Vitamin A

The order of the degradation of trans-retinol in beef liver puree was explored graphically by plotting different functions of the concentration against time of heating at constant temperature. (Actual concentrations of trans-retinol before and after processing are shown in Appendix A.3). A plot of ln c versus time generated a straight line over the entire range of heating time, thus indicating that vitamin A loss on heating could be described by first order kinetics. Such a first order reaction is defined as:

$$\ln c = -kt + \ln c_o$$

where c_o = vitamin A concentration at time t = 0, $\mu g g^{-1}$
c = vitamin A concentration at time t = t, $\mu g g^{-1}$
k = rate constant, s^{-1}
t = time, s.

The reaction rate constant was calculated from the slope of the line. The intercept of this line at time zero was $\ln c_o$ as instantaneous heating and cooling were assumed. The reasons for this assumption are outlined in Section 4.3.1 and 4.3.2. Using the values of c_o determined from the intercepts, plots of $\ln c_o/c$ vs time were prepared (Figure 4.3). As a check that variation from the first order model was due to uncertainty in experimental measurements only, a probability plot (Figure 4.4) of % residuals was constructed, where:

$$\% \text{ residuals} = (c_{pre} - c_{obs})/c_{pre}$$

and c_{pre} = predicted concentration, $\mu g g^{-1}$
c_{obs} = observed concentration, $\mu g g^{-1}$

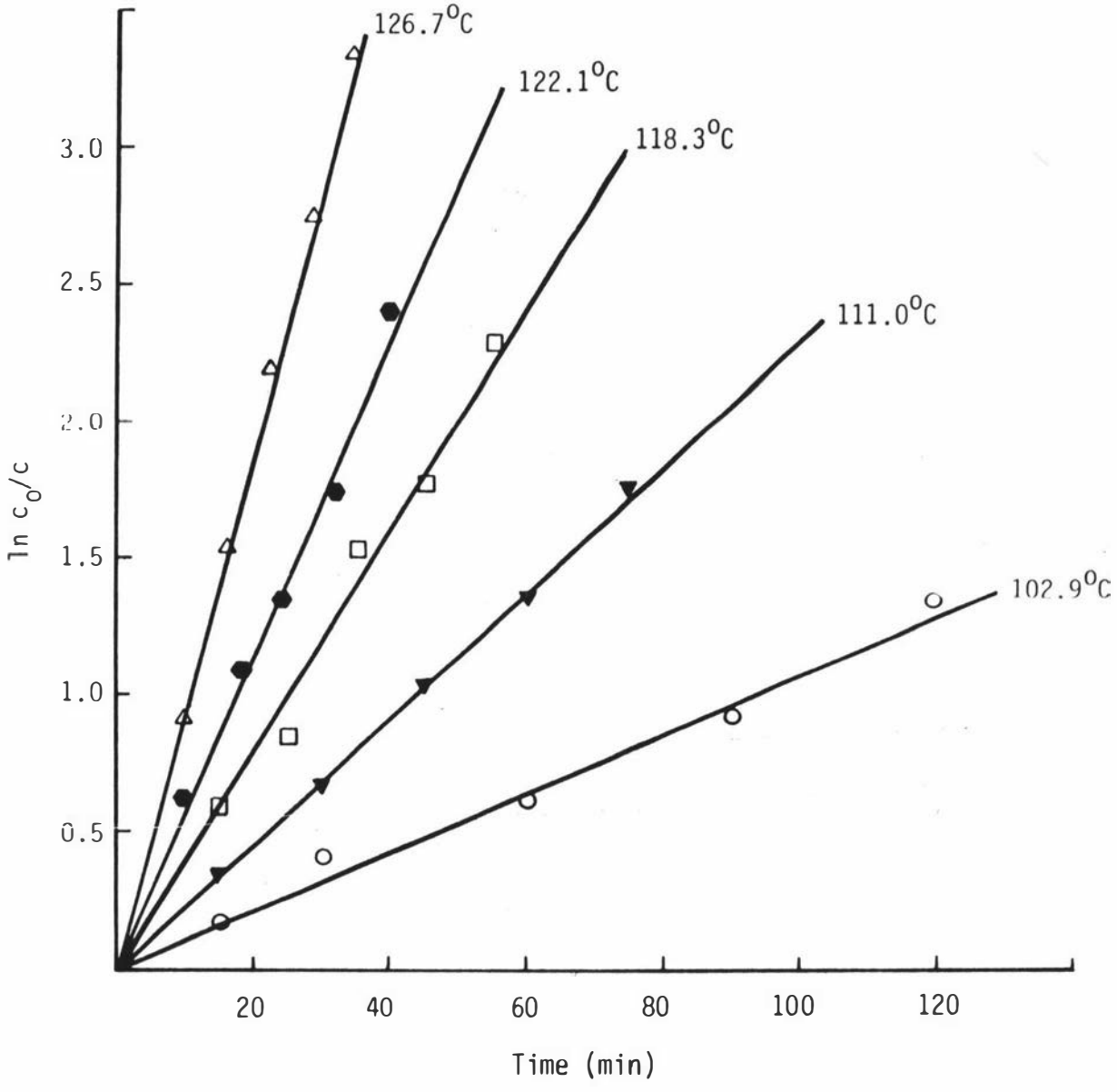


Figure 4.3 Degradation rates for vitamin A in beef liver puree on heating, showing the natural logarithm of the concentration ratio as a function of time for various temperatures

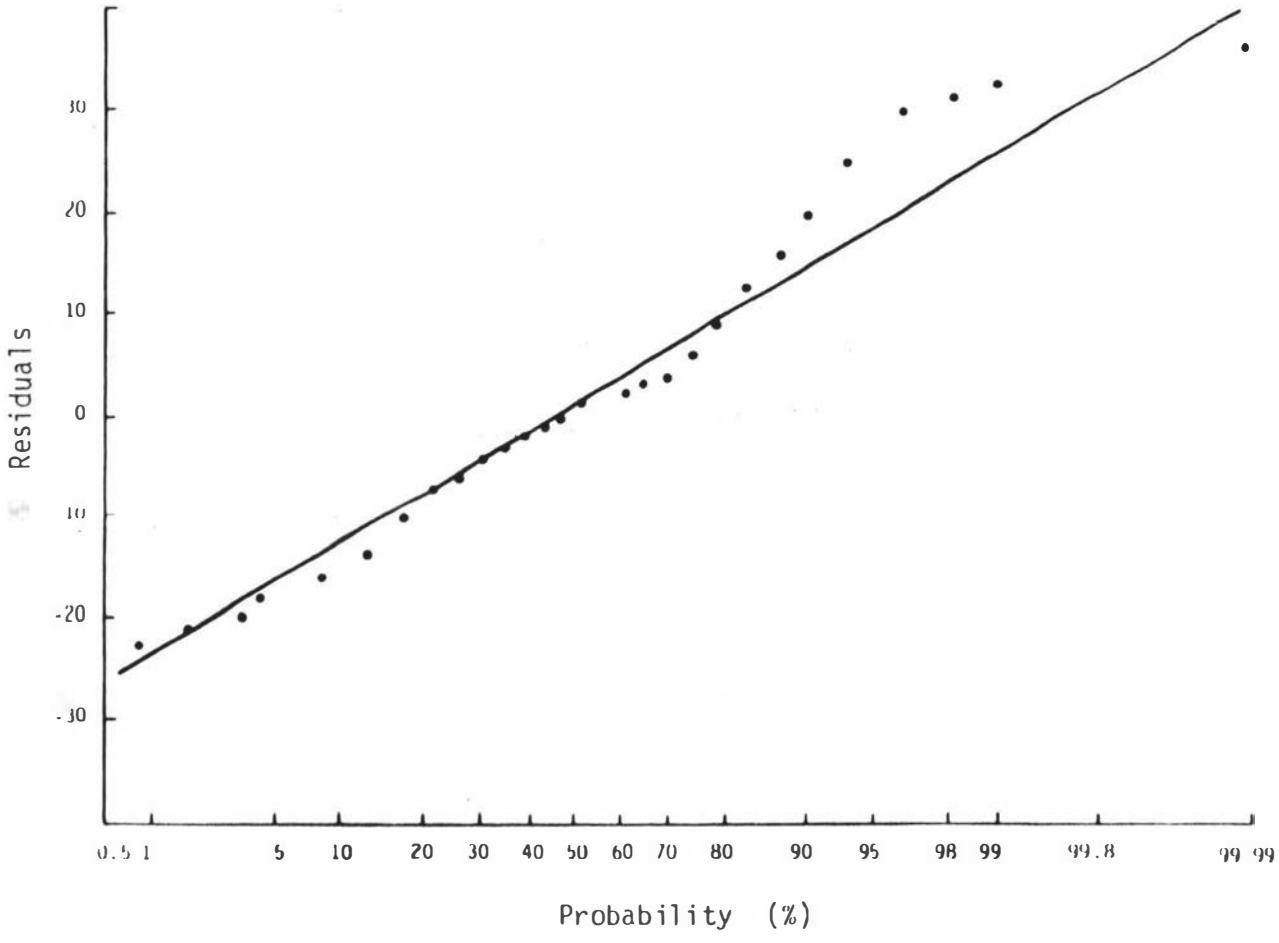


Figure 4.4 Probability plot of calculated percent "residuals" for vitamin A

The probability plot was a straight line indicating that deviations from the first order kinetic model followed a normal distribution. They were therefore randomly distributed and this implies they arose from experimental uncertainty. The major contributing factors were probably variation in each vial and the inherent error in the assay method.

The first order rate constants are shown in Table 4.4. Compared with the first order reaction coefficients calculated from the data of Maqsood et al., (1963), loss of vitamin A was more rapid in heating ghee at 100°C than heating beef liver puree at 102.9°C (109×10^{-5} s^{-1} cf 17.9×10^{-5} s^{-1}).

Table 4.4: First Order Reaction Rate Constants for Trans-Retinol in Natural Beef Liver Puree

Temperature (°C)	k ($\times 10^{-5}$ s^{-1}) [a]	R^2 [b]
102.9	17.9 ± 0.7	0.992
111.0	38.6 ± 1.3	0.993
118.3	68.0 ± 2.8	0.989
122.1	96.1 ± 2.6	0.995
126.7	162.3 ± 1.7	0.999

[a] 95% confidence limits

[b] R^2 = Correlation coefficient squared.

The activation energy and the frequency factor were derived from the Arrhenius equation defined as:

$$k = Ae^{-E_a/RT}$$

where k = rate constant, s^{-1}
A = frequency factor, s^{-1}
E_a = activation energy of the thermal loss, $Jmol^{-1}$
R = universal gas constant, $Jmol^{-1}K^{-1}$
T = absolute temperature, K.

Values of ln k were plotted against the inverse of absolute temperature (Figure 4.5) and E_a determined from the slope of the line and A from the intercept. The activation energy determined from the Arrhenius plot was 112 $\pm$ 9 $kJmole^{-1}$ (26.9 $\pm$ 2.1 $kcalmol^{-1}$) with 95% confidence. An alternative method of data analysis to find E_a was also used. The Arrhenius equation was substituted into the first order model giving:

$$\ln c_o/c = Ae^{-E_a/RT} t$$

After manipulation this yields:

$$\ln \left[\frac{\ln c_o/c}{t}\right] = \ln A - \frac{E_a}{RT}$$

A plot of the left hand side of this equation vs 1/T yields E_a and A from the slope and intercept respectively.

This approach was tried, as linear regression assumes that variance is evenly distributed whereas it was not for the ln k values used in the Arrhenius plot. This can be seen by examination of the data in Table 4.4.

As all concentration data were equally reliable it was considered that the alternative transformation may have created a better distribution of variance than the Arrhenius plot. When the calculations were carried out the calculated activation energy was identical, indicating that the difference in accuracy of the k data had not influenced the Arrhenius plot calculation.

The activation energy for vitamin A in multivitamin tablets of 118 $kJmol^{-1}$ (calculated from the data of Slater et al., 1979) fell within the 95% confidence limits of the experimentally determined E_a and so there was no significant difference between the two values. However this value of E_a for vitamin A in beef liver puree disagreed with Garrett's determined E_a of 61 $kJmol^{-1}$ for vitamin A in a liquid multivitamin preparation. Differences in activation energies were reported for other vitamins when heated in different media. In the case of thiamin, an activation energy of 123 $kJmol^{-1}$ was reported for phosphate buffer and 114 $kJmol^{-1}$ for beef puree (Mulley et al., 1975a).

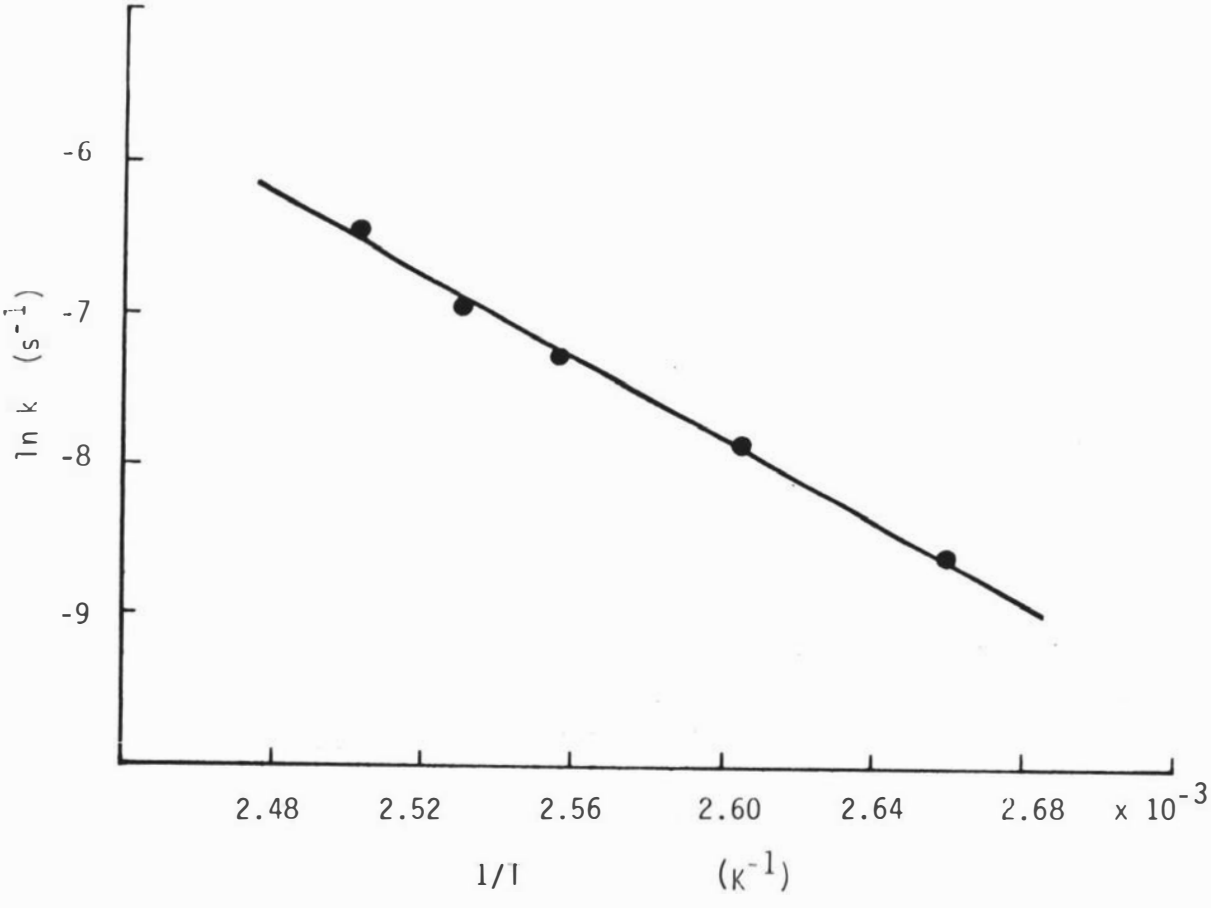


Figure 4.5 Arrhenius plot for loss of vitamin A in beef liver puree, showing the natural logarithm of the first order rate constant as a function of the inverse of absolute temperature

However, this estimated E_a may be considered comparable to E_a parameters in the range 42 - 126 $kJmol^{-1}$ (10-30 $kcalmol^{-1}$) generally recognised for thermal degradation of other vitamins (Lund, 1977).

The frequency factor A, was found to be 8.13×10^{11} s^{-1} whilst for multivitamin tablets A had the value 4.98×10^{11} s^{-1}. This difference could be due to several factors such as difference in state, difference in component composition and other intrinsic properties such as pH.

As the frequency factor is derived from extrapolation of the non-linear Arrhenius equation, it is convenient to use a modified form in which a reference rate constant replaces the frequency factor. This modified form of the Arrhenius equation is:

$$k = k_{ref} \exp \left[\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]$$

In this study k_{ref} was chosen as k_{122} as 122°C was to be near the upper limit of temperatures studied. For an activation energy of 112 $kJmol^{-1}$ and an A of 8.13×10^{11} s^{-1}, k_{122} was 125.0×10^{-5} s^{-1}.

4.4 CONCLUSIONS

The kinetics of vitamin A loss on heat processing were characterised and quantified using a steady state procedure. It was found that for beef liver puree over the temperature range 103-127°C, the observed rate of loss could be described by first order kinetics. The reference rate constant, k_{122}, was 125.0×10^{-5} s^{-1}. The value of the frequency factor was 8.13×10^{11} s^{-1}. The activation energy for the temperature dependence of the rate constant was 112 ± 9 $kJmol^{-1}$.

These kinetic parameters were not identical to those reported for vitamin A in other media. It indicates that other factors apart from temperature and time of processing, affect vitamin A loss on heating. As vitamin A loss followed the Arrhenius expression it would not be necessary to use as many processing temperatures in the subsequent steady state heating experiments.

5. EFFECT OF PROXIMATE COMPOSITION ON THE LOSS OF VITAMIN A DURING STEADY STATE HEATING

5.1 INTRODUCTION

Having established that the observed rate of vitamin A loss on heating could be described by first order kinetics, the next step was to look at other variables - apart from time and temperature - that might affect loss.

Factors reported to influence retinol stability in foods, (as outlined in Section 2.3) include the redox potential of the system; pH; fat, protein, moisture and carbohydrate contents; mineral salts; metal ions; light; peroxides; antioxidants; and nitrites and nitrates. There are also antivitamin A factors in foods such as lipoxidase; thyroxine which aggravates vitamin A deficiency; and citral which acts as an antagonist to vitamin A.

Some of the above factors, though possibly important in retinol degradation, were not considered in this study for one or more of the following reasons:

(i) unlikely to be encountered in normal thermal processing operations such as canning, e.g. light, high levels of nitrite and nitrate, mineral salts.

(ii) their action would be nullified by heat processing, e.g. the antivitamins thyroxine and lipoxidase.

(iii) difficult to measure, e.g. natural antioxidants such as tocopherol.

(iv) difficult to control, e.g. peroxides.

(v) their presence is known to affect another factor which was under study, e.g. nitrite/nitrate and salt have an effect on the redox potential of the system, which was to be measured.

Consequently, the list of factors most likely to affect the rate of vitamin A loss on heat processing in liver was reduced to fat, protein and moisture contents, pH, and the presence of heavy metals such as copper. The effect of carbohydrate was not considered because of the low level in natural liver and formulated liver products. An orthogonal experimental design could not be used to investigate the effect of the five variables (moisture, fat and protein contents, pH and copper) because moisture, fat and protein levels could not be varied independently. In studying compositional factors, a mixture design is normally used. In order to study all five factors simultaneously, it would be necessary to repeat the mixture design at both high and low levels of the other two variables. The amount of work involved in such a design was prohibitively high, so it was decided to set pH and copper at one level and investigate moisture, fat and protein only. The results from this study could then be used to establish whether it was necessary to repeat the full mixture design at different levels of pH and copper. This chapter therefore quantifies the effect of proximate composition on the kinetics of vitamin A loss during steady state heating of beef liver mixtures with copper and pH held constant. Heating was conducted at three temperatures within the range used for canning meat products. This enabled the order of reaction, reaction rate constants and activation energy to be calculated.

5.2 EXPERIMENTAL

5.2.1 Sample Preparation

Fresh beef liver was chilled to ca $1^{o}C$, and connective tissue and visible fat were removed. The liver was then cut into 3.0 cm cubes, pureed in a chilled condition in a Stephan mixer (Model UM 12), packed and then blast frozen at $-40^{o}C$. During processing, the puree was protected from light. A proximate analysis of the liver was made.

The mixtures (as described in Section 5.2.2) were prepared by adding to the liver, fat and/or beef liver protein concentrate (method given in Section 3.3), and mixing in a Waring Blendor for 2-4 mins until an homogeneous puree was obtained. The blended mixture was removed and reweighed. The copper concentration, pH and moisture were then adjusted. The mixtures were analyzed for their fat, protein and moisture contents and their pH and E_h measured. Samples were then filled into vials as described in Section 3.4.3.

5.2.2 Design of Extreme Vertices Mixture Experiment

The extreme vertices design of McLean and Anderson (1966) was used. The specification of the experimental plan is shown in Table 5.1. The proximate composition of the base raw material was:

Extractable Fat	=	1.9%
Crude Protein	=	19.1%
Moisture Content	=	71.6%
Ash and Carbohydrate	=	7.4% (by difference)
Copper	=	6 mg kg^{-1}
pH	=	6.07
E_h	=	-184 mV

The proportions of the first three components were constrained so that they complied with New Zealand legal and practical requirements for canned meat products:

10% $\leq$ fat content	$\leq$	25%
12% $\leq$ protein content	$\leq$	20%
50% $\leq$ moisture content	$\leq$	70%

However, as the base raw material had a carbohydrate and ash content of 7%, the Σ(fat + protein + moisture) was only 93%, not 100%. Therefore this component was to remain approximately at this level and only the effect of fat, protein and moisture contents to be investigated.

Table 5.1: Composition of Mixtures in Extreme Vertices Design

Vertex	Fat (%)	Protein (%)	Moisture Content (%)	Feasible Solution[a]
1	10	12	-	
2	10	20	63	+
3	25	12	56	+
4	25	20	-	
5	10	-	50	
6	10	13	70	+
7	25	18	50	+
8	25	-	70	
9	-	12	50	
10	11	12	70	+
11	23	20	50	+
12	-	20	70	
Centroid[b]	17.33	15.83	59.84	+

[a] + indicates a solution within the legal and practical constraints

[b] Centroid was calculated by averaging the feasible vertices

Of the feasible runs, vertices 6 and 10, and vertices 7 and 11 were very similar in composition and hence were averaged. Thus a final modified design with a new centroid was used. This final design involved investigation of the rate of degradation in five mixtures, four points approximately at the extremes of the constraints and a centre point. The actual compositions are shown in Table 5.2 and also on Figure 5.1.

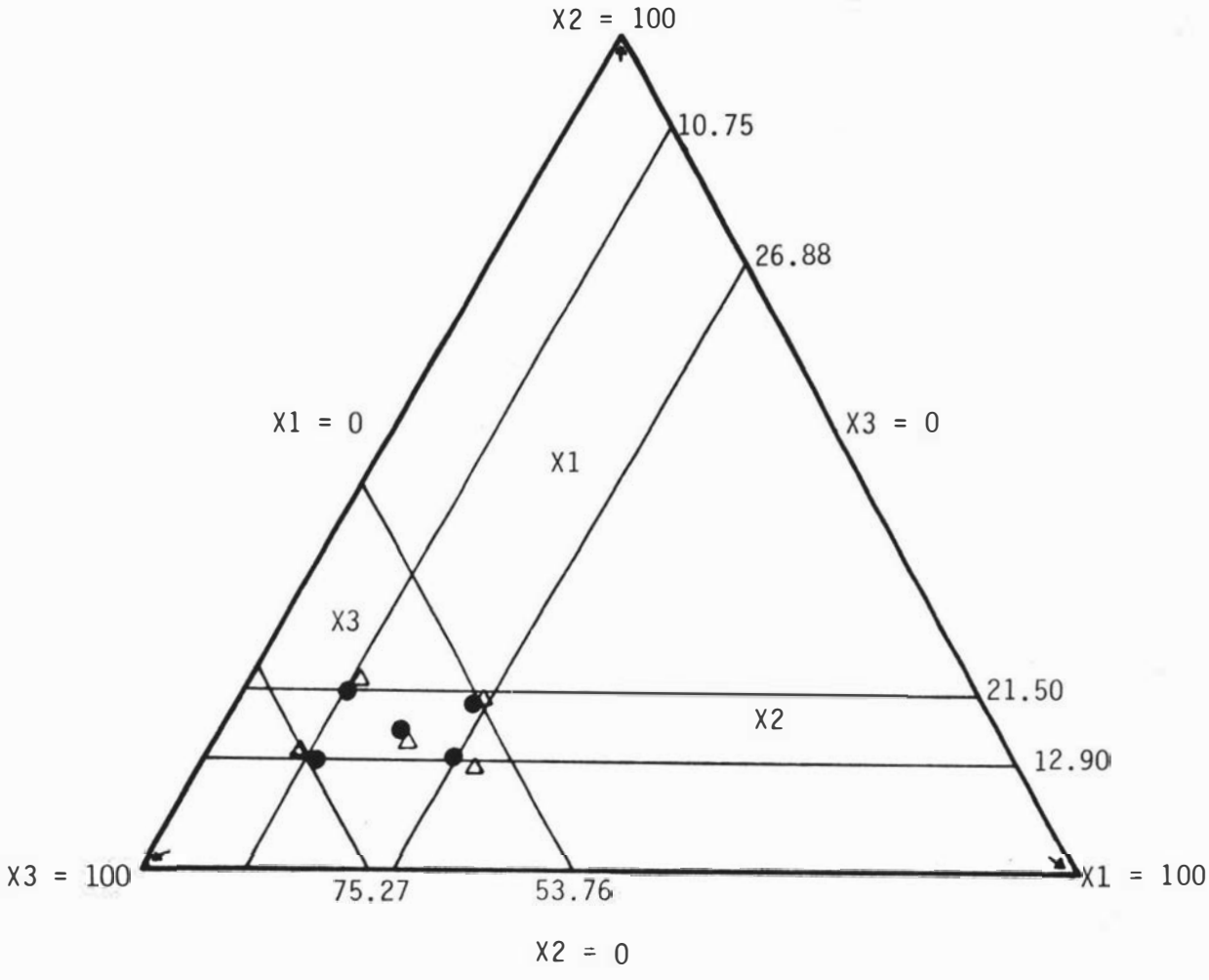


Figure 5.1 Extreme vertices design for beef liver mixtures of different fat, protein and moisture contents

● Desired composition
△ Achieved composition

X1 = coded fat content, %
X2 = coded protein content, %
X3 = coded moisture content, %

Table 5.2: Proximate Analysis of Mixtures in the Modified Extreme Vertices Design

Vertex	Fat (%)	Protein (%)	Moisture Content (%)	Ash and Carbo. (%) (by difference)	E_h (mV)
1	10.9	21.6	63.2	4.3	-147
2	27.9	11.7	56.0	4.4	-142
3	10.0	13.0	72.2	4.8	-150
4	26.1	20.2	51.7	2.0	-161
Centroid	18.6	15.2	61.0	5.2	-145

The pH of canned meats is unlikely to lie outside the range 5.6 to 7.0, so this variable was set at a midpoint value of 6.3. The actual pH values ranged from 6.32 to 6.40, and averaged 6.36. The New Zealand legal limit for copper is 30 $mgkg^{-1}$, and the raw material had a concentration of 6 $mgkg^{-1}$ so the midpoint level was chosen as 18 $mgkg^{-1}$.

Vials of the beef liver mixtures, following the specification of the experimental plan (Table 5.2), were heated for the times and at the temperatures shown in Table 5.3. At each sampling time three tubes were removed. Controlled heating and cooling were carried out as described in Section 3.4.3, with the centroid compositional point replicated at each time-temperature combination to provide data for estimation of experimental uncertainty. Analysis for trans-retinol followed Section 3.1.4.

Table 5.3: Temperature-Time Combinations for Thermal Processing of the Extreme Vertices Mixture Design

Temperature (^oC)	Time (minutes)				
102.1	0	100	200	300	400
112.0	0	70	140	210	280
122.0	0	45	90	135	180

5.3 RESULTS AND DICUSSION

5.3.1 Estimation of Parameters Characterising Thermal Loss of Trans-Retinol in Beef Liver Mixtures

The loss of vitamin A (measured as trans-retinol) in beef liver mixtures on heating, was adequately described by first order kinetics as the amount of variation not explained by this model was small (average R^2 = 0.983). Plots of ln c_o/c versus time at various temperatures, for the design points, are shown in Figures 5.2 - 5.4. (Actual concentrations of retinol before and after processing are shown in Appendix B.1). The calculated apparent first order rate constants are shown in Table 5.4.

Table 5.4: Apparent First Order Rate Constants for Trans-Retinol in Beef Liver Puree as a Function of Composition

Vertex	k (x 10^{-5} s^{-1}) with 95% Confidence Limits at Various Temperatures					
	102.1°C	R^2	112.0°C	R^2	122.0°C	$R^{2\,a}$
1	1.71 ± 0.18	0.986	4.49 ± 0.32	0.993	10.27 ± 0.90	0.992
2	1.12 ± 0.12	0.990	2.32 ± 0.30	0.984	5.80 ± 0.50	0.993
3	2.54 ± 0.08	0.999	6.16 ± 0.32	0.997	12.84 ± 0.40	0.996
4	0.72 ± 0.04	0.998	1.30 ± 0.28	0.925	4.53 ± 0.60	0.979
Centroid	1.12 ± 0.10	0.991	3.44 ± 0.14	0.999	9.22 ± 0.20	0.999
Centroid	1.03 ± 0.22	0.965	3.07 ± 0.24	0.992	7.12 ± 0.70	0.990

[a] R^2 = correlation coefficient squared.

The loss of vitamin A on heat processing at each given temperature was different for each design point (Table 5.4) with vertex 3 (low fat, high moisture content) being the highest and vertex 4 (high fat, low moisture content) the lowest. This indicated that at the pH and copper concentration under study, composition affected the rate of vitamin A loss.

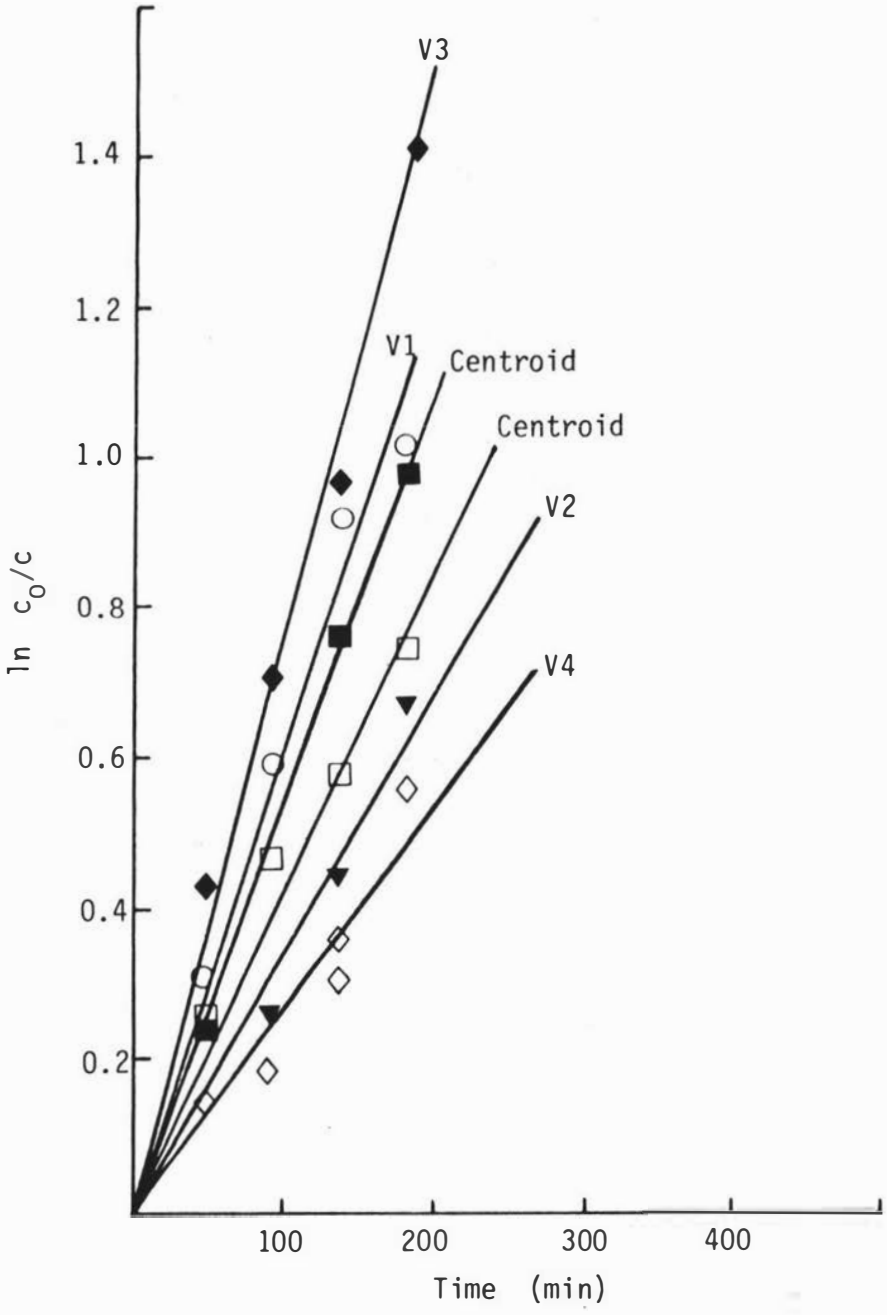


Figure 5.2 Degradation rates for vitamin A in beef liver mixtures at 122.0°C, showing the natural logarithm of the concentration ratio as a function of time

○ = V1 = vertex 1
▼ = V2 = vertex 2
◆ = V3 = vertex 3
◇ = V4 = vertex 4
□ ■ } = Centroid

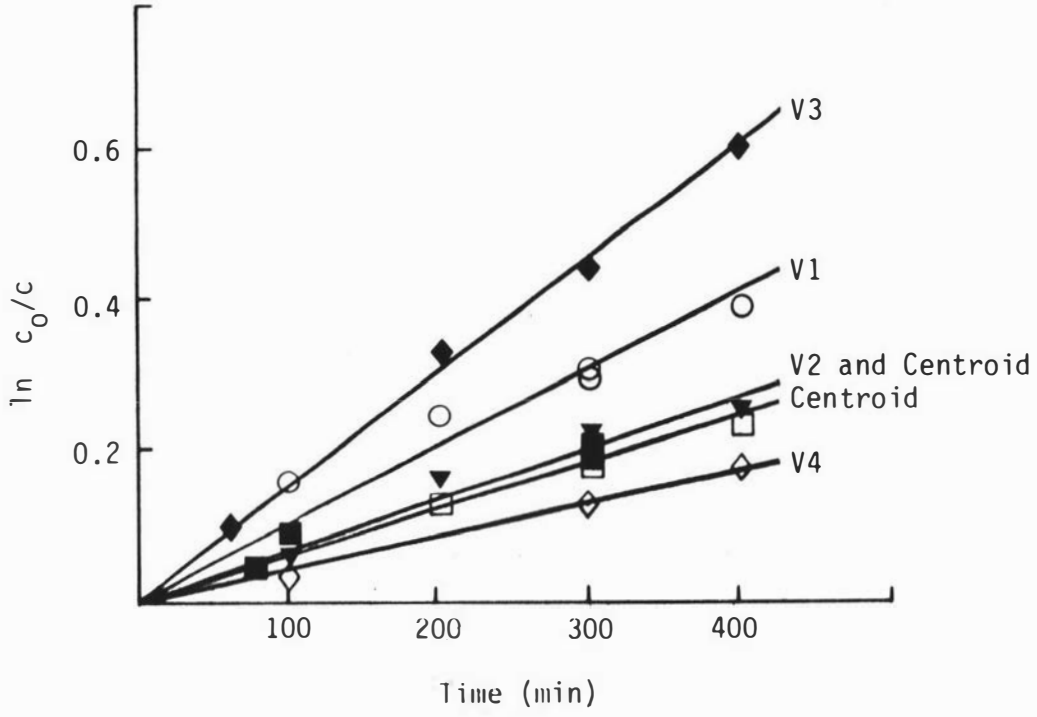


Figure 5.3 Degradation rates for vitamin A in beef liver mixtures at 102.1°C, showing the natural logarithm of the concentration ratio as a function of time

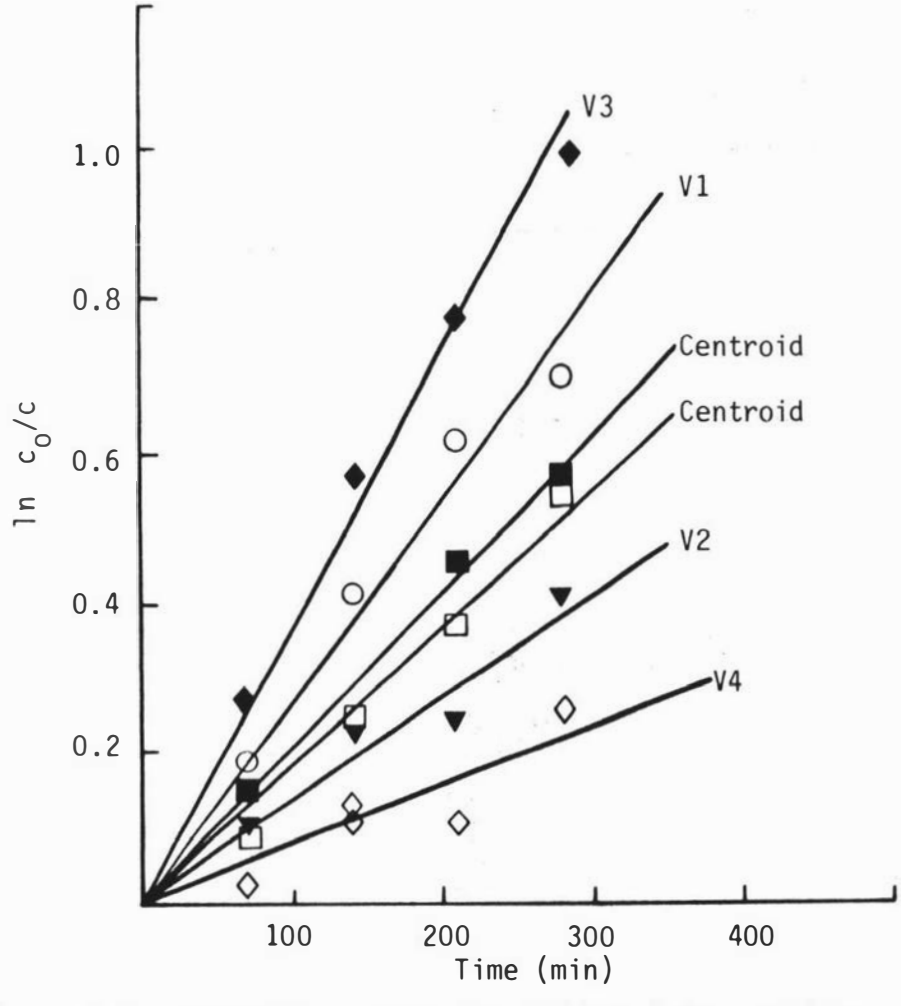


Figure 5.4 Degradation rates for vitamin A in beef liver mixtures at 112.0°C, showing the natural logarithm of the concentration ratio as a function of time

The activation energy and frequency factor was calculated for each compositional point by linear least squares regression. The alternative plot:

$$\ln\left[\frac{\ln c_o/c}{t}\right] = \ln A - \frac{E_a}{RT}$$

where A = frequency factor, s^{-1}
E_a = activation energy, J mol^{-1}
R = gas constant, J K^{-1} mol^{-1}
T = absolute temperature, K

was used as inspection of Table 5.4 showed that variance in ln k was not evenly distributed.

Estimated values of the activation energy and frequency factor are shown in Table 5.5. As can be seen from this table, all values for E_a were close together and it was possible that the differences between them were due to experimental uncertainty only. Therefore the data were reanalyzed to find one average activation energy. The value obtained was 112 $\pm$ 8 $kJmol^{-1}$ at the 95% level of confidence. The Arrhenius plots for the design points using this average activation energy are shown in Figure 5.5. This value for E_a is identical to that found in Section 4.3.3 for vitamin A loss on steady state heating of natural beef liver puree.

Table 5.5: Estimated Values of the E_a and A Parameters of the Arrhenius Equation for the Thermal Loss of Trans-Retinol in Beef Liver Mixtures Over the Temperature Range 102 -122°C

Vertex	E_a ($kJmol^{-1}$)[a]	A (x 10^9 s^{-1})
1	107 $\pm$ 13	17.2
2	99 $\pm$ 13	0.8
3	101 $\pm$ 8	3.5
4	121 $\pm$ 37	443.0
Centroid	122 $\pm$ 12	1148.0

[a] 95% confidence limits

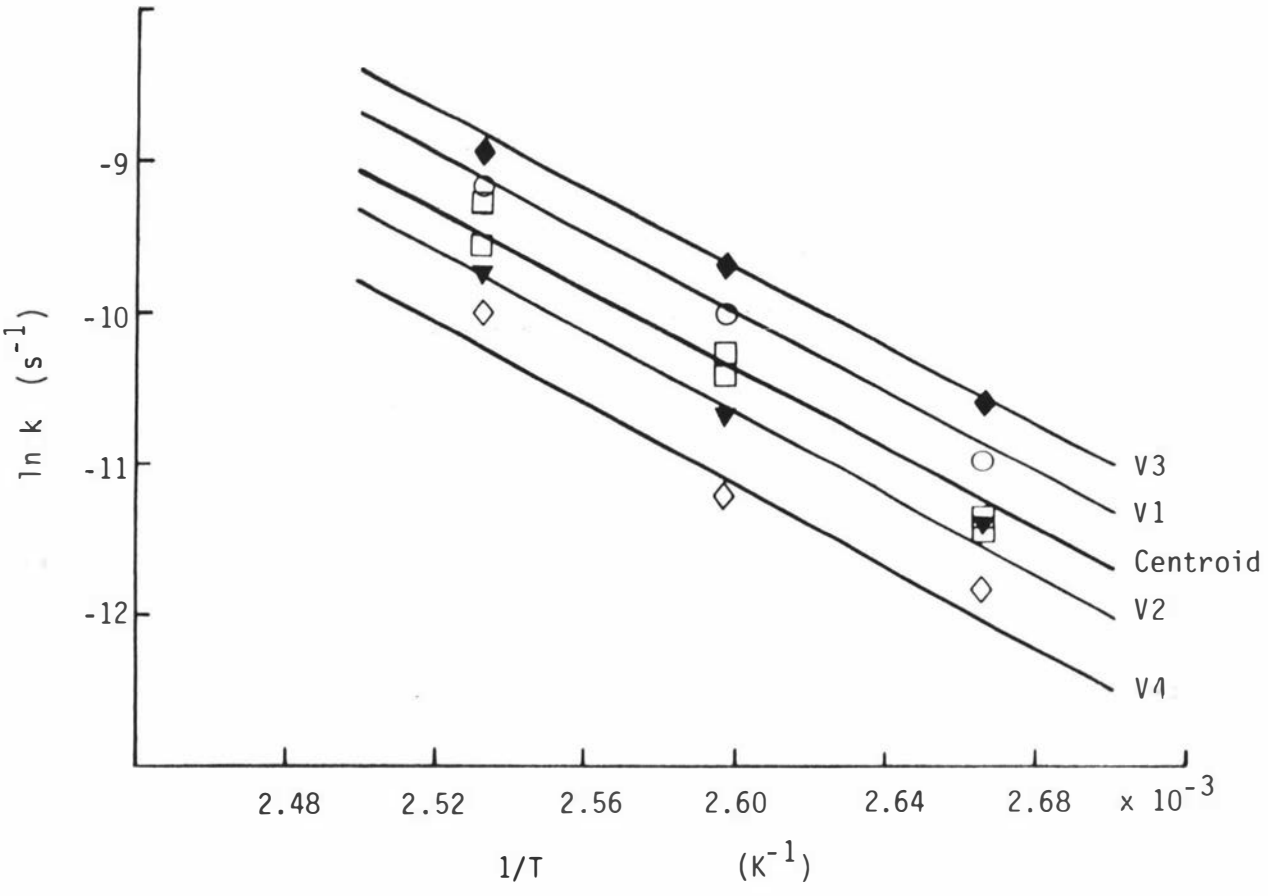


Figure 5.5 Arrhenius plot for loss of vitamin A in beef liver mixtures on heating, showing the natural logarithm of the first order rate constant as a function of the inverse of absolute temperature (average E_a)

Hill and Grieger-Block (1980) investigated the accuracy with which E_a can be found as a function of the uncertainty in the data used. They suggest that to measure E_a over a 10^oC interval to within $\pm$ 0.5% generally requires temperature uncertainties of less than $\pm$ 0.03^oC and rate constant uncertainties of 0.3% which necessitates an analytical precision of $\pm$ 0.1% over an extended range of concentration changes. In the current work, processing temperatures were measured to $\pm$ 0.2^oC, and replication of analysis was within 8 $\mu g g^{-1}$, which would indicate a variation of up to 5% in the concentrations used in the determination of E_a. Therefore, the unavoidable uncertainty in any derived data was expected to be significant. The adequacy of the model using the average E_a (112 $kJmol^{-1}$) was tested by comparing predicted values of the rate constant with the experimental data:

$$\% \text{ deviation} = \frac{k_{measured} - k_{predicted}}{k_{measured}} \times 100$$

Where the predicted rate constants were obtained from the model using the average E_a, the average deviation was 12%; whereas if the individual E_a values were used, the average deviation was 10%. The extra uncertainty introduced by using the average E_a was therefore small. This indicates that E_a was only weakly dependent on composition.

Using the average activation energy of 112 $kJmol^{-1}$, the estimated frequency factors ranged from 4.9 - 100.0 x 10^9 s^{-1} as shown in Table 5.6.

Table 5.6: Estimates of A for Each Design Point Using an Average E_a (112 $kJmol^{-1}$)

Design Point	A (x 10^9 s^{-1})
1	75.0
2	39.9
3	100.0
4	4.9
Centroid	50.8

As for the rate constant, the proximate composition affected the value of the frequency factor with it being highest at vertex 3 (low fat, high moisture content), and lowest at vertex 4 (high fat, low moisture content). At the pH and copper concentration under study, composition affected the value of A. In comparison, the value for the frequency factor determined in Section 4.3.3 was 813 x 10^9 s^{-1}. (Fat content = 2.4%; Moisture content = 69.6%).

5.3.2 Effect of System Composition on Reaction Rate

Linear relationships were fitted for rate constants (at 102.1, 112.0 and 122.0^{o}C) versus the fat content, moisture content, fat: moisture ratio and (moisture - fat content) of the design points. The first two plots of k versus fat content and k versus moisture content, are shown in Figure 5.6 as these are the simplest representations which adequately describe the rate constant as a function of composition.

The rate constant/moisture content relationship was statistically significant at the 99% level of confidence. Increasing the moisture content in beef liver mixtures caused an increase in the rate of loss of vitamin A at the given temperatures. This follows the trend reviewed by Labuza (1980) and could be, as he postulated, caused by viscosity changes or solubilisation of catalysts/reactants, or concentration in the small fat phase. The correlation coefficients for the linear relationships between the rate constant and fat content were lower than that for moisture content, with fat being significant at the 95% level at 102.1^{o}C and at the 99% level at 122^{o}C. As the fat content of the mixture increased, the loss of vitamin A decreased. However, as the fat and moisture contents are aliased, either could explain most of the observed variation, but moisture content was the preferred variable needed to model the effect of composition as it explained a greater proportion of the variation (average R^2 = 0.92 for moisture content cf average R^2 = 0.79 for fat content). No simple linear relationships could be established between rate constants and either the protein content, fat:protein ratio or protein:moisture content ratio.

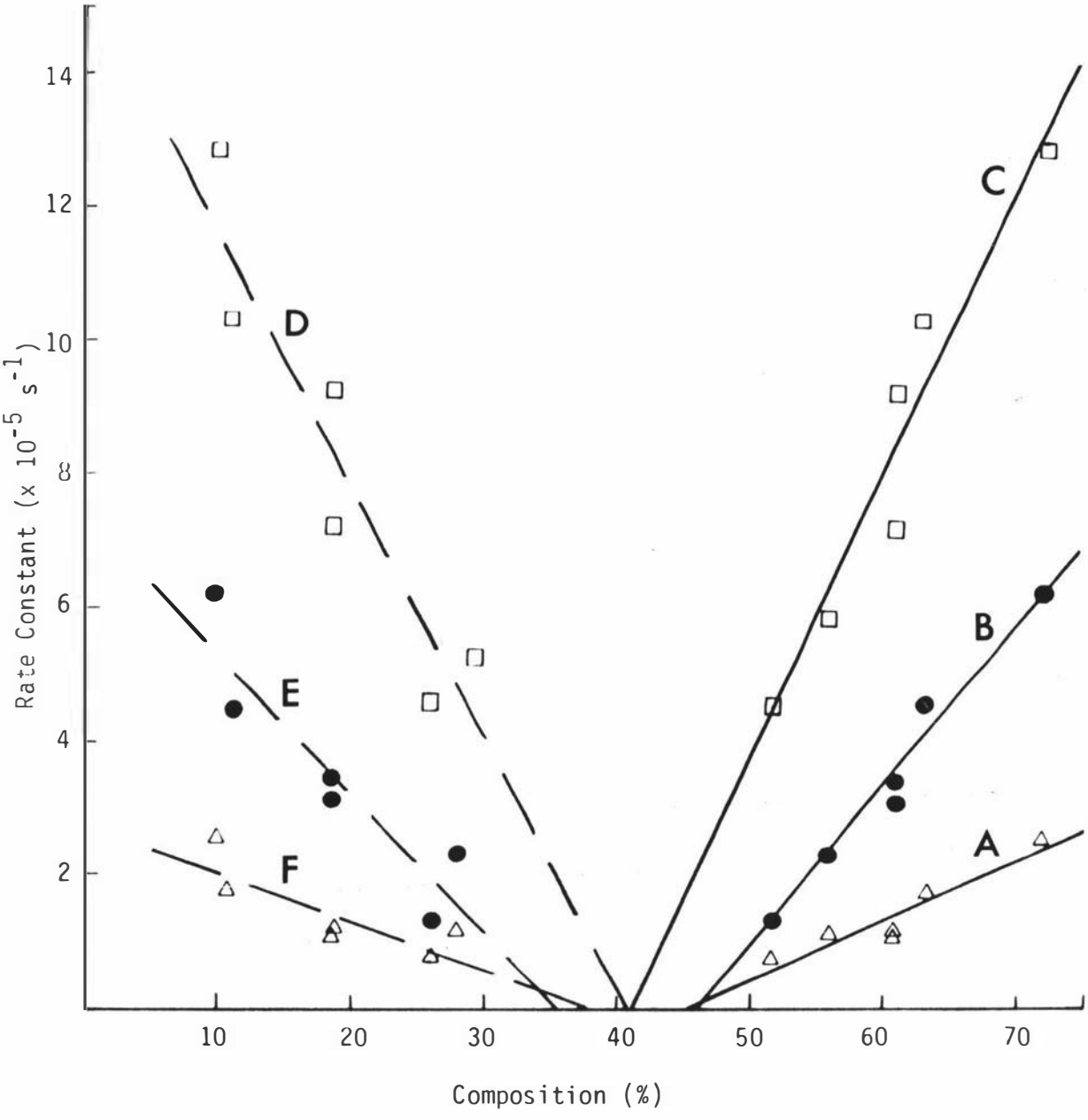


Figure 5.6: Plot of apparent first order rate constants for vitamin A loss as a function of compositional factors at various temperatures

A, $k_{102} = 0.087(W - 45.40) \times 10^{-5}\ s^{-1}$; $R^2 = 0.862$
B, $k_{112} = 0.241(W - 46.43) \times 10^{-5}\ s^{-1}$; $R^2 = 0.971$
C, $k_{122} = 0.425(W - 41.34) \times 10^{-5}\ s^{-1}$; $R^2 = 0.928$
} k vs Moisture Content (W)

D, $k_{102} = 0.072(37.78 - F) \times 10^{-5}\ s^{-1}$; $R^2 = 0.667$
E, $k_{112} = 0.210(35.38 - F) \times 10^{-5}\ s^{-1}$; $R^2 = 0.840$
F, $k_{122} = 0.383(40.39 - F) \times 10^{-5}\ s^{-1}$; $R^2 = 0.861$
} k vs Fat Content (F)

The rate data were also analysed as a coded mixture design according to the methods of Snee (1971; 1973). The data were coded such that $\Sigma(F + P + W) = 100\%$. As the design was non-orthogonal, the best-fit equations were determined by a trial and error method, testing term by term. Generally the best-fit equation was the linear form, as the introduction of other terms did not reduce the total sum of squares of residuals. The decoded linear equations for the three rate constants were:

$k_{102} = (0.043W - 0.040F - 0.030P) \times 10^{-5}\ s^{-1}$, $R^2 = 0.944$, W sig at 98% level

$k_{112} = (0.112W - 0.123F - 0.066P) \times 10^{-5}\ s^{-1}$, $R^2 = 0.991$, F and W sig at 99% level

$k_{122} = (0.216W - 0.209F - 0.059P) \times 10^{-5}\ s^{-1}$, $R^2 = 0.990$, F sig at 98% and W at 99% level

where F = fat content (%)
P = protein content (%)
W = moisture content (%)

Again protein was not significant, indicating that loss was connected with the fat and moisture content compositions, and so this reduced to a two variable mixture design and could be analysed as such.

5.3.3 Effect of System Composition on Activation Energy

As indicated in Section 5.3.1, an average E_a of 112 $kJmol^{-1}$ was found for the different beef liver mixtures. The extra uncertainty introduced by using an average activation energy was small, indicating that E_a was only weakly dependent on composition at a pH of 6.3 and copper concentration of 18 $mgkg^{-1}$.

In fact when the activation energies were considered as different, no significant linear relationships existed between E_a and any of the compositional factors (fat, protein, moisture contents) or compositional ratios (fat:moisture, fat:protein, protein:moisture). The plots were totally random in nature indicating little causal relationship.

5.3.4 Effect of System Composition on Frequency Factor

Linear relationships similar to that for the rate constants (Section 5.3.1) were fitted for the frequency factor, A. This was to be expected from a consideration of the Arrhenius relationship, where $k = Ae^{-E_a/RT}$. It has been shown that the activation energy, under the conditions of the mixture design, was unaffected by composition. Hence, $k \alpha A$, and so factors affecting k, should affect A.

Either moisture content or fat content could explain most of the observed variation but the best fit linear relationship was A against moisture content with an R^2 of 0.948. Increasing moisture content (52 - 72%) caused an increase in the frequency factor for the thermal loss of vitamin A. Other linear relationships for the frequency factor included A vs (moisture content - fat content); and A vs fat:moisture ratio. No linear relationships existed between A and protein content, fat:protein ratio, or protein:moisture ratio.

Mixture design analysis of the data was also done. However, from an examination of all the models, moisture content seemed to be the most significant factor in influencing the value of A, and the magnitude of its effect appeared to swamp other factors which might be important.

As stated in Section 4.3.3, the frequency factor is derived by extraplolation of the non-linear Arrhenius equation and it is convenient to use a modified form in which a reference rate constant replaces the frequency factor. For an average activation energy of 112 $kJmol^{-1}$, a predictive equation for the reference rate constant (k_{122}) as a function of the compositional variables was formulated. The final model adopted for k_{122} was that with a minimum of terms in which the addition of extra parameters did not reduce the total sum of squares of residuals significantly. This was:

$$k_{122} = 0.696\ (W - 48.84) \times 10^{-5}\ s^{-1}$$

where W = moisture content (%) and pH and copper were held at 6.3 and 18 $mgkg^{-1}$ respectively. For this model $R^2 = 0.947$.

5.4 CONCLUSIONS

The kinetics of the loss on heating of vitamin A were determined in liver and fat mixtures using a steady state procedure at 102.1, 112.0 and 122.0°C. Three compositional variables (fat, protein and moisture contents) were studied using a constrained mixture design. Copper concentration (18 $mgkg^{-1}$) and pH (6.3) were held constant. The loss of vitamin A was adequately described by first order kinetics. At the pH and copper concentration under study, composition affected the rate of loss. Increasing the moisture content in beef liver puree mixtures caused an increase in the rate of loss of vitamin A at any of the given temperatures.

The experimentally determined activation energies ranged from 99-122 $kJmol^{-1}$. As E_a was only weakly dependent on composition it was possible to find one average activation of 112 $\pm$ 8 $kJmol^{-1}$ (at the 95% confidence level), that described the data almost as well as the individual values. The average deviation was 12%, whereas if the individual E_a values were used the average deviation was 10%. The extra uncertainty introduced by using the average E_a was therefore small. The average value is identical to that found in Section 4.3.1 for heating natural beef liver under steady state conditions.

In the same way as the rate constant was affected by system composition, so was the frequency factor. As A is found by extrapolation of the Arrhenius plot any errors in E_a are magnified in A. Therefore, a reference rate constant, k_{122}, from within the experimental range was used. The reference rate constant as a function of composition was described by:

$$k_{122} = 0.696(W - 48.84) \times 10^{-5}\ s^{-1}$$

where pH was held at 6.3 and copper concentration at 18 $mgkg^{-1}$, and where composition was constrained as specified in Section 5.2.2.

Moisture content was found to be the only variable needed to model the effect of composition on vitamin A loss during heating as protein content was unimportant and changes in fat content could only be made by changing moisture content as well. Therefore, it would not be necessary to repeat the full mixture design at combinations of pH and copper, the next variables to be studied.

6. EFFECT OF COPPER AND pH ON THE LOSS OF VITAMIN A IN DIFFERENT BEEF LIVER MIXTURES DURING STEADY STATE HEATING

6.1 INTRODUCTION

Steady state heating of beef liver mixtures at midpoint pH and copper levels, indicated that moisture content was the only variable needed to model the effect of composition on vitamin A loss during heating. As pH and copper were thought to be other factors likely to influence stability of this vitamin, they along with moisture content, were more fully investigated in the next experimental stage.

The design of this experiment was a full 2^3 factorial with copper concentration at two levels, 6 and 30 $mgkg^{-1}$, pH at 5.6 and 7.0, and moisture content at 55 and 68%. The maximum legal limit for copper in New Zealand meat products is 30 $mgkg^{-1}$ and the base raw material had a concentration of 6 $mgkg^{-1}$. It is unlikely that meat products have a pH outside the range 5.6 to 7.0. The particular levels for copper concentration and pH were chosen to cover these full ranges. The two levels of moisture content were selected as they were approximately the same as vertices 2 and 3 used in the mixture experiment. Steady state heating at 102 and 122^{o}C for times up to 330 minutes was used to determine the order of reaction, rate constant and the temperature dependence of the rate of loss of vitamin A.

6.2 EXPERIMENTAL

6.2.1 Sample Preparation

Fresh beef liver (the same as that used in Section 5) was chilled to ca $1^{o}C$, and connective tissue and visible fat were removed. The liver was then cut into 3.0 cm cubes. It was pureed in a chilled condition in a Stephan mixer (Model UM 12), packed in plastic bags and blast frozen at $-40^{o}C$. During processing, the puree was protected from light. A proximate analysis of the liver was made.

The mixtures (as described in Section 6.2.2) were prepared by adding lard to the pureed liver (Section 3.3), and mixing in a Waring Blendor for 2-4 minutes until an homogeneous puree was obtained. The blended mixture was removed and reweighed. The pH and moisture were adjusted by adding the correct amounts of the relevant reagents (Section 3.3) and mixing in by hand. The specified amount of copper was added to the liver. The mixtures were analyzed for their fat, protein and moisture contents, and their pH and E_h measured. Samples were then filled into vials as described in Section 3.4.3.

6.2.2 Design of 2^3 Factorial Experiment

In the mixture experiment (Section 5), moisture content was found to be the only variable needed to model the effect of proximate composition on vitamin A loss during heating. Consequently this factor, and the two new factors pH and copper, were chosen to be more fully investigated. The design of this experiment was a full 2^3 factorial with copper concentration at two levels, 6 and 30 $mgkg^{-1}$, pH at 5.6 and 7.0, and moisture content at 55 and 68%. The moisture content and fat were varied, and an attempt was made to keep the protein, ash and carbohydrate components approximately constant. Protein was held at ca 15%, and carbohydrate and ash ca 3% for the low moisture content points and ca 8% for the high moisture content points. The two levels of moisture content were chosen as they were approximately the same as vertices 2 and 3 used in the mixture experiment. The centre point for this design was the same as that in the mixture experiment. The actual specification of the experimental plan is given in Table 6.1 and the proximate analysis of the design points in Table 6.2.

Table 6.1: 2^3 Factorial Design for Beef Liver Mixtures at Different pH and Copper Levels

Design Point[a]	Code Level		
	a	b	c
1	-1	-1	-1
a	+1	-1	-1
b	-1	+1	-1
ab	+1	+1	-1
c	-1	-1	+1
ac	+1	-1	+1
bc	-1	+1	+1
abc	+1	+1	+1

[a] Coding: a = copper concentration; -1 = 6 $mgkg^{-1}$; +1 = 30 $mgkg^{-1}$
b = pH; -1 = 5.6; +1 = 7.0
c = moisture content; -1 = 55%; +1 = 68%

Table 6.2: Proximate Analysis of 2^3 Factorial Design Mixtures

Code	Fat (%)	Moisture Content (%)	Protein (%)	Ash and Carbo. (%) (by difference)	E_h (mV)	pH
1	24.3	57.8	14.8	3.1	-159	5.59
a	26.4	54.8	15.5	3.3	-168	5.57
b	27.2	53.5	16.0	3.3	-116	7.05
ab	26.8	54.1	15.8	3.3	- 97	7.03
c	9.7	67.2	14.5	8.6	-126	5.61
ac	9.7	67.4	14.4	8.5	-122	5.60
bc	9.3	68.6	13.9	8.2	-141	7.06
abc	9.5	67.8	14.3	8.4	-134	7.02

Vials of beef liver mixtures, following the specification of the experimental plan (Table 6.1) were heated for the times at the temperatures shown in Table 6.3,three vials being withdrawn at each sampling time. The 122^{o}C run had different heating times for the low and high moisture content points. It was observed from the 102^{o}C run , that longer heating times were needed for the low moisture content points to obtain significant losses of vitamin A necessary for assay purposes. Controlled heating and cooling were carried out as described in Section 3.4.3.3, and analysis for trans-retinol followed the method given in Section 3.1.4.

Table 6.3: Temperature-Time Combinations for Thermal Processing of 2^3 Factorial Mixtures

Temperature (^{o}C)	Time (mins)				Design Points
102.0	0	110	220	330	All
122.0	0	50	100	150	Low MC[a]: 1,a,b,ab
	0	40	80	120	High MC: c,ac,bc,abc

[a] MC = moisture content, %

NOTE: Because of the length of time required to obtain the experimental data, and the fact that vitamin A loss followed first order kinetics and the Arrhenius expression in previous work (Sections 4 and 5), only two processing temperatures were used.

6.3 RESULTS AND DISCUSSION

6.3.1 Estimation of Parameters Characterising Thermal Loss of Trans-Retinol in Beef Liver Puree as a Function of Copper, pH and Composition

The loss of vitamin A on heat processing was adequately described by first order kinetics as the amount of variation not explained by this model was small (average R^2 = 0.991). Plots of ln c_o/c versus time at various temperatures for the design points, are shown in Figures 6.1 and 6.2. (Actual concentrations of trans-retinol before and after processing are given in Appendix C.1). The values for apparent first order rate constants differed for each design point and are presented in Table 6.4.

Table 6.4: Apparent First Order Reaction Rate Constants for Trans-Retinol in Beef Liver Puree as a Function of Copper, pH and Composition

Design Point[a]	Code Level	$k(\times 10^{-5}\ s^{-1})$ with 95% Confidence Limits at Various Temperatures			
	a b c	102.0°C	R^2	122.0°C	R^2
1	-1 -1 -1	1.11 ± 0.16	0.974	4.55 ± 0.16	0.999
a	+1 -1 -1	1.29 ± 0.10	0.994	2.19 ± 0.14	0.994
b	-1 +1 -1	0.83 ± 0.12	0.980	4.53 ± 0.66	0.975
ab	+1 +1 -1	1.11 ± 0.04	0.998	2.28 ± 0.40	0.971
c	-1 -1 +1	2.75 ± 0.16	0.996	18.36 ± 0.88	0.998
ac	+1 -1 +1	3.78 ± 0.38	0.988	15.53 ± 0.34	0.999
bc	-1 +1 +1	2.57 ± 0.16	0.996	17.82 ± 0.82	0.998
abc	+1 +1 +1	2.43 ± 0.10	0.999	8.38 ± 0.70	0.993

[a] Coding: a = copper concentration, b = pH, c = moisture content.

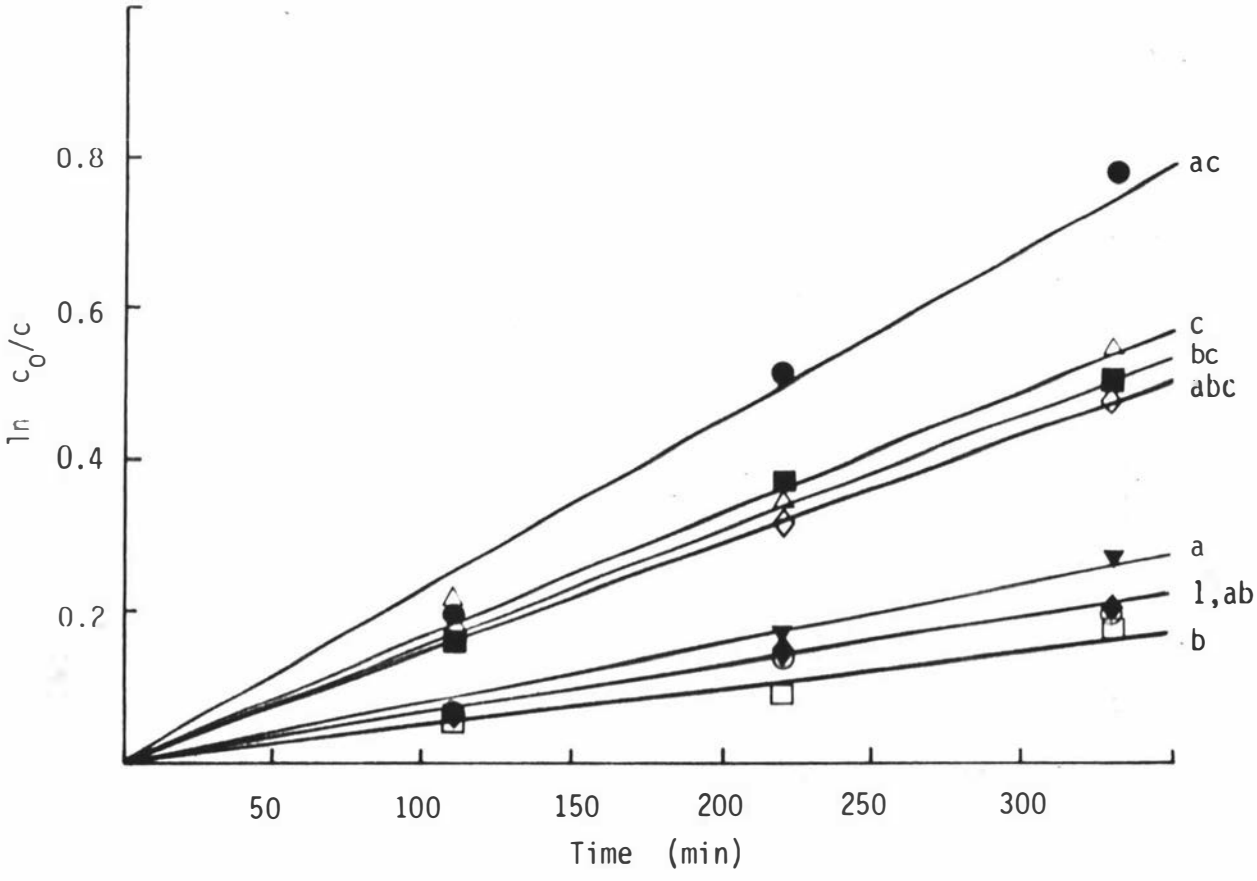


Figure 6.1 Degradation rates for vitamin A in beef liver puree mixtures at differing pH and copper concentrations, showing the natural logarithm of the concentration ratio as a function of time at 102°C

○ = 1
▼ = a
□ = b
◆ = ab
△ = c
● = ac
■ = bc
◇ = abc

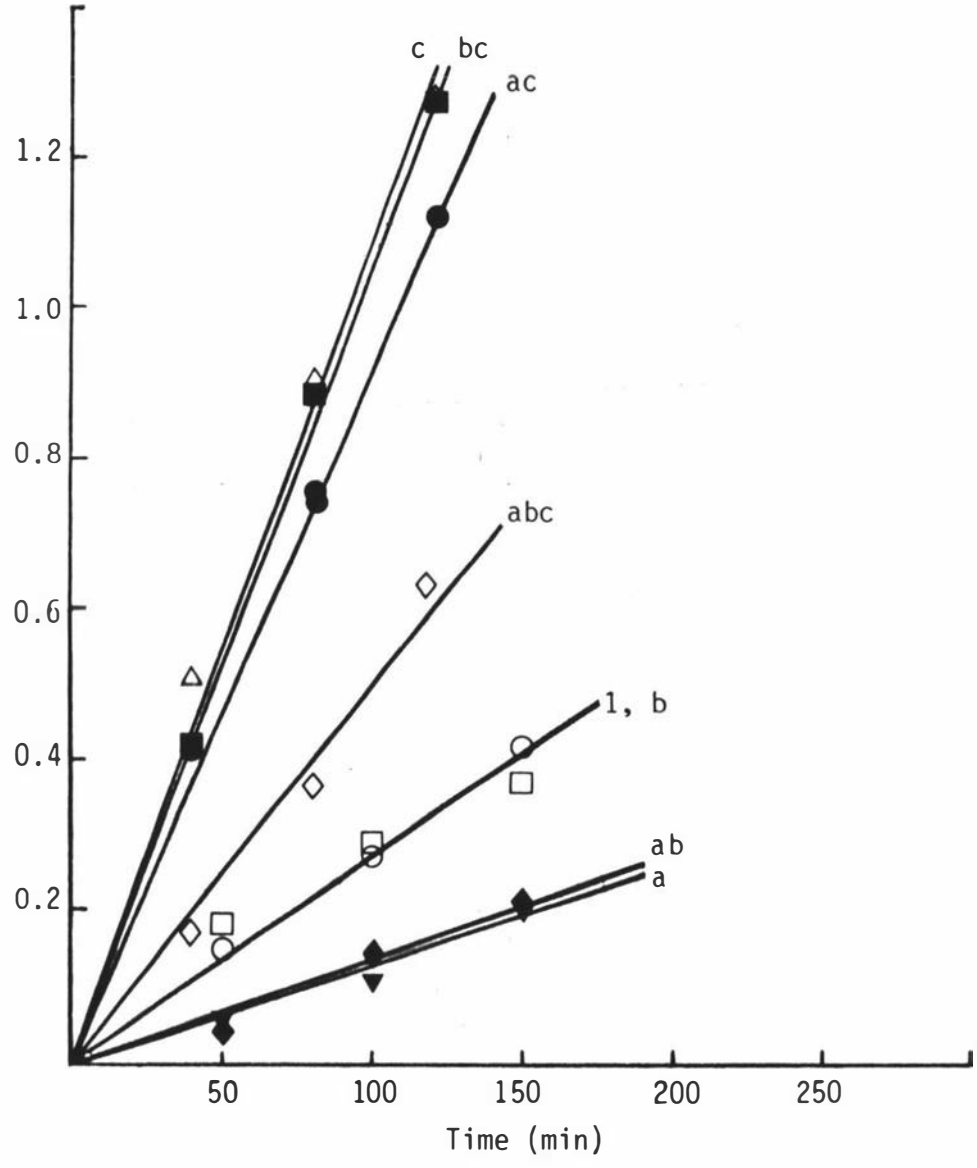


Figure 6.2 Degradation rates for vitamin A in beef liver puree mixtures at differing pH and copper concentrations, showing the natural logarithm of the concentration ratio as a function of time at 122°C

It was assumed that the Arrhenius equation applied. The values for the experimentally determined activation energies ranged from 36 - 121 $kJmol^{-1}$ (8.5 - 29.0 $kcalmol^{-1}$) - Table 6.5.

Table 6.5: Experimentally Determined Activation Energies for Trans-Retinol in Beef Liver Puree as a Function of Copper, pH and Composition

Design Point	E_a ($kJmol^{-1}$)[a]
1	91 ± 7
a	36 ± 12
b	117 ± 18
ab	36 ± 21
c	118 ± 9
ac	97 ± 12
bc	121 ± 7
abc	72 ± 7

[a] 95% confidence limits

As can be seen, there were two distinct groups in this range which had similar E_a values, i.e. design points a and ab; and b, c and bc. Using the logarithmic transformation of the Arrhenius equation (Section 4.3.3) it was possible to determine average activation energies of 36 ± 12 $kJmol^{-1}$ (8.6 ± 2.8 $kcalmol^{-1}$) for design points a and ab, and of 118 ± 7 $kJmol^{-1}$ (28.3 ± 1.8 $kcalmol^{-1}$) for b, c and bc. The other three points, 1, ac, abc were in the middle (91, 97 and 72 $kJmol^{-1}$ respectively). The significant difference in composition between the three groups was in the copper concentration and pH.

The estimated values for the frequency factor, using the average for each group and individual activation energies are given in Table 6.6, and it is apparent that the frequency factor differed for each design point.

Table 6.6: Experimentally Determined Frequency Factors for Trans-Retinol Loss in Beef Liver Puree as a Function of Copper, pH and Composition

Design Point	A (s^{-1}) (Indiv. E_a)	A (s^{-1}) (Avg. E_a)
1	5.80×10^{7}	-
a	1.15	1.28
b	1.60×10^{11}	2.86×10^{11}
ab	1.33	1.18
c	8.61×10^{11}	1.04×10^{11}
ac	1.25×10^{9}	-
bc	2.22×10^{12}	9.48×10^{11}
abc	2.48×10^{5}	-

6.3.2 Effect of Copper, pH and Composition on Reaction Rate

The effect of copper, pH and moisture content on the rate constants was determined by standard Yates analysis (Walpole and Myers, 1972) of a 2^3 factorial experiment. Each main effect and interaction effect has associated with it a single degree of freedom. Therefore it is possible to write $2^3 - 1$ orthogonal single-degree-of-freedom contrasts in the treatment combinations, each representing variation due to some main or interaction effect. Thus, under the usual independence and normality assumptions in the experimental model, tests can be used to determine if the contrast reflects systematic variation or merely chance or random variation.

At 102°C, increased moisture content (55 -68%) and increased copper concentration (6 - 30 $mgkg^{-1}$) increased the rate of vitamin A loss, whereas increased pH (5.6 - 7.0) decreased it, as shown in Tables 6.4 and 6.7.

Table 6.7: Estimated Contrasts (by Yates Analysis) of k_{102} for Vitamin A Loss in Beef Liver Mixtures Following a 2^3 Factorial Experiment

Variable[a]	Estimated Contrast
1	15.87
a	1.35
b	-1.99
ab	-1.07
c	7.19
ac	0.43
bc	-1.07
abc	-1.27

[a] a = copper concentration, b = pH, c = moisture content

As can be seen from Table 6.7, c (moisture content), was the dominant effect. The other two main effects a (copper) and b (pH) had much smaller contrasts. As no replication was done, the only way significance could be determined was by assuming that the interaction terms ab, ac, bc, and abc, were negligible and thus using these as the error term. This meant only main effects could be tested for significance, and in this case only c, moisture content, was significant. It was significant at the 99% level of confidence.

The entire data set was also analyzed in terms of coded values with the centre point from the mixture design (Section 5) used to estimate error. From the regression equation moisture content was the only significant effect, the coefficient being significant at the 90% level. Therefore the best fit decoded model for k_{102} as a function of the experimental conditions was:

$$k_{102} = 0.14\ (W - 47.93) \times 10^{-5}\ s^{-1} \qquad (6.1)$$

where W = moisture content, %

In comparison, the mixture design which was conducted at the midpoint levels of copper and pH used in this experiment, produced a regression equation for k_{102} of the form:

$$k_{102} = 0.087\ (W - 45.40) \times 10^{-5}\ s^{-1} \qquad \text{(Figure 5.6)}$$

Heating identical samples at 122^{o}C again confirmed that high moisture content design points had higher reaction rate constants than the low moisture content points (Table 6.4). This trend follows that established in the mixture experiment (Section 5). Table 6.8 gives the estimated contrasts of k_{122}, and thus an indication of the significance of the experimental variables.

Table 6.8: Estimated Contrasts (by Yates Analysis) of k_{122} for Vitamin A Loss in Beef Liver Mixtures Following a 2^3 Factorial Experiment

Variable[a]	Estimated Contrast
1	73.64
a	-16.88
b	7.62
ab	-6.50
c	46.54
ac	7.66
bc	-7.76
abc	6.72

[a] a = copper concentration, b = pH, c = moisture content

As can be seen from the above table, c (moisture content) was the dominant effect. Unlike k_{102}, a (copper) also has a high contrast value, its effect being to decrease vitamin A loss as it itself is increased in value from 6 to 30 mgkg^{-1}. As for k_{102}, significance was determined by combining the interaction terms and using them as the error term. The significant main effects were copper at the 90% level and moisture content at the 99% level of confidence. From the regression equation for the 2^3 factorial experiment (k_{122} as the

response variable, and using the whole data set and the centre point from the mixture design in the analysis), moisture content was significant at the 99% level and copper concentration at the 95% level. None of the other coefficients was significant. The best fit decoded model was therefore:

$$k_{122} = 0.89\ (W - 0.20\ Cu - 48.20) \times 10^{-5}\ s^{-1} \qquad (6.2)$$

where W = moisture content (%)
Cu = copper concentration ($mgkg^{-1}$)

This last form of the equation is similar to that for k_{102} except that it has an additional term i.e. one for copper.

At high copper concentrations, the effect of temperature on the rate of reaction was different to that at low copper concentrations. This implies a different activation energy and hence possibly a change in the mechanism of reaction, although the measured data still fitted an apparent first order model.

As the experiment was conducted at two temperatures - 102 and 122°C it was possible to analyse the design in terms of a 2^4 factorial. Thus the factors were:

a = copper concentration, $mgkg^{-1}$
b = pH
c = moisture content, %
d = temperature, °C

The contrasts as estimated by Yates analysis are shown in Table 6.9.

Table 6.9: Estimated Contrasts (by Yates Analysis) of the Rate for Vitamin A Loss in Beef Liver Mixtures Following a 2^4 Factorial Experiment

Variable	Estimated Contrast
1	89.51
a	-15.53
b	5.70
ab	7.57
c	53.73
ac	-7.23
bc	6.55
abc	-7.99
d	57.77
ad	-18.23
bd	9.68
abd	-5.43
cd	39.35
acd	-8.09
bcd	8.69
abcd	5.45

Main effects a (copper), c (moisture content), d (temperature) and second order effects ad (Cu * temp) and cd (moisture * temp) have the largest contrasts (Table 6.9). Combining all the three and four factor interaction terms to use as the error term and so determine significance, a and ad were significant at the 90% level, cd at the 99% level and c and d at the 99.9% level of confidence. Temperature had the greatest positive influence on vitamin A loss, followed by moisture content. The increase in rate with increase in moisture content may be partially explained by the fact that increasing a_w (hence moisture content) causes solubilization and concomitantly increased mobilization of catalysts/reactants that may attack vitamin A (Labuza, 1980). As vitamin A is fat soluble an increase in moisture content implies a concentration of the vitamin in a

diminishing fat phase. This trend of moisture content follows that established in the mixture experiment (Section 5.3).

Copper too (estimated contrast of -15.53), was shown to be important in affecting vitamin A loss. At 122^{o}C high copper concentration had an apparent protective effect on vitamin A, as the rate of loss in going from 102 to 122^{o}C was not as great as expected if copper had been at a lower level. This indicates that the mechanism by which vitamin A was destroyed on heating was complex.

It is possible that degradation was lipid mediated and perhaps metal catalyzed. The rate of lipid oxidation increases with an increase in a_w and it is possible that this is related to the increasing loss of vitamin A with increasing moisture content. This apparent anomaly with regards to the effect of copper at 102 and 122^{o}C was similar to that reported by Marcuse and Fredrickson (1971) who showed that for linoleic acid oxidation in emulsions at low oxygen pressure, addition of copper ions generally accelerated the rate of lipid oxidation but as the copper concentration increased, copper ions were shown to have an antioxidative tendency. As vitamin A is fat soluble, the effect of copper on the mechanism may be similar in beef liver mixtures.

According to Harris and Karmas (1975), vitamin A is regarded as being more stable in basic than acidic pH. However, Hannukainen and Niinivaara (1974b) showed that artificially lowering the pH in pork liver from 6 to 5 increased vitamin A retention on cooking. As can be seen from Tables 6.7 - 6.9, pH was not statistically significant in influencing vitamin A stability in beef liver mixtures under the particular conditions of temperature and copper concentration.

6.3.3 Effect of Copper, pH and Composition on Activation Energy

As shown by Table 6.5, the experimental variables affected the activation energy, even when average activation energies were determined for design points a, ab and b, c, bc. To evaluate which of the experimental variables were significant in affecting E_a, the data (using average Ea's where appropriate) were re-analysed in terms of a 2^3 factorial with activation energy as the response variable. Table 6.10 gives the estimated contrasts of E_a.

Table 6.10: Estimated Contrasts (by Yates Analysis) of E_a for Vitamin A Loss in Beef Liver Puree Following a 2^3 Factorial Experiment

Variable[a]	Estimated Contrast
1	686
a	-204
b	2
ab	- 52
c	124
ac	70
bc	- 52
abc	2

[a] a = copper, b = pH, c = moisture content

Main effects a (copper) and c (moisture content) had the highest contrast values. Increasing copper concentration caused a lowering of the activation energy, whilst increasing moisture content had the opposite effect. The other main effect b (pH) on its own did not alter E_a, but its interaction terms ab and bc had high contrast values.

For the coded regression equation for the 2^3 factorial experiment the R^2 value was 0.935 but no factors were significant at the 95% confidence level. In testing this, the full model, there was only one degree of freedom and it was considered that the lack of significant coefficients may have been due to the small amount of information available for checking significance. Therefore an alternative method, a half-normal plot (Daniel, 1959), was used to see if any of the seven coefficients were significant. By this test, moisture content (W) and possible copper were significant.

Moisture content increased E_a, whereas copper decreased the value of the activation energies.

This suggests that vitamin A loss may be lipid mediated and perhaps metal catalyzed, because metal catalyzed lipid oxidation has a lower E_a than lipid oxidation not metal catalyzed (63 - 105 $kJmol^{-1}$ cf 147 - 167 $kJmol^{-1}$, Labuza, 1971). Apart from design point ac , the other design points having high copper concentrations had much lower activation energies. Points a and ab , which had the lowest activation energy, also had a low moisture content i.e. a high fat content (ca 26%). The activation energy changed with moisture content. All the low moisture content design points had on average a lower activation energy than the high moisture content points (70 $kJmol^{-1}$ cf 101 $kJmol^{-1}$). This result is consistent with the findings of Labuza (1980), but not in agreement with the mixture experiment (Section 5) in which moisture content (and the other compositional factors, fat and protein) were found to affect E_a only slightly, when copper and pH were held at their midpoint levels. When copper and pH levels were changed, moisture content had an effect on E_a.

6.3.4 Effect of Copper, pH and Composition on Frequency Factor

As a general trend, the high moisture content points had high frequency factor values (Table 6.6). However, no attempt was made to model the frequency factor as a function of the experimental conditions because this parameter is found by extrapolation of the Arrhenius plot and is very sensitive to changes in E_a. Values for A ranged from 1.18×10^{0} to 1.04×10^{12} s^{-1} for rate constants which varied by only 90%. The variation was highly non linear with respect to the process parameters. This shows the underlying difficulty of using the frequency factor as a reference point when it lies well outside the experimental conditions.

No predictive equations for E_a and a reference rate constant (k_{122}) that included only significant effects, could be found to fit the data, because of the complex manner in which changes in the level of copper changed the rate constant.

6.4 CONCLUSIONS

The kinetics of the loss on heating of vitamin A were determined in liver and fat mixtures of varying pH's and copper concentrations. The rate followed apparent first order kinetics and it varied with the changes in composition, particularly with moisture content but copper also had a significant effect. High moisture content design points had higher reaction rate constants than the low moisture content points. The change in rate of loss of vitamin A in moving from 102 to 122^{o}C was less in runs where copper was present at high concentrations than when it was at a low level. This indicates that the mechanism by which vitamin A was lost on heating was complex.

The activation energies for the temperature dependence of the rate constants ranged from 36 - 122 kJmol^{-1} with changes in composition. Design points b, c and bc (low copper, high pH, low moisture content; low copper and low pH, high moisture content; and low copper, high pH and high moisture content), had an average E_a of 118 $\pm$ 7 kJmol^{-1}, which was similar to that from Section 4 and Section 5. For heating natural beef liver E_a had the value 112 kJmol^{-1}, and for heating beef liver and fat mixtures the average was also 112 kJmol^{-1}. Only in this study where the copper level was changed, did both the moisture content and copper alter the E_a value.

Values for the frequency factor ranged from 1.18 x 10^{0} to 1.04 x 10^{12} s^{-1} and were related to changes in composition. However, no attempt was made to model A as a function of the experimental conditions because this parameter is found by extrapolation of the Arrhenius plot and is very sensitive to changes in E_a.

Under the experimental conditions studied in this 2^3 factorial design, it was not possible to formulate predictive equations for E_a and k_{ref} for vitamin A loss on heating, because of the copper effect. It can be concluded that under these conditions of copper concentration (6 - 30 mgkg^{-1}), pH (5.6 - 7.0) and moisture content (55 - 68%), vitamin A loss on heating was complex. Apart from the effects of heat, loss may also have been lipid mediated and metal catalyzed.

7. EFFECT OF UNSTEADY STATE HEATING ON VITAMIN A LOSS IN BEEF LIVER MIXTURES

7.1 INTRODUCTION

The principle aim of the study was to find a method of predicting vitamin A loss during heat processing of canned products. It was thought that it might be feasible to predict losses on canning by using kinetic data obtained from steady state experiments. The purpose of this part of the study was to prove whether this could be done.

The data from the steady state experiments were obtained under specific food system compositions and heating temperatures. Therefore, the unsteady state study had to be made in the same range of experimental conditions for any useful comparison to be made.

It was decided to use a full 2^3 factorial design to investigate the effects of three variables on vitamin A loss under unsteady state conditions (pilot plant canning procedures) and also to compare the results with steady state procedures under similar conditions. Processing temperature was set at two levels, 112 and 120^{o}C, intended equivalent processing time (F_o) at 2 and 6 minutes, and moisture content at 56 and 69%. Two times and two temperatures were chosen in order that the kinetic parameters could be established. Heating beef liver mixtures was expected to be entirely by conduction, so the higher temperature of 112 and 120^{o}C and relatively low F_o values were used because of the possible deleterious sensory and physical changes that would occur at low temperature-long time processing. The two levels of moisture content were selected as they approximated those used in the mixture experiment (Section 5).

Unsteady state heating was done in a pilot plant scale stationary retort, and the percent retention of vitamin A after processing was determined experimentally and compared with that predicted using the steady state kinetic data in conjunction with finite difference calculations of temperature distribution in the can.

7.2 EXPERIMENTAL

7.2.1 Sample Preparation

Extraneous fat and connective tissue were removed from fresh beef liver. The liver was cut into 3.0 cm cubes and pureed in a chilled condition in a Stephan mixer (Model UM 12), divided into two equal quantities as two mixtures were to be made, packed in plastic containers, frozen and stored at -30^{o}C until required for use. A proximate analysis of the liver was made and it had the following composition:

Extractable Fat	= 2.0%
Crude Protein	= 20.2%
Moisture Content	= 70.1%
Ash and Carbohydrate	= 7.7% (by difference)
Copper	= 16 mgkg^{-1}
pH	= 6.09

The two mixtures (as described in Section 7.2.2) were prepared by adding lard and a copper solution (Section 3.3) to the liver and blending in a Stephan mixer until an homogeneous puree was obtained. The pH and moisture were adjusted by adding the correct amounts of the relevant reagents (Section 3.3). The mixtures were analysed for their fat, protein and moisture contents, and their pH measured. They were then frozen and stored at -30^{o}C until required for canning trials.

7.2.2 Design of 2^3 Factorial Experiment

The three variables investigated in the factorial experiment were processing temperature, F_o, and moisture content. The processing temperature in this case was the retort temperature and it was set at 112 and 120^{o}C, which was within the heating range used for the steady state experiments. Because the temperature of a thermal process normally varies with time, it is convenient and necessary to have some way of comparing the relative sterilising capacities of different heat processes. A unit of lethality, F, which is the heat treatment regarded as necessary for destroying micro-organisms, is used. F_o is the equivalent processing time in minutes at a constant

temperature of 121.1°C, for micro-organisms whose destruction rate with temperature is described by the Arrhenius relationship with an E_a of 290 kJ mol^{-1}. In common canning technology, this is stated as z of 10°C. In this experiment the F_o values were set at 2 and 6 minutes.

Moisture content levels of 56 and 69% were chosen as they were approximately the same as vertices 2 and 3 used in the mixture experiment (Section 5) and also the 2 levels used in the 2^3 factorial steady state experiment (Section 6). Copper and pH were not variables in the design but they were set at the levels used in the mixture experiment, 18 $mgkg^{-1}$ and 6.3 respectively, as it would have been too complicated and time consuming to scale up the steady state experiments of both Sections 5 and 6. The actual specification of the experimental plan is given in Table 7.1 and the proximate analysis of the design points in Table 7.2.

Table 7.1: Layout for 2^3 Factorial Design for Unsteady State Heating of Beef Liver Mixtures

Design Point	Coding			Values		
	a	b	c	Temp (°C)	F_o (min)	MC (%)
1	-1	-1	-1	112	2	56
a	+1	-1	-1	120	2	56
b	-1	+1	-1	112	6	56
ab	+1	+1	-1	120	6	56
c	-1	-1	+1	112	2	69
ac	+1	-1	+1	120	2	69
bc	-1	+1	+1	112	6	69
abc	+1	+1	+1	120	6	69

Table 7.2: Proximate Analysis of 2^3 Factorial Mixtures for Unsteady State

Design Points	Fat (%)	MC (%)	Protein (%)	Ash & Carbo (by diff.) (%)	pH	Copper ($mgkg^{-1}$)
1, a, b, ab	26.9	56.1	14.9	2.1	6.32	18
c, ac, bc, abc	11.6	69.4	15.1	1.9	6.33	18

Cans of beef liver mixtures, following the specification of the experimental plan (Table 7.1), were processed according to the procedure given in Section 3.4.4. Run abc was repeated because the trans-retinol content in the controls i.e. unprocessed cans, was low in comparison with the other high moisture content controls and an error was considered possible. In fact the duplicate agreed closely with the original. Control and processed cans of the beef liver mixtures were analysed for trans-retinol following the method given in Section 3.1.4.

7.2.3 Thermal Diffusivity Determination

To be able to calculate the temperature distribution at different times, a thermal property, the thermal diffusivity, α is needed. The temperature of the beef liver mixtures was measured by a thermocouple placed at the geometric centre of the can. The experimental temperature/time data was used to calculate α, using the analytical solution of the heat transfer equation. To do this, it was assumed that the material heated solely by conduction, the thermal diffusivity remained constant over the entire process, and the external heat transfer resistance was zero.

Taimmanenate (1980) described the technique in detail. In summary:

$$\ln Y = \text{constant} - t\left(\frac{\alpha\beta_1^2}{a^2} + \frac{\alpha\pi^2}{4h^2}\right) \qquad (7.1)$$

where $Y = \dfrac{\theta - \theta_i}{\theta_a - \theta_i}$

t = time, s

α = thermal diffusivity, $m^2\ s^{-1}$

β_1 = positive root of the first order Bessel's function = 2.4048

a = can radius = 0.037 m

h = half-height of the can = 0.058 m

θ = centre temperature, ^{o}C

θ_i = initial temperature, ^{o}C

θ_a = retort temperature, ^{o}C

From a plot of ln Y against time, t, the slope of the regression equation on the linear portion will be equal to $-(\alpha\beta_1/a^2 + \alpha\pi^2/4h^2)$. Knowing β_1 and the dimensions of the can, the thermal diffusivity can be calculated as illustrated below:

$$-\alpha\frac{(2.4048)^2}{(0.037)^2} + \frac{\pi^2}{4(0.058)^2} = \text{slope}$$

$$\alpha = \frac{-\text{slope}}{5011.1}\ m^2\ s^{-1} \qquad (7.2)$$

This method has been used by other workers (Olson and Jackson, 1942; Teixeira, 1969). It should be noted that the thermocouple does not need to be at the centre of the can to obtain the temperature data. In addition, only part of the ln Y vs time plot is straight, and in this portion only one term in the series solution is significant. This straight line section is used to calculate α.

7.3 SIMULATION

A computer program was developed which calculated the bacterial lethality and vitamin A retention in a conduction-heated food. A finite difference approximation to the partial differential equation for unsteady state heat conduction in a finite cylinder was used. This calculated the temperature distribution throughout the container at intervals in time. The rate equation for bacterial lethality and vitamin loss was applied over short time intervals assuming constant temperature in small volumes within the can for the time interval. The final spore destruction and percent vitamin retention was obtained by integration over the container volume and over process time of the spore and vitamin concentrations in different parts of the can.

In determining the temperature distribution, an analytical or a numerical finite difference method could be used. In the heating phase, the analytical solution will give exact temperature predictions provided:

(i) the thermal diffusivity is known exactly and is constant,
(ii) the material is homogeneous and completely fills the can,
(iii) all heat transfer is by conduction,
(iv) the surface temperature of the material in the can equals the retort temperature,
(v) the retort temperature changes instantaneously at time zero to the required value and remains constant at this value.

In contrast, finite differences by definition, approximate the partial differential equation describing heat transfer, and thus involve approximations. Provided the space increments Δx and Δr, and the time increment Δt are chosen wisely, the mathematical approximations are small and insignificant compared with error introduced by the five assumptions above.

In the cooling phase, the analytical method solution superimposes a cooling calculation on the heating profile (Taimmanenate, 1980). This is to approximate the non-uniform temperature distribution at the onset of cooling. In contrast, the finite difference calculation uses the actual temperature profile at the end of heating as its initial condition for cooling.

Another difficulty in the application of the analytical solution at the onset of cooling is that the series solution is slow to converge, and an approximate analytical solution for short times must be applied (Taimmanenate, 1980).

There are slight differences in the predictions by the two methods. Explicit finite differences tend to overpredict the extent of change compared with the analytical solution (Myers, 1971) but the difference between the two methods in heating phase predictions found by Taimmanenate (1980) never exceeded $0.4^{o}C$ and was $0.1^{o}C$ on average.

Errors in temperature prediction will only matter if they lead to errors in the calculation of the vitamin A retention. It was expected that the activation energy would be similar to the steady state experiments (112 $kJmol^{-1}$). The error in a calculated rate constant can be estimated approximately from:

$$\% \text{ Error} = 100 \left(1 - e^{-E_a(\Delta\theta)/RT^2}\right)$$

where T = temperature, K

$\Delta\theta$ = temperature prediction error

An error of $0.5^{o}C$ would lead to a percent error in the rate constant of 5.3% at $80^{o}C$, and 4.3% at $120^{o}C$. For F_o values of 2 min and 6 min, preliminary predictions using the finite difference method and the steady state data suggested that vitamin A retention would be between 70 - 90%. If the vitamin A retention in a process was 80%, then that predicted by a consistent $0.5^{o}C$ temperature overprediction would be 79%. This difference would not be distinguished by the assay method used for vitamin A where differences between replicates were up to 5%.

This, coupled with the five necessary assumptions, meant that both methods (analytical and finite difference) predicted sufficiently accurate temperature/time profiles. The choice between the two methods was therefore made on the basis of which needed less computing resource. Due to the difficulties in applying an analytical solution at the onset of the cooling phase, the analytical method required considerably more computer process time, so the finite difference method was chosen.

7.3.1 Finite Difference Method

The general unsteady state heat transfer equation in a finite cyclinder with constant thermal properties is:

$$C\frac{\partial\theta}{\partial t} = \lambda\frac{\partial^2\theta}{\partial x^2} + \lambda\frac{\partial^2\theta}{\partial r^2} + \frac{\lambda}{r}\frac{\partial\theta}{\partial r} \qquad (7.3)$$

where C = specific heat capacity on a volumetric basis, $Jm^{-3}\,{}^{o}C^{-1}$

θ = temperature, ^{o}C

t = time, s

λ = thermal conductivity, assumed constant, $Wm^{-1}K^{-1}$

x = displacement in axial heat flow, m

r = displacement in radial heat flow, m

Where thermal properties are constant, a simple explicit finite difference scheme for equation (7.3) is:

$$\frac{\theta^{n+1}_{i,j} - \theta^{n}_{i,j}}{\Delta t} = \frac{\lambda}{C}\left(\frac{\theta^{n}_{i+1,j} - 2\theta^{n}_{i,j} + \theta^{n}_{i-1,j}}{(\Delta x)^2}\right) + \frac{\lambda}{C}\left(\frac{\theta^{n}_{1,j+1} - 2\theta^{n}_{i,j} + \theta^{n}_{i,j-1}}{(\Delta r)^2}\right) + \frac{\lambda}{C}\frac{1}{r}\left(\frac{\theta^{n}_{i,j+1} - \theta^{n}_{i,j-1}}{2\Delta r}\right) \qquad (7.4)$$

$$= f(A) + f(B) + f(C')$$

where i = distance along the height, m

j = distance along the radius, m

r = $(n - 1)\Delta r$, m

x = $(m - 1)\Delta x$, m

θ^n = temperature at time t, ^oC

θ^{n+1} = temperature at time $t + \Delta t$, ^oC

α = thermal diffusivity, which is $\frac{\lambda}{C}$, $m^2\ s^{-1}$

It is assumed that the temperature distribution in the can is symmetrical about the geometrical centre. Several special cases must be considered. Where j = 1, equation (7.4) was modified by Albasiny (1960) by assuming:

$$\lim_{r \to 0} \frac{1}{r}\frac{\partial\theta}{\partial r} = \frac{\partial^2\theta}{\partial r^2} \tag{7.5}$$

This yields:

$$\frac{\theta^{n+1}_{i,1} - \theta^n_{i,1}}{\Delta t} = \alpha\left(\frac{\theta^n_{i+1,1} - 2\theta^n_{i,1} + \theta^n_{i-1,1}}{(\Delta x)^2}\right) + 4\alpha\left(\frac{(\theta^n_{i,2} - \theta^n_{i,1})}{(\Delta r)^2}\right) \tag{7.6}$$

Where i = 1, the temperature is calculated by:

$$\frac{\theta^{n+1}_{i,j} - \theta^n_{1,j}}{\Delta t} = 2\alpha\left(\frac{\theta^n_{2,j} - \theta^n_{1,j}}{(\Delta x)^2}\right) + f(B) + f(C') \tag{7.7}$$

The temperature at the centre (i = 1, j = 1) can be calculated by:

$$\frac{\theta^{n+1}_{1,1} - \theta^n_{1,1}}{\Delta t} = 2\alpha\left(\frac{\theta^n_{2,1} - \theta^n_{1,1}}{(\Delta x)^2}\right) + 4\alpha\left(\frac{\theta^n_{1,2} - \theta^n_{1,1}}{(\Delta r)^2}\right) \tag{7.8}$$

The computer program was constructed by incorporating the above equations and assuming the initial temperature at all points was equal throughout the can, while the temperature at the can surface was equal to the retort temperature during heating, and equal to the cooling water temperature during cooling. The program was written in FORTRAN IV language for a Burroughs B6700 computer. Details are given in Appendix D.1.

7.3.2 Kinetic Calculation

Assuming that the changes in bacterial numbers and vitamin concentration can be described by first order kinetics, the concentration change will be:

$$\frac{dc}{dt} = -kt \qquad (7.9)$$

where c = concentration
k = reaction rate coefficient
t = time

Internally, the can is divided into a number of small volumes. Within each of these volumes, it is assumed that the temperature gradient is negligible so that an average temperature for each small volume can be used.

In order to predict the extent of change in each of these small volumes, the rate constant k, is integrated with respect to time.

$$\int_0^t k\,dt = \ln c_0/c = \nabla \qquad (7.10)$$

In unsteady state heating, the temperature varies with time and so does k. Therefore, the left hand side of equation (7.10) must be evaluated numerically. This is done by assuming that for a small interval of time Δt, the temperature in the small elemental volume remains constant and so does k. The product $k\Delta t$ therefore represents the contribution to ∇ occurring in that time step. The variation of k with temperature is assumed to follow the Arrhenius relationship. In the total process time t, the number of time steps N is determined from:

$$N = t/\Delta t \tag{7.11}$$

and ∇ is calculated as:

$$\nabla = \sum_{n=1}^{n=N} k_n \Delta t \tag{7.12}$$

Once ∇ for the process is known, c/c_o which is the fractional retention, can be calculated from equation (7.10).

In this manner, values of ∇ or c can be calculated for each elemental volume Δv within the can. If it is required to find the mass average value of c for the can as a whole at the end of the process, the individual values of c are used in:

$$c_{ma} = \frac{1}{V} \sum_{i=1}^{i=m} \sum_{j=1}^{j=m} c_{i,j} \Delta v_{i,j} \tag{7.13}$$

where $j=2, m-1;\ \Delta v_{i,j} = \pi \Delta x \Delta r^2 \left((j - \tfrac{1}{2})^2 - (j - 1\tfrac{1}{2})^2\right)$ (7.14)

where $j=1;\ \Delta v_{i,1} = \frac{\pi}{4} \Delta r^2 \Delta x$ (7.15)

$$j=m;\ \Delta v_{1,m} = (m - 1\tfrac{1}{4})\pi \Delta x \Delta r^2 \tag{7.16}$$

In special cases where i = 1, i = m, the value of $\Delta v_{i,j}$ calculated from equations (7.14), (7.15), (7.16) is halved prior to use in equation (7.13). These modifications take account of differing volumes associated with central axis and surface nodes.

This method is similar to that used by Taimmanenate (1980).

7.4 RESULTS AND DISCUSSION

7.4.1 Comparison of Predicted and Experimental Time-Temperature Profiles

Experimental temperature/time data were collected at only the geometric centre of the cans. Data collection at other points in the can was considered unnecessary for two reasons. Firstly, the position of the thermocouple is immaterial for thermal diffusivity measurements. Secondly, all predictions of temperature were based on assumptions that α was constant, heating was by conduction only, the material was homogeneous and completely filled the can. If these assumptions are valid, experimental and predicted temperatures will agree irrespective of position. If they are not valid, agreement will not occur at the centre or any other position. Therefore centre temperature measurements repeated in several cans, yield as much information as several measurements at different positions in one can, and the former are easier to carry out.

As the first stage of data analysis, the thermal diffusivity was calculated. The results are shown in Table 7.3.

Table 7.3: Thermal Diffusivities for Beef Liver Mixtures at Various Temperatures

Design Point	Retort Temp. (^{o}C)	Thermal Diffusivity ($\times 10^{-7}$ m^2s^{-1})
1	112.0	1.68, 1.60
a	120.0	1.99, 1.89
b	112.6	1.74, 1.84, 1.72, 1.84
ab	120.0	1.80, 1.84
c	112.0	1.83, 1.79
ac	118.0	2.06, 1.99
bc	112.0	-[a]
abc	120.0	2.06, 1.87, 2.00, 2.01

[a] For run bc there were measurement problems due to thermocouple damage, and hence no reliable temperature data.

For the low moisture content points (1, a, b, ab) the average thermal diffusivity was 1.79×10^{-7} m^2s^{-1} with a standard deviation of 0.11×10^{-7} m^2s^{-1}. The high moisture content points had an average α of $(1.95 \pm 0.11) \times 10^{-7}$ m^2s^{-1}. These values of α were similar to data given by Teixeira et al. (1975) and Lenz (1977). The difference between these two means was tested and found to be significant at the 99% confidence level.

The average thermal diffusivity for the individual design points was also tested against the mean for the low moisture content and high moisture content points. No values were significantly different at the 95% level from the mean thermal diffusivities. It was therefore decided to use the average low and high moisture diffusivities for the relevant beef liver mixtures for the processing conditions studied.

Typical centre temperature-time profiles for the two different liver compositions - run 1 and run c - are shown in Figures 7.1 and 7.2. The processing conditions for these two runs were very similar as shown in Table 7.4.

As can be seen, the calculated temperatures were close to the measured temperatures in the heating phase. In the first ten minutes, the predicted centre temperature was approximately two degrees lower than the experimental temperature but after that, was exactly the same for run 1 and 0.5 - 1.0^{o}C different for run c. Deviations of the predicted temperatures from the experimental temperatures could have been caused by the assumption of constant thermal diffusivity not being valid and by errors in the thermocouple measurements. These errors possibly include inaccurate positioning of the thermocouple junction, and conduction along the wires. This latter case would be highest at the beginning of both the heating and the cooling phase as the temperature difference between the outside of the can and the junction was high, e.g. 110.2^{o}C cf 16.7^{o}C for run 1 at the onset of cooling.

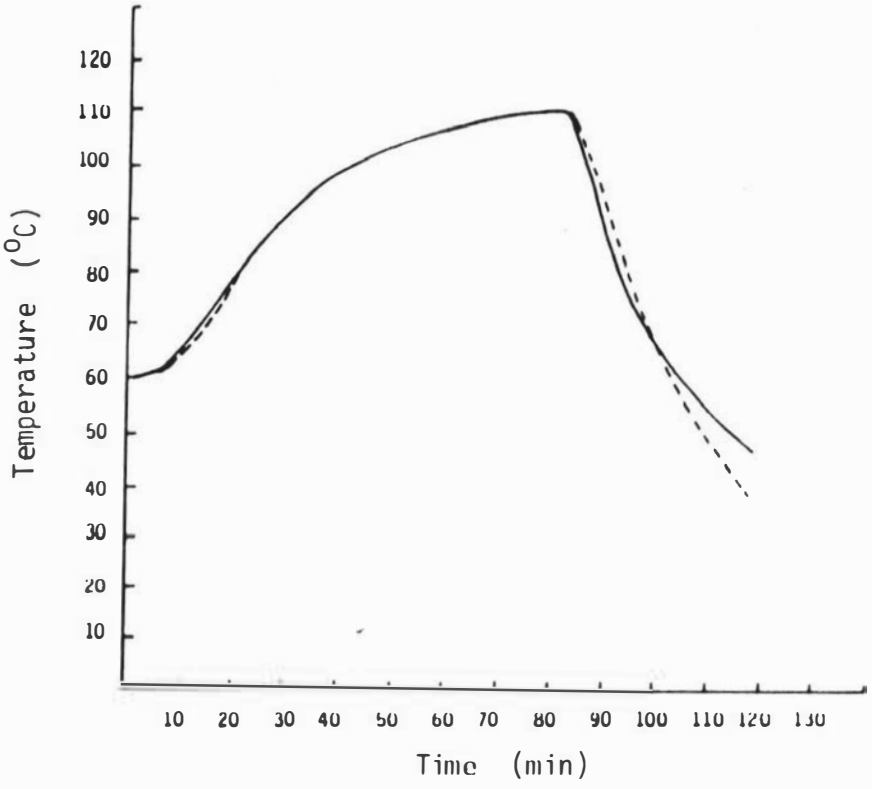


Figure 7.1 Comparison of experimental centre temperatures and predicted centre temperatures for a low moisture mixture at a retort temperature of 112°C (run 1)

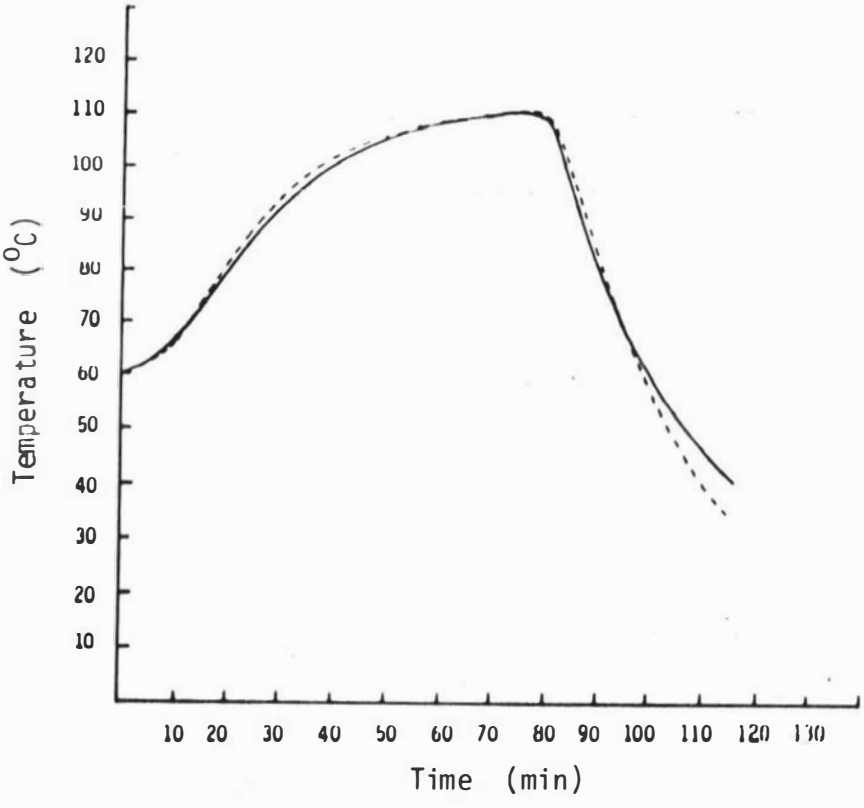


Figure 7.2 Comparison of experimental centre temperatures and predicted centre temperatures for a high moisture mixture at a retort temperature of 112°C (run c)

(——— experimental temperatures)

(-------- predicted temperatures)

At temperatures below 90°C, the vitamin A loss was negligible and so the lack of agreement between experimental and predicted temperatures late in the cooling phase was considered unimportant. It was therefore unnecessary to modify the program and to form fit the latter part of the cooling phase.

Poor agreement at low temperatures was thought to be caused by a change in the mode of heat transfer in the cans. On opening some of the cans, the meat emulsion had destabilised and there was some free liquid present as shown in Figure 7.3 and 7.4. This was more significant for the high than for the low moisture content cans. The presence of free liquid indicates that the assumption of only conduction heat transfer occurring was invalid. The extent of deviation from conduction heat transfer would be expected to be greater in the high moisture content cans. Irregularities in cooling have been reported by other workers (Board et al., 1960; Taimmanenate, 1980).

7.4.2 Comparison of Predicted and Experimental Vitamin A Retentions

Cans of beef liver mixtures were processed according to the conditions shown in Table 7.4, and then analysed for trans-retinol content. The total time taken to complete this work was four months, so the cans were frozen after processing until they could be analysed.

To standardise the initial processing conditions, the temperatures of the liver as it was put into the retort was ~ 60°C. Although the design specified that the retort temperature should be 112 or 120°C, run b was heated at 112.6°C and run ac at 118°C due to problems in controlling the retort pressure accurately.

It was difficult to experimentally obtain the required F_o. These varied, especially for the low values e.g. 1.6 for run a to 3.0 for run ac. This problem arose because it was difficult to ascertain the likely contribution of cooling to F_o, when the length of the heating phase to achieve a certain total F_o value was chosen.

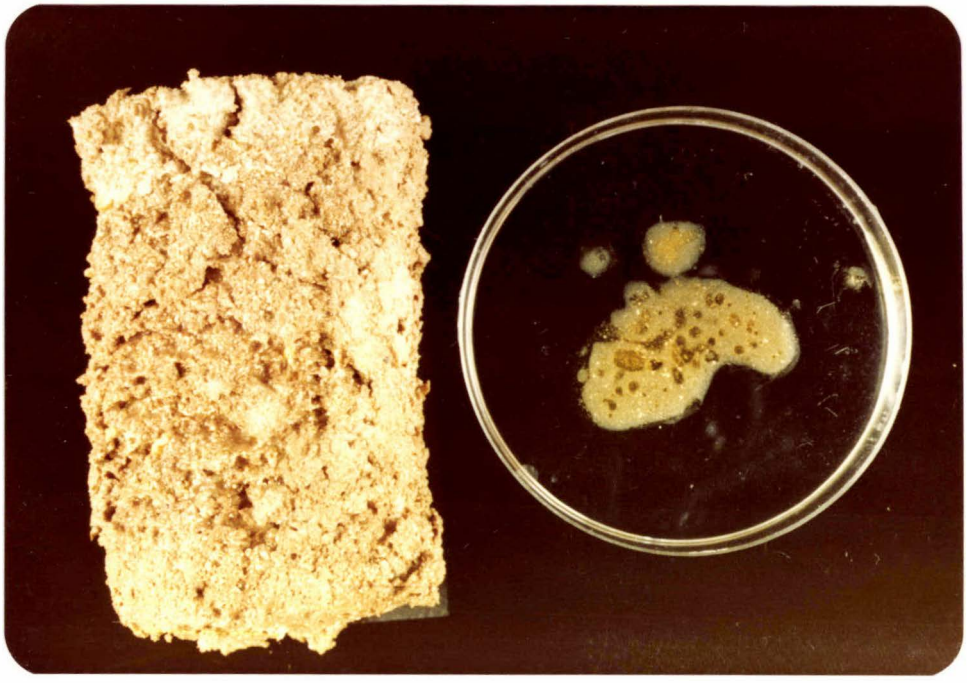


Figure 7.3 Processed low moisture content beef liver showing the meat matrix and exudate (run 1)

Figure 7.4 Processed high moisture content beef liver showing the meat matrix and exudate (run c)

Table 7.4: Processing Conditions for 2^3 Factorial Unsteady State Experiment

Design[a] Point	Initial Temp (^oC)	Retort Temp (^oC)	Heating[b] Time (min.)	Cooling H_2O Temp (^oC)	Cooling[c] Time (min.)	Predicted[d] F_o (min.)
1	59.4	112.0	76.0	16.7	40.0	2.1
a	60.2	120.0	48.5	17.9	40.0	1.6
b	60.3	112.6	102.0	17.2	40.0	5.5
ab	60.5	120.0	64.0	17.6	40.0	6.3
c	60.2	112.0	73.5	16.8	40.0	2.4
ac	61.0	118.0	57.0	17.6	40.0	3.0
bc	60.5	112.0	111.0	17.3	40.0	6.7
abc	60.9	120.0	60.0	17.4	40.0	7.0
abc_{rep}	60.2	120.0	60.0	17.9	40.0	6.9

[a]Coding: a = retort temperature, b = F_o, c = moisture content

[b]The interval between the time when the retort temperature reached 100^oC, to the time when the cooling water was turned on.

[c]The interval between the time when the cooling water was turned on to the end of cooling i.e. after 40 min.

[d]F_o evaluated from simulation program, for the particular processing conditions.

The experimentally determined and the predicted vitamin A retentions as a result of processing are shown in Table 7.5. The predicted vitamin A retentions were from using the finite difference program (Section 7.3) which incorporated the steady state parameters from the mixture experiment (Section 5). The individual steady state parameters (Table 5.5) were used. For the low moisture content liver (equivalent to vertex 2) values were: $A = 0.8 \times 10^9\ s^{-1}$, $E_a = 99\ kJmol^{-1}$. The high moisture content liver mixtures (equivalent to vertex 3 of the mixture experiment) had steady state parameters of $A = 3.5 \times 10^9\ s^{-1}$, $E_a = 101\ kJmol^{-1}$.

Table 7.5: Vitamin A Values and Experimental and Predicted Retentions for Each Design Point

Design Point	Trans-Retinol Concn ($\mu g g^{-1}$)		Exptal. Determ. % Vit A Retent[a]	Pred. % Vit A Retent
	Control	Processed		
1	201.3	172.0	84.7 ± 2.2	90.5
	202.6	170.8		
a	201.1	174.8	86.4 ± 2.3	90.9
	202.4	174.8		
b	209.3	174.6	84.2 ± 2.2	85.7
	200.8	166.4		
ab	201.1	173.8	85.5 ± 2.3	86.7
	200.3	172.0		
c	217.7	132.5	63.5 ± 2.1	79.2
	218.8	141.7		
ac	213.9	119.7	53.5 ± 2.0	78.8
	212.6	111.5		
bc	216.2	86.0	40.0 ± 2.0	67.4
	216.0	86.7		
abc	184.2	105.5	59.5 ± 1.7	72.3
	184.0	110.8		
abc_{rep}	193.3	112.3		72.3
	185.0	115.7		

[a] Mean with 95% confidence limits

Run abc was repeated because of the low trans-retinol content in the controls i.e. unprocessed cans in comparison with the other high moisture content controls (c, ac, bc). As can be seen, the concentration was still very similar for the two abc runs and there appeared to be no difference in the two sets of data. No explanation could be postulated for these low values, but as vitamin A loss on steady state heating was characterised as being first order (Section 4.5 and 6) its percent retention was independent of initial concentration.

Differences between the control concentrations could arise from three sources:

(i) errors in the vitamin A assay procedure,
(ii) sampling errors,
(iii) differences in treatments.

Inspection of Table 7.5 suggested that the last factor was insignificant in comparison with the others. All low moisture content values were virtually the same and all high moisture content values, except for abc, were similar. Within each of the three groups, the variation in control sample values represented analysis and sampling errors only. Therefore, an average control concentration could be used for each group. These values were 202, 216 and 187 $\mu g g^{-1}$. Standard deviations describing the spread around the values were 2.7, 2.3 and 2.5 $\mu g g^{-1}$ respectively. As the analysis method and sampling techniques were the same for each group, the same variation (standard deviation) would be expected within each group. The three different values arose from the small sample sizes. It would be expected that if a greater number of cans were available, the true standard deviation would be close to the mean standard deviation, calculated by weighting the three estimates in proportion to the sample sizes (3 $\mu g g^{-1}$). This was assumed to be the standard deviation describing uncertainty in both the control and processed samples. Using these data, the 95% confidence interval on the initial (control) concentrations were calculated as $\pm$ 2.1, $\pm$ 2.5 and $\pm$ 3.0% respectively. Similarly, the standard deviation in the mean processed values was $\pm$ 4.2% for runs 1 to bc and $\pm$ 3.0% for abc. From these standard deviations, a 95% confidence interval on the percent retentions was calculated. This is the figure shown in Table 7.5.

From Table 7.5 it was clearly evident that moisture content (c) was the most significant factor influencing vitamin A loss. High moisture content liver mixtures (points c, ac, bc and abc) produced the greatest losses of vitamin A on canning. This was consistent with the steady state results (Section 5 and 6) in which it was found that increasing moisture content increased the rate of loss.

The effect of the experimental variables, retort temperature, F_o, and moisture content on the experimental vitamin A retention was determined by standard Yates analysis of a 2^3 factorial experiment as shown in Table 7.6.

Table 7.6: Estimated Contrasts (by Yates Analysis) of % Vitamin A Retention in Beef Liver Mixtures Following a 2^3 Factorial Experiment

Variable	Estimated Contrast
1	557.3
a	12.5
b	-18.9
ab	29.1
c	-124.3
ac	6.5
bc	-16.1
abc	29.9

As can be seen from the above table, c (moisture content) was the dominant effect, its action being to decrease vitamin A retention as it increased in value from 56 to 69%. As the experiment was not replicated, significance was determined by combining the interaction terms and using them as the error term. The only significant term was moisture content at the 99% level of confidence.

7.4.2.1 Differences Between Predicted and Experimental Vitamin A Retentions

Table 7.5 also compares the experimentally determined and the predicted vitamin A retentions. The agreement with the low moisture content points is better than for the high moisture liver mixtures. Two of the low moisture content runs (b, ab) were within the estimated uncertainty. However, for the high moisture content liver mixtures, in all cases retention was over-predicted. Possible reasons for this include:

(i) the actual heat transfer did not match the modelled conditions and

(ii) changes in the kinetic parameters from steady state to unsteady state.

It was observed that in the high moisture content samples, a significant physical change had occurred in the material after heating (Figure 7.4). Free liquid existed and there appeared to be partitioning of the fat. Therefore the assumption that all heat transfer was by conduction was probably invalid. As vitamin A is fat soluble, it would have been expected to have migrated with the liquified fat which moved towards the surface of the can. Here it would have received harsher processing conditions, than if it had remained uniformly distributed throughout the protein matrix. The simulation model assumed no migration and therefore would under-predict retention even if the steady state kinetic parameters held. In the low moisture content cans, only slight destabilisation of the meat matrix had occurred (Figure 7.3).

The pure conduction model gives the maximum retention of vitamin A, as this process gives the lowest average treatment throughout the whole can. Conversely, pure convection gives the minimum vitamin A retention as it gives the highest average heat treatment throughout the can. Table 7.7 shows the predicted retentions for both cases. If the steady state kinetic parameters are also true for unsteady state heating, the actual mass average retention must lie between pure conduction and pure convection. This is not the case for the high moisture runs, but the low moisture data just lie within the range. It can therefore be concluded that there was a definite change in kinetic parameters between steady state and unsteady state for the high moisture material.

Table 7.7: Actual and Predicted Vitamin A Retentions for the Design Points if Heating was Pure Conduction or Pure Convection

Design Point	Predicted % Vitamin A Retention		Exptal. % Vitamin A Retention
	Conduction	Convection	
1	90.5	86.9	84.7 ± 2.2
a	90.9	84.5	86.4 ± 2.3
b	85.7	82.1	84.2 ± 2.2
ab	86.7	80.1	85.5 ± 2.3
c	79.2	72.6	63.5 ± 2.1
ac	78.8	68.3	53.5 ± 2.0
bc	67.4	61.7	40.0 ± 2.0
abc	72.3	60.9	59.5 ± 1.7

In order to evaluate unsteady state parameters, one of the two models, pure conduction or complete mixing, i.e. convection, must be assumed. The nature of the material and the shape of the temperature-time graph (Figures 7.1 and 7.2) suggest that pure conduction is the better model. The method suggested by Taimmanenate (1980) could then be used, but the calculated kinetic parameters would be subject to uncertainty introduced by the nature of the actual heat transfer.

7.4.3 Kinetic Parameter Determination for Unsteady State Heating of Low and High Moisture Content Liver

The vitamin A retention cannot be predicted by the computer program (Section 7.2.3) unless the kinetic parameters, k_{ref} (k_{122}) and E_a are known. Therefore for the unsteady state, these parameters had to be assumed, used in the program to calculate the predicted retention and then compared with the experimental result. An experimental design was used to determine the k_{122} and E_a for each low moisture content point (1, a, b, ab) and each high moisture content design point (c, ac, bc, abc). The predicted retention for each processing run was dependent on only k_{122} and E_a. Therefore, to determine the actual kinetic parameters for vitamin A loss, unsteady state conditions, the relationship between the residual (difference between the experimentally determined and predicted retention) and k_{122} and E_a was evaluated using

multiple regression analysis to obtain a second order response surface model and the residual was minimized by the differentiation of the empirical relationship.

To fit a second order response surface, at least three levels of each variable are required. A central composite design was used as it fulfils these requirements. In this study, k_{122} and E_a were the two variables involved. The design is shown in Figure 7.5, where X1 is a coded variable of E_a and X2 a coded variable of k_{122}.

Values of E_a chosen for the design points encompassed ranges previously established (Section 5 and 6) from steady state work and the centre point, 0, was taken to be 105 $kJmol^{-1}$ and the other design points in increments of $\pm$ 10 $kJmol^{-1}$. The coding for X1 was therefore:

$$X1 = \frac{E_a - 105}{10}$$

and it was used for both the low and the high moisture content points.

From the steady state work (Section 5) for low moisture content (56%) liver, the value for k_{122} was 6.72×10^{-5} s^{-1}. In the canning trials, the actual vitamin A loss averaged 1.46 times more than the predicted using the steady state parameters. Therefore, the centre-point, 0, was taken as 9.02×10^{-5} s^{-1} and the other design point values were in increments of $\pm$ 25% of this, i.e. $\pm$ 2.25×10^{-5} s^{-1}. Therefore the kinetic reaction rate constant at $122^{o}C$ at each level was coded by:

$$X2 = \frac{k_{122} - 9.02 \times 10^{-5}}{2.25 \times 10^{-5}}$$, for low moisture content mixtures

In the case of the high moisture content liver, the steady state k_{122} was 16.11×10^{-5} s^{-1}. The unsteady state work gave an average vitamin A loss 1.79 times more than predicted using the steady state parameters. Therefore the centrepoint, 0, was taken as 27.70×10^{-5} s^{-1}. Other design point values were increments of $\pm$ 25% of this i.e. $\pm$ 6.93×10^{-5} s^{-1}. So the coding was:

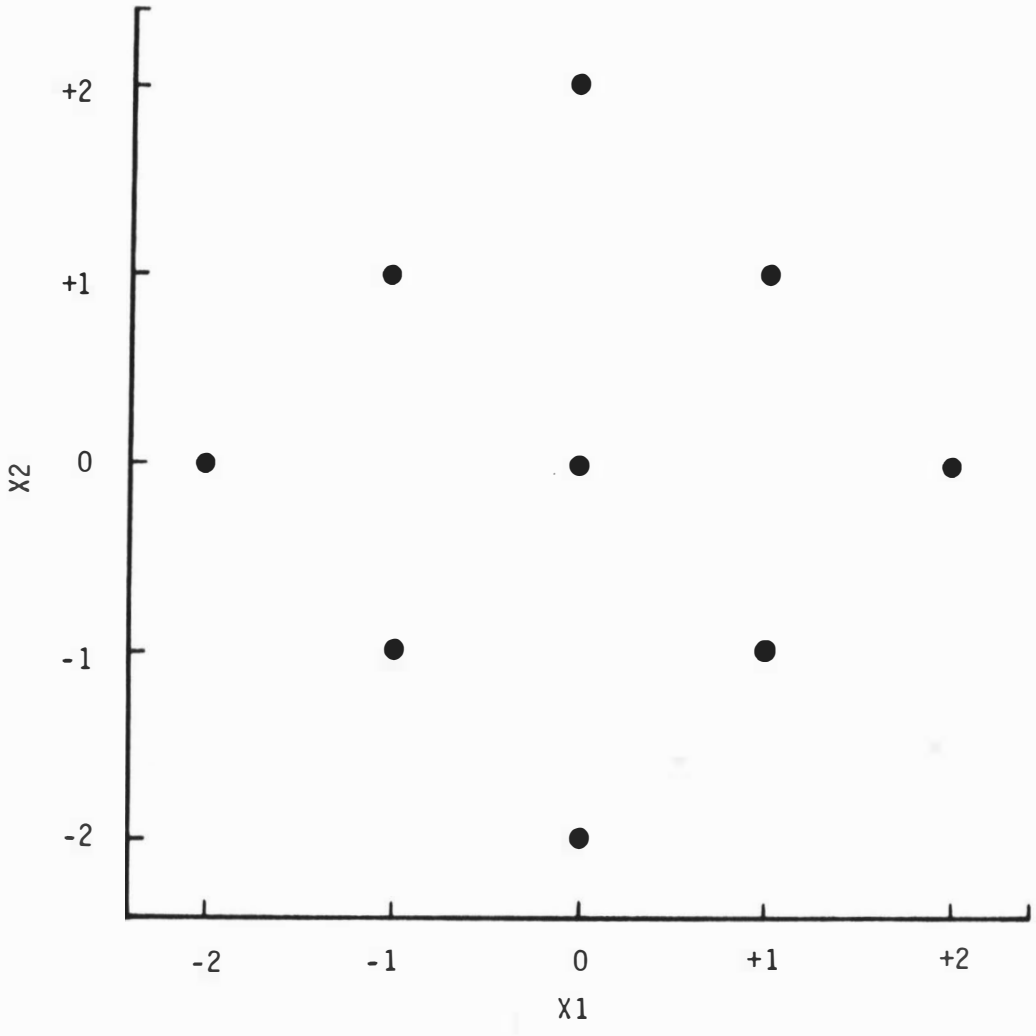


Figure 7.5 A central composite design to fit a second order response surface

$$X2 = \frac{k_{122} - 27.70 \times 10^{-5}}{6.93 \times 10^{-5}}$$, for high moisture content mixtures

The actual central composite design layout for the low and high moisture points is shown in Table 7.8.

Table 7.8: Central Composite Design for Low and High Moisture Content Liver Mixtures

Design Point	X1	X2	E_a ($kJmol^{-1}$)	k_{122} ($\times 10^{-5}$ s^{-1})	
	Coded E_a	Coded k_{122}		Low MC	High MC
1	+1	+1	115	11.27	34.63
2	-1	+1	95	11.27	34.63
3	+1	-1	115	6.77	20.77
4	-1	-1	95	6.77	20.77
5	0	0	105	9.02	27.70
6	0	+2	105	13.52	41.66
7	0	-2	105	4.52	13.84
8	+2	0	125	9.02	27.70
9	-2	0	85	9.02	27.70

The above design was used for each of the low moisture content runs i.e. for 1, a, b and ab; and for each of the high moisture content runs c, ac, bc, and abc_{avg}. The residual (Y) was then calculated for all points in the design where Y was defined as:

$$Y = \%\ Retention_{experimental} - \%\ Retention_{predicted}$$

These residuals are shown in Tables 7.9 and 7.10.

Table 7.9: Retention Residuals for Low Moisture Content Runs

Design Point	Residuals for Runs:				Average Residual
	1	a	b	ab	
1	0.5	2.6	6.5	9.4	4.7
2	3.2	3.8	9.8	10.8	6.9
3	-5.4	-3.4	-2.1	0.9	-2.5
4	-3.7	-2.6	0.1	1.8	-1.1
5	-1.3	0.1	3.7	5.8	2.1
6	4.8	6.0	12.3	14.1	9.3
7	-7.9	-6.3	-6.0	-3.4	-5.9
8	-3.4	-0.8	1.1	4.8	0.4
9	1.1	1.3	6.7	7.0	4.0

Table 7.10: Retention Residuals for High Moisture Content Runs

Design Point	Residuals for Runs:				Average Residual
	c	ac	bc	abc_{avg}	
1	3.1	-3.1	-1.5	13.0	2.9
2	8.6	0.2	4.1	15.3	7.1
3	-10.5	-17.1	-18.9	-3.3	-12.5
4	-6.3	-14.7	-14.0	-1.5	-9.1
5	-0.9	-8.3	-6.8	6.5	-2.4
6	11.7	4.4	7.9	20.5	11.1
7	-17.0	-24.6	-28.3	-13.1	-20.7
8	-5.6	-11.0	-12.3	4.6	-6.1
9	4.3	-5.1	-1.1	8.9	1.5

A quadratic equation was fitted, relating the residual (Y) to E_a and k_{122} by multiple regression. If the average residual was positive, the sign of the individual residuals remained unchanged, and if the average was negative the sign of all individual residuals was changed. The regression equation was fitted using the individual residuals, not the average. Because of the differences within each design point, all had to be taken into consideration. Because of other inherent inaccuracies, such as the assay method and the mode of heat transfer, k_{122} and E_a were not determined for each design point, but were calculated for the whole design. Thus k_{122} and E_a were determined by minimising the residual.

The full coded model for the low moisture content beef liver was:

$$Y = 2.358 - 0.663\ X1 + 1.238\ X2 - 0.016\ X1^2 + 1.328\ X2^2 - 0.888\ X1X2$$

Minimising Y, gave k_{122} and E_a of $7.40 \times 10^{-5}\ s^{-1}$ and $97\ kJmol^{-1}$ respectively.

For the high moisture content beef liver the full model was:

$$Y = 3.908 + 0.650\ X1 - 2.575\ X2 + 0.097\ X1^2 + 3.103\ X2^2 - 1.875\ X1X2$$

Minimising Y, gave a k_{122} of $29.85 \times 10^{-5}\ s^{-1}$ and an E_a of $102\ kJmol^{-1}$.

Table 7.11 compares the unsteady state kinetic parameters for low and high moisture content liver mixtures with those from steady state heating of mixtures of similar composition.

Table 7.11: Kinetic Parameters for Two Different Beef Liver Mixtures

Method of Heating	Low Moisture Content Liver		High Moisture Content Liver	
	E_a ($kJmol^{-1}$)	k_{122} ($\times 10^{-5} s^{-1}$)	E_a ($kJmol^{-1}$)	k_{122} ($\times 10^{-5} s^{-1}$)
Unsteady State	97	7.40	102	29.85
Steady State (indiv. E_a)	99	6.72	101	16.11

For the high and low moisture content liver mixtures, the values for the activation energy were very similar for both steady and unsteady state processing, indicating that E_a was relatively insensitive to the methods of heating. This was not the case for the reference rate constant, k_{122}. There was very little difference for the low moisture content samples, with a k_{122} of 6.72×10^{-5} s^{-1} for steady state heating and 7.40×10^{-5} s^{-1} for unsteady state heating. In the case of high moisture content mixtures, k_{122} for steady state was 1.85 times smaller i.e. underpredicted actual losses found in unsteady state processing. There was certainly a change in the kinetic parameters from steady state to unsteady state for the high moisture content samples. However, it should be noted that Table 7.11 values are not accurate as pure conduction was assumed.

In conclusion, kinetic parameters established using steady state procedures must be verified by canning trials as the parameters may not apply on scale-up and there could be changes in the physical structure of the product, which may lead to changes in the mode of heat transfer.

7.5 CONCLUSIONS

The investigation of the effects of retort temperature, equivalent processing time, F_o, and moisture content on vitamin A retention showed that moisture content was the most significant factor. Vitamin A retention decreased as moisture content increased in value from 56 to 69%. This was consistent with the steady state results (Section 5 and 6).

The predicted vitamin A retentions were higher than the experimentally determined values for the high moisture mixtures. There was much closer agreement with the low moisture samples, and two runs out of four actually fell within the estimated uncertainty. For the high moisture samples, it was postulated that over-predication was caused by a change in mode of heat transfer, vitamin A migration in the fat phase, and probably a change in the kinetic parameters from steady state to unsteady state.

It was observed that in both the low and high moisture content samples, a physical change had occurred in the material, though less pronounced in the former. Free liquid was present, which indicated that the heat transfer was a combination of conduction and convection. When the two extreme limiting cases were considered separately, i.e. pure conduction or pure convection, it was found that the low moisture data lay within the range of predicted retentions, but the high moisture content data did not. This could only occur if there was a definite change in kinetic parameters between steady state and unsteady state for the high moisture material.

The nature of the material and the temperature-time graphs suggested that conduction was the predominant heat transfer mode and pure conduction was therefore a better model to describe the heat transfer than convection. The conduction model was used in determining the unsteady state kinetic parameters. For the low moisture content samples, the unsteady state parameters were very close in value to those established using steady state procedures for a similar compositional liver mixture (Section 5). The activation energy was almost identical, and the reference rate constant established from unsteady state conditions was only 10% higher than the steady state rate constant.

In the case of the high moisture content samples, the E_a values were also similar under both steady and unsteady state heating - 101 cf 102 $kJmol^{-1}$ - but the reference rate constants were appreciable different: $k_{122} = 16.11 \times 10^{-5}\ s^{-1}$ for steady state and $k_{122} = 29.85 \times 10^{-5}\ s^{-1}$ for unsteady state. These values are only approximate due to a mixed heat transfer mode.

If kinetic parameters are established by steady state methods, it is important to verify these by conducting canning trials. There may be changes on scale-up in the kinetic parameters, and the physical state of the system may change, which could lead to complex heat transfer.

8. DISCUSSION AND CONCLUSIONS

Heating beef liver puree in the temperature range 102 - 127^{o}C caused loss of vitamin A (measured as trans-retinol). This loss was adequately described by a first order reaction kinetic model and its temperature dependence followed the Arrhenius expression.

When only the proximate composition (fat, protein and moisture contents) of beef liver was altered, and the pH and copper concentration held constant, the relative rate of reaction changed, but the activation energy did not. Increasing the moisture content from 52 to 72% caused an increase in the rate of loss of vitamin A at any of the studied temperatures. As the fat content of the mixtures increased from 10 - 28%, vitamin A loss decreased. The protein content had no effect, so in fat, protein, moisture content mixtures, it was the percentage fat and moisture contents that were significant in vitamin A loss. An increase in one generally required a decrease in the other, so either alone could explain most of the observed variation. Moisture content was the preferred variable to model the effect of composition as it explained a greater proportion of the variation. Losses in natural beef liver (fat content, 2.4%, moisture content, 69.6%) were higher than those for beef liver and fat mixtures with a similar moisture content. For natural beef liver $k_{122} = 125.0 \times 10^{-5}\ s^{-1}$, and for a liver and fat mixture (fat, 10.0%, moisture content, 72.2%) $k_{122} = 12.84 \times 10^{-5}\ s^{-1}$. The activation energies did not vary greatly between the two systems and were 112 and 101 $kJmol^{-1}$ respectively.

The effect of moisture and fat composition on vitamin A loss is important and requires more detailed study at wider ranges of these two variables in particular between 2 and 10% fat. It could be assumed that the vitamin A, as a fat soluble vitamin, would be present mainly in the fat phase, but if there is a large quantity of moisture, the fat globules could be dispersed throughout the water phase. This could be an explanation of the greater loss of vitamin A at high moisture content. Therefore the physical structure of the food may also affect the loss of vitamin A on heat processing, and further research could be done on different types of dispersion of fat in the food matrix.

When the effects of copper, pH and moisture content on the loss of vitamin A on heat processing were investigated, the kinetic relationships were different. When the copper concentration was changed, the E_a changed significantly from 121 to 36 $kJmol^{-1}$. Again increasing moisture content increased the rate of loss of vitamin A but copper also had an effect. The effect of copper was complex. When copper was held at one level, the activation energies of mixtures with different moisture contents were similar, but different copper concentrations caused changes in E_a. The change in rate of loss of vitamin A moving from processing temperatures of 102 to 122^{o}C was less in runs where copper was present at high concentrations than when it was at a low level. This indicates that changing copper concentration probably brought about changes in the mechanism of vitamin A loss, which is likely lipid mediated and metal catalysed. This is because the activation energy dropped to approximately 30% of previously determined values. As only two levels of copper were investigated, further kinetic studies at different levels of copper, moisture content and fat content, are needed to determine under what conditions the apparent kinetics change. Because vitamin A loss may be a fat initiated mechanism, it could be affected by different types of fat depending on their saturation and level of peroxides. The experiments in these studies need to be repeated in other food systems to see what effect fats and other food constituents may have on the vitamin A loss on heating.

When steady state parameters were used to predict vitamin A losses under unsteady state conditions, it was found that retentions were overpredicted, especially for high moisture content (69%) liver mixtures. This overprediction could not be explained by deviations in the actual heat transfer from the conduction model assumed, and therefore indicated that at the same compositions the kinetic parameters were different in unsteady state and steady state. For the low moisture content mixtures, steady and unsteady state parameters were similar. Although the E_a for the high moisture content samples was similar under both sets of heating conditions, the value of the reference rate constant was appreciably different. Minor changes in the fat and moisture contents and the physical state of the system altered k_{122}. This showed the difficulty in applying steady state results to the unsteady state conditions found in heat processing of cans.

Although it is simpler to use the steady state procedure because of ease of final data analysis and the small amount of raw material required, it is imperative to validate findings using the unsteady state heating method as it is under these conditions that food is normally thermally processed. The food system must have exactly the same form in both the steady and unsteady states to use the kinetic parameters from steady state data in predicting the effects of unsteady state heating. This is an area of food science which requires a great deal more study because of the importance today of not only knowing if a food has been adequately sterilised but that it is still providing the nutrients expected by the consumer.

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NOTE: Chem. Abs. signifies Chemical Abstracts.
FSTA signifies Food Science and Technology Abstracts.

APPENDIX A.1: Published Paper Summarising the Findings in Section 4

Kinetics of Vitamin A Degradation in Beef Liver Puree on Heat Processing

S. A. WILKINSON, M. D. EARLE, and A. C. CLELAND

ABSTRACT

The effects of heating on Vitamin A (measured as trans-retinol) in beef liver puree were investigated. The liver puree was heated in capillary tubes at five temperatures in the range 103-127°C, the typical canning temperature range for meat products. It was found that in this system and over the temperature range studied, the observed rate of degradation followed first order kinetics. The activation energy for the temperature dependence of the rate constant was 112 ± 9 kJ/mole.

INTRODUCTION

VITAMIN A is one of the fat-soluble vitamins. Although no coenzyme role has been demonstrated, it is necessary for the maintenance of proper vision, bone development and growth, epithelial tissue, and for reproduction. It occurs naturally only in animals, being present in highest concentrations in the liver. Its precursors, the carotenoids, do however occur more widely being found in green and yellow pigmented plant foods. Pennington (1976) listed vitamin A as one of the seven index nutrients used in dietary evaluation. Because of the nutritional significance of vitamin A, it is important to know the losses occurring in the processing of foods.

Two recent reviews stated that vitamin A has good stability during cooking and processing operations, but losses do occur when the foods are heated in the presence of oxygen (Lang, 1970; Barratt, 1973). Retentions of vitamin A between 50 and 100% were found for heating of vitamin enriched food products such as cereal products (Bauernfeind and Cort, 1974; Harper, 1978); peanut butter (Willich et al., 1954); and vitamin preparations (Benterud, 1977). Braising liver to an internal temperature of 77°C resulted in 90-100% retention (Kizlaitis et al., 1964) whereas mincing and cooking resulted in 87% retention (Hannukainen and Niinivaara, 1974).

Widely differing retentions for vitamin A in food processing have been reported. Negligible losses of vitamin A were reported in the pasteurisation, sterilization and spray-drying of milk (Ford et al., 1969; Thompson, 1969), and in the spray-drying of whole egg; but there was only 67% retention on the spray-drying of egg yolk (Denton et al., 1944). For canning, Labuza (1974) reported that in soups containing meat, retention was between 80-85% after sterilization, whereas Hellendoorn et al. (1971) showed that greater losses (0-100%) occurred on the canning of meat and vegetable meals. During the preparation and preservation of canned baby foods, it was found that of the six vitamins studied, A and C were the most labile during processing, and after canning the average vitamin A retention was 46 ± 8.5% (Auffray et al., 1978).

Obviously, the differences found in vitamin A retention were caused by different processing conditions, but there are no data in the literature on the effect of vitamin concentration and temperature on the rate of thermal destruction of vitamin A in food systems. Analysis by the present authors of some published data on the heating of ghee (Maqsood et al., 1963) gave a first order reaction for vitamin A degradation at both 100 and 200°C. The rate constants were $k = 109 \times 10^{-5}\ s^{-1}$ (heating ghee in boiling water) and $k = 1870 \times 10^{-5}\ s^{-1}$ (frying ghee at 200°C). Garrett (1956) determined an activation energy for vitamin A in liquid multivitamin preparations of 61 kJ/mol (14.6 kcal/mol). Slater et al. (1979) measured the shelf-life stability of vitamin A in multivitamin tablets and determined that degradation was pseudo-first order. Analysis by the present authors of Slater's data gave an activation energy of 118 kJ/mol (28.1 kcal/mol).

The lack of kinetic data for food systems therefore justified research into the rate of vitamin A degradation during thermal processing. If rate data existed for different food systems, the extent of destruction of this vitamin during commercial heat processing operations could be predicted. Important conditions to study were therefore those liable to be encountered in the sterilization of meat products in the temperature range 103-127°C. Experimental work to determine and characterize the kinetics of vitamin A degradation at five temperatures in this range was therefore done.

EXPERIMENTAL

Sample preparation

Fresh beef liver (pH = 5.81 ± 0.01) was cut into 1.0 cm cubes and pureed in a Waring Blendor for ca 90 sec. Puree (0.5g) was filled in boro silicate glass vials (3 mm internal diameter, 1 mm thick and 130 mm long) with a 45 mm headspace. The vials were heat sealed under vacuum.

Thermal processing system

The vials were heated at specified temperatures (102.9, 111.0, 118.3, 122.1 and 126.7°C) in a thermostatically controlled oil bath (± 0.2°C). Consideration of the heat transfer characteristics through the glass vial indicted that the temperature difference between the center of the liver and the glass-liver interface during the initial heating period was ca 20°C after 3 sec and ca 3°C after 30 sec. In 15 sec, 90% of the desired temperature was reached and so nearly instantaneous heating and cooling was assumed. For each temperature, three vials were removed at five different times and immediately cooled in a crushed ice/water bath. After cooling, the vials were washed and stored in the dark at −18°C until analyzed for retinol content. Control samples, which were not heated were treated in a similar manner to the test ones.

Determination of total trans-retinol in beef liver puree

The assay method used was basically that described by Haggett (1976). This procedure was a development of methods used in the Vitamin Assay Laboratories of Roche Products Pty Ltd., Australia.

The frozen vial was opened and the beef liver puree blown out under a stream of compressed air into a round bottom flask. The beef liver puree and glass tubing were saponified with ethanolic potassium hydroxide (aldehyde free) for 30 min. After cooling, the unsaponifiable matter was extracted with peroxide free diethyl ether and then with petroleum ether (40-60°C). The extract was then washed with ether saturated water and the ether evaporated off to give a concentrated extract.

The retinol was separated from the carotenoids and other inter-

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fering substances by thin-layer chromatography. Kieselgel G (Merck) thin-layer plates were activated at 100°C for 2 hr. The concentrated extract (ca 1 cm^3), dissolved in 1 cm^3 spotting solvent (petroleum ether:absolute ethanol:triethylamine, 20:1:1), was applied as a band to the plate. The flask was washed with 1 cm^3 of spotting solvent and the washings were then loaded onto the plate just below the band. The plate was developed in the dark with cyclohexane: ethyl acetate (3:1) and the trans-retinol band indentified with U.V. light. Trans-retinol was quantitatively recovered from the separated band with chloroform, and was colorimetrically measured according to the Carr-Price method at 610 nm (Carr and Price, 1926).

Treatment of data

The order of the degradation of trans-retinol in beef liver puree on heat processing was explored graphically by plotting different functions of the concentration against time of heating at constant temperature. A plot of $\log_e C_0/C$ (to normalize data) versus time generated a straight line, thus indicating first order kinetics for degradation of trans-retinol during thermal processing. Such a first order reaction is defined as:

$$\log_e(C_0/C) = kt$$

The reaction rate constant was determined from the slope of the line (calculated by regression analysis with all the lines forced through zero as instantaneous heating and cooling was assumed).

The activation energy and the frequency factor were derived from the Arrhenius equation defined as:

$$k = Ae^{-E_a/RT}$$

where k = rate constant (s^{-1}); A = frequency factor (s^{-1}); E_a = activation energy (J mol^{-1}); R = gas constant (J K^{-1} mol^{-1}); T = absolute temperature (K).

To determine the activation energy, $\log_e k$ was plotted against the inverse of absolute temperature and E_a deduced from the slope of the line. The frequency factor was determined from the intercept of the above plot.

RESULTS & DISCUSSION

THE CONCENTRATION relationships with time are shown in Figure 1. The reaction rate constants calculated from the results are shown in Table 1. The high correlation coefficients indicate that over the temperature range studied the kinetics of vitamin A degradation in beef liver puree were consistent with first order kinetics. Compared with the reaction coefficients calculated from the data of Maqsood et al. (1963), degradation of vitamin A was more rapid in heating ghee at 100°C than heating beef liver puree at 100°C.

To estimate the error in the raw data, a probability plot was constructed of % residuals, defined as:

$$(C_{pre} - C_{obs})/C_{pre}$$

where C_{pre} = predicted concentration, and C_{obs} = observed concentration. The probability plot was a straight line indicating that all deviation from the line was random. The deviations were assumed to be due to experimental uncertainty only. The major contributing factors could be variation in composition of the liver between each vial and the inherent error in the assay method.

The activation energy determined from the Arrhenius plot (Fig. 2) was 112 ± 9 kJ/mol (26.9 ± 2.1 kcal/mol) with 95% confidence. The activation energy for vitamin A in multivitamin tablets of 118 kJ/mol (calculated from the data of Slater et al., 1979) fell within the 95% confidence limits so there was no significant difference between the two values.

Differences in activation energies were reported for other vitamins when heated in different media. In the case of thiamin, an activation energy of 123 kJ/mol (29.4 kcal/mol) was reported for phosphate buffer and 114 kJ/mol (27.3kcal/mol) for beef puree (Mulley et al., 1975).

The frequency factor, A, was found to be 8.13 × $10^{11}s^{-1}$. The frequency factor is affected by the molecular structure of the reactants, and consequently there are considerable variations in it from system to system due to changes in structures of the reactants. This is exemplified by comparison of the present data with the pharmaceutical data of Slater et al. (1979). For vitamin A in beef liver puree, A had the value 8.13 × $10^{11}s^{-1}$ whilst for the multivitamin tablets, A was 4.98 × $10^{11}s^{-1}$. This difference could be due to several factors such as difference in component composition and other intrinsic properties such as pH.

—Continued on page 40

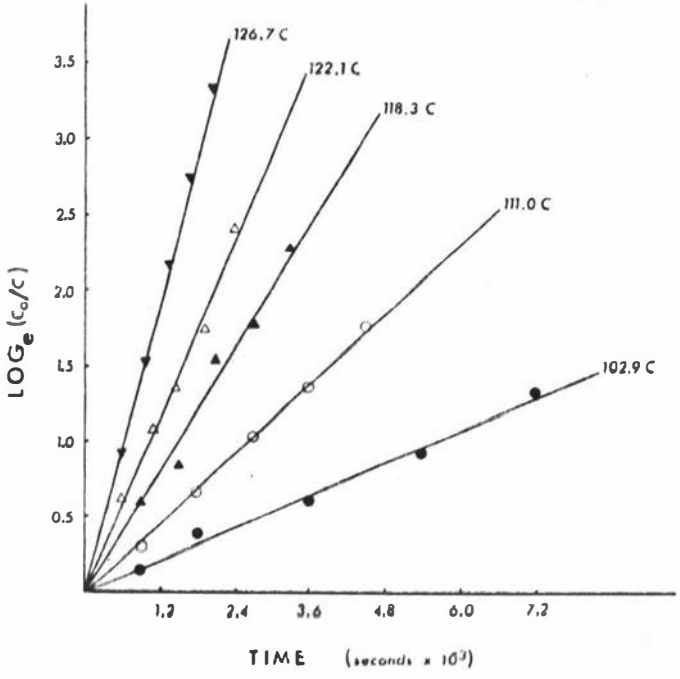

Fig. 1—Degradation rates for vitamin A in beef liver puree on heating, showing the natural logarithm of the concentration ratio as a function of time for various temperatures.

Table 1—First order reaction rate constants for trans-retinol with 95% confidence limits

Temp (°C)	k(s^{-1})	Correlation coefficient
102.9	(17.9 ± 0.7) × 10^{-5}	0.992
111.0	(38.6 ± 1.3) × 10^{-5}	0.993
118.3	(68.0 ± 2.8) × 10^{-5}	0.989
122.1	(96.1 ± 2.6) × 10^{-5}	0.995
126.7	(162.3 ± 1.7) × 10^{-5}	0.999

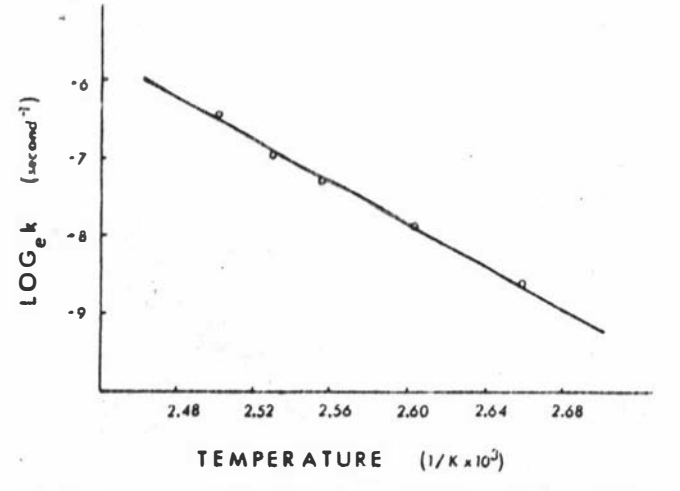

Fig. 2—Arrhenius plot for degradation of vitamin A in beef liver puree on heating, showing the natural logarithm of the first order rate constant as a function of the inverse of absolute temperature.

The comparison of the experimental results with the pharmaceutical data of Slater et al. (1979) and the analogy of thiamin, indicate that other factors apart from those studied, affect the degradation of vitamin A on heat processing. Hence, further work is planned to quantify the variation in kinetics as a function of other factors such as product composition and pH.

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Ms received 3/14/80; accepted 7/26/80.

APPENDIX A.2: Computer Listing for Temperature-Time Profile and Extent of Reaction in Glass Vial Program

```
100     FILE  5(KIND=DISK,TITLE="ECCLES",FILETYPE=7)
200     FILE  6(KIND=PRINTER)
300     DIMENSION K(100),C(100),T(100),TI(100),DEL(100)
350     REAL K
400     READ (5,/) THICK,NODE,NODED,DELT,DELR,TA,TIN,H,TIMTOT,NPRINT
500     DO 10 I=2,NODE+1
600     DEL(I)=0
700     10 TI(I)=TIN
800     TI(1)=TA
820     T(1)=TA
900     DO 20 I=2,NODED-1
1000    20 K(I)=1.09
1100    DO 30 I=NODED,NODE
1200    30 K(I)=0.50
1300    K(1)=H*DELR
1400    DO 40 I=3,NODED-1
1500    40 C(I)=1895500
1600    C(2)=C(3)/2
1700    DO 50 I=NODED+1,NODE
1800    50 C(I)=3605000
1900    C(NODED)=0.5*(C(NODED+1)+C(NODED-1))
2000    TIME=0
2100    NN=0
2200    60 TIME=TIME+DELT
2300    DO 70 I=3,NODE-1
2400    70 T(I)=DELT/DELR/DELR/C(I)*(K(I)*(TI(I+1)-TI(I))-K(I-1)*
2500    *  (TI(I)-TI(I-1)))+DELT/DELR/DELR/C(I)/FLOAT(NODE-I)*
2600    *  (K(I)+K(I-1))/4*(TI(I-1)-TI(I+1))+TI(I)
2610      T(NODE)=DELT/DELR/DELR/C(NODE)*2.*K(NODE-1)*(TI(NODE-1)
2615    *  -TI(NODE))+TI(NODE)
2620    T(2)=TI(2)+DELT/DELR/DELR/C(2)*(K(I)*(TI(1)-TI(2))
2630    *  -K(2)*(TI(2)-TI(3)))+H/THICK*DELT/C(2)*(TI(1)-TI(2))
2700    DO 110 I=NODED,NODE
2800    RATEC=8.13E11*EXP(-13564.225/(TI(I)+273.15))
2900    110 DEL(I)=RATEC*DELT+DEL(I)
3000    120 FORMAT (8F15.10)
3400    NN=NN+1
3500    IF(NN.LT.NPRINT)GO TO 80
3600    WRITE (6,90) TIME,(T(I),I=2,NODE)
3700    90 FORMAT (15F8.2)
3800    NN=0
3900    80 DO 100 I=2,NODE
4000    100 TI(I)=T(I)
4100    IF(TIME.LT.TIMTOT)GO TO 60
4150    WRITE(6,120) (DEL(I),I=2,NODE)
4200    STOP
4300    END
```

APPENDIX A.3: Concentration of Trans-Retinol in Natural Beef Liver Puree Before and After Thermal Processing

Table A.1: Concentration of Trans-Retinol After Heating for Various Times

Temperature (^{o}C)	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
102.9	0	181, 177, 170
	15	152, 147
	30	110, 118, 118, 124, 117
	60	94, 98, 96
	90	71, 78, 66, 65, 73, 76
	120	44, 43, 46, 51, 48
111.0	0	60, 72, 74
	15	46, 52, 52
	30	38, 33
	45	22, 29, 25, 25
	60	19, 16
	75	11, 14, 11, 13
118.3	0	58, 57, 50, 48, 49, 44
	15	29, 31, 25, 29
	25	23, 23, 22, 20
	35	11, 9, 11, 11, 13, 13
	45	8, 9, 9, 9
	55	4, 5, 6
122.1	0	319, 318, 320, 321
	10	168, 171, 180
	18	111, 105, 109
	24	96, 89, 95, 70, 74, 75
	32	56, 57
	40	29, 29
126.7	0	273, 263, 270, 277, 274
	10	108, 110, 111, 106, 105, 110, 111
	16	61, 60, 63, 53, 54, 57
	22	29, 31, 31, 31
	28	18, 18
	34	10, 10, 10

NOTE: There were differences in the initial trans-retinol concentrations. These were due to a loss in efficacy of the Carr-Price reagent over time. It was established that vitamin A loss followed first order kinetics and so the reaction was independent of initial concentration. Because of the length of time needed to obtain the data it was decided not to repeat the experiment.

APPENDIX B.1: Concentration of Trans-Retinol in Beef Liver Mixtures Before and After Thermal Processing (Mixture Design)

Table B.1: Concentration of Trans-Retinol After Heating for Various Times at 102.1°C

Vertex	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
1	0	282
	100	240
	200	221
	300	210, 209
	400	191
2	0	218
	100	207
	200	186
	300	176
	400	170
3	0	265
	60	240
	200	190
	300	169
	400	145
4	0	237
	100	229
	300	209
	400	198
Centroid	0	276
	80	264
	100	253
	300	223, 230
Centroid	0	272
	200	239
	300	229
	400	219

Table B.2: Concentration of Trans-Retinol After Heating for Various Times at 112.0°C

Vertex	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
1	0	291, 282
	70	237
	140	189
	210	155
	280	142
2	0	249
	70	225
	140	198
	210	196
	280	166
3	0	265, 265
	70	199
	140	148
	210	122
	280	98
4	0	227, 233, 222
	70	223
	140	199, 203
	210	205
	280	176
Centroid	0	299
	70	260
	210	190
	280	170
Centroid	0	290, 276
	70	259
	140	221
	210	196
	280	164

Table B.3: Concentration of Trans-Retinol After Heating for Various Times at 122.0°C

Vertex	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
1	0	285
	45	209
	90	157
	135	114
	180	103
2	0	247, 249
	90	191
	135	159
	180	127
3	0	253
	45	164
	90	125
	135	96
	180	62
4	0	241
	45	209
	90	201
	135	177, 167
	180	138
Centroid	0	332
	45	261
	135	155
	180	124
Centroid	0	282
	45	217
	90	177
	135	158
	180	135

APPENDIX C.1: Concentration of Trans-Retinol in Beef Liver Mixtures Before and After Thermal Processing (2^3 Factorial Design)

Table C.1: Concentration of Trans-Retinol After Heating for Various Times at 102°C

Design Point[a]	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
1	0	179, 192
	110	174
	220	161
	330	152, 146
a	0	217, 218
	110	204
	220	185
	330	167
b	0	231, 236
	110	221
	220	214
	330	195
ab	0	213, 211
	110	198
	220	182
	330	171
c	0	267, 270
	110	217
	220	191
	330	155
ac	0	256, 243
	110	209, 205
	220	149
	330	114
bc	0	258, 254
	110	220
	220	177
	330	156
abc	0	259, 252
	110	215
	220	186
	330	158

[a] Coding: a = copper concentration, b = pH, c = moisture content

Table C.2: Concentration of Trans-Retinol After Heating for Various Times at 122°C

Design Point[a]	Time (min)	Trans-Retinol Concentration ($\mu g g^{-1}$)
1	0	162, 160
	50	139
	100	123
	150	107
a	0	172, 172
	50	162
	100	154
	150	140, 141
b	0	201, 202
	50	167, 167
	100	151
	150	140
ab	0	179, 170
	50	167
	100	151
	150	141
c	0	259, 268
	40	160
	80	106
	120	73
ac	0	252, 249
	40	166
	80	119, 118
	120	82
bc	0	220, 239
	40	150
	80	94
	120	65
abc	0	180, 171
	40	147
	80	121
	120	93

[a] Coding: a = copper concentration, b = pH, c = moisture content

APPENDIX D.1: Computer Listing for Temperature-Time Profiles and Vitamin A Retention Program for Unsteady State Heating

```
      FILE  5(KIND=DISK,TITLE="INDATA",FILETYPE=7)                                  00000100
      FILE  6(KIND=PRINTER)                                                         00000200
      $SET FREE                                                                     00000300
      DIMENSION T(20,20),TI(20,20),DEL(20,20),DELL(20,20)                           00000400
      REAL K,M,N,KR,KRA                                                             00000500
      READ (5,*) DELR,DELX,NR,NX,K,C,DELT,THEAT,TCOOL,TIN,TAH,TAC,DELTT,           00000600
     * EA,A,R,NPRINT,EAA,AA                                                        00000700
       WRITE (6,*) DELR,DELX,NR,NX,K,C,DELT,TIN,DELTT,EA,A,R                      00000710
      DO 10 I=1,NX-1                                                              00000800
      DO 10 J=1,NR-1                                                              00000900
   10 TI(I,J)=TIN                                                                 00001000
      NN=0                                                                        00001100
      NH=THEAT/DELT                                                               00001200
      NC=(TCOOL-100.*DELTT)/DELT                                                  00001300
      DO 20 I=1,NX                                                                00001400
      T(I,NR)=TAH                                                                 00001500
   20 TI(I,NR)=TAH                                                                00001600
      DO 30 J=1,NR                                                                00001700
      T(NX,J)=TAH                                                                 00001800
   30 TI(NX,J)=TAH                                                                00001900
      DO 40 NN=1,NH                                                               00002000
      DO 50 I=2,NX-1                                                              00002100
      DO 50 J=2,NR-1                                                              00002200
   50 T(I,J)=DELT/DELR/DELR*K/C*(TI(I,J+1)-2.*TI(I,J)+TI(I,J-1))+                 00002300
     * TI(I,J)+0.5*DELT/DELR/DELR*K/C*(TI(I,J+1)-TI(I,J-1))/FLOAT(J-1)           00002400
     * +DELT/DELX/DELX*K/C*(TI(I+1,J)-2.*TI(I,J)+TI(I-1,J))                       00002500
      DO 60 J=2,NR-1                                                              00002600
   60 T(1,J)=DELT/DELR/DELR*K/C*(TI(1,J+1)-2.*TI(1,J)+TI(1,J-1))+                 00002700
     * TI(1,J)+0.5*DELT/DELR/DELR*K/C*(TI(1,J+1)-TI(1,J-1))/FLOAT(J-1)           00002800
     * +DELT/DELX/DELX*K/C*2.0*(TI(2,J)-TI(1,J))                                  00002900
      DO 70 I=2,NR-1                                                              00003000
   70 T(I,1)=DELT/DELR/DELR*K/C*4.0*(TI(I,2)-TI(I,1))+DELT/C/DELX                 00003100
     * /DELX*K*(TI(I+1,1)-2.*TI(I,1)+TI(I-1,1))+TI(I,1)                          00003200
      T(1,1)=DELT/DELR/DELR*K/C*4.0*(TI(1,2)-TI(1,1))+TI(1,1)+2./C*               00003300
     * DELT/DELX/DELX*K*(TI(2,1)-TI(1,1))                                         00003400
      DO 80 I=1,NX                                                                00003500
      DO 80 J=1,NR                                                                00003600
      KR=A*EXP(-EA/R/((T(I,J)+TI(I,J))/2.+273.16))                                00003700
      KRA=AA*EXP(-EAA/R/((T(I,J)+TI(I,J))/2.+273.16))                             00003720
      DELL(I,J)=KRA*DELT+DELL(I,J)                                                00003740
   80 DEL(I,J)=KR*DELT+DEL(I,J)                                                   00003800
      NN=NN+1                                                                     00003900
      IF(NN .LT. NPRINT) GO TO 110                                                00004000
```

```
      NN=0                                                                  00004100
      TIME=FLOAT(MM)*DELT                                                   00004200
      WRITE (6,90) TIME                                                     00004300
      DO 100 I=1,NX                                                         00004400
      WRITE (6,120) (T(I,J),J=1,NR)                                         00004500
      WRITE(6,125) (DELL(I,J),J=1,NR)                                       00004550
  100 WRITE (6,125) (DEL(I,J),J=1,NR)                                       00004600
   90 FORMAT(' ',//,' TIME= ',F6.1,//)                                      00004700
  125 FORMAT(11G10.4)                                                       00004800
  120 FORMAT(11F10.4)                                                       00004900
  110 DO 130 I=1,NX-1                                                       00005000
      DO 130 J=1,NR-1                                                       00005100
  130 TI(I,J)=T(I,J)                                                        00005200
   40 CONTINUE                                                              00005300
      TIME=THEAT                                                            00005310

      NN=0                                                                  00005320
      DO 510 I=1,NX                                                         00005330
      WRITE(6,120) (T(I,J),J=1,NR)                                          00005340
      WRITE(6,125) (DELL(I,J),J=1,NR)                                       00005350
      WRITE(6,125) (DEL(I,J),J=1,NR)                                        00005360
  510 CONTINUE                                                              00005370
      DO 135 I=1,NX                                                         00005400
      T(I,NR)=TAC                                                           00005500
  135 TI(I,NR)=TAC                                                          00005600
      DO 145 J=1,NR                                                         00005700
      T(NX,J)=TAC                                                           00005800
  145 TI(NX,J)=TAC                                                          00005900
      DO 140 MM=1,100                                                       00006000
      DO 150 I=2,NX-1                                                       00006100
      DO 150 J=2,NR-1                                                       00006200
  150 T(I,J)=DELTT/C/DELR/DELR*K*(TI(I,J+1)-2.*TI(I,J)+TI(I,J-1))+          00006300
     * TI(I,J)+0.5*DELTT/C/DELR/DELR*K*(TI(I,J+1)-TI(I,J-1))/FLOAT(J-1)    00006400
     * +DELTT/C/DELX/DELX*K*(TI(I+1,J)-2.*TI(I,J)+TI(I-1,J))                00006500
      DO 160 J=2,NR-1                                                       00006600
  160 T(1,J)=DELTT/C/DELR/DELR*K*(TI(1,J+1)-2.*TI(1,J)+TI(1,J-1))+          00006700
     * TI(1,J)+0.5*DELTT/C/DELR/DELR*K*(TI(1,J+1)-TI(1,J-1))/FLOAT(J-1)    00006800
     * +DELTT/C/DELX/DELX*K*2.0*(TI(2,J)-TI(1,J))                           00006900
      DO 170 I=2,NR-1
```

```
      170 T(I,1)=DELTT/C/DELR/DELR*K*4.0*(TI(I,2)-TI(I,1))+DELTT/C/DELX
     * /DELX*K*(TI(I+1,1)-2.*TI(I,1)+TI(I-1,1))+TI(I,1)
      T(I,1)=DELTT/C/DELR/DELR*K*4.0*(TI(I,2)-TI(I,1))+TI(I,1)+2./C*
     * DELTT/DELX/DELX*K*(TI(2,1)-TI(1,1))
      DO 180 I=1,NX
      DO 180 J=1,NR
      KR=A*EXP(-EA/R/((T(I,J)+TI(I,J))/2.+273.16))
      KRA=AA*EXP(-EAA/R/((T(I,J)+TI(I,J))/2.+273.16))
      DELL(I,J)=KRA*DELTT+DELL(I,J)
  180 DEL(I,J)=KR*DELTT+DEL(I,J)
      NN=NN+1
      IF(NN .LT. NPRINT) GO TO 210
      NN=0
      TIME=THEAT*FLOAT(MM)*DELTT
      WRITE (6,90) TIME
      DO 200 I=1,NX
      WRITE (6,120) (T(I,J),J=1,NR)
      WRITE(6,125) (DELL(I,J),J=1,NR)
  200 WRITE (6,125) (DEL(I,J),J=1,NR)
  210 DO 230 I=1,NX-1
      DO 230 J=1,NR-1
  230 TI(I,J)=T(I,J)
  140 CONTINUE
      IF(NN  .NE. 0) GO TO 520
      NN=0
      TIME=THEAT*DELTT*100.
      DO 530 I=1,NX
      WRITE(6,120) (T(I,J),J=1,NR)
      WRITE(6,125) (DELL(I,J),J=1,NR)
      WRITE(6,125) (DEL(I,J),J=1,NR)
  530 CONTINUE
  520 CONTINUE
      DO 240 MM=1,NC
      DO 250 I=2,NX-1
      DO 250 J=2,NR-1
  250 T(I,J)=DELT/DELR/DELR*K/C*(TI(I,J+1)-2.*TI(I,J)+TI(I,J-1))+
     * TI(I,J)+0.5*DELT/DELR/DELR*K/C*(TI(I,J+1)-TI(I,J-1))/FLOAT(J-1)
     * +DELT/DELX/DELX*K/C*(TI(I+1,J)-2.*TI(I,J)+TI(I-1,J))

      DO 260 J=2,NR-1
  260 T(1,J)=DELT/DELR/DELR*K/C*(TI(1,J+1)-2.*TI(1,J)+TI(1,J-1))+
     * TI(1,J)+0.5*DELT/DELR/DELR*K/C*(TI(1,J+1)-TI(1,J-1))/FLOAT(J-1)
     * +DELT/DELX/DELX*K/C*2.0*(TI(2,J)-TI(1,J))
      DO 270 I=2,NR-1
  270 T(I,1)=DELT/DELR/DELR*K/C*4.0*(TI(I,2)-TI(I,1))+DELT/C/DELX
```

```
11100     * /DELX*K*(TI(I+1,1)-2.*TI(I,1)+TI(I-1,1))+TI(I,1)                    00011100
11200     T(1,1)=DELT/DELR/DELR*K/C*4.0*(TI(1,2)-TI(1,1))+TI(1,1)*2./C*        00011200
11300     * DELT/DELX/DELX*K*(TI(2,1)-TI(1,1))                                 00011300
11400     DO 280 I=1,NX                                                        00011400
11500     DO 280 J=1,NR                                                        00011500
11600     KR=A*EXP(-EA/R/((TI(I,J)+TI(I,J))/2.+273.16))                        00011600
11620     KRA=AA*EXP(-EAA/R/((TI(I,J)+TI(I,J))/2.+273.16))                     00011620
11640     DELL(I,J)=KRA*DELT+DELL(I,J)                                         00011640
11700     280 DEL(I,J)=KR*DELT+DEL(I,J)                                        00011700
11800     NN=NN+1                                                              00011800
11900     IF(NN .LT. NPRINT) GO TO 310                                         00011900
12000     NN=0                                                                 00012000
12100     TIME=THEAT*100.*DELTT*FLOAT(MM)*DELT                                 00012100
12200     WRITE (6,90) TIME                                                    00012200
12300     DO 300 I=1,NX                                                        00012300
12400     WRITE (6,120) (T(I,J),J=1,NR)                                        00012400
12450     WRITE(6,125) (DELL(I,J),J=1,NR)                                      00012450
12500     300 WRITE (6,125) (DEL(I,J),J=1,NR)                                  00012500
12600     310 DO 330 I=1,NX-1                                                  00012600
13000     DO 330 J=1,NR-1                                                      00013000
13100     330 TI(I,J)=T(I,J)                                                   00013100
13200     240 CONTINUE                                                         00013200
13300     TIME=THEAT*TCOOL                                                     00013300
13400     WRITE(6,90) TIME                                                     00013400
13500     DO 400 I=1,NX                                                        00013500
13600     WRITE (6,120) (T(I,J),J=1,NR)                                        00013600
13650     WRITE(6,125) (DELL(I,J),J=1,NR)                                      00013650
13700     400 WRITE (6,125) (DEL(I,J),J=1,NR)                                  00013700
13800     DO 410 I=1,NX                                                        00013800
13900     DO 410 J=2,NR-1                                                      00013900
14000     CA=EXP(-DEL(I,J))                                                    00014000
14100     CB=EXP(-DELL(I,J))                                                   00014100
14200     V=3.1415926*DELX*DELR*DELR*((FLOAT(J)-0.5)**2-(FLOAT(J)-1.5)**2)     00014200
14220     IF(I .EQ. NX)V=V/2.                                                  00014220
14240     IF(I .EQ. 1)V=V/2.                                                   00014240
14300     TOT=TOT+V*CA                                                         00014300
14400     TOTA=TOTA+V*CB                                                       00014400
14450     410 CONTINUE                                                         00014450
14500     DO 420 I=2,NX-1                                                      00014500
14600     CA=EXP(-DEL(I,1))                                                    00014600
14700     CB=EXP(-DELL(I,1))                                                   00014700
14800     V=3.1415926/4.*DELX*DELR*DELR                                        00014800
14900     TOT=TOT+V*CA                                                         00014900
15000     420 TOTA=TOTA+V*CB                                                   00015000
15100     CA=EXP(-DEL(1,1))                                                    00015100
15200     CB=EXP(-DELL(1,1))                                                   00015200
15300     V=V/2.                                                               00015300
15400     TOT=TOT+V*CA                                                         00015400
15500     TOTA=TOTA+V*CB                                                       00015500
15600     CA=EXP(-DEL(NX,1))                                                   00015600
15700     CB=EXP(-DELL(NX,1))                                                  00015700
15800     TOT=TOT+V*CA                                                         00015800
15900     TOTA=TOTA+V*CB                                                       00015900
16000     DO 430 I=2,NX-1                                                      00016000
16100     V=3.1415926*DELX*DELR*DELR*9.75                                      00016100
```

```
      CA=EXP(-DEL(I,NR))                                                00016200
      CB=EXP(-DELL(I,NR))                                               00016300
      TOT=TOT+V*CA                                                      00016400
      TOTA=TOTA+V*CB                                                    00016500
  630 CONTINUE                                                          00016600
      V=V/2.                                                            00016700
      CA=EXP(-DEL(NX,NR))                                               00016800
      CB=EXP(-DELL(NX,NR))                                              00016900
      TOT=TOT+V*CA                                                      00017000
      TOTA=TOTA+V*CB                                                    00017100
      CA=EXP(-DEL(1,NR))                                                00017200
      CB=EXP(-DELL(1,NR))                                               00017300
      TOT=TOT+V*CA                                                      00017400
      TOTA=TOTA+V*CB                                                    00017500
      V=3.1415926*DELX*DELR*DELR*1000.                                  00017600
      CA=TOT/V                                                          00017700
      CB=TOTA/V                                                         00017800
      WRITE(6,7) CA,CB                                                  00017900
      STOP                                                              00018000
      END                                                               00018100
```